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Research

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Quantification and identification of bacterial presence in salted eggs

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Abstract

Background Salted eggs, typically made from duck eggs, are subjected to a preservation process by the addition a high concentration of salt. However, the possibility of bacterial contamination remains a significant concern, as it can potentially to impair the quality of the eggs.

Objective This study aimed to quantify the total bacterial count in salted eggs and identify bacterial species that may affect their quality.

Methods Fifty samples consisted of 10 fresh salted eggs that passed the candling test, 10 portions of pasta dough at 0, 3, and 6 hours, and 10 fresh salted eggs that had failed the candling test (black egg yolk). Each sample was tested in triplicate. The total bacterial count was determined using the plate count agar method, and bacterial identification was based on phenotypic analysis, which included Gram staining and biochemical tests.

Results The total bacterial load in fresh salted eggs was below the maximum limit set by SNI 7388:2009 (1×10^5 CFU/g), whereas the pasta dough and black egg yolk exceeded this limit. The identified bacteria included *Escherichia* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp.

Conclusion Although the bacterial count in salted eggs meets SNI standards, the presence of potentially harmful bacteria highlights the need for enhanced hygiene and sanitation measures to be implemented during the production of salt eggs.

Keywords bacterial contamination | black egg yolk | microbial identification | salted eggs | total bacteria count

Introduction

Eggs are a widely consumed animal product due to their high nutritional value, primarily attributed to their protein content. One particularly popular variation is salted eggs, typically made from duck eggs through a salting and preservation process. The consumption of salted eggs has been steadily rising each year (Putri, 2019), with significant popularity in Indonesia and neighboring countries such as Singapore, where they are incorporated into various culinary dishes and preparations. Singapore imports salted eggs from various countries, including Indonesia, to meet the growing demand for this delicacy (Ditjen PKH, 2018).

Eggs are highly vulnerable to quality degradation caused by physical, chemical, and biological factors (Kumaji, 2019). Spoilage is predominantly attributed to microbial contamination from various sources, such as diseased poultry, contaminated bedding materials, feces, or inadequate handling practices during farm processing,

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storage, and hygiene procedures. Additionally, microbial infiltration can occur through cracks in the eggshell, compromising its natural barrier function (Yuniati, 2011).

In the context of salted eggs, bacterial contamination can occur at various processing stages, often exacerbated by poor hygiene practices among workers, which constitute a critical source of contamination (Zuzana *et al.*, 2014). The traditional salting process is widely recognized for its efficacy in preserving food by creating a hyper-saline environment that inhibits microbial growth (Taormina, 2010). However, certain factors can still permit bacterial survival and proliferation. For example, improper salting techniques, such as uneven salt distribution, can result in localized areas of reduced salinity, facilitating bacterial survival. Furthermore, some bacteria exhibit adaptive capabilities, enabling them to thrive in high-salt conditions (Gunde-Cimerman *et al.*, 2018).

The selection of duck eggs before processing is also crucial, as contamination can occur through within the ovary, via the vitelline membrane during passage through the oviduct, and through the eggshell as the egg exits the cloaca (Gantois *et al.*, 2009). Salting preserves duck eggs and prolongs their shelf life (Novidar *et al.*, 2018). This process can be done by immersing the eggs in a saline solution or using other mediums such as rice husk ash or red brick mixed with salt. Salt is both as a preservative and an antimicrobial agent, inhibiting bacterial growth (Suprapti, 2002).

Introducing bacteria into the egg can result in spoilage and degrade the quality of salted eggs, potentially leading to economic losses for producers. According to Fakhruddin (2008), microbial spoilage of eggs can be caused by various bacteria, including *Pseudomonas sp*., *Micrococcus*, *Clostridium botulinum*, *Bacillus*, and *Cladosporium*. Therefore, this study aimed to quantify the total bacterial count in salted eggs and identify the bacterial species present that may affect the quality of salted eggs.

Methods

Study time and location

The research was conducted from December 2023 to March 2024. Samples were collected from a salted egg producer in Karawang, West Java. The microbiological testing was performed at the Medical Microbiology Laboratory, School of Veterinary Medicine and Biomedical Sciences (SKHB), IPB University.

Sample collection

The pasta dough was made from ash from rice husk dried, burned, and ground, mixed with reverse osmosis (RO) water and 15% salt. The black yolk describes egg yolks that darken due to spoilage or bacterial contamination. A black yolk in a salted duck egg clearly indicates spoilage and potential microbial contamination.

Samples used in this study were obtained from a salted egg producer in Karawang. All fifty samples were taken from the same poultry cage (Code: A6). The samples comprised 10 fresh salted eggs that passed the candling test (**Figure 1A**), which were randomly selected, as well as pasta dough samples (**Figure 1B**), and 10 fresh salted eggs that had failed the candling test (black egg yolk) (**Figure 1C**). The pasta dough samples were collected at 0 hours (initial), 3 hours, and 6 hours (with ten samples for each time) to evaluate the aging process of the dough and its potential as a source of bacterial contamination over time. All samples, including eggs, pasta dough, and black yolk, were transported to the laboratory for analysis. Each sample type was collected in triplicate, representing different production batches.

Pooling of egg samples

Typically, the yolk of a salted duck egg should range from yellow to orange; however, when the yolk turns black, it suggests the egg has undergone significant decomposition, often caused by bacterial or fungal activity. Because of this, we chose to collect egg yolk for

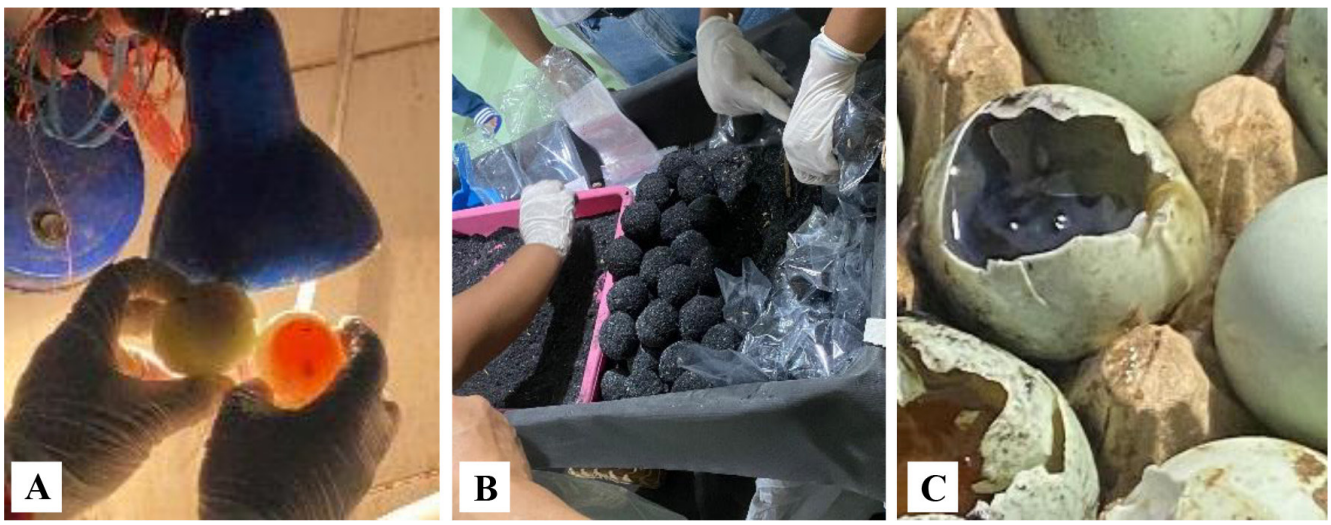

Figure 1 Salted egg production process: (A) example of eggs passing candling, (B) salting paste used for shell coating, and (C) example of black egg yolk that failed the candling process.

analysis. Egg whites have natural antimicrobial properties, so isolating bacteria from them can yield low bacterial counts, especially for harmful pathogens like *Salmonella*. Isolating bacteria from egg whites makes it a less practical approach for research or diagnostic purposes.

The egg samples were combined into pools of two egg yolks each. Pooling was conducted as the samples were considered homogeneous, originating from the same source, cage, dough, and environmental conditions. This approach was also designed to enhance efficiency. The eggs were cracked open, and the yolks were separated from the whites using an egg separator. The yolks were then placed in an Erlenmeyer flask and homogenized. From the pooled samples, five fresh salted eggs that passed candling, five that failed, and three pasta samples were obtained for each replication.

Total bacterial count

The total bacterial count was determined using the plate count method based on Indonesian National Standard (SNI) No. 2897:2008 regarding the microbiological testing of meat, eggs, milk, and their derivatives (BSN, 2008). For salted eggs, 10 mL of pooled egg yolk was added to 90 mL of 0.1% buffer peptone water (BPW) in an Erlenmeyer flask and homogenized for 1 minute, yielding a 10^{-1} dilution. Subsequently, 1 mL of this dilution was transferred to 9 mL of 0.1% BPW for a 10^{-2} dilution. This process was repeated for dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. For each dilution, 1 mL of the suspension was poured into a petri dish, and 15–20 mL of cooled plate count agar (PCA) was added. The plates were homogenized in a figure-eight motion and allowed to solidify. The plates were incubated at 37°C for 24 hours in an inverted position, with each test performed in duplicate (BSN, 2008).

For pasta dough samples, 10 mL was homogenized with 90 mL of 0.1% BPW, followed by serial dilutions as described above. After incubation, the colonies were counted, excluding spreader colonies, and the results were reported as CFU/g based on the average of the duplicate plates multiplied by the dilution factor:

$$\text{The total microbial count (CFU/g)} = \text{average colony count} \times \text{dilution factor}$$

$$\text{where dilution factor} = \frac{1}{\text{Dilution level}}$$

If colony counts fell below 25–250, they were labeled as estimates. The total microbial count (CFU/g) is the average colony count multiplied by the dilution factor, and the dilution factor is 1/(dilution level).

Bacterial isolation and identification

In order to isolate bacteria, two to three of the most prominent colonies from each agar plate were subcultured onto slanted Tryptic Soy Agar (TSA), Blood Agar (BA), and MacConkey Agar (MCA). MCA is a selective medium for Gram-negative bacteria that distinguish between lactose fermenters, which form pink colonies, and non-lactose fermenters, which produce colorless colonies. Subcultures were incubated at 37°C for 24 hours. Pure bacterial isolates were identified based on macroscopic and phenotypic characteristics, including Gram staining and biochemical assays, using the protocols described by Cowan & Steel (1993).

For Gram-negative bacteria, a series of biochemical tests were conducted, including MCA, the IMViC series (indole, methyl red, Voges-Proskauer, and citrate utilization tests), the Sulfide Indole Motility (SIM) test, the Triple Sugar Iron Agar (TSIA) test, and carbohydrate fermentation tests (glucose, sucrose, lactose, and mannitol). The IMViC assessed specific metabolic pathways, while SIM media was utilized to determine indole production and bacterial motility. The TSIA test was conducted to assess the ability of bacteria to ferment sugars and produce hydrogen sulfide.

In order to identify the Gram-positive bacteria, a series of morphological evaluations were conducted to ascertain accurate identification. These evaluations included colony shape, size, and pigmentation. Additionally, a series of tests were conducted, including catalase and oxidase assays, and further biochemical analyses on Mannitol Salt Agar (MSA) plates.

Data analysis

Data were analyzed descriptively based on literature, using tables and figures to conclude.

Results

Total bacterial count

Colony enumeration was performed using a colony counter after incubating samples at 37°C for 24 hours on PCA (**Figure 2**). The average microbial count of fresh duck eggs that passed the candling process, the pasta mixture at time points 0, 3, and 6 hours, and the black egg yolk are presented in **Table 1**.

Figure 2 Example of bacterial colonies growing on plate count agar (PCA) medium.

Table 1 Average total microbial count on fresh duck eggs passing candling, salting paste, and fresh salted eggs with black yolk

Sample	Time (h)	Batch	Average Microbial Count (CFU/g)	Maximum Limit (SNI 7388:2009)
Fresh duck eggs		1	$<1\times10^3$ *est*	1×10^5
		2	$<1\times10^3$ *est*	
		3	$<1\times10^3$ *est*	
Salting paste	0	1	1.9×10^4	-
		2	3.2×10^3	
		3	1.8×10^5	
	3	1	3.3×10^4	
		2	2.7×10^3*	
		3	$1{,}9\times10^6$	
	6	1	2.1×10^5	
		2	1.8×10^6*	
		3	1.6×10^6*	
Fresh salted eggs with black yolk		1	2.7×10^6	1×10^5
		2	2.6×10^5	
		3	$5{,}1\times10^8$*	

Est: estimate if the plate contains fewer than 25 colonies; *denotes spreaders on the plate.

The average microbial count of fresh duck eggs that passed the candling process was found to be less than 1×10^3 CFU/g in all three batches (**Table 1**), falling under the "estimated" category (est.), as the colony count on the plates was less than 25. This result indicates that the salted eggs met the microbial contamination standards outlined by the Indonesian National Standard (SNI 7388:2009), which sets the maximum allowable limit for microbial contamination in processed meat, poultry, and game products at 1×10^5 CFU/g (BSN, 2009). Thus, the microbial count of fresh duck eggs that passed candling suggests that these eggs are safe for consumption.

The bacterial counts in all three batches increased over time (**Table 1**). The first batch demonstrated a gradual rise, the second batch experienced fluctuations due to spreaders on the plate, and the third batch exhibited a significant increase, particularly at later time points. These results suggest a progressive growth in bacterial load across the batches, with some variability in the contamination patterns.

Specifically, the first batch showed bacterial counts of 1.9×10^4 CFU/g at 0 hours, 3.3×10^4 CFU/g at 3 hours, and 2.1×10^5 CFU/g at 6 hours. Similarly, the second batch recorded counts of 3.2×10^5 CFU/g at 0 hours, 2.7×10^3 CFU/g at 3 hours (with spreaders on the plate), and 1.8×10^6 CFU/g at 6 hours. The third batch also exhibited an increase in bacterial load, with counts of 1.8×10^5 CFU/g at 0 hours, 1.9×10^6 CFU/g at 3 hours, and 1.6×10^6 CFU/g at 6 hours (also with spreaders at the final time point). The increase in bacterial count in the pasta mixture, particularly at the 6-hour mark, suggests that the mixture acts as a growth medium for bacteria, which could compromise the quality of the salted eggs.

Black-yolk salted eggs are characterized by discoloration and spoilage. The results showed varied bacterial contamination across the batches (**Table 1**). The first batch recorded a count of 2.7×10^6 CFU/g, while the second and third batches recorded counts of 2.6×10^5 CFU/g and $<1\times10^6$ CFU/g, respectively, with spreaders on the plates. According to SNI 7388:2009, the maximum allowable limit for microbial contamination in food products is 1×10^5 CFU/g (BSN, 2009). Based on these results, the microbial counts in all three batches of black egg yolk salted eggs exceeded the permissible limits, indicating that these products are not fit for consumption.

Bacterial identification

Bacterial identification was conducted on samples from the fresh duck eggs that passed candling, the pasta, and the black egg yolk (**Table 2**), revealing a diverse range of bacterial genera. Fresh duck eggs that passed candling were found to harbor *Staphylococcus* spp. and *Bacillus* spp. The pasta mixture used for coating the eggs contained five genera: *Staphylococcus* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp., and *Bacillus* spp. In contrast, black-yolk salted eggs were contaminated with six bacterial genera: *Escherichia* spp., *Enterobacter* spp., *Proteus* spp., *Bacillus* spp., *Pseudomonas* spp., and *Staphylococcus* spp.

Detailed Gram staining of the bacteria identified is provided in **Figure 3.** The biochemical analysis is provided for Gram-negative bacteria in **Table 3** and Gram-positive bacteria in **Table 4**. *Proteus* spp. is a Gram-negative rod-shaped bacterium characterized by its swarming morphology on PCA. No hemolysis was observed on blood agar, and no glucose fermentation occurred on MacConkey agar. *Proteus* spp. tested positive for sugar fermentation, indole production, urease, and H_2S production on TSIA, with positive results in the MR test. *Staphylococcus* spp. was identified as a Gram-positive, cocci-shaped bacterium that appeared as small, translucent colonies on PCA. Culturing *Staphylococcus* spp. on TSA slants produced positive results in the coagulase test and showed a yellow color change on MSA, indicating positive mannitol fermentation. *Bacillus* spp. was identified as a Gram-positive, rod-shaped bacterium capable of

Table 2 Bacterial identification in fresh duck eggs passing candling, salting paste, and fresh salted eggs with black yolk

Sample	Time (h)	Batch 1	Batch 2	Batch 3
Fresh duck eggs		*Staphylococcus* spp.	*Staphylococcus* spp.	*Staphylococcus* spp. *Bacillus* spp.
Salting paste	0	*Staphylococcus* spp. *Enterobacter* spp. *Proteus* spp. *Pseudomonas* spp. *Bacillus* spp.	*Staphylococcus* spp. *Bacillus* spp. *Proteus* spp. *Pseudomonas* spp.	*Staphylococcus* spp. *Proteus* spp. *Pseudomonas* spp *Bacillus* spp.
	3	*Staphylococcus* spp. *Enterobacter* spp. *Proteus* spp. *Pseudomonas* spp. *Bacillus* spp.	*Staphylococcus* spp. *Bacillus* spp. *Proteus* spp. *Pseudomonas* spp.	*Proteus* spp. *Pseudomonas* spp *Bacillus* spp.
	6	*Enterobacter* spp. *Proteus* spp. *Pseudomonas* spp. *Bacillus* spp.	*Staphylococcus* spp. *Bacillus* spp. *Proteus* spp. *Pseudomonas* spp.	*Staphylococcus* spp. *Proteus* spp. *Pseudomonas* spp *Bacillus* spp.
Fresh salted eggs with black yolk		*Staphylococcus* spp. *Enterobacter* spp. *Proteus* spp. *Escherichia* spp.	*Enterobacter* spp. *Proteus* spp. *Escherichia* spp. *Pseudomonas* spp. *Bacillus* spp.	*Enterobacter* spp. *Proteus* spp. *Pseudomonas* spp. *Staphylococcus* spp. *Bacillus* spp.

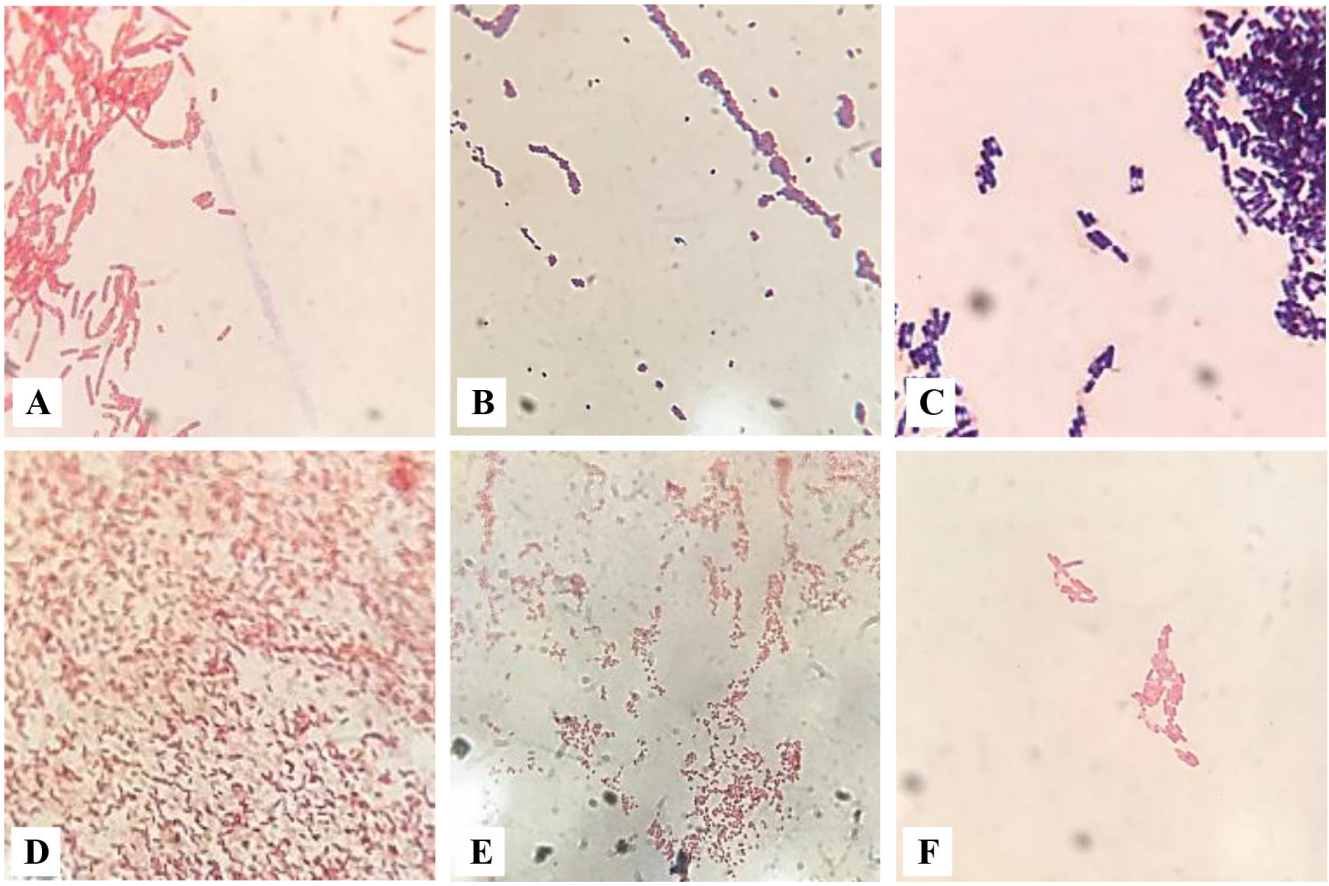

Figure 3 The bacteria identified in salt eggs: (A) *Proteus* spp. (Gram-negative, rod-shaped), (B) *Staphylococcus* spp. (Gram-positive, cocci-shaped), (C) *Bacillus* spp. (Gram-positive, rod-shaped), (D) *Escherichia* spp. (Gram-negative, rod-shaped), (E) *Enterobacter* spp. (Gram-negative, rod-shaped), and (F) *Pseudomonas* spp. (Gram-negative, rod-shaped). Gram staining, magnification 1000×.

Table 3 Biochemical results of identification of Gram-negative bacteria from salted eggs

Sample	Batch	Code	TSIA	MCA	Mo	In	MR	VP	Ci	Ur	Gl	La	Mn	Ml	Su	Identification*
Fresh duck eggs	1–3				No Gram-negative bacteria were detected											-
Salting Paste	1	3.1 KB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		3.1 SW	K/A/-/-	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 KB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		3.2 SW	K/A/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.3 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.3 BB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		3.4 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 SE	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.6 SW	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
	2	3.1 SW	K/K/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 SW	K/K/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 SE	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.2 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.3 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.3 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.4 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 SE	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.6 SE	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
	3	3.1 SW	K/K/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 SW	K/K/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		3.2 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.2 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.3 SE	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		3.3 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.4 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 SE	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.5 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		3.6 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
Fresh salted eggs with black yolk	1	2.1 BB	A/A/-/-	LF	+	-	+	-	+	-	+	-	+	+	-	*Escherichia* spp.
		2.1 KB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		2.1 SW	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.2 SW	K/A/+/+	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		2.2 BB	A/A/-/-	LF	+	-	+	-	+	-	+	-	+	+	-	*Escherichia* spp.
		2.3 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.3 SE	K/K/+/+	NLF	-	-	-	-	+	+	+	+	+	+	+	*Proteus* spp.
		2.4 SW	K/K/+/+	NLF	+	-	+	-	+	+	+	-	-	+	-	*Proteus spp*
		2.5 SE	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
		2.5 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
	2	2.1 SW	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.1 KB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		2.1 SW	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.2 SW	K/A/-/-	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		2.2 BB	A/A/-/-	LF	+	-	+	-	+	-	+	-	+	+	-	*Escherichia* spp.
		2.3 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.3 SE	K/K/-/-	NLF	-	-	-	-	+	+	+	+	+	+	+	*Proteus* spp.
		2.4 SW	K/K/-/-	NLF	+	-	+	-	+	+	+	-	-	+	-	*Proteus spp*
		2.5 SE	K/K/+/-	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.
	3	2.1 SW	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.1 SE	K/A/-/-	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		2.1 SW	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.2 SE	K/A/-/-	NLF	+	+	+	-	+	+	+	-	-	-	-	*Proteus* spp.
		2.2 BB	A/A/-/+	LF	-	-	+	-	+	+	+	+	+	+	+	*Enterobacter* spp.
		2.3 BB	K/K/+/-	NLF	-	-	-	-	+	+	+	+	+	-	-	*Pseudomonas* spp.
		2.3 SE	K/K/+/+	NLF	-	-	-	-	+	+	+	+	+	+	+	*Proteus* spp.
		2.3 SW	K/K/+/+	NLF	+	-	+	-	+	+	+	-	-	+	-	*Proteus* spp.
		2.5 SE	K/K/+/+	NLF	+	-	+	-	+	+	+	-	+	+	+	*Proteus* spp.

* Based on Cowan & Steel (1993). +: hasil uji positif; -: hasil uji negatif; TSIA: Triple Sugar Iron Agar with A/K,+,+: acidic/alkaline, gas production, H2S production; MCA: MacConkey Agar; LF: Lactose fermenter, NLF: Non-lactose fermenter; Mo: Motility; Ur: Urea; MR: Methyl Red; VP: Voges Praskeur; Ci: Citrate; In: Indol; Gl: Glucose; La: Lactose; Mn: Mannitol; Ml: Maltose; S: Sucrose.

Table 4 Microscopic observation and biochemical results of identification of Gram-positive bacteria from salted eggs

Sample	Batch	Code	Microscopic observation		MSA	Identification*
			Shape	Spores		
Fresh duck eggs	1	1.1 KB	Coccus	-	+	*Staphylococcus* spp.
		1.2 BK	Coccus	-	+	*Staphylococcus* spp.
		1.3 BK	Coccus	-	+	*Staphylococcus* spp.
		1.4 BK	Coccus	-	+	*Staphylococcus* spp.
		1.5 KB	Coccus	-	+	*Staphylococcus* spp.
	2	1.1 KB	Coccus	-	+	*Staphylococcus* spp.
		1.2 BK	Coccus	-	+	*Staphylococcus* spp.
		1.2 BB	Basil	+	ND	*Bacillus* spp.
		1.3 BK	Coccus	-	+	*Staphylococcus* spp.
		1.4 BK	Coccus	-	+	*Staphylococcus* spp.
		1.4 KB	Coccus	-	+	*Staphylococcus* spp.
		1.4 BB	Basil	+	ND	*Bacillus* spp.
		1.5 BB	Basil	+	ND	*Bacillus* spp.
	3	1.1 KB	Coccus	-	+	*Staphylococcus* spp.
		1.2 BK	Coccus	-	+	*Staphylococcus* spp.
		1.3 BK	Coccus	-	+	*Staphylococcus* spp.
		1.4 BK	Coccus	-	+	*Staphylococcus* spp.
		1.4 KB	Coccus	-	+	*Staphylococcus* spp.
		1.5 BB	Basil	+	ND	*Bacillus* spp.
		1.5 BK	Coccus	-	+	*Staphylococcus* spp.
Salting Paste	1	3.1 KB	Coccus	-	+	*Staphylococcus* spp.
		3.1 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BB	Basil	+	ND	*Bacillus* spp.
		3.4 BB	Basil	+	ND	*Bacillus* spp.
		3.4 BK	Coccus	-	+	*Staphylococcus* spp.
		3.5 BK	Basil	+	ND	*Bacillus* spp.
		3.6 BK	Basil	+	ND	*Bacillus* spp.
	2	3.1 KB	Coccus	-	+	*Staphylococcus* spp.
		3.1 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BB	Basil	+	ND	*Bacillus* spp.
		3.4 BB	Basil	+	ND	*Bacillus* spp.
		3.4 BK	Coccus	-	+	*Staphylococcus* spp.
		3.5 BK	Basil	+	ND	*Bacillus* spp.
		3.6 BK	Basil	+	ND	*Bacillus* spp.
	3	3.1 KB	Coccus	-	+	*Staphylococcus* spp.
		3.1 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BK	Coccus	-	+	*Staphylococcus* spp.
		3.2 BB	Basil	+	ND	*Bacillus* spp.
		3.3 BK	Coccus	-	+	*Staphylococcus* spp.
		3.4 BB	Basil	+	ND	*Bacillus* spp.
		3.5 BB	Coccus	-	+	*Staphylococcus* spp.
		3.6 BK	Basil	+	ND	*Bacillus* spp.
		3.6 BB	Coccus	-	+	*Staphylococcus* spp.
Fresh salted eggs with black yolk	1	2.1 KB	Coccus	-	+	*Staphylococcus* spp.
	2	2.1 BB	Basil	+	ND	*Bacillus* spp.
		2.2 BB	Basil	+	ND	*Bacillus* spp.
	3	2.1 KB	Coccus	-	+	*Staphylococcus* spp.
		2.1 BK	Coccus	-	+	*Staphylococcus* spp.
		2.3 BB	Basil	+	ND	*Bacillus* spp.

MSA: Mannitol Salt Agar; ND: not done, because the identification of Gram-positive bacilli (rods) bacteria generally does not use MSA media such as Gram-positive coccus bacteria.

forming endospores. It was β-hemolytic on blood agar, catalase-positive, and did not grow on MacConkey agar. *Escherichia* spp. was isolated as a Gram-negative, rod-shaped bacterium that fermented lactose on MacConkey agar. It tested positive for sugar fermentation, MR, and indole production. *Enterobacter* spp. was another Gram-negative rod that produced pink colonies on MacConkey agar, indicating lactose fermentation. It tested positive for VP and citrate utilization. *Pseudomonas* spp. was a Gram-negative rod-shaped bacterium identified based on its inability to ferment lactose and its positive result in the Simon's citrate test.

Discussion

Eggs are highly perishable food products, and their quality can deteriorate rapidly due to bacterial contamination, which can occur both before and after processing. The findings of this study indicate that fresh duck eggs that passed candling were found to have microbial contamination levels below the maximum permissible limits. In contrast, salted eggs with black yolk and pasta mixtures exhibited total bacterial counts that exceeded the maximum threshold established by Indonesian National Standard (SNI) No. 7388:2009**,** which sets the microbial contamination limit at 1×10^5 CFU/g (BSN, 2009). The elevated bacterial counts may be attributed to several factors, including storage duration, temperature, humidity, gas pressure, light, and environmental contaminants (Dora *et al.*, 2018).

Bacterial contamination in eggs can occur via two main routes: horizontal and vertical transmission. Horizontal transmission involves contamination from external environmental sources, such as poultry feces, and the conditions in the barn where the eggs are stored. This finding is consistent with the results of previous studies by Surahmaida & Nurhatika (2018) and Fatayahi *et al.* (2023), which indicated that environmental contamination may originate from soil, manure, and unclean barns, allowing bacteria to penetrate through the eggshell. Therefore, proper handling of eggs after laying is essential to prevent bacterial contamination. In contrast, vertical transmission occurs when the pathogen is passed from the infected hen to the egg through the reproductive tract. This route of contamination has been highlighted in previous studies, where diseased poultry have been shown to produce contaminated eggs (Shaji *et al*., 2023).

Environmental factors such as cleanliness, barn temperature, and storage conditions play critical role in bacterial contamination**.** Risdayanti *et al.* (2023) reported that inadequate sanitation on poultry farms can result in elevated rates of bacterial contamination. Bedding material in barns must be replaced regularly to avoid becoming reservoirs for bacterial growth, and poultry feces should be removed frequently to prevent the horizontal spread of pathogenic bacteria (Adegunloye & Adejumo, 2020). In this study, duck eggs were stored for a period of between one and three days prior to the salting process, which may have increased the risk of contamination. Hence, proper storage conditions are critical for minimizing bacterial growth. It is essential to ensure that eggs are thoroughly washed with water before salting to prevent contamination. Additionally, the risk of dough contamination should be carefully examined, as research indicates susceptibility to yolk contamination. Optimal storage conditions, including pH, humidity, oxygen, and temperature, are crucial for maintaining dough quality and safety. Duck eggs, with a longer and deeper cuticle than chicken eggs, are at higher risk of external bacteria entering the yolk. Thus, maintaining strict hygiene and appropriate storage is critical to reducing contamination risks.

The identification of bacterial genera such as *Staphylococcus* spp. and *Bacillus* spp. in all sample types suggests that these halophilic bacteria, which thrive in high-salt environments, could survive the salting process (DasSarma & Arora, 2002). These bacteria are commonly found in air, food, and eggs, with *Staphylococcus* spp. capable of penetrating the eggshell and causing foodborne diseases (Argudín *et al.,* 2010). The ability of these bacteria to tolerate high salt levels further emphasizes the importance of sanitation throughout the production process (Hastuti, 2010; Argudín *et al*., 2010).

Bacterial species identified in the pasta mixture and black-yolk salted eggs, such as *Enterobacter* spp., *Proteus* spp., and *Pseudomonas* spp., are commonly associated with water, soil, and barn environments. These bacteria are known to contaminate poultry barns and food storage areas (Amer *et al.*, 2013; Kebede, 2010; Jambalang *et al.*, 2017). The presence of *Escherichia* spp. in black-yolk salted eggs, which was not detected in the pasta mixture, may indicate cross-contamination from the pasta mixture during the salting process, mainly due to the lack of regular pasta mixture changes during processing.

Notably, the genus *Proteus* spp. has been associated with discoloration of egg yolks, turning them black, particularly in environments with elevated temperatures (Shebuski & Freier, 2009). The ability of these bacteria to persist in poultry farming environments highlights the need for strict hygiene protocols to prevent contamination of egg products. Furthermore, *Pseudomonas* spp. and *Escherichia* spp. have been identified as bacteria capable of vertical transmission, with *Pseudomonas* spp. being part of the poultry gastrointestinal flora (Mehdi *et al.*, 2018). These bacteria are particularly implicated in the spoilage of eggs, with *Pseudomonas* spp. known to cause internal egg decomposition, resulting in off-odors and semi-liquid yolk masses (Novidar *et al.*, 2018). In poultry, *Escherichia* spp. can lead to colibacillosis, a significant disease affecting egg-laying birds (Shaji *et al*., 2023).

In light of these findings, it is evident that environmental factors such as temperature, humidity, and sanitation play a pivotal role in bacterial growth and transmission. Identifying a diverse range of bacterial species across all sample types highlights the necessity for enhanced hygiene practices throughout all production steps. This necessity includes sanitation of egg storage areas, cleaning production equipment, ensuring worker hygiene, and maintaining clean barn conditions from egg collection to distribution.

Comprehensive animal health management and sanitation protocols should be implemented to optimize egg quality. Specifically, attention should be given to barn hygiene, animal health, and bedding replacement to minimize bacterial reservoirs. During egg storage, appropriate temperature control should be ensured, both in the pre-salting phase and during the salting process itself. Cleaning eggs before salting can further reduce bacterial contamination and enhance salt penetration (Engelen *et al.*, 2017).

Salt concentrations of 10−15% in the salting mixture have effectively eliminate most spoilage bacteria, as previously researched by Lee *et al.* (2022) and Zang *et al.* (2020). However, halophilic bacteria such as *Staphylococcus* spp. and *Bacillus* spp. may still survive these conditions, necessitating the regular replacement of the salting paste to minimize bacterial contamination. Furthermore, worker hygiene during egg processing is essential, as Zuzana *et al.* (2014) highlighted, where inadequate worker hygiene can contribute significantly to bacterial contamination during processing. Improving egg quality requires a holistic approach encompassing animal health management, sanitation, processing, storage, and distribution.

Conclusion

This study demonstrates that salted eggs are susceptible to bacterial contamination, which can lead to a decline in their quality. Fresh duck eggs that passed candling were found to have total bacterial counts within the permissible limits set by Indonesian National Standard (SNI) No. 7388:2009, which is 1×10^5 CFU/g. However, both the salted eggs with black yolk and the salting paste samples exceeded the maximum allowable bacterial count. The identified bacteria belonged to six genera, namely *Escherichia* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp.. These findings highlight the importance of maintaining stringent hygiene protocols during the production, storage, and processing of salted eggs to minimize bacterial contamination and ensure product safety.

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References

Adegunloye DV, Adejumo FA. 2020. Microbial assessment of turkey (*Meleagris ocellata L.*) and duck (*Anas platyrhynchos L.*) faeces (droppings) in Akure metropolis. *Applied Ecology and Environmental Research*, 18(1):1489– 1507. DOI: 10.4236/aim.2014.412085.

Amer MM, Dahshan AHM, Hassan HS, Mohamed A A. 2013. Studies on the prevalence of *Enterobacteriaceae* in chickens and chicken eggs. *Journal of Veterinary Medical Research* 22(1): 136–144. DOI: 10.21608/jvmr.2013.77696.

Argudín ***MÁ, Mendoza MC, Rodicio MR.*** 2010. Food Poisoning and *Staphylococcus aureus* ***Enterotoxins.*** *Toxins*, 2(7): 1751-1773. DOI: 10.3390/toxins2071751.

BSN [Badan Standardisasi Nasional]. 2008. *SNI Nomor 2897:2008 Metode Pengujian Cemaran Mikroba Dalam Daging, Telur dan Susu, serta Hasil Olahannya* Jakarta: Badan Standardisasi Nasional.

BSN [Badan Standardisasi Nasional]. 2009. *SNI Nomor 7388:2009 Batas maksimum cemaran mikroba dalam pangan*. Jakarta (ID): Badan Standardisasi Nasional.

Cowan ST, Steel KJ. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria.* London (UK): Cambridge University Press.

DasSarma S, Arora P. 2002. *Halophiles. Encyclopedia of Life Science*. London (GB): Nature Publishing Group.

Ditjen PKH [Direktorat Jenderal Peternakan dan Kesehatan Hewan]. 2018. Singapura borong telur asin. Link: https://ditjenpkh.pertanian.go.id/berita/776-singapura-borong-telur-asin- indonesia. Downloaded: 11 October 2023.

Dora R, Ferasyi TR, Ismail, Hamzah A. 2018. Jumlah cemaran mikrob pada telur asin mentah yang dijual di Pasar Ulee Kareng Banda Aceh. *Jurnal Ilmiah Mahasiswa Veteriner,* 2(4): 435–441.

Engelen A, Umela S, Hasan AA. 2017. Pengaruh lama pengasinan pada pembuatan telur asin dengan cara basah. *Jurnal Agroindustri Halal*, 3(2): 133–135. DOI: 10.30997/jah.v3i2.831.

Fakhruddin U. 2008. Studi penggunaan edible coating dari campuran *kappa karaginan* dan natrium alginat terhadap daya simpan telur asin rebus pada suhu ruang dan suhu refrigerator. [Thesis]. Malang (ID): Universitas Brawijaya.

Fatayahi I, Amanda AC, Nurhayati E, Djohan H, Sutriswanto, Komara. 2023. Gambaran cemaran mikroba terhadap masa simpan dan kebersihan penyimpanan telur ayam ras. *SENTRI Jurnal Riset Ilmiah*. 2(5): 1674–1678. DOI: 10.55681/sentri.v2i5.850.

Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, Immerseel FV. 2009. Mechanisme of egg contamination by Salmonella Enteritidis. *FEMS Microbiology Reviews* 4(33): 718–738. DOI: 10.1111/j.1574-6976.2008.00161.x.

Gunde-Cimerman N, Plemenitaš A, Oren A. 2018. Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. *FEMS Microbiology Reviews*, 42(3): 353–375. DOI: 10.1093/femsre/fuy009.

Hastuti S. 2010. Pengaruh perendaman dalam larutan kapur dan lama penyimpanan terhadap kualitas telur asin. *AGROINTEK Jurnal Teknologi Industri Pertanian* 4(1): 37–81.

Jambalang AR, Buys EM, Botha FS. 2017. Bacterial species from retailed poultry eggs in Tshwane, South Africa: Implication for consumers. *South African Journal of Science,* 11(3): 29–36. DOI: 10.17159/sajs.2017/20160232.

Kebede F. 2010. *Pseudomonas* infection in chickens. *Journal of Veterinary Medicine and Animal Health*, 2(4): 55–58.

Kumaji SS. 2019. Pengaruh lama penyimpanan telur ayam ras pada suhu refrigerator terhadap jumlah bakteri. *Jurnal Ilmu Pendidikan Nonformal AKSARA.* 5(2): 119–128. DOI: 10.37905/aksara.5.2.119-128.2019.

Lee S, Kim D, Son Y, Le H, Jo SW, Lee J, Song Y, Kim H. 2022. Effects of salt treatment time on the metabolites, microbial composition, and quality characteristics of the soy sauce moromi extract. *Foods*, 11(1): 63. DOI: 10.3390/foods11010063.

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Côté C, Ramirez AA, Godbout S. 2018. Use of antibiotics in broiler production: global impacts and alternatives. *Animal Nutrition*, 4(2): 170– 178. DOI: 10.1016/j.aninu.2018.03.002.

Shebuski JR, Freier TA. 2009. Microbiological spoilage of eggs and egg products. *In*: Sperber W, Doyle M (eds), Compendium of the microbiological spoilage of foods and beverages. Food microbiology and food safety. New York (US): Springer. DOI: 10.1007/978-1-4419-0826-1_5.

Novidar R, Rastina R, Razali R. 2018. Perbedaan lama penyimpanan telur itik asin mentah terhadap jumlah *Pseudomonas* sp.. *Jurnal Ilmiah Mahasiswa Veteriner*, 2(3): 311–317.

Putri MF. 2019. Telur asin sehat rendah lemak tinggi protein dengan metode perendaman jahe dan kayu secang. *Jurnal Kesejahteraan Keluarga dan Pendidikan,* 6(2): 94–97. DOI: 10.21009/JKKP.062.03.

Risdayanti, Latif UTA, Wirawan HP. 2023. Deteksi keberadaan bakteri pengkontaminasi pangan *Salmonella sp*. pada telur. *Filogeni: Jurnal Mahasiswa Biologi*, 3(3): 117–120. DOI: 10.24252/filogeni.v3i3.29811.

Shaji S, Selvaraj RK, Shanmugasundaram R. 2023. Salmonella infection in poultry: A review on the pathogen and control strategies. *Microorganisms*, 11(11): 2814. DOI: 10.3390/microorganisms11112814.

Suprapti IML. 2002. *Pengawetan Telur.* Yogyakarta (ID): Kanisius.

Surahmaida S, Nurhatika S. 2018. Perhitungan lempeng total bakteri pada telur ayam ras. *STIGMA Jurnal Matematika dan Ilmu Pengetahuan Alam UNIPA,* 11(1): 33–36. DOI: 10.36456/stigma.vol11.no01.a1506.

Taormina PJ. 2010. Implications of salt and sodium reduction on microbial food safety. *Critical Reviews in Food Science and Nutrition*, 50(3): 209–227. DOI: 10.1080/10408391003626207.

Yuniati H. 2011. Efek penggunaan abu gosok dan serbuk bata merah pada pembuatan telur asin terhadap kandungan mikroba dalam telur. *Nutrition and Food Research*. 34(2): 131–135. DOI: 10.22435/pgm.v34i2.3103.

Zang J, Xu Y, Xia W, Regenstein JM. 2020. Quality, functionality, and microbiology of fermented fish: a review. *Critical Reviews in Food Science and Nutrition*. 60(7): 1228–1242. DOI:10.1080/10408398.2019.1565491.

Zuzana HK, Kejlova J, Sosnovcova D, Jírova A, Vavrous, Janousek S, Sycová, Špelina V. 2014. Microbial contamination of paperbased food contact materials with different contents of recycled fiber. *Czech Journal of Food Sciences*. 33(4): 308–312. DOI: 10.17221/645/2014-CJFS.