



Research Article

## Effect of rinses on microbial quality of commercially available eggs and its components before processing from Ilorin in western Nigeria

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### Abstract

This study examined the effect of rinses on the microbial safety of whole egg and its components, commonly consumed in the Ilorin west area, to unrinsed eggs. The microorganisms isolated during this study include: *Serratia* spp., *Providencia* spp., *Citrobacter* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp., while the fungus includes *Cladosporium* spp., *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Penicillium* spp. and *Mucor* spp. From this study the rinsed eggs have the lowest amount of microbial load at  $0.6 \times 10^2$  cfu/ml, which was less than the accepted 6.0 log<sub>10</sub> CFU/ml recommended by the International Commission on the Microbiological Specification for Food. Therefore, it is recommended that table eggs should not be consumed raw.

**Keywords:** Rinses, microbial safety, fresh egg, isolated microorganisms, Nigeria

### 1. Introduction

The livestock industry is very important in the Nigerian economy because it provides a good source of animal protein such as meat, milk and eggs that are rich in the essential amino acids required for bodily functions. Excess released from such products could be exported for foreign exchange (Folorunsho & Onibi 2005). The poultry industry has become a diverse industry with a variety of business interests such as egg production, broiler production, hatcher, and poultry equipment (Amos 2006).

Chicken eggs are familiar, versatile, nutritious, economical and an easy to prepare food, as they provide a well balanced source of nutrients for man world-wide (McNamara 2003; Matt et al. 2009). Moreover, their high quality protein, low caloric value and ease of digestibility make eggs valuable in many therapeutic diets for adults (Bufano 2000).

Modern operations allow for the washing and packaging of thousands of eggs an hour (Klippen 1990). Since large scale operations became prevalent in the 1970s, there have been many modifications to the process (Hutchison et al. 2003).

Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms (Ellen et al. 2000; Smith et al. 2000). Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells (Schoeni et al. 1995), and also through the transovarian route (Bruce & Drysdale 1994). Other factors such as environmental temperature and humidity influence the bacterial penetration and thus, enhance the infection and spoilage (Theron et al. 2003).

Food-borne diseases caused by microorganisms are a large and growing public health problem. Contamination

of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission.

Over the years multiple experiments have been made to increase the preservation period of eggs for public consumption without depreciating the quality of their component parts (Clavijo et al. 2006). When discussing the quality of consumption eggs we have in mind the complex character of the quality which is given by various groups of technological and technical characteristics, and psycho-sensorial, sanitary, aesthetic, nutritional and economical features which must be evaluated to receive a quality certificate (Ahlboorn & Sheldon 2005; De Ketelabere et al. 2004).

Washing eggs with water colder than the egg, with water heavily contaminated with bacteria, with water containing large amounts of soluble iron, or in machines whose surfaces are contaminated with large numbers of microorganisms are established factors that increase chances of bacterial cross-contamination during egg washing (Baker & Bruce 1994; Zeidler 2002; Hutchison et al. 2003). Such conditions are addressed in AMS guidelines (USDA 2000). Appropriate detergents, sanitizers, sanitizer levels, defoamers, the prompt drying of washed eggs, changing of the water used to wash the eggs at least every four hours, and prohibition of soaking are other washing conditions addressed by the guidelines. When attention is given to these conditions, modern commercial egg shell washing operations result in improved microbiological egg quality (Baker & Bruce 1994). This program guarantees to consumers that shelled eggs produced by AMS graded facilities will meet quality and size standards (USDA 2000).

During this present research we investigated the effect of rinses on the microbiology of wet poultry eggs before being processed in an oven.

## 2. Material and methods

### 2.1. Source of eggs

Fresh good quality eggs were obtained from the poultry farm located at the back of Nigerian stored product research institute at Ilorin, Nigeria. The eggs collected were processed, while some were not processed (unrinsed). All the pooled egg samples were examined for microbial quality, for the presence of *Listeria*, *E. coli* O157:H7, *Campylobacter* sp. and *Salmonella* sp. in their albumen, yolk and their mixtures (Jones et al. 2004).

### 2.2. Sample preparation

Upon reaching the laboratory, each egg was aseptically transferred to a sterile zip-lock bag and 10 ml of phosphate buffered saline (PBS) was added. A rinse sample was obtained by shaking the bag by hand for one minute. Rinsates were stored overnight at 4°C until microbiological analyses were performed.

### 2.3 Cultural techniques

Enterobacteriaceae, Nenumerated by duplicate plating of 1 ml aliquots of egg rinsate, were put on Violet red bile glucose agar (VRBG). Plates were poured with an overlay of VRBG to assist in the recovery of injured organisms. Plates were incubated overnight at 37°C and observed for colony formation. Dark red to purple colonies with red-purple haloes were counted and converted to log<sub>10</sub> CFU/ml samples. Up to five isolates for each positive sample were randomly selected for further analysis. A numbered circular grid (10 cm diam with 1 cm<sup>2</sup> divisions) and random number tables were used to select isolates from plates with greater than 20 colonies. Each selected isolate was streaked for purity on plate count agar plates (PCA) and incubated overnight at 37°C. Slants were then stored at 4°C. Using an isolated colony the procedure was repeated twice to ensure purity. An isolate from the third streak plate was saved on brain-heart infusion agar slants at 37°C and protect beads (Technical Service Consultants Ltd., The Ropewalk, Schofield St., Heywood, Lancashire OL10 1DS) at -20°C until further identification analyses.

Total mould count on egg samples was estimated on dichloran rose bengal chloramphenicol agar (Difco-DRBC) and the plates were then incubated at 25°C for 7 to 10 days.

For *Salmonella* isolation, egg samples were enriched in Rappaport-Vassilidis broth (RV, Oxoid, UK), followed by recovery on xylose lysine dextrose agar (XLD - Scharlau, Barcelona, Spain). For *Listeria* isolation, two stage enrichment procedures were done using *Listeria* enrichment broth (LEB - Oxoid) followed by isolation on palcam agar plates (Oxoid). For *E. coli* O157:H7, tryptone soya broth (TSB, Difco, Detroit, MI, USA) supplemented with 20 mg/L novobiocin (Sigma, Germany) were used. Isolation was done on MacConkey sorbitol agar plates. Thermophilic *Campylobacter* were isolated directly or after enrichment on Karmali media at 42°C (BK + BS, Biokar Diagnostic, Beauvais Cedex - France).

The methods used were of the Association of Official Analytical Chemists (AOAC 1995) and in the compendium of methods for the microbiological examination of foods (Downes & Ito 2001). Identification of Enterobacteriaceae and other species was made by

commercially available biochemical tests, while taxonomic identification of the different genera and species was made according to microscopic criteria in accordance with appropriate keys (Pitt & Hocking 1997; Klich 2002).

## 3. Results and Discussion

From this study the rinsed eggs have the lowest amount of microbial load of 0.6×10<sup>2</sup> cfu/ml (Table 1), which was less than the accepted 6.0 log<sub>10</sub> CFU/ml recommended by the International Commission on the Microbiological Specification for Food (ICMSF 1998).

**Table 1.** Microbial analysis of rinsed wet egg and its components

Microbial isolates	Yolk	Albumin	Mixture of Yolk and Albumin
<b>Total plate count</b>	1.03 × 10 <sup>2</sup>	2.0 × 10 <sup>2</sup>	2.3 × 10 <sup>2</sup>
<b>Enterobacteriaceae</b>	-	0.6 × 10 <sup>2</sup>	1.0 × 10 <sup>2</sup>
<i>E. coli</i> O157:H7	-	-	-
<i>Salmonella</i>	-	-	-
<i>Campylobacter</i>	-	-	-
<i>Listeria</i>	-	-	-
<b>Fungi</b>	1.1 × 10 <sup>5</sup>	1.7 × 10 <sup>5</sup>	1.8 × 10 <sup>5</sup>

Microbial contamination of eggs is a well-known problem that has important economic implications and poses a serious obstacle to the well-being of consumers (Wong & Kitts 2003). Contaminants could be a spoilage microorganism, a commensal bacterium or a pathogen.

The bacteria species that were isolated include the following: *Serratia* spp., *Providencia* spp., *Citrobacter* spp., *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. The fungus includes: *Cladosporium* spp., *A. fumigatus*, *A. niger*, *A. flavus*, *Penicillium* spp. and *Mucor* spp. Mycological examination carried out in the current work revealed four genera, which agrees with published reports where *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Mucor* spp. have been recovered from eggs or their wash water (Obi & Igbokwe 2007; Salem et al. 2009).

Microbial contamination of table eggs in the process of production, handling and marketing has been, therefore, of a major public health concern. Until recently, little is known regarding microbial quality of table eggs and most studies are concerned with the quality of hatching eggs (Board & Tranter 1995; Favier et al. 2000; Knape et al. 2002).

**Table 2.** Microbial analysis of unrinsed wet egg and its components

Microbial isolates	Yolk	Albumin	Mixture of Yolk and Albumin
<b>Total plate count</b>	3.1 × 10 <sup>2</sup>	3.3 × 10 <sup>2</sup>	3.8 × 10 <sup>2</sup>
<b>Enterobacteriaceae</b>	1.0 × 10 <sup>2</sup>	0.9 × 10 <sup>2</sup>	1.8 × 10 <sup>2</sup>
<i>E. coli</i> O157:H7	-	-	-
<i>Salmonella</i>	-	-	-
<i>Campylobacter</i>	-	-	-
<i>Listeria</i>	-	-	-
<b>Fungi</b>	2.3 × 10 <sup>5</sup>	2.7 × 10 <sup>5</sup>	2.9 × 10 <sup>5</sup>

The microbial analysis of the total bacteria count present in the unrinsed fresh egg components varied from 3.8 to  $3.1 \times 10^2$  cfu/ml, whilst the yolk had the lowest number of  $3.1 \times 10^2$  cfu/ml (Table 2). When compared with the rinse fresh egg components it varied from 2.3 to  $1.03 \times 10^2$  cfu/ml whilst the yolk had the lowest number of  $1.03 \times 10^2$  cfu/ml (Table 1). Some researchers (Musgrove et al. 2005; Hutchison et al. 2004) reported that rinsed eggs had a lower bacterial count compared to the unwashed eggs.

Contamination with Enterobacteriaceae was used to evaluate the sanitary or hygienic quality of raw foods and also during food processing. The microbial analysis of Enterobacteriaceae present in the unrinsed fresh egg components varied from 1.8 to  $0.9 \times 10^2$  cfu/g, whilst the yolk had the lowest number of  $0.9 \times 10^2$  cfu/ml (Table 2). When compared with the rinse fresh egg components it varied from 0.6 to  $1.0 \times 10^2$  cfu/ml whilst the albumin had the lowest number at  $1.03 \times 10^2$  cfu/ml (Table 1). Similarly, other studies reported low detection level of Enterobacteriaceae; the highest concentration detected was 0.6 log<sub>10</sub> cfu/g (Jones et al. 2004). Rodenburg et al. (2006) and De Reu et al. (2007) found that the log average Enterobacteriaceae egg shell contamination of table eggs were 1.5 log<sub>10</sub> cfu/eggs. In 1999, Cox & colleagues stated that *L. monocytogenes* had not been detected in whole eggs during their studies (Cox et al. 1999). The pathogenic microorganisms that were not detected during microbial analysis from both the rinsed and unrinsed egg were *E. coli* O157:H7, *Salmonella*, *Campylobacter* and *Listeria*. (Table 1 and 2), showing that the eggs used during this study were in good condition. Moreover, several pathogenic microorganisms have been isolated from the surface of chicken egg shells and contents. Amongst them, *Listeria monocytogenes*, *Yersinia enterocolitica*, *E. coli* O157:H7, *Salmonella* and *Campylobacter* were detected (Farber et al. 1992; Moore & Madden 1993; Schoeni & Doyle 1994; Hope et al. 2002; Adesiyun et al. 2005).

The fungi count present in the microbial analysis of the unrinsed fresh egg components varied from 2.9 to  $2.3 \times 10^2$  cfu/ml, whilst the yolk had the lowest number of  $2.3 \times 10^2$  cfu/ml (Table 2). When compared with the rinse fresh egg components it varied from 1.8 to  $1.1 \times 10^2$  cfu/ml whilst the yolk had the lowest number of  $1.1 \times 10^2$  cfu/ml (Table 1). Moreover, a higher fungal count had been reported from egg and its components (Ahmed et al. 2002; Suba et al. 2005; Salem et al. 2009) which was reported to be >5 log<sub>10</sub> CFU/g. Jones et al. (2004) found an average fungal concentration of 1.5 log cfu/ml in the day of egg collections while averaged 0.1 log CFU/ml in the content of unwashed eggs. In conclusion, the results showed that eggs and their components used in this study are generally of a good quality when examined. The rinsed eggs had a microbial count below the value recommended by the food standard organization compared to the unrinsed eggs. Therefore, it is very important to rinse egg before being processed to minimize the risk of food-borne infection or intoxication to consumers when unrinsed eggs are consumed.

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