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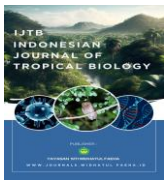

Original Research Paper

Detection of Aerobic Bacterial Contamination in Frozen Chicken Meat Using the TPC (Total Plate Count) Method

Annisa Alifiya Mutmainnah[1*], Lili Suharli[1], Kusdianawati[1], Ardiyanto Chandra Wijaya[2]

[1] Bioteknology Study Program, Faculty of Life Sciences and Technology, University Technology of Sumbawa, Jl. Raya Olat Maras, Batu Alang, Moyo Hulu, Sumbawa Besar 84371, West Nusa Tenggara, Indonesia,

[2] Animal Quarantin Laboratory, Class I Sumbawa Agricultural Quarantine Station, Jl. Sultan Hasanuddin, Sumbawa Besar 84311, West Nusa Tenggara, Indonesia

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Corresponding Author: Annisa Alifiya Mutmainnah
Author Name: Annisa Alifiya Mutmainnah
Email: annisaalifiya50@gmail.com
Number Hp: +62 8523 9161 655

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Abstract

This study aims to evaluate the biological quality of frozen chicken meat through pH, early spoilage detection, and total microbial contamination, supporting food safety monitoring. According to SNI No. 01-6366 (2000), the maximum microbial contamination limit (BMCM) for chicken meat is 1×10^4 CFU/g. The study utilized pH tests with litmus paper, the Eber test with Eber reagent, and total plate count (TPC) using the MC-Media Pad ACplus™ method, involving buffer preparation, serial dilution, and colony counting. Results showed all three samples had a normal pH range (5–6), meeting the standard. The Eber test indicated all samples were negative for spoilage, as no NH_4Cl compound was formed. However, TPC results revealed that sample A1 (1×10^4 CFU/ml) met the microbial threshold, while samples A2 (3×10^5 CFU/ml) and A3 (5×10^5 CFU/ml) exceeded the BMCM after 48 hours. The findings suggest that although pH and spoilage indicators were within normal limits, microbial load in some frozen chicken samples surpassed safety thresholds. These results underscore the importance of strict cold chain management and routine microbial testing to ensure food safety and consumer health.

Keywords: Aerobic bacterial, frozen chicken meat, microbial, TPC

INTRODUCTION

Indonesia is one of the largest chicken meat producing countries in the world. In 2022, Indonesia's broiler production reached 3.39 million tons, ranking 6th globally with a contribution of 2.97% of the total world production of 114.32 million tons (Saputra & Ali, 2022). Demand for chicken meat also increased significantly, especially during wedding seasons and religious holidays, with a need increase of around 10–20% compared to regular days, according to a BAPOK BPS survey. Population growth, urbanization, and the development of the food processing industry are also driving the diversification of processed chicken products such as nuggets, sausages, and ready-to-eat chicken (Pramudya, 2017), making chicken meat one of the national strategic food commodities (Chidi et al., 2022).

Chicken meat is an excellent source of animal protein at a relatively affordable price. Every 100 grams of chicken contains approximately 74% water, 22% protein, and a number of minerals such as calcium (13 mg), phosphorus (190 mg), and iron (1.5 mg). However, the high water and protein content makes chicken meat a very supportive medium for the growth of contaminating microorganisms from the environment (Zelpina et al., 2019; Sipayung et al., 2022). As a result, chicken meat is considered a perishable food item that spoils quickly if not handled properly (Hajrawati et al., 2016; Putri et al., 2024).

This phenomenon poses a serious challenge to the food distribution and quality control chain. The Indonesian government, thru SNI No. 01-6366 of 2000, has set the microbial contamination limit for Animal Origin Materials (BAH) such as fresh, frozen, and minced chicken at 1×10^4 colonies/gram. Some microorganisms frequently associated with food poisoning outbreaks include Staphylococcus aureus, Salmonella sp., Escherichia coli, and Pseudomonas aeruginosa (Mustika, 2019). Infections resulting from the consumption of contaminated chicken meat can cause various serious symptoms, ranging from digestive issues to systemic disorders that endanger public health.

Reflecting on the high risks associated with the circulation of poultry products, this study was designed to evaluate the microbiological quality of frozen chicken meat transported to the Class I Agricultural Quarantine Station in Sumbawa. Poultry meat is highly perishable and can serve as a medium for the growth of pathogenic and spoilage microorganisms if not handled under proper hygiene and temperature control. Therefore, monitoring the microbial load of frozen chicken meat is a critical step to ensure that only safe products enter the food chain. In this study, the Total Plate Count (TPC) method was employed using the MC-Media Pad

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ACplus™ medium to detect and quantify the presence of contaminating microbes.

The use of this method allows for a reliable and efficient assessment of microbial contamination levels in meat samples. The outcomes of this research are expected to contribute significantly to strengthening the national food safety surveillance system, enhancing the role of quarantine services in safeguarding public health, and providing scientific evidence to support regulatory actions aimed at preventing the distribution of contaminated poultry products.

METHODS

Time and location of the research

The research was conducted for 1 month, from July 24th to August 24th, 2023, at the Animal Quarantine Laboratory of the Agricultural Quarantine Station Class 1 Sumbawa Besar.

Tools and materials

The tools used in this study include 1000 ml erlenmeye tubes, test tubes, measuring cups, test tubes with rubber stoppers, threads, mortars, pestles, medical scissors, glass mixer rods, beaker cups, micropipette tips, vertical laminar air flow, analytical scales, Marchery-nagel litmus paper, stationery, sterilizing cupboards, incubators, wooden tube racks and source foam. The materials used in this study included 3 samples of frozen chicken meat (1 gr, 10 gr, and 25 gr), 800 ml of buffer solution, 25 ml of eber reagent, 12 MC-Media Pad ACplusTM, 9 ziplock plastics, tissue, and aluminum foil.

Working procedure

pH test

Working Procedure pH Test 10 grams of frozen chicken meat sample was sliced and ground with a mortar and pestle. Then, in a beaker, the ground chicken meat was mixed with 10 milliliters of sterile distilled water and stirred with a stirring rod until the solution became homogeneous or the solution became more concentrated. Next, the pH test was conducted by dipping litmus paper into the solution for 15 to 30 seconds. Adjusting the color that appears between the litmus paper and the pH color chart shows the results. This procedure is repeated for two additional samples, A2 and A3.

Eber test

Eber's reagent is made by mixing concentrated HCl, 96% alcohol, and an ether solution in a 1:3:1 ratio (5ml:15ml:5ml). The Eber's reagent is then poured into 3 test tubes, with each tube containing 3-5 ml of the reagent. Next, the three frozen chicken meat samples, each weighing 1 gram, were tied with thread, placed in test tubes, and the test tube caps were sealed at the mouth of the tubes. After that, the samples were allowed to sit for 20-30 minutes before observation. Positive Eber samples are characterized by the formation of gas/white cloud adhering to the test tube walls on the chicken meat due to the reaction of protein and amino acid products with strong acid (HCl) to form NH4Cl compound (Afdal et al., 2017; Tolba et al., 2022).

Total Microbial Count (Total Plate Count)

In the TPC test, the surface/spread plate method is used, where the diluted sample is spread onto the surface of the solid medium. A total of 8 PBS (Phosphate Buffered Saline) tablets were dissolved in 800 ml of distilled water in an Erlenmeyer flask. For the initial dilution, 225 ml of PBS solution was mixed with 25 g of previously sliced and ground frozen chicken meat sample (A1). Then the solution is stirred with a spatula until it is homogeneous or the solution becomes more concentrated. Next, 3 test tubes containing 9 ml of PBS solution were prepared for serial dilutions of 102, 103, and 104. The PBS and chicken meat mixture was homogenized first, then 1 ml of the solution was taken with a micropipette and placed into the test tube (102). Subsequently, 1 ml of the 102 dilution was added to test tube 103, and 1 ml of the 103 dilution was added to test tube 104. A total of 1 ml of the 101, 102, 103, and 104 dilutions was taken using a micropipette and then poured onto solid media and labeled. This serial dilution was performed on the other two samples (A2 and A3). Then, all 14 media pads were incubated at 35°C for 2x24 hours. After incubation, observations were made. If there are red spots on the fiber layer in the solid medium, the sample is considered positive for aerobic bacteria and the number of microbial colonies is then counted.

RESULTS AND DICCUSSION

pH test

Microorganisms can grow well in both neutral (pH 7.0) and slightly alkaline environments (Susilo et al., 2019). Additionally, pH value can be an indicator to determine whether a product is fresh or spoiled (Triyannato et al., 2021). After the animal is slaughtered (the animal is dead), biochemical processes occur in the muscle tissue and other tissues due to the cessation of the heart pump. One of the processes that occurs in muscle tissue after death (the first 36 hours postmortem) is anaerobic glycolysis or postmortem glycolysis. In this anaerobic glycolysis, in addition to energy (ATP) being produced, lactic acid is also generated. This lactic acid will accumulate in the tissues and cause a decrease in the pH value of muscle tissue (Hidayat, 2020). The results of the muscle tissue pH test can be seen in Table 1.

Table 1. Results of pH testing on frozen chicken meat samples

No	Sample	pH	Standard
1	A1	5	MS
2	A2	5	MS
3	A3	6	MS

***Description:** BMS = Does Not Meet Standards, MS = Meets Standards

Table 1: All three samples met the standards because the test results showed a pH range of 5-6, and none exceeded or fell below this range, thus they were categorized as meeting the standards with normal chicken meat pH. During frozen meat storage, the meat is already in the postmortem stage, so the ultimate pH of the meat has been reached (Kariang et al., 2023).

Eber test

The Eber test is one method for determining the production of NH3 as a result of the biochemical activity of microorganisms in meat. If decomposition occurs, this test is indicated by the emission of smoke on the tube walls, where

the amino acid chain will be broken by the strong acid (HCl), resulting in the formation of gaseous NH4Cl (Hidayat, 2021). The results of the Eber test can be seen in Table 2.

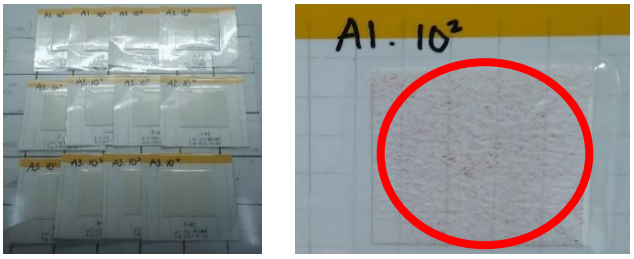
Table 2. Results of the Eber test on frozen chicken meat samples

No	Sample	Eber	Note
1	A1	Negative	MS
2	A2	Negative	MS
3	A3	Negative	MS

***Description:** BMS = Does Not Meet Standards, MS = Meets Standards

Total Microbial Count (Total Plate Count)

MC-Media Pad ACplus™ or *Rapid Aerobic Count (RAC)* media pad is coated with growth medium and three redox indicators to detect aerobic bacterial colonies. The method used on the solid medium is a quantitative sheet with a special media composition and a specific chromogenic substrate for β-galactosidase. The media will reform after the liquid sample is inoculated onto the test pad, and the sample will diffuse throughout the pad by capillary action. The media will automatically reform. If there are target organisms, red-colored colonies will grow on the test pad (Komsta, 2011; Hassanien et al., 2016; Teramura et al., 2018; Teramura et al., 2019). After 2x24 hours, all the agar plates showed positive results, indicating the presence of aerobic bacteria, characterized by red spots on the surface of the agar plates for samples A1-A3. The results of the total microbial contamination test can be seen in (Figure 1).



(a) **(b)**

Figure 1. *MC-Media Pad ACplus*™

*** Description:** A. Before incubation, B. After incubation marked by a red circle 2x24 hours.

Figure 1 (a) shows the solid medium used for the aerobic bacterial contamination test on samples A1-A3 after the spread method was performed. Figure 1 (b) shows the presence of red spots after 2x24 hours of incubation. Coliform bacteria are Gram-negative bacteria with many rods that can ferment lactose. Gram-negative bacteria are anaerobic and aerobic, capable of growing in media containing bile salts. Gram-negative bacteria can ferment lactose to produce acid and gas within 48 hours at a temperature of 35-37ºC when incubated, forming red spots on solid media (Masoumbeigi et al., 2017; Jufri & Rahman, 2022). Beside MC-Media Pad ACplusTM, there is also MC-Media Pad EC, which is a quantitative sheet method with a special media composition and specific chromogenic substrate for β-galactosidase and β-glucuronidase to specifically detect the growth of E. coli bacteria. Although rapid use can be applied to processed foods and other items after verification by the user, it does not apply to foods containing large amounts of lactic acid bacteria or psychrophilic bacteria (Teramura et al., 2018; Teramura et al., 2019; Morshdy et al., 2023). Fera's (2021) research also showed a positive reaction after 2x24 hours of incubation. This indicates a significant amount of contamination in the sample, leading to observable biochemical reactions (Khotimah, 2016).

TPC (Total Plate Count) is a method for counting the number of microbes present in food samples and agricultural products. Its principle involves growing live microbial cells on agar media, allowing the microbes to multiply and form colonies that can be seen directly and counted with the naked eye without using a microscope (CFU/ml) (Mostofa et al., 2016; Wati, 2018). After 2x24 hours of incubation, the test results using the TPC (Total Plate Count) method were obtained as shown in (Table 3).

Tabel 3. Result of TPC Test

No	Sample	Descriptions		
		10^4	Aerobic Bacteria Index	SNI No.01 -6366 year 2000
1	A1	1	$1x10^4$ CFU/ml	
2	A2	30	$5x10^5$ CFU/ml	$1x10^4$ CFU/ml
3	A3	50	$5x10^5$ CFU/ml	

Table 3 Results of TPC Test after 2x24 Hour Incubation at Temperature Based on the data in Table 3, sample A1 is above the maximum microbial contamination limit, while A2 and A3 exceed the maximum microbial contamination limit (BMCM) according to SNI No. 01-6366 year 2000, which is 1x104. Sample A1 was 1x10^4 CFU/ml, sample A2 was 3x10^5 CFU/ml, and sample A3 was 5x10^5 CFU/ml. In the study by Rizaldi et al. (2022), it was found that out of 13 chicken meat samples tested from vendors in the Tamiang Layang traditional market, 8 vendors (61.53%) did not meet the TPC standard. Similarly, in other traditional markets in East Barito Regency, 3 vendors (60%) did not meet the maximum TPC contamination limit.

The results of the contamination test (TPC) observation of frozen chicken meat samples A2 and A3 showed higher contamination factors such as the slaughter location and the relatively long storage of frozen chicken meat before it was finally transported, as well as poorly monitored temperatures, causing the frozen chicken meat to thaw (Zelpina et al., 2020; Desokey et al., 2020). Then there are several internal and external factors that increase the risk of aerobic bacterial contamination in chicken meat, particularly internal factors such as: the water used in carcasss washing, cutting boards, knives, plucking areas, and other equipment (Setyawan et al., 2017; Eissawy et al., 2023).

CONCLUSION

Of the three samples, sample A1 had 1x104 CFU/ml, sample A2 had 5x105 CFU/ml, and sample A3 had 5x105 CFU/ml. Sample A1 was below the limit for aerobic bacterial contamination, while the other two frozen chicken meat samples exceeded the maximum microbial contamination limit (BMCM) for aerobic bacteria according to SNI No. 01-

6366 year 2000 based on TPC value, which is 1x104 CFU/ml after 2x24 hours of incubation. In the next examination, a confirmation test needs to be conducted to determine whether the increased contamination contains pathogenic or non-pathogenic bacteria that are contaminating the frozen chicken meat sample, making it unsafe for consumption. This confirmation test can be performed by culturing the bacteria in a special medium for pathogenic bacteria such as *E. coli* and *Salmonella.*

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