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Study of microbial changes in albumen during storage of salted white leghorn eggs

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Abstract
A study was conducted on microbial changes during storage in albumen of salted chicken eggs. A total of 320 white leghorn eggs were divided into four groups (salted eggs at room temperature, control eggs at room temperature, salted eggs at refrigeration temperature and control eggs at refrigeration temperature). 20 eggs from each group were subjected to microbial study (total plate counts, anaerobic counts, staphylococcal counts, yeast and moulds counts and coliform count) at 0, 5th, 7th and 10th day of storage. During storages of eggs at room and refrigeration temperature the counts of total plate, anaerobic, staphylococcal and yeast and moulds in treated groups remain lower as compared to the control group. Additionally, a significant ($p \leq 0.01$) increase in microbial counts was observed as the storage period advanced. However, No colifom counts was observed in the albumen in any sample at any stage of the storage period. It could be inferred that salted eggs were microbiologically safer than the normal control eggs during the period of study.

Keywords: Egg, white leghorn, total plate count, anaerobic count, staphylococcal counts, yeast and moulds counts, coliform count

1. Introduction
Like all natural organic matter, eggs can eventually spoil through the action of spoilage organisms. The bacteria belonging to genera Streptococcus, Staphylococcus, Micrococcus and Bacillus, etc. may be found on eggshell surfaces because all these species can tolerate dry conditions. As the egg ages, these bacteria tend to decline and are replaced by Gram-negative spoilage bacteria, such as coliforms and Flavobacterium, and several types of Pseudomonas etc.

Miller (1954) [6] observed that eggs yielding plate count less than 10 million/ml generally do not manifest unpleasant odours. Panda and Panda (1969) [8] also isolated various fungal agents belonging to Mucor, Aspergillus, Penicellium etc. genera from eggs during cold storage and marketing. They found washing of eggs with CFTRI egg washing powder, prior to storage, was effective in preventing spoilage of eggs during cold storage by said fungal agents. Brown *et al*. (1970) [4] noted early bacterial growth in eggs with broad end up than the eggs with broad end down.

Anand *et al*. (1995) [2] carried out microbiological studies on the shelf-life of pasteurized liquid whole egg, albumen and yolk in ultra-violet irradiated LDPE (250 G) pouches at refrigeration (4 plus/minus 1 ºC) and room (28 plus/minus 1 ºC) temperatures. While none of the sample contained salmonellae, the coliforms, staphylococci, and yeast and mould were encountered. The microorganisms were found to multiply much faster at room temperature as compared to refrigeration with a shelf life of 6 and 12 days under these two storage conditions, respectively. The most common route of infection is considered to be by penetration through the shell (Adler, 1965) [1]. Bacteria on shells may under certain conditions penetrate through the shells into the interior and cause spoilage (Moats, 1980) [7]. Contaminants originating in faecal material or dust move through the shell pores before ultimately colonising the membranes and yolk (Bruce and Drysdale, 1994) [5]. Gram-positive microorganisms are dominant contaminants on the shell surface whereas Gram- negative microflora are the major internal contaminants of eggs (Board, 1996) [3].

However, the available information on the microbial evaluation and shelf life of salted chicken eggs is scanty. Hence, it is deemed imperative to conduct systematic scientific studies on microbial changes during storage of salted chicken eggs which is also likely to help in generating rural entrepreneurship.

2. Materials and methods

160 Eggs were dipped in synthetic vinegar solution for 40 minutes followed by their dipping in saturated solution of salt for 42 hrs.

2.1 Microbiological quality evaluation

Microbiological evaluations of salted and control egg albumen were done as per methods of ICMSF (1982) in fresh as well as stored egg. Egg albumin was separated from egg yolk aseptically and then 5 ml of albumin was mixed with 5 ml of normal saline solution and transferred to 90 ml of buffered peptone water and incubated at 37 ºC for 18 h. Following parameters were recorded and counts were expressed as log10cfu/g of egg albumen 1. Total plate count 2. Anaerobic plate count 3. Staphylococci 4. Coliforms 5. Yeast and mould.

2.2 Statistical Analysis

The data obtained were subjected to statistical analysis. Means and standard errors were calculated following the standard statistical procedures.

3. Result and discussion

3.1 Total plate count

Total plate counts in the control (untreated) and salted (treated) egg albumen during the storage are shown in Table 1. Total plate counts in control as well as salted group eggs increased significantly ($P \leq 0.05$) during the storage periods in both room and refrigeration conditions. However, total plate counts found in the salted eggs were significantly ($P \leq 0.05$) lower than the control group during both room and refrigeration conditions. It is presumed that treatment with salt solutions might have resulted in the lower total plate count in salted eggs.

Table 1: Effect of storage on Total plate counts (log_{10} cfu/g) of salted egg albumen

Duration of storage (days)	Treatments			
	Control		Salted	
	Room	Refrigeration	Room	Refrigeration
0	0.85 ± 0.36^{Ad}		0.74 ± 0.38^{Bc}	
5	1.54 ± 0.46^{Ab}	1.10 ± 0.40^{Bc}	0.96 ± 0.41^{Ca}	0.95 ± 0.41^{Cb}
7	1.78 ± 0.46^{Aa}	1.45 ± 0.30^{Bb}	1.02 ± 0.43^{Ca}	1.03 ± 0.29^{Cb}
10	-	1.72 ± 0.45^{Aa}	-	1.24 ± 0.24^{Ba}

Identical capital superscripts indicated insignificant difference between treatments, whereas unidentical small superscripts indicated significant difference between duration of storage. *$p \leq 0.05$, **$p \leq 0.01$

3.2 Anaerobic counts

Anaerobic counts in egg albumen of the control and salted eggs during the storage are given in Table 2. Significant ($P \leq 0.05$) increase in the counts was observed in the control and salted eggs in both room and refrigeration temperatures, but the increase in counts was more rapid in eggs kept at room than at refrigeration condition in both salted and unsalted group. Salted egg albumen were having significantly ($P \leq 0.05$) lower counts as compared to the unsalted eggs. The application of salt could be attributed to the decreased counts observed in salted eggs.

Table 2: Effect of storage on Anaerobic plate counts (log_{10} cfu/g) of salted egg albumen.

Duration of storage (days)	Treatments			
	Control		Salt	
	Room	Refrigeration	Room	Refrigeration
0	0.63 ± 0.33^{Ac}		0.42 ± 0.28^{Bc}	
5	0.99 ± 0.26^{Ab}	0.92 ± 0.39^{Bb}	0.54 ± 0.27^{Cb}	0.50 ± 0.33^{Db}
7	1.14 ± 0.40^{Aa}	0.96 ± 0.26^{Bb}	0.84 ± 0.36^{Ca}	0.65 ± 0.35^{Da}
10	-	1.43 ± 0.43^{Aa}	-	0.71 ± 0.25^{Ba}

Identical capital superscripts indicated insignificant difference between treatments, whereas unidentical small superscripts indicated significant difference between duration of storage. *$P \leq 0.05$, **$P \leq 0.01$

3.3 Staphylococcal counts

The results of studies on Staphylococcal counts in the control and salted egg albumen are given Table 3. No staphylococcal counts were found on zero day of storage. The staphylococcal counts significantly ($p \leq 0.05$) increased then to 0.42 and 0.26cfu/g on 7th day of storage in control and salted eggs respectively kept at room temperature. The count was lower in salted eggs as compared to unsalted eggs in room temperature storage. But no counts were observed in both salted and unsalted eggs till 10th day of storage in either room or refrigeration conditions. Salt treatment is presumed to play its role in reducing the Staphylococcal counts.

Table 3: Effect of storage on Staphylococci counts (log_{10} cfu/g) of egg albumen

Duration of storage (days)	Treatments			
	Control		Salt	
	Room	Refrigeration	Room	Refrigeration
0	0.00 ± 0.000^{Ab}		0.00 ± 0.000^{Ab}	
5	0.00 ± 0.000^{Ab}	0.00 ± 0.000^{Ab}	0.00 ± 0.000^{Ab}	0.00 ± 0.000^{Ab}
7	0.42 ± 0.028^{Aa}	0.00 ± 0.000^{Cb}	0.26 ± 0.025^{Ba}	0.00 ± 0.000^{Cb}
10	-	0.00 ± 0.000^{Ab}	-	0.00 ± 0.000^{Ab}

Identical capital superscripts indicated insignificant difference between treatments, whereas unidentical small superscripts indicated significant difference between duration of storage. *$p \leq 0.05$, **$p \leq 0.01$

3.4 Yeast and mould counts
The result of yeast and mould counts in the control and salted eggs are given in Table 4. Yeast and moulds count registered a significant increase in both salted and unsalted eggs in both conditions. The growths were significantly ($P \leq 0.05$) more in unsalted eggs with respect to salted eggs in either condition. Presence of salt in salted eggs might have produced detrimental effect on yeast and moulds growth.

Table 4: Effect of storage on Yeast and mould counts ($\log_{10}$ cfu/g) of salted egg albumen

Duration of storage (days)	Treatments			
	Control		Salt	
	Room	Refrigeration	Room	Refrigeration
0	0.29 ± 0.10^{Ac}		0.17 ± 0.06^{Bc}	
5	0.59 ± 0.19^{Ab}	0.41 ± 0.18^{Cb}	0.51 ± 0.16^{Ba}	0.39 ± 0.14^{Cb}
7	0.71 ± 0.12^{Aa}	0.45 ± 0.16^{Cb}	0.55 ± 0.12^{Ba}	0.42 ± 0.10^{Dab}
10	-	0.61 ± 0.14^{Aa}	-	0.46 ± 0.17^{Ba}

Identical capital superscripts indicated insignificant difference between treatments, whereas unidentical small superscripts indicated significant difference between duration of storage. $^*P \leq 0.05$, $^{**}P \leq 0.01$

3.5 Coliform count
No coliform count was found in the egg albumen in any sample at any stage of storage in any condition.

4. Conclusion
The microbial counts of total plate, staphylococcal, and yeast and molds in the salt treated eggs remained consistently lower compared to the control group during storage of eggs at both room and refrigeration temperatures. Notably, coliform counts were absent in the albumen of any sample at any stage of storage of salted chicken eggs. However, it is important to note that an increase in the counts of these microbes was observed as the storage period advanced. These results suggest that salting effectively inhibits the growth of harmful microorganisms in eggs, thereby extending their shelf life and enhancing their safety for consumption.

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