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Validity of cold storage and heat treatment on the deactivation of *Vibrio parahaemolyticus* isolated from fish meat markets

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Abstract
Vibrio parahaemolyticus is a zoonotic disease transmitted to humans when handling or consuming improperly cooked fish meat. This study aimed to evaluate the effect of thermal treatment on *V. parahaemolyticus* isolates. Different heat treatment methods are used to determine the best methods for controlling *V. parahaemolyticus*, isolated from fish meat, which include microwave, low-temperature long time, and high-temperature short time methods. The *V. parahaemolyticus* isolates significantly declined in bacteria count when they were kept at 4°C, and 25°C for a long time, and the *V. parahaemolyticus* isolates significantly declined in bacteria count manner when they were kept at -20°C for a long time. The high temperature and long-time exposure at 75°C/25 minutes by moist heat, 87°C/5 minutes by dry heat, and 70°C/20 minutes by frying heat were enough to kill *V. parahaemolyticus* isolates. This work can be useful to decrease the hazards of infections related to *V. parahaemolyticus* and reduce the causes of fish-borne pathogens.

Introduction
Fish farming is one of the best ways to solve 21st-century problems like feeding a rising population. (Muringai *et al*., 2022). The United Nations (UN) reported that the current world population of 7.6 billion is expected to reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion in 2100 (UN, 2022).
The growing global population and longer life expectancy have raised concerns about food availability. Food from terrestrial animals has been available to humans via agriculture for at least 5000 years (Modlinska and Pisula, 2018). Protein-rich fish items are a popular human food choice. In the 21st century, the importance of the fishing and farming industries in ensuring everyone has access to healthy food is becoming more widely acknowledged. In 2020, it is predicted that the world will produce 178 million metric tons of aquatic animals (FAO, 2022).
From fish to crabs to octopus, aquatic animal source foods (AASFs), which constitute the majority of aquatic foods, provide critical nutrients that support human health and development. AASFs enhance human health in at least three ways: being the primary omega-3 fatty acid supply, long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (hereafter referred to jointly as DHA+EPA), which may protect against cardiovascular disease and improve cognitive function, help people eat less of the less-healthy red and processed meats that have been linked to negative health outcomes, and help people get enough of the micronutrients (like vitamin A, calcium, and iron) they need to prevent disease (Iannotti *et al*., 2022). The evidence of protein hydrolysate's physiological benefits, including its antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial, and hypocholesterolemia effects, is limited (Kamaruddin *et al*., 2021).
Despite their great nutritional content, aquatic goods nonetheless face several safety issues associated with their production methods and environmental dangers (Castello *et al*., 2023). People are understandably worried about the safety of the food supply owing to the prevalence of dangerous bacteria in the marine environment across the globe. The study designed by Carella *et al*. (2010) in Italy reported that marteiliosis caused by the genus *Marteilia* has been shown to cause important production losses at the national and international level in many of the susceptible species. Also, Smaldone *et al*. (2020) confirmed that the presence of *Anisakis larvae* in fish represents a major public health concern. On the other hand, Anastasio *et al*. (2014) confirmed that the treatment with Rosmarinus extends the shelf life of vacuum-packed refrigerated swordfish steaks.
However, *Vibrio parahaemolyticus*, a type species of the genus *Vibrio* that belongs to the family *Vibrionaceae*, serves as an excellent illustration (Taragusti *et al*., 2020). The Gram-negative halophilic

bacteria *V. parahaemolyticus* is ubiquitous in estuarine, marine, and coastal ecosystems. In humans, *V. parahaemolyticus* is the most common cause of acute gastroenteritis after eating raw, undercooked, or improperly handled seafood. Wound infections, ear infections, and septicemia caused by *V. parahaemolyticus* are very dangerous, especially for those with compromised immune systems (Baker-Austin *et al*., 2018). Fujino *et al.* (1953) initially identified *V. parahaemolyticus* in 1950 as the cause of foodborne illness during a significant epidemic in Japan that resulted in 272 cases of sickness and 20 fatalities due to shirasu intake.

In particular, since these microorganisms are resistant to most medications, the risk of infection is increased. The presence of thermostable toxins is thought to contribute to *V. parahaemolyticus'* pathogenicity.These toxine is the main virulence factors of *V. parahaemolyticus* including direct hemolysin and thermostable direct hemolysin-related hemolysin , which are encoded by the *tdh* and *trh* genes (Raghunath, 2015; Ha *et al*., 2023).

Several environmental conditions, such as water temperature, salt, and oxygen concentrations, contact with plankton, the presence of sediment, organic materials in suspension, and marine creatures, are known to affect the prevalence and dispersion of *V. parahaemolyticus* (Haque *et al*., 2022).

From most to least significant, environmental markers for the occurrence of *V. parahaemolyticus* are sea surface temperature, salt, pH, chlorophyll, and turbidity (Namad and Deng, 2023).

Vibrio species have the highest rates of growth among bacteria, multiplying in response to favorable circumstances such as high temperature, salt, and dissolved oxygen nearly instantly (Abioye *et al*., 2021).

The study conducted by Zhang *et al*. (2021) aimed to reduce the risks of infections caused by *V. parahaemolyticus;* results revealed that *V. parahaemolyticus* in alkaline peptone water (APW)-salt broth decreased gradually at 0 and 5˚C. while increasing rapidly when the temperature was greater than 15˚C. There is some evidence that storing *V. parahaemolyticus* at temperatures between -18 and -30°C for 15 to 30 days may decrease the pathogen to undetectable levels (3 MPN/g). The number of *V. parahaemolyticus* bacteria in APW-salt broth might drastically decrease if frozen. *V. parahaemolyticus* was more effectively inactivated by storage at -18°C than by storage at -30°C. As a bonus, *V. parahaemolyticus* was killed off from a concentration of 10,000 MPN/g to undetectable levels after being heated to 60°C for 5 minutes, 70°C for 2 minutes, or 80°C or higher for 1 minute. In light of the paucity of research on the topic, we investigated the effects of exposing *V. parahaemolyticus* isolated from Yemeni fish meat to room temperature (21°C), refrigeration (4°C), freezing (-20°C), and various cooking methods.

Materials and Methods

Isolation of Vibrio parahaemolyticus from fish meat

Fish tissue samples were taken in clean plastic bags. Sterile blades were used to mince fish into tiny bits, releasing any attached germs into the enrichment broth. A clean environment was used for this procedure. 10 g of fish flesh were reconstituted in 90 mL of APW and incubated for 24 hours at 37ºC in an incubated atmosphere for enrichment broth. Subsequently, a loopful [1 µl] of enrichment broth was streaked onto thiosulfate citrate bile salt sucrose media (TCBS) agar plates. Identification of suspected colonies was conducted by conventional biochemical tests. After the growth of *V. parahaemolyticus* colonies on selective media, another subculture was made on the same media, and the plates were incubated at 37°C for 24 hours to purify the colony. The pure *V. parahaemolyticus* colonies on TCBS media were described morphologically (shape, size, surface texture, edge and elevation, color, opacity, *etc*.), and then the suspected colonies were subjected to the identification procedures described. The bacterial smears were prepared from suspected colonies and stained with Gram stain. The suspected *V. parahaemolyticus* isolates appear as pink-colored, curved, rod-shaped, Gram-negative bacteria. Typical colonies of *V.*

parahaemolyticus were identified using biochemical tests including catalase, oxidase, urease, indole, motility, MR-VP, citrate, and TSI (Hi-Media, India). Tubes of nutrient broth (0%, 3%, 6%, 8%, and 10% NaCl) were inoculated with the suspected colonies and incubated at 37°C for 24 hours. *V. parahaemolyticus* showed growth only in nutrient broth containing 3%, 6%, and 8% NaCl, but no growth occurred in nutrient broth without NaCl and there was also no growth in nutrient broth containing 10% NaCl. All media and reagents were obtained from Hi-Media, India, and the biochemical tests were done according to Hassan *et al*. (2012).

Preparation of Vibrio parahaemolyticus

Isolates of *V. parahaemolyticus* were cultured on TCBS selective agar aerobically at 37ºC for 24 hours . The pure isolates were inoculated in APW and incubated at 37ºC for 24 hours. The density of bacteria was measured using a 0.5 MacFarland stander tube then it was confirmed using a spectrophotometer at the optical wavelength 480 nm. Each mL contained 108 colony-forming unit (CFU)/mL (Yeung and Thorsen, 2016).

Preparation of fish meat samples

The soft fish meat pieces that did not give any growth of the *V. parahaemolyticus* were used in the current study. A 25 gm for each piece, where inoculated with 108 cells/mL of *V. parahaemolyticus* on the surface and inside of each meat piece with a sterile disposable syringe. All the pieces were incubated in polyethylene bags and divided into two parts.

Study the survival of Vibrio parahaemolyticus at room temperature, cooling, and freezing

Room temperature (21°C)

After 3 days at 21°C, the fish tested for spoilage of *V. parahaemolyticus* at 0 time and after 1, 2, and 3 days. CFU was counted as previously described in duplicate plates by using TCBS agar (Yeung and Thorsen, 2016).

Cooling temperature (4°C)

The number of CFU was determined at 0 time and after 1, 2, and 3 days of incubation in the refrigerator at 4°C, each piece was mixed well after cutting with 250 mL of APW broth, and the loop full was streaked on TCBS agar at 37ºC for 24 hours in duplicate plates (Almashhadany, 2020).

Freezing temperature (-18°C)

Samples were taken from inoculated polyethylene bags after 0, 1, 2, 3, 4, 5, 6, and 7 weeks after being stored at -18°C. CFU was measured on triplicate plates using the methods previously described, by using TCBS agar (Almashhadany, 2020).

Study the impact of cooking, microwaving, and frying on Vibrio parahaemolyticus

Cooking (moist heat temperature)

The inoculated pieces of fish meat were transferred and inserted in a sterile Erlenmeyer flask containing 300 mL of sterile distilled water, which was closed with aluminum foil. The flasks were incubated separately in a water bath in different temperature conditions (50°C/5 minutes; 55°C/10 minutes; 65°C/15 minutes; 70°C/20 minutes; and 75°C/25 minutes). The internal temperature of fish meat was measured by

a special wire thermometer. The CFU was counted on duplicate plates in TCBS agar (Almashhadany, 2020).

Microwave (dry temperature)
Five pieces of fish meat experimentally contaminated with 108 CFU/mL were put in sterile polyethylene bags. Then, each piece was exposed to short radiation inside the microwave. Five conditions were used in microwave temperature conditions (55°C/1 minute; 63°C/2 minutes; 71°C/3 minutes; 79°C/4 minutes; and 87°C/5 minutes). Each piece was mixed well after cutting with 250 mL of APW broth and the loop full was streaked on TCBS agar at 37°C for 24 hours in duplicate plates (Geedipalli *et al*., 2007).

Frying (dry temperature)
Pieces of fish meat were contaminated with *V. parahaemolyticus* were placed in the frying oil. A special wire thermometer was used to determine the internal temperature of the fish meat. Five different conditions were used in the frying temperature test (50°C/5 minutes, 55°C/10 minutes, 65°C/15 minutes, 70°C/20 minutes, and 75°C/25 minutes). The CFU was calculated in duplicate plates in TBS (Saad *et al*., 2015).

Results
Survival of Vibrio parahaemolyticus at room temperature (21°C)
The highest bacterial count was found on day 1 after incubating fish meat at room temperature (21°C), while the lowest bacterial count was found on day 3 (Table 1). There is a highly significant difference between the time of fish meat preservation and the count of bacteria.

Survival of Vibrio parahaemolyticus at a cooling temperature (4°C)
The highest bacterial count was found on inoculating day (day 0) while the lowest bacterial count was found on day 3 (Table 2). There is a highly significant difference between time exposure to 4°C temperatures and *V. parahaemolyticus* bacterial count.

Survival of Vibrio parahaemolyticus at freezing temperature (-18°C)
The weekly bacterial count in fish meat at freezing temperature -18°C is illustrated in Table 3. The highest bacterial count was found in fish meat in the first week after incubating at a freezing temperature of -18°C, while no bacterial growth was recorded at week 7. Long preservation of fish meat at freezing temperature was a weekly signficantly decline in bacterial count ($p=0.000$) compared with the week of inoculation.

Impact of cooking (moist heat)
The cooking temperature affected *V. parahaemolyticus* bacterial count depending on the time of fish meat exposure to cooking and temperature degree. Long-time exposure to increasing temperature is more effective in declining bacterial count (Table 4). There are highly significant ($p=0.000$) differences and a negative correlation between the time and cooking temperature.

Impact of microwave (dry heating)
The effect of microwave on *V. parahaemolyticus* count depends on the time exposure and the temperature degree. Long-time exposure to increasing temperature is more effective in declining bacterial count (Table 5). There are high significant ($p=0.000$) differences between the time and temperature exposure of fish meat to the microwave.

Impact of frying

The long-time exposure with increasing the frying temperature is more effective in declining bacterial count as shown in Table 6. There are high significant (p=0.001) differences between the time and temperature exposure of fish meat.

Discussion

The United States Center for Disease Control and Prevention produced a surveillance study covering the years 2009-2018, and it found that fish was the food category most often linked to single-food-category foodborne outbreaks (Centers for Disease Control and Prevention, 2018; Ahmed, 2021).

V. parahaemolyticus is the most common bacterial cause of food poisoning in humans and originates in seafood. This bacterium was first identified in 1950 and has since been isolated from both epidemic and isolated cases of gastroenteritis across the globe (Matsuda *et al*., 2020; Beshiru and Igbinosa, 2023).

Several methods of food preservation have been developed to fight the spread of disease-causing microbes. However, one of the most common methods for killing spoilage and harmful bacteria is cold storage followed by heating treatment (Agriopoulou *et al*., 2022).

In our study, the results of room temperature and cooling (4°C) were accompanied by a gradual decrease in the counts of *V. parahaemolyticus* until the end of the experiment period (Tables 1 and 2). From Table 2, we show that there is a highly significant difference between the time exposure of fish meat at 25ºC and the count of bacteria. The room temperature was shown to be more effective in reducing the bacterial count than the cooling temperature. Physical, biological, and chemical treatment options are all viable options for preservation. Techniques such as high-pressure processing, vacuum sealing, and vacuum freezing are all examples of physical preservation; fermentation, which inhibits the growth of spoilage and harmful microbes, is an example of a biological therapy, whereas the use of antimicrobial drugs is an example of a chemical treatment (Chiozzi *et al*., 2022).

V. parahaemolyticus may live in shell stock oysters for at least three weeks at 4°C and then proliferate in as little as 2 or 3 days at 35°C. Similarly, 48 hours of storage at 5°C decreased the number of cells of *V. parahaemolyticus* in cooked fish mince and surimi, whereas storage at 25°C resulted in growth. According to the results of these analyses, naturally occurring vibrios may successfully reproduce in unshelled shell-stock oysters (DePaola *et al*., 2003).

Both *V. parahaemolyticus* and *V. vulnificus* required a minimum of 13°C and 11°C, respectively, to develop in broth. At temperatures between 13 and 30°C, flounder and salmon sashimi had significantly different lag times and specific growth rates (p=0.05) (Kim *et al*., 2012). *V. parahaemolyticus* in the concentration of APW in salt broth rose sharply above 15°C, dropped progressively between 0 and 5°C, then rose again above 15°C (Zhang *et al*., 2021)

However, the review published by Sheikh *et al*. (2022) states that almost half of the publications found growth to be correlated with rising temperatures, and nearly a third found the link to be more nuanced. There were additional reports of stunted development (6%), as well as no impact (9%). The majority of *Vibrio* spp. tested favorably responded to an increase in temperature, although others did not.

Regarding the freezing technique (-18°C), the results show that the highest bacterial count was found in fish meat in the first week after incubating at a freezing temperature of -20°C. While no bacterial growth was recorded at week 7 (Table 3).

Fresh aquatic products have a high nutrient density, but they are readily perishable. As an effective preservation method, freezing could help aquatic products maintain their quality and safety. Nonetheless,

mechanical damage caused by ice crystals and the oxidation of proteins and lipids would inevitably result in a decline in the frozen product's quality during freezing and preservation (Qiao *et al*., 2022)

Lund *et al*. (2000) mentioned that freezing generally causes a reduction in levels of vibrio during storage by 3 to 4 $\log^{10}$ units or greater. Recoveries after storage for 8 days at -20°C have been reported for *V. parahaemolyticus* in seafood, after 3 months in rock lobster tails inoculated at 10^4 to 10^6 per sample and stored at -18°C and, broth *V. parahaemolyticus* was recovered from pre-sterilized shrimp homogenate inoculated at approximately 10^8 g^{-1}.

Johnston and Brown (2002) reported that the number of cells was not diminished by freezing. Research into pasteurization at low temperatures revealed that *V. parahaemolyticus* was very resistant to heat. All three pathogens were killed by pasteurization at 70°C for 2 minutes. The heat tolerance of non-cultivatable cells was either the same or greater than that of normal cells.

Liu *et al*. (2009) showed that bacteria in seafood may be killed by storing it at 3°C for 7 days, freezing it, or subjecting it to low-temperature pasteurization and that at -18°C or -24°C, live cells can be inactivated for 15-28 weeks.

Mudoh *et al*. (2014) showed that *V. parahaemolyticus* may be killed or its development slowed by thermal treatment and cold storage (including refrigeration, icing, and freezing). Keeping meals at a low temperature (10°C) was an efficient way to prevent the spread of *V. parahaemolyticus*.

V. parahaemolyticus might be eliminated (3 MPN/g) after being frozen at -18 and -30°C for 15-30 days. The number of *V. parahaemolyticus* bacteria in APW-salt broth might be drastically reduced if frozen. They demonstrated that storing at -18°C was more effective than storing at -30°C in deactivating *V. parahaemolyticus*.

However, freezing is one of the most popular procedures since it prevents the development of most bacteria and the action of enzymes, two factors that may hasten spoilage in meat. Also, Wang *et al*. (2023) chicken breasts purchased frozen must be thawed and returned to temperatures between 2 and 4°C before being used for any other purpose.

However, the danger of quality decline is increased during tempering, therefore it is important to choose a technique that minimizes damage to the chicken breast. The structure of meat and other properties are severely altered after freezing, and the "fresh-keeping" effect is not fully realized (Cao *et al*., 2023).

While fresh aquatic goods are abundant in nutrients, they quickly go bad. The quality and safety of aquatic items may be better preserved by the use of freezing as a preservation technique. However, the quality of frozen food will certainly decrease during freezing and preservation due to mechanical damage induced by ice crystals and the oxidation of proteins and lipids (Qiao *et al*., 2022).

The survival of *V. parahaemolyticus* in moist heat temperature (cooking) is more effective in declining bacterial count. There are highly significant (p=0.000) differences and a negative correlation between the time and cooking temperature exposed to fish meat and *V. parahaemolyticus* bacterial count (Table 4).

According to Andrews *et al*. (2000), shell stock oysters infected with 105 MPN/g of *V. parahaemolyticus* were found to have non-detectable levels of the pathogen after being subjected to a 10-minute heat treatment at 50°C; this finding revealed that *V. parahaemolyticus* exhibited significant heat resistance. Virulence-causing *vibrios* may be killed by boiling shellfish to an interior temperature of at least 60°C for several minutes. After 10 seconds of heating, interior and external oyster flesh temperatures were found to be identical, as shown by temperature and pressure profiles. The venting pressure was 52% lower in the 100 g package despite taking 1.53 times as long as in the 50 g package. Total inactivation of both pathogens was accomplished after microwave, heating at 100% power for 50 seconds on 50 g and 60 seconds on 100 g of oyster flesh, respectively. The research proved the steam-venting package combined with microwave

heating could inactivate microorganisms via uniform heating, avoidance of overheating, and temperature maintenance (Espinoza Rodezno *et al*., 2023).

Yeung and Boor (2004) showed that at 48-50°C for 5 minutes, *V. parahaemolyticus* cells are no longer identifiable, indicating that the bacteria may be rendered harmless by adequate cooking of shellfish products. The amount of *V. parahaemolyticus* in APW-salt broth significantly dropped after being heated to 50°C, demonstrating the efficacy of heating therapy in reducing *V. parahaemolyticus* levels. However, after 10 minutes of heating the culture broth at 50 or 55°C, there were still 1034 CFU/mL of viable cells following the heat treatment. No viable cells were found in the culture broth at 60°C for 5 minutes, and at 70°C or higher for 1 minute while heating the culture cocktail at higher temperatures (60, 70, 80, 90°C) could accomplish a larger than log -6 decreases. *V. parahaemolyticus* infection may be reduced from 106 MPN/mL to a non-detectable level by cooking at 60°C for 5 minutes, 70°C for 2 minutes, and 80°C or higher for 1 minute.

V. parahaemolyticus was killed from 10,000 MPN/g to undetectable levels when exposed to temperatures of 60°C for 5 minutes, 70°C for 2 minutes, or 80°C or above for 1 minute, as specified. Because *V. parahaemolyticus* is so easily killed by heat, any trace of it in cooked food indicates that it was either undercooked or decontaminated (Zhang *et al*., 2021).

The microwave (dry temperature) effect on *V. parahaemolyticus* bacterial count depends on the time fish meat is exposed to dry heat and the temperature degree. Long-time exposure with increasing the dry temperature is more effective in declining bacterial count as shown in Table 5. There are high significant ($p=0.000$) differences between the time and temperature exposure of fish meat. To store a wide range of raw or partially pre-cooked meals, microwaveable containers with active venting technology have been created for fresh and frozen goods (Regier, 2014). However, microwave heating has the potential to replace traditional heating methods for killing off *V. vulnificus* and *V. parahaemolyticus*. Although microwaves may be used to prepare food, they are most often used as a fast-heating technique (Kubo *et al*., 2020).

After just 10 seconds of heating, the interior and external temperatures of the oyster flesh were found to be the same. The 100 g package required 1.53 times as much time to vent as the 50 g package, yet the venting pressure was 52% lower. Both viruses were completely inactivated after 50, and 60 seconds of microwave heating at 100% power on 50, and 100 grams of oyster flesh, respectively. A combination of steam venting and microwave heating shown to inactivate germs, heat evenly and avoid overheating the research (Espinoza Rodezno *et al*., 2023).

The final part of this study includes the impact of frying on *V. parahaemolyticus*. The results confirmed that the bacterial count depended on the time of exposing fish meat to frying and the degree of temperature. The long time exposure with increasing frying temperature is more effective in reducing bacterial count (Table 6). There are high significant ($p=0.001$) differences between the time and temperature.

In the study performed by Ong *et al*., 2023, the log range at which pathogens are killed by pan-frying is wide. It was determined to be between 1.1 and 7.0 on a scale used in virtual kitchens, achieved by Ye *et al*. (2012) a more cautious assessment of risk when compared to the cooking log decrease range of -2.5 to -7.5 estimated by Tan *et al*. (2019).

Conclusions

The presence of *Vibrio* species in seafood is a public health concern, particularly as long as seafood is used as a vector for their spread. Every year over the last decade, a new *Vibrio* species has emerged, posing a potential environmental health risk to humans. Monitoring and epidemiology, as well as studying the validity of different methods, including cold storage and heat treatment, are essential for establishing

efficient control measures to limit the risk of infection by this bacterium and assure the safety of foods. According to the results obtained in this study, the occurrence rate of *V. parahaemolyticus* in fish samples collected from Dhamar city was higher than recommended.

The *V. parahaemolyticus* isolates significantly declined in bacteria count when they were kept at 4°C and 25°C for a long time, and the *V. parahaemolyticus* isolates significantly declined in bacteria count manner when they were kept at -20°C for a long time. The high temperature and long-time exposure at 75°C/25 minutes by moist heat, 87°C/5 minutes by dry heat, and 70°C/20 minutes by frying heat were enough to kill *V. parahaemolyticus* isolates. In summary, consuming seafood after one of the above storage methods or heat treatments proved to be a good technique to reduce the risk of infections related to *V. parahaemolyticus* and reduce the causes of fish-borne pathogens.

References

Abioye OE, Osunla AC, Okoh AI, 2021. Molecular detection and distribution of six medically important *Vibrio* spp. in selected freshwater and brackish water resources in Eastern Cape Province, South Africa. Front Microbiol 12:617703.

Agriopoulou S, Stamatelopoulou E, Skiada V, Varzakas T, 2022. Nanobiotechnology in food preservation and molecular perspective. In: Parameswaranpillai J, Krishnankutty RE, Jayakumar A, Rangappa SM, Siengchin S, eds. Nanotechnology-enhanced food packaging. Wiley, Weinheim, Germany, pp 327-59.

Ahmed AR, 2021. Evaluation of the heavy metal content in the muscle tissue of common carp Cyprinus carpio L. reared in groundwater in Basrah province, Iraq. Iraqi J Vet Sci 35:157-61.

Almashhadany DA, 2020. Monitoring of antibiotic residues among sheep meats at Erbil city and thermal processing effect on their remnants. Iraqi J Vet Sci 34:217-22.

Anastasio A, Marrone R, Chirollo C, Smaldone G, Attouchi M, Adamo P, Sadok S, Pepe T, 2014. Swordfish steaks vacuum-packed with rosmarinus officinalis. Ital J Food Sci 26:390-7.

Andrews LS, Park DL, Chen YP, 2000. Low-temperature pasteurization to reduce the risk of vibrio infections from raw shell-stock oysters. Food Addit Contam 17:787-91.

Baker-Austin C, Oliver JD, Alam, M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J, 2018. Vibrio spp. infections. Nat Rev Dis Primers 4:1-19.

Beshiru A, Igbinosa EO, 2023. Surveillance of Vibrio parahaemolyticus pathogens recovered from ready-to-eat foods. Sci Rep 13:4186.

Cao R, Yan L, Xiao S, Hou B, Zhou X, Wang W, Bai T, Zhu K, Cheng J, Zhang J, 2023. Effects of different low-temperature storage methods on the quality and processing characteristics of fresh beef. Foods 12:782.

Carella F, Aceto S, Marrone R, Maiolino P, De Vico G, 2010. Marteilia refringens infection in cultured and natural beds of mussels (Mytilus galloprovincialis) along the Campanian coast (Tirrenian sea, South of Italy). Bull Eur Ass Fish Pathol 30:189-96.

Castello A, Alio V, Sciortino S, Oliveri G, Cardamone C, Butera G, Costa A, 2023. Occurrence and molecular characterization of potentially pathogenic Vibrio spp. in seafood collected in Sicily. Microorganisms 11:53.

Centers for Disease Control and Prevention, 2018. National outbreak reporting system (NORS). Available from: https://www.cdc.gov/nors/index.html.

Chiozzi V, Agriopoulou S, Varzakas T, 2022. Advances, applications, and comparison of thermal [pasteurization, sterilization, and aseptic packaging] against non-thermal ultrasounds, UV radiation, ozonation, high hydrostatic pressure technologies in food processing. Appl Sci 12:2202.

DePaola A, Nordstrom JL, Bowers JC, Wells JG, Cook DW, 2003. Seasonal abundance of total and pathogenic Vibrio parahaemolyticus in Alabama oysters. Appl Environ Microbiol 69:1521-6.

Espinoza Rodezno LA, Bonilla F, Reyes V, Janes M, Sathivel S, 2023. Inactivation of Vibrio vulnificus and Vibrio parahaemolyticus in cryogenically frozen oyster meat using steam venting technology. J Food Eng 340:111-28.

FAO, 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. Available from: https://doi.org/10.4060/cc0461en.

Fujino T, Okuno Y, Nakada D, Aoyama A, Mukai T, Ueho T, 1953. On the bacteriological examination of shirasu food poisoning. Med J Osaka Univ 4:299-304.

Geedipalli SSR, Rakesh V, Datta AK, 2007. Modeling the heating uniformity contributed by a rotating turntable in microwave ovens. J Food Eng 82:359-68.

Ha PTH, Thi QVC, Thuy NP, Luan NT, 2023. Multi-antibioticsresistance phenotype of pathogenicVibrio parahaemolyticus isolated from acute hepatopancreatic necrosis disease inLitopenaeus vannamei farmed in the Mekong Delta. J World Aquacult Soc 54:1070-87.
Haque N, Parveen S, Tang T, Wei J, Huang Z, 2022. Correction: Haque et al. Marine natural products in clinical use. Mar Drugs 20;528-636.
Hassan HS, Sule MI, Musa AM, Musa KY, Abubakar MS, Hassan AS, 2012. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. Afr J Tradit Complement Altern Med 9:250-5.
Iannotti LL, Blackmore I, Cohn R, 2022. Aquatic animal foods for nutrition security and child health. Food Nutr Bull 43:127-47.
Johnston MD, Brown MH, 2002. An investigation into the changed physiological state of Vibrio bacteria as a survival mechanism in response to cold temperatures and studies on their sensitivity to heating and freezing. J Appl Microbiol 92:1066-77.
Kamaruddin A, Nurhudah M, Rukmono D, Wiradana PA, 2021. Potential of probiotics Bacillus subtilis to reduce ammonia levels, *Vibrio* spp abundance, and increased production performance of Seaworm (Nereis sp) under laboratory scale. Iraqi J Vet Sci 35:757-63.
Kim YW, Lee SH, Hwang IG, Yoon KS, 2012. Effect of temperature on growth of Vibrio parahaemolyticus corrected and Vibrio vulnificus in flounder, salmon sashimi and oyster meat. Int J Environ Res Public Health 9:4662-75.
Kubo MTK, Siguemoto ES, Funcia ES, Augusto PED, Curet S, Boillereaux L, Sastry SK, Gut J, 2020. Non-thermal effects of microwave and ohmic processing on microbial and enzyme inactivation: a critical review. Curr Opin Food Sci 35:36-48.
Liu C, Lu J, Su YC, 2009. Effects of flash freezing, followed by frozen storage, on reducing Vibrio parahaemolyticus in Pacific raw oysters (Crassostrea gigas). J Food Prot 72:174-7.
Lund BM, Baird-Parker TC, Gould GW, 2000. The microbiological safety and quality of food. Aspen Publishers, Gaithersburg, MD, USA.
Matsuda S, Hiyoshi H, Tandhavanant S, Kodama T, 2020. Advances on Vibrio parahaemolyticus research in the postgenomic era. Microbiol Immunol 64:167-81.
Modlinska K, Pisula W, 2018. Selected psychological aspects of meat consumption - a short review. Nutrients 9:10-4.
Mudoh MF, Parveen S, Schwarz J, Rippen T, Chaudhuri A, 2014. The effects of storage temperature on the growth of Vibrio parahaemolyticus and organoleptic properties in oysters. Front Public Health 2:45.
Muringai RT, Mafongoya P, Lottering RT, Mugandani R, Naidoo D, 2022. Unlocking the potential of fish to improve food and nutrition security in sub-Saharan Africa. Sustainability 14:318.
Namad P, Deng Z, 2023. Optimum environmental conditions controlling prevalence of vibrio parahaemolyticus in marine environment. Mar Environ Res 183:105828.
Ong HMG, Zhong Y, Hu CC, Ong KH, Khor WC, Schlundt J, Aung KT, 2023. Quantitative risk evaluation of antimicrobial resistant Vibrio parahaemolyticus isolated from farmed grey mullets in Singapore. Pathogens 12:93.
Qiao Z, Yin M, Qi X, LI Z, Yu Z, Chen M, Xiao T, Wang X, 2022. Freezing and storage on aquatic food: underlying mechanisms and implications on quality deterioration. Food Sci Technol 42:e91322.
Raghunath P, 2015. Roles of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in Vibrio parahaemolyticus. Front Microbiol 5:805.

Regier M, 2014. Microwavable food packaging. In: Jung HH, ed. Innovations in food packaging. 2nd ed. Academic Press, Cambridge, MA, USA.
Saad SM, Samir MM, Abd El Maksod HE, 2015. Incidence of Vibrio species in fish with special emphasis on the effect of heat treatments. BVMJ 29:38-44.
Sheikh HI, Najiah M, Fadhlina A, Laith AA, Nor MM, Jalal KCA and Kasan NA, 2022. Temperature upshift mostly but not always enhances the growth of Vibrio species: a systematic review. Front Mar SCI 9:959830.
Smaldone G, Abollo E, Marrone R, Bernardi CEM, Chirollo C, Anastasio A, del Hierro SP, 2020. Risk-based scoring and genetic identification for anisakids in frozen fish products from Atlantic FAO areas. BMC Vet Res 16:65.
Tan CW, Malcolm TTH, Premarathne JMKJK, New CY, Kuan CH, Thung TY, Chang WS, Loo YY, Rukayadi Y, Nakaguchi, Y, 2019. Preliminary quantitative microbial risk assessment of pathogenic Vibrio parahaemolyticus in short mackerel in Malaysia. Microb Risk Anal 12:11-9.
Taragusti A, Prayogo P, Rahardja BS, 2020. The effect of stocking density and the application of Nitrobacter as ammonia decomposer in aquaponics system of Clarias gariepinus with water spinach (Ipomoea aquatic). Iraqi J Vet Sci 35:217-22 .
UN, 2022. World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100. Available from: https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100.
Wang D, Fletcher GC, On SLW, Palmer JS, Gagic D, Flint SH, 2023. Biofilm formation, sodium hypochlorite susceptibility and genetic diversity of Vibrio parahaemolyticus. Int J Food Microbiol 16:385:110011.
Ye M, Huang Y, Chen H, 2012. Inactivation of Vibrio parahaemolyticus and Vibrio vulnificus in oysters by high-hydrostatic pressure and mild heat. Food Microbiol 32:179-84.
Yeung M, Thorsen T, 2016. Development of a more sensitive and specific chromogenic agar medium for the detection of Vibrio parahaemolyticus and other Vibrio species. J Vis Exp 8:54493.
Yeung PS, Boor KJ, 2004. Epidemiology, pathogenesis, and prevention of foodborne Vibrio parahaemolyticus infections. Foodborne Pathog Dis 1:74-88.
Zhang Y, Li F, Yao Y, He J, Tang J, Jiao Y, 2021. Effects of freeze-thaw cycles of Pacific white shrimp (Litopenaeus vannamei) subjected to radio frequency tempering on melanosis and quality. Innov Food Sci Emerg Technol 74:102860.

Table 1. *Vibrio parahaemolyticus* count after preservation of fish meat at room temperature (21°C) for 3 days.

Dependent variable	Day	N	Mean of bacterial count n=(n ×10^3)	SD	p
Time of preservation	Day 0	6	198.2	9.24	0.000
	Day 1	6	192.8	9.64	
	Day 2	6	176.0	7.80	
	Day 3	6	152.0	5.25	

SD, standard deviation.

Table 2. *Vibrio parahaemolyticus* count after preservation of fish meat at Refrigerator (4°C) for three days with one day sampling interval.

Dependent variable	Day	N	Mean of bacterial count = (n ×10^3)	SD	p
Time of exposure	Day 0	6	198.2	9.24	0.004
	Day 1	6	192.8	10.89	
	Day 2	6	182.5	5.65	
	Day 3	6	178.0	9.53	

SD, standard deviation.

Table 3. Weekly count of *Vibrio parahaemolyticus* in fish meat at freezing (-18°C)

Dependent variable	Week	N	Mean of bacterial count =(n ×10^3)	SD	p
Time of exposure	Week 0	6	198.2	9.24	0.000
	Week 1	6	180.8	13.48	
	Week 2	6	146.2	7.19	
	Week 3	6	96.3	9.22	
	Week 4	6	44.2	16.68	
	Week 5	6	11.7	4.76	
	Week 6	6	1.0	1.55	
	Week 7	6	0.0	0.00	

SD, standard deviation.

Table 4. Impact of cooking (moist heat) on *Vibrio parahaemolyticus*.

Dependent variable	Moist heat temperature	N	Mean of bacterial count =(n ×10^3)	SD	p
Temperature of exposure	50°C/5 minutes	6	164.3	16.76	0.000
	55°C/10 minutes	6	128.7	12.89	
	65°C/15 minutes	6	106.8	6.76	
	70°C/20 minutes	6	58.5	22.06	
	75°C/25 minutes	6	22.3	24.71	

SD, standard deviation.

Table 5. Impact of microwave (dry heating) on *Vibrio parahaemolyticus.*

Dependent variable	Dry heat temperature	N	Mean of bacterial count =(n ×10^3)	SD	p
Temperature of exposure	55°C/1 minute	6	126.0	19.30	0.000
	63°C/2 minutes	6	85.0	16.55	
	71°C/3 minutes	6	34.5	14.04	
	79°C/4 minutes	6	2.5	1.38	
	87°C/5 minutes	6	0.0	0.00	

SD, standard deviation.

Table 6. Impact of frying on *Vibrio parahaemolyticus.*

Dependent variable	Frying heat temperature	N	Mean of bacterial count =(n ×10^3)	SD	p
Temperature of exposure	50°C/5 minutes	6	25.3	20.59	0.001
	55°C/10 minutes	6	10.0	9.08	
	65°C/15 minutes	6	4.8	5.38	
	70°C/20 minutes	6	0.0	0.00	
	75°C/25 minutes	6	0.0	0.00	

SD, standard deviation