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Original Article

Assessment of the Microbial Safety and Quality of Eggs from Small and Large-Scale Hen Breeders

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ABSTRACT

Egg is considered a nutritionally complete food and an excellent source of protein. The objectives of this study were (i) to assess the level of hygienic practices in small and large scale hen breeders, (ii) to evaluate the microbiological safety and quality of eggs along the production chain and (iii) to compare the shelf-life of eggs stored at ambient and refrigerated temperatures. The post-laying hygienic practices of farmers were assessed by a survey. Eggs obtained at post-laying and at retail were microbiologically analyzed for TVC, *Salmonella*, *Staphylococcus* and Yeasts and Molds. Eggs were also stored at ambient (ca. 22°C) or chilled temperature (4 ± 1°C) over a period of 23 days and analyzed every 6 days. Parameters tested included TVC, yolk index, Haugh unit and pH of the albumen. The survey revealed that neither small nor large-scale hen breeders washed the eggs before sale; however inspection for cracks and dry removal of dirt on egg surfaces were performed. The mean population of TVC, *Staphylococcus* spp. and Yeasts and Molds just after laying was ca. 4.6, 3.1 and 2.8 log cfu/g (egg shell) and 3.1, 2.5 and < 1.0 log cfu/g (egg content) respectively and ca. 4.8, 4.6 and 3.5 log cfu/g (egg shell) and 3.2, 3.0 and <1 log cfu/g (egg content) at retail. No *Salmonella* was detected on either egg shell or content at the post-laying and retail stages. Storage of shell eggs at ambient storage resulted in a decrease in the yolk index and Haugh unit and an increase in the pH of the albumen as well as a significant increase in the TVC (P<0.05). Findings from this study indicated that the microbiota of eggs increased steadily along the farm-to-kitchen continuum and highlight the importance of chilled storage to preserve egg freshness.

Keywords: Egg, Pathogens, Shelf-Life, Storage, pH, Yolk Index

INTRODUCTION

Since prehistory, egg has formed part of human diets worldwide (Musgrove et al., 2005). Egg is considered as a nutritionally complete food and an excellent source of protein (Ruxton et al., 2010). Most eggs (ca. 90%) have been found to be sterile when laid, but they have the potential to become occasionally contaminated (Egg Safety Center, 2010). Unfortunately, egg is also an ideal source of nutrients for proliferation of both spoilage and pathogenic contaminating microorganisms. The rate of spoilage of egg depends on nutrient availability, temperature, storage and handling (Al-Bahry et al., 2012). Eggs can be contaminated with micro-organisms such as bacteria and fungi. These microorganisms can evade the defense mechanism of eggs and penetrate inside the egg, thus increasing the risk of food-borne illnesses or product spoilage. The most prevalent pathogen of eggs is *Salmonella*. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequent *Salmonella* serotypes found inside shell eggs that caused food poisoning (Tan et al., 2012). The Centers for Disease Control and Prevention (CDC) specifies that egg is among the commodities with the highest number of outbreak- related illnesses in the US (CDC, 2013b). In Mauritius, 53 cases of salmonellosis were reported in 2008, after the consumption of contaminated egg-containing product called 'Marlin mousse'. The microorganism detected was *Salmonella enterica* serovar Typhimurium (Gaungoo et al., 2013). Even though eggs can be contaminated with pathogenic or spoilage microorganisms, hygienic practices among hen breeders are important to prevent microbial contamination at the initial stage of production. In Mauritius, eggs are produced in the conventional cage systems, cage free and free range systems. Good handling and appropriate storage conditions are essential to minimize egg contamination and deterioration in egg interior quality. Storage time and temperature play an important role in shelf life. The objectives of this study were three-fold: (i) to assess the level of hygienic practices adopted by small and large scale hen breeders, (ii) to determine the microbiological safety and quality of eggs throughout the chain of production and (iii) to determine the shelf-life of eggs stored under different storage conditions.

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MATERIALS AND METHODS

Survey on the level of hygiene prevailing among hen breeders

Five small and five large scale-hen breeders were selected for the survey. The survey aimed at assessing the level of hygiene prevailing at the site designated for egg laying and post-laying treatment of eggs which included inspection of egg, washing and storage before sale. A survey was administered to shed light on the hygienic practices adopted by the breeders from the time of laying to sale as well as the storage conditions of the eggs.

Microbiological analysis of eggs collected at different times in the chain of production

Ten random eggs were purchased from hen breeders soon after laying and also at the time of sale in markets and supermarkets. The eggs were aseptically transported to the laboratory for analysis. Five composite samples of two eggs were analyzed. The rinse method adapted from Musgrove et al. (2005) was employed to recover microorganisms from the shell of eggs. Briefly, the eggs were placed in stomacher bag containing diluent peptone water and surface-rinsed for 5 minutes. The surface rinsate was considered as the mother sample. The egg was then broken using a sterile knife and the egg contents were mixed evenly in a beaker using a sterile spatula. Twenty-five ml of the egg was placed in a sterile stomacher bag to which 225ml of buffered peptone water was added. The sample was homogenized in the stomacher for one minute to obtain a homogeneous primary sample. Decimal serial dilutions of the primary sample for both shell and egg content were set up using test tubes containing 9ml of the diluent of 0.1% peptone water. Serial dilutions of the rinsate were pour plated on plate count agar (BS EN ISO 4833:2003) and the plates incubated at 30°C for 72±2hrs. For the presumptive enumeration of *Staphylococcus* spp, 0.1 ml of the primary sample and its serial dilutions were spread-plated in Baird-Parker agar in duplicates (ISO6888-1:1999). Plates were allowed to incubate at 37°C for 24±2 hrs. Enumeration of *Escherichia coli* was carried out by spread-plating the primary sample and its serial dilutions on Eosin Methylene Blue (EMB) agar which were then incubated at 30°C for 24±2 hrs (Leininger, 2001). Detection of *Salmonella* species was done in accordance with ISO 6579: 2002 method. Briefly, the sample was pre-enriched in sterile buffered peptone water followed by selective enrichment in Rappaport-Vassiliadis broth (RVS), streaking on XLD agar and incubating at 37°C for 24 ± 3hrs. Enumeration of Yeast and Moulds was performed on the Dichloran Rose-Bengal Chloramphenicol agar incubated at 30°C for 72±2 hrs (ISO 21527-1:2008).

Assessment of quality of eggs stored at ambient and refrigeration temperatures

For the shelf life study, eggs were bought and stored at ambient (~22°C) or chilled (4 ±1°C) temperatures for a period of 30 days. Indices of egg quality assessed were Total Viable Counts, Yolk index, Haugh unit and pH of albumen (Hasan and Aylin, 2009). Yolk index is the ratio of average height of yolk to average diameter of yolk. For the Haugh unit, the weight of the egg was first measured before breaking it and then the height of albumen was measured at the widest expanse of the thick albumen. Haugh unit was then calculated using the formula; Haugh Unit = 100 x log $(H + 7.57 – 1.7\ W^{0.37})$, where H is the height of the albumen and W is the weight of the egg.

Statistical Analysis

All experiments were conducted in two independent trials. Where appropriate, statistical analyses were conducted using Minitab® Release 17. A single factor analysis of variance (ANOVA) and Tukey’s one-way multiple comparisons were conducted to determine differences in the population of the different bacterial species. Significant differences were considered at the 95% confidence level ($P<0.05$).

RESULTS AND DISCUSSION

Post laying practices among hen breeders

It was generally observed that none of the hen breeders washed the eggs after laying. After visual inspection, eggs contaminated with faecal matter were dry cleaned by wiping with a clean cloth. It should be noted that washing eggs is believed to contribute to a general hygienic improvement of the products and a decrease in the potential for cross contamination during food preparation (EFSA, 2005). Current USDA regulations (1999) specify wash water temperature and time allowed between water changes in the tank. According to the USDA, wash water temperature must be at least 90°F (32.2 °C) to provide appropriate cleaning. Furthermore, wash water should be at least 20°F (11°C) warmer than internal egg temperature to help prevent microbial penetration. Eggs must be dried immediately after washing to complete the process and to avoid the surface microorganisms from being drawn through the egg shell into the internal content during the cooling phase. Many studies have been carried out to confirm the decontamination efficacy of egg washing (Hutchison et al., 2004; EFSA, 2005; Messens et al., 2011). On the other hand, several studies have demonstrated that washing eggs can favour trans-shell contamination with microorganisms and moisture loss if subsequent drying and storage conditions are sub-optimal (Chousalkaret al. 2010, 2013; Vaibhavet al. 2014). Even though washing damages the cuticle, a waxy deposit on the shell surface known to act as a sealant against microbial contamination, it should be noted that washing considerably lowers microbiological populations on the shell (Olayemi and Adetunji, 2013). In several developed countries, properly maintained and operated modern equipment are available to wash eggs with minimal removal of the cuticle, thereby preserving the eggs’ defenses against microbial penetration (Wabeck, 2002). Small-scale egg breeders were found to store eggs at ambient temperature in open crates, thus increasing the chances for contamination by microorganisms or other environmental contam-inants.

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According to the Code of Hygienic Practices for Eggs and Egg Products (1976), eggs should be stored in closed places so as to minimize damage to the eggshell and avoid the introduction of contaminants, or growth of existing microorganisms in or on eggs, giving consideration to time and temperature conditions.

Table 1. Post laying hygiene practices among hen breeders

Post laying practices	**Hen-breeders**	
	Small-scale	**Large-scale**
Visual inspection of cracks/dirts	Yes	Yes
Cleaning of eggs contaminated with fecal matter	Yes	Yes
Washing	No	No
Oiling	No	No
Marking for expiry date	No	Yes
Closed packing	No	Yes
Storage temperature of eggs	Ambient	Ambient

Microbial load of eggs collected after laying and at retail

Microbiological analysis of eggs shells and egg content revealed Total viable Counts in the range of 4.7-4.9 log cfu/g and 2.9-3.5 logcfu/g respectively (Table 2, 3 and 4). Other authors have also reported high levels of microflora on commercial shell eggs. Researchers have found 46% and 54% of isolates from commercial eggs to be gram-negative and gram-positive respectively, and totaling more than thirty species. Gram-positive isolates including *Lactobacillus*, *Bacillus* and *Staphylococcus* spp. and gram-negative species including *Salmonella*, *Escherichia coli* and *Pseudomonas* have been identified (Zeidler, 2002). Kanpe et al. (1999) found that the shell surface of unwashed eggs harboured a high population of TVC exceeding 5 log cfu/egg. Bell (2002) reported that unwashed eggs from off-line systems tend to carry higher microbial load than industrially washed eggs obtained from in-line systems (Bell, 2002). Indeed, washing and sanitizing eggs under optimal conditions has the potential to reduce the microbial load by 2-3 log cfu/egg (Bell, 2002). A microbial load of less than 2 log cfu/packaged egg is considered an excellent commercial standard (Bell, 2002), whereas viable counts of 100,000 or more cfu/egg is considered unacceptable. Since the average population of TVC determined in this study is 4.6 log cfu/g and the average weight of an egg is 60g, then the average population of aerobic mesophilic bacteria is ca. 6.5 log cfu/egg. Hence, we can infer that the microbial quality of the tested eggs is commercially unacceptable. The high TVC count observed can be attributed to lack of washing compounded by contamination during handling by retailers or during storage. The TVC count was also statistically higher on the shell compared to the content (P<0.05). This may be due to the antimicrobial defense mechanism of eggs, which may either be physical (the shell and its membrane) or chemical (the membrane or albumen) (Joseph and Babatunde, 2006).

A relatively high load of *Staphylococcus* species of approximately 4.6 log cfu/g was recovered from the shell of eggs collected from the market and supermarket (Tables 2, 3 and 4).

Table 2. Microbial analysis of eggs collected from three different sellers in the Central Market of Mauritius

Market	TVC		*S. aureus*		Yeast & Molds		*E. coli*		*Salmonella*	
	Shell	Content	Shell	Content	Shell	Content	Shell	Content	Shell	Content
Seller 1	4.8 ± 0.2^{a}	3.5 ± 0.2^{a}	4.4 ± 0.3^{a}	3.2 ± 0.4^{a}	3.1 ± 0.3^{a}	< 1.0	< 1.0	< 1.0	(0/2)	(0/2)
Seller 2	4.7 ± 0.2^{a}	3.3 ± 0.3^{a}	4.7 ± 0.4^{a}	3.0 ± 0.3^{a}	3.3 ± 0.3^{a}	< 1.0	< 1.0	< 1.0	(0/2)	(0/2)
Seller 3	4.8 ± 0.3^{a}	3.1 ± 0.1^{a}	4.5 ± 0.5^{a}	2.9 ± 0.2^{a}	3.4 ± 0.4^{a}	< 1.0	< 1.0	< 1.0	(0/2)	(0/2)

Means within the same column followed by common superscripts letters are not significantly different (P >0.05); Limit of detection by the plating methodology is < 1 log cfu/g; (n/2): Number of samples testing positive out of two

Table 3. Microbial analysis of eggs collected from three different supermarkets of Mauritius

Supermarket	**TVC**		***S. aureus***		**Yeast & Molds**		***E. coli***		***Salmonella***	
	Shell	Content	Shell	Content	Shell	Content	Shell	Content	Shell	Content
Seller 1	4.9 ± 0.4^{a}	3.1 ± 0.1^{a}	4.7 ± 0.3^{a}	3.0 ± 0.7^{a}	3.6 ± 0.2^{a}	< 1.0	< 1	< 1	(0/2)	(0/2)
Seller 2	4.8 ± 0.2^{a}	2.9 ± 0.3^{a}	4.7 ± 0.5^{a}	2.7 ± 0.2^{a}	3.8 ± 0.1^{a}	< 1.0	< 1	< 1	(0/2)	(0/2)
Seller 3	4.9 ± 0.3^{a}	3.1 ± 0.2^{a}	4.6 ± 0.7^{a}	2.9 ± 0.1^{a}	3.5 ± 0.3^{a}	< 1.0	< 1	< 1	(0/2)	(0/2)

Means within the same column followed by common superscripts letters are not significantly different (P >0.05); Limit of detection by the plating methodology is < 1 log cfu/g; (n/2): Number of samples testing positive out of two

Table 4. Microbial analysis of eggs collected from two different farms of Mauritius immediately after laying

Farms	**TVC**		***S. aureus***		**Yeast & Molds**		***E. coli***		***Salmonella***	
	Shell	Content	Shell	Content	Shell	Content	Shell	Content	Shell	Content
Farm A	4.9 ± 0.1^{a}	3.2 ± 0.1^{a}	3.2 ± 0.9^{a}	2.8 ± 0.3^{a}	2.6 ± 0.4^{a}	< 1	< 1	< 1	(0/2)	(0/2)
Farm B	4.6 ± 0.7^{a}	3.0 ± 0.0^{a}	3.0 ± 0.3^{a}	2.3 ± 0.4^{a}	3.1 ± 0.1^{a}	< 1	< 1	< 1	(0/2)	(0/2)

Means within the same column followed by common superscripts letters are not significantly different ($P > 0.05$); Limit of detection by the plating methodology is < 1 log cfu/g; (n/2): Number of samples testing positive out of two

This high population of *Staphylococcus* species can partly reflect the extensive manual manipulation of eggs and the poor hygienic conditions of storage. Stepien-Pysniak et al. (2009) similarly reported a high population density of *Staphylococcus* recovered from table eggs. Wieneke et al. (1993) reported that in Great Britain, between 1969 and 1990, 3.5% of cases of staphylococcal food poisoning were caused by eating eggs contaminated by *S. aureus*. In France, 11% of cases of food poisoning, during the period of 1999-2000, resulted from eating eggs and egg products contaminated with staphylococci (Haeghebaert et al., 2002). In Poland, 2.8 % of sporadic isolate cases of food poisoning were linked to eggs contaminated with *S. aureus*. In 2001, this figure soared to 6.9 % (Przybylska,2002, 2003), and in 2009 it was reported that as high as 25 % of food poisoning cases incriminated table eggs contaminated with *S. aureus* (Baumann-Popczyk and Sadkowska-Todys, 2011). *Staphylococcus* spp. has also been isolated from eggs by several other researchers (Bell, 2002). Pyzik and Marek (2012) isolated 45 strains of staphylococci from the shell and the content, and 7 out of 45 were *S. aureus*. Pyzik and Marek (2013) also isolated 105 bacterial strains of *Staphylococcus*, 55.5 % of which were isolated from the shell, 27.8 % from the yolks and 16.7 % from the albumen.

Yeasts and molds can withstand harsh environment. They can thus proliferate on eggs under conditions of high moisture and oxygen conducive for growth, thus accelerating egg spoilage (Ansahet al., 2009). Optimal storage temperature and relative humidity can favour the growth of these microorganisms (Joseph and Babatunde, 2006). Results obtained from this study indicated a high Yeast and Molds count of 3.1-3.8 log cfu/g on shells of eggs bought from markets and supermarkets. This high charge could suggest that the eggs were kept under relatively high humid conditions. However, no fungal microorganism was recovered from the egg content, which is in accordance with the results of Joseph and Babatunde (2006). One possible explanation could be because molds are obligate aerobes and the content is devoid of oxygen thus explaining the absence of molds from content. Also, yeasts and molds have a lower minimum water activity requirement for growth than bacteria and can therefore grow to higher numbers on the shell. It is also possible that antimicrobials present in the egg content such as lysozyme could inhibit the growth of yeasts and molds. No *Escherichia coli* was detected from any of the samples analyzed. Florian and Trussell (1957) classified egg spoilage bacteria as primary and secondary egg invaders. They considered *Pseudomonas*, *Alcaligenes* and *Proteus* as primary invading species while *Escherichia coli* was classified as a secondary invader. In more recent studies, *E. coli* was also considered as less frequently invading compared to other spoilage bacteria such as *Pseudomonas aeruginosa* (De Reuet al., 2006; Al-Bahry et al., 2012). Board (1994) also indicated that *E. coli* was less prevalent at the egg surface relative to other genera. *E. coli* originates primarily from the intestines of birds and to a lesser degree, from workers in the processing plant. Since *E. coli* serves as an indicator of sanitary quality as well as an index organism of pathogens (Kornacki and Johnson, 2011), their numbers represent a measure of the efficacy of sanitation and disinfection procedures in the plant and the degree of contamination and cross-contamination during processing (Kornacki, 2010). The results imply that appropriate measures were most likely taken by farmers to prevent or reduce fecal contamination.

No *Salmonella* was detected in eggs sourced from either the small-scale or large-scale egg farms. From the study conducted by Ansah et al. (2009), no *Salmonella* was isolated from the samples tested either. However, poultry is widely acknowledged to be a reservoir for *Salmonella*. Egg contents have been shown to be contaminated with salmonellae by 2 routes: transovarian (vertical transmission) or trans-shell (horizontal transmission) (Ziedler, 2002). In vertical transmission, *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes associated with poultry reproductive tissues that are of public health concern include *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg. Among the different serotypes, *Salmonella* Enteritidis may be better able to achieve invasion, and as a consequence, may be found more frequently in reproductive tissues (Zeidler, 2002). Theoretically, horizontal transmission usually originates from fecal contamination of the egg shell or contamination of the eggs during passage through the cloaca. Other routes of transmission include contamination through environmental vectors, such as farmers, pet and rodents. *Salmonella* may be able to contaminate egg contents by migration through the egg shell and membranes. Such route is facilitated by moist egg shells, storage at ambient temperature and shell damage. The absence of *Salmonella* in the current study could be attributed to the relatively small sample size. A survey of eggs destined to British retail markets indicated that *Salmonella* Enteritidis contamination rate ranged from 0.04 to 0.11%, with overall contamination rate for all salmonellae ranging from 0.15 to 0.27% (ICMSF, 1996). This was attributed to the fact that poultry farmers practice strict medication and care.

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Influence of storage temperatures on the quality and shelf-life of eggs

The yolk index helps to determine the quality of an egg. A fresh laid egg has a firm round yolk and a strong yolk membrane (Fariset al., 2011). From the results represented in Figure 1., it can be observed that the yolk index decreased from 0.46 to 0.14 when stored for 23 days at ambient room temperature. However the decrease in yolk index occurred less rapidly when the eggs were stored at chilled temperatures. The results were in agreement with Hasan and Aylin (2009) who reported that the yolk and albumen index and Haugh units were affected by storage periods and temperature. The results showed that yolk and albumen index considerably decreased during storage at 20ºC.

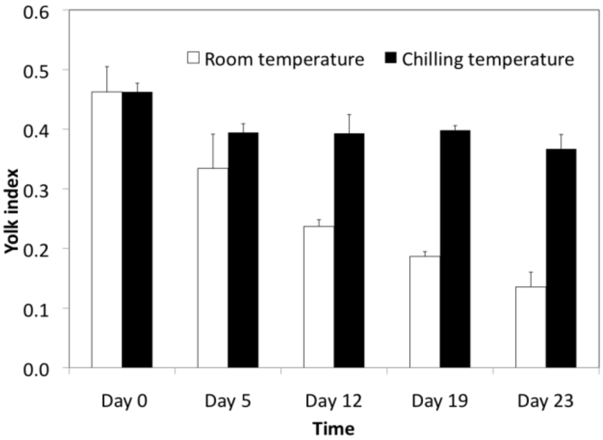

Fig 1. Variation in yolk index of eggs stored at ambient and chilled temperatures for up to 23 days

The albumen condition and quality can be determined by measuring the Haugh unit. A high Haugh unit represents a better albumen quality of egg. There was a significant decline in the Haugh unit after 23 days of storage (P<0.05). From the results presented in Fig. 2, it can be observed that the Haugh unit decreased from 76.3 to 21.4 when stored for 23 days at ambient room temperature and decreased to 26.11 when stored at chilled temperature. It can hence be inferred that storage time significantly reduced the Haugh unit of eggs and this decline was exacerbated at room temperature. These results are in agreement with those of Ihsan (2012), who reported that during storage at 20ºC, Haugh unit decreased from 72.68-52.11. This decrease in Haugh unit takes place due to loss of carbon dioxide and moisture from the albumen of eggs. At high temperature, it was also observed that the albumen became thinner and watery. The change in consistency is attributed to movement of water into the yolk causing it to enlarge hence increasing yolk index of eggs.

The pH of the albumen can be used to determine the freshness of eggs (Heath, 1977). A freshly laid egg has a pH of 7.6 to 8.2 and upon storage the pH can increase to 9.5 (Heath, 1977). This is in accordance with the results presented in Fig.3. The pH of a freshly laid egg was 8.3 and increased to a pH of 9.3 when stored for 23 days at ambient room temperature. The pH of eggs stored at chilled temperature over the same period was 9.0. It can hence be observed that that the combined effect of storage time and temperature can significantly affect pH of the albumen after 23 days of storage ($P < 0.05$). These results agree with Hasan and Aylin (2009) who reported that albumen and yolk pH increased to a greater extent at 20°C than at 4°C. Albumen pH was reported to increase at 22 and 50 weeks of storage, from 7.9 to 9.2 and from 8.2 to 9.3 at 0 and 14 days of storage respectively. But the increase occurred mainly during the first 3 days of storage. This change in pH is attributed to a loss of carbon dioxide from the pores of the egg shell (Stadelman et al., 1996), which increases the alkalinity of the albumen. However the change in pH took place less rapidly when eggs are stored at chilled temperature.

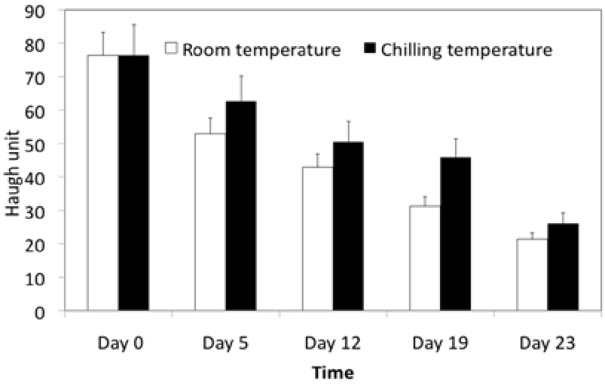

Fig. 2. Variation in Haugh unit of eggs stored at ambient and chilled temperatures for up to 23 days

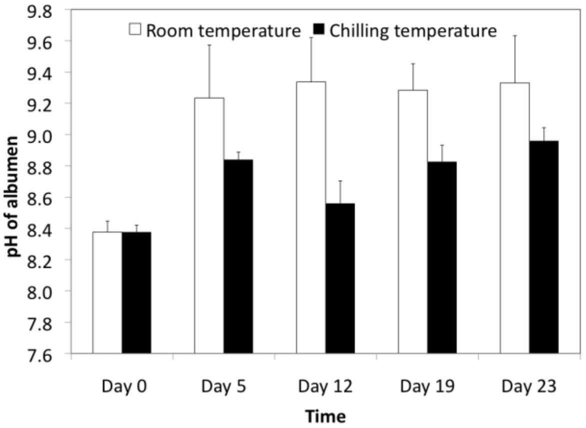

Fig. 3. Variation in pH of albumen of eggs stored at ambient and chilled temperatures for up to 23 days.

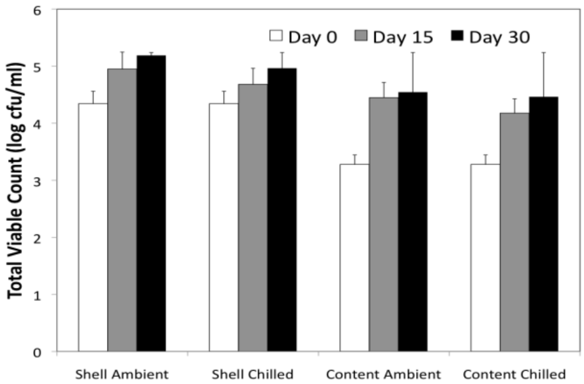

Fig. 4. Development of TVC as a function of storage temperature of eggs during 30 days of storage

The microbial charge of eggs was also determined to assess its shelf-life. From the results, it can be observed that the total viable counts on egg shells increased by a maximum of 0.9 log cfu/g at ambient temperature and by 0.7 log cfu/g after chilled storage. For egg content, the total viable count increased by 1.2 log cfu/g following ambient or chilled temperature after 30 days of storage.

Overall, it can be noted that storage temperature did not significantly affect the rate of development of mesophilic aerobes on egg shells or egg content ($P > 0.05$).

CONCLUSION

From this study it can be observed that the post laying practices such as inspection of cracks, egg washing and maintenance of proper storage conditions are important to maintain a lower microbial load of eggs and hence a better egg quality. It can also be concluded that eggs harbour a diverse microflora including pathogens or spoilers. However with appropriate handling and storage conditions, microbial development can be better controlled. Egg is a perishable food whose quality can deteriorate rapidly during storage and this can be slightly exacerbated during ambient storage. To preserve its freshness, egg should be kept at chilled temperature. At this low temperature, the egg quality is maintained and growth of microorganisms is inhibited.

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