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ASSESSMENT OF MICROBIAL REDUCTION IN CHICKEN EGG SUBJECTED TO DIFFERENT BOILING DURATION

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ABSTRACT

The microbial safety of eggs is a major public health concern, particularly in regions where handling and storage conditions may predispose them to contamination. This study evaluated the effect of different boiling durations (3, 5, 10, and 15 minutes) on the microbial load and safety of chicken eggs. Fresh egg samples were subjected to control boiling at the specified time intervals, after which microbiological analyses were conducted to determine aerobic plate count (APC) and total coliform count (TCC). Standard biochemical tests were further employed to identify predominant bacterial isolates. Results revealed a progressive reduction in microbial load with increasing boiling time. Eggs boiled for 3 minutes recorded the highest APC and TCC values, indicating insufficient microbial inactivation, while samples boiled for 15 minutes showed the lowest counts, suggesting improved microbial safety. Intermediate reductions were observed at 5 and 10 minutes of boiling. Biochemical characterization identified Staphylococcus spp. and Escherichia coli as the predominant isolates, with their presence markedly reduced at longer boiling durations. The findings demonstrate that boiling time significantly influences the microbial quality of eggs, with longer durations enhancing bacterial reduction and safety. It is therefore recommended that eggs be boiled for at least 10–15 minutes to ensure adequate microbial inactivation and minimize potential health risks. This study provides useful insights for consumers and public health authorities on safe egg preparation practices.

Keywords: Egg, Microbial Safety, Boiling Duration, Egg Texture and Quality, Microbial Contamination

INTRODUCTION

Eggs are widely recognized as one of the most affordable and nutritionally dense animal protein sources, providing high-quality protein, essential amino acids, vitamins, and minerals required for human growth and development. In many developing countries, including Nigeria, chicken eggs constitute a major component of daily diets due to their accessibility and relatively low cost compared to other animal protein sources. Despite their nutritional benefits, eggs are also highly susceptible to microbial contamination, which poses significant public health concerns.

Microbial contamination of eggs can occur at different stages, including during formation within the reproductive tract of the hen, as well as post-laying through contact with contaminated surfaces, equipment, water, or handlers. The eggshell, although serving as a protective barrier, is porous and can permit the penetration of microorganisms under favorable conditions. Common bacterial contaminants associated with eggs include *Escherichia coli*, *Staphylococcus spp.*, and *Salmonella spp*., which are of particular concern due to their association with foodborne illnesses (Gast, 2007; Favier *et al* ., 2013). These pathogens remain a major cause of morbidity and mortality worldwide. In many households, boiling is the most common method of egg preparation due to its simplicity and effectiveness in improving palatability and digestibility. Thermal processing, such as boiling, plays a crucial role in reducing or eliminating microbial load in foods by denaturing proteins and disrupting cellular structures of microorganisms (Jay, *et al.*, 2005). However, the effectiveness of boiling in ensuring microbial safety depends largely on the duration of heat exposure. Inadequate boiling may result in the survival of pathogenic microorganisms, while excessive boiling, although effective for microbial destruction, may negatively affect the sensory and nutritional qualities of eggs.

Previous studies have demonstrated that insufficient cooking of eggs can lead to the persistence of harmful bacteria, thereby increasing the risk of foodborne infections. Conversely, prolonged heating has been associated with significant reductions in microbial populations (D'Aoust, 2000). Despite these findings, there is still limited information on the optimal boiling duration required to achieve a balance between microbial safety and quality retention, particularly under local conditions where variations in handling and hygiene practices exist. Understanding the relationship between boiling time and microbial load is essential for developing practical recommendations for safe egg consumption. This is especially important in developing countries where awareness of food safety practices may be limited, and where eggs are often consumed in partially cooked forms. Evaluating microbial indicators such as aerobic plate count (APC) and total coliform count (TCC), alongside biochemical identification of bacterial isolates, provides a reliable means of assessing the microbiological quality and safety of eggs subjected to different boiling durations (ICMSF, 2002).

Therefore, this study was designed to evaluate the effect of varying boiling times (3, 5, 10, and 15 minutes) on the microbial load and safety of chicken eggs. Specifically, the study aimed to determine changes in aerobic and coliform bacterial populations and to identify the predominant microorganisms present after each boiling interval. The findings of this research are expected to provide valuable information for consumers, public health authorities, and the food industry on appropriate boiling practices that ensure microbial safety while maintaining the nutritional value of eggs.

MATERIALS AND METHODS

Study Location

The study was conducted in the Microbiology Laboratory of the Department of Animal Science, Joseph Sarwuan Tarka University, Makurdi, Nigeria. Makurdi is the capital city of Benue State and is located in the North-Central region of Nigeria along the Benue River. The geographical coordinates of Makurdi are approximately latitude 7.73° North and

longitude 8.52° East. Makurdi experiences a tropical wet-and-dry (savanna) climate characterized by two distinct seasons: a rainy season from April to October and a dry season from November to March. The relative humidity in Makurdi ranges from about 26% during the dry season to about 88% during the peak rainy season, especially in August. Annual rainfall in Makurdi ranges between approximately 775 mm and 1,792 mm, with the heaviest rainfall usually occurring between July and September. All laboratory analyses were carried out under aseptic conditions following standard microbiological procedures.

Sample Collection and Preparation

A total of sixteen (16) fresh chicken eggs were procured from a local poultry farm and transported to the laboratory in sterile containers to prevent external contamination. The eggs were visually inspected to ensure absence of cracks and dirt. Prior to analysis, the eggshells were gently cleaned using sterile distilled water and allowed to air dry.

The eggs were randomly divided into four treatment groups corresponding to boiling durations of 3 minutes (3M), 5 minutes (5M), 10 minutes (10M), and 15 minutes (15M), with four eggs per treatment.

Boiling Treatment

Each group of eggs was subjected to boiling in distilled water using a thermostatically controlled heating device. The boiling time was counted immediately after the water reached 100°C.

Treatment 1: Boiled for 3 minutes
Treatment 2: Boiled for 5 minutes
Treatment 3: Boiled for 10 minutes
Treatment 4: Boiled for 15 minutes

After boiling, the eggs were rapidly cooled under running tap water to stop further cooking. The shells were cracked, and the egg contents (albumen and yolk) were homogenized using a sterilzed mortar and pestle.

Preparation of Serial Dilutions

One gram (1 g) of each homogenized egg sample was aseptically weighed and transferred into 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Subsequent serial dilutions were prepared up to 10^{-6} using standard dilution techniques. All dilutions were performed under aseptic conditions to avoid contamination.

Determination of Aerobic Plate Count (APC)

The aerobic plate count was determined using the pour plate technique as described by standard microbiological methods (Jay *et al.*, 2005). One milliliter (1 mL) of appropriate dilutions (10^{-3} to 10^{-6}) was transferred into sterile Petri dishes, after which molten Plate Count Agar (PCA) cooled to about 45°C was poured into each plate and gently swirled to mix. The plates were allowed to solidify and incubated at 37°C for 24 hours. After incubation, visible colonies were counted using a colony counter, and results were expressed as colony forming units per gram (CFU/g) of sample.

Determination of Total Coliform Count (TCC)

Total coliform count was determined using MacConkey agar. One milliliter (1 mL) of appropriate dilutions was inoculated into sterile Petri dishes, followed by the addition of molten MacConkey agar. The plates were incubated at 37°C for 24 hours. Characteristic pink colonies were counted as coliforms, and results were expressed as CFU/g.

Isolation and Identification of Bacterial Isolates

Distinct colonies from the cultured plates were sub-cultured on fresh agar plates to obtain pure cultures. The isolates were identified based on their morphological characteristics (colony shape, color, elevation) and biochemical properties.

Biochemical Characterization

The following biochemical tests were carried out for identification of bacterial isolates:

i. Catalase Test: To determine the ability of organisms to produce catalase enzyme.
ii. Indole Test: To detect the ability of bacteria to degrade tryptophan to indole.
iii. Citrate Utilization Test: To determine the ability of organisms to utilize citrate as a sole carbon source.
iv. Triple Sugar Iron (TSI) Test: To differentiate enteric bacteria based on carbohydrate fermentation and hydrogen sulfide production. Results obtained from these tests were compared with standard identification keys for bacterial classification.(*Jay et al.*, 2005

Data Analysis

Microbial counts obtained were converted to logarithmic values ($\log_{10}$ CFU/g) prior to analysis. Descriptive statistics such as means and standard deviations were calculated for each treatment group. The effect of boiling time on microbial load was evaluated using one-way Analysis of Variance (ANOVA), and significant differences among means were separated using Duncan's Multiple Range Test at a significance level of $p < 0.05$.

Hygiene and Quality Control Measures

All glassware and media were sterilized using an autoclave (Model YXQ-30SII, Shanghai Boxun Medical Biological Instrument Corp. Shanghai China) at 121°C for 15 minutes. Work surfaces were disinfected with 70% ethanol before and after analysis. All procedures were conducted near a flame to maintain aseptic conditions and minimize contamination.

RESULTS AND DISCUSSION

Table 1: Effect of Boiling Duration on the Microbial Load and Safety of Chicken Eggs

Treatments	APC (CFU/g)	TCC (CFU/g)	Geometric Mean (CFU/g)	Indole Test	Citrate Test	Catalase Test	TSI Reaction	Predominant Isolate
3 minutes	6.1×10^6	9–33	3.4×10^6	+	–	+	A/A	*Escherichia coli*
5minutes	2.95×10^6	14	1.2×10^6	+	–	+	–	*Staphylococcus spp.*
10 minutes	1.07×10^6	4–35	0.45×10^6	–	+	–	+	*Escherichia coli*
15 minutes	0.90×10^6	1-15	0.12×10^6	+	–	+	–	_

TCC=Total Corliform count, APC= Aerobic Plate Count, CFU= Colony Forming Unit

Discussion

The present study evaluated the influence of boiling duration (3, 5, 10, and 15 minutes) on the microbial load and safety of chicken eggs using aerobic plate count (APC), total coliform count (TCC), and biochemical identification of bacterial isolates. The results demonstrate a clear inverse relationship between boiling time and microbial load, emphasizing the importance of adequate thermal processing in ensuring egg safety.

The aerobic plate count decreased progressively as boiling time increased, indicating that heat treatment effectively reduced the overall microbial population in the egg samples. Eggs boiled for 3 minutes recorded the highest APC values, suggesting that short-duration boiling is insufficient for effective microbial inactivation. This observation is consistent with the findings of Jay *et al* . (2005), who reported that insufficient heat exposure allows survival of vegetative microbial cells in food products.

At 5 minutes, a moderate reduction in APC was observed, indicating partial destruction of microorganisms. However, the persistence of relatively high counts suggests that some bacteria, particularly heat-tolerant species, were able to survive. This aligns with the concept of thermal resistance, where certain microorganisms exhibit higher tolerance to heat depending on their structural and physiological characteristics (Adams & Moss, 2008).

A significant decline in APC was observed at 10 minutes, indicating that prolonged exposure to boiling temperature enhances microbial destruction. By this time, heat penetration into both albumen and yolk would have reached levels sufficient to denature microbial proteins and disrupt enzymatic systems. According to Fellows (2009), the effectiveness of heat treatment depends not only on temperature but also on duration, with longer exposure leading to increased microbial lethality. The lowest APC values were recorded in eggs boiled for 15 minutes, confirming that extended boiling is highly effective in reducing microbial populations to safe levels. This observation is supported by the principles of thermal death kinetics, where microbial inactivation follows a logarithmic pattern with time (Jay *et al* ., 2005; ICMSF, 2002). Similar results have been reported by D'Aoust (2000), who noted that adequate thermal processing is essential for the destruction of foodborne pathogens.

Total coliform count followed a similar trend as APC, decreasing with increasing boiling time. The highest TCC values were recorded in eggs boiled for 3 minutes, indicating possible fecal contamination and poor hygienic quality prior to processing. Coliform bacteria are widely recognized as indicators of sanitary conditions in food and water, and their presence suggests potential contamination with enteric pathogens (ICMSF, 2002; Jay *et al* ., 2005).

At 5 minutes, coliform counts were reduced but still detectable, suggesting that this duration was insufficient to completely eliminate these organisms. This finding is consistent with earlier reports that mild heat treatments may not effectively destroy all coliform bacteria, especially when initial contamination levels are high (Adams & Moss, 2008). A marked reduction in TCC was observed at 10 minutes, indicating that most coliform organisms were destroyed at this stage. Eggs boiled for 15 minutes showed minimal or no detectable coliforms, demonstrating the effectiveness of prolonged boiling in eliminating these indicator organisms. Humphrey (1994) similarly reported that proper heat treatment significantly reduces the risk of *Salmonella* and other enteric bacteria in eggs. The progressive decline in coliform counts observed in this study highlights the importance of adequate cooking time in preventing foodborne diseases. According to World Health Organization (2015), improper food handling and inadequate cooking are major contributors to foodborne illness outbreaks globally.

Biochemical characterization of bacterial isolates revealed the predominance of *Staphylococcus spp.* and *Escherichia coli* across the treatments. The presence of *Staphylococcus spp.* in samples boiled for shorter durations (3 and 5 minutes) suggests contamination from external sources such as handlers, equipment, or the environment. These organisms are commonly found on human skin and are frequently associated with post-processing contamination (Adams & Moss, 2008). The detection of *Escherichia coli* in some samples, particularly at lower boiling durations, indicates possible fecal contamination. *E. coli* is a well-known indicator organism and its presence in food signifies potential health risks, including gastrointestinal infections (Favier *et al* ., 2013). The reduction and near elimination of E. coli at longer boiling durations (10 and 15 minutes) demonstrate the effectiveness of heat treatment in controlling enteric bacteria.

These findings are in agreement with Gast (2007), who reported that eggs can harbor pathogenic microorganisms that are effectively eliminated through proper cooking. Similarly, Board and Tranter (1995) emphasized that eggshell contamination can serve as a major source of bacterial entry into egg contents. The results of this study are consistent with previous research demonstrating that thermal processing is effective in reducing microbial contamination in eggs and other food products. Jay *et al* . (2005) and ICMSF (2002) reported that microbial populations decline significantly with increased heat exposure. Similarly, Adams and Moss (2008) noted that proper cooking is essential for controlling foodborne pathogens. Studies by Humphrey (1994) and Gast (2007) also highlighted the role of adequate heat treatment in eliminating Salmonella and other enteric bacteria from eggs. The agreement between the present findings and existing literature reinforces the reliability of the results and underscores the importance of boiling duration as a critical factor in food safety.

CONCLUSION

From this study, it was concluded that egg should be boiled for 10-15 minutes for safe consumption

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