ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 5 (8): 772-775, 2006
ISSN 1682-8356

Bacteriological Quality of Cracked Eggs Sold for Consumption in Abeokuta, Nigeria

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Abstract: Cracked eggs are eggs with cracks on the surfaces of egg shells. The procedure in developed countries is usually to divert eggs with cracks, chips or breaks (which encourage bacteria to pass through the shell) away from the human food supply. But in developing countries such as Nigeria, they are sorted and sold at about half the prices of whole un-cracked eggs. The presence of cracks on egg shells was found to significantly ($P < 0.05$) increase the load of bacterial groups such as salmonellae, pseudomonads, staphylococci and coliforms examined. No organisms were isolated from un-cracked egg samples at purchase. Salmonellae were not detected in samples of cracked on days one and two; neither were they found in the un-cracked eggs throughout the experiment. A total of 17 bacterial isolates belonging to 13 genera were identified from the samples. *Bacillus licheniformis* and *Micrococcus* spp were only isolated from the shells while *Bacillus subtilis, Enterobacter, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhii, Serratia* spp., *Shigella* spp, *Staphylococcus epidermidis* and *Streptococcus feacalis* were isolated from cracked eggs alone. *Aeromonas, Alcaligenes Escherichia coli, Pseudomonas fluorescens* and *Staphylococcus aureus* were found on both eggs and the shells of samples examined. No organisms were isolated from cakes baked with both cracked and un-cracked egg samples. Steaming for 20 to 30 minutes was effective in removing pseudomonads and staphylococci but not coliforms while steaming for 30 minutes totally removed salmonellae from un-cracked egg samples. It was observed that frying did not remove any of the groups of bacteria examined from the egg samples. Based on the findings in this study, the use of cracked eggs for baking purposes alone is recommended.

Key words: Cracked egg, *Salmonella*, *Pseudomonas*, total aerobic bacteria

Introduction

Shell eggs without cracks have many natural, built-in chemical and physical properties that help prevent bacteria from entering and growing. These protect the egg on its way from the hen to the home. Examples of these barriers to bacterial growth are the lysozyme present in the egg white, the egg shell and the membranes between the shell and the white, and between the white and the yolk. Although the porous shell helps, it is not in itself a foolproof bacterial barrier. For further safety, government regulations in some countries require that eggs be carefully washed with special detergent and sanitized. Then, the hen's original protective shell coating is generally replaced by a thin spray coating of a tasteless, odorless, harmless, natural mineral oil. However, eggs are susceptible to bacterial growth once the shell membranes are broken. The egg becomes exposed to oxygen, and the nutrients from the white and yolk are mixed. The microorganisms of particular importance to eggs and egg products include *Salmonella*, *Staphylococcus*, *Pseudomonas* and members of the coliform group. These bacteria are typically found in the gastrointestinal tract of warm-blooded animals, manure and the soil. Although *Salmonella* may not make the carrier animal ill, if it gets in the human food supply it can make people ill. The risk of getting a foodborne illness from eggs is very low. However, the nutrients that make eggs a high-quality food for humans are also a good growth medium for bacteria. Any food, particularly protein-rich animal foods such as eggs, can carry microorganisms that cause disease or spoil the food (Frazier and Westhoff, 1986). Bacteria, if they are present at all, are most likely to be in the white and will be unable to grow, mostly due to lack of nutrients. As the egg ages, however, the white thins and the yolk membrane weakens. This makes it possible for bacteria to reach the nutrient-dense yolk where they can grow over time if the egg is kept at warm temperatures. But, in a clean, un-cracked, fresh shell egg, internal contamination occurs only rarely.

In developed countries, cracked eggs are considered contaminated and their use is discouraged. The safety of shell eggs for human consumption in such countries is therefore ensured by diverting eggs with cracks, chips or breaks away from human food supply. The high rate of poverty and malnutrition in developing countries however makes in difficult to discard cracked eggs. They are therefore sold at about half the price of whole eggs within a few days of collection. The aim of the present study therefore was to investigate the incidence of some foodborne, potentially pathogenic bacteria in cracked eggs.

Materials and Methods

Sample collection: The study was conducted in Abeokuta, South-western Nigeria. Samples of cracked eggs were randomly purchased from whole-sale egg distributors located at market places and other specific locations in the metropolis. This category of egg sellers usually sell cracked eggs along with un-cracked eggs. Four locations were chosen for the study. All samples were aseptically collected using sterile polythene bags in triplicate and taken to the laboratory for analyses in ice buckets. Whole un-cracked eggs were also purchased to serve as control. Samples were analyzed immediately on the day of purchase, while the remaining samples were kept on room temperature (29±2^{o}C) for a week. One set of samples were thereafter taken daily for analysis. On day four, one set of samples each were processed by frying, steaming for 20 mins and 30 mins separately as well as for baking small queen's cakes. On cooling, the processed samples were subjected to microbiological evaluation as for raw samples

Determination of pH: The pH values of the raw eggs were determined by a combined glass electrode and a pH meter (Mettler-Toledo, Essex M3509 Type 340) (A.O.A.C, 1990).

Microbiological analyses

Isolation and enumeration of microflora: Replicate portions of ten-fold dilutions of samples in sterile peptone water were made for all samples collected. The preparations were homogenized and 0.1 ml each of appropriate dilutions was plated using the pour plate method (Harrigan and McCance, 1976). Enumeration of the total viable counts (aerobic mesophiles) was carried out using Plate Count Agar (Oxoid CM325, Hampshire, UK). Total coliform counts and were done according to the standard procedures described in APHA (1992) using MacConkey broth and Eosin Methylene Blue agar. Counts of staphylococci were made on Mannitol salt agar and Baird-Parker medium as well as the coagulase test were used to differentiate *Staphylococcus aureus* from the other staphylococci isolated. *Salmonella-Shigella* (SS) agar was used for the isolation and enumeration of *Salmonella* and *Shigella* after enrichment of samples in tetrathionate broth. Plates were incubated at 37^{0}C for 48 - 72 h. Surface swabs of shells of the cracked eggs were also taken and cultured on the same media.

Characterization of isolates: At intervals, all colonies from a sector of incubated plates were picked, purified by repeated sub-culturing before being examined microscopically for Gram reaction (Claus, 1992), cell morphology (using 24 h old cultures), motility, pigmentation and sporulation (Harrigan and McCance, 1976). Biochemical analysis included catalase and oxidase activities, nitrate reduction, patterns of sugar utilization as well as urea and starch hydrolysis (Christensen, 1946; Harrigan and McCance, 1976).

Identification of Isolates: The isolates were identified on the basis of the results obtained from biochemical characterization complemented with the API identification kits (API System, France). The results were analyzed using Bergey's manual of systematic bacteriology (Sneath *et al.*, 1986).

Statistical analyses: The data obtained were subjected to statistical analysis (means, correlation and ANOVA) using SPSS10.0 for Windows.

Results

The bacteriological quality of cracked eggs sold for consumption in Abeokuta was evaluated in comparison with that of whole un-cracked eggs. The bacterial groups examined as indicators of quality were salmonellae, pseudomonads, staphylococci and coliforms. Total counts of aerobic mesophiles were also taken. Fig. 1 shows the total viable counts of the bacterial groups examined on the shells of cracked and un-cracked eggs at purchase. Counts were in the order of 10^{6} cfu/ml of samples. The counts of bacterial groups in samples of raw eggs from day one of purchase through the four days of storage are presented in Tables 1 to 4. Counts were in the order of 10^{6} cfu/ml from day one to day two, increasing consistently during storage at ambient temperature, until the experiment was terminated on day four when the cracked eggs were already spoilt, producing offensive odor and obviously un-fit for consumption. No organisms were isolated from un-cracked egg samples at purchase. Salmonellae were not detected in samples of cracked eggs on days one and two; neither were they found in the un-cracked eggs throughout the experiment. Significant positive correlations were observed throughout the study among counts of aerobic mesophiles, staphylococci and pseudomonads at 0.01 (2-tailed) by Pearson's correlation coefficient. A total of 17 bacterial isolates belonging to 13 genera were identified from the samples. They were *Aeromonas, Alcaligenes, Bacillus licheniformis, B. subtilis, Enterobacter, Escherichia coli, Micrococcus* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhii, Serratia* spp., *Shigella* spp., *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus feacalis*. Their occurrence in egg samples and on the surfaces of shells are as presented in Table 5. *Bacillus licheniformis* and *Micrococcus* spp were only isolated from the shells and were not found in both cracked and un-cracked egg samples. Cracked eggs had significantly higher numbers of all bacterial groups examined than un-cracked eggs. They also had more types of bacterial species both in and on their shells.

Table 1: Counts of bacterial groups (10^6 cfu/g) in samples of raw eggs at purchase (day 1)

Bacterial type	Location 1	Location 2	Location 3	Location 4	Control (un-cracked eggs)	Shell of cracked eggs
Salmonella	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.3^{b}
Pseudomonas	1.6^{b}	3.6^{c}	2.9^{bc}	3.0^{bc}	0^{a}	2.6^{bc}
Staphylococci	1.6^{b}	2.4^{c}	1.4^{b}	1.1^{b}	0^{a}	1.0^{b}
Coliform	1.3^{b}	2.4^{c}	2.2^{c}	1.1^{b}	0^{a}	2.6^{c}
Aerobic mesophiles	4.5^{bc}	5.2^{c}	3.2^{b}	3.2^{b}	0^{a}	6.0^{c}

Values along rows with different subscripts are significantly different by Duncan's Multiple Range Test at 5% confidence level

Table 2: Counts of bacterial groups (10^6 cfu/g) in samples of raw eggs on day 2 of storage at room temperature

Bacterial type	Location 1	Location 2	Location 3	Location 4	Control (un-cracked eggs)	Shell of cracked eggs
Salmonella	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.8^{b}
Pseudomonas	3.4^{bc}	5.7^{c}	5.0^{bc}	5.1^{bc}	0^{a}	2.8^{b}
Staphylococci	2.0^{c}	2.5^{d}	1.6^{bc}	1.4^{b}	0.3^{a}	1.2^{b}
Coliform	1.5^{b}	2.6^{c}	2.4^{c}	1.4^{b}	0.3^{a}	2.7^{c}
Aerobic mesophiles	5.8^{b}	7.0^{b}	4.4^{b}	6.7^{b}	1.0^{a}	5.0^{b}

Values along rows with different subscripts are significantly different by Duncan's Multiple Range Test at 5% confidence level

Table 3: Counts of bacterial groups (cfu/g) in samples of raw eggs on day 3 of storage at room temperature

Bacterial type	Location 1	Location 2	Location 3	Location 4	Control (un-cracked eggs)	Shell of cracked eggs
Salmonella	$0.7 \times 10^{6}_{ab}$	2.3×10^{6b}	0.5×10^{6ab}	1.9×10^{6b}	0^{a}	0.6×10^{6ab}
Pseudomonas	$1.2 \times 10^{7}_{b}$	3.1×10^{7c}	1.3×10^{7b}	1.4×10^{7b}	0.6×10^{6a}	1.5×10^{7b}
Staphylococci	$1.5 \times 10^{7}_{b}$	1.8×10^{7b}	1.4×10^{7b}	1.8×10^{7b}	2.0×10^{6a}	4.0×10^{7c}
Coliform	$2.7 \times 10^{7}_{d}$	3.6×10^{7e}	2.2×10^{7c}	2.5×10^{7cd}	1.0×10^{6a}	1.1×10^{7b}
Aerobic mesophiles	$2.1 \times 10^{7}_{b}$	3.2×10^{7c}	2.0×10^{7b}	2.3×10^{7b}	2.0×10^{6a}	4.9×10^{7d}

Values along rows with different subscripts are significantly different by Duncan's Multiple Range Test at 5% confidence level

Table 4: Counts of bacterial groups (cfu/g) in samples of raw eggs on day 4 of storage at room temperature

Bacterial type	Location 1	Location 2	Location 3	Location 4	Control (un-cracked eggs)	Shell of cracked eggs
Salmonella	3.9×10^{6c}	9.2×10^{6d}	3.5×10^{6bc}	3.2×10^{6bc}	0^{a}	$0.8 \times 10^{6}_{ab}$
Pseudomonas	3.1×10^{7cd}	3.5×10^{7}	3.0×10^{7bcd}	2.9×10^{7bc}	0^{a}	$2.5 \times 10^{7}_{b}$
Staphylococci	1.9×10^{7b}	2.4×10^{7c}	2.0×10^{7b}	1.9×10^{7b}	0.3×10^{6a}	$4.1 \times 10^{7}_{d}$
Coliform	3.0×10^{7d}	3.8×10^{7f}	3.5×10^{7e}	2.7×10^{7c}	0.6×10^{6a}	$1.3 \times 10^{7}_{b}$
Aerobic mesophiles	2.4×10^{7b}	3.7×10^{7b}	2.7×10^{7b}	3.2×10^{7b}	0^{a}	$5.1 \times 10^{7}_{c}$

Values along rows with different subscripts are significantly different by Duncan's Multiple Range Test at 5% confidence level

Table 5: List of isolated bacterial groups from eggs and shells

Bacterial isolates	Cracked eggs	Un-cracked eggs	Shell of cracked eggs	Shell of un-cracked eggs
Aeromonas				
Bacillus subtilis	+	+	+	+
Bacillus licheniformis	+	-	-	+
Streptococcus feacalis	+	-	-	+
Micrococcus spp.	+	-	+	+
Staphylococcus aureus	+	-	+	+
Staphylococcus epidermidis	-	-	+	+
Salmonella typhii	+	-	+	+
Pseudomonas aeruginosa	+	-	+	-
Pseudomonas fluorescens				
Alcaligenes	+	+	+	+
Proteus mirabilis	+	-	-	+
Escherichia coli	+	-	+	+
Serratia	+	-	+	+
Enterobacter	+	+	+	+

The following species were isolated only on cracked egg samples: *Bacillus subtilis, Enterobacter, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhii, Serratia spp., Shigella spp, Staphylococcus epidermidis* and *Streptococcus feacalis*. *Aeromonas, Alcaligenes, Escherichia coli,* P*seudomonas fluorescens* and *Staphylococcus aureus* were found on both eggs and the shells of samples examined. Samples of both cracked and un-cracked eggs were subjected to four different treatments on the third day after purchase and the effects of the treatments on counts of the bacterial groups are presented in Table 6. No organisms were isolated from cakes baked with both cracked and un-cracked egg samples. Steaming for 20 to 30 minutes was effective in removing pseudomonads and staphylococci but not coliforms while steaming for 30 minutes totally removed salmonellae from un-cracked egg samples. It was observed that frying did not remove any of the groups of bacteria examined from the egg samples.

Table 6: Counts of bacterial groups in processed cracked and un-cracked eggs

Bacterial/egg type	Frying	Steaming for 20 mins	Steaming for 30 mins	Baking
Salmonella Cracked eggs				
Salmonella Un-cracked eggs	3.9×10^{6c}	9.2×10^{6d}	3.5×10^{6bc}	3.2×10^{6bc}
Pseudomonas Cracked eggs				
Pseudomonas Un-cracked eggs	3.1×10^{7cd}	3.5×10^{7d}	3.0×10^{7bcd}	2.9×10^{7bc}
Staphylococci Cracked eggs				
Staphylococci Un-cracked eggs	1.9×10^{7b}	2.4×10^{7d}	2.0×10^{7b}	1.9×10^{7b}
Coliform Cracked eggs				
Coliform Un-cracked eggs	3.0×10^{7d}	3.8×10^{7f}	3.5×10^{7e}	2.7×10^{7c}
Aerobic mesophiles Cracked eggs				
Aerobic mesophiles Un-cracked eggs	2.4×10^{7b}	3.7×10^{7b}	2.7×10^{7b}	3.2×10^{7b}

Values along rows with different subscripts are significantly different by Duncan's Multiple Range Test at 5% confidence level

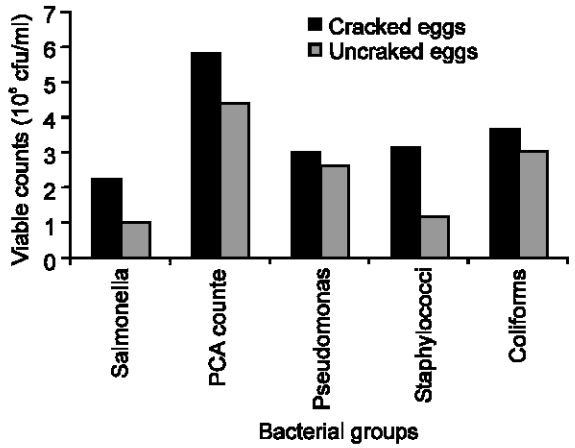

Fig. 1: Counts of bacterial groups (10^6 cfu/g) on shells of cracked and un-cracked eggs at purchase

Discussion

Microorganisms are found everywhere in our environment - in soil, water, air, animals, and insects. Foods which come into contact with dirt and manure (eggs and produce grown with manure as a fertilizer) will contain a large number of microorganisms. It is therefore almost impossible to eliminate all microorganisms, but measures can be taken to control the growth of harmful microorganisms. Northcutt *et al*. (2004) found coliforms and pseudomonads on surfaces of egg processing equipments and facilities. Proper storage and handling are important in maintaining egg quality. Eggs kept at room temperature (or above 68°F) may lose more quality in one day than in one week under refrigeration. In recent years, *Salmonella enteritidis* has adapted itself to survive in the reproductive tract of the hen. Its occurrence on the shell of some of the eggs was therefore not unexpected. The inside of an egg was once considered almost sterile. But in recent years, *Salmonella enteritidis* has been found inside a small number of eggs. Cracks on the surfaces of egg shell could however increase the chances of the organism within the egg. Perishable foods such as, meat, fish, eggs, milk and milk products are susceptible to microorganism growth. This is why sanitation must be practiced continuously from the farm to the table. It also serves to substantiate measurements of food temperature and spot checks on intrinsic inhibitory attributes. When eggs are handled with care, they pose no greater food-safety risk than any other perishable food. The scientific knowledge obtained in this study should be supported and validated by Public Health Authorities who would then ensure communication with the public. The risk associated with contaminated eggs can be reduced by proper chilling before use and eliminated by proper cooking. In developing countries, it may be impossible to prevent the consumption of cracked eggs as obtainable in developed countries; however, we recommend that they can be safely used for baking purposes.

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