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Effect of Storage Conditions on External and Internal Quality of Table Eggs

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Authors' contributions

This work was carried out in collaboration between both authors. Author NUA designed the study, performed the statistical analysis and wrote the protocol. Author UUNN wrote the first draft of the manuscript, managed the analyses of the study and performed literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The shelf life of table eggs from poultry farm, open market and supermarket stored in the refrigerator, open shelf and cupboard for 21days were determined using standard microbiological and weight reduction techniques. The changes in pH, weight loss and bacterial load of the eggs were monitored as quality indices. Out of 216 eggs collected, only 24 (11%) were observed to be microbiologically spoilt. The bacterial isolates and their frequencies of occurrence were *Micrococcus sp.* 1(10%), *Sarcina sp.* 2(20%), *Bacillus sp.* 3(30%), *Streptococcus sp.* 1(10%), *Staphylococcus sp.* 2(20%), *Salmonella sp.* 1(10%) within the internal surfaces and *Micrococcus sp.* 4(13.3%), *Sarcina sp.* 3(10%), *Bacillus sp.* 5(16.7%), *Streptococcus sp.* 3(10%), *Staphylococcus sp.* 8(26.7%), *Salmonella sp*. 2(6.7%), *Flavobacterium sp.* 1(3.3%), *Corynebacterium sp.* 2(6.7%), *Proteus sp.* 1(3.3%) *and E. coli* 1(3.3%) from the external surfaces respectively. Under the different storage conditions, the pH of the eggs were highly alkaline but progressively declined over the 21 days of storage. Similarly, there was a significant weight loss ($p<0.05$) over the storage period. Also, the microbial load on the external surface of the refrigerated eggs which was actually lower than those on the open shelf and cupboard differed significantly

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(p=0.05). From the correlation analysis, there existed a combined effect of pH and weight on the overall microbial load/quality of the eggs investigated. Eggs stored in the refrigerator over time generally recorded lower bacterial count and relatively no significant changes in weight loss and pH values. There was a positive correlation between the bacterial load and the storage time in all the samples. Refrigeration was therefore recommended as the best storage condition for eggs in order to increase their shelf life.

Keywords: Shelf life; microbial spoilage; weight reduction; refrigeration.

1. INTRODUCTION

Egg is an inexpensive and highly nutritious food and have been described as a cheap, rich and acceptable source of protein for rapidly increasing protein requirements of the human population [1]. They have been a staple in the human diet for centuries and are considered functional food because they offer moderate calories, protein of excellent quality, great culinary versatility and low economic cost. They also contain many vitamins and minerals essential for normal growth and development [2].

An egg is basically composed of three parts – a shell, egg white and egg yolk. The shell consists of mainly calcium carbonate and calcium phosphate and has about 10,000 pores through which gases (O_2, CO_2) are exchanged with the environment [3]. The egg white represents approximately 60% of the egg's weight consisting of 88% water, 12% dry weight and primary proteins. The egg yolk represents about 28% of the weight of the egg and consists of mainly fat and protein. Eggs have a number of microbe-retarding properties which include physical barriers such as cuticle, eggshell and membrane as well as antimicrobial compounds (lysozyme, ovotransferrin) naturally present in the egg white [4,5].

Egg is naturally alkaline in pH and despite the high nutritional value and antimicrobial properties of eggs, various studies have reported the contamination and subsequent spoilage of eggs by microorganisms [5,6,7].

Although, egg contents are naturally sterile, they become contaminated when microorganisms on the shell surface gain entrance into the egg via the numerous pores on the shell surface [4]. The contamination of egg shells is largely dependent on the surfaces with which it comes into contact with after it is laid. These possible sources include faecal material, water, hands, other broken eggs, packaging material, etc. [8] According to Board and Tranter [9], the type and quantity of microbes, storage conditions and egg shell quality generally influences the penetration of microbes into the egg and thus the egg quality.

Egg weight and pH are also useful parameters in the determination of egg quality [10,11] as egg weight reduces over time due to loss of moisture to the atmosphere.

In Nigeria, eggs are mostly stored under ambient temperature conditions and the effect of storage conditions on their shelf life has been given inadequate attention [12]. This is worsened by the belief that the shell is protective against all contamination. This study therefore seeks to assess the effect of different storage conditions on the shelf life of eggs by measuring pH changes and weight loss as well as the microbial load of fresh eggs hence providing baseline information regarding their influence on the quality of eggs.

2. MATERIALS AND METHODS

2.1 Sources and Collection of Eggs

A total of 216 table eggs were randomly sourced from poultry farms, open markets and supermarkets within Uyo, Nigeria. Seventy-two (72) eggs were randomly picked/selected from different crates at each source and they were appropriately labelled and carried in packaging containers (crates) to the laboratory for analysis.

2.2 Storage of Eggs

From each source of egg collection, 24 eggs were picked respectively to form 3 groups of 24 eggs. They were exposed to 3 different storage conditions: refrigeration, open shelf and cupboard for a period of 21 days.

2.3 Determination of Egg Quality

The quality of egg samples were determined by estimating the bacterial load (using culture-

dependent techniques) as well as measuring their weight using a digital balance and adopting the methods of [4] for pH determination. Their values were recorded as means of replicates obtained at weekly intervals of days 0, 7, 14 and 21 respectively.

2.4 Culture-dependent Bacteriological Analysis

To assess the bacterial load on the egg shell (external), the swab method of [13] was adopted with slight modifications. The surface of the whole egg was swabbed aseptically with sterile cotton wool previously moistened with 0.1% peptone water. This was dipped in sterile water, allowed to stand for few hours and further diluted serially using 10-fold dilution factor. One (1) ml aliquot of appropriate dilution was inoculated into petri plates, the plates were incubated aerobically at 35 – 37℃ for 24 – 48 hours.

To assess the internal bacterial load, some eggs were dipped in 75% ethanol and allowed to air-dry. In an upright position, the upper end of the egg was flamed and holed using sterile forceps. The whole egg was emptied into a sterile beaker, mixed and serially diluted before plating using the pour-plate technique.

The media used were nutrient agar and MacConkey agar. Following incubation, discrete colonies were enumerated, isolated and identified based on their colonial morphology and biochemical characteristics using Bergey's Manual of Determinative Bacteriology [14].

2.5 Statistical Analysis

The means of microbial counts obtained from the replicates under each storage conditions were tested for significant differences at the 0, 7, 14 and 21 days of storage. Analysis of variance using single factor ANOVA were employed to determine their significant differences. Correlation analysis was also carried out to determine the relationship/influence of pH changes and weight loss on the microbial load.

3. RESULTS AND DISCUSSION

The measured weight and pH of eggs at different storage conditions are presented in Table 1. A reduction in weight of the eggs from different sources at different storage conditions was recorded over time. This weight loss as previously reported by [10] could be attributed to loss of moisture or gases to the atmosphere. Generally, eggs stored in the refrigerator had a significant reduction ($p<0.05$) in weights (comparatively 1.1 times higher) than those on open shelf and cupboard.

The pH of the eggs was highly alkaline ranging from 8.50 to 10.24 under the different storage conditions. This corroborates the report of [5] however, the storage conditions had no significant effect on the pH of the eggs. Nonetheless, this pH dropped over storage time of 21 days and contradicts the findings of [15], who reported a significant increase in pH of eggs after 23 days of storage at both ambient and chilling temperatures. It could be deduced that storage conditions and time have a significant combined effect on the pH and weight of eggs. Meanwhile, correlation analysis revealed a weak relationship ($r=0.31, 0.27$) between the pH of eggs and the microbial load. On the contrary, the weight of the egg had a strong influence ($r=0.71, 0.66$) on the microbial burden of the stored eggs.

The average bacterial load of the external surface and internal contents of the eggs stored under different conditions are presented in Table 2. The results indicated that except in few cases, bacterial contamination of the internal (egg) contents was not detected. This agrees with the report of [4] that egg contents are naturally sterile. However, the bacterial load of the external (egg shell) was observed to increase over time, with the highest counts of 8.0×10^3cfu/ml recorded on day 21 from eggs stored in open shelf and was statistically significant at $p<0.05$. This suggests that the storage conditions greatly influenced the proliferation of the organisms on the egg shell. Although the bacterial load on the external (egg shell) surfaces increased over time, [8] reported that microbial contamination of egg shells is largely dependent on the surface it comes in contact with.

The study revealed that the source(s) of the eggs had great influence on the quality as well because eggs sourced from supermarkets with presumably better sanitary conditions had lower microbial load than those from the open markets and poultry farms with typically poor sanitary conditions. With respect to storage conditions, eggs stored in the refrigerator recorded the lowest rate of microbial proliferation on the external (egg shell) surface. This could be attributed to the bacteriostatic nature of the refrigerating temperature [15].

It was also observed that eggs with higher bacterial load on the external (egg shell) surface had deteriorated internal (egg content) quality. This again corroborated the findings of [6] that increasing numbers of microorganisms on the eggshells consequently increased the risk of microbial penetration and hence contamination and subsequent spoilage of egg contents.

A total of ten isolates belonging to the genera *Staphylococcus*, *Sarcina*, *Micrococcus*, *Salmonella*, *Bacillus*, *Streptococcus*, *Corynebacterium*, *Proteus*, *Escherichia* and *Flavobacterium* were characterized and identified. Their distribution and frequency of occurrence on both the external (eggshell) surface and internal egg contents are shown in Table 3. *Bacillus* spp (30%) predominated the internal contents while *Staphylococcus aureus* (26.7%) predominated the external surface. The high percentage occurrence of *Staphylococcus aureus* (Table 3) is of concern as studies have reported Staphylococcal food poisoning caused by the consumption of contaminated eggs [16,17]. Although no *Escherichia coli* was detected in the internal content of the eggs, this was however not the case on the egg shell and may originate from the

Table 1. Observed weight (g) and pH of the egg samples

Egg	Storage	Refrigerator		Open shelf		Cupboard	
Source	**Period (days)**	**Weight (g)**	**pH**	**Weight (g)**	**pH**	**Weight (g)**	**pH**
Poultry farm	0	70.05	9.97	61.02	9.78	68.69	10.24
	7	69.48	9.82	60.00	9.52	68.64	9.02
	14	67.63	9.86	59.91	9.28	63.30	8.62
	21	64.63	9.60	58.08	9.21	50.72	8.50
Open market	0	63.54	10.04	61.06	9.88	70.01	10.11
	7	62.79	10.00	61.01	9.80	69.03	9.91
	14	60.00	9.97	61.40	9.76	68.04	9.72
	21	57.22	9.96	61.59	9.74	57.52	9.63
Super market	0	53.95	9.83	55.07	10.61	61.34	9.44
	7	53.76	9.68	53.57	9.96	60.05	9.21
	14	53.39	9.55	50.41	9.32	58.66	9.00
	21	50.26	9.47	46.81	9.04	52.64	8.92

Table 2. Bacterial load (×10^3 cfu/ml) of stored egg samples

Source of eggs	Storage	Storage conditions					
	Period (days)	Refrigerator		Open shelf		Cupboard	
		INT	**EXT**	**INT**	**EXT**	**INT**	**EXT**
Poultry farm	0	ND	3.0±0.2	ND	3.0±0.6	ND	3.0±0.1
	7	ND	3.5±0.1	ND	5.0±0.2	ND	4.0±0.4
	14	ND	4.0±0.1	ND	6.0±0.2	0.2±0.1	6.0±0.1
	21	ND	5.0±0.3	ND	7.0±0.1	0.3±0.1	6.5±0.7
Open market	0	ND	2.0±0.2	ND	2.0±0.6	ND	2.0±0.2
	7	ND	3.0±0.1	ND	5.0±0.1	ND	2.0±0.1
	14	ND	4.0±0.1	ND	6.0±0.1	0.1±0.3	5.0±0.3
	21	ND	4.0±0.5	ND	8.0±0.8	0.2±0.1	6.0±0.2
Super market	0	ND	0.1±0.7	ND	0.1±0.1	ND	0.1±0.3
	7	ND	0.1±0.1	ND	0.3±0.1	ND	0.1±0.5
	14	ND	2.3±0.3	ND	0.4±0.1	ND	0.2±0.2
	21	ND	0.4±0.4	ND	0.6±1.0	ND	0.3±0.1

**Values are means of duplicate measurements with standard deviation.*
INT = Internal/egg content
EXT = External or egg shell surface
ND = Not detected

intestine of birds during passage of egg from the cloaca. The isolation of *Salmonella* sp is in consonance with the report of [18] and suggests faecal contamination. Board and Tranter [9] reported that gram positive bacteria especially Staphylococcus are the major contaminants of egg because they predominate in the surrounding environment. The ratio of occurrence and distribution of gram positive to gram negative bacteria in this study was 5:1 and 6:4 on both the internal egg shell surface and external egg contents respectively.

Table 3. Bacterial distribution and occurrence in egg samples

Isolates	Internal (Egg content)	External (Egg shell)
Staphylococcus aureus	20%	26.7%
Bacillus sp.	30%	16.7%
Micrococcus sp.	10%	13.3%
Sarcina sp.	20%	10%
Streptococcus sp.	10%	10%
Salmonella sp.	10%	6.7%
Corynebacterium sp.	ND	6.7%
Proteus sp.	ND	3.3%
Escherichia coli	ND	3.3%
Flavobacterium sp.	ND	3.3%
	100%	100%

ND = Not Detected

4. CONCLUSION AND RECOMMENDATIONS

The contamination of the external (egg shell) surfaces is influenced by the microbial quality of the storage environment. Thus the deterioration of the internal quality is a direct consequence of microbial contamination of egg contents which is largely dependent on the microbial proliferation on the external (egg shell) surface and its storage condition.

Comparatively, refrigeration temperature provided the best storage condition as it produced eggs of better quality as evinced in their bacterial load, weight loss and pH changes. It can therefore be opined that hygienic handling and proper storage of eggs would ensure preservation of egg quality and shelf life elongation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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