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Analysis of Total Microbes, *Coliform, Escherichia coli*, and *Salmonella* spp. of Smoked Fish Sold in Denpasar City, Bali, Indonesia

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ABSTRACT

Fish is a source of animal protein with high nutritional content such as protein, minerals, and vitamins, as well as health benefits. Fish are highly perishable food, so a process is needed to prevent spoilage in fish products, one of which is the smoking process. Smoking fish prevents post-harvest losses by removing the water content and inhibiting bacterial growth. Improper handling, processing, and storage of smoked fish can lead to contamination of microorganisms, especially pathogenic bacteria. This study aims to determine the total microbes, *Coliform*, *E. coli*, and *Salmonella* spp. of smoked fish in accordance with the food safety standards Indonesia (BPOM). Samples were obtained from smoked fish merchants in four sub-districts in Denpasar, namely West Denpasar, South Denpasar, East Denpasar, and North Denpasar. The research was conducted using streak and spread plate methods on agar media and using Petrifilm. The result shows the highest Total Plate Count (TPC) average number was $139{,}4 \times 10^4$ CFU/g from the South Denpasar, total highest average *Coliform* number was 55×10^3 CFU/g from South Denpasar, total highest average number of *Escherichia coli* was 238×10^1 CFU/g and some of the samples showed positive result for *Salmonella* spp.. According to the BPOM safety standard, the maximum total plate count for smoked fish is 10^5 CFU/g and for Salmonella is negative/25 g. In conclusion of this study, some of the smoked fish samples exceeded the BPOM safety standard.

Key words: *Indicator, Food, Pathogen, Safety standard.*

Introduction

Fish is one of the animal protein sources with high nutritional content and has many health benefits (Emmett *et al.,* 2013). Fish are perishable food due to the activity of enzymes and microorganisms (Laluraa *et al.*, 2014). The smoking process can reduce the quality decrease in fish. The fish smoking process is one of the methods to prevent post-harvest losses by removing the water content, adding a distinctive taste, and can inhibit the growth of bacteria in fish (Akerina, 2018; Tutuarima, 2016). Smoke fish is a processed fish product that many people are interested in, including people in Denpasar. Smoked

1. Student

fish sellers in the area of the City of Denpasar generally sell on the side of the road with conditions where fish are smoked and stored open. Fish smoking in Denpasar City is still traditionally carried out on a small scale; hence the tools used are still simple and sanitation in processing needs to be more observed.

Processing fish products traditionally pays less attention to hygiene, quality, and the freshness of the fish used. In addition, inadequate handling, processing site, and storage of smoked fish can also cause contamination of microorganisms, especially pathogenic bacteria (Akerina, 2018). Contamination by microorganisms in seafood products that can cause disease has rapidly increased in the last decade (Kim *et al.,* 2017). Fish products are one of the food ingredients that can be a medium of foodborne disease transmission (Iwamoto *et al.,* 2010). Foodborne disease is any disease caused by consuming food contaminated with pathogenic bacteria, viruses, and parasites (Adley and Ryan., 2016).

Based on research by Akerina (2018), *E. coli* bacteria were found to contaminate smoked tuna, which is 23 CFU/g, and in the Laluraa et al. study (2014), contamination of *E. coli* was found to be 9 × 103 CFU / g. In addition, in the research, Susanti et al. (2016) also found the presence of bacterial contamination of *Salmonella* spp. in smoked fish products sold in the market. If consumed by humans, contamination of pathogenic bacteria in foods can cause health problems and even death (Ali *et al*., 2020). The bacterial pathogen *E. coli* is the leading cause of infection in the human digestive tract (Makvana and Krilov, 2015). *Escherichia coli*, including *Coliform*, is generally used as a parameter of hazardous conditions during fish processing (Aberounmand, 2010). Another bacterium that can contaminate fish products is *Salmonella* spp., which these bacteria can cause gastrointestinal disease, typhoid fever, and in some cases can cause death (Nugraha *et al.,* 2020).

The increased consumption of fish products by society causes food safety to become a health concern (Atwill and Jeamsripong, 2021). The quality of processed fish through microbiological testing is essential to know the safety of products and can prevent food poisoning due to microbial contamination. Therefore, the study aims to detect microbial contamination, bacteria *Coliform*, *E. coli*, and *Salmonella* spp. of smoked fish sold in the area of the City of Denpasar.

Methods

Methods of Data Collection

Time and place of research

The study was conducted in PT. Seafood Inspection Laboratory, Sidakarya, Denpasar, Bali, from December 2022 until January 2023. Smoked fish samples are obtained by purchasing directly from the Seller in each district in Denpasar, Bali, namely, Western Denpasar, Southern Denpasar, Northern Denpasar, and Eastern Denpasar. Smoke fish were sampled by proportional random sampling method in four districts in Denpasar.

Preparation of samples

Smoked fish samples were obtained directly from sellers in four districts: Northern Denpasar, Eastern Denpasar, Southern Denpasar, and Western Denpasar. Sampling was carried out according to National Standardization Agency of Indonesia (SNI) 19-0428-1998. Each sample of smoked fish obtained is placed in a sterile plastic that has already been labeled and put in a cool box containing an ice pack. The sample is taken to the laboratory and tested.

Total Plate Count (TPC) test

A sample of smoked fish from a 10^{-2} dilution of 100 ìl is inoculated using the spread plate method on a Petri dish containing media Plate Count Agar (PCA). The sample is spread over the entire surface of the medium using a sterile Drigalsky rod. The sample in the Petri dish was incubated for 24 hours at a temperature of 37 °C. Colonies formed from the test results are counted and multiplied ten times to obtain results per ml (Jamilatun, 2022).

Coliform and *E. coli* number test

Coliform and *E. coli* test using Petrifilm

The 10^{-1} dilution sample of 1 mL of smoked fish is inoculated into the 3M™ Petrifilm™ *E. coli*/*Coliform* Count Plate, and the petrifilm is closed. The sample is spread by shaking the petrifilm slowly. The petrifilm is incubated for 24 hours at a temperature of 37 °C (Agustini *et al*., 2020). Colonies formed from the test results are counted or read using colony counters. *Coliform* bacterial colonies are represented by colonies colored red with gas, while *E. coli* colonies colored blue to red blue, and there are trapped gases (3M, 2017).

Confirmation test for *E. coli*

Colonies of blue to blue red with gas growing on Petrifilm were inoculated on the EMBA media. The Petri dish was then incubated at 37°C for 24 hours. A positive sample is said to contain *E. coli* if the EMBA media is formed a metallic green color with (Ivani *et al.*, 2019).

The presence of *Salmonella* spp. test

The bacteria *Salmonella* spp.

The sample of 10 g smoked fish is pre-enriched, inserted into the stomacher bag by pouring 90 ml of Buffered Peptone Water (BPW), then homogenized and incubated for 24 hours at 37 °C. The sample from BPW is taken 100 µl and pour into 10 mL of Rappaport Vassiliadis Soy Broth (RVS) media, then incubated in a water bath for 24 hours at a temperature of 42 °C. Samples on the RVS media were then taken using Ose and inoculated using the streak method on a Petri dish containing Xylose Lysine Deoxycholate (XLD) media, then incubated for 24 hours at a temperature of 37 °C. A positive result is characterized by a specific Salmonella colony, i.e., characteristic of a colony of red color with a black center, small size, and round (Ivani *et al.*, 2019).

The confirmation test for *E. coli* and *Salmonella* spp. was carried out with catalase test and Gram staining procedure.

Data analysis

The data obtained from the test results of Total Plate Count (TPC), the presence test of *Coliform*, *E. coli*, and *Salmonella* spp. are processed using Microsoft Excel software as well as analyzed descriptively and compared with the food safety standards of Indonesia (BPOM).

Results

Total Plate Count (TPC) test on smoked fish

Testing the total plate numbers on nine smoked fish samples showed different results each week. The S2 sample showed the highest TPC value in the first week at $54{,}8 \times 10^4$ CFU/g. In the second week, it was found in the S2 sample that was 57×10^3, and in the third week, the highest TPC value was shown in the S3 sample, which was $41{,}6 \times 10^5$ CFU /g. Total plate numbers with the highest average were shown by the S3 sample of $139{,}4 \times 10^4$ CFU/g. The U1 sample $13{,}6 \times 10^2$ CFU / g showed the lowest average. Test results shown in Table 1.

The test results of the Total Plate Count Number showed the number of bacterial colonies on the Plate Count Agar media, as seen in Figure 1.

Fig. 1. Bacterial colonies from the Total Plate Count result on Plate Count Agar (PCA)

***Coliform* bacteria in smoked fish**

Based on the test results of the total number of *Coliform* bacteria in smoked fish using Petrifilm, ob-

Table 1. Total Plate Count result in smoked fish

Sub-district	Sample Code	Total Plate Count (CFU/g)				BPOM standard (CFU/g)
		Week 1	Week 2	Week 3	Average	
North Denpasar	U1	1×10^2	3×10^3	1×10^3	13.6×10^2	
	U2	35×10^2	54×10^3	65×10^3	40.8×10^3	
East Denpasar	T1	26×10^2	84×10^2	31×10^3	140×10^2	
West Denpasar	B1	32.8×10^3	3×10^4	37×10^3	33.2×10^3	
	B2	94×10^2	12×10^2	131×10^3	47.2×10^3	10^5
South Denpasar	S1	28×10^2	28×10^3	2×10^3	109.3×10^2	
	S2	54.8×10^4	57×10^3	81×10^3	228.6×10^3	
	S3	98×10^2	13×10^3	41.6×10^5	139.4×10^4	
	S4	42×10^2	51×10^2	3×10^2	32×10^2	

tained the results as in Table 2. In the first week, there were four samples containing *Coliform*, with the highest value shown by the S2 sample of 139 × 10^2 CFU/g. In the second week, there were five samples containing *Coliform*, and the greatest value found in the S2 sample of 28 × 10^1 CFU/g. In the third week, six samples were contaminated with *Coliform*, with the greatest value found in the S3 sample 165 × 10^3 CFU / g. The highest average was obtained in the S3 sample of 55 × 10^3 CFU/g, and the lowest average in the T1 sample was not found *Coliform*.

The *Coliform* test results showed the macroscopic characteristics of the *Coliform* colony on the 3M™ Petrifilm™*E. coli*/*Coliform* Count Plate, a red-colored colony surrounded by gas bubbles, as shown in Figure 2.

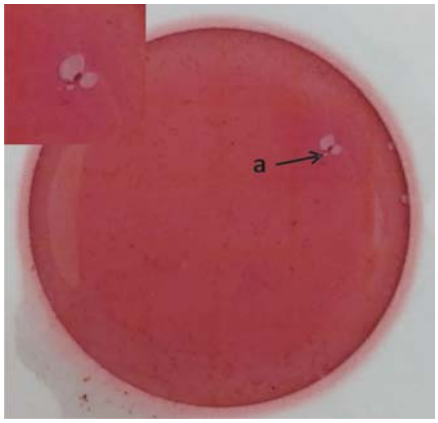

Fig. 2. *Coliform* colony on petrifilm
Description: (a) *Coliform* showed red-colored colony surrounded by gas.

Escherichia coli bacteria in smoked fish

The number of *E. coli* bacteria in smoked fish showed different weekly results. In the first week, there was one sample containing *E. coli*, which in the S2 sample was 68 × 10^2 CFU/g. In the second and third weeks, two samples contained *E. coli*, with the highest value in the second week found in the S2 sample of 8 × 10^1 CFU/g, while in the third week 27 × 10^1 CFU/g. The number of *E. coli* bacteria with the highest average is shown by the S2 sample of 238 × 10^1 CFU/g which shown in Table 3.

The *E. coli* number test results showed the macroscopic characteristics of the *E. coli* colony on the 3M™ Petrifilm™ *E.coli/ Coliform* Count Plate, a red-blue colored colony surrounded by gas bubbles, as shown in Figure 3

The *E. coli* number test results showed the macroscopic characteristics of the *E. coli* colony on the 3M™ Petrifilm™ *E.coli/ Coliform* Count Plate, a red-blue colored colony surrounded by gas bubbles, as shown in Figure 3.

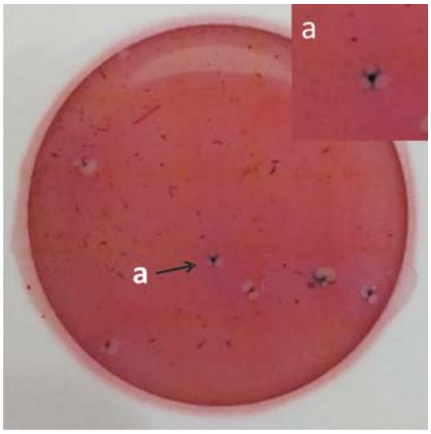

Fig. 3. *E. coli* colony on petrifilm
Description: (a) *E. coli* showed blue-red colored colony surrounded by gas

Positive *E. coli* colony results on the 3M™ Petrifilm ™*E. coli/ Coliform* Count Plate, then re-iso-

Table 2. *Coliform* number result in smoked fish

Sub-district	Sample code	Total *Coliform* (CFU/g)			
		Week 1	Week 2	Week 3	Average
North Denpasar	U1	30	0	0	10
	U2	0	20	43 × 10^1	15 × 10^1
East Denpasar	T1	0	0	0	0
West Denpasar	B1	10	0	0	3.3
	B2	0	10	30 × 10^1	10.3 × 10^1
South Denpasar	S1	0	10	10	6.7
	S2	139 × 10^2	28 × 10^1	78 × 10^1	49.8 × 10^2
	S3	0	60	165 × 10^3	55 × 10^3
	S4	10	0	30	13

Table 3. *E. coli* number result in smoked fish

Sub-district	Sample code	Total *E. coli* (CFU/g)			
		Week 1	Week 2	Week 3	Average
North Denpasar	U1	0	0	0	0
	U2	0	0	0	0
East Denpasar	T1	0	0	0	0
West Denpasar	B1	0	0	0	0
	B2	0	0	60	20
South Denpasar	S1	0	0	0	0
	S2	68×10^2	8×10^1	27×10^1	238×10^1
	S3	0	30	0	10
	S4	0	0	0	0

lated on the Eosin Methylene Blue Agar (EMBA) medium using the streak plate method. Colonies of *E. coli* macroscopically show the metallic green color seen in Figure 7. The colony resulted from the EMBA media, and the Gram staining procedure was carried out. The results showed the rod-shaped bacterial cells of *E. coli*, which are Gram-negative bacteria, as seen in Figure 4.

Salmonella spp. in smoked fish

Based on the results of testing for the presence of *Salmonella* spp. Six samples of smoked fish were contaminated with the *Salmonella* spp. in three weeks. In the first week, there was one positive sample containing *Salmonella* spp. In the second week, there were two positive samples of *Salmonella* spp., then in the third week, there were three positive samples of

Table 4. *Salmonella* spp. test result in smoked fish

Sub-district	Sample code	*Salmonella* (Positive/Negative)			BPOM standard
		Week 1	Week 2	Week 3	
North Denpasar	U1	Negative	Negative	Negative	Negative
	U2	Negative	Positive	Positive	
East Denpasar	T1	Negative	Negative	Negative	
West Denpasar	B1	Negative	Negative	Negative	
	B2	Negative	Positive	Positive	
South Denpasar	S1	Negative	Negative	Negative	
	S2	Positive	Negative	Positive	
	S3	Negative	Negative	Negative	
	S4	Negative	Negative	Negative	

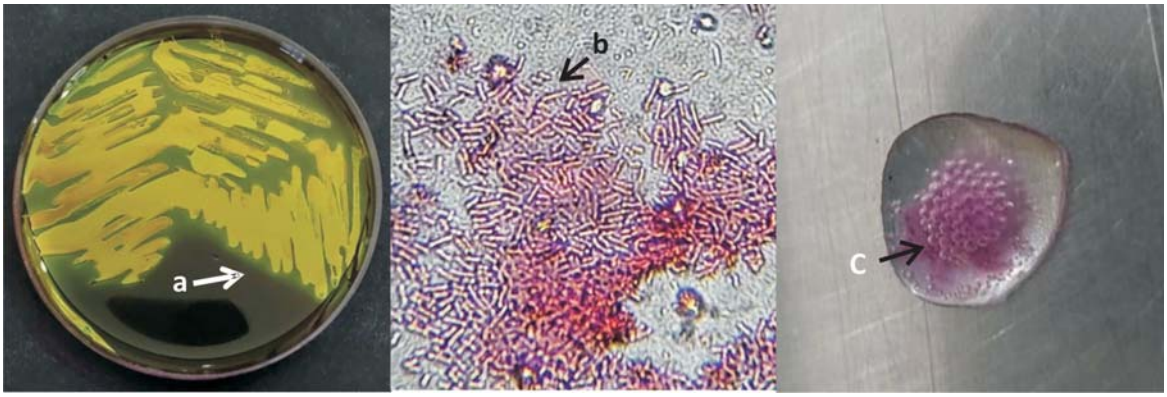

Fig. 4. *E. coli* colony
Keterangan: (a) macroscopic on EMB Agar showed green metallic color (b) microscopic on 1000x magnification showed rod-shaped and Gram-negative bacterial cells (arrow) (c) catalase positive showed gas bubbles

Salmonella spp. Test results shown in Table 4.

The test results for the presence of *Salmonella* spp. in smoked fish were shown with the macroscopic characteristics of a red-colored with a black center colony on the XLD selective medium, as in Figure 4. The colonies on the XLD media then underwent a Gram coloring process, with the results showing the rod-shaped *Salmonella* bacterial cells and Gram-negative, as seen in Figure 5.

Bacteria *Escherichia coli* and *Salmonella* spp. catalase test and Gram stain

Results of catalase test and Gram stainin *E. coli* and *Salmonella* spp. colonies found in several samples showed positive catalase results by the presence of gas bubbles and Gram stain showed red color (Gram-negative) as seen in Table 5.

Discussion

Based on the parameters of the BPOM Regulation Number 13 of the Year 2019, the TPC limit value for smoked fish is 10^5 CFU / g. The test results of the Total Plate Count (TPC) in smoked fish shown in Table 2. The test results showed that 11.11% of the smoked fish tested exceeded the quality requirements for TPC according to BPOM Regulation Number 13 of the Year 2019.

High TPC values in the sample may be influenced by the water content in the specimen. According to Hagos (2021), this may be because the fish still have high water levels. Bacteria such as Salmonella and *E. coli* have a range of water activity between 0.94 – 0.99 for their growth (Graziani *et al.*, 2017; Ross *et al*., 2003). Smoked fish can be a suitable substrate for the growth of bacteria when processed at inappropriate temperatures or procedures and carried out by people who ignore sanitation and hygiene (Dutta *et al.*, 2018). The presence of bacteria can be caused by high levels of contamination in the raw material, and these microbes do not completely disappear during the smoking process (Marc et al., 2014). Conditions of smoked fish that are not dry

Table 5. Catalase test and Gram stain result

Sample code	Week-	Bacteria	Catalase Test	Gram stain
S2	1	*E. coli*	Positive	Negative
S2	2		Positive	Negative
S3	2		Positive	Negative
S2	3		Positive	Negative
B2	3		Positive	Negative
S2	1	*Salmonella* spp.	Positive	Negative
U2	2		Positive	Negative
B2	2		Positive	Negative
U2	3		Positive	Negative
B2	3		Positive	Negative
S2	3		Positive	Negative

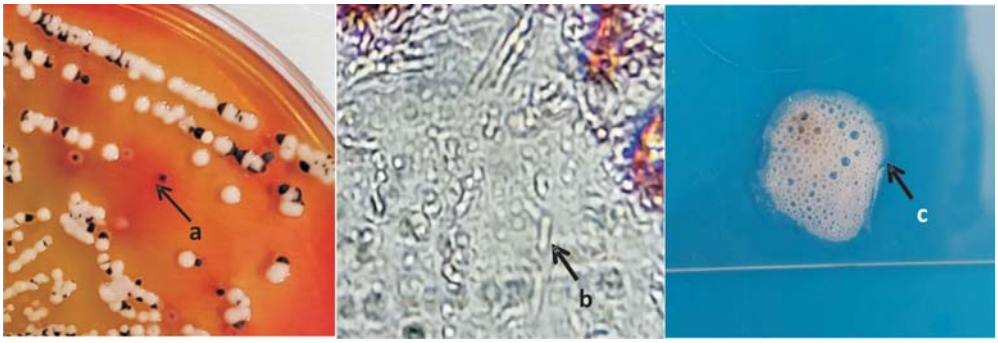

Fig. 5. *Salmonella* spp. colony
Description: (a) macroscopic on XLD medium showed red-colored colony with a black center (b) microscopic on 1000x magnification showed rod-shaped and Gram negative bacterial cell(arrow) (c) catalase positive showed gas bubbles

enough, unclean containers, and selling sites on the side of the street can increase the likelihood that bacteria can still grow (Mailoa *et al*., 2019).

Results of the *Coliform* and *E. coli* showed 18.5% of smoked fish contaminated with *E. coli*, as seen in Table 2 and Table 3. *Coliform* and *E. coli* in smoked fish indicate fecal contamination and the possibility of other pathogenic bacteria (Olaleye and Abegunde, 2015; Akerina, 2018). Food contaminated with *E. coli* can cause foodborne diseases (Zhou *et al*., 2022). These bacteria can cause diarrhea and gastrointestinal disorders in children and adults (Olaleye and Abegunde, 2015). *E. coli* contamination indicates poor sanitation and hygiene (Akerina, 2018). According to research by Akinwumi and Adegbehingbe (2015), *E. coli* contamination in fish is caused by poor individual hygiene of fish processors. Inappropriate handling during and after the smoking process can cause smoked fish to be exposed to bacteria (Likongwe *et al.*, 2018).

The presence of *Salmonella* spp. in smoked fish is shown in Table 5 is 22.2%. Positive results on smoked fish samples exceeded the test limit of *Salmonella* spp., established by BPOM Regulation Number 13 the Year 2019, SNI 2725:2013, and FDA Circular 2013-010, which is negative/25 g sample. *Salmonella* spp. Contamination in fish products may occur due to poor hygiene during the process, equipment, and improper food handling (Fernandes *et al.*, 2018). The use of dirty water is also known to be a carrier of *Salmonella* spp. and a several enteric diseases. Using fecal contaminated water in processing causes contamination of *Salmonella* spp. in food products (Traore *et al.*, 2015).

In addition, contamination can arise due to poor sanitation of food processing individuals who suffer from salmonellosis and become carrier individuals after symptoms disappear. Individual carriers can still release *Salmonella* spp. from the stool and as a result of poor hygiene, can give rise to a health risk. These individuals can cause contamination in fish when handling food during processing (Fernandes *et al*., 2018). The condition of some smoked fish sold is not appropriately cleaned, so there are still gills and inside parts of the fish's stomach. Previous research also showed that *E. coli* and Salmonella were isolated from the gut, intestines, and skin, with the highest numbers found in the skin (Sujatha *et al*., 2011). The presence of bacteria on the surface of the skin and internal organs is caused by cross contamination in the fish processing (Fernandes *et al.*, 2018).

Fish processing should pay attention to the handling, storage, preservation, transportation, and commercialization of fish products because these factors determine the product's final quality (Fernandes *et al.*, 2018). Adequate sanitary conditions during the process, including individual hygiene, as well as surfaces used to process fish, equipment, and using clean water during the treatment process phase is essential to avoid cross-contamination (Fernandes *et al*., 2018). Cooling or freezing after catching fish becomes a critical action to maintain the quality of fish. Properly handling fresh fish raw materials with attention to sanitation and applying low-temperature storage systems can prevent contamination and bacterial activity (Pasue *et al*., 2016).

Conclusion

The Total Plate Count of 11.11% of smoked fish in the Denpasar City area exceeded the Food and Drug Administration standard threshold, as much as 18.5% of smoked fish was contaminated with *E. coli*, while 22.5% was contaminated with *Salmonella* spp., so that the smoked fish did not meet BPOM food eligibility standards.

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References

3M. 2017. *E. coli/Coliform Interpretation Guide*. 3M Food Safety. Canada.

Aberoumand, A. 2010. Edible Gelatin from Some Fishes Skins as Affected by Chemical Treatments. *World Journal of Fish and Marine Sciences*. 2(1): 59-61.

Adley, C.C. and Ryan, M.P. 2016. *The Nature and Extent of Foodborne Disease*. Elsevier. Amsterdam.

Agustini, N. K. S., Putra, I N.K. dan Permana,D.G.M. 2020. Studi Cemaran Mikroba Pada Produk Pangan Tradisional "Lawar Putih Daging Babi" di Kecamatan Denpasar Utara. *Jurnal Ilmu dan Teknologi Pangan.* 9(1): 20-29.

Akerina, F.O. 2018. Cemaran Mikroba pada Ikan Tuna Asap di Beberapa Pasar Tradisional Tobelo, Halmahera Utara, Indonesia. *Akuatikisle: Jurnal Akuakultur, Pesisir, dan Pulau-pulau Kecil*. 2(1): 17-21.

Akinwumi, F.O. and Adegbehingbe, K.T. 2015. Microbiological Analysis of Three of Smoked Fish Obtained

from the Ondo State, Nigeria. *Food and Public Health.* 5(4): 122-126.

Ali, A., Parisi, A., Conversano, M.C., Lannacci, A., D'Emilio, F., Mercurio, V. and Normanno, G. 2020. Food-Borne Bacteria Associated with *Seafood*s: A Brief Review. *Journal of Food Quality & Hazards Control*. 7: 4-10.

Atwill, E.R. and Jeamsripong, S. 2021. Bacterial Diversity and Potential Risk Factors Associated with *Salmonella* Contamination of *Seafood* Products Sold in Retail Markets in Bangkok, Thailand. *Peer J.* 1-20.

Dutta, M., Majumdar, P.R., Islam, Md.R.UI. and Saha, D. 2018. Bacterial and Fungal Population Assessment in Smoked Fish During Storage Period. *Journal of Food Microbiology, Safety & Hygiene.* 3(1): 1-7.

Emmett, R., Akkersdyk, S., Yeatman, H. and Meyer, B.J. 2013. Expanding Awareness of Docosahexaenoic Acid During Pregnancy. *Nutrients*. 5: 1098-1109.

Fernandes, D.V.G.S., Castro, V. S., Neto, A.C. and Figueiredo, E.E.S. 2018. *Salmonella* spp. in The Fish Production Chain: A Review. *Ciencia Rural*. 48(8): 1-11.

Food and Drug Administration. 2013. *FDA Circular 2013-10 Revised Guidelines for the Assessment of Micribiological Quality of Processed Foods.* Department of Health Food and Drug Administration. Philippines.

Graziani, C., Losasso, C., Luzzi, I., Ricci, A., Scavia, G. and Pasquali, P. 2017. *Foodborne Disease – Salmonella.* Elsevier. Amsterdam

Hagos, L. 2021. Smoking Methods and Microbiological Characteristics of Smoked Fishes: A Review. *Journal of Food and Nutrition Sciences.* 9(5): 113-116.

Ivani, D., Kawuri, R. and Yulihastuti, D.A. 2019. Keberadaan bakteri patogen pada sampel pangan jajanan anak sekolah dasar di Pulau Sapeken, Sumenep, Jawa Timur. *Jurnal Biologi Udayana.* 23(2): 68-79.

Iwamoto, M., Ayers, T., Mahon, B.E. and Swerdlow, D.E. 2010. Epidemiology of *Seafood*-Associated Infections in the United States. *Clinical Microbiology Reviews*. 23(2): 399-411.

Jamilatun, M. 2022. Analisis Cemaran Mikroba Angka Lempeng Total (ALT) pada Kue Jajanan Pasar. *ULIL ALBAB*: *Jurnal Ilmiah Multidisiplin.* 1(5): 1243-1248.

Kim, H.W., Hong, Y.J., Jo, J.I., Ha, S.D., Kim, S.H., Lee, H.J. and Rhee, M.S. 2017. Raw Ready-To-Eat *Seafood* Safety: Microbiological Quality of the Various *Seafood* Species Available in Fishery, Hyper and Online Markets. *Letters in Applied Microbiology*. 64: 27-34.

Laluraa, L.F.H., Lohoo, H.J. and Mewengkang, H. 2014. Identifikasi Bakteri *Escherichia coli* pada Ikan Selar (*Selaroides* sp.) Bakar di Beberapa Resto di Kota Manado. *Jurnal Media Teknologi Hasil Perikanan*. 2(1): 5-8.

Likongwe, M. C., Kasapila, W., Katundu, M. and Mpeketula, P. 2018. Microbiological Quality of Traditional and Improved Kiln Smoked Catfish (*Clarias gariepinus*; Pisces; Clariidae) in Lake Chilwa Basin. *Wiley Food Science & Nutrition.* 7(1): 281-286.

Mailoa, M. N., Lokollo, E., Nedissa, D.M. and Harsono, P.I. 2019. Karakteristik Mikrobiologi dan Kimiawi Ikan Tuna Asap. *Jurnal Pengolahan Hasil Perikanan Indonesia.* 22(1): 89-99

Makvana, S. and Krilov, L.R. 2015. *Escherichia coli* Infections. *Pediatrics in Review*. 36(4): 167-171.

Marc, K., Philippe, S., Eustache, H., Boniface, Y., Dominique, S. and Souaibou, F. 2014. Microbiological Quality of Smoked Mackerel (*Trachurus trachurus*), Sold in Abomey-Calavi Township Markets, Benin. *Journal of Microbiology Research.* 4(5): 175-179.

Nugraha, R., Nurilmala,M., Nurjanah and Pratama,P. 2020. Detection of *Salmonella* sp. in Fisheries Product Using Real-Time PCR. *IOP Conf. Series: Earth and Environmental Science*. 404 (2020): 1-5

Olaleye, O.N. and Abegunde, T.A. 2015. Microbiological Safety Assessment of Selected Smoked Fish in Lagos Metropolis. *British Microbiology Research Journal.* 9(3): 1-5.

Pasue, R., Dali, F.A. and Mile, L. 2016. Uji Salmonella sp. pada Yellowfin Tuna (*Thunnus albacores*) yang Dipasarkan di Kota Gorontalo. *Nike: Jurnal Ilmiah Perikanan dan Kelautan*. 4(2): 56-63.

Ross, T., Ratkowsky, D.A., Mellefont, L.A. and McMeekin, T.A. 2003. Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli*. *International Journal of Food Microbiology.* 82(2003): 33-34.

SNI 19-0428-1998. 1998. *Petunjuk Pengambilan Contoh Padatan*. Badan Standarisasi Nasional. Jakarta.

SNI 2725:2013. 2013. *Ikan Asap dengan Pengasapan Panas.* Badan Standarisasi Nasional. Jakarta.

Sujatha, K., Senthilkumar, P., Sangeeta, S. and Gopalakrishnan, M.D. 2011. Isolation of Human Pathogenic Bacteria in Two Edible Fishes, *Priacanthus hamrur* and *Megalaspis cordyla* at Royapuram Waters of Chennai, India. *Indian Journal of Science and Technology*. 4(5): 539-541.

Susanti., A., Fusvita and Janhar, I. A. 2016. Identifikasi *Salmonella* sp. pada Ikan Asap di Pasar Tradisional Kota Kendari. *Biowallacea*. 3(2): 467-473.

Traore, O., Nyholm, O., Siitonen, A., Bonkoungou, I.J.O., Traore, A.S., Barro, N. and Haukka, K. 2015. Prevalence *and* Diversity of *Salmonella* Enterica in Water, Fish and Lettuce in Ouagadougou, Burkina Faso. *BMC Microbiology*. 15(151): 1-7.

Tutuarima, T. 2016. Angka Lempeng Total pada Ikan Lele Asap di Pasar Panorama Kota Bengkulu Selama Penyimpanan Suhu Ruang. *Jurnal Agroindustri.* 6(1): 28-33.

Zhou, F., Wang, D., Hu, J., Zhang, Y., Tan, B.K. and Lin, S. 2022. Control Measurements of *Escherichia coli* Biofilm: A Review. *Foods.* 11: 1-11.