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Effect of Egg Shells Washing on the Keeping Quality and Microbial Load of the Egg Contents stored at 4^0C

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Abstract
The effect of washing eggshells on the keeping quality of the egg contents refrigerated at 4^0C and their resultant microbial load were examined. Thirty-seven-day old egg samples were brought from two cage layer poultry farms. The shell samples from each of the farms were grouped into four for the different washings. The shell eggs were treated with different washing conditions. One egg sample (egg content) from each of the farms was assessed microbiologically in order to know the exact state of the content before storage. All the eggs in each of the groups were refrigerated at 4^0C and were analyzed microbiologically fortnightly. The shell after cleaning with methylated spirit was cracked with sterile surgical forceps; the contents were pooled and homogenized in a sterile beaker. The contents were serially diluted, and the 10^{-3} dilutions were cultured onto four different media. The microbial load count (< 1 x 10^3xcfu/ml) from both the washed and unwashed eggs after two weeks of storage was within the limits (< 1 x 10^5cfu/g) of APHA (1992). The microbial load count for both the washed and unwashed eggs after four (4) weeks of storage ranged from 1.5 to 4.6 x 10^3 cfu/ml and 5.0 to 9.0 x 10^3cfu/ml after 6 weeks of storage. The egg contents whose shells were subjected to different washing conditions had reduced microbial load count unlike the unwashed ones. On the whole four types of organisms: *Listeria sp* (46%), *Staphylococcus aureus* (33%), *Klebsiella* sp (13%) and *Salmonella* sp (08%) were identified. The study showed that freshly laid eggs are safe for consumption. Also washing especially with cold water and storage at 4^oC can extend the shelf life of eggs for up to 3 weeks.

Keywords: Microbial loads, Poultry, Egg storage, Egg washing, Refrigeration temperature (4^0C).

Introduction
Eggs are excellent source of proteins. They are of high biological value as they contain all the essential amino acids needed by the human body (RSCSA, 2000). Eggs therefore complement other food proteins of lower biological value by providing the amino acids that are in short supply in those foods. In view of the relative high consumption of eggs and egg products and their association with outbreaks of food poisoning, preservation of this valuable products becomes paramount (Suba *et al*., 2005). Although majority of freshly laid eggs are sterile inside, the shells soon become contaminated with liter, droppings, dust and other environmental substances. These contaminants and other un-conducive environmental conditions may cause their spoilage. Thus, the egg becomes unfit for consumption and can spread infection to consumers (USDA, 2003).
According to WHO (2005), food borne illnesses caused by microorganisms are of public health importance. The increased incidence of food borne diseases due to microbiological hazards is the result of multiplicity of factors, all associated with our fast-changing world (RSCSA, 2000). Most countries with systems for reporting cases of food borne illnesses have documented significant increase over the past decades in the incidence of diseases caused by microorganisms in food (USFDA, 2004). To ensure that eggs are safe for human consumption, USDA (2003) requires that all shell eggs be stored at 45^0 F (7^0 C) or lower after processing. This is because Salmonella, the organism often associated with food borne diseases and egg spoilage and other bacteria do not grow well at refrigerated temperature (USDA, 2008). Most cases of Salmonellosis in human beings in the United States are associated with consumption of contaminated food from animal sources, meat, raw milk and

raw or under cooked eggs (Tauxe, 1991)

Several studies had been carried out on preservation of eggs below 8^0C in a refrigerator, washing eggs with cold water (11^0C higher than the temperature of the egg), washing eggs with warm water, washing with chemicals, etc and the use of proper sanitizing agent had been conducted as means of maintaining egg shell cleanliness and preventing microbial contamination of eggs in other parts of the world (APHA, 1992; AOAC, 2000; ICMSF, 2002; OSPBHC, 2005; Suba *et al*., 2005). But in developing African countries including Nigeria, there is paucity of information on such studies. Considering the relative high human consumption of eggs, their high nutritive value and the fact that they are easily prone to microbial contamination, there is a need for quality control especially on microbial safety in order to improve their keeping quality.

Hence, this study was carried out to investigate the effect of cold, warm and lukewarm water washing of eggshells in order to control bacterial growth and subsequent contamination of eggs. This was aimed at determining the best state for the preservation of eggs in the refrigerator in order to avoid or at least reduce microbial contamination of eggs and prevent food poisoning incidences through consumption of eggs.

Materials and methods

Sample collection

Two cage layer poultry farms located at two different towns in Awka south Local Government Area of Anambra State were randomly selected. A total of thirty-seven eggs were bought from each of the farms (74 eggs altogether). The eggs were carried in sterile plastic buckets to the Department of Applied Microbiology and Brewing Laboratory of Nnamdi Azikiwe University Awka.

Sample preparation

The eggs were examined on the day of collection for cracks and those with cracks were replaced. Two eggs one from each farm was processed and cultured in order to have idea of the exact state of the eggs. The remaining thirty-six eggs from each farm were divided into four groups. Three groups were washed with either cold water, warm water, or lukewarm water respectively then later in sterile cold water. The last group was not washed at all. The washed ones were air dried and labeled according to the farm from which they were collected. The eggs in each groups were then stored in a refrigerator at 4^0C for six weeks. However, the eggs from each of the washing methods were assessed fortnightly for microbial contamination. Freshly laid eggs (about a day old) collected in the morning of the day of analysis were used as control. These were neither washed no refrigerated.

Microbiological procedures

The eggshells were cracked with sterile surgical forceps and the contents were dropped into a sterile beaker. According to AOAC (2000) as used by Olutiola *et al*. (2003), the contents of the eggs were homogenized and 1 ml of the content was added in a test tube containing 9 ml of sterile saline solution to make a ten-fold serial dilution of the contents. It was thoroughly mixed through the remaining tubes discarding 1 ml from the last tube. Dilutions 10^{-3} were cultured on to different culture media plates; Nutrient agar, MacConkey agar, Simmon's Citrate agar and Salmonella- Shigella agar for microbial counts using pour plate technique in duplicates. The whole procedure lasted for 10-15 minutes inside a sterile inoculation chamber. All plates were incubated at 37^0C for 24 hours and visible growths after overnight incubation were counted. After the 24 hours of incubation the bacterial colonies were enumerated and multiplied by the dilution factor and expressed as number of colony forming units per ml of egg material. Representative colonies that grew were sub- cultured onto fresh corresponding agar plates until pure culture of each isolate were obtained. The isolates were identified by their characteristic morphologies, Gram reaction and biochemical tests such as Motility test, Catalase, Coagulase, Indole, Urease and Citrate tests using the method of Cheesebrough, (2010). Gene sequencing of the isolates were also carried out for confirmation.

Results

The results of the study were as presented below. It was observed that the total bacteria counts from the eggs obtained from the farm A ranged from < 1 to $1x10^3$: > 1 to 4×10^3 and 5 to 9×10^3 cfu/ml for the periods of 2, 4 and 6 weeks respectively (Table 1). Similarly for the periods of 2, 4, and 6 weeks egg samples from farm B had total bacterial counts of < 1 to 1×10^3: 2 to 5×10^3 and 5 to 7×10^3, respectively (Table 1). In all categories, the bacterial counts of some isolates were found to be $< 1 \times 10^3$cfu/ml especially for storage less than 2 weeks. According to APHA (1992), $< 1x10^5$ cfu/g represents plates with no colonies. The microbiological analysis of the control egg samples from both farms at the very first time of collection without washings and storage and the ones (a day old freshly laid eggs) collected on each day of the analysis were found to be < 1 to 1×10^3 cfu/ml (Table 3). However, the egg samples collected from both farms during the 4 weeks period of analysis were found to be more 3×10^3 and 2×10^3 respectively as shown in Table 1.

Biochemical profiles of the isolates were presented in Tables 2A and B. The frequencies of the bacteria identified were shown in Table 3. Three organisms: *Listeria* spp (7), *Staphylococcus aureus* (3) and *Klebsiella* spp (2) were identified from the egg samples collected from farm A. Of these organisms *Listeria* spp was the most frequent isolate from the washed (cool) and unwashed eggs (Table 2). The most prominent organisms identified from the egg samples obtained from farm B was *Staphylococcus aureus* (5), followed by *Listeria* spp (4) besides *Salmonella* spp (2) and *Klebsiella* sp (1) (Table 2).

Table 1: Total Microbial counts of the eggs with different washing methods and the unwashed eggs stored at 4^0C in a fridge.

Different washing method	Period of storage in weeks	Total microbial counts of the washed/ unwashed (x 10^3 cfu/ml)	
		Farm A	Farm B
Washed with cold water	2	0.4	0.6
	4	1.5	2.0
	6	6.0	5.0

Washed with warm water	2	0.2	0.4
	4	1.6	2.3
	6	5.6	5.4
Washed with lukewarm water	2	1.4	0.3
	4	2.5	3.2
	6	8.0	6.2
Without washing	2	1.0	0.6
	4	4.0	4.6
	6	9.0	6.5
Control (Freshly laid)	2	0.2	0.3
	4	3.0	2.0
	6	0.5	0.4

Table 2A: Biochemical profile of the isolates from the egg samples subjected to different washings and stored at 4^0C (Farm A).

Method of washing	Period of storage (weeks)	Gram reaction	Shape	Motility test	Catalase test	Indole test	Urease test	Probable organism
Washing with cool water	2	+	Rod	+	+	-	-	*Listeria*
	4	+	Rod	+	+	-	-	*Listeria*
	6	-	Rod	+	+	-	x	*Klebsiella*
Washing with warm water	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Cocci	-	+	-	-	*Staphylococcus*
	6	+	Rod	+	+	-	x	*Listeria*
Washing with warm/cool water	2	+	Rod	+	+	-	-	*Listeria*
	4	+	Cocci	-	+	-	-	*Staphylococcus*
	6	-	Rod	-	+	-	x	*Klebsiella*
Without washing	2	+	Rod	+	+	-	-	*Listeria*
	4	+	Rod	+	+	-	-	*Listeria*
	6	+	Rod	+	+	-	x	*Listeria*
Control	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Rod	+	+	-	-	*Listeria*
	6	+	Cocci	-	+	-	x	*Staphylococcus*

Keynote: + = Positive - = Negative x= Test Not carried out
Note; all the isolates were citrate negative except for Salmonella, Klebsiella.

Table 2B: Biochemical profile of the isolates from the egg samples subjected to different washings and storage at 4^0C (Farm B).

Method of washing	Period of storage (weeks)	Gram reaction	Shape	Motility test	Catalase test	Indole test	Urease test	Probable organism
Washing with cool water	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Rod	+	+	-	-	*Listeria*
	6	-	Rod	-	+	-	x	*Klebsiella*
Washing with warm water	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Cocci	-	+	-	-	*Staphylococcus*
	6	+	Rod	+	+	-	X	*Listeria*
Washing with warm/cool water	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Cocci	-	+	-	-	*Staphylococcus*
	6	-	Rod	+	+	-	X	*Salmonella*
Without washing	2	+	Rod	+	+	-	-	*Listeria*
	4	+	Rod	+	+	-	-	*Listeria*
	6	-	Rod	+	+	-	X	*Salmonella*
Control	2	+	Cocci	-	+	-	-	*Staphylococcus*
	4	+	Cocci	-	+	-	-	*Staphylococcus*
	6	+	Cocci	-	+	-	x	*Staphylococcus*

Keynote: + = Positive - = Negative x= Not carried out
Note; all the isolates were citrate negative except for Salmonella, Klebsiella.

Table 3: Frequency of bacteria identified from the egg samples stored at 4^0C after the six weeks interval.

Washing Methods	Period of storage	Total no of eggs examined	Type and frequency of bacteria							
			Staphylococcus		*Listeria*		*Klebsiella*		*Salmonella*	
			Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B
Washing with cool water	2	2	-	1	1	-	-	-	-	-
	4	2	-	-	1	1	-	-	-	-
	6	2	-	-	-	-	1	1	-	-
Washing with warm water	2	2	1	-	-	-	-	-	-	-
	4	2	1	1	-	-	-	-	-	-
	6	2	-	-	1	1	-	-	-	-

Washing with warm/cool water	2	2	-	1	1	1	-	-	-	-
	4	2	1	1	-	-	-	-	-	-
	6	2	-	-	-	-	1	-	-	1
Without washing	2	2	-	-	1	1	-	-	-	-
	4	2	-	-	1	1	-	-	-	-
	6	2	-	-	1	-	-	-	-	1
Total no		**24**	**3**	**5**	**7**	**4**	**2**	**1**	**0**	**2 =24**

Discussion

The microbial count of < 1 x10^3 cfu/ml after storage for 2 weeks in this study indicated that the maximum storage periods of eggs in the refrigerator at 4^0C should not exceed 2 weeks.

The findings of this study disagree with the recommendation of USDA (2003) and that of Suba *et al*. (2005) who postulated that fresh shell eggs should be used within 3-5 weeks after laying. This could be attributed to the fact that in Nigeria epileptic power supply is the order of the day and this hinders adequate and safe refrigeration of food items. The result of this study disagrees also with the work reported by Sauba *et al.*, (2005) who recommended a storage temperature of 12^0C for table eggs. According to APHA (1992) < 1x10^5cfu/g represents plates with no colonies. The microbial count after 2 weeks of storage is in line with the APHA, 1992 recommendations pertaining to the microbial contamination of the eggs. From 4 to 6 weeks storage for both washed and unwashed eggs, the microbial load count increased from 1.5 x 10^3 to 9.0 x 10^3 cfu/ml. *Salmonella* spp was only isolated from eggs washed with lukewarm water and from that of unwashed ones from farm B after 6 weeks of storage as seen in Table 2B. Gast and Beard (1992) reported that 3% of freshly laid eggs, 4 % of eggs held for 7 days at 7.2^0C and 16% of eggs from 25^0C held for 7 days were contaminated with *Salmonella* spp.

However, in all the storage periods (2-6 weeks) the unwashed eggs had higher microbial load compared to washed ones. Information has it that washed eggs had lower microbial counts on the shell surface than unwashed eggs and there was no movement of microbes from the shell to the content provided the shell cuticle was not damaged (OSPBHC, 2005). The microbial load counts from the eggs cultured on the first day of collection and the subsequent ones collected on the day of each analysis (<1x10^3 cfu/ml) except for the eggs collected during the analysis of the 4 weeks storage (3.0(A) and 2.0 (B) x 10^3 cfu/ml) were within the tolerable limits of APHA, (1992). Hence, freshly laid eggs from both farms are microbiologically safe for human consumption when they are freshly laid.

From the result, it can be deducted that the type of bacteria and their microbial loads differed according to the farm, washing method and the period of storage though not significant. It was observed that washing of eggs shells before storage in a refrigerator and the period of storage has effect on the types, microbial load, and keeping quality of the eggs. Hence, egg samples washed with cold, warm and lukewarm water can be stored up to 3 weeks as against the ones without washing (Table 1), but none of the eggs washed or unwashed could be stored up to 6 weeks.

The differences in the frequencies and types of organisms isolated from the contents of the eggs collected from the two farms as presented in Table 3 could reflect differences in the handling of eggs and also on the extent to which the shell integrity has been compromised as it has been reported that numerous factors affect the general functional quality of the eggshell (OSPBHC, 2005). The frequency of Listeria (46%) in the study especially from the unwashed eggs could mean that this organism is taking the lead over Salmonella spp. Salmonella organisms especially *S. enteritidis* have been the most frequently isolated organisms from eggs (both shell and the contents) irrespective of the treatment and storage methods (USDA, 2004: OSPBHC, 2005; Science Daily, 2008; Pelzer, 2009). The isolation of microorganisms from both the washed and unwashed eggs refrigerated at 4^0C could mean that washing of shell eggs has no impact on the microbial contamination of eggs. Saced and Koons (1993) and Chen *et al*. (1996) reported minimal or no growth in refrigerated eggs at 4^0C and such storage was stated to be necessary to reduce microbial growth and rate of penetration into eggs. Report has it that the use of egg coating oil in preservation of eggs at room temperature gave satisfactory results in reducing the microbial contamination during a storage period of 5 weeks as compared with the eggs without oil coating stored at room temperature over the period of 2 weeks (Suba *et al*., 2005). They also observed that preservation of oil coated eggs at refrigerated condition over a period of 12 weeks showed almost similar bacterial, yeasts and moulds count with the eggs preserved without oil coating over the same period of storage. It has been reported that eggshells may be contaminated through cracks or shell defects. Salmonella *enteritidis*, however, is shed in the yoke via trans-ovarian transmission, prior to shell deposition (Gast and Beard, 1990).

In conclusion, farm fresh eggs in Awka South Local Government of Anambra State, Nigeria can be said to be microbiologically safe for human consumption. Fresh shell eggs refrigerated at 4^0C should be used within 2 weeks or at least 3 weeks after lay. Washing of eggshells in cool, warm or lukewarm water can only minimize microbial load counts provided the shell is intact, but not eradicate microbial contamination.

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