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Influence of Sodium Chloride Treatment and Storage Temperature on the Microbial and Physicochemical Quality of Garden Egg (*Solanum aethiopicum L.*)

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Abstract

This study investigated the effect of 7% Sodium Chloride treatment on the shelf life of garden eggs under distinct storage conditions (ambient and refrigerated) by examining the microbial dynamics, proximate composition, and mineral content. Standard microbiological and proximate methods were employed. Results indicated significant variations in microbial counts across different storage periods and conditions. The total heterotrophic bacterial count of unsalted garden eggs ranged from $1.29x10^6$ CFU/g to $1.3x10^8$ CFU/g, showcasing a notable increase over the storage duration. *Staphylococcus* counts fluctuated, with ambient temperature storage showing no growth at the final stage but refrigerated samples exhibiting counts of $1.5x10^2$ CFU/g. Similarly, Total Coliform counts varied from $1.37x10^4$ CFU/g to $3.6x10^5$ CFU/g for unsalted garden eggs stored at different temperatures. Counts were less for Garden egg samples treated with 7% Sodium Chloride compared to those not treated across all storage conditions. Frequencies of occurrence differed among these organisms, with *Staphylococcus* spp. and Bacillus spp. being the most prevalent at 28.2% and 16.5%, respectively. Others were *Escherichia coli* 20%; *Klebsiella* spp. 2.3%; *Proteus* spp. 9.4%; *Pseudomonas* spp. 7.1% *Lactobacillus* 11.8 %; *Corynebacterium* 1.2%; and *Salmonella* spp. 3.5%. Fungal counts exhibited variations as well, ranging from $7.15x10^3$ CFU/g to $5.95x10^4$ CFU/g for unsalted garden eggs stored at room temperature. Predominant fungal isolates included: *Yeast* 24.1%; *Aspergillus niger* 24.1%; *Mucor* 22.2%; *Fusarium* 9.3%; *Aspergillus flavus* 7.4 %; *Penicillium* 11.1% and *Trichoderma* 1.9%. Moreover, proximate composition analyses displayed fluctuations in ash, moisture content, lipid, crude protein, crude fiber, and carbohydrates between day 0 and day 15 of storage, indicating changes in nutritional constituents during the shelf life. Additionally, the mineral content, Phosphorus, Calcium, and Potassium showed alterations in concentrations from day 0 to day 15 for both salted and unsalted samples across different storage conditions. These findings underscore the importance of refrigeration storage in controlling microbial proliferation, emphasizing the need for stringent quality control measures, awareness campaigns, and the potential incorporation of antimicrobial agents to extend the shelf life of garden eggs. This study provides valuable insights into optimizing storage conditions and enhancing food safety, quality, and nutritional retention of garden eggs.

***Keywords*:** *Garden Egg, Sodium Chloride, Storage temperature, Microbial and Physiochemical Quality*

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I. INTRODUCTION

The phrases garden egg" comes from the appearance of certain sorts of this natural product, which take after chicken eggs (Opara and Udourioh, 2023). Having a place to the Solanaceae family and the Solanum class, around 25 species of plant eggs are found in Nigeria, where they are either developed or developed in the wild. The clear and natural products of these species are both eaten as vegetables and utilized in conventional pharmaceuticals (Bonsu *et al*., 2008). In Nigeria, garden eggs are alluded to as "gauta" in Hausa, "afufa" or "anara" in Igbo, and "igba" in Yoruba.

The garden egg is experimentally classified as *Solanum aethiopicum L.,* which is socially and culinarily noteworthy. These natural products can be eaten either crudely or cooked, particularly in soups and stews (Edem *et al*., 2009). Although they are fundamentally developed in northern Nigeria, plant eggs are moreover well known in the southern and western districts (Chinedu *et al*., 2011). They come in an assortment of shapes and colors, counting little yellow sorts with green stripes, bigger yellow and white assortments, and level, ribbed green shapes. By and large, they are picked amid the blustery season, with the most noteworthy accessibility happening from Admirable to October, although a few

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developments exterior of this season are conceivable through irrigation.

In terms of nutrients, Garden eggs are comparable to tomatoes but have lower vitamin C content. The fruit's flesh can be either cream-colored or green and has a light consistency (Chinedu *et al*., 2011). Both the roots and the natural products are recognized for their restorative benefits, which incorporate narcotic properties and the treatment of sicknesses such as hypertension and colic (Grubben and Denton, 2004). The deterioration of garden eggs in Nigeria is affected by several factors, including animal activity, insect damage, contact with soil, and human handling practices like inadequate harvesting (Schwartz and Gent, 2007). Furthermore, natural conditions altogether impact microbial advancement and deterioration. This research intends to explore the shelf life of garden eggs to minimize waste and spoilage, enhance food safety, and improve marketability, particularly examining the disinfectant effects of sodium chloride (NaCl) and the effectiveness of washing fruits.

Garden eggs are one of the most commonly consumed fruit vegetables in tropical Africa, following tomatoes and onions but preceding okra. They are classified as both vegetables and fruits, valued for their diverse horticultural traits. Rich in essential vitamins and minerals, garden eggs are among the top ten vegetables for antioxidant potential, thanks to phenolic compounds such as caffeic acid and chlorogenic acid. Their nutritional importance also encompasses traditional medicine, where they are used to improve digestion, relieve constipation, combat cancer, regulate blood pressure, and lower cholesterol levels.

Sodium chloride (NaCl) is a preservative widely used in food, due to its antibacterial properties. For garden eggs, NaCl treatment can inhibit the growth of pathogenic and putrefactive microorganisms, which have effectiveness depending on the concentration and duration of exposure. The microbial quality of garden eggs during storage is strongly influenced by storage conditions, including temperature, humidity and storage atmosphere (aerobic or anaerobic).

Microbial spoilage of stored garden eggs can be caused by pathogenic or saprophytic microorganisms, which can invade healthy fruit through stomatal openings, growth cracks, or surface lesions. Common spoilage agents associated with garden eggs *include Enterobacter spp., Salmonella spp., Bacillus spp., Escherichia coli, Listeria monocytogenes, Penicillium spp*., and *Aspergillus spp*. (Schwartz and Gent, 2007). The effects of these pathogens, especially *Aspergillus niger*, known for its high ability to form spores and produce toxins, highlight the challenges facing garden eggs storage and safety. Sodium chloride treatment and different storage temperatures can be used for garden eggs (*Solanum aethiopicum L*). Understanding how sodium chloride treatment interacts with different storage conditions will provide valuable information to improve post-harvest management and reduce spoilage of this important fruit-bearing vegetable. The study is aimed at determining the influence of sodium chloride treatment and storage temperature on the microbial and physicochemical quality of garden eggs (*Solanum aethiopicum L).*

II. Materials And Methods

A. Sample collection

Solanum aethiopicum L. (the green-striped round-shaped garden egg) were bought from Choba market in Port Harcourt Rivers State Nigeria, packaged in sterile Ziploc bags and then taken to the laboratory immediately for analysis.

B. Sample preparation / Treatment

The garden egg samples obtained from the market were placed in a clean plastic bowl and rinsed under runny water. Some of the garden egg samples were separated and placed in another bowl, and 7g of sodium chloride was weighed and put inside 100ml of distilled water making 7% sodium chloride, the sodium chloride solution was stirred vigorously and poured inside of the bowl containing the separated garden egg samples. The treated garden egg samples were divided into two parts. One portion of the treated garden egg sample was put inside a sterile Ziploc bag and this sample was placed inside a refrigerator of temperature 4°C and stored for 15 days. This sample was labelled as TGRT-For treated garden egg sample at refrigerated temperature the second portion of the treated garden egg sample was placed inside a sterile tray and stored for 15 days at ambient temperature. This sample was labelled as AT-For treated garden egg samples at ambient temperature the remaining garden egg samples were divided into two portions, one portion was put into a sterile ziploc bag and placed in the refrigerator at a temperature 4°C and stored for 15 days. This sample was labelled as UGRT-For untreated garden egg sample at refrigeration temperature. The second garden egg sample was placed inside a sterile tray and stored at ambient temperature for 15 days. This sample was labelled as UGAT-For untreated garden egg sample at ambient temperature on day 0 samples UGAT and TGAT were analyzed, while on days 3, 6, 9, 12, and 15 after storage samples UGAT, TGAT, UGRT and TGRT were analyzed as seen in table 1.

Table 1. Different sample types and sample codes

Sample code	TREATMENT
UGAT	Untreated garden egg sample kept at ambient temperature
TGAT	Garden egg sample treated with 7% sodium chloride kept at ambient temperature
UGRT	Untreated garden egg samples kept at refrigerated temperature
TGRT	Garden egg sample treated with 7% sodium chloride kept at refrigerated temperature

C. Microbial analysis of the samples

25 g of garden eggs were chopped with a sterile knife and transferred to a sterile stomacher for 2 min. The crushed samples were then placed into separate sterile stomachers to which 225 ml of peptone water was added. These bags were then mixed and homogenized in a homogenizing machine. A 1 ml aliquot of the sample was further diluted by adding it to 9 ml of saline in the tube, reaching a dilution of 10^{-2}, and further dilutions were made to 10^{-6}. For culturing, 0.1 ml of samples at dilution factors of 10^{-2} and 10^{-4} were spread on agar plates for duplicate counting. Furthermore, 0.1 ml samples at dilution factors of 10^{-2} and 10^{-3} were spread on a selective medium for bacterial isolation.

D. Microorganism isolation

From the diluted samples, 0.1 ml aliquots with dilution factors of 10^{-2} and 10^{-4} were plated on plate count agar for the total viable heterotrophic count. Similarly, aliquots were plated on Mannitol Salt Agar for *Staphylococcus spp*. count and on MacConkey Agar for coliform count. The plates were incubated at 37°C for 24 hours to allow bacterial growth. For fungal count, 0.1 ml of the diluted sample was plated on Potato Dextrose Agar and incubated at 25-27°C for 2-5 days. The spread plate method was employed, and inoculation was performed aseptically using sterile hockey sticks. Plates were labelled according to their dilutions and incubated. After incubation, colonies were counted and recorded.

The number of bacterial and fungal colonies was determined for each plate. The total heterotrophic bacterial count was obtained from plate count agar culture after 24 hours of incubation. The colony counts were expressed as colony-forming units per gram (CFU/g).

E. Sub-culturing of bacterial isolates

Individual bacterial colonies were selected from the previously incubated media based on their morphological features. These were then sub-cultured onto freshly prepared nutrient agar plates using the streak plate method and incubated for 24 hours to obtain pure cultures. Biochemical tests were conducted for further identification and confirmation of bacterial isolates, as described by Cheesbrough (2006).

F. Physicochemical analysis

Proximate analysis

The proximate analysis procedure including the percentage of moisture content, crude protein, ash contents and crude fiber in the garden egg sample was determined by The Association of Official Analytical Chemists methods (AOAC, 2023).

G. Mineral analysis

Sample digestion

The garden egg sample was digested using a mixture of nitric and perchloric acids. The digested sample was then diluted with distilled water. The diluted sample was analyzed for Calcium (Ca), Phosphorus (P), and Potassium (K), using Atomic Absorption Spectroscopy (AAS). Calibration was done using standard solutions. The mineral analysis procedure of the garden egg sample was determined by The Association of Official Analytical Chemists methods (AOAC 2023).

III. RESULTS

A. Total heterotrophic bacteria count of salted and unsalted garden eggs stored in ambient and refrigerated temperature

The total heterotrophic bacterial count (THBC) of salted and unsalted garden eggs stored at ambient and refrigeration temperatures exhibited significant variation over time. As shown in Figure 1. Day 0, the unsalted garden egg recorded a THBC of 1.29×10^6 CFU/g, whereas the salted garden egg sample demonstrated a count of 8.6×10^5 CFU/g. On subsequent days (days 3, 6, 9, and 12), unsalted garden eggs stored at ambient temperature showed an increase in the microbial load, with the count reaching 2.6×10^6 CFU/g. In contrast, the THBC of unsalted garden eggs stored at refrigerated temperatures was lower at 1.23×10^6 CFU/g. For salted garden egg samples, the counts observed at 3, 6, 9, and 12 days at room temperature ranged from 2.85×10^6 CFU/g to 6.55×10^6 CFU/g. Conversely, salted samples stored in the refrigerator had levels ranging from 1.75×10^6 CFU/g to 3.6×10^6 CFU/g over the same days.

On the last day of assessment (day 15), both salted and unsalted garden eggs had significantly higher bacterial levels. Unsalted garden eggs stored at ambient temperature showed a count of 1.3×10^8 CFU/g, while the refrigerated unsalted sample showed a count of 2.26×10^8 CFU/g. Similarly, salted garden eggs at ambient temperature yielded a count of 1.8×10^8 CFU/g, whereas the refrigerated salted sample reached a count of 2.36×10^8 CFU/g.

B. Staphylococcus counts of salted and unsalted garden eggs stored in ambient and refrigerated temperature

The Staphylococcus counts for salted and unsalted garden eggs stored at ambient and refrigerated temperatures displayed significant variability throughout the storage period as illustrated in Figure 2.

On day 0, the unsalted garden egg recorded a Staphylococcus count of 7.0×10^3 CFU/g, while the salted garden egg showed a slightly higher initial count of 8.4×10^3 CFU/g.

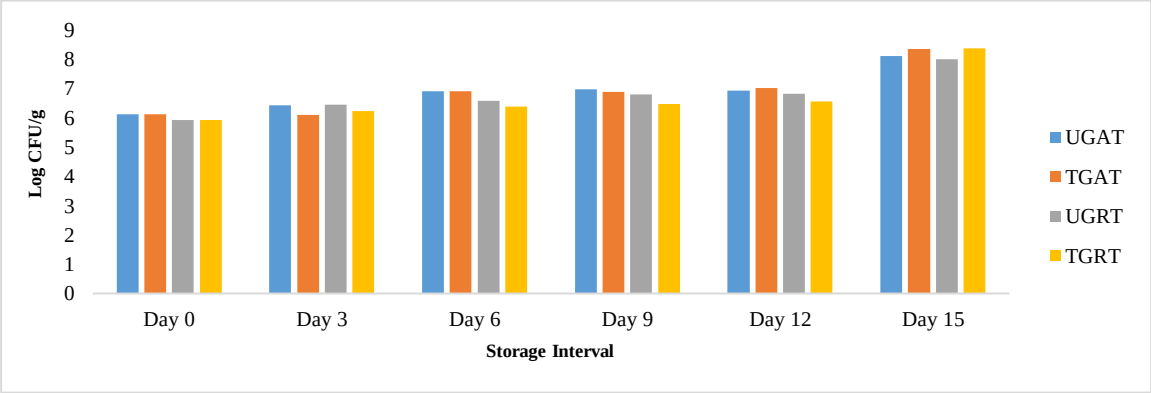

Figure 1. Total heterotrophic bacteria count of salted and unsalted garden eggs stored in ambient and refrigerated temperature.

Key: UGAT = Untreated Garden samples at Ambient Temperature, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature.

On days 3, 6, 9, and 12, the unsalted garden egg stored at ambient temperature exhibited Staphylococcus counts ranging from 4.1 × 10^3 CFU/g to 3.25 × 10^4 CFU/g. In contrast, the unsalted garden egg kept at refrigeration temperature had lower Staphylococcus counts, varying from 2.1 × 10^3 CFU/g to 9.5 × 10^3 CFU/g during the same time frame.

The salted garden egg stored at ambient temperature displayed Staphylococcus counts ranging from 6.2 × 10^3 CFU/g to 3.94 × 10^4 CFU/g over Days 3, 6, 9, and 12. Meanwhile, the salted sample stored under refrigeration showed counts ranging from 3.1 × 10^3 CFU/g to 8.4 × 10^3 CFU/g during this period. By day 15, there was a notable reduction in Staphylococcus counts. The unsalted garden egg stored at ambient temperature showed no detectable Staphylococcus growth, while the refrigerated unsalted sample had a count of 1.5 × 10^2 CFU/g. The salted garden egg stored at ambient temperature still exhibited a count of 2.86 × 10^4 CFU/g, while the one stored under refrigeration showed a reduced count of 2.25 × 10^3 CFU/g.

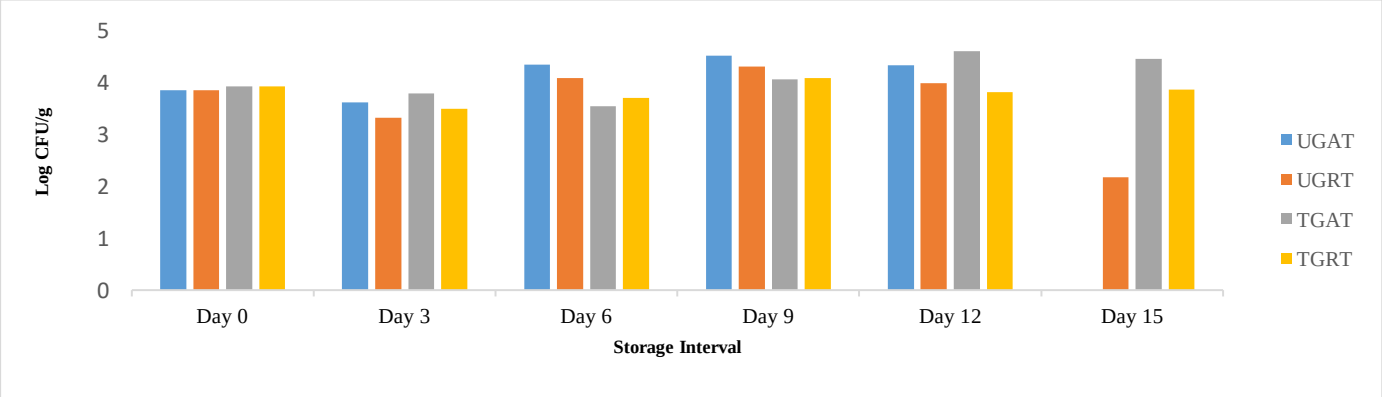

Figure 2. Staphylococcus counts of salted and unsalted garden eggs stored in ambient and refrigerated temperature.

Key: UGAT = Untreated Garden samples at Ambient Temperature, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature.

C. Total coliform counts of salted and unsalted garden eggs stored at ambient and refrigerated temperature

The total coliform counts for salted and unsalted garden eggs stored at ambient and refrigerated temperatures varied over the storage period as presented in Figure 3. On day 0, the unsalted garden egg recorded an initial coliform count of 1.37 × 10^4 CFU/g, while the salted sample exhibited a lower count of 5.8 × 10^3 CFU/g.

On days 3, 6, 9, and 12, the unsalted garden egg stored at ambient temperature showed coliform counts ranging from 6.7 × 10^3 CFU/g to 3.6 × 10^5 CFU/g. In contrast, the unsalted sample kept under refrigeration had counts varying from 7.45 × 10^3 CFU/g to 1.37 × 10^4 CFU/g over the same period.

The salted garden egg stored at ambient temperature exhibited coliform counts between 3.35 × 10^3 CFU/g and 4.75 × 10^4 CFU/g throughout Days 3, 6, 9, and 12, while the

refrigerated salted sample had counts ranging from 3.1×10^3 CFU/g to 1.85×10^4 CFU/g.

By day 15, coliform counts increased across all samples. The unsalted garden egg stored at ambient temperature reached a coliform count of 3.6×10^5 CFU/g, while the refrigerated unsalted sample had a count of 2.25×10^5 CFU/g. The salted garden egg stored at ambient temperature recorded 1.95×10^5 CFU/g, whereas the one stored under refrigeration reached a higher count of 2.70×10^5 CFU/g.

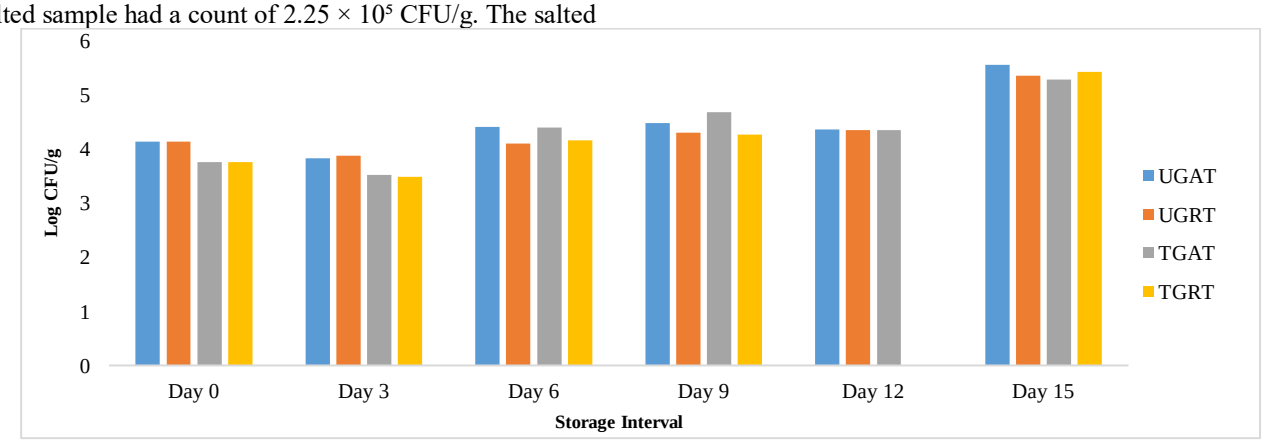

Figure 3. Total coliform counts of salted and unsalted garden eggs stored in room and refrigerated temperature.
Key: UGAT = Untreated Garden samples at Ambient Temperature, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature.

D. Total fungi counts of salted and unsalted garden eggs stored in ambient and refrigerated temperature

The fungal counts of salted and unsalted garden eggs stored at ambient and refrigeration temperatures showed variability across the storage duration as shown in Figure 4. On Day 0, the unsalted garden egg exhibited a fungal count of 7.15×10^3 CFU/g, while the salted sample had a lower count of 2.2×10^3 CFU/g.

For Days 3, 6, 9, and 12, the unsalted garden egg stored at ambient temperature displayed fungal counts ranging from 1.15×10^3 CFU/g to 7.75×10^4 CFU/g. The unsalted sample stored under refrigeration conditions exhibited a fungal count range from 2.255×10^3 CFU/g to 6.1×10^4 CFU/g.

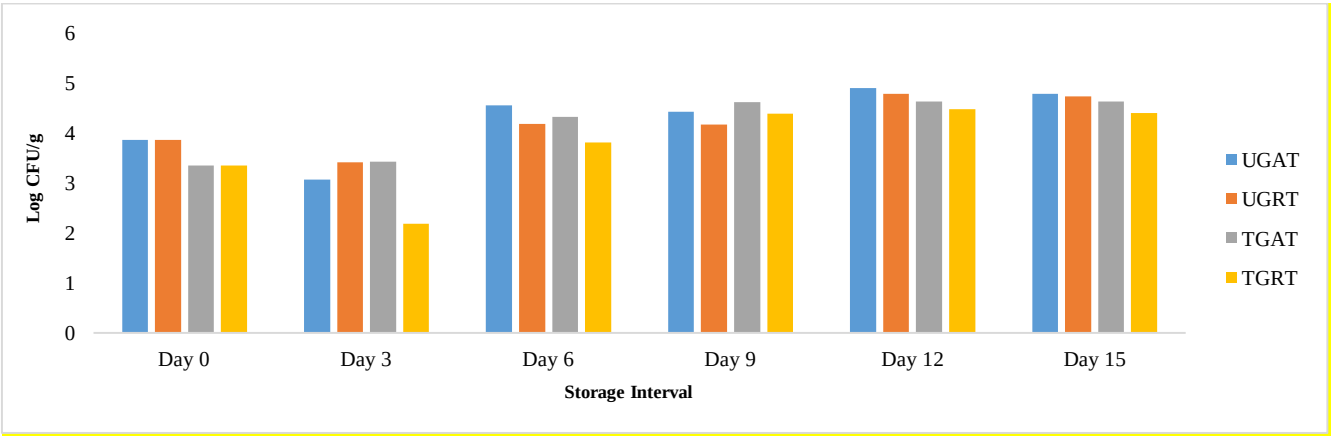

Figure 4. Total fungi count of salted and unsalted garden eggs stored in ambient and refrigerated temperatures.
Key: UGAT = Untreated Garden samples at Ambient Temperature, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature.

The salted garden egg stored at ambient temperature recorded fungal counts between 2.65×10^3 CFU/g and 4.2×10^4 CFU/g over the same period. Meanwhile, the salted sample stored under refrigeration conditions had fungal counts ranging from 1.5×10^3 CFU/g to 3.0×10^4 CFU/g.

On the final day of analysis (Day 15), increased fungal counts were observed. The unsalted garden egg stored at ambient temperature reached 5.95×10^4 CFU/g, while the one stored under refrigeration had a count of 5.35×10^4 CFU/g. The salted garden egg stored at ambient temperature exhibited a fungal count of 4.2×10^4 CFU/g, whereas the refrigerated salted sample had a count of 2.50×10^4 CFU/g.

Table 2. Frequency of occurrence of bacteria isolated from salted and unsalted garden eggs on different days.

Bacteria genera	Salted n (%)	Unsalted n (%)	Total N (%)
Staphylococcus spp.	13 (32.5)	11 (24.4)	24 (28.2)
Bacillus spp.	6 (15.0)	8 (17.8)	14 (16.5)
Corynebacterium spp.	0	1 (2.2)	1 (1.2)
Escherichia coli	8 (20.0)	9 (20.0)	17 (20.0)
Klebsiella spp.	0 ()	2 (4.4)	2 (2.3)
Pseudomonas spp.	3 (7.5)	3 (6.7)	6 (7.1)
Lactobacillus spp.	4 (10.0)	6 (13.3)	10 (11.8)
Salmonella spp.	1 (2.5)	2 (4.4)	3 (3.5)
Proteus spp.	5 (12.5)	3 (6.7)	8 (9.4)
Total	40 (100)	45 (100)	85 (100)

E. Frequency of occurrence of fungi isolated from salted and unsalted garden eggs on different days

The frequency of occurrence showed that the most frequent fungal species across all different treatments and storage conditions studied were Yeast 24.1%; *Aspergillus niger* 24.1%; *Mucor* 22.2%; *Fusarium* 9.3%; *Aspergillus flavus* 7.4 %; *Penicillium* 11.1% and *Trichoderma* 1.9% (Tables 2 and 3).

Table 3. Frequency of occurrence of fungi isolated from salted and unsalted garden eggs on different days.

Fungi identity	Salted n (%)	Unsalted n (%)	Total N (%)
Aspergillus niger	7 (26.9)	6 (21.4)	13 (24.1)
Aspergillus flavus	3 (11.5)	1 (3.6)	4 (7.4)
Fusarium spp	2 (7.7)	3 (10.7)	5 (9.3)
Yeast	6 (23.1)	7 (25.0)	13 (24.1)
Mucor spp	6 (23.1)	6 (21.4)	12 (22.2)
Trichoderma spp	0	1 (3.6)	1 (1.9)
Penicillium spp	2 (7.7)	4 (14.3)	1 (11.1)
Total	26 (100)	28 (100)	54 (100)

F. Proximate composition of salted and unsalted garden eggs stored at room and ambient temperature

The proximate composition of salted and unsalted garden egg samples studied showed that one Day 0, Ash content of the respective samples studied was 0.40 and 0.75%; Moisture content was 90.71% and 87.12%; Lipid content was 0.60 and 0.95%; Crude protein 3.00 and 3.28% and Carbohydrate 4.89 and 7.15% respectively. The values for unsalted garden egg stored at ambient temperature for Ash, Moisture content, Lipid, crude protein, crude fiber and carbohydrates respectively were 0.18%, 81.89%, 0.25%, 5.42%, 9.24% and 4.02 while that of unsalted samples stored at refrigeration temperature were 0.72%, 79.95%, 1.45%, 8.82%, 4.82%, and 4.26%. The values of salted garden egg samples stored at ambient temperature for Ash, Moisture content, Lipid, crude protein, crude fiber and carbohydrates respectively were: 0.34%, 80.42%, 0.25%, 8.33%, 8.48% and 2.19% while the salted garden egg stored at refrigeration temperature were 0.49%, 85.21%, 0.15%, 3.80%, 6.42% and 3.39%. as shown in Table 4.

Table 4. Proximate composition of salted and unsalted garden eggs stored in a room and refrigerated temperature.

Sample Code	Ash (%)	Moisture Content (%)	Lipid (%)	Crude Protein (%)	Crude Fiber (%)	Carbohydrate (%)
Day 0						
UG	0.75	87.12	0.95	3.28	0.40	7.15
TG	0.40	90.71	0.60	3.00	1.40	4.89
Day 15						
UGAT	0.18	81.89	0.25	5.42	9.24	4.02
UGRT	0.72	79.95	1.45	8.82	4.82	4.26
TGAT	0.34	80.42	0.25	8.33	8.48	2.19
TGRT	0.49	85.21	0.15	3.80	6.42	3.39

Key: UG= Untreated Garden samples TG= Treated Garden egg samples, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature, UGRT= Untreated Garden egg samples kept at Refrigerated Temperature.

G. Mineral composition of salted and unsalted garden eggs stored at room and refrigerated temperature

The mineral content of the salted and unsalted garden eggs stored at ambient and refrigeration temperatures showed that the unsalted sample on day zero contained 310.00 mg/Kg; 16.52 mg/kg and 136.00 mg/kg for Phosphorus, Calcium and Potassium respectively while the salted sample had 308.00mg/kg, 18.85mg/kg, and 134.07mg/kg respectively. On the 15th day of storage, the unsalted samples stored at ambient and refrigeration temperature had values of 305.00mg/kg and 301.00mg/kg for Phosphorus; 15.52 and 16.19mg/kg for calcium; 132.00 and 132.00mg/kg for Potassium. The salted sample had 300 and 308mg/kg for Phosphorus, 18.05 and 17.11mg/kg for calcium and 130.07 and 131.20mg/kg for potassium. As shown in Table 5

Table 5. mineral composition of salted and unsalted garden eggs stored in the room and refrigerated temperature.

Sample Code	Phosphorus (mg/Kg)	Calcium (mg/Kg)	Potassium (mg/Kg)
Day 0			
UG	310.00	16.52	136.00
TG	308.00	18.85	134.07
Day 15			
UGAT	305.00	15.52	132.00
UGRT	301.00	16.19	132.00
TGAT	300.00	18.05	130.07
TGRT	308.00	17.11	131.20

Key: UG= Untreated Garden samples TG= Treated Garden egg samples, TGAT= Treated Garden egg samples kept at Ambient Temperature UGRT= Untreated Garden egg sample kept at Refrigerated Temperature, TGRT= Treated Garden samples kept at Refrigerated Temperature, UGRT= Untreated Garden egg samples kept at Refrigerated Temperature.

IV. DISCUSSION

A. Microbial quality of sodium chloride treated and untreated garden egg stored at ambient and refrigeration temperature

The storage life of garden eggs is an important aspect of their post-harvest management, affecting their quality and safety for consumption. This study investigates how different storage conditions, in particular ambient and refrigerated temperatures, affect the microbial dynamics and biochemical

composition of horticultural eggs, thereby providing relevant information for optimizing storage practices in the agricultural and food sectors.

This study examined the shelf life of garden eggs under different storage conditions, namely ambient temperature and refrigeration temperature. The total heterotrophic bacterial counts (THBC) of salted and unsalted garden eggs varied depending on the storage conditions as seen in Figure 1. Initially, on day 0, the unsalted garden eggs showed a THBC of $1.29x\ 10^6$ CFU/g, while the salted samples showed a lower count of $8.6x\ 10^5$ CFU/g. By the final day of analysis (day 15), higher counts were recorded. Unsalted garden eggs stored at ambient temperature had a count of $1.3x\ 10^8$ CFU/g, while the refrigerated samples had a count of $2.26x\ 10^8$ CFU/g. Similarly, the salted garden egg stored at ambient temperature had a count of $1.8x\ 10^8$CFU/g, while the refrigerated sample had a count of $2.36x10^8$ cfu/g.

The initially high microbial count may reflect poor food handling practices, inadequate storage conditions, and suboptimal sample processing and selling practices. This contrasts slightly with the findings of Yaji *et al*. (2016) and Aguoru *et al*. (2015), who investigated microorganisms associated with fresh garden eggs and reported microbial counts ranging from 1.9×10^5 to 1.1×10^5cfu/g and 5.0×10^7 to 6.4×10^7 cfu/g, respectively.

The THBC results reveal a significant increase in bacterial count over 15 days in both salted and unsalted garden egg samples, and the trend observed when refrigerated storage is consistently compared to ambient conditions underscores the effectiveness of refrigeration in suppressing bacterial growth and thereby extending the shelf life of garden eggs.

Staphylococcus counts of both salted and unsalted garden eggs stored at ambient and refrigerated temperatures exhibited varying levels as illustrated in Figure 2. Initially, on day 0, the unsalted garden egg displayed a staphylococcus count of $7.0x10^3$ CFU/g, while the salted sample showed a count of $8.4x10^3$ CFU/g. By the final day of analysis (Day 15), the unsalted garden egg stored at ambient temperature showed no staphylococcus growth, whereas the refrigerated sample exhibited a count of $1.5x10^2$ CFU/g. Similarly, the salted garden egg stored at ambient temperature had a count of $2.86x10^4$ CFU/g, while the refrigerated sample had a count of $2.25x10^3$ CFU/g.

Like the total heterotrophic bacterial count (THBC), staphylococcus count increased over the study period and refrigerated storage generally inhibited growth compared to ambient temperature storage. On the 3rd day, refrigerated unsalted samples highlighted the effectiveness of refrigeration in suppressing certain bacterial species and showed a minimum staphylococcus count. However, by Day 15, both ambient and refrigerated samples showed varying staphylococcus count, indicating the persistence of this microbial species even under refrigeration.

The presence of these bacteria poses health risks to consumers who may consume fruit without proper cleaning. For example, the presence of *Staphylococcus aureus* in garden eggs can lead to gastroenteritis in individuals who consume the fruit without proper washing. Isolation of Staphylococcus spp. It may indicate unhygienic handling practices by fruit sellers (Yaji *et al*., 2016).

The total coliform count of salted and unsalted garden eggs stored at ambient and refrigeration temperatures showed varying levels as presented in Figure 3. Day 0, unsalted garden eggs showed a total coliform count of $1.37x10^4$ Cfu/g, while salted samples showed a count of $5.8X10^3$ CFU/g. By Day 15 unsalted garden eggs stored at ambient temperature showed a coliform count of $3.6x10^5$ CFU/g, while refrigerated samples showed a count of $2.25x10^5$ CFU/g. Similarly, salted garden eggs stored at ambient temperature had a count of $1.95x10^5$ CFU/g, while refrigerated samples had a count of $2.70x10^5$ CFU/g.

The total coliform count also showed a noticeable increase over 15 days, with ambient temperature storage, resulting in a higher count compared to refrigeration conditions. The last day of the analysis showed significant coliform numbers for all samples, suggesting potential fecal contamination. This highlights the important importance of maintaining proper storage conditions to reduce the risk of pathogenic bacterial growth in garden eggs. The presence of *E. coli* suggests the use of fecal-contaminated water during fruit washing, which is consistent with the findings of Yaji *et al*. (2016). They noted that the isolation of *E. coli* indicates poor hygiene of the water source used during the fruit cleaning process.

The fungi count in salted and unsalted garden eggs stored at ambient and refrigerated temperatures showed varying levels as seen in Figure 4. Day 0, unsalted garden eggs showed a fungal calculation of $7.15x10^3$CFU/g, while salted samples showed a calculation of $2.2x10^3$CFU/g. By the last day of analysis (Day 15), unsalted garden eggs stored at ambient temperature had a fungal count of 5.95x107CFU/g, while chilled samples had a count of 5.35x104CFU/g. Similarly, salted garden eggs stored at ambient temperature had a count of $4.2x10^4$ CFU/g, while refrigerated samples had a count of 2.50x104 CFU/g.

Fungal numbers followed a similar pattern, and ambient temperature preservation promoted higher fungal growth compared to refrigeration. The presence of certain fungal species, such as *Mucor spp. Aspergillus flavus, Aspergillus niger, Yeast, Trichoderma spp*. and *Penicillium spp*. It emphasizes the diversity of fungi in the garden egg samples. Refrigeration reduced the number of fungi counts, but did not eliminate their presence, emphasizing the importance of vigilant storage practices.

Salt has been identified as an effective preservative in various foods, including vegetables, such as garden eggs. A high salt concentration can inhibit the growth of putrefactive microorganisms and prolong the shelf life of garden eggs. Morphological and biochemical characterization of the isolates from the samples revealed the presence of nine genera of bacteria, including *Staphylococcus, Klebsiella, Bacillus, Lactobacillus, Corynebacterium, Pseudomonas, Escherichia coli, Salmonella* and *Proteus* as seen Table 2.

These findings provide valuable insights into the diversity of microbes present in garden eggs. The presence of potential pathogens like *E. coli* and *Salmonella* underscores the importance of proper storage and handling to prevent foodborne diseases.

Macroscopic and microscopic identification of fungal isolates from salt-treated and unsalted samples, as shown in Table 3. The predominant fungal isolate was *Mucor spp. Aspergillus flavus, Aspergillus niger, Yeast, Trichoderma spp*, Fusarium and *Penicillium.* The frequency of the occurrence of isolates varied depending on the treatment and storage conditions studied.

The frequency obtained was *Staphylococcus spp*. 28.2%; *Bacillus spp*. 16.5%; *E. coli* 20.0%; *Klebsiella spp*. 2.3%; *Proteus*. 9.4%; *Pseudomonas spp*. 7.1% lactic acid bacteria 11.8%; *Corynebacterium* 1.2%; and *Salmonella.* 3.5% as seen in table 2. Frequency of occurrence showed that there were the most frequent fungal species across all the different samples studied: yeast 24.1%; *Aspergillus niger* 24.1%; *Mucor* 22.2%; *Fusarium* 9.3%; *Aspergillus flavus* 7.4%; *Penicillium* 11.1% and *Trichoderma* 1.9% as seen in table 3. Most of the isolated bacterial species are similar to the study of Nasiru and Dalhatu, (2020) and Mike-Anosike *et al*., (2019).

Several species of bacteria and fungi have been identified, including *Staphylococcus aureus, Escherichia coli* and the genus *Salmonella. Aspergillus* and *Penicillium spp*. *Staphylococcus aureus* poses a significant risk to public health due to its ability to produce toxins that can cause food poisoning (Balaban *et al*., 2000). Symptoms of *E. coli* are known to induce foods derived from foods characterized by symptoms such as diarrhea and more serious infections (Kaper *et al*., 2004). Similarly, the genus Salmonella. It has been associated with foodborne diseases that result in symptoms such as diarrhea, fever, and abdominal cramps (Majowicz *et al*., 2010). Fungal species such as *Aspergillus spp.* and *Penicillium spp.* produce mycotoxins that can contaminate food, posing health risks upon ingestion (Pitt *et al*., 2000).

Sodium chloride (salt) can inhibit the growth of spoilage and pathogenic microorganisms, extending shelf life although higher concentrations of salt may be more effective in reducing microbial load but can affect the overall quality and taste of the garden egg. Lower temperatures generally reduce microbial growth rates. Storing garden eggs in a refrigerator can significantly prolong their freshness. Higher temperatures can accelerate microbial growth, leading to spoilage and reduced safety.

B. Proximate and mineral composition of sodium chloride treated and untreated garden egg stored at ambient and refrigeration temperature

The proximate composition of the salted and unsalted garden egg samples as seen in Table 4 showed that on day 0, the ash contents of the respective samples examined were 0.40 and 0.75%, which are very similar to the values of 0.87 and 0.47% obtained for *S. aethiopicum L* and *S. macrocarpon L*. respectively, whereas round green *S. aethiopicum* and sweet white *S. macrocarpon* cultivars recorded lower values of 4.06 and 5.58%. respectively, and 23.78% in fresh *S. incanum*. as reported by Showemimo and Olarewaju (2004), Chinedu *et al*. (2011), Auta *et al*. (2011). The low ash content reflects the low mineral content of the fruit samples.

The fruit exhibited high moisture levels of 90.71% and 87.12%, akin to the values of 89.27% and 92.50% respectively found in *S. aethiopicum* L and *S. macrocarpon L* varieties by (Edem *et al*..2009) and 89.0% for Gboma fruit (*S. macrocarpon*). However, these levels were lower compared to the 95.13% moisture content observed in raw *S. incanum* and 94.8% and 94.6% in *S. gilo* and *S. aubergine* respectively by Showemimo and Olarewaju (2004), Edem *et al*. (2009), Auta *et al*. (2011), and AOAC (2012). The fruit's high moisture content suggests freshness, aiding digestion and promoting overall health. Nevertheless, it also renders the fruit susceptible to bacterial activity, leading to short shelf life and rapid spoilage (Adepoju and Oyewole, 2008). The fruit exhibited lipid contents of 0.60% and 0.95%, which are comparable to the 1.0% reported for Gboma eggplant or African eggplant but surpass the 0.1% noted for *S. aethiopicum*, as well as the 0.52% and 0.17% for *S. aethiopicum* and *S. macrocarpon* respectively by Edem *et al*. (2009), Grubben and Denton (2004), and Chinedu *et al*. (2011). This relatively low-fat content suggests limited suitability for commercial oil extraction purposes.

Regarding crude protein content, levels of 3.00% and 3.28% were observed in untreated and treated garden egg respectively. These values exceed those of 1.6% and 1.40% for *S. aethiopicum* and *S. macrocarpon* respectively, and are higher than the 2.24% and 1.33% reported for the same by Chinedu *et al*. (2011), and 1.5% for *S. aethiopicum* by Showemimo and Olarewaju (2004), Grubben and Denton (2004), Chinedu *et al*. (2011). However, they fall short of the 8.90% recorded for raw *S. incanum* by Auta *et al*. (2011), as well as the 14.87% and 15.75% for *S. gilo* and *S. aubergine* respectively reported by Edem *et al*. (2009) and Auta *et al*. (2011). Protein serves a crucial role as a source of amino acids, contributing to the organoleptic characteristics of food, and facilitating the formation of enzymes and hormones essential for bodily functions. Moreover, it aids in the production of antibodies vital for combating infections (Orech *et al*., 2005; Brosman, 2003).

In terms of carbohydrate content, both treated and untreated garden eggs exhibited values of 4.89% and 7.15% respectively. These figures exceed the 4.14% and 4.42% recorded for *S. aethiopicum* and *S. macrocarpon* by Chinedu *et al*. (2011), as well as the 4.0% for *S. aethiopicum* reported by Norman (1992) and Chinedu *et al*. (2011). However, the relatively low carbohydrate content suggests that garden eggs may not be an ideal energy source in feed formulations.

Regarding crude fiber content, untreated and treated garden eggs showed levels of 0.40% and 1.40% respectively. These values were slightly lower than the 2.96% recorded for *S.*

aethiopicum but were slightly higher than the 1.11% for *S. macrocarpon* reported by Chinedu *et al*. (2011). The high fiber and low carbohydrate content of garden eggs make them conducive for weight loss by inducing satiety and reducing the intake of high-calorie foods. Additionally, the fiber content aids in lowering cholesterol levels, thereby protecting heart health. Dietary fiber intake has also been associated with a decreased risk of various health conditions including coronary heart diseases, hypertension, diabetes, colon and breast cancer, as well as conditions like piles and appendicitis (Omale and Ugwu, 2011).

All samples exhibited varying values compared to their respective day 0 measurements by day 15. The values for unsalted garden egg stored at ambient temperature for ash, moisture content, lipid, crude protein, crude fiber, and carbohydrates were as follows: 0.18%, 81.89%, 0.25%, 5.42%, 9.24%, and 4.02% respectively. On the other hand, unsalted samples stored in the refrigerator displayed figures of 0.72%, 79.95%, 1.45%, 8.82%, 4.82%, and 4.26%. For salted garden egg samples stored at ambient temperature, the values for ash, moisture content, lipid, crude protein, crude fiber, and carbohydrates were 0.34%, 80.42%, 0.25%, 8.33%, 8.48%, and 2.19% respectively. Conversely, the refrigerated salted samples exhibited values of 0.49%, 85.21%, 0.15%, 3.80%, 6.42%, and 3.39%.

The higher nutritional value and moisture content of garden eggs may create an environment conducive to microbial growth, which may significantly contribute to the increased microbial diversity observed in the different samples studied. The results obtained in the proximate composition analysis indicate that the nutritional composition of garden eggs changed significantly during the study period. The decrease in ash and moisture content, associated with changes in lipid, crude protein, crude fiber, and carbohydrate levels, suggests ongoing biochemical processes, potentially influenced by microbial activity and environmental conditions. Changes in these parameters highlight the dynamic nature of garden egg composition during storage (Akpanabiatu and Essien, 2014).

The mineral content analysis for both salted and unsalted garden eggs stored at ambient and refrigerated temperatures as seen in Table 5 which showed initial levels on day zero and the unsalted sample exhibited concentrations of 310.00mg/kg, 16.52mg/kg, and 132.00mg/kg for phosphorus, calcium, and potassium respectively, whereas the salted sample displayed 308.00mg/kg, 18.85mg/kg, and 134.07mg/kg respectively. By the 15th day of storage, the unsalted samples-maintained values of 305.00mg/kg and 301.00mg/kg for phosphorus, 15.52mg/kg and 16.19mg/kg for calcium, and 132.00mg/kg for potassium in both ambient and refrigerated conditions. Conversely, the salted sample showed levels of 300.00mg/kg and 308.00mg/kg for phosphorus, 18.05mg/kg and 17.11mg/kg for calcium, and 130.07mg/kg and 131.20mg/kg for potassium under the same conditions. These results show changes in phosphorus, calcium and potassium levels over the storage period. Although there is some variability, the overall retention of mineral content, especially in refrigerated samples, suggests that garden eggs have the potential to retain their nutritional value if properly stored. The combined effects of sodium chloride treatment and storage temperature may have a significant impact on the mineral composition of garden eggs. Optimal conditions will improve nutritional value and extend shelf life, while poor storage conditions and excess salt can lead to nutrient loss.

V. Conclusion

Scrutinizing the shelf life of garden eggs under varying storage conditions, this study discerned pivotal insights into the dynamic microbial and biochemical transformations these agricultural commodities undergo. The totality of findings underscores the multifaceted influences of ambient and refrigeration temperatures on the quality and safety of garden eggs. The diverse array of bacterial genera and fungal isolates, alongside their morphological and biochemical characterizations, reveals the complexity of microbial interactions during storage. Proximate composition alterations, including changes in ash, moisture, lipid, crude protein, crude fiber, and carbohydrates, emphasize the ongoing biochemical transformations during storage. The mineral content variations highlight the potential for garden eggs to retain nutritional value under controlled conditions.

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