Total plate count and *Salmonella* spp. in de-boned milkfish (*Chanos chanos*) in Palu City, Indonesia

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**ABSTRACT**

High total plate count (TPC) and the presence of *Salmonella* spp. in food products can cause health problems for consumers. De-boned milkfish products are popular with consumers in Palu City, Central Sulawesi, Indonesia, but there is a lack of data on their safety. Therefore, this study aimed to investigate TPC levels and detect contamination by *Salmonella* spp. in these products. Samples of fresh and processed milkfish were collected from two de-boned milkfish processing sites: the Technical Implementation Unit for the Application of Fishery Product Quality Control (TIU-AQFP) and the Melona Micro, Small and Medium Enterprise (MSME) Group in Palu City. Microbiological assays included counting the number of bacterial colonies (TPC) as well as the isolation and identification of *Salmonella* spp. through biochemical tests. The study applied a completely randomized factorial design with three replicates per site and per product (12 experimental units). De-boning had a significant (P<0.05) effect on TPC (1.26×10^3 to 2.20×10^3 CFU/g for de-boned milkfish compared to 4.28×10^3 to 2.94×10^3 CFU/g for fresh unprocessed milkfish). However, the types of bacteria identified in fresh and de-boned milkfish, including *Klebsiella*, *Enterobacter* and *Citrobacter*, were present at non-pathogenic levels. No *Salmonella* spp. contamination was found in the test samples. These results indicate that de-boned milkfish products from the TIU-AQFP and Melona MSME Group in Palu City are safe and suitable for human consumption.

**Introduction**

Fisheries resources play an important role in food security and meeting the nutritional needs of people around the world. Milkfish (*Chanos chanos* Forsskål 1775), called *ikan bandeng* in Indonesian, is a popular fisheries commodity because it is tasty, has a high nutritional value and is generally affordable for most people. Milkfish is classified as a high protein and low-fat fish with a high vitamin and mineral content (*Aji et al., 2022*). The Omega-3 (ω3) content is higher than that of other fish such as sardines, mackerel, salmon, and tuna (*Rofiqi et al., 2018*), making milkfish especially good for infants and children, as it can help nervous system development (*Diana, 2013*). The fisheries statistics for Central Sulawesi Province (BPS, 2023) show that in 2021 milkfish production exceeded 14,000 metric tons, ranking third (after seaweed and shrimp) among aquaculture commodities in terms of both volume and value.

Milkfish can be processed to produce many added-value milkfish products; however, processing skills are needed to realise this economic opportunity. Milkfish can be processed with various spices and other ingredients. In Indonesia, popular milkfish products include boneless (de-boned) milkfish (*bandeng tanpa duri* or *bandeng cabut duri* in Indonesian), smoked milkfish, dumpling-like milkfish snacks wrapped in banana leaves called *otak-otak*, crispy milkfish, and pressure-cooked milkfish called *bandeng presto* (*Abriana et al., 2021*).

De-boned milkfish is a relatively recent product that is increasingly popular; it is a semi-prepared product, in which the bones have been removed from fresh (raw, gutted but otherwise whole)
milkfish. The advantage of de-boned milkfish is that the nutritional content of the fresh fish is maintained, but it can easily be consumed without the need to individually remove the many fine bones that can make eating milkfish time-consuming and challenging (Hasnidar and Tamsil, 2019).

In general, fish can be considered a highly perishable food, vulnerable to degradation due to both biochemical processes and microbiological activity, even with good handling (FAO, 2020; Hua et al., 2019). It has long been recognised that additional handling and processing tends to increase total bacterial counts in raw fish products (Gillespie and Macrae, 1975), with a risk of contamination during each handling and processing stage (Novoslavskij et al., 2016). The high water content promotes enzymatic biochemical reactions, while the protein content is intrinsically perishable, and offers a substrate for microbial growth. However, proper handling and processing can inhibit decomposition processes, keeping the fish safe to eat for longer and prolonging the product shelf life.

Pathogens of the genus *Salmonella* are of particular concern, as they can readily be transmitted to human hosts from many sources (Jenikova et al., 2000), including from pets, the faeces of infected people or animals, and contaminated water or food; they can survive for weeks outside of a living host, and are not killed or inactivated by freezing (Kananub et al., 2020). Pathogenic *Salmonella* species that can infect humans include *Salmonella typhi*, *S. typhimurium* and *S. enteritidis* (Strugnell et al., 2014).

It is vital that fisheries produce should be free from contamination with *Salmonella* spp., to avoid infection with salmonellosis, a disease with symptoms including acute gastroenteritis, enteritic fever, faecal infection, and sequelae (Wen et al., 2017). The National Standard for de-boned milkfish microbiological safety (SNI 7316.1) includes several important limits including maximum Total Plate Count (TPC) of 5.0 x 10³ colonies/g and a negative *Salmonella* spp. test result on a 25 g sample. The TPC and presence/absence of *Salmonella* spp. are important in order to protect consumers for potential health risks. However, there is a lack of studies on the TPC and *Salmonella* spp. contamination of de-boned milkfish to evaluate whether, in general, these products are safe for human consumption or not.

As the capital of Central Sulawesi Province, Indonesia, Palu City is a hub for marketing and processing in the province (Khairil, 2018; Aggarwal, 2022), including fisheries and aquaculture produce for local consumption and to supply wider domestic and international markets. De-boned milkfish has gained in popularity; however to date there are no data on the food safety aspects of this growing processing industry, which involves a number of micro, small and medium enterprises.

Therefore, the purpose of this research was to evaluate the food safety of de-boned milkfish in Palu City, based on the Total Plate Count (TPC) and presence/absence of *Salmonella* spp., specifically the de-boned milkfish produced by two production units. These were the Technical Implementation Unit for the Application of Fishery Product Quality Control (TIU-AQFP) and the Melona Micro, Small and Medium Enterprise (MSME) Group.

**Materials and Methods**

**Study site, time-frame and sample collection**

This research was performed in Palu over three months from February to April 2021. Fresh and de-boned milkfish samples were collected from two sites: the Melona Micro, Small and Medium Enterprise (MSME) Group (henceforth referred to as the Melona MSME) and the Technical Implementation Unit for the Application of Fisheries Product Quality Control (henceforth referred to by the acronym TIU-AQFP). These sites were chosen as representative of the range of units producing de-boned milkfish in Palu. While the TIU-AQFP is a government-run unit that should represent best practices, the Melona MSME is a community group that should represent practices within the small-scale private sector. Total Plate Count (TPC), testing for *Salmonella* spp., isolation and identification were performed at the Palu Fish Quarantine, Quality Control and Safety of Fishery Products Station Laboratory. Samples were collected at random at three different times from each of the two study sites. Each sample comprised one de-boned milkfish from the current production run and one fresh milkfish awaiting processing. The TIU-AQFP obtained milkfish from brackish-water aquaculture ponds in Parigi, Parigi Moutong Regency, while the Melona MSME purchased milkfish from the Masomba Market in Palu City.

**Materials used**

Materials used in the laboratory included the following: distilled water, plate count agar (PCA), lactose broth, Butterfield’s phosphate buffered solution, 70 % ethanol, carbohydrate broth (lactose, sucrose, dextrose), Hektoen Enteric Agar (HEA), Xylose Lysine Deoxycholate agar (XLDA), Bismuth Sulphite Agar (BSA), Lysine Iron Agar (LIA), Triple Sugar Iron Agar (TSIA), Voges-Proskauer (MR-VP) broth, glucose-phosphate medium, methyl red reagent, tryptone broth, Simmons citrate medium,
Kovac’s reagent, tetrathionate broth, and selenite cystine broth.

**Microbiological testing procedures**

The microbiological tests performed included Total Plate Count (TPC) following the Indonesian National Standard SNI 01-2332.3-2015 (Microbiological Test Methods Part 3: Determination of Total Plate Count (TPC) in Fisheries Products), total Salmonella spp. assay, and Salmonella spp. detection. Biochemical tests were performed following the Indonesian National Standard SNI 01-2332.2-2006 (Microbiological Test Methods Part 2: Determination of Salmonella in Fishery Products).

**Preparation of media, reagents and samples**

All equipment used during the microbiological analyses was sterilized in an autoclave at 15 psi and 121°C for 15 minutes. Agar media was prepared by placing 3.68 g plate count agar (PCA) in an Erlenmeyer glass with 160 mL of distilled water. The mixture was homogenized, heated to boiling point and sterilized in the autoclave for 15 minutes at 121°C and 15 PSI. Butterfield's phosphate buffered solution stock was prepared by homogenizing 34 g KH₂PO₄ with 500 mL of distilled water and adjusting the pH to 7.2 with 1 N NaOH. The volume of the solution was made up to 1 L with distilled water. The solution was sterilized for 15 minutes at 121°C and then stored in a refrigerator. For use in the assays, 10 mL of stock solution was made up to 1 L with distilled water and sterilized for 15 min at 121°C.

Each milkfish sample weighed between 1 and 4.5 kg. The flesh of each sampled fish was cut into pieces weighing 25 g using aseptic techniques. One 25 g piece was selected at random, placed in a sterile stomacher bag with 225 mL Butterfield's phosphate buffered solution, and homogenised for 2 minutes. This homogenised solution had a dilution rate of 10⁻¹. A sterile pipette was used to add 10 mL of the homogenised product to 90 mL of Butterfield's phosphate buffered solution to obtain a 10⁻³ dilution rate. Homogenised solutions at 10⁻³, 10⁻⁴, 10⁻⁵ and so on were prepared in the same manner, shaking the mixture at least 25 times after each dilution.

**TPC assay**

For each of the diluted sample solutions, 1 mL was transferred to a sterile Petri dish using a pipette with two replicates for each dilution level. PCA was added (12 to 15 mL) to each Petri dish, and the dish was rotated to-and-fro and side-to-side to ensure the diluted sample and PCA were well mixed. The Petri dishes were incubated upside-down at 35°C±1°C in an incubator for 48 ± 2 hours to promote the growth of mesophilic bacteria. Only colonies in Petri dishes with 25-250 colonies and no spreading were counted. The dilution level and the total colony count were recorded. The TPC was calculated following SNI 01-2332.3-2015 using the equation:

$$N = \frac{\sum C}{[(1 \times n1) + (0.1 \times n2)] \times (d)}$$

where:

- N is the number of colonies (TPC), in colonies per mL or colonies per g;
- ΣC is the number of colonies in all counted Petri dishes;
- n1 is the number of petri dishes with the first dilution level counted;
- n2 is the number of petri dishes with the second dilution level counted;
- d is the first dilution level used

**Total Salmonella assay**

The total Salmonella assay followed similar procedures to the TPC. The difference was that Bismuth Sulphite Agar (BSA) was used instead of PCA, and was prepared by placing 6.4 g BSA in an Erlenmeyer then adding 160 mL of distilled water. The mixture was heated to boiling point, then sterilised and autoclaved for 15 min at 121°C and 15 psi. The colonies growing on the inoculated and incubated BSA with traits specific to Salmonella spp. (grey, brown or black colour, sometimes with a metallic sheen) were counted to give the total Salmonella count in TVC/g following the Indonesian National Standard (SNI 01-2332.2-2006).

**Salmonella isolation**

The first stage in this process was enrichment. A 25 g piece from each milkfish sample was mixed with an enrichment medium in a 1:9 ratio. Each 25 g sample was placed in a sterile container with 225 mL of lactose broth and homogenised for 2 min. Sterile (aseptic) conditions were maintained while transferring the solution to a sterile container which was then sealed hermetically and incubated at room temperature for 60 min. The mixture was then shaken gently, and the pH checked; if necessary, the pH was adjusted to 6.8 ± 0.2. The mixture was then shaken thoroughly, and the lid loosened as necessary to release pressure before incubation for 24 ± 2 h at 35° ± 1°C. The lid was tightened before shaking vigorously again. A pipette was used to place 1 mL of the mixture into 10 mL Selenite Cystine Broth (SCB) and 10 mL Tetrathionate Broth (TTB). The TTB and SCB mixtures were then incubated for 24 ± 2 h at 35° ± 1°C.
The isolation procedure began by vortexing the tube with the TTB enriched mix. The three incubation media (HEA, XLDA and BSA) which had been prepared in Petri dishes the day before were then scored with the mix using a 3mm loop. The inoculated BSA, HEA and XLDA Petri dishes were incubated for 24 h at 35°C ± 1°C. Each dish was then observed to look for signs of *Salmonella* colonies, with the following typical traits: a) HEA: bluish-green to blue colonies with or without a central black spot, or mostly black; b) XLDA: pink colonies with or without a central black spot, or mostly black; c) BSA: brown, grey or black colonies, sometimes with a metallic sheen. Colonies can look like flat "rabbit's eyes", black or with a black margin and metallic sheen (Atlas, 2010).

The centre of each colony was carefully removed using a sterile inoculation needle to inoculate triple sugar iron agar (TSIA) by scoring the surface of the medium on the slant and stabbing the medium vertically with the needle. The same needle and medium were then used to inoculate lysine iron agar (LIA) media by first stabbing the medium vertically then scoring the surface on the slant. The media with colonies removed to provide material for the inoculation were incubated at 5°C – 8°C. The TSIA and LIA media were incubated for 24 ± 2 h at 35°C ± 1°C, covered with a loosely closed lid to avoid excessive build-up of the gas hydrogen sulphide (H₂S). On the TSI media, typical *Salmonella* spp. cultures will cause an alkaline (red) reaction on the slanted scored and acid (yellow) reaction on the vertical stab holes, with or without H₂S (blackish colouration) on the agar. On the LIA media, typical *Salmonella* cultures will cause an alkaline (purple) reaction across the whole culture dish. Truly yellow colour in the stab holes is counted as a negative culture, although discoloration of the stab holes is not sufficient to declare the culture negative, and H₂S is usually produced by *Salmonella* spp. cultures on LIA media.

**Salmonella** identification

*Salmonella* spp. colonies isolated can be identified using biochemical reactions and serological assays following Barrow and Feltham (1993), Holt *et al.* (1994), and the relevant Indonesian national Standard (SN1 01-2332.2-2006). The assays used are briefly described in Table 1. Tests 8-10 were only performed if all earlier tests were negative or inconclusive. Where possible, isolates testing negative as *Salmonella* spp. were identified to genus level based on diagnostic traits (Barrow and Feltham, 1993).

### Table 1. *Salmonella* spp. identification assays on enriched/isolated samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Assay Name/type</th>
<th>Isolate Type</th>
<th>Volume</th>
<th>Medium</th>
<th>Incubation Hours</th>
<th>Incubation °C</th>
<th>Positive signs (+)</th>
<th>Negative signs (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urease</td>
<td>TSI</td>
<td>1 ose</td>
<td>Urea broth</td>
<td>24±2</td>
<td>35±1</td>
<td>Gas in Durham tube, acid pH (yellow)</td>
<td>No gas, red (phenol red) or purple</td>
</tr>
<tr>
<td>2</td>
<td>Phenol red/</td>
<td>TSI</td>
<td>1 ose</td>
<td>Purple broth base</td>
<td>48±2</td>
<td>35±1</td>
<td>Produces TB isolate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>dulcitol</td>
<td></td>
<td></td>
<td>+ 0.5% dulcitol</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Tryptone</td>
<td>TSI</td>
<td>1 ose</td>
<td>Tryptone broth</td>
<td>24</td>
<td>35±1</td>
<td>+ Growth (cloudiness)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>KCN</td>
<td>TB</td>
<td>1 ose</td>
<td>KCN broth</td>
<td>48±2</td>
<td>35±1</td>
<td>+ change to blue colour</td>
<td>- change to green colour</td>
</tr>
<tr>
<td>5</td>
<td>Malonate</td>
<td>TB</td>
<td>1 ose</td>
<td>Malonate broth</td>
<td>48±2</td>
<td>35±1</td>
<td>- change to green colour</td>
<td>+ red ring on the surfaces (orange or pink ring inconclusive)</td>
</tr>
<tr>
<td>6</td>
<td>Indole</td>
<td>TB</td>
<td>5 mL</td>
<td>Kovac’s reagent (0.2-0.3 mL)</td>
<td>None</td>
<td>None</td>
<td>+ lumps form in the culture solution and not in the control</td>
<td>- no lumps in the culture solution or the control</td>
</tr>
<tr>
<td>7</td>
<td>Polyvalent</td>
<td>TSI</td>
<td>1 ose</td>
<td>Place on a microscope slide, emulsify with a drop of 0.85% sterile saline solution; place a drop of <em>Salmonella</em> polyvalent somatic (O) antiserum beside the emulsion, mix and spread, observe over a dark background</td>
<td>None</td>
<td>None</td>
<td>+ lumps form in the culture solution and not in the control</td>
<td>- no lumps in the culture solution or the control</td>
</tr>
<tr>
<td></td>
<td>Somatic Serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Methyl Red</td>
<td>TSI</td>
<td>1 ose</td>
<td>MR-VP broth (1 mL)</td>
<td>48±2</td>
<td>35±1</td>
<td>+ possible colours change to red (pale eosin to ruby)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Methyl Red (MR)</td>
<td>TSI (slanted scores)</td>
<td>1 ose</td>
<td>MR-VP broth then add 5-6 drops MR</td>
<td>96</td>
<td>35±1</td>
<td>+ diffused red colour throughout mediumb</td>
<td>- yellow colour</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td>- green to blue colour</td>
</tr>
<tr>
<td>10</td>
<td>Simmons</td>
<td>TSI (slanted scores)</td>
<td>1 ose</td>
<td>Score and stab to inoculate the agar</td>
<td>96±2</td>
<td>35±1</td>
<td>- very little growth and no colour change</td>
<td></td>
</tr>
<tr>
<td></td>
<td>citrate agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Then add 0.6 mL alpha naphthol and shake, add 0.2 mL 40% KOH solution and shake again, then add a small quantity of creatine crystals and shake; observe after 4 hours

b Cultures that are positive for KCN and VP as well as negative for MR are diagnosed as not being *Salmonella*.

### Data analysis

The TPC data were analysed as factorial fully randomised data with two levels, site (Melona MSME...
and the TIU-AQFP) and treatment (de-boned milkfish and fresh un-processed milkfish), after logarithmic transformation. The two-factor (site and product) analysis of variance (ANOVA) with replication was conducted in the Minitab 16 software package using site factor codes L1 (Melona MSME) and L2 (TIU-AQFP) and product factor codes P1 (fresh milkfish) and P2 (de-boned milkfish). Total *Salmonella* spp. count and other assay data were tabulated and analysed descriptively.

**Results**

**Total Plate Count (TPC)**

The mean total plate count (TPC) data per sample (Table 2) ranged from $1.264 \times 10^3$ to $2.200 \times 10^4$ CFU/g for the unprocessed milkfish and $2.045 \times 10^3$ to $2.940 \times 10^4$ CFU/g for the de-boned milkfish. These data show that, in general, the de-boned milkfish TPC was higher than that of the raw material (unprocessed fish).

**Table 2.** Mean total plate count (TPC) of fresh and de-boned milkfish from two sites.

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Sample Type</th>
<th>Code</th>
<th>Mean TPC (CFU/g)</th>
<th>Site No.</th>
<th>Sample Type</th>
<th>Code</th>
<th>Mean TPC (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fresh milkfish</td>
<td>B1</td>
<td>1.995×10³</td>
<td>2</td>
<td>Fresh milkfish</td>
<td>B7</td>
<td>2.200×10⁴</td>
</tr>
<tr>
<td>1</td>
<td>De-boned milkfish</td>
<td>B2</td>
<td>4.279×10³</td>
<td>2</td>
<td>De-boned milkfish</td>
<td>B8</td>
<td>1.054×10⁴</td>
</tr>
<tr>
<td>1</td>
<td>Fresh milkfish</td>
<td>B3</td>
<td>2.086×10³</td>
<td>2</td>
<td>Fresh milkfish</td>
<td>B9</td>
<td>1.264×10⁴</td>
</tr>
<tr>
<td>1</td>
<td>De-boned milkfish</td>
<td>B4</td>
<td>2.313×10⁴</td>
<td>2</td>
<td>De-boned milkfish</td>
<td>B10</td>
<td>2.045×10⁵</td>
</tr>
<tr>
<td>1</td>
<td>Fresh milkfish</td>
<td>B5</td>
<td>2.082×10³</td>
<td>2</td>
<td>Fresh milkfish</td>
<td>B11</td>
<td>1.305×10⁴</td>
</tr>
<tr>
<td>1</td>
<td>De-boned milkfish</td>
<td>B6</td>
<td>2.168×10⁴</td>
<td>2</td>
<td>De-boned milkfish</td>
<td>B12</td>
<td>2.940×10⁴</td>
</tr>
</tbody>
</table>

**Fully randomised factorial TPC analysis**

The two-way ANOVA factorial analysis of the fully-randomised design (Table 3) shows that, although in Table 2 the TPC values were higher at the Melona MSME than at the TIU-AQFP, the effect of site on TPC was not significant \( (P>0.05) \). However, there was a significant effect of product \( (P<0.05) \) on TPC, with higher TPC in de-boned milkfish compared to fresh unprocessed milkfish. There was no significant interaction between the two factors \( (P>0.05) \).

**Total Salmonella Assay**

No bacterial colonies grew on the bismuth sulphite agar (BSA) media. This indicates that both fresh (unprocessed) and de-boned milkfish sampled had a total *Salmonella* spp. count of 0 CFU/g.

**Table 3.** Two-factor ANOVA results, mean and standard deviation (SD) values of TPC for factorial treatments and aggregated factors.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>0.75457</td>
<td>0.4103</td>
<td>5.317655</td>
</tr>
<tr>
<td>Product</td>
<td>14.09143</td>
<td>0.0056</td>
<td>5.317655</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.00385</td>
<td>0.9520</td>
<td>5.317655</td>
</tr>
</tbody>
</table>

* Significant at the 95% confidence level

![Figure 1](image-url) **Figure 1.** Bacterial colonies growing on BSA. A. fresh (unprocessed) milkfish from the Melona MSME (no *Salmonella* spp. detected); B. de-boned milkfish from the TIU-AQFP (no *Salmonella* spp. detected); C. Positive control.

![Figure 2](image-url) **Figure 2.** Bacterial colonies growing on HEA media. A. fresh (unprocessed) milkfish from the Melona MSME (no *Salmonella* spp. detected); B. de-boned milkfish from the TIU-AQFP (no *Salmonella* spp. detected); C. Positive control.
processing can also influence the composition of the bacterial communities in fresh fish products, either promoting or inhibiting the growth, and therefore the absolute and proportional abundance, of pathogenic or spoilage causing bacteria (Gillespie and Macrae, 1975).

The TPC values obtained in this study were not a cause for concern with regards to public health, because all samples were within food safety limits, and not likely to cause health problems in consumers. The maximum allowable TPC according to the relevant National Standard (SNI 7316.1:2009) is $5.0 \times 10^5$ CFU/g, considerably higher (by at least an order of magnitude) than the TPC of any samples in this study. Furthermore, the lack of a significant between-site effect means that both production centres sampled appear to be producing de-boned milkfish fit for human consumption, in terms of the total bacterial load.

Nonetheless, the increase in TPC after compared to before processing indicates a need for vigilance with regards to hygiene, and to carefully monitor and seek opportunities to optimise sanitary practices at both sites. Although not statistically significant, the TPC tended to be higher at the community enterprise group Melona MSME than at the TIU-AQFP. This difference could indicate a less stringent adherence to best practices, or a less optimal processing environment.

**Total Salmonella spp. and Salmonella spp. identification**

The relevant National Standard (SNI 7316.1:2009) is a negative result for a 25g sample. The negative results of all total Salmonella assays (no typical Salmonella spp. colonies growing on the BSA cultures) indicate that Salmonella spp. contamination was absent or undetectably low on all samples. This result indicates that the de-boned milkfish produced at both sites met the food safety standard for Salmonella spp. This was confirmed by the biochemical assays. Typical Salmonella spp. colonies cultured on XLD media are pink, with or without a shiny black centre (Rabins et al., 2018), because Salmonella can ferment xylose, decarboxylate lysine, and produce hydrogen sulphide from natrium thiosulfate. Fermentation can alter the pH of the XLD media making it more basic, resulting in the pink coloration, while the black colour is caused by the hydrogen sulphide (Abd et al., 2018). Selective isolates produced on XLD media produced single colonies that were almost all yellow. This shows that the bacteria growing on the media were, unlike Salmonella spp., unable to ferment xylose.
A positive TSIA assay would be marked by yellow colouration of the stab holes and red colouration on the slanted score marks, with or without H₂S gas. The yellow colouration is caused by the ability of Salmonella spp. to ferment glucose in order to grow and reproduce, while the red colour arises from the inability of Salmonella spp. to ferment lactose and sucrose. The release of H₂S gas by bacteria indicates the decomposition of sulphurous amino-acids which also results in a release of the black-coloured compound ferrous sulphate (FeS) (Lay, 1994). However, these signs were not observed in this study.

A positive lysine iron agar (LIA) assay is marked by a stable purple colouration or no colour change, with or without the release of H₂S. Salmonella spp. react positively with lysine, and the LIA media also contains natrium thiosulfate, a substrate for producing H₂S and the black-coloured FeS (Haryani., 2012). Out of the 72 isolates, 37 isolates had this trait typical of Salmonella spp., while the other 35 isolates changed to a yellow colour.

The indole assay for Salmonella spp. is negative if a yellow ring is formed on the surface of the media. This occurs because Salmonella spp. cannot produce indole using tryptophan as a source of carbon (Sridevi and Mallaiah, 2007). In this assay, 44 isolates exhibited a positive reaction with a violet or purple colouration of the media surface.

The urease assay produced 66 isolates with positive reactions and only six with negative reactions. Salmonella spp. have a negative reaction to the urease assay, with no colour change or a stable yellow colour, because Salmonella spp. do not produce the urease enzyme that can break the carbon and nitrogen bonds in urea to form ammonia which changes the pH of the media (Loharch and Berlicki, 2022).

Salmonella spp. react positively to the Simmons citrate assay, with a colour change to blue. This change is caused by the use of citrate as a source of carbon for bacterial growth and results in an alkaline condition which changes the media colour to blue (Sari and Apridamayanti, 2015). In this study, 69 isolates were positive and only three were negative for this assay. Salmonella spp. react negatively to the VP test because of its inability to ferment the 2,3-butandiol in MR-VP media (Puspadewi et al., 2017). In this study, 47 isolates had a negative reaction. In the methyl red assay, Salmonella spp. react positively, causing a spreading red stain on the MR-VP media. There were 23 isolates displaying a positive reaction, meaning that the bacteria were able to ferment the acids produced from the fermentation of a medium containing glucose.

The combined results of the biochemical assays did not identify any of the 72 isolates from fresh (unprocessed) and de-boned milkfish as Salmonella spp. This demonstrates that milkfish can be de-boned by hand using tweezers without causing Salmonella spp. contamination, with the caveat that hygiene protocols are observed for hands and tools, and only clean water is used. The bacterial colonies other than Salmonella spp. growing on the three selective media (BSA, XLDA and HEA) probably grew because they were formed by bacteria well-adapted to these media. However, these bacteria are not considered pathogenic at the levels observed.

Conclusion
From the results of this study, it can be concluded that processing fresh fish to produce de-boned milkfish has a significant effect on total plate count (TPC), but despite the increased TPC the de-boned milkfish produced at the two study sites, Melona MSME and the TIU-AQFP in Palu City remained within food quality and safety guidelines and fit to eat. No Salmonella spp. contamination was found in fresh or de-boned milkfish at either site, although three other bacterial genera were identified (Klebiella, Enterobacter and Citrobacter) at non-pathogenic levels.

The increase in TPC between the raw material and de-boned milkfish product highlights the need for constant vigilance with respect to sanitation and hygiene protocols during the processing, as well as before and after. This includes the supply chains and the sales chain as well as handling by the end consumer. Based on the results, the de-boned milkfish from the two study sites can be recommended as a safe, practical and nutritious food, with the proviso that they are handled and cooked properly within a suitable timeframe. Further research is recommended to test for additional pathogens as well as Salmonella spp. in processed milkfish products, ideally combining classic and molecular biology methods.

References


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Strugnell, A., M. Terentjeva, I. Eizenberga, O. Valciņa, V. Loharch
Khairil Hua Haryani, Gillespie FAO
Ndobe et al
Kartika Jurnal Kesehatan Khatulistiwa, (2023). Dissemination for Community Development), Community, Gresik Regency
in Puducherry, India
public health significance of salmonella from major meat sources
Manuntung, Staphylococcus aureus
15.
Bartkevičs, and A. Bērziņš. 2016. Major foodborne pathogens in
ureases inhibitors
Persada, pulling investors interest in commercial raw pet foods Vet
Contamination factors associated with surviving bacteria in Thai
Microbiology,
Salmonella sources in aquaculture diets
2019
C. Wilkins, Jurnal Aplikasi Teknik dan Pengabdian Masyarakat,
oleh isolat
Applied Bacteriology,
Masyarakat,
Province in Figures
for the Identification of Medical Bacteria
44
dan Pengabdian Masyarakat, IRPI Publisher, Pekanbaru, pp. 444-
448.
Atlas, R. M. 2010. Handbook of Microbiological Media. CRC Press,
Boca Raton.
for the Identification of Medical Bacteria. Cambridge University
BPS. 2023. Provinsi Sulawesi Tengah dalam Angka - Sulawesi Tengah
Province in Figures. Badan Pusat Statistik Provinsi Sulawesi
Tengah, Palu.
Masyarakat, 7(2): 82–8.
FAO. 2020. The State of World Fisheries and Aquaculture 2020:
Sustainability in action. Food and Agriculture Organization of the
United Nations, Rome.
Queensland Fish and its Ability to cause Spoilage. Journal of
Applied Bacteriology, 39(2): 91–100.
Haryani, Y. Chainuliffah and Rustiana. 2012. Fermentasi karbohidrat
oleh isolat Salmonella spp. dari daianan pingir jalan. Jurnal
Indonesian Chemia Acta, 3(1): 50–53.
Hasnidar and A. Tamsil. 2019. Pengolahan ikan bandeng tanpa dari
Kelurahan Lakkang, Kecamatan Tallo, Kota Makassar Jati Emas.
1994. Bergey's manual of determinative bacteriology. William and
Wilkins, Baltimore.
Hua, K., J. M. Cobercroft, A. Cole, K. Condon, D. R Jerry., A. Mangott,
2019. The future of aquatic protein: implications for protein
sources in aquaculture diets. One Earth, 1(5) 316–329.
Salmonella in food samples by the combination of immunomagnetic
Kananub S., N. Pinniam, S. Phothithcheerabut and P. Krajanglikit.
2020. Contamination factors associated with surviving bacteria in Thai
Khairil, M. 2018. Promotion strategy by Palu City Government to
pulling investors interest in Palu special economic zone. Jurnal
Persada, Jakarta.
ureases inhibitors. The Chemical Record, 22(8).
Novoslavskaï, A., M. Terentjeva, I. Eizenberga, O. Valcina, V.
Bartkevičs, and A. Bērziņš. 2016. Major foodborne pathogens in
15.
Srđević, M., and K. V. Malliaiah. 2007. Bioproduction of indole acetic
acid by Rhizobium strains isolated from root nodules of green
manure crop, Sesbania sesban (L.) Merr. Iranian Journal of
Puspawedi, R., P. Adirestuti and A. Abdullahsith. 2017. Deteksi
Staphylococcus aureus dan Salmonella pada jajanan sirup. Jurnal Ilmu
Rabins, S. L., A. Bhattacharya, V. A. Kumar, P.N. Antony., V. B.
Rokha and S. V Perumal. 2018. An exploration on animal and
public health significance of salmonella from major meat sources
in Puducherry, India. Journal of Entomology and Zoology Studies,
6(4): 1691-1699.
Rofig, R. S., P. R. Dwi, B. Prastika, Y. C. Nanda, A. Susanto, I. Rosadi,
A.R. Rahim. 2018. Milkfish fish as a business opportunity Kalirejo
makanan laut yang beredar di pasar tradisional Kota Pontian.
Strugnell, R. A., T. A. Scott, N. Wang, C. Yang, N. Peres, S. Bedosse
and A. Kapz. 2014. Salmonella vaccines: lessons from the mouse
model or bad teaching? Current Opinion in Microbiology, 17(1):
99-105.

infections in children: Review of literature and recommendations for
936–941.

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