International Journal of Applied Agricultural Sciences
2023; 9(4): 100-105
http://www.sciencepublishinggroup.com/j/ijaas
doi: 10.11648/j.ijaas.20230904.12
ISSN: 2469-7877 (Print); ISSN: 2469-7885 (Online)

Deterioration of Soiled Eggs Stored at Room Temperature

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To cite this article:

Modupe Esther Adeolu, Olayinka Hannah Asolo, Olamide Tawa Owolabi. Deterioration of Soiled Eggs Stored at Room Temperature. *International Journal of Applied Agricultural Sciences*. Vol. 9, No. 4, 2023, pp. 100-105. doi: 10.11648/j.ijaas.20230904.12

Received: May 22, 2023; **Accepted**: July 6, 2023; **Published**: July 24, 2023

Abstract: Poultry eggs serve as food and for reproduction, and their quality depends on their physical and chemical composition. Contamination can occur during egg development or after laying. Microorganisms, such as bacteria and fungi, play a role in spoiling the egg content due to their mobility, ability to penetrate the egg shell and membranes, resistance to growth inhibitors, and enzymatic activities that break down nutrients in the egg fluids. This research aimed to identify the specific microorganisms responsible for degrading dirty eggs stored at room temperature. Samples of recently laid eggs, clean and soiled, were obtained from a chicken farm and analyzed at Rufus Giwa Polytechnic's Science Laboratory Technology Department. The eggs were kept at room temperature in the laboratory for four weeks, with microbial deterioration monitored weekly. Nutrient agar (NA), salmonella-shigellosis agar, and potato dextrose agar (PDA) were used for culture. Eight isolates, including *Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Klebsiella pneumonia, Enterobacter spp., Proteus spp., and Pseudomonas aeruginosa,* were found. The soiled egg samples had bacterial counts of 11.3x10^3cfu/g and fungal counts of 4.1x10^3cfu/g which increases as the week progresses. Moisture on the egg shell's surface promoted microbial growth, leading to egg contamination and degradation at room temperature. To prevent pathogenic spread, it is advised to use battery cages to prevent fresh droppings on litter and maintain regular cleaning and hygiene in the enclosures.

Keywords: Poultry, Soiled Eggs, Clean Eggs, Deterioration, Fungi and Bacteria

1. Introduction

Poultry egg is a vehicle for reproduction; it also serves as a source of food for human consumption [1]. Eggs are laid by female animals of many different species, including birds, reptiles, amphibians, a few mammals, and fish, and many of these have been eaten by humans for thousands of years [2]. Other poultry eggs including those of duck and quail also are eaten. [3]

Egg yolks and whole eggs store significant amounts of protein and choline, and are widely used in cookery [4]. Due to their protein content, eggs are categorized as *Meats* within the Food Guide Pyramid. Despite the nutritional value of eggs, there are some potential health issues arising from cholesterol content, salmonella contamination, and allergy to egg proteins. Chicken sand other egg-laying creatures are kept widely throughout the world and mass production of chicken eggs is a global industry [5]. In 2009, estimated 62.1million metric tons of eggs were produced worldwide from a total laying flock of approximately 6.4 billion hens [6].

The size and shape of eggs differs among the various species of birds, but all eggs have three main parts: yolk, albumen and shell [1]. The physical makeup and chemical content of an egg's parts determine its quality. Due to the variety of uses for poultry eggs and subsequent consumer demands, it is quite difficult to quantify egg quality [7].

The egg content may be contaminated both during egg development in the diseased hens' genital tracts and after the eggs are laid. The former sort of contamination is possible when the hen's reproductive tissues are seriously contaminated; however, it is less common, intermittent, and of a smaller degree than contamination that happens after laying. The latter is a reference to the eggshell surface becoming polluted with hen faeces as well as with flora found in hen houses and egg conditioning facilities [8]. The microbiota, which is diverse, occasionally contains pathogenic bacteria

(most commonly Salmonella enteritidis) and food-rotting microbes [9]. Gram-positive bacteria that dominate the microflora of the eggshell, such as *Staphylococcus, Streptococcus, Aerococcus, Bacillus,* and *Micrococcus,* as well as gram-negative bacteria like Salmonella, Escherichia, and *Alcaligene ssp*., which represent additional minor pollutants, are characteristics and species that cause the rotting of shell eggs. Gram-negative bacteria are thought to be more able to withstand the natural defenses of eggs than gram-positive bacteria, which make up the majority of the flora on the eggshell surface and are mostly responsible for contamination of the eggs' inside [10]. It is understood that the traits of bacteria involved in the spoilage of egg contents include the capacity to resist the growth-inhibiting effects of albumen and a variety of enzymatic activities that result in the breakdown of complex nitrogen and carbon sources present in the egg fluid. The egg fluid is the perfect matrix for promoting bacterial growth because of these properties [11].

The principal instance of egg spoilage is referred to as a rotten egg, which often emits an unpleasant stench and takes the form of a colored egg (black, blue, pink, red, or green) [12]. The bacteria implicated are *Pseudomonas, Proteus, Alcaligenes, Enterobacter, Serratia, Stenotrophomonas, Acinetobacter, Moraxella, and Citrobacter spp.* Other egg-spoiled events are reported to involve Flavo bacterium or *Cytophaga* species, which turn the shell's membranes yellow. It is not immediately obvious how the frequency of spoiling occurrences and the extent of eggshell surface contamination are connected [13].

According to [14], the type and level of egg contamination on the eggshell surface are influenced by the sanitary circumstances, in which the chickens are raised, as well as the breeding environment, breeding procedures, housing system, geographic location, and season. The environment in which the eggs are produced, how they are produced, how they are housed, where they are produced, the time of year, and the region all have an impact.

Contamination can occur during egg transport and/or packaging in fields or in the conditioning center, either through the environment or from one egg to another. Though the microflora of the eggshell surface varies, the intrinsic egg barriers have a substantial impact on the invasiveness of rotting bacteria. The rotting flora of the egg's interior, in comparison, is typically less diversified [15]. To begin with, the cuticle, shell, and shell membranes serve as barriers to prevent microorganisms from entering the interior of the egg. Even so, handling eggs or wearing them out over time might cause the cuticle, which is impermeable to water and microbial infiltration, to rupture. Thus, the usefulness of this protective covering is limited. Although the calcified proteinaceous shell of the eggshell serves as a physical barrier, it is inadequate because it may allow microbes to get through the pores, especially if the eggshell has condensed water on it [13]. Fissures or micro cracks in the eggshell increase the risk of contamination. Egg handling, especially in conditioning facilities, increases the likelihood of eggs shattering. The exterior and internal shell membranes are made of anti-bacterial glycoprotein fibers, which operate as effective filters and may aid in preventing penetration [16]. In addition to these physical barriers, egg white, which resembles intracellular fluid and represents an unfavorable environment for microbial growth (nutrient-poor, exhibiting an alkaline pH, having a high viscosity, and being heterogeneous), is a crucial line of defense against invading bacteria. It contains several molecules expressing antimicrobial activities, such as lysozyme, ovotransferrin, and proteinase inhibitors (cystatin, ovomucoid and ovoinhibitor), and vitamin binding proteins (riboflavin binding protein, avidin and thiamin binding proteins) [17]. According to [11], maintaining the integrity of these barriers—the cuticle, shell, shell membranes, egg white, and vitelline membrane—is crucial to preventing microbial invasion and growth.

1.1. General Objective of the Study

The general objective of the study is to determine the rate of deterioration of soiled eggs stored at room temperature.

1.2. Specific Objectives

The specific objectives are to determine

i) To identify the specific microorganism (bacteria and fungi) in soiled eggs stored at room temperature.
ii) To assess the microbial population in soiled eggs stored at room temperature.
iii) To determine the physiological changes on soiled eggs stored at room temperature especially in weight, colour and odour of the eggs.

2. Materials and Methods

2.1. Sample Collection

Freshly laid eggs were gotten from a poultry farm in locality; clean and soiled samples were taken from the farm and transported to the Microbiology Laboratory of Science Laboratory Technology Department of Rufus Giwa Polytechnic, Owo immediately for analysis. The eggs were stored at room temperature in the laboratory for 4 weeks and the microbial spoilage was monitored at weekly interval.

2.2. Microbial Analysis

2.2.1. Sterilization Procedures

The glass wares were rinsed in large quantity of clean water and finally with distilled water to remove salt content of the tap water, were air dried and then sterilized in hot air oven for 2 hours at 160°C. Flaming Bunsen burner was used to sterilize Inoculating wire loop until red hot and then allowed to cool before using. Alcohol of 75% was used to sterilize the work bench before and after each working period [18].

2.2.2. Culture Media Preparation

The media used for this research work include nutrient agar (NA), *Salmonella-Shigella* agar and Potato Dextrose agar (PDA). They were all of analytical grade and were gotten from the Department of Science Laboratory Technology, Rufus

Giwa Polytechnic, Owo.

Preparations of all culture media were strictly according to the manufacturer's specification.

They were all autoclaved at 121^0C for 15minutes. They were all allowed to cool to about 45^0C before dispensing into Petri-dishes and Mac Cartney bottles and allowed to set [19].

2.2.3. Microbiological Assay

(i). Isolation of Bacterial Species from Egg Samples

Nine milliliters of distilled water was pipette into 5 clean test tubes each, they were covered with cotton wool and aluminum foil, and the autoclaved at 121^0C for 20minutes. The surface of the egg shell was cleaned with cotton wool moistened with ethanol then, the yolk samples were taken with the aid of sterile needle and syringes after the shell was punctured. One ml of the content was added to one of the test tubes to make10^{-1}. The mixture was shaken well to suspend the propagules then a sterile pipette was used to measure 1ml from the supernatant into another test tube containing 9ml sterile distilled water. The mixture was shaken to homogenize this making 10^{-2}. This was done in sequential order until the last test tube10^{-6} [22]. 0.1ml of suitable dilutions was transferred aseptically into Petri dishes and molten sterile agar at (45^0C) was aseptically poured and the mixture was swirled gently to ensure even distribution of inoculates. The medium was allowed to set after which plates were inverted and incubated at appropriate temperatures [20].

(ii). Characterization and Identification of Isolates

The isolates were identifying from pure cultures and biochemical tests. It includes cultural and morphological Characteristics of the Colonies. The cultural and morphological characteristics of the colonies were observed based on the criteria of Berger's Manual of Determinative Bacteriology. These include the following shape of the colonies: circular, irregular or rhizoid the elevation: flat, raised, convex or umbonate the edge: entire, undulate, lobate or dentate optical characteristics: transparent, transluscent or opaque consistency: butyrous, viscid, granular or membraneous pigmentation: yellow, white, red, pink, blue etc.

2.2.4. Biochemical Tests

The following were the biochemical tests carried out on the isolates.

(i). Gram Staining

A differential staining procedure, which divides bacteria into two classes-gram negative and gram positive, reflects a basic structural difference in the cell-wall between the two cell types. A smear of each isolate was made on a clean grease-free glass slide with a sterile inoculating loop. It was air dried and then heat fixed by passing over a Bunsen burner flame 3 times. The slide was allowed to cool and then flooded with crystal violet solution for 30seconds. It was washed off in running tap water and again was flooded with gram's iodine for 30 seconds. It was again washed off in running tap water and then washed with ethanol for few seconds to decolourize the smear; it was then washed off in running water. The smear was then counter stained with safranin solution for 30 seconds. This was also washed off gently under the tap and the smear was allowed to air dry [21].

The stained smear was then examined under the microscope using X100 oil immersion lens. Purplish blue indicated gram positive while pink to red indicates gram negative.

(ii). Sugar Fermentation

This test shows the ability of bacteria to effectively ferment sugar with acid and/gas production. Peptone water containing 1% of the sugars (Mannitol, Arabinose, Fructose, Sucrose, Fructose, galactose, lactose, mannose, ribose, sorbitol, inosotol) was dispensed in separate test tubes. 0.5ml of an indicator (3% phenol red) was incorporated into the medium and Durham tube was introduced into the set up. The media was then sterilized by autoclaving at 121^0C for 15mins after which the indicator-sugar-broth was inoculated with the isolate and incubated at 35^0C for 3days. The same organism was introduced into different test tubes containing different sugars while some tubes were left un-inoculated to serve as control. Yellow colour indicated acid production while gas production was indicated by bubble in the Durham tube [22].

2.2.5. Catalase Test

Catalase, an enzyme that converts hydrogen peroxide to water and oxygen on contact with hydrogen peroxide leads to production of bubbles. A smear of the isolate was made on a clean slide and then 2 drops of hydrogen peroxide was added to the smear. Production of bubbles within 5 seconds signified positive catalase [23].

2.2.6. Coagulase Test

This test differentiates the pathogenic *S. aureus* from non pathogenic staphylococci. A discrete colony was emulsified in a drop of sterile normal saline which had been placed on a clean oil-free slide. The mixture was homogenized and the drop of human plasma was added to it. Clumping within 10 seconds indicated positive result [24].

2.2.7. Citrate Test

Some organisms are able to utilize citrate as their only source of carbon and ammonium as their only source of nitrogen. The citrate is metabolized to aceto in and CO_2. A light suspension of the isolate is made in saline and is stab in oculated on Simmon's citrate agar. A growth of blue colour indicates positive result [25].

2.2.8. Indole, Urease and Motility Test

A sloppy Motility-Indole-Urease medium is inoculated with the isolate with the aid of straight wire. Then, an indole paper strip was placed in the neck of the tube and corked tight and the set up was incubated at 35^0C overnight. Motility was shown by diffused turbidity in the medium. Indole paper turns red if positive while a pink colour in the medium indicates positive urease production [26].

3. Results and Discussion

3.1. Data Presentation and Analysis

Table 1. *Microbial Counts on the Clean and soiled Egg Sample.*

Weeks	Sample	Weight	Total bacterial count (x10^3cfu/ml)	Total fungal count (x10^3sfu/ml)
Week1	A1 (Clean)	A1=77.7	27	23
	A2 (Clean)	A2=68.4	28	22
	A3 (Clean)	A3=69.9	31	19
Average			28.6	21.3
	B1 (Soiled)	B1=69.0	35	26
	B2 (Soiled)	B2=71.1	37	23
	B3 (Soiled)	B3=66.5	32	21
Average			34.6	23.3
Week2	A1 (Clean)	A1=76.7	34	9
	A2 (Clean)	A2=67.4	36	10
	A3 (Clean)	A3=68.9	33	13
Average			34.3	10.6
	B1 (Soiled)	B1=68.0	39	12
	B2 (Soiled)	B2=70.0	39	14
	B3 (Soiled)	B3=63.1	40	13
Average			39.3	13
Week3	A1 (Clean)	A1=60.0	43	5
	A2 (Clean)	A2=65.1	47	7
	A3 (Clean)	A3=61.1	40	5
Average			43.3	5.6
	B1 (Soiled)	B1=69.1	54	9
	B2 (Soiled)	B2=66.5	50	9
	B3 (Soiled)	B3=71.3	57	11
Average			53.6	9.6
Week4	A1 (Clean)	A1=58.3	63	4
	A2 (Clean)	A2=58.8	60	5
	A3 (Clean)	A3=60.0	67	5
Average			63.3	4.6
	B1 (Soiled)	56.9	83	3
	B2 (Soiled)	58.9	89	7
	B3 (Soiled)	60.0	89	7
Average			87	5.6

The table 1 above shows the microbial count on the egg samples. The bacterial and fungal counts for the both soiled and clean eggs were identified and are covered. Also, it was found that micro organisms' population was higher in the soiled eggs than the clean one.

Table 2. *Identification of Fungal Isolates on Eggs Sample.*

Description of isolates	Organisms
Velutinous colonies that covers the plate surface with my celia bearing closely packed, dark brown to black conidia with a yellow reverse	*Aspergillus niger*
The colonies are wrinkled, velutinous and dense with white mycelia bearing dark green-grey conidia. The reverse is greyish.	*Aspergillus flavus*
The colonies are centrally velutinous with pale yellow mycelia bearing abundant greyish green. The reverse is brown.	*Penicillium oxalicum*
The colonies are spread across the Petri dish possessing deep grey coloured sporangia with colourless reverse.	*Mucor plumbeus*

Table 2 Shows the identified fungal isolates on eggs sample. The isolates identified were carefully described based on the characteristics. These isolates includes *Aspergillus niger, Aspergillus flavus, Penicillium oxalicum* and *Mucor plumbeus.*

Table 3. *Morphological and Biochemical Identification of Isolates from Egg Sample.*

Isolates/Morphology	1	2	3	4	5	6	7
Colour	Golden yellow	Green with metallic sheen	White	Black	Pink	Creamy	Bluish
Margin	Entire	Entire	Entire	Round	Entire	Crenated	Entire
Consistency	Dry	Butyrous	Dry	Dry	Mucoid	Mucoid	Dry
Opacity	Opaque	Opaque	Opaque		Opaque	Opaque	Opaque
Gram staining	+	-	+	-	-	-	-
Shape	Cocci	Rod	Cocci	Rod	Rod	Rod	Rod
Catalase	+	+	+	+	+	+	+

Isolates/Morphology	1	2	3	4	5	6	7
Coagulase	+	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Lactose	+	+	-	+	-	+	-
Maltose	+	+	-	-	-	-	+
Fructose	-	+	+	+	-	-	+
Organisms	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus epidermidis*	*Salmonella* spp	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Pseudomonas aeruginosa*

Table 4. *Distribution of the Isolates on the Samples.*

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella spp*	*Klebsiella spp*	*Proteus vulgaris*
Week1Clean egg	-	-	-	+	-	-
Soiled egg	-	+	-	+	+	+
Week2 Clean egg	+	-	-	-	-	+
Soiled egg	-	+	+	+	+	+
Week 3 Clean egg	-	-	-	-	-	+
Soiled egg	-	+	+	-	+	+
Week 4 Clean egg	-	-	+	-	-	+
Soiled egg	-	+	+	-	-	+

Table 4. *Continued.*

Sample	*Staphylococcus epidermidis*	*Aspergillus flavus*	*Aspergillus niger*	*Penicilliu moxalicum*	*Mucor plumbeus*
Week1Clean egg	-	+	-	-	-
Soiled egg	-	-	-	-	-
Week2Clean egg	+	+	+	-	+
Soiled egg	-	+	+	-	+
Week 3 Clean egg	-	+	+	-	+
Soiled egg	-	+	+	-	+
Week 4 Clean egg	-	+	+	-	+
Soiled egg	-	+	+	-	+

3.2. Discussion

Table 1 shows the microbial counts on each samples of the soil. The bacterial and fungal counts for the soiled egg samples were found to be 11.3×10^3cfu/g and 4.1×10^3cfu/g respectively. Also, the bacterial and fungal counts for the clean egg samples were found to be 208×10^3cfu/g and 156×10^3cfu/g respectively. It was found that micro organisms' population was higher in the soiled egg samples than the clean one. This implies that the activities of microbes in the soiled egg sample Suggest that the micro organisms have an adverse effect on the soiled egg hence promoting contamination of the eggs. Interestingly, it was discovered that the microbes on the soiled eggs were as a result of the chicken litters on the shell of the eggs. The growth of the micro organisms on the egg shell were as a result of the moisture content of the litters on the shell which encourage the growth of the micro organisms, thereby contaminating the eggs and causes deterioration of the soiled eggs samples under room temperature.

It was also discovered that the rate of deterioration of the soiled egg samples were moderately slow because of the temperature under investigation.

Table 3 depicts the morphological and biochemical identification of bacteria isolates in the two samples. Eight isolates were identified including *Staphylooccus aureus, Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Klebsiella pneumonia, Enterobacter* spp, *Proteus s*pp and *Pseudomonas aeruginosa*.

Table 4 shows the distribution of the organisms in both the soiled and clean egg samples. It was found that all the isolate organisms were present (+) in both the soiled egg samples but were absent in clean egg samples. The absence of these micro organisms in the clean egg samples was due to chicken litters absence on the egg shell and reduces the activities of the microbes there by reducing the degrading ability of the microbes.

4. Conclusion

The bacterial and fungal counts for the boths oiled and clean eggs were identified and discovered. Also, it was found that microorganism's population was higher in the soiled eggs than the clean one. Eight isolates were identified including *Staphylococcus aureus, Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Klebsiella pneumoniae, Enterobacter* spp, *Proteus* spp and *Pseudomonas aeruginosa*. The growth of the micro organisms on the egg shell were as a result of the moisture content of the chicken litters on the shell which encourage the growth of the micro organisms, thereby contaminating the eggs and causes deterioration of the soiled eggs samples under room temperature.

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