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MICROBIAL CONTAMINATION OF STORED SHELL EGGS OF CHICKEN UNDER TEMPERATURE AND RELATIVE HUMIDITY INTERACTION

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ABSTRACT

Contamination of food products by microbial organisms is a significant health and safety issue. The study was to investigate the microbial contamination of stored chicken eggs under temperature and relative humidity interaction. To achieve this, from a total of 192 freshly laid eggs collected for the study, 48 eggs were randomly assigned to each of the four treatments of temperature and relative humidity of 29^0Cx60%RH(T_1); 29^0Cx80%RH(T_2); 10^0Cx60%RH(T_3) and 10^0Cx80%RH(T_4). After that, three eggs were collected weekly from these four treatment groups for bacterial and fungal isolation. This examination was carried out for eight weeks. The microbial isolates examined were *E. coli, Pseudomonas, Salmonella, Staphylococcus (bacteria) and, Aspergillus, Penicillium (fungi).* The presence and frequency of these microbial isolates were recorded. The emerging results were that *Salmonella* growth at 10^0C for 60%RH and 80%RH were marginally recorded for up to week 3 of storage. Refrigeration at 10^0C, irrespective of relative humidity, generally reduced the frequency and presence of these bacterial isolates. For the fungal isolates, at either 10^0C or 29^0C, more fungi isolates were generally recorded at a higher R.H. of 80%. It is concluded from this study that storage at combined temperature and R.H. of 10^0C and 60% will provide an ideal condition that would limit microbial contamination of chicken eggs in storage. This storage environment will ensure safe and suitable egg products.

Keywords: *Microbial contamination, Temperature, Relative humidity, Storage, Interaction.*

INTRODUCTION

The egg is an important food commodity of trade. The egg market's revenue is estimated at US$130.70bn in 2024 and is expected to grow annually by 8.47 percent at a compounded annual growth rate for 2024 to 2028 (Statista.com). Globally, the average egg consumption per capita is 180 (International Egg Commission, 2018). Eggs are a vital food product and are often considered nutritionally complete (Ruxon et al., 2010). This condition makes it most suitable for microbial organisms to contaminate the egg (Cader et al., 2014). Consequently, egg safety is central to trade, livelihood support and human health.

Microbial organisms on eggshells can emanate from fecal matter, feed, air, nesting material, farm personnel, and farm/storage equipment (Techer et al., 2015). However, the most prevalent contaminating microbial organisms on eggs include *Pseudomonas, Alcaligens, Proteus mirabilis, Salmonella typhimurium, Escherichia coli, Enterobacter cloacae, Klebsiella, Enterococcus faecalis, Staphylococcus species* (Chousalkar et al. 2021; Cedar et al. 2014). Some fungi are also found to cause spoilage of eggs or be pathogenic. When microbial organisms invade the eggshell's defense system, they may spoil eggs or lead to food-borne infection or intoxication in humans (Aygun, 2017). Consequently, the food product's quality and eventual market value are reduced. It

has been acknowledged that increased air temperature and humidity are critical factors that favor the proliferation of these bacteria and fungi on the egg's surface and its internal content (Al-Bahry et al., 2012; Chousalkar et al., 2021). Temperature causes the decrease of shell thickness that eventually facilitates the penetration of germs inside the egg,

Like in other tropical environments, high temperatures and relative humidity naturally promote microbial organisms' activities in Nigeria. How temperature and relative humidity as environmental factors independently influence the contamination of eggs by microbial organisms is widely known. However, to enhance the safety and suitability of stored eggs, understanding the combined or interactive effects of these factors in relation to microbial contamination is critical. Therefore, the study's main objective is to examine microbial contamination of stored shell eggs of chicken under temperature and relative humidity interaction. The study's findings will contribute to practical knowledge of techniques for safely and appropriately handling eggs during storage. This outcome will further advance the egg trade and livelihood of actors in the value chain.

MATERIALS AND METHOD

Experimental Area

The experiment was conducted at the Animal Science Laboratory of Federal College of Education (Tech) Umunze, Anambra state. Umunze is located within latitude 5.9622^0N and longitude 7.238^0E. The area is situated within the tropical rainforest belt. Microbial analysis of eggs was carried out by Animal Care's Poultry Diseases and Diagnosis Laboratory Asaba, Delta state.

Egg Collection and Storage Conditions

Eggs used for this study were collected from 34-week-old Bovan brown layers raised on a commercial farm at Awka. The layers were housed in deep litter and fed a commercial layer ration. The birds received additional light to provide 16hrs of light and 8hrs of darkness. A total of 192 eggs were collected within 2 hours after they were laid with sterile hand gloves. Eggs were divided equally into four groups of 48 eggs each and placed in separate plastic basins. Two of these basins were placed in plastic bowls containing 2 liters of water each. These giant bowls were then tightly covered with their lids. This was done to provide the moisture necessary for higher relative humidity. After that, a set of these eggs involving one only in a plastic bowl but not immersed in water and one immersed in the giant moist bowl were stored in a refrigerator. A second set, in like manner, was stored in a ventilated room at ambient temperature. The plan resulted in four treatments:

1. Ambient temperature + normal RH (T_1)
2. Ambient temperature + high RH (T_2)
3. Refrigerated temperature + normal RH (T_3)
4. Refrigerated temperature + high RH (T_4)

Three (3) eggs were sampled weekly from each treatment combination for the microbial analysis. The storage period was eight weeks, giving storage durations of 7, 14, 21, 28, 35, 42, 49, and 56 days.

Temperature and Relative Humidity Measurements

The instrument for measuring temperature and relative humidity was the 4 1 professional digital meter (Anemometer, thermometer, hygrometer and light meter). The model specification is LUTRON LM 8000. Ambient temperature was determined as the mean of the maximum temperature readings of the thermometer at three consecutive weekly samplings when the 4 1 digital meter was placed at the level of the egg basins in the ventilated storage room. This value was calculated as 29^{o}C. The refrigeration temperature was determined as the thermometer reading when the 4 1 digital meter was placed inside the compartment. The value recorded was 10^{O}C.

Normal R.H. was determined as the mean of the maximum R.H. readings of the hygrometer at three consecutive weekly samplings when the instrument was placed inside the egg basin without water. The mean value of R.H. determined was 60%. High R.H. was determined as the mean of the maximum R.H. readings of the hygrometer at three consecutive weekly samplings. The 4 1 digital meter was lowered into the big bowl containing water, and an egg basin was immersed. The value was determined to be 80% (All measurements were according to the specifications of LUTRO LM 8000).

Microbial Examination

The eggs were collected each with sterile hand gloves. Each egg was rubbed with a sterile swab moistened with lactose broth on half of the shell's surface. One swab was used for three eggs sampled weekly from each of the four treatment groups, and after that, it was used to inoculate the media. This method aligns with the approved procedure for the bacteriological culturing of eggshells (USDA, 1994; Sacco *et al*., 1989). MacConkey and Sabouraud dextrose agar media were used to isolate bacteria and fungi.

Ultimately, the plates were labeled correctly according to their treatment groups. Isolates from the MacConkey agar were incubated at 37^{o}C for 24 hours, while the plates from the Sabouraud dextrose agar (S.D.A.) media were incubated for six days. The S.D.A. used for fungal isolation was fortified with 0.05% chloramphenicol to inhibit bacterial contamination.

The bacterial isolates were presumptively identified using macroscopic, microscopic and some biochemical characterization and then compared using Bergey's manual of determinative bacteriology (Buchanan and Gibbons 1974). Morphological features, slide culture techniques, and slide mounts in lactophenol-cotton-blue for each fungal isolate were carried out according to Barnett and Hunter (1972). The microbial isolates on the eggshell from the four treatment groups were analyzed weekly, expressing them as a percentage of occurrence on treatment samples and their viable counts per plate.

MacConkey agar and Sabouraud dextrose agar media were purchased from commercial media manufacturers as original recipes. The MacConkey Agar, 500g EA Difco brand, was used. This is a selective and differential medium for isolating and differentiating lactose-fermenting organisms from lactose-fermenting enteric gram-negative bacteria. The preparation method follows MacConkey (1905) and the manufacturer's instructions.

Sabouraud dextrose agar single wrapped 90mm prepared plates; product code 12967138; supplier code P001160A of Oxoid Brand was procured. S.D.A. is used for the cultivation and differentiation of fungi. The preparation followed the manufacturer's instructions.

RESULTS

The effects of temperature and relative humidity and their interaction on bacterial and fungal growth on eggs during storage are shown from Tables 1 to 8. From week 1 (Table 1) to week 2 (Table 2), eggs under ambient temperature storage had scanty growth (1-20 colonies/plate (+)) of *E. coli* on their shells. This count increased to 51-120 colonies (+++) in week 4 (Table 4) and week 5 (Table 5). After that, it steadily decreased from 21-50 colonies/plate (++) in week 6 (Table 6) to 1-20 colonies/plate (+) in week 7 (Table 7). The contamination of shells from week 1 to week seven by *E. coli* was not affected by the level of storage R.H. By Week 8, at ambient temperature, no *E. coli* survived under high R.H. conditions. In contrast, scanty growth between 1- 20 colonies/plate (+) was still observed on those eggs in normal R.H. conditions. Generally, there were no growths of *E. coli* observed on eggs under refrigeration storage except in weeks 4 and 5 (at normal R.H.) and in week 8 (at high R.H.) conditions where marginal growth of between 1-20 colonies/plate (+) were recorded (Tables 4 and 5).

Pseudomonas spp was found (Table 1) to have contaminated the shell of eggs under ambient temperature and normal R.H. by the end of week one at scanty growth of between 1-20 colonies/plate (+). No growth of this genera was observed on eggshells between weeks 2 – 3 for all four temperature and relative humidity storage conditions. There was an increased growth at ambient temperature (for both standard and high R.H.) to counts too numerous to count (TNTC) in week 4 (Table 4) and week 5 (Table 5). By week 6 (Table 6), there was a steady decrease in the counts of *Pseudomonas* from 21- 50 colonies/plate (++) to 1-20 colonies/plate (+) observed in week 8 (Table 8). At refrigeration temperature generally, no growths of *Pseudomonas spp* were observed on eggs except at a scanty growth of 1-20 colonies/plate (+) at week one from eggs on high R.H. condition (Table 1) and week 8 for those on normal R.H. condition (Table 8).

Table 1: Microbial isolates from eggshell in week 1

Treatments	Bacteria				Fungi	
	E. coli	*Pseudomonas* sp	*Salmonella* sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* Sp
T_1	+	+	+	+	1cfu	38cfu
T_2	+	–	–	+	14cfu	142cfu
T_3	–	–	–	+	–	1cfu
T_4	–	+	–	+	1cfu	8cfu
Occurrence of Microbes (%)	50	50	25	100	75	100

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; cfu: colony forming units.

Table 2: Microbial isolates from eggshell in week 2

Treatments	Bacteria				Fungi	
	E. coli	*Pseudomonas* sp	*Salmonella* sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* sp
T_1	+	–	–	++	1cfu	38cfu
T_2	+	–	–	++	12cfu	160cfu
T_3	–	–	–	–	–	6cfu
T_4	–	–	–	–	1cfu	20cfu
Occurrence of Microbes (%)	50	0.00	0.00	50	75	100

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; (++): 21 – 50 colonies/plate; cfu: colony forming units.

Table 3: Microbial isolates from eggshell in week 3

Treatments	Bacteria				Fungi	
	E. coli	*Pseudomonas* sp	*Salmonella* sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* Sp
T_1	++	–	++++	++++	1cfu	87cfu
T_2	–	–	–	–	167cfu	TNTC

T_3	–	–	–	++	–	8cfu
T_4	–	–	+	+	1cfu	TNTC
Occurrence of Microbes (%)	25	0.00	50.00	75	75	100

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; (++): 21 – 50 colonies/plate; (+++): 51 – 120 colonies/plate; (++++): 121 and more colonies/plate; TNTC: Too numerous to count; cfu: colony forming units.

Table 4: Microbial isolates from eggshell in week 4

Treatments	**Bacteria**				**Fungi**	
	E. coli	*Pseudomonas* sp	*Salmonella* sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* Sp
T_1	+++	TNTC	–	++	16cfu	120cfu
T_2	+++	TNTC	–	++	TNTC	TNTC
T_3	+	–	–	+	–	12cfu
T_4	–	–	–	–	2cfu	TNTC
Occurrence of Microbes (%)	75	50	0.00	75	75	100

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; (++): 21 – 50 colonies/plate; (+++): 51 – 120 colonies/plate; TNTC: Too numerous to count; cfu: colony forming units.

Table 5: Microbial isolates from eggshell in week 5

Treatments	**Bacteria**				**Fungi**	
	E. coli	*Pseudomonas* sp	*Salmonella* sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* Sp
T_1	+++	TNTC	–	+++	–	280cfu
T_2	+++	TNTC	–	TNTC	TNTC	TNTC
T_3	+	–	–	–	4cfu	TNTC
T_4	–	–	+	+	6cfu	TNTC

Occurrence of Microbes (%)	75	50	25	75	75	100

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; (++): 21 – 50 colonies/plate; (+++): 51 – 120 colonies/plate; TNTC: Too numerous to count; cfu: colony forming units.

Table 6: Microbial Isolates from Eggshell in Week 6

Treatments	**Bacteria**				**Fungi**	
	E. coli	*Pseudomonas* sp	*Salmonella* Sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* sp
T_1	++	++	–	++	–	120cfu
T_2	++	+	–	–	2cfu	–
T_3	–	–	+	+	–	–
T_4	–	–	–	–	2cfu	156cfu
Occurrence of Microbes (%)	50	50	25	25	50	50

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH
(–): O colonies /plate; (+): 1 – 20 colonies/plate; (++): 21 – 50 colonies/plate; cfu: colony forming units.

Table 7: Microbial Isolates from Eggshell in Week 7

Treatments	**Bacteria**				**Fungi**	
	E. coli	*Pseudomonas* sp	*Salmonella* Sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* sp
T_1	+	+	–	–	1cfu	10cfu
T_2	+	–	–	–	8cfu	45cfu
T_3	–	–	–	–	–	–
T_4	–	–	–	–	2cfu	12cfu
Occurrence of Microbes (%)	50	25	0.00	0.00	75	75

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH

(–): O colonies /plate; (+): 1 – 20 colonies/plate; cfu: colony forming units.

Table 8: Microbial Isolates from Eggshell in Week 8

Treatments	Bacteria				Fungi	
	E. coli	*Pseudomonas* sp	*Salmonella* Sp	*Staphylococcus* sp	*Aspergillus* Sp	*Penicillium* sp
T_1	+	+	–	–	–	–
T_2	–	+	–	–	–	36cfu
T_3	–	+	–	–	–	2cfu
T_4	+	–	–	–	2cfu	12cfu
Occurrence of Microbes (%)	50	75	0.00	0.00	25	75

T_1: ambient temperature with normal RH; T_2: ambient temperature with high RH; T_3: refrigeration temperature with normal RH; T_4: refrigeration temperature with high RH (–): O colonies /plate; (+): 1 – 20 colonies/plate; cfu: colony forming units.

Salmonella spp were isolated by week 1 (Table 1) from eggs stored at ambient temperature with normal R.H. at marginal growth of 1-20 colonies/plate (+). After that, the viable counts of this genera increased to >120 colonies/plate (++++) by week three on the same temperature and R.H. condition of storage. After week 3, there were no surviving *Salmonella* on the shells of eggs at ambient temperature in both normal R.H. and high R.H. conditions. Refrigeration temperature, on the other hand, inhibited the multiplication of *Salmonella*. Though *Salmonella* was isolated from eggs on refrigeration temperature and high R.H. condition in week 3 (Table 3) and week 5 (Table 5), and at normal R.H. in week 6, their growth levels were limited to 1-20 colonies/plate (+). However, by week 7 (Table 7), all Salmonella across the four treatments had died since no counts were further recorded.

Staphylococcus was isolated by the end of week one at scanty viable counts of 1-20 colonies/plate (+) on shells of eggs from all four temperatures and R.H. storage conditions (Table 1). There were steady increases in counts in the ambient temperature storage condition to > 120 colonies (++++) in week 3 for normal R.H. (Table 3) and to viable count too numerous to count (TNTC) in week five at high R.H. Afterwards, there was a decrease to 21-50 colonies/plate (++) in week 6 (Table 6) observed only under normal R.H. condition. By week 7 (Table 7), all the *Staphylococcus* on the eggs across all four temperature and R.H. storage conditions were dead, as no growths were further recorded. At refrigeration temperature, eggshell contamination by *Staphylococcus* increased to 21- 50 colonies/plate (++) under normal R.H. in week three, and thereafter, the number reduced to 1-20 colonies/plate (+) in week 6. At high R.H., scanty growths of 1-20 colonies/plate (+) were observed again by week 3 (Table 3) and week 5 (Table 5).

For the fungal isolates, there was generally higher growth of *Aspergillus spp* on shells of eggs in high R.H. conditions compared to normal R.H. for both ambient temperature and refrigeration temperature storage conditions. In week 1, at ambient temperature and average R.H. storage, shell contamination by *Aspergillus spp* was 1cfu. At the same time, 14cfu was recorded for the high R.H. By week 2 (Table 2) and week 3 (Table 3), whereas the viable counts on shells under normal R.H. were at 1cfu for both weeks, the viable counts for high R.H. were higher and increased from 12cfu to 167cfu in the respective weeks. By week 4 (Table 4) and week 5 (Table 5), further increases in the viable counts of *Aspergillus* were recorded: Eggs under high R.H. consistently maintained higher numbers of viable counts (TNTC) in both weeks when compared with 16cfu and 0cfu at normal R.H. for identical periods. Thereafter, there was a decline to 1cfu in normal R.H. and 8cfu in high R.H. at week 7 (Table 7). By week 8 (Table 8), all the *Aspergillus spp* at ambient storage (for both levels of R.H.) had died, leaving no trace of this genus in the treatment media. Except at week 5 (Table 5), no contamination of shells by Aspergillus was observed at normal R.H. conditions in refrigeration storage. By the same week, five 4cfu were isolated from eggs on normal R.H. condition while 6cfu were isolated from those on high R.H. condition. This suggests that fungal contamination is a more common problem with storage at high humidity conditions. Results indicate that the exponential growth phase of *Aspergillus* is from week 1 to week 5. This is shown by the rates of increase in the viable counts of the two fungi shown in Tables 1 to 4.

In week 1 (Table 1), the contamination of shells of eggs by Penicillium spp was higher at ambient temperature than at storage temperature in refrigeration. However, at the same temperature, contamination was more at high R.H. than normal R.H. At an ambient temperature of storage with normal R.H., 38cfu were isolated, whereas 142cfu was the viable counts of *Penicillium* found on shells of eggs in high R.H. condition. By the same week, under refrigeration temperature of storage with normal R.H./high R.H. conditions, 1cfu and 8cfu were the levels of *Penicillium* contamination of shells of eggs, respectively. This pattern continued though at increasing rates of contamination up to week 5 (Table 5), where 280cfu were the viable counts under ambient temperature and normal R.H., whereas *Penicillium* isolated on shells of eggs on the three other treatment groups were too numerous to count (TNTC). From week 6, Penicillium counts on eggshells in all treatments steadily decreased. However, it maintained a similar pattern in the previous weeks according to temperature and relative humidity. By week 8 of storage (Table 8), there were still measurable or significant populations of *Penicillium* isolated from the culture of 3 treatment groups. There were 36cfu for ambient temperature with high R.H., 2cfu for refrigerated temperature with normal R.H. and 12cfu for refrigerated temperature with high R.H. storage conditions.

DISCUSSION

The isolation of *E. coli*, *Pseudomonas* sp, *Salmonella* sp and *Staphylococcus* sp as microflora on eggshells agrees with Obi and Igbokwe (2009) Oliveira (2022) reports. Some of these bacterial species evade the shell and eventually gain entry into the content, causing spoilage of stored shell

eggs. The uniformity in the growth of *E. coli* observed under ambient temperature at both normal R.H. and high R.H. up to week 7 of storage (Table 7) suggests that high temperature independent of R.H. promoted the growth of this microbe. In contrast, its inability to grow at refrigeration temperature observed within three weeks of storage underpins the importance of refrigeration as a method for inhibiting *E. coli* growth. It is, therefore, important for shell egg producers and handlers in tropical environments to provide cooling systems for egg storage. *E. coli* is significantly associated with faecal contamination. Therefore, their presence on eggshells should be of great concern since species of this bacteria cause diarrhea and fever in human hosts (Arthur and Osei-Asamoah 2001).

This study's low frequency of Salmonella isolated from shell surfaces is consistent with the report that *Salmonella enteritidis* egg contamination is shallow (Davis and Brelin, 2000). Egg storage at low-temperature regimes can control the multiplication of *Salmonella*; for instance, storage below 10^{o}C was reported to eliminate its growth (Humphrey, 1994). This could be explained by the fact that much lower temperature conditions may be required to inhibit the growth of *Salmonella.* The presence of *Salmonella* on the eggshell in this study could result from eggs coming in contact with dirt after laying, faecal contaminated water and feed, consistent with many research findings (Messens *et al.*, 2007; Smith *et al*., 2000).

One of the observations in the current investigation is the high frequency of occurrence of *Staphylococcus* across treatments and their survival up to 6 weeks (Table 6) under ambient and refrigeration temperatures. This points to how pervasive this species exists around the poultry house and how easily it can attach to the surface of the shells. Du Reu *et al.* (2006) found that natural eggshell contamination of table eggs was dominated by gram-positive *Staphylococcus* sp. The results presented in this work agree with those of previous studies. Again, this may partly be due to the tolerance of these species for dry conditions that mainly originate from dust, soil or faeces. Likely, many of these bacterial isolates from the shell surfaces of the egg may eventually penetrate the content, leading to various forms of deterioration, decay or rots. This could have contributed to various rots observed after week 4 of egg storage under ambient temperature. Rotten eggs typically contain a mixed infection of gram-negative and a few gram-positive organisms. Among many other food spoilage organisms, *Pseudomonas*, *Escherichia* and *Staphylococcus* have been implicated in egg rots (Awny et al. 2018; Oliveira et al. 2022).

The findings of this study showed that at both storage temperatures when R.H. is high, *Aspergillus* and *Penicillium* contaminations of shell surfaces were very rapid. This indicates that high humidity conditions would increase the risk of mold spoilage even when eggs are kept under refrigeration. This result confirms previous reports that the shells are clothed in mycelium under humid storage conditions, a condition known as 'whiskers' (Stadelman and Cotterill, 1995). Under such conditions, these hyphae could penetrate the pore canals and spread throughout the egg content. Penetration of fungi into egg's content and their multiplication could contribute to black rot and other rots noticeable during storage. The result of this study is also consistent with the report that spoilage fungi such as *Aspergillus* and *Penicillium* predominate in warm and humid conditions (D'mello, 2001).

CONCLUSION

The study practically demonstrated the effect of temperature and relative humidity interactions on bacterial and fungal growth on the eggshell. Conclusively, it showed that storage at refrigeration temperature (10^{o}C) generally inhibits bacterial and fungal growth, whereas high R.H. storage condition (80%) increases fungal growth on the eggshell.

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