# MICROBIAL QUALITY OF STORED EGGSHELLS FOR POTENTIAL FEEDSTUFF APPLICATION

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**ABSTRACT**
*Eggshells from three (3) different sources (hatchery, bakery and food outlet) were treated and stored over a period of time and their resultant microbial loads were examined. The eggshells were randomly grouped into four (4) treatments namely, the Control group which was examined immediately after heat treatment, Treatment A was stored for 2 weeks, Treatment B was stored for 4 weeks, Treatment C was stored for 6 weeks while Treatment D was stored for 8 weeks before microbial examination. The bacteria, salmonella, coliform and yeast counts were examined. All parameters were significantly ($p<0.05$) different across the storage periods, eggshell sources and their interaction effect. It was observed that bacteria count increased as the weeks of storage progressed, eggshells from the bakery had the highest bacteria count [57.70 (Cfu/g) x$10^3$], those from the hatchery had the highest coliform count [12.40 (cfu/g) ×$10^3$] while those from the food outlet had the highest yeast count [7.20 (cfu/g)×$10^3$]. It was concluded that heat-treated eggshells should not be stored beyond two (2) weeks prior to use as feedstuff.*
**Keywords:** Microbial loads, Bacteria count, Salmonella count, Coliform count, Yeast/mould count, Waste-to-wealth

## INTRODUCTION
Eggshells are by-products of the poultry industry. They are mostly composed of calcareous outer shells highlighted by proteinaceous membranes and the proteins from egg white that adsorb to these membranes (Hincke *et al*., 2012). Swamiappan and Vijayaraghavan (2006) estimated that the eggshell makes up around 11% of the weight of the egg. Eggshell is composed of high levels of calcium and other minerals (Ali and Badawy, 2017) as well as some proteins (Gautron *et al*., 2001) making it possible to utilize them in numerous applications. Studies in the field of animal nutrition demonstrate the potential of eggshells as a calcium source for diets intended for cocks, cockerels and layers (Maranan *et al.,* 2021).
Eggshells contamination is widely studied worldwide. In general, eggs including their shells are contaminated after laying by dust, faeces, and handling. Microbes can contaminate the shell in a short period and penetrate the egg interior (Adegbenro *et al*., 2020) and bacterial charge on the eggshell depends on several factors, including the diet of the laying hens, the environmental conditions of the egg during and after laying, and the bacterial charge of the air (De Reu *et al*., 2005). In this study, the microbial status of eggshells from three (3) sources, intended as potential feedstuff is assessed.

## MATERIALS AND METHODS
### Collection and Treatment of Eggshells
Fresh eggshells were collected from (3) three different sources which are the hatchery (Chi farms, Ibadan), food outlets (Glofes eatery, Akure) and bakery (Cake and pastries, Akure). The eggshells were washed to clean off dirt, boiled in hot water at 100°C for 15 minutes (all eggshells were submerged about 1 inch below water level) and sun-dried for 3 days (Nahla, 2015). Thereafter, the control group were taken immediately for microbial analysis while the others were stored in polythene bags at room temperature. The storability of the treated eggshells was determined forth-nightly over a period of 8 weeks (2, 4, 6 and 8 weeks respectively).
### Microbial Analysis of Eggshells
The pour plate method was used. Bacterial, *Salmonella*, coliform and fungi counts were enumerated on nutrient, salmonella-shigella, macConkey and potato dextrose agars respectively. Sterilized molten agars were aseptically poured into inoculated petri dishes containing $10^{-3}$ and $10^{-4}$ diluted samples respectively. The plates were incubated in an inverted position at 37°C for 24 hours for bacteria growth and 25°C for 2-4 days for fungi growth. Colonies were counted in order to obtain the total viable count, discrete colonies were purified by sub-culturing into new prepared agar media and growth was observed under the microscope and then characterized using standard method.
### Statistical Analysis
Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS statistical package (version 21). Means were compared usong Duncan multiple range test where significant differences were observed.

## RESULTS AND DISCUSSION

Table 1 shows the microbial loads of the eggshells stored over a period of 8 weeks. The results showed that the bacteria, salmonella, coliform and yeast counts were all significantly ($p<0.05$) different. Week 8 (Treatment D) had the highest bacteria (108.33 cfu/g $\times 10^3$) and salmonella (8.33 cfu/g $\times 10^3$) counts, the coliform count was highest in Week 4 (Treatment B) and Week 6 (Treatment C) having the same values of 14.00 cfu/g $\times 10^3$ and yeast count was highest in Week 4 (Treatment B). Based on eggshell sources, significant ($p<0.05$) differences were also recorded for all microbial counts. Bacteria count was highest on eggshells sourced from the bakery (57.70 cfu/g $\times 10^3$). salmonella (10.10 cfu/g $\times 10^3$), coliform (12.40 cfu/g $\times 10^3$) and yeast (9.80 cfu/g $\times 10^3$) counts were highest on eggshells sourced from the hatchery. All eggshells irrespective of their source were contaminated with bacteria, which is in agreement with Fahim *et al*. (2021). The bacterial count on the eggshell varied across the sources and periods of storage. De Reu *et al*. (2008) suggested that for eggshells to be considered of hygienic quality, the bacterial load present on their surface must remain below 5log CFU (Colony-forming unit).

**Table 1: Microbial loads of eggshells stored over a period of time**

| Treatment effect (No. of weeks) | Source of egg shell | Bacteria count (Cfu/g)x10$^3$ | Salmonella count (Cfu/g)×10$^3$ | Coliform count (cfu/g)×10$^3$ | Yeast count (Cfu/g)×10$^3$ |
|---|---|---|---|---|---|
| Control (0) | | 10.50$^e$ | 0.00$^e$ | 0.00$^d$ | 3.83$^b$ |
| A | | 25.50$^d$ | 5.67$^b$ | 7.00$^c$ | 8.67$^a$ |
| B | | 31.50$^c$ | 1.33$^d$ | 14.00$^a$ | 9.17$^a$ |
| C | | 53.00$^b$ | 3.00$^c$ | 14.00$^a$ | 9.33$^a$ |
| D | | 108.33$^a$ | 8.33$^a$ | 9.17$^b$ | 9.00$^a$ |
| SEM | | 0.83 | 0.28 | 0.52 | 0.47 |
| P-value | | 0.00 | 0.00 | 0.00 | 0.00 |
| | Hatchery | 42.70$^b$ | 10.10$^a$ | 12.40$^a$ | 9.80$^a$ |
| | Bakery | 57.70$^a$ | 0.00$^b$ | 6.60$^b$ | 7.00$^b$ |
| | Food outlet | 36.90$^c$ | 0.00$^b$ | 7.50$^b$ | 7.20$^b$ |
| | SEM | 0.65 | 0.22 | 0.40 | 0.37 |
| | P-value | 0.00 | 0.00 | 0.00 | 0.00 |

$^{abcde}$Means with different superscripts along the same column differed significantly (P< 0.05), SEM = Standard Error of Mean, A = Week 2, B = Week 4, C= Week 6, D = Week 8

In this study, salmonella counts increased significantly in treatment A (week 2), fluctuated slightly in treatment B (week 4) and treatment C (week 6) and had its peak in treatment D (week 8), which indicates an increase with storage time. The hatchery source had a high risk of salmonella contamination which increased rapidly as the weeks of storage increased while the bakery and food outlet sources showed no detectable salmonella counts across the treatment (weeks of storage).

**Table 2: Interaction effect of microbial loads of eggshells stored over a period of time**

| Treatment effect (No. of weeks) | Source of egg shell | Bacteria count (Cfu/g)x10$^3$ | Salmonella count (Cfu/g)×10$^3$ | Coliform count (cfu/g)×10$^3$ | Yeast count (Cfu/g)×10$^3$ |
|---|---|---|---|---|---|
| Control (0) | Hatchery | 10.50$^e$ | 0.00$^e$ | 0.00$^d$ | 4.00$^c$ |
| | Bakery | 12.50$^d$ | 0.00$^c$ | 0.00$^d$ | 4.00$^c$ |
| | Food outlet | 8.50$^e$ | 0.00 | 0.00$^c$ | 3.50$^d$ |
| A | Hatchery | 59.00$^b$ | 17.00$^b$ | 21.00$^a$ | 13.00$^a$ |
| | Bakery | 6.50$^e$ | 0.00$^c$ | 0.00$^d$ | 7.00$^b$ |
| | Food outlet | 11.00$^d$ | 0.00 | 0.00$^c$ | 6.00$^c$ |
| B | Hatchery | 29.00$^d$ | 2.50$^d$ | 15.00$^b$ | 9.50$^b$ |
| | Bakery | 44.50$^c$ | 1.50$^b$ | 10.00$^b$ | 7.00$^b$ |
| | Food outlet | 21.00$^c$ | 0.00 | 17.00$^a$ | 11.00$^a$ |
| C | Hatchery | 39.00$^c$ | 6.00$^c$ | 19.00$^{ab}$ | 13.00$^a$ |
| | Bakery | 74.00$^b$ | 3.00$^a$ | 7.00$^c$ | 6.00$^b$ |
| | Food outlet | 46.00$^b$ | 0.00 | 16.00$^b$ | 9.00$^b$ |
| D | Hatchery | 76.00$^a$ | 25.00$^a$ | 7.00$^c$ | 9.50$^b$ |
| | Bakery | 151.00$^a$ | 0.00$^c$ | 16.00$^a$ | 11.00$^a$ |
| | Food outlet | 98.00$^a$ | 0.00 | 4.50$^b$ | 6.50$^c$ |
| | SEM | 1.44 | 0.48 | 0.90 | 0.82 |
| | P-Value | 0.00 | 0.00 | 0.00 | 0.00 |

$^{abcde}$Means with different superscripts along the same column differed significantly (P< 0.05), SEM = Standard Error of Mean, A = Week 2, B = Week 4, C= Week 6, D = Week 8

Despite the fact that pasteurization was predicted to be effective for reducing *Salmonella spp*., it was still observed that salmonella count increased. Therefore, the way to avoid *Salmonella* contamination is to eliminate the infectious agent through several cooking methods (Nahla, 2015). The high coliform counts observed on hatchery eggshells might be an indicator of poor hygienic handling or processing and greater exposure to contamination. According to Musgrove *et al*. (2008), coliform counts are the traditional indicator of microbial quality, safety and reflect the hygiene standards adopted in the food operation.

The interaction between the weeks of storage and the source of eggshells presented in Table 2 showed that the microbial loads were significantly ($p<0.05$) different. The highest bacteria count was recorded on week 8 for eggshells sourced from the bakery (151.00 cfu/g $\times10^3$). There was no salmonella and coliform in the control group immediately after heat treatment irrespective of the eggshell source. Eggshells sourced from food outlets also had no salmonella count throughout the 8 weeks of storage. Eggshells from the hatchery had the highest coliform count at week 2 (21.00 cfu/g $\times10^3$). Several factors contributing to the selective transfer and survival of specific bacterial species on eggshells probably include the different ability of certain bacteria to adhere to or survive on the eggshell surface (Trudeau *et al*., 2020).

## CONCLUSION AND RECOMMENDATION

This study revealed that the longer the period of storing eggshells, the more the microbial load increased. Freshly treated eggshells did not have salmonella and coliform, thus, it was concluded that eggshells should not be stored more than 2 weeks prior to usage in animal feed. Longer heat treatment time is also recommended.

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