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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 2.94×10^4 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)

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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 10^5 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, dulcitol), 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 KH_2PO_4 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^{-2} 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;

$\sum C$ 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 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 in an autoclave 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° ± 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 colony 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° ± 1°C, covered with a loosely closed lid to avoid excessive build-up of the gas hydrogen sulphide (H_2S). On the TSI media, typical *Salmonella* spp. cultures will cause an alkaline (red) reaction on the slanted scores and acid (yellow) reaction on the vertical stab holes, with or without H_2S (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 discolouration of the stab holes is not sufficient to declare the culture negative, and H_2S 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 (SNI 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.

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

[a] 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

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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 × 10^3 to 2.200 × 10^3 CFU/g for the unprocessed milkfish and 2.045 × 10^3 to 2.940 × 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.

Site No.	Sample Type	Code	Mean TPC (CFU/g)	Site No.	Sample Type	Code	Mean TPC (CFU/g)
1	Fresh milkfish	B1	1.995×10^3	2	Fresh milkfish	B7	2.200×10^3
1	De-boned milkfish	B2	4.279×10^3	2	De-boned milkfish	B8	1.054×10^4
1	Fresh milkfish	B3	2.086×10^3	2	Fresh milkfish	B9	1.264×10^3
1	De-boned milkfish	B4	2.313×10^4	2	De-boned milkfish	B10	2.045×10^3
1	Fresh milkfish	B5	2.082×10^3	2	Fresh milkfish	B11	1.305×10^3
1	De-boned milkfish	B6	2.168×10^4	2	De-boned milkfish	B12	2.940×10^4

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.

Source of Variation	*F*	*P-value*	*F crit*
Site	0.75457	0.4103	5.317655
Product	14.09143	0.0056*	5.317655
Interaction	0.00385	0.9520	5.317655

* Significant at the 95% confidence level

Treatment	Mean Log (TPC)[b]	SD Log (TPC)
L1P1	3.30[a]	0
L2P1	3.10[a]	0.174
L1P2	4.10[b]	0.428
L2P2	3.92[b]	0.585
	Factor 1 = Site	
L1	3.70[a]	0.512
L2	3.51[a]	0.592
	Factor 2 = Product	
P1	3.20[a]	0.155
P2	4.01[b]	0.468

[a] Site codes: L1 = Melona MSME, L2 = TIU-AQFP; Product codes: P1 = fresh milkfish, P2 = de-boned milkfish)
[b] For each section, different superscripts indicate mean values differ significantly at the 95% confidence level

Salmonella spp. isolation and identification

Bacterial colonies growing on three incubation media (BSA, Figure 1; HEA, Figure 2; XLDA, Figure 3) yielded 72 colonies with traits typical of *Salmonella* spp. These colonies were inoculated on TSIA to provide 72 bacterial isolates for the biochemical assays. The assays (Table 4) did not find any trace of *Salmonella* spp.

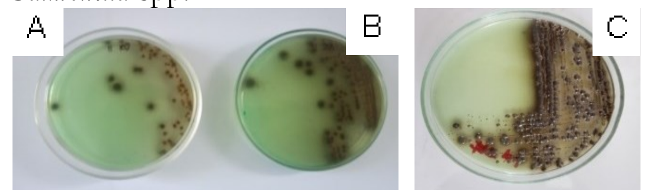

Figure 1. Bacterial colonies growing on BSA. A. fresh (unprocessed) milkfish from the Melona MSME (no *Salmonella* spp. detected); B. de-boned milkfish from the TIU-AQFP (no *Salmonella* spp. detected); C. Positive control.

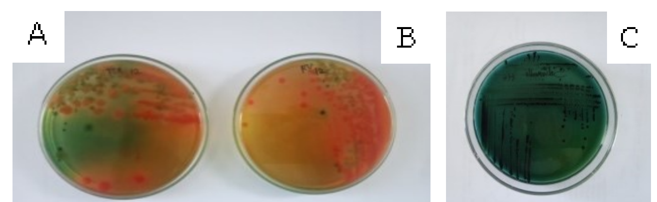

Figure 2. Bacterial colonies growing on HEA media. A. fresh (unprocessed) milkfish from the Melona MSME (no *Salmonella* spp. detected); B. de-boned milkfish from the TIU-AQFP (no *Salmonella* spp. detected); C. Positive control.

Figure 3. Bacterial colonies growing on XLD 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

Table 4. Biochemical assays with positive isolates.

No.	Assay	Positive	
		No	%
1	LIA	37	51.39
2	Urease	66	91.67
3	Indole	44	61.11
4	Methyl Red	23	31.94
5	Simmons Citrate	69	95.83

Based on the dominant characteristics from the laboratory biochemical assay results and the guidelines in Barrow and Feltham (1993), three genera in the Enterobacteriaceae family were identified (*Enterobacter*, *Klebsiella*, and *Citrobacter*). Although potentially pathogenic, these bacteria are not considered pathogenic at the levels observed. Of the 72 isolates, 39 were identified as *Klebsiella*, 23 as *Enterobacter*, one as *Citrobacter*, and 9 remained unidentified.

Discussion

Total Plate Count (TPC)

The significantly higher TPC of de-boned milkfish samples compared to unprocessed fresh milkfish (Table 3) indicates that some aspect of the processing involved in producing de-boned milkfish increases the bacterial load. One likely source for the additional bacteria in de-boned milkfish is contamination, albeit most likely at a low level, as the TPC counts were still not high. Bacterial contamination could come from many sources, including the workers (with bare or even with gloved hands), the tools and surfaces used at all stages, as well as the water used during the de-boning process (Novoslavskij *et al*., 2016).

The TPC could also be due to the natural growth of bacteria already on or in the fish before the processing began. In this situation, the higher counts could be the result of bacteria from one part of the fish (e.g. the skin) being spread to another part of the fish (e.g. the flesh), for example through handling or water used for cleaning, thereby increasing the substrate area (Novoslavskij *et al*., 2016). The 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 XLDA 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 XLDA 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 XLDA 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 H_2S 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 H_2S 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 H_2S. *Salmonella* spp. react positively with lysine, and the LIA media also contains natrium thiosulfate, a substrate for producing H_2S 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-butanadiol 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 (*Klebsiella*, *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.

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