Asia-Pacific Journal of Science and Technology :Volume :25 .Issue :04 .Article ID :.APST-25-04-06. Research Article

APST

Asia-Pacific Journal of Science and Technology

https//:www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Microbiological evaluation of Thai fermented fish (pla-ra) production contact surfaces

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Received 4 August 2020
Revised 8 November 2020
Accepted 11 November 2020

Abstract

Pla-ra is a lactic acid fermented fish product in Thailand, typically consumed as a side dish. Multiple outbreaks have been linked to the consumption of fermented foods, including pla-ra. During the fermentation process, pla-ra is in direct contact with a food container for an extended period which is also potentially contaminated with a high number of microorganisms. This research determined the prevalence of pathogenic bacteria on the surface of pla-ra production containers. From three different pla-ra manufacturers in Khon Kaen province, Thailand, 110 swab samples were collected from 25 pla-ra clay and polyethylene (PE) production containers. The samples, cultivated in selective media, were contaminated with *Bacillus cereus* (7.64 ± 0.13 $\log_{10}$ colony forming unit (CFU)/cm^2), *Clostridium perfringens* (6.53 ± 0.21 $\log_{10}$ CFU/cm^2), *Escherichia coli* (8.23 ± 0.15 $\log_{10}$ CFU/cm^2), *Salmonella* spp. (5.73 ± 0.22 $\log_{10}$ CFU/cm^2) and *Staphylococcus aureus* (7.81 ± 0.16 $\log_{10}$ CFU/cm^2). The 16s rRNA sequencing analysis confirmed the presence of *Klebsiella aerogenes, Clostridium perfringens*, *Escherichia coli*, *Listeria innocua*, and *Staphylococcus aureus*. The population of each bacteria found on the surfaces was, respectively, 7.64 ± 0.18, 7.79 ± 0.14, 8.40 ± 0.24, 7.82 ± 0.14, and 8.18 ± 0.17 $\log_{10}$ CFU/cm^2 in clay containers and 7.39 ± 0.24, 7.39 ± 0.26, 8.12 ± 0.08, 8.32 ± 0.15, and 8.46 ± 0.26 $\log_{10}$ CFU/cm^2 in PE containers. The specific environmental conditions and the handling of raw materials, including contact time during fermentation and sanitation, affect the possibility of cross-contamination. These results highlight the importance of pasteurization of pla-ra to ensure its safety.

Keywords: Pla-ra, Pathogenic bacteria, Container, Clay, Polyethylene, Contact surfaces

1. Introduction

Fermentation is an ancient and economical method of food preparation. It is defined as a food preservation method because of its by-products, such as organic acids and other anti-microbial metabolites [1]. Fermented products also offer health benefits, such as reducing blood cholesterol levels, increasing immunity, and others. However, there have been reported cases of poisoning from fermented foods caused by pathogenic bacterial toxins such as those from *Clostridium botulinum* or pathogenic *Escherichia coli* [1,2]. In northeastern Thailand, one popular fermented product is Thai fermented fish (pla-ra), widely used as a seasoning in papaya salad and listed as one of Thailand's national treasures by the Ministry of Culture of Thailand [2]. The first preparation step is removing the scales and tails of the freshwater fish, then mixing it overnight with salt in a ratio of 3:1. The fish is then packed tightly in containers, and saturated brine and ground roasted rice is added at an appropriate ratio for at least six months until the characteristic taste of pla-ra is attained [1,3,4]. The complete flow chart of the pla-ra production can be seen in Figure 1 [1]. The fermentation is commonly performed in containers with lids, such as earthen or clay jars, polyethylene, or even a cement pond [1,3]. In 2000, Thailand's pla-ra production approached 40,000 tons per year, worth up to THB 800 million. Consumption has been estimated at 15-40 gr/day/person [3].

According to the Thai Agricultural Standard for pla-ra (TAS 7023-2018), a satisfactory product must meet specific requirements for physical characteristics, salt content, food additives, and contaminants such as lead

(Pb), inorganic arsenic (As), mercury (Hg), and bacteria. Various bacteria may contaminate pla-ra due to traditional fermentation processing, starting from raw material handling to hygiene practices [1-3]. There have been reports of microbial pathogens in many fermented foods, including fermented fish, sausages, cheese, and fermented cereals [2,4-6]. *Bacillus cereus*, *C. perfringens*, *E. coli*, *Salmonella* spp., and *Staphylococcus aureus* are specific pathogenic bacteria that must be restricted according to the Thai Agricultural Standard for pla-ra.

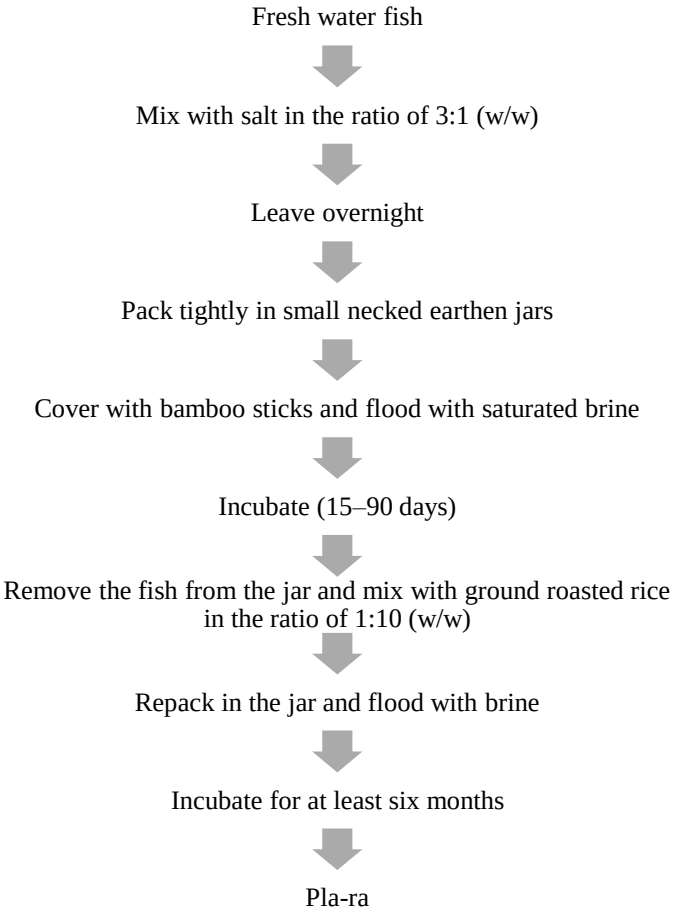


Figure 1 Flow sheet of pla-ra production.

One fermentation step potentially causing contamination involves interaction between the food product and container surface. Microorganisms can attach, multiply, and develop on the surface and form a biofilm [7]. Microbes inhabiting contact and environmental sites such as through biofilms in food processing can affect public health because of their role in certain infectious diseases and their importance in various device-related infections. Bacteria can establish colonies in critical locations and thereby contaminate the end product [7, 8]. Transmission or cross-contamination has been studied in many processing environments, and various factors can increase the potential of bacterial transfer to the product [9]. In pla-ra manufacturing plants, earthen jar or clay and polyethylene are the most common materials used in the fermentation process. Bacterial contamination of these production containers has not yet been determined. This study aims to determine the pathogenic bacterial prevalence on clay and polyethylene (PE) pla-ra production containers that may lead to cross-contamination.

2. Materials and methods

2.1 Sample preparation

Samples were collected from three different pla-ra manufacturers in Khon Kaen province, Thailand, from production containers made of clay (70 cm in diameter, 85 cm in height) and polyethylene (50 cm in diameter, 100 cm in height) materials. Swab samples were collected from 25 containers (13 clay and 12 PE) that had not received any cleaning or washing treatment after being emptied. A total of 110 samples were obtained randomly from the top, middle, or bottom area of the inner containers by the perpendicular swab (10 × 10 cm^2) method.

The cotton tip of each sample swab was soaked in 10 ml of 20.00 g/L sterile buffered peptone water (Himedia, Mumbai, India). The sample solutions were transported in a cooler to the refrigerator (4 ˚C) at the microbiological laboratory, Faculty of Technology, Khon Kaen University.

2.2 Isolation, identification, and confirmation of bacteria

The sample's solution from refrigerator (4 ˚C) was taken to 1 mL and diluted with 9 mL of sterile tryptone soya broth (TSB) (Himedia, Mumbai, India). The solution was mixed thoroughly by a vortex for 30 s. Five ten-fold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were prepared, and 0.1 mL of the 10^{-3}, $10^{-4,}$ and 10^{-5} dilutions were poured into respective selective media that had been sterilized at 121 ˚C for 15 min. Samples were then incubated at 37 ˚C for 24-48 h. The selective media were phenol red egg yolk polymyxin (PREYP) [10], tryptose sulfite cycloserine (TSC) [11], eosin methylene blue (EMB) [12], xylose-lysine deoxycholate (XLD) [13], PALCAM [14], and mannitol salt agar [15] (Himedia, Mumbai, India) respectively used for the selection of *B. cereus*, *C. perfringens*, *E. coli*, *Salmonella* spp., *Listeria* spp. and *S. aureus.*

The morphology and cultural characteristics of suspected colonies from each selective media were analyzed. Samples suspected to contain *S. aureus* were further analyzed by a coagulase test. A motility test was used to confirm samples suspected of containing *Listeria* spp. Nutrient agar slants were used for subculturing colonies for confirmation. Inoculated slants were incubated at 37 ˚C for 24-48 h, then stored in the refrigerator at 4 ˚C until use.

2.3 Confirmatory test by 16s rRNA analysis

The preliminarily characterized isolates were confirmed by 16s rRNA analysis at the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. The PCR amplified the 16s rRNA gene of unknown bacteria strain with universal primers 27F (5'- AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). The DNA template for PCR was prepared and purified using the DNeasy Tissue Kit (Qiagen, Germany). The PCR cycling conditions were as follows: 94 ˚C for 3 min, 30 cycles of 94 °C for 60 s, 55 ˚C for 60 s, and 72 ˚C for 2 min, with a final extension at 72 ˚C for 3 min.

2.4 Statistical analyses

Data were analyzed using Microsoft Excel 2010 and SPSS (version 20). The prevalence of pathogenic bacteria was summarized in Microsoft Excel and analyzed by an independent sample t-test. Descriptive statistics (mean ± SD) and analysis of variance (ANOVA) were used to assess the relationship between the prevalence of bacteria ($\log_{10}$ values) and the type of containers.

3. Results and discussion

3.1 Types of containers and presumptive identification of pathogenic bacteria

Photographs of representative sample surfaces from both container types are shown in Figure 2. The samples from which specific colonies were isolated are presented in Figure 3. The results highlighted the presence of suspected samples containing *B. cereus*, *C. perfringens*, *E. coli*, *Listeria* spp., *Salmonella* spp., and *S. aureus.*

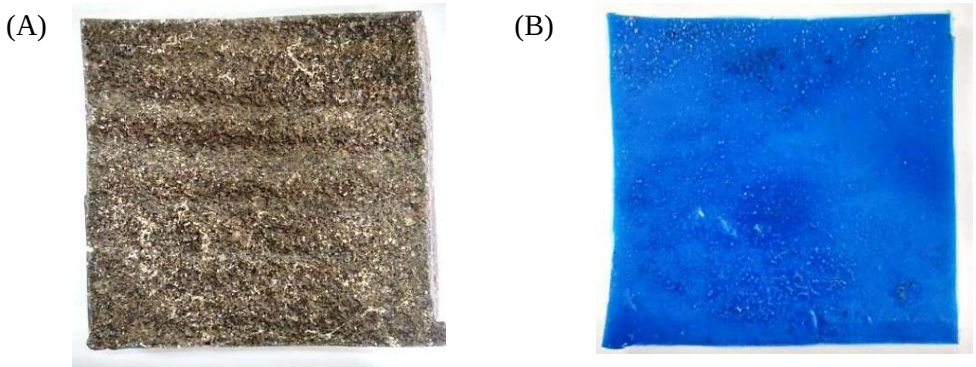


Figure 2 Representative pla-ra production container materials: (A) clay and (B) polyethylene (PE).

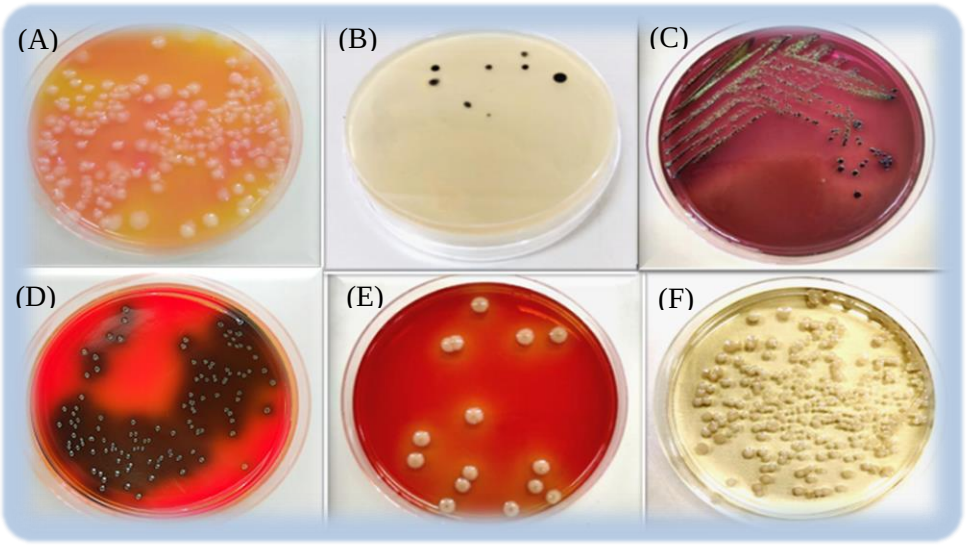


Figure 3 Samples containing suspected colonies: (A) *B. cereus*, (B) *C. perfringens*, (C) *E. coli*, (D) *Listeria* spp., (E) *Salmonella* spp., (F) *S. aureus*.

E. coli was the major contaminant of both containers (100 %). The contamination of clay-based container samples by specific bacteria was as follows: *Listeria* spp. (95 %), *S. aureus* (90 %), *Salmonella* spp. (85 %), *C. perfringens* (34 %), and *B. cereus* (30 %). PE container samples were contaminated as follows: *S. aureus* (98 %), *Listeria* spp. (94 %), *Salmonella* spp. (73 %), *C. perfringens* (20 %), and *B. cereus* (20 %). Although the contamination of containers with specific pathogenic bacteria was variable (%), there were no significant differences between container material types. Only *B. cereus* and *C. perfringens* contaminated fewer than 50 % of both clay and PE-based surfaces.

Table 1 Prevalence of pathogenic bacteria on clay and polyethylene (PE) container surfaces.

Isolated bacteria	Clay*	Prevalence (%)	Polyethylene*	Prevalence (%)	*p*-Value**
B. cereus	18/61	30%	10/49	20%	0.131
C. perfringens	21/61	34%	8/49	20%	0.128
E. coli	61/61	100%	49/49	100%	
Listeria spp.	58/61	95%	46/49	94%	0.314
Salmonella spp.	52/61	85%	36/49	73%	0.128
S aureus	55/61	90%	48/49	98%	0.254

*Number of positive samples/total swab samples from clay and polyethylene (PE) containers
**No significance at $p < 0.05$ for all samples

3.2 16s rRNA confirmation of the isolated pathogenic bacteria

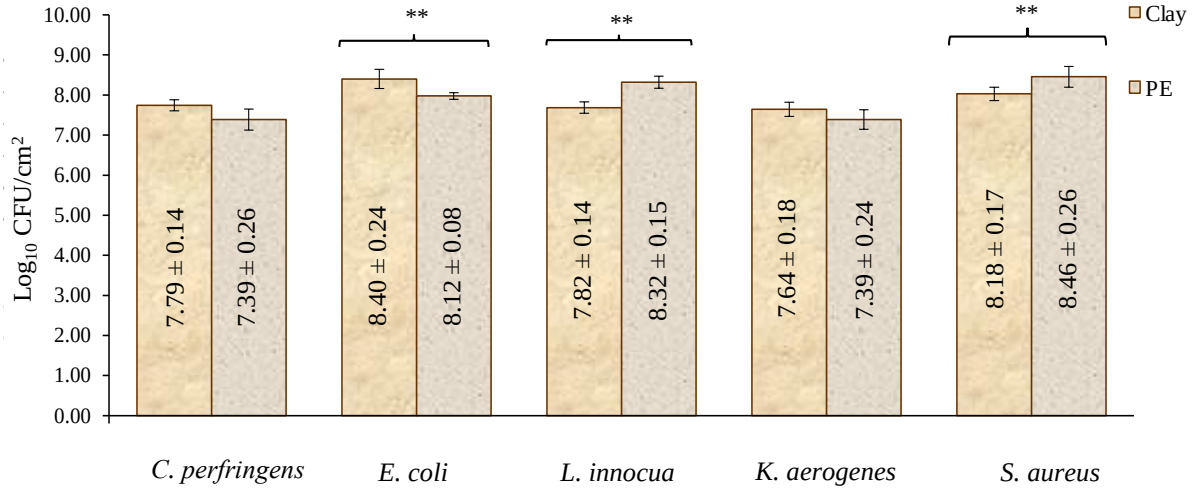
16s rRNA sequencing assessment results are shown in Table 2. *Klebsiella aerogenes*, *C. perfringens*, *E. coli*, *L. innocua*, and *S. aureus* were confirmed to inhabit the inner surfaces of both pla-ra production container types. *B. cereus* and *Salmonella* spp., detected in preliminary testing, were not confirmed by the 16s rRNA sequencing analysis.

Table 2 Results of the 16S rRNA sequencing analysis of the isolated bacteria.

Suspected bacteria	Primer sequence*	Result	% Similarity
B. cereus	• 27F (5'-AGAGTTTGATCATGGCTCAG-3')	*K. aerogenes*	99.57%
C. perfringens		*C. perfringens*	99.38%
E. coli		*E. coli*	99.49%
Listeria spp.	• 1492R (5'-TACGGTTACCTTGTTACGACTT-3')	*L. innocua*	99.52%
Salmonella spp.		*K. aerogenes*	99.63%
S. aureus		*S. aureus*	99.75%

* The two universal primers (both 27F and 1492R) are applied or used for all the six bacteria.

Confirmed bacteria were also counted. The populations of *C. perfringens* on clay and PE-based container surfaces were not significantly different: 7.79 ± 0.14 and 7.39 ± 0.26 $\log_{10}$ CFU/cm^2, respectively. The average population of *E. coli* on the surface of clay containers was also high, 8.40 ± 0.24 $\log_{10}$ CFU/cm^2, whereas on the surface of PE, it was 8.12 ± 0.08 $\log_{10}$ CFU/cm^2. The total number of *L. innocua* bacteria contained on the PE containers surface was significantly higher (7.82 ± 0.14 $\log_{10}$ CFU/cm^2) than in clay containers (8.32 ± 0.15 $\log_{10}$ CFU/cm^2). The average population of *K. aerogenes* on the clay container surfaces was 7.64 ± 0.18 $\log_{10}$ CFU/cm^2, which was higher than the number of bacteria on the surface of PE containers, 7.39 ± 0.24 $\log_{10}$ CFU/cm^2. This difference was not significant. The *S. aureus* population on the surface of PE containers was significantly greater than on the clay containers, which was 8.46 ± 0.26 $\log_{10}$ CFU/cm^2 vs. 8.18 ± 0.17 $\log_{10}$ CFU/cm^2, respectively.



**Significant at $p < 0.05$ (2-sided) based on the comparison between clay and PE-based containers
Figure 4 Contamination levels of the pathogenic bacteria in clay and PE container surfaces.

There was a significant difference in the number of *E. coli, L. innocua*, and *S. aureus* colonies between clay containers and PE-based containers. As seen in Figure 3, *C. perfringens*, *E. coli*, and *K. aerogenes* were more common on clay containers, whereas PE containers had more *L. innocua* and *S. aureus*. The US Public Health Service recommends no more than 100 CFUs bacteria per 50 cm^2 sampled from food contact surfaces [16], which is substantially less than found inside pla-ra production containers, potentially leading to cross-contamination of the pla-ra during fermentation.

Table 1 and Figure 3 show the total bacteria found in 25 clay and PE production containers for pla-ra among three manufacturers in Khon Kaen province, Thailand. The 13 clay and 12 PE containers are problematic as they had visible food residue after a batch of pla-ra was removed. As seen in Table 1, the clay and PE containers had the same high potential for microbial exposure. Furthermore, both container types were exposed at the same intensity to five pathogenic bacteria: *E. coli*, *C. perfringens*, *L. innocua*, *K. aerogenes*, and *S. aureus.* The average population of each bacteria shown in Figure 2 was within the range of 7.00-8.50 $\log_{10}$ CFU/cm^2.

Swab sampling was carried out at the container's top, middle, and bottom area. The inoculation results showed that all *C. perfringens* was isolated from samples collected at the bottom of the container, indicating the species gathered there to avoid oxygen [17,18]. Other isolates were found randomly at all positions and in both types of containers. Contamination on the surfaces can come from the raw material, especially in freshwater fish habitats. Freshwater fish often used for making pla-ra include mud gourami, snakehead, catfish, and tilapia [1, 17].

In many previous studies, *E. coli*, *C. perfringens*, and *Listeria* species were commonly found in freshwater resulting from contamination by combined sewer outflows in estuaries or groundwater [17,18]. The sites of pla-ra production are largely still ground floors without pavement that may lead to pathogen contamination. Many pathogens can be transported from surface to sub-surface water [17,18], and the sources of water in pla-ra production are the freshwater from fish habitats and the manufacturers' water for mixing ingredients and cleaning processes [18]. Controlling groundwater pathogen contamination is a growing concern in many countries, as pathogens can survive up to 400 days, depending on the soil temperature [19].

Soil can be a reservoir for various pathogenic bacteria, especially when contaminated by animal or human feces [19]. For decades, it has been speculated that gut bacteria appear in soil and other environments due to human and livestock manure pollution [18, 19]. Other studies have shown that *Enterococcus* spp. and *E. coli* are

equally common in soil under various climatic and geographic conditions [20]. In addition, *E. coli* and *C. perfringens* are edaphic, meaning these species are easily found in soil or sediment. They are also common in the intestines and feces of many animals [21]. Soil is a major reservoir and contributes to other parts of the environment, leading to possible indirect transmission. *C. perfringens* has also been found in soil at different seasons, supporting the argument that these bacteria have lived and adapted in the soil environment [18-21].

Early ecological studies revealed that *Listeria* spp could be easily isolated from urban land, agricultural fields, and natural aquatic environments [19,20]. In Serbia, 58 of 470 fish samples (12.34 %) contained *Listeria* spp., and 8.51 % of samples were specifically contaminated by *L. innocua* [19]. *Listeria innocua* has been isolated from soil, rotting vegetation, plants, groundwater, surface water, compost, and other biological waste [21]. Previous studies have shown that the transmission of pathogens to the soil was through the application of low-quality organic fertilizers after spreading them on land [20,21]. The presence of *E. coli*, *C. perfringens*, and *L. innocua* can be significantly influenced by pla-ra production containers, especially those made of clay as it is essentially composed of hardened soil and minerals co-deposited over time [22]. Certain impurities may include organic matter and promote contamination [8,21].

K. aerogenes has been reported to cause a high incidence of diseases in Southeast Asia [23]. It is an opportunistic pathogen present in soil and aquatic environments. A total of 55 isolates of *Klebsiella* species were isolated from seven out of the eight stations along the Sepetang, Sangga Besar, and Selinsing rivers of the Matang mangrove estuary in Malaysia, with *K. aerogenes* being the most predominant species in freshwater, brackish water, and saltwater [19,23].

In contrast, *S. aureus* is present in airways, soil, surfaces, and elsewhere on the human body, such as the skin and mucous membranes of most healthy people. This bacterium survives well in the dust, on fabrics, tile, glass, and flooring [9]. It does not normally cause infection on healthy skin; however, it may cause various potentially serious infections if allowed to enter the bloodstream or internal tissues [17,18]. Finding *S. aureus* in all types of containers was expected since the making of pla-ra is a manual process. According to many previous studies, *S. aureus* was the dominant bacteria isolated from ngari, hentak, and tungtag as fermented fish products of northeast India [24].

Similarly, *S. aureus* was the dominant bacteria isolated from lanhouin and cassava fish, a traditionally processed fermented fish product in the Republic of Benin [25] and hout-kasef traditional salted fermented fish from Saudi Arabia [24]. The presence of *S. aureus* in pla-ra, which has high salt concentration, is influenced by the fact that *S. aureus* can tolerate elevated levels of NaCl in food and control the overall expression of genes such as the sigma factor, which could result in increased resistance to sub-lethal stresses. It can grow in a broad range of pH values (4-10) and salt concentrations as high as 20 % [21]. Therefore, *S. aureus* could survive under pla-ra conditions, which has a pH range from 4.3 to 6.2 and NaCl concentration of approximately 8-18 % in fermentation at room temperature (28-30 ˚C) [15,21].

The detection of *E. coli*, *C. perfringens L. innocua*, *K. aerogenes*, and *S. aureus* in the pla-ra production containers proves that they can survive in low pH and high salt environments. *Escherichia coli* O157:H7 and *S. aureus* was found to have remained active at a high salt concentration (17.5 % NaCl) [17,18,21]. Two identical mutation genes (rpsG and sspA) have been found in each *E. coli* isolate that evolved to tolerate increased salt concentrations in limiting organic acid accumulation [26]. *L. innocua* also maintains its viability, along with other *Listeria* species, in model brine solutions (6.0-14.0 % NaCl, pH 4.5) [17,19,20]. Studies have shown that for *L. monocytogenes* in particular, survival rates are greater in concentrated salt solutions when the temperature is lower. Four proteins (DnaK, CysK, CcpA, and Gap) were found to be over-expressed in the cold stress of other bacteria among the salt-stress proteins recognized. In conclusion, the research showed that one protein, GbuA, was over-expressed by *L. monocytogenes* in the presence of salt [27].

C. perfringens is also moderately salt tolerant, allowing it to survive the curing process in several meat products. The pla-ra production container provides the essential nutrients needed for the organism to grow and a favorable oxidation-reduction potential or low aerobic environment for *C. perfringens* survival. It can optimally grow in temperatures between 25-45 ˚C with sufficient generation or doubling time [17,21]. In contrast, *B. cereus* and *Salmonella* were not found in pla-ra production containers. Both of the bacteria were identified during confirmation as *K. aerogenes*, and substantial research supports that *Klebsiella* species are more easily found in nature. Furthermore, their characteristics are similar to those of *Salmonella* spp. [21,28]. However, there is potential for both *B. cereus* and *Salmonella* to grow in pla-ra manufacturing plants. *B. cereus* and *Salmonella* isolation require more enrichment steps than *Klebsiella* [21,23]. Other studies have reported that *Klebsiella* species could tolerate high salt and low pH conditions [23]. They can survive in cheese with a pH of 4.5 and 6-8 % salt concentration [29].

The prevalence and number of bacteria were not significantly different in the two different materials. Isolated bacteria measurements presented in Figure 1 and Figure 2 showed that clay containers tended to have higher bacterial populations. Of the five isolated bacterial species, populations of *C. perfringens*, *E. coli*, and *K. aerogenes* were greater in clay-based containers than PE-based containers. A clay container's surface characteristics are not recommended because the food contact surfaces should be easily cleaned and disinfected

according to the Codex Alimentarius [8,21,22]. The surface with direct contact with food should be smooth, non-absorbent, inert to food, and cleaned with detergents and disinfectants under normal operating conditions [7,8,9]. The clay container surface was uneven and higher in porosity, which could entrap the food debris and other materials, enhancing the possibility of food contamination. Moreover, cleaning residues will easily remain in food processing when the cleaning and washing process is not appropriate [7-9,21]. The adherence of microorganisms to surfaces is a very complex process, with many variables affecting the outcome. In general, increased adherence will occur most readily on rougher surfaces that are more hydrophobic, have increased flow velocity, and provide a nutrient-rich environment [7,8,21].

Additionally, the processors can contaminate the fermented fish with bacteria from the mouth, nose, or skin by talking, coughing, sneezing over the fish, or handling it with dirty hands or skin. The prevalence of bacterial isolates in this study is similar to previous reports indicating that the lack of hygiene is the major source of food contamination [8,17,21]. Even though classified as a fermented product, the safety of pla-ra as the final product cannot be guaranteed due to multiple factors, especially contamination of contact surfaces [5,10]. As seen in Table 1, the bacterial concentration indicates that any containers could contain bacteria and contaminate the food products. This contamination is exacerbated when pla-ra is consumed without heat treatment or pasteurization before eating, such as in a papaya salad mixture.

This study's results emphasize that some pathogenic bacteria remain on the food contact surfaces and potentially cause bacterial cross-contamination from surface exposure [8,9]. Cross-contamination refers to the transfer, direct or indirect, of bacteria or virus from a contaminated product to a non-contaminated product. Furthermore, the longer the contact time, the greater the opportunity of infected surfaces to transfer pathogens through direct contact and even be derived from a thin layer of organic matter left on the surfaces [8,9,29]. In terms of contact time, several studies found that other factors, including moisture of the food and the surface, were greater importance for instantaneous transfer (less than 1 s), disproving the "five-second rule" of bacterial transmission [29,30].

In a study analyzing 688 samples, *Listeria* spp. were found to contaminate environmental sponge samples collected from food contact surfaces (tables and knives), non-food contact surfaces (drains, floors, sinks, door handles), and the finished product [18]. High concentrations of *E. coli* (6.40 $\log_{10}$ CFU/ml), *S. aureus* (8.20 $\log_{10}$ CFU/mL), and *L. innocua* (7.80 $\log_{10}$ CFU/mL) were found in a refrigerator-stored traditional fermented Turkish beverage. *E. coli*, *Listeria* spp., and *S. aureus* are well-known to occur during food preparation by cross-contamination due to poor personal hygiene and contaminated tools and equipment [8].

The presence of pathogens on pla-ra production containers at such high rates, as shown in this study, can lead to pla-ra contamination. The US Public Health Services have stated that 100 CFU per 50 cm^2 should be the maximum number of bacteria on food contact surfaces [16]. The discovery of a high population for all five bacteria on each container showed that pla-ra production containers should be washed beforehand, and proper hygiene and sanitation exercised to prevent cross-contamination. Studies revealed that cleaning with hot water and detergent had little effect in removing some pathogenic bacteria from food preparation areas [9,20]. Proper handling should start from handling the raw fish and cleaning the container effectively by detergent or disinfectant, continued by scrubbing the surfaces with particular tools. Thus, the top management of the food operation must understand the urgency of this issue to decrease the contamination in the operational environment. The results of this study also highlight the necessity of the pla-ra pasteurization process before consumption. Furthermore, additional studies are needed to understand the potential sources of cross-contamination in the pla-ra industry.

4. Conclusion

The surface characteristic of container material used in this study, clay and PE-based material, did not significantly affect the number of isolated bacteria. Both types of containers are potentially a source of *C. perfringens*, *E. coli*, *L. innocua*, *K. aerogenes,* and *S. aureus*. The handling of raw materials, contact time during fermentation, and environmental conditions affect the number of each bacteria present in the surface and cause cross-contamination. It is recommended to wash pla-ra production containers beforehand with proper hygiene and consistent sanitation practices. Pla-ra, as the final product, must be pasteurized to ensure safety, as it will be consumed directly or be used as a condiment for papaya salad and other foods. Future studies should observe the points of potential cross-contamination from the production container to pla-ra as the end product.

5. Acknowledgements

Authors gratefully acknowledge the Faculty of Technology, Khon Kaen University, for funding and providing consent to undertake this research.

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