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Microbiological quality analysis of grouper salted fish (Epinephelus Fuscoguttatus) in several traditional markets in ambon

Gratia Talakua, Melda Yunita*, Anggun Lestary Husein

Faculty of Medicine, Universitas Pattimura, Indonesia

*Corresponding author: meldayunita22@gmail.com

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ABSTRACT

Salted fish is one of the food ingredients that is in demand by the community and has great potential in almost all traditional markets in Maluku, especially in the city of Ambon. One of the salted fish is grouper fish (*Epinephelus fuscoguttatus*) great potential, so it is necessary to conduct food safety checks from the microbiological aspect. This study aimed to analyze the total plate count of bacteria in grouper salted fish sold in several traditional markets in Ambon City, Indonesia. The research method used was quantitative descriptive with an experimental laboratory approach. The results of the total bacterial plate in the grouper salted fish sample from the Mardika market ranged from 1,55 x 103 CFU/mL to 1,05 x 104 CFU/mL, the origin of the Rumah Tiga market ranged from 3,85 x 103 to 3,5 x 104 and the origin of the Waiheru market ranged from 9,5 x 102 to 4,5 x 103 CFU/mL. In conclusion, the three samples of grouper salted fish do not exceed the maximum limit of the total plate count according to the Indonesian National Standard (INS) of 1x105 CFU/g so considered safe for consumption from a microbiological aspect.

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INTRODUCTION

Geographically, Indonesia is the largest archipelago in the world and has considerable potential to be utilized optimally in the fisheries sector. Maluku Province has a sea area of 92.4%, with many islands including Ambon Island, which is one of the islands (Tuapetel et al, 2022). The coral reef ecosystem around Ambon Island is home to various marine animals, including reef fish. There are many types of reef fish found in the waters of Ambon Island, one of which is grouper (*Epinephelus fuscoguttatus*) which is an important food source and has economic value (Aznardi et al, 2020). Grouper fish is also easy to find in traditional markets in Ambon city. However, fish do tend to spoil easily compared to other foods (Jeujanan, 2022; Rini et al, 2020). To prevent

spoilage, fish products are processed and preserved. One of the methods of preservation of fish that is often used is salting. Fish that are decent, safe, and meet the requirements for cleanliness and health are fish that meet the Indonesian National Standard (INS) based on low microbial contamination levels (Nurliana et al, 2023).

Bacteria that are often found in fish are *Escherichia coli* and *Salmonella* sp. *Escherichia coli* generally lives in the digestive system of mammals, including humans (Sinaga et al, 2022). *E. coli* also often colonizes the surface of fish meat (Chotimah et al., 2023) and fishing water (Larawo et al., 2019). Other studies have shown that *Vibrio harveyi* was also found to contaminate grouper fish from the Maros cultivation center (Seniati et al., 2019). These bacteria can contaminate not only fresh fish but also other food sources and if they are contaminated and consumed, they will be very dangerous to the health of consumers because they can cause foodborne disease.

According to the World Health Organization (WHO), Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420.000 deaths. 30% of foodborne deaths occur among children under 5 years of age. Food poisoning causes diarrhea and kills about 3 million children under the age of 5 every year (BPOM, 2015). Based on data obtained from the Food and Drug Supervisory Agency (BPOM), it shows that in 2009-2013 there were 10,700 cases of extraordinary food poisoning in Indonesia with 411,500 people getting sick and 2,500 people dying (BPOM, 2018). Therefore, it is necessary to carry out food safety inspections that are reviewed from the microbiological aspect.

In a study conducted by Hernanda et al (2023), which identified bacteria in salted fish sold at the market in the suburbs of Samarinda City, the results showed that all samples that had been studied were contaminated with bacteria. Another study was also conducted by Tuhumury (2022), which analyzed the total of *Salmonella* spp bacteria. In the samples of smoked skipjack fish products sold at the Mardika Market, there was contamination with *Salmonella* spp. bacteria, while the smoked skipjack fish samples taken at the Modern Market and the Small Hative Market, it was not contaminated or contaminated by *Salmonella* sp. However, in the city of Ambon itself, there is still no research that analyzes the total plate count of bacteria in grouper salted fish (*Epinephelus fuscoguttatus)* marketed in several traditional markets. Therefore, the current study aimed to analyze the total plate count of bacteria in grouper salted fish (*Epinephelus fuscoguttatus*) sold in several traditional markets in the City of Ambon.

RESEARCH METHODS

Research Design

The research was quantitative descriptive research using the true experimental laboratory approach method. The stages in this study were using serial dilution then isolated with spread plate technique, and analyzed the total plate count of bacteria in grouper salted fish (*Epinephelus fuscoguttatus*) sold in several traditional markets in the City of Ambon. The results of the total plate count were then compared with the Indonesian national standard (INS) 1x 105 colony/g (BSNI, 2009).

Population and Samples

In this study, the samples used were grouper salted fish taken from 3 traditional markets in Ambon City, namely Mardika Market, Rumah Tiga Market, and Waiheru Market. There are 13 traditional markets in Ambon City, of which 8 markets are still active, while the other 5 markets are inactive or not operating. In 8 traditional markets that are still active, it was found that there were only 3 markets that sold grouper salted fish, so the sampling technique in this study used the purposive random sampling technique.

Instruments

The tools used in this study were autoclaves, incubators, stirrers, mortars, analytical scales, measuring cups, beaker glasses, pens, books, cameras, erlenmeyers, microscopes, vortex, ose needles, micropipettes, aluminum foils, petri dishes, test tubes, colony counters. Meanwhile, the ingredients were grouper salted fish, 0.85% NaCl solution, PCA media, Nutrient Agar (NA) media, gram staining reagents, and sterile aquadest.

Procedures

Sterilization of Tools and Materials

The tools and materials used such as petri dishes wrapped in paper, Erlenmeyer, and media covered with aluminum foil, test tubes, and glasses containing micropipettes were then sterilized in an autoclave at 121°C for 15 minutes.

Sample Preparation

Samples of grouper salted fish were taken from 3 traditional markets in the city of Ambon (Mardika Market, Rumah Tiga Market, and Waiheru Market). The sample was then cut into small pieces, then pounded until smooth using mortar.

Preparation of Plate Count agar

A total of 22.5 grams of plate count agar (PCA) media was mixed with sterile aquadest until it reached a volume of 1000 mL. The medium was further simmered on a hot plate until completely dissolved. The medium was then sterilized in an autoclave with a pressure of 1 atm (temperature 121°C), this process takes 15 minutes at a pressure of 1 atm. The media was then incubated for 24 hours and made sure there was no contamination before starting the isolation process.

Bacterial Isolation

Bacterial isolation was carried out by serial dilution. Five grams of fish samples were put into a test tube containing 45 mL of sterile distilled water as a pre-dilution and homogenized by vortex. Dilution 10^{-2} was performed by taking 1 ml of suspension from the first dilution, transferring it into a test tube containing 9 ml of aquades, and homogenizing. Then, dilute 10^{-3}, 10^{-4}, and 10^{-5} in the same way. Then, the PCA media was inoculated using the plate spread technique and incubated at a temperature of 37 °C for 24-72 hours.

Total Plate Count Analysis

The number of bacterial colonies that grow per petri dish was then counted on each petri dish. Calculation of the number of bacterial colonies to find out the total plate count of bacteria in 1 mL of sample, by the following formula (Yunita et al., 2022a; Yunita et al., 2022b):

$$\text{Total Plate Count} = \text{number of bacterial colonies x } \frac{1}{\text{dilution}}$$

RESULTS

The results of the analysis of the Total Plate Count are presented in Table 1, where in the salted fish sample from the Mardika market the number of colonies at dilution 10^{-1} was 1.55 x 10^{3} CFU/mL, then dilution 10^{-2} was 1.05 x 10^{4} CFU/mL. In the salted fish sample from the Rumah Tiga market, the number of colonies at the 10^{-1} dilution was 3.85 x 10^{3} CFU/mL, the 10^{-2} dilution was 5.45 x 10^{4} CFU/mL, and the 10^{-2} dilution was 3.5 x 10^{4} CFU/mL with. In the salted fish sample from the Waiheru market, the number of colonies at 10^{-1} dilution was 9.5 x 10^{2} CFU/mL, and at 10^{-2} dilution was 4.5 x 10^{3}. The comparison of total plate count and INS is presented in Figure 1.

Table I. Results of Total Plate Count Analysis of Grouper Salted Fish (*E. fuscoguttatus*)

No.	Traditional Market	Total Plate Count (CFU/mL)			INS
		10^{-1}	10^{-2}	10^{-3}	
1.	Mardika	$1.55 \times 10^{3} \pm 7$	$1.05 \times 10^{4} \pm 21$	0	1×10^{5}
2.	Rumah Tiga	$3.85 \times 10^{3} \pm 35$	$5.45 \times 10^{4} \pm 7$	$3.5 \times 10^{4} \pm 21$	1×10^{5}
3.	Waiheru	$9.5 \times 10^{2} \pm 21$	$4.5 \times 10^{3} \pm 7$	0	1×10^{5}

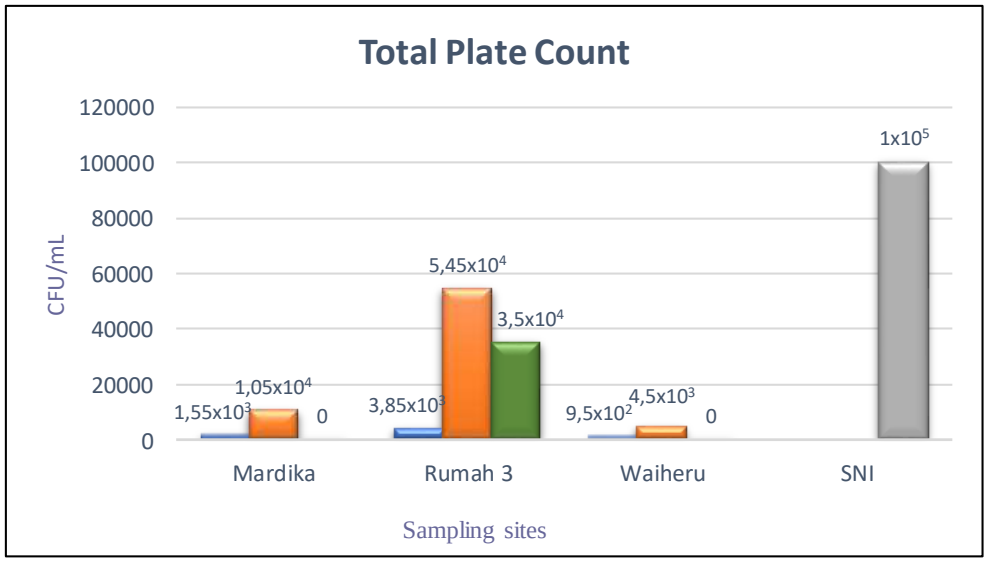

Figure 1. Comparison Chart of Total Plate Count of Grouper Salted Fish (*E. fuscoguttatus*)

DISCUSSION

According to the study, the total count from the three samples of grouper salted fish examined has several bacterial colonies below Indonesian National Standards (INS) and is suitable for marketing and consumption. In a study conducted by Sukmawati (2022) which analyzed the total plate count of salted anchovy microbes at the Remu Market, Sorong City, West Papua, the results were that of the nine salted fish samples tested, all of them were below the INS standard so that they were still suitable for consumption. The salted fish samples used in the study were in an open condition starting from the production process to distribution.

Another study was also conducted by Huda (2022) which analyzed the Total Plate Count of Terasak Fish (*Escualosa thoracata*) Salting Dry Household Industry in the District Lekok Pasuruan Regency obtained the results that of the 6 salted fish samples examined, 4 samples met the INS standard requirements, while the other 2 samples did not meet the INS standard. This can be because the samples taken from the three markets use clean plastic packaging. This is by research conducted by Nurhasanah et al. (2022), which said that packaging has an important role in preventing or reducing damage to a product to be packaged, especially food products. In addition, the effect of preservation using salt affects the growth of bacteria.

According to Ahillah et al. (2017), said that salt has a high enough osmotic pressure that causes osmotic processes in the body of fish and plasmolytic processes in microorganism cells. These processes can inhibit the growth of bacteria. This is also to the understanding of Muhammad et al. (2019), where salt can reduce microbial activity in salted fish products because the salting and drying processes reduce and replace moisture and free water in salted fish products. Microorganisms and bacteria are also inhibited by desiccation, which reduces moisture content and inhibits microbial and enzyme activity.

The total amount of bacteria in the grouper salted fish samples sold at the Rumah Tiga market is suspected to be due to the length of storage compared to the other 2 samples. This is in line with research conducted by Ruksanan (2020), which showed that the length of storage affects the total microbes of dried salted cepa fish produced. Although the Rumah Tiga market is relatively empty of visitors and vehicles and is quite clean, it is possible that the food sold can be contaminated with bacteria. The processing process also affects bacterial contamination such as the hygiene of processors who do not pay attention to hand hygiene and the equipment used when processing salted fish. The drying process by drying in the sun can also be contaminated with various dust, dirt, and even flies that can land on the salted fish. This is also in line with research conducted by Nasution (2020), which said that the main sources of contamination in food come from food handlers or workers, garbage, equipment, rats, insects, and environmental factors such as air and water. Of all the sources of food contamination, food handlers are the most affected by contamination. The health and hygiene of food processors have a considerable influence on the quality of the products they produce, so they need attention.

According to the study findings, salted fish sold in an open condition does not necessarily cross the safe limit according to Indonesian national standards. Thus, the biggest contamination factor in salted fish is during the production process where contamination can come from the environment food handlers, and unclean equipment during the production process (Sipriyadi et al., 2022).

CONCLUSION

Grouper salted fish sold in three traditional markets in Ambon City, namely Mardika market, Rumah Tiga market, and Waiheru market are considered safe for consumption from a microbiological perspective with a total plate count of bacteria in the sample of grouper salted fish from Mardika market ranged from 1.55 x 10^2 to 1.05 x 10^4, from the Rumah Tiga market ranged from 3.85 x 10^3 to 3.5 x 10^4 and from Waiheru market ranged from 9.5 x 10^2 to 4.5 x 10^3. Therefore, this study implies that Grouper salted fish sold in several traditional markets in Ambon City, namely Mardika market, Rumah Tiga market, and Waiheru market are considered safe for consumption from a microbiological aspect thus people do not need to worry about buying and consuming grouper salted fish sold in traditional markets because they have been tested for safety according to INS.

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