

## MICROBIAL PROFILE OF FROZEN FISHERIES PRODUCTS AT UPT PMP2KP SURABAYA

Fadiya Furuujihim Rohsarifuddin<sup>1\*</sup>, Yolandha Sephiani Nurhafifah<sup>2</sup>, Puspa Maharani<sup>2</sup>, Kismiyati<sup>3</sup>, Rahma Hendriyanti<sup>4</sup>, Woro Hastuti Satyantini<sup>3</sup>

<sup>1</sup>Program Magister Bioteknologi Perikanan dan Kelautan, Fakultas Perikanan dan Kelautan, Universitas Airlangga, Kampus C Jl. Dr. Ir. H. Soekarno, Surabaya, 60115

<sup>2</sup>Program Studi Teknologi Hasil Perikanan, Fakultas Perikanan dan Kelautan, Universitas Airlangga, Surabaya

<sup>3</sup>Departemen Akuakultur, Fakultas Perikanan dan Kelautan Universitas Airlangga. Surabaya

<sup>4</sup>Asisten Pembina Mutu Hasil Kelautan dan Perikanan Penyelia, Unit Pelaksana Teknis Pengujian Mutu dan Pengembangan Produk Kelautan dan Perikanan, Jl. Pagesangan II, Surabaya

\*Corresponding author email: fadiyaa.fr@gmail.com

Submitted: 04 July 2024 / Revised: 05 March 2025 / Accepted: 05 March 2025

<http://doi.org/10.21107/juvenil.v6i1.26337>

### ABSTRACT

*Microbial contamination of food products is one of the significant hazards in food safety because the nature of food-polluting bacteria is generally pathogenic. This research was conducted at the Microbiology Laboratory in UPT PMP2KP Surabaya. Samples tested in this research including frozen fish, frozen shrimp, and frozen value added products with microbiological parameters tested are Total Plate Count, Salmonella, and Escherichia coli. The results obtained in this research showed that 100% of 21 frozen fish and shrimp samples met SNI safety standards, namely  $< 1 \times 10^5$  CFU/g for TPC,  $< 3$  MPN / g for E. coli, and negative for Salmonella.*

**Keywords:** E. coli, Food Safety, Frozen Fisheries Product, Salmonella

### INTRODUCTION

The growth of fisheries production in Indonesia is known to continue to increase. Even in 2020, the number of fisheries production in Indonesia reached 23.16 million tons. The capture fisheries sector took 7.7 million tons of the total output, while the other 15.46 million tons came from aquaculture (KKP, 2021). As for the nature of fishery products, perishables are still a challenge for producers. The main problem in product quality and safety is microbial contamination. Frozen fishery products generally contain high water, protein, and fat levels. These components are favorable substrates for microbes to grow and develop. This factor is the main factor causing microorganism contamination in fishery products. Other factors that cause microbial contamination in fishery products are improper handling of materials, poor personal hygiene of producers, and implementation of Good Manufacturing Practices and sanitation that are not adhered to (Ristyanti & Masithah, 2021). Microbial contamination in frozen fishery products is detrimental to consumers and producers because it can reduce product

competitiveness in the market. Not only that, if the product is an export commodity, the biological quality of the frozen product must comply with the standards of the destination country. Simbolon et al. (2020) stated that more than 350 cases of rejection of Indonesian exported crab products by the United States in 2003 - 2012 due to contamination in the products. About 15% of export products from Indonesia also experienced rejection due to microbiological issues from the European Union, Canada, and South Korea (Pradianti et al., 2019).

Food-polluting bacteria are generally pathogenic. Microbial contamination of food products was the primary cause of almost 800 foodborne disease outbreaks in the United States (Qiu et al., 2021). The highest case was caused by Salmonella contamination. Salmonella is known for causing food poisoning, such as cholera, salmonellosis, and typhoid infection (Billah & Rahman, 2024). Li et al. (2019) reported that Salmonella is present in shellfish and fish products. The leading cause of Salmonella infestation in raw fishery ingredients is the water quality of the fish's living

environment, which is supported by the wrong handling methods (Ali *et al.*, 2020). Another bacterium that causes foodborne disease is *Escherichia coli*. *E. coli* is a Gram-harmful bacteria commonly found in fishery products because these bacteria generally pollute the water used as the primary medium during production. *E. coli* contaminates food and has been widely known as a pathogenic agent that causes foodborne illnesses such as diarrhea, nausea, and others (Selfiana *et al.*, 2017).

Knowing the dangers and risks caused by microbial contamination of frozen fishery products, the National Standardization Agency of the Republic of Indonesia has regulated their character specifications. SNI yang mengatur permasalahan terkait adalah SNI No. No. 7388 of 2009. The Indonesian National Standard (SNI) that regulates related issues is SNI No. 7388 of 2009. In order to monitor and ensure the quality of frozen fishery products distributed in Surabaya and for export purposes, the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia established the Technical Implementation Unit for Quality Assurance and Development of Marine and Fishery Products (UPT PMP2KP) in Surabaya. This research was conducted at the Microbiology Laboratory of UPT PMP2KP to determine the microbial contamination profile of various frozen fishery products obtained from several markets in Surabaya.

## MATERIAL AND METHODS

### Materials

The main ingredient in the observation of microbial contamination is frozen fishery products obtained from commercialized products found in the fish market in Surabaya, including a variety of frozen fish filet products, frozen shrimp, frozen squid, and products processed fish bases such as fish cake, dumplings, and many others with a total of 21 samples. The samples in this study were confidential and disclosed from a disguised sample code. Supporting materials in the study are bacterial growth media such as Plate Count Agar (PCA), Xylose-Lysine-Deoxycholate (XLD) Agar, Rappaport Vassiliadis (RV) and Muller-Kauffmann Tetrathionate novobiocin (MKTTn) broth, CCA (Chromocult *et al.*) media, and some others under SNI testing *E. coli*, Salmonella, and Total Plate Count.

### Methods

Microbiology testing methods in this study done by following the methods explained in SNI, including SNI 2332.3: 2015 for Determination of

Total Plate Number (ALT) in Fishery Products, SNI 2332.1: 2015 for Determination of the Most Probable Number (MPN) of *Escherichia coli* in Fishery Products, and SNI ISO 6579-1: 2015 for Horizontal Method for Detection of Salmonella spp. on Food Product.

### Total Plate Count (TPC)

The TPC testing process on fishery products includes sample preparation by homogenizing 25 grams of samples on 225 ml Butterfield Phosphate Buffer (BFP) media, which is then carried out serial dilution up to a dilution factor of 10<sup>-5</sup>. Each dilution level is then inoculated on PCA media with as much as 1 ml. Each plate is then incubated at 35°C for 48 hours. The incubation results are then calculated as the number of colonies formed and calculated using the formula listed on SNI.

### Most Probable Number of *Escherichia coli*

The most Probable Number testing of *E. coli* also involves the same process as the beginning of ALT testing, namely homogenizing a 25 g sample on 225 ml of BFP media, which then enters the stage of estimating Coliform by inoculating a mixture of samples on LTB media equipped with Durham tube inside the test tube. The sample in LTB media is then incubated for 48 hours at 35°C. This stage results in the LTB media becoming cloudy and air bubbles in the Durham tube if a Coliform is suspected to grow on the media. If turbidity is found, an *Escherichia coli* estimation test is carried out by inoculating positive LTB media results on EC broth media, which enters the incubation stage as the previous stage. The *E. coli* estimation stage results are the same as the Coliform estimation stage, which is positive if turbidity is found in the media and air bubbles form in the Durham tube. Positive results from estimating *E. coli* then carried out an *E. coli* affirmation test with positive tube inoculation of EC broth on CCA agar media, which is then from the media if *E. coli* bacteria are detected, the colony color formed is dark blue to purple.

### Detection of *Salmonella spp.*

Salmonella detection includes a pre-enrichment stage by mixing samples on Lactose Broth media, which is then incubated at 37 ° C for 24 hours. Then, the enrichment stage with inoculation of 1 ml and 0.1 ml of RV and MKTTn media samples was incubated in a 45°C water bath for 24 hours, which was then inoculated on XLD and CHROMagar Salmonella selective media Plus. The suspected result of Salmonella is the formation

of pink colonies with or without black dots on XLD media and purple on Salmonella Plus CHROMagar media. Suppose a suspected colony of Salmonella is formed. In that case, a confirmatory test is carried out using the somatic polyvalent (O) anti-serology method, while if no suspected colony is formed, it is reported as negative Salmonella.

## RESULT AND DISCUSSION

### Result

Fishery-based products are known as perishable products, so microbiological parameters are essential to determine their safety for consumption. The results of the microbial contamination profile are shown in **Table 1**.

**Table 1.** Observation of Microbial Contamination in Frozen Fishery Products

Sample Type	TPC (CFU/g)	<i>E. coli</i> (MPN/g)	<i>Salmonella</i> spp.
Frozen value-added crab	1,9x10 <sup>4</sup>	< 3	Negative
Frozen value-added squid	1,1x10 <sup>4</sup>	< 3	Negative
Frozen cakalang	1,0x10 <sup>4</sup>	29*	Negative
Frozen tuna	1,9x10 <sup>4</sup>	460*	Negative
Frozen wild caught parrot fish	1,9x10 <sup>4</sup>	< 3	Negative
Frozen shrimp	2,2x10 <sup>5</sup>	< 3	Negative

Remarks:

\* does not meet standards of SNI No. 7388: 2009

Result of microbial profile of frozen fishery products (**Table 2**) showed that 66,67% of the samples met the standards set in SNI 7388:2009, while the frozen cakalang and frozen tuna samples did not meet the safe limit for the *Escherichia coli* parameter.

### Discussion

The Indonesian National Standardisation Agency stipulates in SNI No. 7388:2009 that the requirements for frozen fish products must have a maximum TPC value of  $5 \times 10^5$  CFU/g, *E. coli* < 3 MPN/g, and negative for Salmonella. The microbial contamination profile analysis results in this study showed that 66.67% of the 6 samples observed had met the specification requirements set out in SNI. The frozen tuna and skipjack samples were found to have MPN values for *E. coli* of 460 MPN/g and 29 MPN/g which exceeded the safe limit for the amount of *E. coli* in frozen fish.

The presence of *E. coli* in frozen fish has also been found in several studies. Atwill & Jeamsripong (2021) found that 85% of 335 seafood samples sold in retail markets in Bangkok, Thailand had *E. coli* counts exceeding 3 MPN/g. The prevalence of *E. coli* in seafood products is closely related to Salmonella's prevalence. This result is inversely proportional to this study, where the presence of Salmonella was not detected in all samples analyzed. The prevalence of *E. coli* in seafood products can be caused by improper product handling conditions, especially after capture. In general, fish, after being caught, will be stacked with ice. Ice can be a good vehicle for pathogenic microorganisms such as *E. coli*,

Salmonella, Aeromonas, and fecal Coliform. This is because ice melts easily at room temperature, spreading microorganisms quickly from one seafood to another (Parlapani et al., 2023).

In addition, the placement of seafood for display during buying and selling can cause cross-contamination between the display and the product or between products. Cross-contamination can also occur due to incorrect handling methods of the seller. Some examples include not using gloves to handle the product and not changing gloves after prolonged use. Hygiene in food processing practices is not only related to maintaining sanitation and hygiene of the tools used but also the personal hygiene of food handlers, who are the main actors in processing a product, is necessarily guarded. The completeness of food handlers' protective equipment is crucial in preventing pathogenic microbial contamination of fishery products. Personal protective equipment includes masks, head covers, and gloves (Girma & Aemiro, 2022). Also, constantly washing hands and sanitizing hands and feet before handling raw materials must always be maintained to minimize microbial contamination. Woh et al. (2017) found that from the results of hand swab tests on food handlers, 20.8% were positive for Coliform / *E. coli* and 63.4% positive for *Staphylococcus aureus* which emphasized the importance of maintaining personal hygiene practices a food handler during the processing/production process.

Similarly, the condition of the processing environment the environment is far from sources of contamination, such as far from

residential areas, treatment sites must be far from waste disposal sites, and it must be ensured that all rooms are tightly closed so that rodents that can contaminate food cannot enter the production environment. Not only that but the sanitary condition of the facilities or equipment involved during the processing process must also be considered. Some of them separate the tools used to process meat and vegetable raw materials, select adequate or appropriate sanitizer materials, always clean the tool after every use, clean the parts folds of equipment, and many others. Garmini et al. (2020) state that the condition of production or processing facilities dramatically affects the quality and safety of the products produced, which is stated in the provisions regarding Good Manufacturing Practices (GMP), Hazard Analysis Critical Control Point (HACCP), and others. Therefore, personal hygiene practices and conditions for fish storage must be maintained to prevent contamination of pathogenic bacteria in frozen seafood products. Thus, the two samples (frozen tuna and skipjack) are unfit for distribution and use as raw materials for producing fishery products, even if the TPC value and the Salmonella result are negative.

### CONCLUSION AND SUGGESTION

Microbial contamination profile analysis shows that 66,67% of 6 samples met the safe limits set by the National Standardization Agency in SNI, namely for ALT a maximum of  $1 \times 10^5$  CFU/g, < 3 MPN/g for E. coli, and negative for Salmonella. However, 2 out of 6 samples have exceeded the safe limits of the E. coli value of SNI 7388:2009. It shows that the supplier mostly has good manufacturing practices that have been done well and can motivate other fishery product producers to maintain product safety and quality, making it profitable for both producers and consumers.

### ACKNOWLEDGMENT

This paper and its research would not have been possible without the exceptional support of UPT PMP2KP Surabaya, field supervisor, and Faculty of Fisheries and Marine Airlangga University.

### REFERENCES

Ali, A., Parisi, A., Conversano, M. C., Iannacci, A., D'Emilio, F., Mercurio, V., & Normanno, G. (2020). Food-borne bacteria associated with seafoods: A brief review. *Journal of Food Quality and Hazards Control*.

- Atwill, E. R., & Jearnsripong, S. (2021). Bacterial diversity and potential risk factors associated with Salmonella contamination of seafood products sold in retail markets in Bangkok, Thailand. *PeerJ*, 9, e12694.
- Billah, M. M., & Rahman, M. S. (2024). Salmonella in the environment: A review on ecology, antimicrobial resistance, seafood contaminations, and human health implications. *Journal of Hazardous Materials Advances*, 100407.
- Garmini, R., Kohri, K., Shatriadi, H., & Maftukhah, N. A. (2020). Analysis of Application Hygiene Principles of Food and Safety Employees Tofu Factory in Padang Selasa, Bukit Besar Palembang. *Journal of Physics: Conference Series*, 1477(7), 072017.
- Girma, A., & Aemiro, A. (2022). Prevalence and associated risk factors of intestinal parasites and enteric bacterial infections among selected region food handlers of Ethiopia during 2014–2022: a systematic review and meta-analysis. *The Scientific World Journal*, 2022(1), 7786036.
- Li, Y., Pei, X., Yan, J., Liu, D., Zhang, H., Yu, B., Li, N., & Yang, D. (2019). Prevalence of foodborne pathogens isolated from retail freshwater fish and shellfish in China. *Food Control*, 99, 131–136.
- Parlapani, F. F., Boziaris, I. S., & DeWitt, C. A. M. (2023). Pathogens and their sources in freshwater fish, sea finfish, shellfish, and algae. In *Present Knowledge in Food Safety* (pp. 471–492). Elsevier.
- Pradianti, O. S., Rahayu, W. P., & Dewanti-Hariyadi, R. (2019). Kajian Kesesuaian Standar Cemaran Kimia (Logam Berat dan PAH) pada Produk Perikanan di Indonesia dengan Standar Negara Lain dan Codex. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, 14(1), 45–62.
- Qiu, Q., Dewey-Mattia, D., Subramhanya, S., Cui, Z., Griffin, P. M., Lance, S., Lanier, W., Wise, M. E., & Crowe, S. J. (2021). Food recalls associated with foodborne disease outbreaks, United States, 2006–2016. *Epidemiology & Infection*, 149, e190.
- Ristyanti, E., & Masithah, E. D. (2021). Penerapan SSOP (Sanitation Standard Operating Procedure) pada Proses Pembekuan Cuttlefish (Sepia officinalis) di PT. Karya Mina Putra, Rembang, Jawa Tengah. *Journal of*

*Marine and Coastal Science*, 10(1), 1–17.

- Selfiana, D. R., Rastina, R., Ismail, I., Thasmi, C. N., Darniati, D., & Muttaqien, M. (2017). Jumlah cemaran *Escherichia coli* pada daging ayam broiler di pasar Rukoh, Banda Aceh. *Jurnal Ilmiah Mahasiswa Veteriner*, 1(2).
- Simbolon, D., Nugroho, T., Fajrin, W. A., & Tarigan, D. J. (2020). Penanganan rajungan oleh pelaku rantai pasok, kaitannya dengan penerapan sistem traceability dalam perikanan skala kecil di Cirebon, Indonesia. *ALBACORE Jurnal Penelitian Perikanan Laut*, 4(3), 353–370.
- Woh, P. Y., Thong, K. L., Lim, Y. A. L., Behnke, J. M., Lewis, J. W., & Mohd Zain, S. N. (2017). Microorganisms as an indicator of hygiene status among migrant food handlers in Peninsular Malaysia. *Asia Pacific Journal of Public Health*, 29(7), 599–607.