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Total Plate Count, Coliform, and Salmonella in Skipjack Tuna (Cakalang) Products from a Medium-Scale Fish Processing Industry

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ABSTRACT
Microbiological quality is a key determinant of the safety of processed fish products, yet data from operating medium-scale industries remain limited. This study evaluated the microbiological quality of three skipjack tuna (cakalang)-based products, namely Daging Cakalang Frozen, Steamed Cakalang Flakes, and Filet Cakalang Pasteurisasi, produced at a medium-scale fish processing industry with a capacity of 750–800 kg/day. A descriptive cross-sectional design was used, with one sample unit per product from a single production batch. Total Plate Count (TPC) was determined by the pour plate method with duplicate plates, presumptive coliform by the three-tube Most Probable Number (MPN) method, and Salmonella by culture on selective-differential media (HE, XLD, BSA), which was performed only on the pasteurized fillet. Estimated TPC values were 1.4 × 10^2 to 3.2 × 10^2 CFU/g, below the company requirement of ≤1.0×10^4 CFU/g. Presumptive coliform was <3 MPN/g in all products, and Salmonella was not detected in 25 g of the pasteurized fillet. Under the conditions tested, all products met the specified requirements for these parameters. Because only one batch per product was examined, the findings describe the tested batch and are not a comprehensive assessment of food safety. Multi-batch studies with confirmed Escherichia coli and additional pathogens are recommended.

INTRODUCTION

Indonesia is an archipelagic nation with vast fisheries resources, and the fisheries sector is a mainstay of the national economy for both domestic and export markets (Fitria et al., 2024). Skipjack tuna (locally known as cakalang, *Katsuwonus pelamis*) is a leading commodity that is widely processed into value-added forms such as frozen loin, steamed products, and pasteurized products (Siregar et al., 2026). Like other fishery products, however, it is highly perishable because its high water content, near-neutral pH, and rich nutrient composition support the growth of both spoilage and pathogenic microorganisms (Roy et al., 2024).

Total Plate Count (TPC), coliform, and *Salmonella* are commonly used to assess the microbiological quality and safety of fishery products. TPC reflects the general population of mesophilic aerobic microorganisms and indicates hygiene and handling effectiveness during

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production (Sulistiani & Hafiludin, 2022). Coliforms, including *Escherichia coli*, are sanitation indicators that signal possible fecal contamination or an unhygienic processing environment (Hadji et al., 2025). *Salmonella* is among the pathogens most frequently associated with foodborne illness linked to fishery products worldwide (Odeyemi et al., 2023; Roy et al., 2024).

Contamination levels in traditional or informally processed fishery products are often high. Anihouvi et al. (2019) reported high bacterial loads in smoked fish in Benin, and Birie et al. (2024) found that most sun-dried fish samples from Lake Tana, Ethiopia, exceeded international microbial limits. In Indonesia, Jannah et al. (2025) and Khairunnisa et al. (2023) reported salted and smoked fish samples that failed national microbial standards. In contrast, studies of more controlled processing systems, such as frozen and canned products at BPMHP Semarang (Sulistiani & Hafiludin, 2022) and de-boned milkfish in Palu (Ndobe et al., 2023), tended to report compliance with quality requirements.

Cakalang processing also occupies a strategic position in Indonesia's fishery exports. Meeting the microbiological entry requirements of importing markets depends on raw-material freshness and on consistent processing, including freezing rate, thermal intensity, and post-process handling (Duanmu et al., 2024; Fitria et al., 2024; Siddiqui et al., 2024). Rapid freezing air-blast freezing (ABF), steaming, and pasteurization are widely applied for this purpose. Rapid freezing at very low temperatures preserves quality and limits microbial activity in tuna flesh during frozen storage (Chakma et al., 2020; Duanmu et al., 2024), steaming reduces aerobic bacterial counts in fish products (Chew et al., 2024; Wang et al., 2020), and pasteurization at 80–85°C is a standard method for ready-to-eat seafood (FDA, 2021; Siddiqui et al., 2024).

In Indonesia, microbiological requirements for fishery products are set by product-specific standards and food regulations. Frozen tuna loin is covered by SNI 4104:2015 (Badan Standardisasi Nasional, 2015), cooked tuna products are referenced in SNI 7968:2014 (Badan Standardisasi Nasional, 2014), and maximum microbial limits for processed food are regulated by BPOM Regulation No. 13/2019 as amended by BPOM Regulation No. 3/2026 (Badan Pengawas Obat dan Makanan, 2019, 2026). Medium-scale industries usually combine these requirements with internal quality specifications, and compliance depends heavily on consistent cold chain implementation from raw-material receiving to final storage (Siregar et al., 2026).

Quality assurance systems such as Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) are widely adopted by medium-scale fish processors in Indonesia (Fitria et al., 2024). Nevertheless, much of the prior research has focused on traditional or informal fishery products, such as smoked, salted, and traditional-market fish, in Indonesia and elsewhere (Bardoe et al., 2023; Obasi & Danladi, 2023; Jannah et al., 2025). Microbiological data on modern processed products from operating medium-scale industries remain limited. This study therefore aimed to evaluate the microbiological quality of three cakalang-based processed products, namely Daging Cakalang Frozen, Steamed Cakalang Flakes, and Filet Cakalang Pasteurisasi, produced at one medium-scale fish processing industry, based on TPC, presumptive coliform, and *Salmonella* under their respective processing conditions.

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METHODS

1. Study Location, Products, and Processing Flow

This study was conducted at the internal quality-control laboratory of a medium-scale fish processing industry, hereafter referred to as PT AFA, with a raw-material capacity of 750–800 kg/day. The company processes fresh cakalang (*Katsuwonus pelamis*) into three value-added products. All product lines share the same initial stages (raw-material receiving, sorting, and preparation) and then branch into different freezing or thermal stages (Figure 1).

The three products were: (1) Daging Cakalang Frozen, a frozen product processed by rapid freezing in an ABF system at −35°C to −40°C; (2) Steamed Cakalang Flakes, a ready-to-cook product processed by steaming at 100°C for 30 minutes to reach a minimum product core temperature of 70°C; and (3) Filet Cakalang Pasteurisasi, a ready-to-eat product processed in a thermostatically controlled water bath at 80–85°C for 30 minutes. After these stages, the products were cooled or frozen, packaged, and tested at the company's internal laboratory.

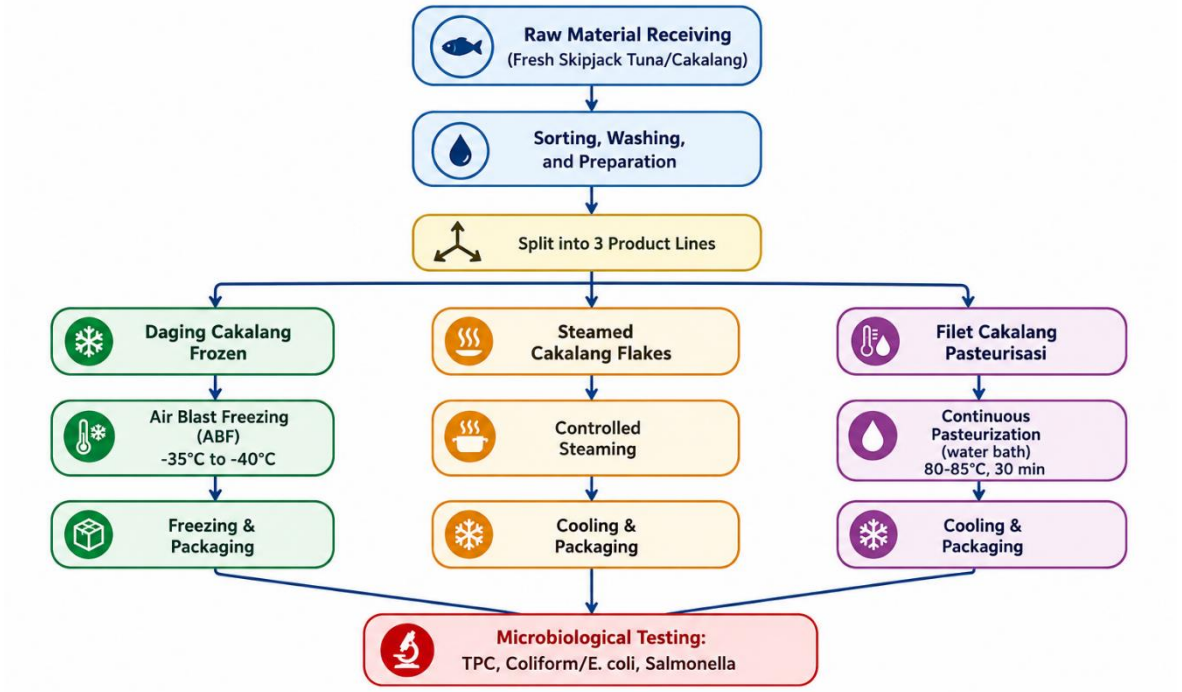


Figure 1. Process Flow Diagram and Microbiological Sampling Points for the Three Cakalang-Based Processed Products at PT AFA

2. Study Design and Sampling

This study used a quantitative descriptive, cross-sectional design. One sample unit per product was taken from a single production batch at a single sampling time. The replicates in this study were technical: duplicate plates for TPC and the three-tube design of the Most Probable Number (MPN) method for coliform. Because only one batch per product was tested, the results describe the microbiological status of the tested batch and do not allow statistical generalization to the whole product population.

TPC testing was performed on one sample unit (10 g) per product from a single production batch, with duplicate plates (technical replicates) at 10^{-2} and 10^{-3} dilution levels. The 10^{-1} dilution

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was not plated for TPC. Coliform testing used the three-tube MPN design at the 10^{-1}, 10^{-2}, and 10^{-3} dilution levels. *Salmonella* testing was performed on one 25 g sample unit of Filet Cakalang Pasteurisasi from the same batch and sampling time.

Salmonella testing was limited to Filet Cakalang Pasteurisasi for operational and risk-based reasons. First, this product is ready-to-eat and is marketed under a mandatory customer requirement of negative *Salmonella* per 25 g, whereas the frozen and steamed products are marketed as ready-to-cook with less stringent requirements. Second, it receives a milder thermal treatment than steaming and undergoes no further microbial inactivation after pasteurization, which makes it the most susceptible to post-process cross-contamination. Third, *Salmonella* analysis takes 5–7 days and requires relatively costly media; the company therefore tests the ready-to-eat product more frequently and the other products periodically.

3. Total Plate Count (TPC) Testing Procedure

TPC was determined by the pour plate method following the principle of SNI 2332.3:2015. A 10 g sample was homogenized in a stomacher with 90 mL of Butterfield's Phosphate Buffer (BFP) and serially diluted in BFP up to 10^{-3}. From the 10^{-2} and 10^{-3} dilutions, 1 mL was transferred into each of two sterile petri dishes. Molten Plate Count Agar (PCA) at approximately 45°C was added and mixed with a figure-eight motion, and the agar was allowed to solidify. Plates were incubated at 35°C for 48 hours, and colonies were counted with a colony counter.

TPC was calculated with the weighted formula of SNI 2332.3:2015 (below), where ΣC is the sum of colonies on all counted plates, n_1 and n_2 are the numbers of plates counted at the first and second dilutions, and d is the first dilution counted. Results are expressed in CFU/g. Because colony counts per plate were below the countable range of 25–250 colonies, the values are reported as estimated counts in accordance with ISO 7218 and SNI 2332.3:2015.

$$\text{TPC (CFU/g)} = \Sigma C \,/\, [(n_1 + 0.1\, n_2) \times d] \qquad (1)$$

4. Presumptive Coliform Testing Procedure

Coliform was determined by the three-tube MPN method following the principle of SNI 2332.1:2015. A 10 g sample was homogenized in 90 mL of BFP and serially diluted up to 10^{-3}. One milliliter from each of the 10^{-1}, 10^{-2}, and 10^{-3} dilutions was inoculated into each of three tubes of Lauryl Tryptose Broth (LTB) fitted with Durham tubes (nine tubes per product). Tubes were incubated at 35°C for 48 hours. A tube was recorded as presumptively positive when turbidity and gas formation in the Durham tube were observed.

Confirmatory tests (Brilliant Green Lactose Bile broth or EC broth) are performed only on presumptively positive tubes. Because all LTB tubes were negative, no confirmatory test was carried out and *E. coli* was not identified separately. The results are therefore reported as presumptive coliform. The 1 mL inocula from the 10^{-1}, 10^{-2}, and 10^{-3} dilutions correspond to 0.1, 0.01, and 0.001 g of sample per tube. In the standard three-tube MPN table, a combination of no positive tubes at all three levels is reported as <3 MPN/g.

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5. *Salmonella* Testing Procedure

Salmonella testing followed the principle of SNI 01-2332.2. A 25 g sample was homogenized in a stomacher, dissolved in Lactose Broth (LB), and incubated at 35°C for 24 hours for pre-enrichment. The pre-enriched culture was then streaked onto three selective-differential agars, namely Hektoen Enteric (HE) Agar, Xylose Lysine Deoxycholate (XLD) Agar, and Bismuth Sulfite Agar (BSA), and incubated at 35°C for 24 hours. Suspected *Salmonella* colonies would be confirmed by preliminary biochemical tests on Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants. A negative result was recorded when no colonies with typical *Salmonella* morphology were found on any of the three media.

6. Data Analysis

Results were analyzed descriptively and compared with the acceptance criteria used by the company. Frozen products were evaluated against SNI 4104:2015 for frozen tuna loin (TPC ≤5.0×10^5 CFU/g, *E. coli* <3 MPN/g, and *Salmonella* negative per 25 g). Steamed and pasteurized products were evaluated against BPOM Regulation No. 13/2019 as amended by BPOM Regulation No. 3/2026 on maximum microbial limits in processed food, with SNI 7968:2014 as the reference for cooked tuna products.

The company also applies stricter internal requirements: TPC ≤1.0×10^4 CFU/g, coliform <3 MPN/g, and *Salmonella* negative per 25 g. Results were compared with these internal requirements, which are stricter than the national TPC limit. Because coliform was examined only at the presumptive stage, comparison with the *E. coli* criterion is indirect: the absence of presumptive coliform suggests that *E. coli* was not detectable under the test conditions. Data are presented in tables and figures.

RESULTS

1. Total Plate Count (Tpc) Results

Estimated TPC values ranged from 1.4×10^2 to 3.2×10^2 CFU/g (Table 1, Figure 2). Steamed Cakalang Flakes had the highest estimated count and Filet Cakalang Pasteurisasi the lowest. Colony counts were 0–3 per plate, below the countable range of 25–250 colonies, so all values are estimated counts from one sample unit per product. All values were below the company requirement of ≤1.0×10^4 CFU/g.

Table 1. Colony Counts and Estimated Total Plate Count (TPC) of Three PT AFA Cakalang-Based Processed Products

Product	Colonies per plate at 10^{-2} (a, b)	Colonies per plate at 10^{-3} (a, b)	Estimated TPC (CFU/g)	Company requirement (CFU/g)
Daging Cakalang Frozen	2, 3	0, 0	2.3×10^2	≤ 1.0×10^4
Steamed Cakalang Flakes	3, 3	1, 0	3.2×10^2	≤ 1.0×10^4
Filet Cakalang Pasteurisasi	1, 2	0, 0	1.4×10^2	≤ 1.0×10^4

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Note: Colony counts are from duplicate plates (a, b) at each dilution. TPC was calculated as $\Sigma C / [(n_1 + 0.1 n_2) \times d]$ (SNI 2332.3:2015) and is an estimated count because colonies per plate were <25.

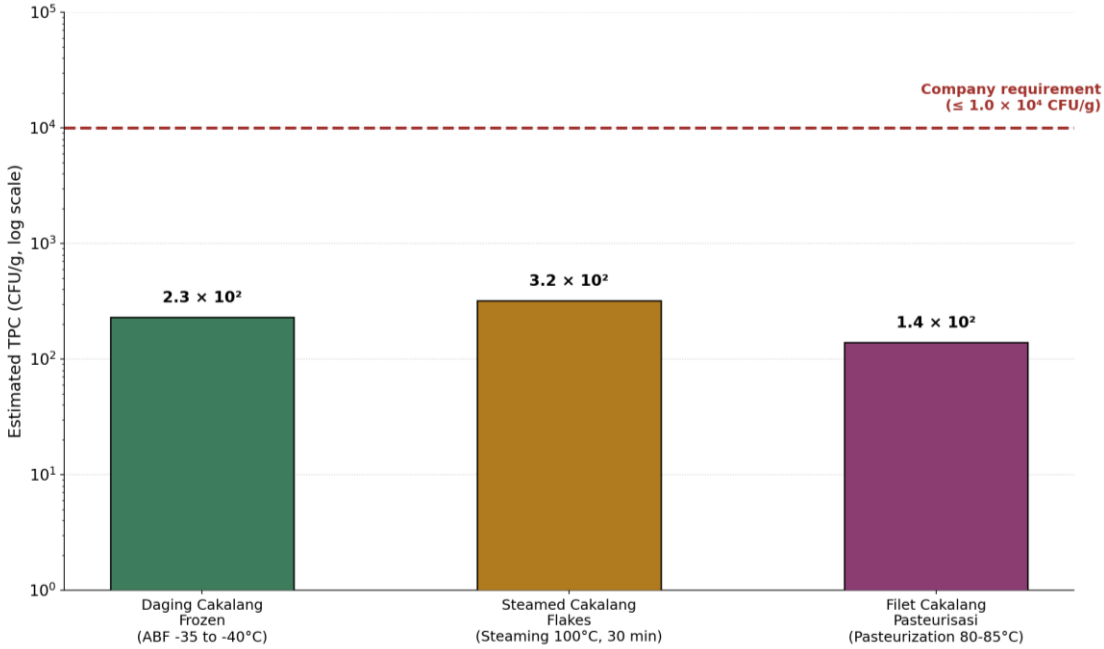


Figure 2. Estimated Total Plate Count (TPC, logarithmic scale) of the Three PT AFA Cakalang-Based Processed Products Compared with the Company Requirement.

2. Presumptive Coliform Results

No turbidity or gas formation was observed in any of the nine LTB tubes (three tubes at each of three dilution levels) of any product (Table 2). The MPN value was therefore <3 MPN/g for all three products.

Table 2. Presumptive Coliform Results (Three-Tube MPN Method) for Three PT AFA Cakalang-Based Processed Products

Product	Dilution levels tested	Result in LTB tubes	MPN value	Requirement	Status
Daging Cakalang Frozen	10^{-1}, 10^{-2}, 10^{-3} (3 tubes/level)	Negative in all tubes	< 3 MPN/g	Max < 3 MPN/g	Compliant
Steamed Cakalang Flakes	10^{-1}, 10^{-2}, 10^{-3} (3 tubes/level)	Negative in all tubes	< 3 MPN/g	Max < 3 MPN/g	Compliant
Filet Cakalang Pasteurisasi	10^{-1}, 10^{-2}, 10^{-3} (3 tubes/level)	Negative in all tubes	< 3 MPN/g	Max < 3 MPN/g	Compliant

3. *Salmonella* Results

Salmonella was not detected in the 25 g sample of Filet Cakalang Pasteurisasi on any of the three selective-differential media (HE, XLD, and BSA; Table 3). No colonies with typical *Salmonella* morphology were found, so biochemical confirmation was not required.

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Table 3. *Salmonella* Test Results for the Filet Cakalang Pasteurisasi Product at PT AFA

Product	HE Agar	XLD Agar	BSA	Result	Requirement	Status
Filet Cakalang Pasteurisasi	Negative	Negative	Negative	Negative	Negative/25 g	Compliant

DISCUSSION

All three products met the requirements for the parameters tested. TPC was well below the company limit, presumptive coliform was not detected in any product, and *Salmonella* was not detected in the pasteurized fillet. The estimated TPC values were about 1.5 to 1.9 $\log_{10}$ units below the company limit of ≤1.0×10^4 CFU/g, which is itself stricter than the national TPC limit, so the products can be described as having low aerobic counts relative to their requirements. Since the counts are estimates from 0–3 colonies per plate, they indicate a low microbial load rather than a precise quantification.

The differences in TPC among the three products are small and are based on estimated counts from a single batch. They should therefore not be ranked or attributed to a specific technology. The low counts are consistent with the known effects of each process. Freezing is mainly bacteriostatic and does not by itself eliminate microorganisms (Duanmu et al., 2024), whereas heat treatments such as steaming and pasteurization reduce vegetative cells (Chew et al., 2024; FDA, 2021; Wang et al., 2020). Chakma et al. (2020), who studied skipjack tuna loins, reported that frozen storage at very low temperatures preserved microbiological quality. This study did not measure microbial counts before and after each process; the contribution of individual steps was therefore not quantified, and raw-material hygiene and cold chain control are likely to influence the final counts as well (Siregar et al., 2026).

Presumptive coliform was not detected in any product (<3 MPN/g), which suggests a low level of fecal contamination and sanitation failure in the tested samples. A result below the detection limit means that the organisms were not detected under the test conditions; it does not prove their absence throughout the production process. Coliforms reflect process-water quality, cleanliness of product-contact surfaces, and worker hygiene (Roy et al., 2024). Because no confirmatory test was performed, *E. coli* was not identified separately.

Salmonella was not detected in Filet Cakalang Pasteurisasi, a ready-to-eat product that is not cooked again by the consumer and therefore carries a higher risk if post-process contamination occurs (Roy et al., 2024). Contamination incidents in ready-to-eat fishery products are generally linked to failures in time-temperature control or post-processing cross-contamination. The negative result is consistent with the principle that pasteurization at 80–85°C for 30 minutes inactivates vegetative *Salmonella* cells (FDA, 2021). A single 25 g sample from one batch, however, cannot exclude low-level contamination in other batches.

The findings are in line with studies of processed products from formal quality-control systems. Ndobe et al. (2023) reported negative *Salmonella* results in de-boned milkfish with good hygienic handling, and Siregar et al. (2026) reported that frozen tuna slices from an export-oriented industry met the national microbiological standards. Fitria et al. (2024) emphasized the role of critical control points in raw-material receiving, packaging, and frozen storage. In contrast, higher

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contamination, in some cases above national limits, was reported in informally handled products, including fresh tuna at a landing base (Hadji et al., 2025), smoked fish (Anihouvi et al., 2019; Bardoe et al., 2023; Khairunnisa et al., 2023), dried fish (Birie et al., 2024), and salted fish (Jannah et al., 2025). Serrano Heredia et al. (2023) and Indio et al. (2024) likewise showed that the cumulative effect of sequential control points, rather than a single step, determines final microbiological quality. These studies differ from the present one in species, product type, and methods, so the comparison is indicative only.

These findings suggest that combining freezing and thermal control points (ABF, steaming, and pasteurization) with sanitation and hygiene practices under GMP and HACCP can keep indicator organisms low in a medium-scale facility, without the need for costlier non-thermal technologies such as high-pressure processing (Siddiqui et al., 2024). Thermal processing may also reduce the load of antibiotic-resistant bacteria in seafood chains (Assogba et al., 2025; Odeyemi et al., 2023). This is a theoretical implication only, because antimicrobial resistance was not tested in this study and it requires further investigation.

This study has several limitations. First, it was based on a single sampling event per product; the results describe the microbiological status of the tested batch and do not capture temporal or batch-to-batch variability. Second, TPC values are estimated counts because colonies per plate were below the countable range of 25–250. Third, *Salmonella* was tested only in Filet Cakalang Pasteurisasi, so generalization of *Salmonella* risk to the frozen and steamed products requires further investigation. Fourth, coliform was determined only at the presumptive stage, and other pathogens such as *Staphylococcus aureus* and *Vibrio parahaemolyticus* were not examined. Finally, the products differ in type and processing history, so the study cannot test the effect of a specific technology.

Several recommendations follow. First, critical process parameters, namely ABF temperature (−35°C to −40°C), steaming temperature and time with product core temperature, and pasteurization temperature and time, should be documented routinely as part of CCP monitoring within a HACCP framework (Fitria et al., 2024). Second, regular monitoring of process-water quality and product-contact surfaces through SSOP should be maintained even when current results are favorable (Roy et al., 2024). Third, periodic *Salmonella* testing should be extended to the frozen and steamed lines following a risk-based approach (Odeyemi et al., 2023; Siddiqui et al., 2024). Future studies should use several batches and a longitudinal design, confirm *E. coli*, and include additional microbiological parameters (Siregar et al., 2026).

CONCLUSIONS

Under the conditions tested, the three cakalang-based products from PT AFA met the specified requirements for the parameters examined. Estimated TPC values were below the company limit of $\leq 1.0 \times 10^4$ CFU/g, presumptive coliform was <3 MPN/g in all products, and *Salmonella* was not detected in 25 g of Filet Cakalang Pasteurisasi, the only product tested for this pathogen.

These results suggest that combining freezing or thermal control points with sanitation practices is associated with low indicator-organism levels in this medium-scale facility. Because only one batch per product, presumptive coliform, and *Salmonella* in one product were examined, the

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results do not constitute a comprehensive declaration of food safety. Studies with multiple batches, confirmed *E. coli*, *Salmonella* testing in all product lines, and additional pathogens are recommended.

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