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Microbiological Safety of Smoked Fish: A Review of *Escherichia coli* and *Salmonella spp*. Contamination in Traditional Markets of Kendari City, Indonesia

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ABSTRACT

The safety and quality of fisheries products, particularly smoked fish, are critical public health concerns. Smoked fish sold in traditional markets are prone to contamination by pathogenic microorganisms such as *Escherichia coli* and *Salmonella spp.*, major causes of foodborne illnesses. This study aimed to assess the safety of smoked fish sold in Kendari City's traditional markets based on the presence of *E. coli* and *Salmonella spp.* using microbiological tests compliant with Indonesian National Standards (SNI) 2526:2013 for hot-smoked fish. A total of 26 smoked fish samples were collected from seven traditional markets in Kendari City. The results revealed that none of the samples tested positive for *E. coli*. However, 17 samples (65.4%) were contaminated with *Salmonella spp.* Contamination likely occurred post-smoking due to unsanitary production environments contaminated by livestock waste and during sales, where smoked fish came into contact with fresh products such as chicken and meat. The high percentage of *Salmonella spp.* contamination highlights a significant food safety risk for consumers of smoked fish in Kendari City.

Key words: *Escherichia coli*, *Salmonella. spp*., Smoked fish safety, Traditional market, Kendari.

INTRODUCTION

Fish is an essential source of animal protein in Indonesia, including in Kendari City and Southeast Sulawesi (Fawzya and Irianto 2020). As a coastal area, Kendari has abundant access to fish resources, making fish a primary food commodity for its residents (Dudi et al. 2019). One of the most common processing methods used is smoking, a traditional technique that enhances shelf life and imparts a distinctive flavor to fish (Indrayani et al. 2024). Various smoked fish are sold in traditional markets, serving as an affordable protein source for the local community (Chintagari et al. 2017).

Smoked fish products are prone to contamination by microorganisms, primarily due to processing, handling, and distribution practices that often lack proper hygiene and sanitation standards (Karimela and Mandeno 2019). Previous studies have shown that smoked fish from traditional markets is at risk of contamination by pathogenic microorganisms such as *Escherichia coli* and *Salmonella spp.*, which indicate poor sanitation throughout the distribution chain. This contamination poses significant health risks to consumers, such as foodborne illnesses and intestinal infections caused by these pathogens (Setiyono and Sulistyorin 2019).

Previous studies have shown that the oversight of smoked fish safety is often neglected, especially in traditional markets in developing countries (Swastawati et al. 2018). Globally, data reported by the World Health Organization (WHO) in 2022 indicate that foodborne illnesses caused by pathogenic bacteria such as *Escherichia coli* and *Salmonella spp.* remain a significant public health threat, particularly in developing nations. Annually, 600 million cases of foodborne diseases are reported, resulting in 420,000 deaths, most of which are attributed to pathogenic bacteria in inadequately processed or stored

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food products (WHO 2022). A study conducted in India revealed a prevalence of *Salmonella* contamination in smoked fish products reaching 15%, whereas in Brazil, *E. coli* contamination was detected in 20% of tested samples (Swastawati et al. 2018). In Indonesia, a report by the Food and Drug Supervisory Agency (BPOM) in 2021 disclosed that approximately 30% of reported food poisoning cases were associated with bacterial contamination in food products sold in traditional markets, including smoked fish (BPOM 2021).

In Kendari City, smoked fish is a popular fishery product among the community, both as a side dish accompanying main meals and as an ingredient for various traditional dishes. Smoked fish is typically sold in traditional markets and by street vendors, either as ready-to-eat products or as ingredients that require further preparation, such as frying or cooking with regional spices. Some residents consume it directly after the smoking process without additional cooking, while others choose to cook it further (Mailoa et al. 2019). The widespread and daily sale of smoked fish makes it an affordable protein source for many families. However, with its popularity and high consumption frequency, the risk of microbiological contamination, such as *E. coli* and *Salmonella*, becomes a significant concern. Fish that is not handled properly during processing, storage, or distribution, particularly under poor sanitary conditions—can increase the likelihood of contamination by these pathogenic bacteria, posing health risks to consumers (Setiyono and Sulistyorin 2019).

Given the susceptibility of smoked fish to contamination, this study was conducted to assess the safety of smoked fish sold in traditional markets in Kendari City. The research focused on identifying the presence of *E. coli* and *Salmonella spp*., both of which serve as general indicators of contamination and poor sanitation during food processing. The findings are expected to provide more comprehensive data on the microbiological quality of smoked fish in Kendari and offer recommendations for improving processing and handling practices with following food safety standards.

MATERIALS AND METHODS

Ethical approval

This study has received ethical approval from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural University, with approval number: 142/KEH/SKE/XII/2023.

Study area, design and sample size

This study was conducted in Kendari City, Southeast Sulawesi, from June to September 2024. Traditional market locations were selected to represent a diverse range of market conditions in the city. The research design used was descriptive with a quantitative approach. A total of 26 smoked fish samples were collected from seven traditional markets, selected using purposive sampling. The sample selection was based on considerations such as different locations, the number of smoked fish sellers at each market, and the willingness of sellers to participate in the study. The smoked fish samples were aseptically collected and placed in sterile plastic bags labeled according to their sampling location for further examination at the laboratory. The sample testing was conducted at the Biomedical Laboratory, Faculty of Medicine, Halu Oleo University, Kendari.

Procedures

Identification of *Escherichia coli*

The identification was carried out using the *Most Probable Number* (MPN) method. A 25-gram sample of smoked fish from each sample was mixed with 225mL of 0.1% *Buffered Peptone Water* (BPW) and homogenized to obtain a 10^{-1} dilution. Serial dilutions were then made in triplicate tubes (10^{-1}, 10^{-3}, and 10^{-4}), where 1mL from each dilution was added to 9mL Lauryl *Sulfate Tryptose Broth* (LSTB) with Durham tubes and incubated at 35°C for 24-48 hours. Positive results were indicated by gas production and turbidity. The positive culture from LSTB was then inoculated into *Escherichia Broth* (ECB) with Durham tubes and incubated at 45°C for 24 hours, and gas formation was observed. The MPN value for fecal coliforms was calculated using the MPN table and positive cultures from ECB were streaked onto *eosin methylene blue agar* (EMBA) to identify colonies with a metallic green appearance and dark centers, which were subsequently tested biochemically using IMViC.

Biochemical tests were performed using the IMViC method to identify the suspected colonies. The indole test was conducted using a tryptone medium incubated at 35°C for 24 hours, with a positive result indicated by the formation of a red ring after adding *Kovac's reagent*. The methyl red test was performed using an MRVP medium incubated for 48 hours, followed by the addition of an MR indicator, with a positive result showing a red color. The Voges-Proskauer test was conducted by adding α-naphthol and KOH to the MRVP solution, with a positive result indicated by a pink color within 2 hours. The citrate test was done using *Koser Citrate Broth* medium, incubated at 35°C for 96 hours, with a positive result indicated by turbidity in the medium.

Identification of *Salmonella spp.*

The process began with pre-enrichment, where 25 grams of smoked fish sample was added to 225mL of 0.1% BPW, homogenized and incubated at 35–37°C for 16–20 hours. Next, 0.1mL of the inoculum from BPW was transferred into 10mL of *Rappaport-Vassiliadis* Medium (RV-Medium) and incubated at 42°C for 24 hours. The inoculation was followed by two streaks on selective media (XLD agar), which was incubated at 35–37°C for 24 hours and suspected *Salmonella* spp. colonies were then subjected to biochemical testing using TSIA and LIA media, which were incubated at 35–37°C for 24 hours. The results of color changes after incubation determined the positive outcome, as outlined in Fig. 1.

Data analysis

The data were analyzed descriptively, presenting the results in the form of tables and figures. Each result was interpreted to provide a deeper understanding of the relationships or implications of the data.

RESULTS

Distribution of *E. coli* contamination

This study revealed that none of the 26 smoked fish

Table 1: Prevalence and Distribution of *E. coli* and *Salmonella spp.* Contamination in Smoked Fish from Traditional Markets in Kendari City

Location	Sample (n=26)	Identication of *E. Coli*			Identication *Salmonella* spp.			
		IMViC for *Escherichia coli* (++--)	Notes	(%)	TSIA (Slant, button and H2S)	LIA (Slant, button and H2S)	notes	(%)
Andounohu market	2	++-+	No	0	yellow/black/+	violet/black/+	Yes	100
		++-+	No		yellow/black /+	violet/black /+	Yes	
Pedismark (PKL Mart)	2	++-+	No	0	black/black/-	violet/black /-	No	0
		++-+	No		black/black/-	violet/black /-	No	
Mall basah	8	++-+	No	0	yellow/black /+	violet/black /-	Yes	62,5
		++-+	No		black / black /-	Ungu/ungu/-	No	
		++-+	No		black / black /-	Ungu/ungu/-	No	
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/ yellow /-	black/black/-	No	
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	
Auction market	4	++-+	No	0	yellow/black /+	violet/black /+	Yes	50
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		black/black/-	violet/black /-	No	
		++-+	No		black/black/-	violet/black /-	No	
Baruga market	2	++-+	No	0	yellow/black /+	violet/black /+	Yes	100
		++-+	No		yellow/black /+	violet/black /+	Yes	
Korem market	4	++-+	No	0	black/black/-	violet/black /-	No	75
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	
Antasari market	4	++-+	No	0	black/black/-	violet/black /-	No	75
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	
		++-+	No		yellow/black /+	violet/black /+	Yes	

Note: TSIA and LIA Test results depending on the color indicator seen.

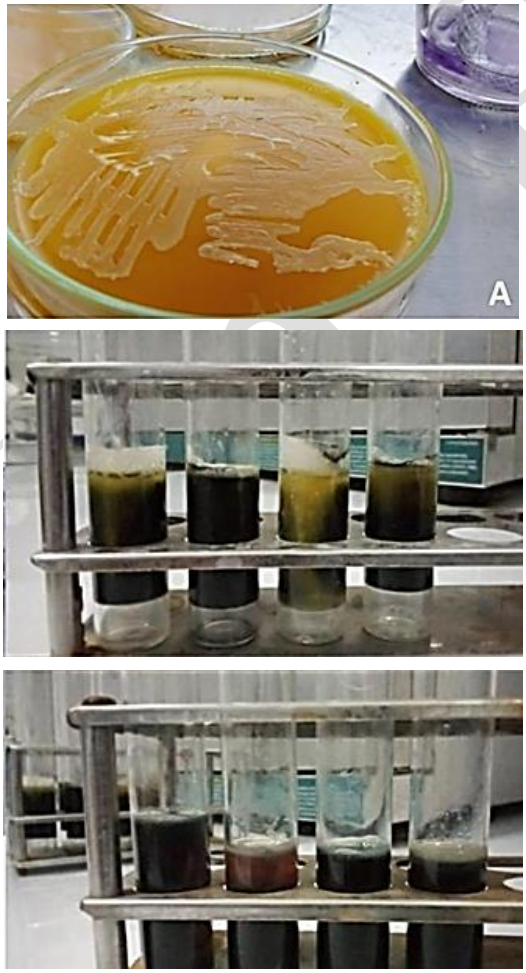

Fig. 1: *Salmonella* biochemical test with TSIA and LIA.

samples examined from various traditional markets in Kendari City were contaminated with *E. coli*. The absence of *E. coli* in smoked fish may be attributed to the fact that *E. coli* naturally inhabits warm-blooded animals such as birds and mammals (Bentancor et al. 2023). As fish are cold-blooded animals, their likelihood of harboring *E. coli* is minimal. Contamination in fresh fish, the raw material for smoked fish, might occur due to environmental pollution from the fecal waste of humans or animals (Lehel et al. 2020). The thorough smoking process also likely eliminates any *E. coli* contamination introduced through fecal pathways (Kannan et al. 2021).

Distribution of *Salmonella* spp. contamination

Examination of the 26 smoked fish samples revealed that 17 samples (65.4%) tested positive for *Salmonella spp.* The distribution of *Salmonella*-positive samples varied across sampling locations. At Anduonohu Market and Baruga Market, all samples from vendors were positive for *Salmonella spp.* (100%). Meanwhile, out of 8 samples from Mandonga Mall, 5 (62.5%) tested positive for *Salmonella* spp., while the remaining 3 tested negative.

At the Fish Auction Market, 2 out of 4 samples (50%) tested positive for *Salmonella spp.*, while in Korem Market and Antasari Market, the contamination rate reached 75%, with 3 out of 4 samples testing positive in each location. This variation indicates differences in contamination levels across locations, likely influenced by disparities in sanitation, handling practices, and environmental conditions during smoking, storage and distribution processes.

These findings highlight significant microbiological risks in smoked fish from the region, as contamination

levels exceed the safety standards outlined by the Indonesian National Standard (SNI 2725:2013), which mandates the absence of *Salmonella spp.* in 25g of sample. The presence of *Salmonella spp.* in food products poses a serious public health threat, linked to improper processing, poor storage conditions, and substandard sanitary practices in market environments (Astrini 2014). Previous studies have shown that handling smoked fish in unsanitary conditions can elevate the risk of pathogenic bacterial contamination, including *Salmonella spp.* (Alfarego et al. 2022). Considering the public health risks associated with *Salmonella spp.*, particularly in causing salmonellosis, it is crucial to enforce better hygiene practices and stronger monitoring systems for smoked fish processing and sale in traditional markets (Safira et al. 2024).

DISCUSSION

Smoked fish is a food product that is widely consumed by the public because of its distinctive flavor (Tamtama et al. 2023). However, the potential for microbiological contamination, particularly by pathogenic bacteria such as *E. coli* and *Salmonella* spp. is a major concern in ensuring food safety (Farahitan and Junianto 2024). The study focused on the presence of these two bacteria, which are considered biological hazards. The study's results can provide an overview of the safety of smoked fish by considering the smoking process, as well as the sanitation practices applied in the production and distribution of these products.

Several previous studies reported results that *E. coli* was not detected in processed fish products including smoked fish. Research by Wibowo et al. (2021) showed that although the fish samples examined contained a number of other pathogenic microbes, *E. coli* was not found in smoked tuna obtained from traditional markets in Pontianak City. This finding is in line with the results of a study by Aristawati et al. (2024), which emphasized that good fish processing, including adequate smoking, can limit pathogenic microbial contamination, including *E. coli*, although other bacteria may still be detected in fresh fish. In addition, another study showed the absence of *E. coli* in other processed fish products such as pempek (Hasanah et al. 2021). This study indicates that successful control of *E. coli* contamination is strongly influenced by other factors, such as cleanliness in the fish handling process, storage temperature and sanitation practices applied at the processing site (Setiyono and Sulistyorin 2019).

E. coli exhibits a greater sensitivity to external environmental changes compared to *Salmonella* spp. One factor that affects the survival of *E. coli* is changes in pH extremes. A significant decrease in pH can cause damage to the cell membrane and destabilize the internal structures of the bacteria, such as enzymes necessary for metabolism (Geraldo et al. 2024)). In addition, *E. coli* is also sensitive to environmental conditions containing high salt levels and drought (Sarwini 2022). High salt levels can damage the osmotic balance in cells, while drought inhibits growth and reproduction processes, worsening its survival in unfavorable environments (Suryani 2023).

In contrast to *E. coli*, *Salmonella* spp. has better adaptability to non-ideal environmental conditions. One of the main factors supporting the survival of *Salmonella* spp. is its ability to survive in a wider range of pH conditions and its tolerance to high salt levels. This is because *Salmonella* spp. has efficient osmotic regulation mechanisms and the ability to adapt to osmotically challenging environments. *Salmonella* spp. also can form biofilms that protect the bacteria from harsh environmental conditions, such as drought or exposure to antimicrobial agents.

Salmonella spp. are pathogenic bacteria that cause gastrointestinal illness, typhoid fever, and in severe cases, death. These bacteria are frequently implicated in seafood-related disease outbreaks in various countries, including the European Union (EFSA 2014) and the United States (Barret et al. 2017). In Indonesia, Salmonella spp. contamination in fish products is frequently reported, such as a study in Surabaya that found 93% of seafood contaminated with these bacteria (Pramono et al. 2019). Contamination can reduce product quality, affect consumer satisfaction, and trigger product recalls (Nugraha et al. 2020).

The smoking process in smoked fish uses high temperatures during the drying and smoking process itself. High temperatures can kill pathogenic bacteria such as *E. coli* (Geraldo et al. 2024). The optimal temperature required in smoking, which is ± 80°C, can damage the bacterial cell membrane and inhibit its growth. Another reason is that wood smoke produced from the smoking process contains antimicrobial compounds such as phenols, aldehydes, and organic acids (Suryani 2023). These compounds have bactericidal properties, so the presence of *E. coli* in smoked fish can be eliminated.

Smoked fish sold in traditional markets and street markets in Kendari are generally poorly packaged and sold openly, which can increase the risk of microorganism contamination. Unpackaged processed food products involve direct exposure to air, dust, and various elements from the surrounding environment (Oktavianty et al. 2021). In traditional markets, smoked fish is often sold alongside other products such as fresh fish, chicken and vegetables, creating the potential for cross-contamination (Widyaningrum et al. 2024). Dirty environmental conditions, with accumulated garbage and lack of hygiene management, increase the likelihood of pathogenic acteria such as *Salmonella* spp. (Kopper et al. 2023). Dust and particles present in the market can also carry harmful microorganisms that could potentially contaminate the fish sold (Tong et al. 2023).

In addition, fish handling practices by vendors also pose a high risk of contamination. Some sellers were also seen doing other activities, such as playing cards, smoking, and sometimes leaving their merchandise and doing other activities without paying attention to hand and tool hygiene. These actions can increase the risk of *Salmonella spp.* entering the smoked fish product. The absence of the use of personal protective equipment such as gloves or clean clothes is also an important factor that facilitates the spread of bacteria. These unhygienic habits are particularly risky in the already dirty environment of traditional markets, making smoked fish sold there more susceptible to microbiological contamination.

The main factor of contamination is poor sanitation in the fish processing environment. Unhygienic tools and

tables can transfer bacteria between products (Arifah et al. 2024). Another study showed that poor environmental sanitation is directly correlated with an increase in the number of pathogenic bacteria in fish products (Chintagari et al. 2017). In addition, the use of water contaminated by animal waste or feces during the washing process also increases the risk of bacterial transmission (FAO 2023; Sanches et al. 2024). Some of the above reasons are indications of why Salmonella spp. can easily migrate and contaminate smoked fish sold in traditional markets in Kendari City.

Conclusion

The safety level of smoked fish sold in traditional markets in Kendari City is low with the discovery of *Salmonella spp*. as much as 65.4%. The smoking process is expected to minimize the risk of contamination with pathogenic microorganisms, so based on this study, thorough supervision of the production process, including environmental sanitation, smoking temperature control, and post-processing practices should be carried out to protect public health. In addition, it is necessary to improve healthy market management by not placing fresh food and processed ready-to-eat food in the same location, the packaging process on smoked fish and smoked fish needs to be properly processed before consumption by the public.

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