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Identification of *Salmonella* spp. Bacteria in Fishery Products at the Testing Laboratory of the Marine and Fishery Products Quality Control and Supervision Agency (BPPMHKP) Surabaya II

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ABSTRACT

Fish is a crucial source of animal protein and a primary commodity in the fisheries industry. Fishery products include fresh fish and frozen fish. Fish is a type of food that is susceptible to biological damage and decay. One of the causes of spoilage and microbial contamination in fishery products by pathogenic bacteria is Salmonella spp. The purpose of this study was to determine the results of the identification of Salmonella spp. in fishery products at the Testing Laboratory of the Fisheries Product Quality Control and Supervision Agency (BPPMHKP) Surabaya II. This study employed the SNI ISO 6579-1:2017 reference method and was analyzed descriptively to determine the presence of Salmonella spp.. Based on the identification results, out of the four samples tested, only one sample was positive for Salmonella spp., namely dried himego products.

INTRODUCTION

Indonesia has abundant fishery resources and is the country with the longest coastline in the world. For the Indonesian people, fish is a crucial source of animal protein and a primary commodity in the fisheries industry (Muchtar et al., 2024). According to data from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia, Indonesia's fisheries production in 2024 totaled 11.43 million tons. The data on capture fisheries and aquaculture production were 5.06 million tons and 6.37 million tons, respectively. Fishery products include fresh fish and frozen fish. Fish is a type of food that is susceptible to biological damage and spoilage (Fikriyah et al., 2024).

Spoilage in fish occurs due to enzymes in the fish's body as well as from the environment that causes microbial activity. The high moisture content of fish can accelerate the development of decaying microorganisms, resulting in the durability of fresh fish lasting only approximately 6 to 7 hours after capture (Fikriyah et al., 2024). The process of spoilage in this fish can hinder the marketing of fishery products, so it is not uncommon for large losses to occur when fish production is abundant (Christanti & Azhar, 2019).

One of the causes of decay and microbial contamination in fishery products by pathogenic bacteria is *Salmonella* spp. *Salmonella* spp. is a short, straight rod-shaped bacterium that has a size of 1-2 μm, belongs to the gram-negative group, does not form spores, and generally moves using flagella peritrichously. This bacterium belongs to the Enterobacteriaceae family because its primary habitat is in the digestive tract of humans and animals. *Salmonella* spp. is a facultative anaerobic bacterium and is biochemically characterized by its ability to ferment glucose, which produces acids and gases, as well as ferment mannitol, arabinose, maltose, dulcitol, xylose, mannose, and rhamnose, but cannot utilize lactose or sucrose. *Salmonella* spp. can decarboxylate the amino acids lysine, ornithine, and arginine, but not glutamic acid. Growth can take place at low water activity (aw ≤ 0.93), with abilities varying depending on the strain and type of food. *Salmonella* can grow in the pH range of 3.6–9.5, with optimal conditions at a pH close to neutral (Umarudin et al., 2023).

Bacterial contamination *Salmonella* spp. often known as a foodborne disease. In the presence of pathogenic bacteria such as *Salmonella* in food, consumer health may be compromised because *Salmonella* spp. can cause salmonellosis. Salmonellosis is a term for infection caused by the bacterium *Salmonella* spp. Diseases that can occur in humans when exposed to bacteria *Salmonella* spp. include typhoid fever, which causes high fever accompanied by vomiting (Ihsan & Abdiani, 2018), diarrhea, headache, and the presence of blood in the stool (Hutomo et al., 2020).

In general, infections caused by pathogens *Salmonella* spp. resulting in millions of cases each year, affecting both humans and animals. Worldwide, the number of salmonellosis infections in humans is estimated at 93.8 million cases per year (Zelpina et al., 2020). Therefore, strict quality control is needed to ensure the quality and safety of fishery products are maintained. One of the agencies tasked with supervising the quality and safety of fishery products is the Marine and Fisheries Products Quality Control and Supervision Agency (BPPMHKP) Surabaya II. The Surabaya II Marine and Fishery Products

Quality Control and Supervision Agency (BPPMHKP) is an agency to support the government's efforts to ensure the safety and quality of fishery products entering and exiting the Surabaya area.

The Marine and Fisheries Products Quality Control and Supervision Agency (BPPMHKP) Surabaya II received several samples of fishery products, including frozen fish, fresh fish, and canned products. Based on previous research, bacterial testing *Salmonella* in fishery products that have been carried out, namely research Putra (2022) Bacterial testing *Salmonella* on fresh milkfish, research Akbar & Diansyah (2016) researching anchovies, research Ihsan (2021) Researching Flying Fish and Milkfish, Research Musa et al. (2024) researching Vannamei shrimp, and research Melawati et al. (2019) Researching salted fish in gutters. The above research indicates that fishery products commonly used in research include fresh fish and frozen fish.

Research on dried himego products has not been conducted previously. This fishery product undergoes drying and processing, making it important to examine the potential presence of *Salmonella* spp. bacteria. Therefore, this study aims to identify *Salmonella* spp. in dried himego products at the Testing Laboratory of the Marine and Fisheries Products Control and Quality Supervision Agency (BPPMHKP) Surabaya II.

RESEARCH METHODS

Method

The research method used is laboratory-based qualitative descriptive to determine the presence of *Salmonella* spp.. This approach is carried out by directly observing the color changes that appear as a result of the interaction between microorganisms and the test media.

Materials and Equipment

The equipment used for testing *Salmonella* spp. included an autoclave, oven, incubator, analytical balance, and magnetic stirrer. Additional laboratory items comprised Petri dishes, test tubes, syringe needles, a test tube rack, and a stomacher. Other supporting tools consisted of beakers, Erlenmeyer flasks, micropipettes, blue tips, a hotplate stirrer, and Schott bottles.

The media used in *Salmonella* spp. testing included BPW (Buffered Peptone Water), RV (Rappaport Vassiliadis), MKTTN (Muller Kauffman Tetrathionate Novobiocin), XLD (Xylose Lysine Desoxycholate), TSA (Tryptone Soya Agar), TSIA (Triple Sugar Iron Agar), LIA (Lysine Iron Agar), TB (Tryptophan broth), Kovacs reagents, sterile aquatics, Urea, and polyvalent antiserum O&H. The samples used were Milkfish, Mackerel Fish, and Dried Himego, obtained from the Fishery Marine Products Control and Quality Control and Supervision Agency (BPPMHKP) in Surabaya II.

Procedure

Sterilization Equipment

Sterilization is carried out by wrapping the tools to be used in paper, then placing them in an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes. After that, the appliance is dried using an oven at 100°C for 1 hour.

Sample Preparation

Salmonella spp. bacteria testing refers to methods based on the SNI ISO 6579.01:2017 standard. This test used four samples: Milkfish, Mackerel, dried himego, and Mackerel, obtained at the Marine Fisheries Product Control and Supervision Agency (BPPMHKP) in Surabaya II. The sample was dissected and separated from the body parts of the fish, including the meat, the head, the fins, and other organs of the fish. This test uses the meat part of the fish. The sample weighed up to 25 grams and was placed in plastic and pre-coded.

Pre-enrichment

225 mL of BPW (Buffered Peptone Water) medium is added to the plastic container containing the sample, and then the sample is homogenized using a stomacher tool. These homogenates are incubated in an incubator for 18 hours ± 2 hours at temperatures of 34 °C to 38 °C.

Selective Enrichment

The culture results of the homogenate were obtained by inoculating 0.1 mL of the homogenate into 10 mL of RV media and 1 mL of 9 mL of MKTTN media using micropipettes. After that, the RV is incubated at 41.5°C ± 1°C for 24 ± 3 hours. Meanwhile, MKTTN is incubated at a temperature of 34°C to 38°C for 24 ± 3 hours.

Test Plating Out

Bacterial inoculation from RV and MKTTN media on XLD selective media to determine the presence of *Salmonella* spp bacterial colonies. Then, it was incubated at a temperature of 34°C to 38°C for 24 ± 3 hours.

Confirmation Test

This confirmation test uses *a* non-selective medium, namely Triptone Soya Agar (TSA). Inoculation of suspected single colonies of *Salmonella* spp. from XLD media on TSA media. After that, it is incubated at a temperature of 34 °C to 38 °C for 24 hours ± 3 hours.

Biochemical Confirmation Test

Colonies of suspected *Salmonella* spp. bacteria were taken from XLD or TSA media using a sterile needle, then punctured and scratched onto TSIA and LIA media, while in Urea media, scratch techniques were used only, and TB media was inoculated using sterile needles. After that, it is incubated at a temperature of 34°C to 38°C for 24 hours ± 3 hours. After incubation, the TB media was added with three drops of the Kovacs solution.

Serological Confirmation Test

This test was conducted by inoculating a colony of suspected *Salmonella* spp. bacteria from the TSA medium were then inoculated onto a glass object. Afterward, physiological NaCl at 0.85% was added, and the mixture was homogenized using a needle. If there is no autoagglutination, it is followed by the addition of polyvalent antiserum O/H, and then homogenizing the polyvalent antiserum and bacterial isolates using a syringe needle.

Data Collection

The data for the *Salmonella* spp. bacterial tests is presented in a table to provide a clear overview of the results. The table outlines the outcome of each assay to support systematic interpretation. The reactions in the selective media are shown through color changes that indicate the presence or absence of the bacteria.

Data Analysis

The data obtained from the identification of *Salmonella* spp. bacteria will be analyzed in a qualitative descriptive manner to identify the presence of *Salmonella* spp. bacteria based on the SNI ISO 6579.1:2017 reference.

RESULTS AND DISCUSSION

The safety of fishery products against pathogenic bacterial contamination requires microbiological testing to detect the presence of *Salmonella* spp. in specific fish product samples. This test aims to assess whether the production and handling process of the product meets applicable food safety standards. Research on *Salmonella* spp. was carried out at the BPPMHKP Surabaya II Testing Laboratory, and the results are shown in **Table 1**.

Table 1. Bacterial Identification Results *Salmonella spp.*

Sample	Selective Enrichment		Plating Out		NO	H2S	LIA	H2S	Urea	TB	O	H	Vi	Result
K (+)			P/H		N/A	+	+	+	-	-	+	+	+	(+) *Salmonella* spp.
Dried Himego	RV	✓	XLD	PH	N/A	+	+	+	-	-	+	+	+	(+)
	MKTTN	✓	XLD	K										*Salmonella* spp.
Frozen Milkfish	RV	✓	XLD	PH	N/A	+	+	-	+	-				(-)
	MKTTN	✓	XLD	KH										*Salmonella* spp
Mackerel	RV	✓	XLD	NG	N/A	-	-	-	+	-				(-)
	MKTTN	✓	XLD	PH										*Salmonella* spp.
Mackerel	RV	✓	XLD	K										(-)
	MKTTN	✓	XLD	K										*Salmonella* spp

Information:
PH (Black Pink), KH (Black Yellow), K (Yellow), NG (No Growth), K/A (Alkaline/Acid)
Suspected *Salmonella* spp. (P/PH), TSIA (K/A) and H2S (+), LIA (+) and H2S (+), Urea (-), TB (-), O (+), H (+), Vi (+)

(a) (b) (c)

Figure 1. XLD Media Results. (a) The initial condition of the XLD media; (b) Suspected positive *Salmonella* spp.; (c) suspected negative *Salmonella* spp.

Table 1 shows three negative samples of *Salmonella* spp. bacteria, namely frozen milkfish, Mackerel, and mackerel fishery products. Meanwhile, the Dried Himego sample showed a positive result for *Salmonella* spp. bacteria marked on pink black XLD media, TSIA K/A media with H2S, LIA media reacted positively with H2S, Urea media, TB reacted negatively, polyvalent antiserum O, H, and Vi positive

Testing of *Salmonella* spp. bacteria at the plating-out stage (plate media) were carried out with XLD (Xylose Lysine Desoxycholate) media. This medium is a type of selective medium, so it can inhibit the growth of bacteria other than *Salmonella* spp. that can grow in the medium (Yusmila et al., 2025). The change in reaction at the plating-out stage is evident in **Figure 1**.

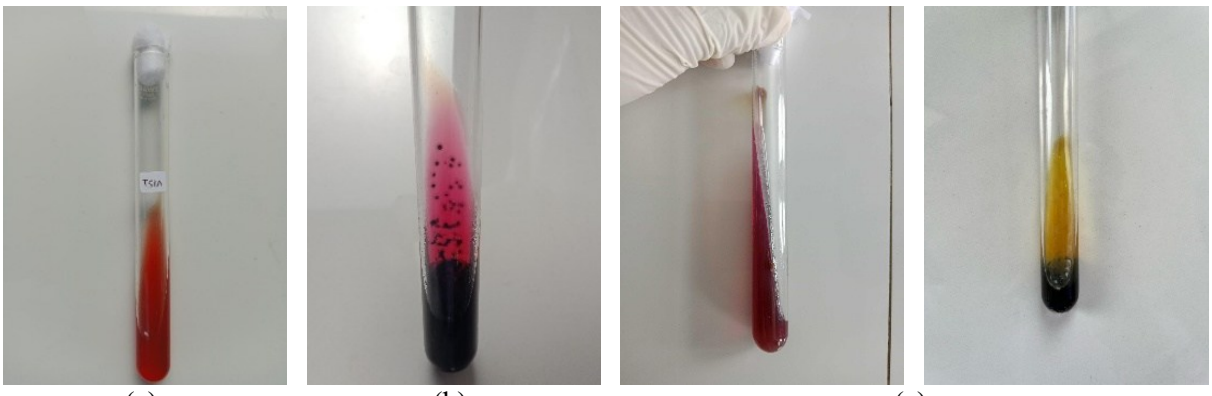
Unexpected positive reactions *Salmonella* spp. on XLD media are marked with pink with or without black dots (H2S) which can be seen in Figure (b). It is due to the bacteria *Salmonella* spp. cannot ferment xylose, but can degrade lactose so that the pH of the medium in the alkaline condition will change the pH indicator Phenol Red i.e. red color becomes pink (Mathew et al., 2024). Bacterial colonies on black XLD media indicate the presence of *Salmonella* spp. carry out metabolic activities so as to produce Hydrogen Sulfide (Rudin et al., 2021). Production of Hydrogen Sulfide (H_2S) caused by the formation of iron sulfide (FeS) deposits (Aini, 2018). A negative reaction is indicated if, in acidic conditions, a change from red to yellow occurs, as shown in Figure (c). It indicates that bacteria can ferment lactose, sucrose, or xylose, which is generally characterized by the presence of bacteria such as *Escherichia coli* (Ummamie et al., 2017).

In samples of dried himego, frozen milkfish, and Mackerel in XLD media, a pinkish-black reaction was observed, indicating a suspected positive sample of

Salmonella spp. while in Mackerel, the XLD media showed a yellow reaction, indicating a suspected negative sample of *Salmonella* spp. The next stage is to ensure the presence of *Salmonella* spp. bacteria is to carry out biochemical and serological confirmation tests.

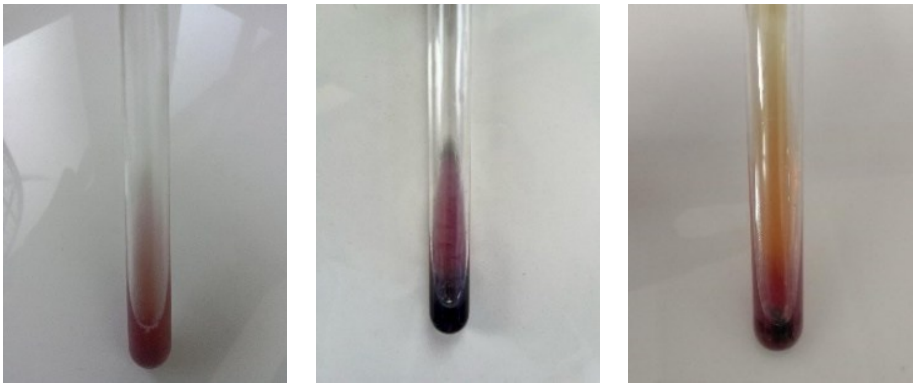
In the confirmation stage, the biochemical test uses TSIA, LIA, Urea, and TB media. Based on the SNI Method ISO 6579-1: 2017, it shows that the reaction to the TSIA media is positive suspected of *Salmonella* spp., namely in the upright part (butt) to yellow (acid) and the oblique part (slant) remains red (alkaline) or called K/A with black dots or H_2S. It is due to the inability of *Salmonella* spp. bacteria in lactose and sucrose fermentation, *Salmonella* spp. bacteria are only able to ferment glucose. Once the glucose is depleted, the bacteria metabolize proteins, producing ammonia and causing an increase in pH in the slope. If the TSIA media is upright and slanted with a red color, it indicates that the bacteria are alkaliphilic (K/K) or can ferment lactose and sucrose, as caused by bacteria. While the upright and sloping part is yellow or A/A indicates that bacteria are unable to ferment glucose, sucrose, and lactose, and the presence of black dots indicates the presence of H_2S.

TSIA media contains 1% lactose and sucrose, 0.1% glucose, as well as the indicator Phenol Red, which changes the color of the media from red-orange to yellow in acidic conditions. On the other hand, in an alkaline condition, the red-orange color will turn pink. This medium also contains sodium thiosulfate as an H_2S-producing substrate, which reacts with iron sulfate to form black FeS, making it easier to identify H_2S-producing bacteria from other types of bacteria (Burhana et al., 2024). The change in media reaction is evident in **Figure 2**.



(a) (b) (c)

Figure 2. Biochemical Test Results on TSA Media. (a) The initial condition of the TSIA media; (b) Suspected positive *Salmonella* spp.; (c) Suspected negative *Salmonella* spp.



(a) (b) (c)

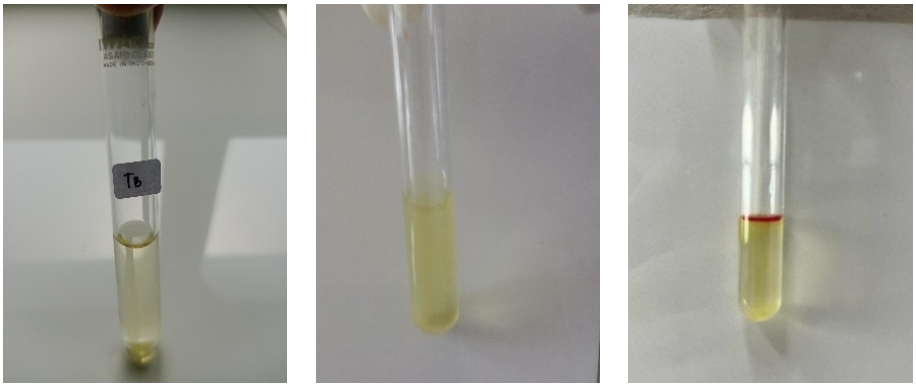
Figure 3. Biochemical Test Results on LIA Media. (a) The initial condition of the LIA media; (b) Suspected positive *Salmonella* spp.; (c) Suspected negative *Salmonella* spp.

In the media LIA the unexpected positive reaction *Salmonella* spp. are characterized by no discoloration (remaining purple) with or without black dots (H_2S). This is because the bacterium *Salmonella* spp. can decarboxylate lysine, leading to an increase in pH and the subsequent formation of cadaverine. Cadaverine can increase alkalinity and alter the pH indicator bromocresol purple to a purple color, whereas in acidic conditions, the color changes to yellow (Nissa et al., 2023).

A black dot marks the formation of H_2S in LIA media. If bacteria do not produce H_2S, this indicates that bacteria cannot reduce sulfur-containing amino acids. The discoloration of the LIA to yellow indicates that the bacteria are unable to decarboxylate lysine due to glucose fermentation (Hidayati, 2016). The change in media reaction is evident in **Figure 3.**

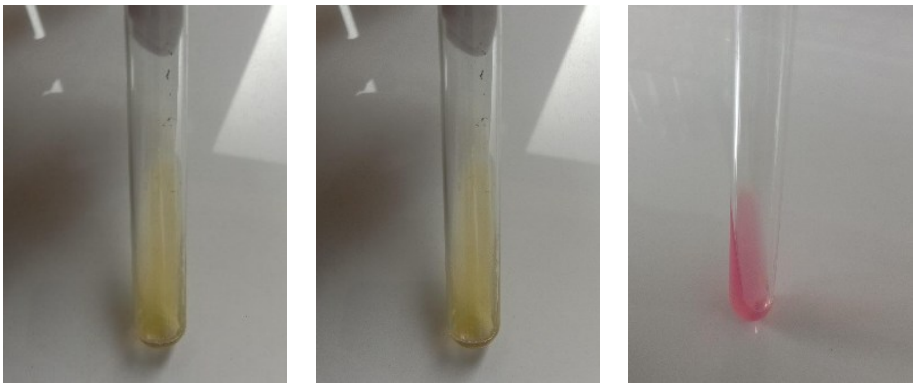
The indole test was carried out on TB media that had been incubated by adding three drops of kovacs reagent and observing the media. The indole test is used to detect the enzyme Tryptophanase in bacteria capable of hydrolyzing the amino acid Tryptophan into indole and pyruvic acid. If the indole test is positive, it is characterized by the formation of a red ring on the surface of the media.

A negative reaction is indicated by the absence of a red or yellow ring, while the presence of such a ring suggests *Salmonella* spp. It occurs due to the inability of the bacterium *Salmonella* enterica to hydrolyse the amino acid Tryptophan (Denis & Hepiyansori, 2024). The presence of indole is known by the addition of Kovacs reagent, which produces a red color on the surface of the media; this indicates a positive indole test. This red color change occurs due to the formation of a complex between indole and P-methyl aminobenzaldehyde contained in Kovacs reagents in acidic conditions (Kepel et al., 2020). Changes in reactions to TB media are evident in **Figure 4.**



(a) (b) (c)

Figure 4. Results of Biochemical Tests on TB Media. (a) Initial condition of TB media; (b) suspected positive *Salmonella* spp.; (c) suspected negative *Salmonella* spp.



(a) (b) (c)

Figure 5. Results of Biochemical Tests on Urea Media. (a) Initial condition of Urea media; (b) Suspected positive *Salmonella* spp.; (c) Suspected negative *Salmonella* spp.

Urea biochemical tests are conducted to determine the reaction of urease enzymes, which do not break the bonds of carbon and nitrogen, resulting in changes in pH. A positive reaction to urea is characterized by a change in color from yellow to pink. The presence of ammonia causes an alkaline State in the media, so Phenol Red turns pink. It indicates the occurrence of a positive reaction and the production of urea (Aini, 2018). At the same time, negative reactions do not occur with color changes (such as yellow). It indicates that the suspect is positive for *Salmonella* spp. Due to the inability of bacteria of the *Salmonella* spp. to hydrolyse the enzyme urease (Shofia et al., 2023). Changes in urea media reactions are visible in **Figure 5.**

In the plating-out test with XLD media, which showed the characteristics of *Salmonella* spp. bacteria, there were three samples, but when the next stage was carried out, namely the biochemical confirmation test, it was unexpected *Salmonella* spp. Bacteria, this occurred due to the growth of other bacteria, but not *Salmonella* bacteria. Based on the confirmation of the biochemical test, as shown in **Table 1**, the

sample of fishery products that tests positive is suspected of containing *Salmonella* spp., specifically dried himego. It is marked in pink, with black XLD media, TSIA K/A media reacting positively to H_2S, LIA media reacting positively to H_2S, and Urea, and TB media reacting negatively. So, to confirm the presence of *Salmonella* spp. bacteria, it is necessary to carry out a serological confirmation test.

Bacterial examination in serological tests uses the principle of reaction between antigens and antibodies. If antibodies are present in the serum, agglutination will occur (Syamsuddin et al., 2021). In this serological test, Polyvalent Antiserum was used against *Salmonella* O and H. Polyvalent O and H are types of antiserum that contain antibodies against different types of O antigens and H antigens from the bacterium *Salmonella* spp. Based on serological tests of fishery product samples, Dried Himego indicates that agglutination occurs after administration of Polyvalent O and H antiserums. It can be observed that the colonies of the sample Dried Himego exhibit a reaction with the serum, resulting in the formation of antibodies, and this sample is positive for the suspected bacterium *Salmonella* spp.

Indicated bacteria *Salmonella* spp. on the product dried himego. It is caused by several factors related to hygiene, processing, and product handling. It is possible that contamination can occur in the early stages, i.e., from fish raw materials obtained from polluted waters that allow the existence of *Salmonella* spp.. Additionally, the post-harvest handling process is less hygienic, often involving the use of unclean tools or workers who are not working in sterile conditions. Other causes can also occur during the drying process that are not carried out in a clean State. Therefore, the combination of poor sanitation, substandard processing processes, and unhygienic post-harvest handling is the leading cause of contamination with *Salmonella* spp. on dried himego (Yennie et al., 2017).

Negative results were obtained in bacterial testing for *Salmonella* spp. in three samples: milkfish, Mackerel, and Mackerel. It, contrary to research Courtesy & Abdiani, (2018), that milkfish sold at the Tarakan City Gusher Market showed positive results for bacteria *Salmonella* tuition and research Putra (2022), indicates test results *Salmonella* spp. on eight samples of milkfish sold at TPI Gadukan Lumpur, Gresik Regency, East Java Province were detected with contamination *Salmonella* so that this study has not met health and food safety standards. However, based on research by Viola (2025), frozen mackerel products have shown negative results in testing for *Salmonella* spp. Additionally, research by Mulyana & Yanti (2018) has found that frozen mackerel samples obtained from UD

Company also yielded negative results. The Sorong I Cup also showed negative results for the presence of the bacterium *Salmonella*. These results indicate that the product meets food safety standards and demonstrate the implementation of effective sanitation systems and hygienic production processes.

Negative results on bacterial testing of *Salmonella* spp. on fishery products tested at the BPPMHKP Surabaya II testing laboratory show that fishery products are safe for consumption and have met food safety standards. No indication of bacteria *Salmonella* spp. shows that the production process for the three products has been carried out in accordance with hygiene standards, including hygienic handling during processing, proper water quality free from microorganisms, and effective supervision of the entire production chain. The implementation of food safety systems, such as HACCP (Hazard Analysis Critical Control Point), can effectively prevent contamination by *Salmonella*, among other pathogens, during the processing and distribution process (Viola, 2025).

In this case it is very important to implement sanitation. Sanitation plays a crucial role in determining the level of bacterial contamination, as its purpose is to prevent the entry of bacteria into food and equipment used in the food processing process. Contamination prevention efforts can be achieved by improving individual hygiene and ensuring that the equipment used in fish processing is kept clean. In addition, proper handling and sanitation are essential to maintain the freshness of fish, because the longer the fish are exposed to open air, the fresher they will be. As a perishable food ingredient (Highly perishable food), fish requires fast, clean, careful, and cold handling in cold temperatures, namely in the temperature range of 0–4°C, so that the quality is maintained from the moment it is caught until it reaches the consumer. Washing hands is an important step in maintaining product cleanliness, as hands can be a source of contamination, carrying dirt, chemicals, or microorganisms (Rahmi et al., 2021).

Based on research by Lokollo & Mailoa (2020) and Rahmi et al. (2021), it is known that during the process of distributing and handling fish in the market, traders generally wash the fish with seawater from the surrounding market area. In fact, the water has the potential to contain pathogenic bacteria such as *Salmonella* sp., *Shigella*, *V. cholera,* and *E. coli*. Therefore, traders must pay attention to the quality of the water used to wash fish in order to prevent pollution.

CONCLUSION

The identification results showed that, out of four samples tested at the Testing Laboratory of the Fishery Product Control

and Quality Supervision Agency (BPPMHKP) Surabaya II, only one sample was positive for *Salmonella* spp., which was a dried himego product.

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