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Microbiological and Chemical Studies on Edible Fresh Table Eggs

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Abstract

The aim of the present study was to assess the quality of table eggs produced in battery and floor production systems. Storage of table eggs in refrigerator is a popular practise; it may lead to increase the risks of eggs contamination. A total of 100 table eggs were collected from battery and floor farms in Sharkia province and then stored in refrigerator at 4°C. The collected eggs were divided into two groups (50 of each). Each group was divided into five sub-groups for examination: at laying time, the 7th, 14th, 21st, and 28th days. They were subjected to physical, chemical, and microbiological examination of eggshells and internal contents. The results of the current study indicated that the storage had a major effect on the egg quality parameters including eggs, albumen, and yolk weights and internal contents pH, in both battery and floor eggs. In addition, the microbiological examination showed that the eggshells, particularly in the floor system, were higher in bacterial contamination with *E. coli* and *Salmonella* than the internal contents. The egg quality traits that were done by this study help to protect consumers against foodborne diseases.

KEYWORDS
Table eggs, Egg quality, Eggshells, Egg contents, Egg contamination, Storage, Foodborne diseases.

INTRODUCTION

Egg is one of the major poultry economical products. It is one of the most nutritive, low caloric value, ease of digestibility, and complete foods known to man (Al-Obaidi *et al*., 2011; Awny *et al*., 2018). It provides an excellent source of protein for man in all ages. Eggs contain all vitamins and minerals needed by human beings except vitamin C. They contain about 65% water, 12% proteins, and 11% fat (Mansour *et al*., 2015; Sadek *et al*., 2016; Paul *et al*., 2017).

The fresh eggshell has outer waxy and inner shell membranes. These egg protective barriers are effective against entry of microorganisms before laying, trans-ovarian, and after laying. Freshly laid eggs are generally devoid of organisms. The different sources of egg contamination included the vent, floor liter, fecal matter, dirty nesting materials, soil, dust, improper handling and washing, the type of detergent used, pH of the washing solution, and inadequate sanitization of the equipment. Some other factors such as environmental temperature and humidity due to poor storage conditions of the fresh eggs influence the bacterial penetration, and thus enhance the infection and spoilage (Mansour *et al*., 2015; Sadek *et al*., 2016).

Contamination of eggs with microorganisms may affect their quality and transmit pathogens or intoxication to consumers, causing food-borne diseases and public health hazards (Awny *et al*., 2018). Ever since some people take raw eggs to enhance their blood synthesis process principally among malnourished and anemic patients. At the same time, the many nutrient substances present in eggs create an excellent environment for the development of bacterial microflora, including pathogenic bacteria (Mansour *et al*., 2015).

Several pathogenic microorganisms have been isolated from the surface of eggshells and contents and could cause outbursts of foodborne diseases. The most common pathogens are *Listeria monocytogenes*, *Yersinia enterocolitica*, *E. coli*, *Salmonella* and *Campylobacter*, Fungi, and *Staphylococci*. Among all bacterial pathogens, egg-borne *Salmonella* appears to be the most essential cause of foodborne diseases (Adesiyun *et al*., 2005; Stępień-Pyśniak *et al*., 2010; Adesiyun *et al*., 2020).

Eggs deterioration commences soon after lay. Thus, egg handling and storage rehearses have a significant influence on the eggs quality before reaching the consumers (Okoleh and Eze 2016; Hisasaga *et al*., 2020). Albumen quality is a standard measure of the egg quality. To evaluate egg quality, several considerations used to determine the egg size and contents consistency, moreover the structural integrity of the eggshell and the yolk membrane (Silversides and Scott, 2001; Samli *et al*., 2005).

Regarding the measures of egg quality used in this study, they are essential for evaluating the effect of storage and the different production systems (battery and floor production systems) on the quality of commercial table eggs. These traits are important to protect consumers against health hazards caused by food-borne infection and intoxication.

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MATERIALS AND METHODS

Collection of samples

A total of 100 table eggs were collected from private farms in Sharkia Governorate, Egypt, during November 2022. The eggs were obtained from Hy-Line white hens, aged 40 weeks. The collected samples were packed in a sterile plastic bag and transferred directly to the laboratory for physical, chemical, and microbiological examination. The eggs were stored in refrigerator at 4°C until analysed. They were divided into two groups (50 of each), the first group of eggs from battery farms and the second group of eggs from floor farms. Each group was divided into five sub-groups with different storage periods for examination: at the laying day (fresh eggs), the 7th, 14th, 21st, and 28th days.

Physical analysis

Weight of the eggs

The weight of each egg was taken and recorded by using a digital weighing scale (model: AX 1000). After breaking the egg on a flat surface, the yolk was separated from the albumen, and both were distributed into two glass beakers. Then, the weights of the albumen and yolk were recorded by the digital weighing scale.

Thickness of eggshells

The eggshell thickness was measured by micrometer (Series 293-IP65, Mitutoyo Corp. Kanagawa, Japan).

Chemical analysis

The yolk was separated from the albumen, and each was dispersed into glass beaker.

pH determination

The pH of the albumen and yolk were measured with a pH meter (Electronic Instrument Ltd). About 2 g of the sample was homogenized in 20ml of de-ionized water in a beaker. Firstly, the pH meter was standardized by using buffer solution (of 4.01 and 9.20 pH). Then, the electrode was rinsed with de-ionized water and dipped into the homogenate, with allowing sufficient time for stabilization before taking the reading.

Microbiological analysis

Preparation of samples for microbiological examination

The previously mentioned five groups of collected samples were examined for microbial examination. The eggshells were studied by the surface rinse method, as described by Moats (1979). The eggs contents were prepared for examination by evacuating their contents, according to Bailey and Scott (1998(.

Isolation and identification of *E. coli* was carried out in accordance with apadopoulou *et al.* (1997).

Isolation and identification of *Salmonella* was performed according to Cox (1988).

Statistical analysis

The values were presented as means±standard error (SE). The data were subjected to the statistical package for social sciences (SPSS-16.; Chicago, IL, USA) software and one-way analysis of variance (ANOVA) at 95 % level of confidence. Significant differences among the means were determined by Tukey's Kramer HD test, considering $P < 0.05$ as significant (Lee and Lee, 2018).

RESULTS

These results showed the effect of storage period on all parameters that assess the internal and external egg quality. Tables 1-4, revealed that there was a bad effect of storage period on all studied physical and chemical parameters, including egg weight, eggshell thickness, albumen weight, yolk weight, and albumen and yolk pH. In addition, the incidences of *E. coli* and *Salmonella* in the eggshell and eggs contents in the battery and floor systems, during different storage periods were illustrated in (Tables 5 and 6, and Figs. 1 and 2).

Table 1. Physical characters of the egg of the examined battery eggs and floor eggs during storage period (n. = 50, 10 eggs of each group).

		Egg – weight (g)	
		Battery eggs	Floor eggs
Fresh (0 day)	Min	53.71	63.9
	Max	54.34	66.2
	Mean±SE	54.00±0.06^{a}	65.11±0.28^{a}
7th day	Min	53.49	62.88
	Max	54.01	65.18
	Mean±SE	53.72±0.05^{b}	64.27±0.22^{b}
14th day	Min	53.49	64.18
	Max	53.71	64.35
	Mean±SE	53.60±0.02^{b}	64.29±0.02^{b}
21st day	Min	53.18	63.81
	Max	53.57	64.18
	Mean±SE	53.34±0.04^{c}	64.01±0.04^{b}
28th day	Min	52.87	63.71
	Max	53.3	63.95
	Mean±SE	53.03±0.05^{d}	63.83±0.03^{b}

Min: Minimum; Max: Maximum; SE: Standard error of mean
Means within the same column carrying different superscripts are significantly different at ($p < 0.05$) based on Tukey's Kramer HD test.

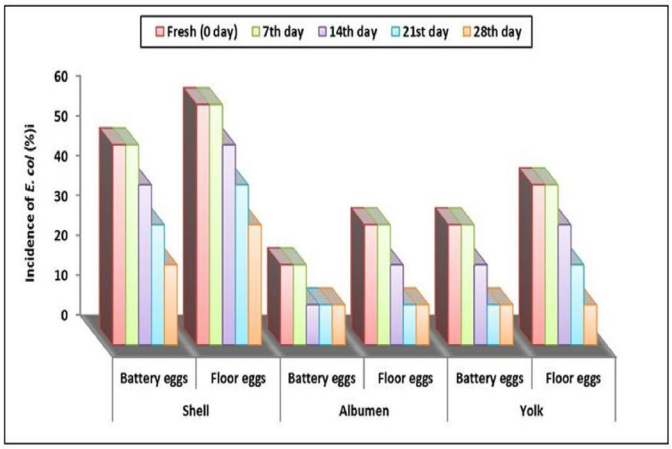

Fig. 1. Incidence of *E. coli* isolated from examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group).

Table 2. Physical characters of the shell of the examined battery eggs and floor eggs during storage period (n. = 50, 10 eggs of each group)

		Shell - Thickness (mm)	
		Battery eggs	Floor eggs
Fresh (0 day)	Min	0.34	0.33
	Max	0.35	0.34
	Mean±SE	0.35±0.001[a]	0.34±0.001[a]
7th day	Min	0.33	0.32
	Max	0.34	0.33
	Mean±SE	0.34±0.001[b]	0.33±0.001[b]
14th day	Min	0.32	0.31
	Max	0.33	0.32
	Mean±SE	0.33±0.001[c]	0.31±0.001[c]
21st day	Min	0.31	0.3
	Max	0.32	0.31
	Mean±SE	0.32±0.0009[d]	0.31±0.001[d]
28th day	Min	0.3	0.29
	Max	0.32	0.3
	Mean±SE	0.31±0.001[e]	0.30±0.001[e]

Min: Minimum; Max: Maximum; SE: Standard error of mean; %: Percentage
Means within the same column carrying different superscripts are significantly different at ($p < 0.05$) based on Tukey's Kramer HD test.

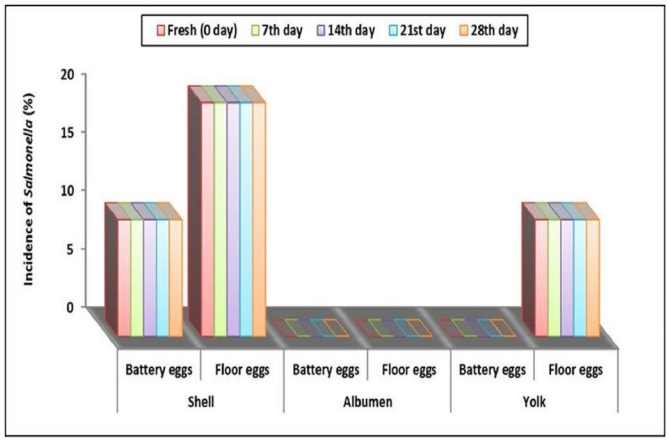

Fig. 2. Incidence of *Salmonella* isolated from examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group).

DISCUSSION

Concerning the physical characters of the examined eggs during different storage period, the egg- weight means showed different significant variations between types of eggs. The egg weight (mean±SE) of the battery and floor eggs at zero day (fresh eggs) was significantly higher than other examined periods (54.00±0.06 for battery and 65.11±0.28 for floor eggs). Thus, there was a decrease in egg weight when storage time increased. This result agreed with the result of Okoleh and Eze, (2016) who showed increased egg weight loss from 1.91 g at the 7th day to 3.60 g at 21st day of storage. Also, Walsh *et al.* (1995) noted that the egg weight decreased within 7 and 14 days of storage (0.36 and 0.57 g, respectively). Similarly, Kralik *et al.* (2014) noted that both fresh and stored eggs for 28 days at 4°C had loss in weight and 65.85 g, respectively).

Decrease in egg weight due to storage was attributed to decrease of albumen weight. This result was coincided with those mentioned by Scott and Silversides (2000). In addition, Eke *et al.* (2013) said that there was a significant loss of weight of eggs during the four weeks of storage. They endorsed this to the increase in shell pores as the egg aged. The increase in shell pores causes easier escape of moisture and gases from the eggs.

The breakdown of the carbonic acid inside the egg-white produces carbon dioxide and water. The carbon dioxide escapes through the shell pores and the egg-white thickness loses and becomes watery, this results in loss of egg weight (Samli *et al.*, 2005) mentioned that egg weight was not significantly decreased by storage for zero to ten days. Also, Lee *et al.* (2016) mentioned that storage time was negatively correlated with the egg weight.

In this study, the statistical analysis of the albumen weight revealed that, there was no significant variation observed in the weight of albumen in battery eggs at zero, 7th, and 21st days and between the 21st and 28th days. While there was a significant difference noticed at zero, 7th, and 21st days in floor eggs. Additionally, the 28th day of storage was significantly lower than other periods in both battery and floor eggs (29.10±0.99 and 34.49±0.01 respectively). The decrease in albumen weight when storage time

Table 3. Physical and chemical characters of the albumen of the examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group

		Albumen			
		Battery eggs		Floor eggs	
		Weight (g)	pH	Weight (g)	pH
Fresh (0 day)	Min	31.54	8.19	35.1	8.08
	Max	31.65	8.25	38.17	8.9
	Mean±SE	31.61±0.01[a]	8.22±0.01[e]	36.86±0.31[a]	8.19±0.08[e]
7th day	Min	31.09	8.38	35.71	8.75
	Max	31.34	8.47	36.41	8.84
	Mean±SE	31.22±0.03[a]	8.43±0.01[d]	36.04±0.07[b]	8.80±0.01[d]
14th day	Min	30.79	8.61	35.65	8.93
	Max	31.14	8.72	35.73	9.03
	Mean±SE	30.99±0.04[a]	8.67±0.01[c]	35.69±0.01[b]	8.98±0.01[c]
21st day	Min	30.28	8.83	34.73	9.16
	Max	30.51	8.91	34.82	9.25
	Mean±SE	30.38±0.02[ab]	8.87±0.01[b]	34.79±0.01[c]	9.21±0.01[b]
28th day	Min	20.25	9.1	34.45	9.41
	Max	30.32	9.18	34.52	9.49
	Mean±SE	29.10±0.99[b]	9.13±0.01[a]	34.49±0.01[c]	9.45±0.01[a]

Min: Minimum; Max: Maximum; SE: Standard error of mean
Means within the same column carrying different superscripts are significantly different at ($p < 0.05$) based on Tukey's Kramer HD test.

increased was corresponded to the result of Okoleh and Eze, (2016) who noticed decrease in albumen weight from zero day (22.6 g) to the 21st day of storage (19.8 g). In addition, Kralik *et al.* (2014) noted that both fresh and stored eggs for 28 days showed loss in albumen weight (41.12 g and 39.96 g, respectively). While Akyurek and Okur (2009) reported that the changes in the storage period did not affect the albumen weight.

Moreover, there was different significance found in yolk weight of battery eggs at the different storage periods, while no significant variations observed in yolk weight in the floor eggs. The yolk weight increased gradually from fresh day (15.71±0.07 and 21.14±0.16) until the 28th day of storage (16.51±0.07 and 22.61±0.03) in battery and floor eggs, respectively. Therefore, the storage affected the loss in yolk weight. This result was in agreement with those mentioned by Kucukkoyuncu *et al.* (2017). Generally, the loss of yolk weight disagreed with Kralik *et al.* (2014) who noted that the yolk weight did not affect by changes in storage period (15.53 g and 15.78 g of fresh and stored for 28 days, respectively). While the obtained results agreed with Okoleh and Eze (2016) who noticed that the yolk weight decreased when storage time increased, from 17.1g at zero day to 15.6g at the 21st day of storage.

Regarding the eggshell-thickness (mean±SE) in both battery and floor eggs, there was a high significant difference between the different storage periods. Among the different storage periods, the highest eggshell-thickness (mm) was noticed at zero day in both battery and floor eggs (0.35±0.001 and 0.34±0.001, respectively), while the 28th day exhibited the lowest eggshell-thickness in both battery and floor eggs (0.31±0.001 and 0.30±0.001, respectively). These findings were differed from the results of Kralik *et al.* (2014) who noted that the storage period did not affect the eggshell thickness. In our result, the production system (battery and floor eggs) had the same effect to some extent on the eggshell-thickness during the whole storage period. This result was matched with those mentioned by Yenice *et al.* (2016) who noticed similar values of eggshell thickness in two different production systems (0.39±0.002 for the battery production system and 0.39±0.003 for the free range system).

Albumen pH is used to determine albumen quality. Accordingly, it measures essentially the freshness of the egg; this principle was mentioned by Scott and Silversides (2000). The results of this study revealed that statistical analysis of the albumen pH showed different significant variations between battery and floor eggs at different storage periods. The 28th day was significantly higher in pH than other periods in both battery and floor eggs (9.13±0.01 and 9.45±0.01, respectively), whereas the battery and floor eggs at zero day showed lower significance in pH (8.22±0.01 and 8.19±0.08, respectively). Consequently, albumen pH increased significantly with increasing storage time. This result was in accordance with findings of Okoleh and Eze (2016) who recorded that albumen pH was higher in eggs stored for 7 and 21 days (0.44 to 0.80%, respectively), than in fresh eggs. Gavril and Usturoi (2012) noted an increase of albumen pH from 7.6 to 9.7 due to storage. Moreover, Kralik *et al.* (2014) noted that

Table 4. Physical and chemical characters of the yolk of the examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group).

		Yolk			
		Battery eggs		Floor eggs	
		Weight (g)	pH	Weight (g)	pH
Fresh (0 day)	Min	15.48	6.11	20.33	5.75
	Max	16.12	6.2	21.96	5.82
	Mean±SE	15.71±0.07^{c}	6.15±0.01^{e}	21.14±0.16^{a}	5.78±0.01^{e}
7th day	Min	15.64	6.34	20.28	5.93
	Max	16.05	6.42	22.26	6.02
	Mean±SE	15.89±0.04^{bc}	6.37±0.01^{d}	21.34±0.19^{a}	5.98±0.01^{d}
14th day	Min	15.87	6.47	21.63	6.05
	Max	16.29	6.94	21.88	6.13
	Mean±SE	16.09±0.04^{b}	6.55±0.04^{c}	21.78±0.03^{a}	6.09±0.01^{c}
21st day	Min	16.02	6.58	2.27	6.14
	Max	16.75	6.68	22.63	6.22
	Mean±SE	16.43±0.07^{a}	6.63±0.01^{b}	20.34±2.01^{a}	6.18±0.01^{b}
28th day	Min	16.28	6.7	22.49	6.22
	Max	16.97	6.79	22.78	6.3
	Mean±SE	16.51±0.07^{a}	6.75±0.01^{a}	22.61±0.03^{a}	6.27±0.01^{a}

Min: Minimum; Max: Maximum; SE: Standard error of mean
Means within the same column carrying different superscripts are significantly different at ($p < 0.05$) based on Tukey's Kramer HD test.

Table 5. Incidence of *E. coli* isolated from examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group).

E. coli	Shell				Albumen				Yolk			
	Battery eggs		Floor eggs		Battery eggs		Floor eggs		Battery eggs		Floor eggs	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh (0 day)	5	50	6	60	2	20	3	30	3	30	4	40
7th day	5	50	6	60	2	20	3	30	3	30	4	40
14th day	4	40	5	50	1	10	2	20	2	20	3	30
21st day	3	30	4	40	1	10	1	10	1	10	2	20
28th day	2	20	3	30	1	10	1	10	1	10	1	10
Total (No. = 50)	19	38	24	48	7	14	10	20	10	20	14	28

No.: Number, %: Percentage
% of each group was calculated according to the number of the examined eggs in each day group (No. = 10), Total % was calculated according to the number of the examined eggs type (No. = 50)

Table 6. Incidence of *Salmonella* isolated from examined battery eggs & floor eggs during storage period (n. = 50, 10 eggs of each group).

Salmonella	Shell				Albumen				Yolk			
	Battery eggs		Floor eggs		Battery eggs		Floor eggs		Battery eggs		Floor eggs	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh (0 day)	1	10	2	20	0	0	0	0	0	0	1	10
7th day	1	10	2	20	0	0	0	0	0	0	1	10
14th day	1	10	2	20	0	0	0	0	0	0	1	10
21st day	1	10	2	20	0	0	0	0	0	0	1	10
28th day	1	10	2	20	0	0	0	0	0	0	1	10
Total (No. = 50)	5	10	10	20	0	0	0	0	0	0	5	10

No.: Number, %: Percentage

% of each group was calculated according to the number of the examined eggs in each day group (No. = 10), Total % was calculated according to the number of the examined eggs type (No. = 50)

values of pH in albumen increased from 8.66 to 9.01. In contrast Walsh *et al.* (1995) reported that the storage time did not influence albumen pH. Increase in alkalinity of the egg albumen due to egg storage was caused by water loss by evaporation through the shell pores and the escape of carbon dioxide from albumen, this was explained by Eke *et al.* (2013).

Furthermore, different significant variations detected in yolk pH of battery and floor eggs at different storage periods. In addition, the 28th day showed higher significance of pH in both battery and floor eggs (6.75±0.01 and 6.27±0.01, respectively). While the battery and floor eggs at zero day showed lower significance in pH (6.15±0.01 and 5.78±0.01, respectively). Therefore, the yolk pH increased significantly with increasing storage time, as recorded by Okoleh and Eze (2016). Additionally, yolk pH was higher in eggs stored for 7 and 21 days (0.83 to 1.64%, respectively), than in fresh eggs. Moreover, Kralik *et al.* (2014) noted that values of pH in yolk increased from 5.99 to 6.06 due to storage. In addition, Lee *et al.* (2016) illustrated an increase in the egg yolk pH along with the storage time, but the changes of the yolk pH were not as large as that of the albumen pH.

The net effect of all previous changes in egg quality was due to storage, especially the loss in egg weight and decrease in albumen quality. Therefore, the maximum egg quality values were detected in the fresh eggs and are decreased with increasing storage time. These observations were coincided with the findings of Lee *et al.* (2016) and Singh *et al.* (2011).

The obtained results in this study showed the incidence of *E. coli* isolated from battery and floor eggs in the eggshells, albumen, and yolk) during different storage periods. The eggshell possessed the highest percent of *E. coli* (38 and 48 % in battery and floor eggs, respectively). Nevertheless, the egg contents had lower incidence of *E. coli*; 14 and 20% in albumen and 20 and 28 % in yolk of battery and floor eggs, respectively. Our results agreed with Adesiyun *et al.* (2005) who found *E. coli* on 58.7% of shells and in 4.3% of egg contents of farm eggs. Besides, Sadek *et al.* (2016) recorded that *E. coli* incidence was 6.7% in battery eggshells and 53.3 % in floor eggshells.

Besides, our findings showed that the floor eggs had higher contamination with *E. coli* than battery eggs. This result disagreed with Awny *et al.* (2018) who noticed failure of *E. coli* isolation from floor egg contents, and in agreement with Sadek *et al.* (2016) who found 6.7 % incidence of *E. coli* in floor eggs contents. However, they noticed negativity of *E. coli* isolation from battery egg contents. This was attributed to better hygienic measures and strict control measures against bacteria during production and handling in battery system than the floor one.

As *E. coli* is a normal microflora of intestinal tracts of birds, it can contaminate the eggshell and penetrate it to contaminate the egg contents, so this bacterium is the major microorganism isolated from both eggshell and the content. It can cause diarrhea and other public health hazards, as mentioned by Awny *et al.* (2018). Presence of *E. coli* in eggs is a good indicator to fecal contamination of eggs and the probability of presence of other enteric pathogens agents, which constitute public health hazards to consumers, as reported by Sadek *et al.* (2016).

The statistical analysis revealed that, *Salmonella* was found on the shells of 10 % of battery eggs and 20 % of floor eggs (Total No.= 50 eggs), with failure of isolation of the *Salmonella* from the egg contents (only 10% isolated from floor eggs yolk). The results of the present study were similar to data published by Mansour *et al.* (2015) who found the frequency of *Salmonella* occurrence on the shell was 4.7% and it was significantly higher than of the egg contents (1.2%). In addition, Awny *et al.* (2018) showed that the lowest incidence of *Salmonella* is found in battery egg contents. Moreover, Adesiyun *et al.* (2005) isolated *Salmonella* from 6.5% of shells and 6.5% of fresh egg contents, and from 2.8% of stored eggshells and 7.5% of egg contents. While Sadek *et al.* (2016) could not isolate *Salmonella* from the eggshell and contents of battery and floor eggs, they attributed that to competition effect of aerobic contaminant or using the antibiotic as a treatment or as a growth promoter, might inhibit *Salmonella* isolation. From the public health point of view, *Salmonella*e remained a potential threat to human health; it had public health importance ranged from gastroenteritis to typhoid, as stated by Awny *et al.* (2018).

From the previously mentioned data, we concluded that the eggshell is highly contaminated with *E. coli* and *Salmonella* than the egg contents, especially in floor eggs production system. This was attributed to exposure to bad environmental conditions and variations in procedures of production, handling, and storage. The eggshell contamination increases the risk of microbial penetration through the eggshell pores and thus causing egg content contamination, as noticed by Mansour *et al.*, (2015). Thus, the eggshell contamination could be important for the shelf life of eggs and the food safety due to consumption eggs and their products, as those mentioned by Sadek *et al.* (2016).

CONCLUSION

There is a significant effect for the storage on the quality of eggs produced in the battery and floor systems. In addition, the eggshells collected from the floor production system show higher microbial contamination than the battery system. The presence of the pathogenic microbes on the chicken table eggs prevents the human consumers to use raw eggs. Hence, the consumption of eggs without proper cooking increases the probability of occurrence of health problems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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