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Microbiological Quality of Cold-smoked Herring (*Clupea harengus*) Roe

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Abstract

Smoked fish roes within cold smoked herring are considered a very popular ready-to-eat food in Egypt. Therefore, the microbial quality of fish roes should be of concern. The purposes of this study were to conduct bacterial analysis and the possibility of detection of foodborne pathogens. With limited application of dry heating at 85°C for 1 and 5 minutes to improve the safety of roes. To study the biogenic amines (BA) including spermine, putrescine, cadaverine, histamine, tyramine, and spermidine in fresh, heated at 85°C for 5 min, and stored at 0°C for 6 months smoked roes. *Vibrio* spp. including *Vibrio vulnificus*, *Vibrio furnissii*, and *Vibrio carcbariae* were found in 5 samples (9.6%). One *Salmonella* spp. (1.9%) and one *Listeria monocytogenes* (1.9%), 4 *Staphylococcus aureus* (7.7%), and 3 *Bacillus cereus* (5.8%) pathogens. While *Clostridium botulinum* (vegetative forms) was not detected. None of the aerobic, anaerobic, or selective bacterial counts existed after heating smoked roes at 85°C for 5 minutes. The average of each BA in all samples was below 3mg/100g, which is less than the 5.0 mg/100g acceptable limit recommended by the US Food and Drug Administration. Histamine and tyramine only appeared after cold storage of roes at 0°C for 6 months. Cold smoked herring roe was low in acidity (pH: 5.92), high in moisture (68.5%), protein (61.37%), and lipid content (28.7%), thus it could support the bacteria growth. Additional measures are needed to reduce the possible health risks for fish roe consumers.

KEYWORDS
Cold smoked, Herring, Roe, Bacteria, Histamine

INTRODUCTION

Smoked roe within the cold-smoked herring (*Clupea harengus*) is one of the common fish roes processed seafood products in Egypt (Amin *et al*., 2022). Fish roe has been a delicacy food since ancient history, as it is rich in proteins, lipids, calcium, iron, and selenium. With a high content of vitamins B, A complex, C, and E, and is full of omega-3 fatty acids (Sidhu, 2003; Rosa *et al*., 2013; Piras *et al*., 2014). Fish roe product quality depends mainly on the roe's size and maturity. Both are influenced by the size, age, spawning stage, and fishing season of the female fish. Egg size particularly can distinguish caviar from non-caviar fish roe products, as the latter has a diameter of less than 1.4 mm (Bekhit, 2010).

Like other fish products, it might be spoiled and transmit foodborne pathogens. These bacteria might be initiated from the fish gut flora or contaminate the roes during handling and processing in poor hygienic measures (Voidarou *et al*., 2011). According to Egyptian standards, fish roe should be free from pathogens including *Clostridium*, *Salmonella*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *E. coli* (EOS, 2005). Because of a lack of hygiene, Voidarou *et al.* (2011) reported that several pathogenic bacteria were isolated from salted grey-mullet roes like *V. parahaemolyticus*, *Salmonella* spp., Aeromonas hydrophila, *Clostridium perfringens* (vegetative forms), *Listeria* spp., and *E. coli*.

Furthermore, fish roes could have the potential for multiple food safety risks. Studies of histamine toxicity caused by fish roe ingestion were reported, especially when not properly processed or stored (Kung *et al*., 2008; Lapa-Guimarães *et al*., 2011). Elevated histamine level in foods causes vasoactive effects and severe toxicity in humans might lead to death (Lehane and Olley, 2000; Yu *et al*., 2018; Zhernov *et al*., 2023). Biogenic amines are formed by the decarboxylation of free amino acids by decarboxylase enzymes released from bacterial contamination (Rawles *et al*., 1996). Both Scrombroid and non-scombroid fish have also been related to histamine poisoning, especially after temperature abuse (Ahmed, 2019; Taylor, 1986).

Seafood might be contaminated with bacteria during handling or processing. Plus other factors such as storage, temperature abuse, infected food handlers, and cross-contamination. seafood is also frequently eaten raw or processed in ways that do not eliminate bacteria. In these previous circumstances, fish roe could support bacterial growth and cause serious food safety risks. In this study, we aimed to examine fish roe for bacteria inside cold-smoked herring and the possibility of detection of *Vibrio* spp., *Salmonella*, *Listeria* spp., *Clostridium botulinum*, *Staphylococcus aureus* pathogens. Apply limited thermal processing at 85°C for 1 and 5 minutes to advance the safety of the fish roe product. Study the presence of 6 biogenic amines (BA): spermine, putrescine, cadaverine, histamine, tyramine, and spermidine in fresh smoked roes, heated at 85°C for 5 min., and roes stored at 0°C for 6 months.

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MATERIALS AND METHODS

Sampling

Smoked roe samples (n.= 52) were collected from commercial cold-smoked herring during summer, winter, and spring seasons from 2021-2022. Samples were divided into three sets of 17 each. All fish were transferred to the College of Fish Recourses laboratory, Suez Canal University for analysis. Roes were aseptically extracted, refrigerated under complete aseptic conditions, and analyzed immediately.

Physicochemical analysis

pH

All cold-smoked herring fish roes (52 samples) were diluted (1:10) with distilled deionized water and measured separately using a pH meter (OHAUS, USA) (Bunga *et al*., 2022).

Proximate analysis

Protein, fat, ash, and moisture contents of samples were analyzed in triplicates (AOAC, 2007). Moisture contents were estimated by dry heating the sample in a vacuum oven at 105˚C/24 h. The moisture percentage was calculated as [(wet sample–dried sample)/wet sample]*100. Samples were incinerated at 650˚C/5 h in a muffle furnace to analyze the ash content by (Weight after ashing/Weight before ashing)*100. The micro-biuret method was used to determine the protein percentage, while the Soxhlet extraction method using hexane at 60°C/9 h was used to estimate the fat content (Boyer, 1993; V'uorela *et al*., 1979; Hajji *et al*., 2014).

Bacterial analysis

Detection of *Vibrio* spp.

Roes (5 g) were placed in a sterilized plastic bag with 1:10 dilution of alkaline peptone (lab M, UK) with NaCl (1%- Biotech, Egypt). Samples were stomached (2 min) (Seward 400 circulator, UK), incubated (37°C for 24 h), and platted on thiosulphate-citrate bile salts sucrose with 1% NaCl (TCBS, TPC, India). After incubation, green and yellow colonies were cultured on trypticase soy agar slants containing 1% NaCl (TSA-Lab M, UK). The isolates were tested biochemically by Indole (I), Methyl red (MR, Condalab, Madrid), Vogues Proskauer (VP, Condalab, Madrid), and detection of growth under different salt conc. (0, 6 and 8%). Suspected *Vibrio*s were confirmed by API 20E strips (BioMérieux, France), and results codes were translated from a computer database provided by the manufacturer (Voidarou *et al*., 2011; Kaysner *et al*., 2004).

Detection of Salmonella spp.

Samples (5 g) were diluted with 45ml of 0.1% sterile peptone water for pre-enrichment then incubated at 35±2°C/24 h and cultured in selective enrichment in tetrathionate broth (Lab M, UK). Platted on xylose lysine desoxycholate citrate agar (XLD, 7166A acumedia). Suspect colonies with characteristic reactions on triple sugar iron (TSI), I, MR, VP, and citrate (Lab M, UK) were confirmed by API 20E (BioMérieux, France) (Voidarou *et al*., 2011; González-Rodríguez *et al*., 2002).

Detection of *Listeria monocytogenes*

Roes (5 g) were enriched in buffered *Listeria* enrichment broth base, UVM-Fraser (EcoBio, bio-lab) for 24h/35°C, then plated on *Listeria* Oxford medium agar (Himedia, M1145-500G). Suspect colonies were plated in Trypticase soy agar with yeast extract (0.6%-TSAYE). Then identified by catalase activity, Rhamnose fermentation on purple carbohydrate fermentation broth containing 0.5% rhamnose, and β hemolysis on blood agar (Oxoid, England) containing 5% sterile blood (González-Rodríguez *et al*., 2002; Hitchins *et al*., 2022).

Detection of *Staphylococcus aureus*

Samples (5 g) were enriched in 45ml of 0.1% sterile peptone water, incubated at 35±2°C/24h, and plated on mannitol salt agar (Lab M, UK). Suspected colonies were identified with Gram staining, catalase, and β hemolysis on blood agar containing 5% sterile blood (Scano *et al*., 2013).

Detection of *Clostridium botulinum*

Cooked meat medium (Alpha chemica, India) was used as enrichment broth. The medium was steamed for 10-15 min and cooled rapidly without agitation before inoculation. Roe samples (2 g) were added to the enrichment broth (15 ml) and incubated at 35°C/5 days in anaerobic jars supplied with CO_2 packs. Turbid broth media with gas production was plated on anaerobic egg yolk agar (TPC, India) and incubated at 35°C/48 h under anaerobic conditions. The morphology of suspected colonies was examined by Gram staining to observe typical tennis racket-shaped clostridial cells (Solomon and Lilly, 2001).

Smoked fish roes (11 samples) were heated in the oven at 85°C for 1 and 5 minutes. Aaerobic plate count was performed in samples before thermal processing as control and after thermal processing for 1 and 5 minutes. Samples (5 g) of smoked roe with 1:10 dilutions with 0.1% peptone water were stomached for 2 minutes. Homogenate was further diluted up to 5 dilutions. Dilutions were spread-plated on plate count agar media (Lab M, UK) in duplicates and incubated at 35±2°C/24 h. (Maturin and Peeler, 2001). While other selective media such as TCBS, XLD, *Listeria* Oxford medium agar, mannitol salt agar, and MacConkey Agar are used for *Vibrio*, *Salmonella*, *Listeria*, *Staphylococcus*, and *Enterobacteriaceae* count, respectively. Anaerobic plate count was performed using anaerobic egg yolk agar and incubated at 35°C/48 h under anaerobic environments.

Identification of bacteria by PCR and 16S rRNA gene sequencing

Identified Gram-positive isolated bacteria were confirmed using PCR and rRNA gene Sequencing.

DNA extraction

According to Azwai *et al*. (2016), DNA was extracted using DNA extraction kit (Bioscience, Germany). 16S rDNA was amplified using universal primers- (forward: 5′-GAGTTTGATCCTGGCTTAG-3′ and reverse: 5′-GGTTACCTTGTTACGACTT-3′).

DNA sequencing

QIAquick Kit (Qiagen, Germany) was used to purify the PCR products, and the second PCR was made using the BigDye-Terminator v3.1 cycle, sequencing kit. Genetic Analyzer (3500, Applied

Biosystems, Massachusetts, USA) was used for DNA sequencing. The obtained sequences were blasted through NCBI- Blast search and concluded by the Mega program (7.0.20). The genetic sequences were sent to the NIH GenBank (the National Library of Medicine database), and their accession numbers were documented (Iwatsuki *et al*., 2021).

Biogenic amines analysis

Cold smoked herring roes including fresh cold smoked (control), immediately heated in the oven at 85°C/5 min and smoked and stored at 0°C/6 months were analyzed for biogenic amines in duplicates. according to Deabes *et al*. (2018), Six Biogenic amines involving spermine, putrescine, cadaverine, histamine, tyramine, and spermidine were extracted.

Biogenic amines were determined using high-performance liquid chromatography (HPLC) prepared with a "Waters 600" delivery system. Using reverse phase C18 Nucleosil column: 250 x 4 mm, 10 µm packing, (Macherey - Naggl), and a UV detector (waters 486) at 254 nm wavelength. Constant solvent flow rate was performed using a linear program of 25 min period and 1 ml/min. Data were analyzed using Millennium Chromatography, Manger Software 2010 (Waters, Milford MA 01757).

Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics version 25 (IBM Corporation, NY, USA). Results were expressed as the mean±SD.

RESULTS AND DISCUSSION

Smoked fish roes are considered highly nutritious food. It is rich in protein (61.37±1.84) and lipids (28.7±2.83), while it has low carbohydrate content (4.3±0.42) (Table 1). The mean value of the pH of all cold-smoked roes samples was recorded as 5.92±0.08. The pH results for all samples were within the limits (5.5-6) permitted by Egyptian for fish roe (EOS, 1996). In the same way, Caredda *et al*. (2018) previously recorded the pH of salted and dried *Mugil cephalus* roes as 5.46. Similarly in Taiwan, the pH of salted mullet roe fish products was reported as 4-5.8 (Kung *et al*., 2008). The moisture content of roes in this study was recorded as 68.5±2.12 (Table 1). Salmon roe was low in acidity with high water activity with high nutrient content, thus it can support the growth of different bacteria (Bledsoe *et al*., 2003). Therefore, with bad handling, processing, and unsanitary conditions, fish roe could transmit foodborne pathogens.

Table 1. Mean values of physicochemical properties in cold-smoked herring fish roes.

	*A*verage
pH	5.92 ± 0.08
Moisture %	68.5 ± 2.12
Dry weight analysis	
Protein%	61.37 ± 1.84
Lipids%	28.70 ± 2.83
Carbohydrate%	4.30 ± 0.42
Ash %	5.63 ± 0.17
Total	100%

In this study, several pathogens were detected in the microbiological analysis of smoked fish roes' samples that were extracted from commercial cold smoked herring fish. *Vibrio* spp. including *Vibrio vulnificus*, *Vibrio furnissii*, and *Vibrio carcbariae* were found in 5 samples (9.6%). One *Salmonella* spp. (1.9%) and one *Listeria monocytogenes* (1.9%) were documented, plus 4 *Staphylococcus aureus* (7.7%), and 3 *Bacillus cereus* (5.8%) pathogens. In addition, several *Enterobacter cloacae* (11.5%) were recorded, and *Clostridium botulinum* was not found (Table 2).

Table 2. The number and proportion of positive samples for selected bacteria species in cold-smoked herring roes (N = 52).

Bacteria spp.	N (%)	Confirmation
Vibrio vulnificus	1 (1.9)	API 20E
Vibrio furnissii	3 (5.8)	API 20E
Vibrio carcbariae	1 (1.9)	API 20E
Salmonella spp.	1 (1.9)	API 20E
Listeria monocytogenes	1 (1.9)	-
Clostridium botulinum	not detected	-
Staphylococcus aureus	4 (7.7)	-
Bacillus cereus	3 (5.8)	Genbank accession numbers for nucleotide sequences: ON332031, ON332032, and ON332033.
Enterobacter cloacae	6 (11.5)	API 20E

Similarly, Voidarou *et al*. (2011) analyzed salted grey-mullet roe and detected *Vibrio* spp., Aeromonas hydrophila, and *Salmonella* spp. in one sample (2%). Three samples (6%) had *Clotriduim perfringens* (vegetative forms), *E. coli*, and spores of *Bacillus* spp. *Listeria* spp. and *Staphylococcus aureus* were identified from 10%, and 8%, respectively of the samples. In other salted roe samples (194) of the gray *Mugil cephalus*, *Clostridium perfringens*, *Enterobacteriaceae*, and *Enterococcus* spp. were identified (Brandas *et al*., 2015).

S. aureus (7.7%) contamination in fish roe samples in this study (Table 2) might be attributed to human contact. Fish roes were previously recorded associated with *S. aureus* 4% by Brandas *et al*. (2015). During handling and processing, and with human contact, or contaminated processing equipment contact, contamination in bottarga was documented by Brandas *et al*. (2015), and Shimizu *et al*. (2007). Food processing-plants have also been reported associated with *L. monocytogenes* contamination of RTE seafood (Autio *et al*., 1999). Fish roe fish products processing usually require more handling compared to other raw seafood products, which increase the possibilities of cross-contamination. Proven by previous studies documented the high contamination frequencies of *L. monocytogenes* in fish roes up to 10.0 to 11.4% (Handa *et al*., 2005). In Japan, *L. monocytogenes* contamination revealed up to 12.1% in salmon fish roe RTE products, and cell number increased rapidly under inappropriate storage temperatures (Miya *et al*., 2010).

Pathogenic *Vibrio* spp. cause outbreaks and illnesses usually recovered from raw or underdone seafood (Iwamoto *et al*., 2010). In Maryland, USA retail stores, total *Vibrio* species prevalence was 4.5% in many seafood including shrimp, tilapia, and catfish (Elbashir *et al*., 2023). *V. parahaemolyticus* were identified from 1.3% of herring salted roe samples (Chiţu *et al*., 1977). Likewise, Five *Vibrio* species (9.6%) were identified in this study from smoked fish roe including one *Vibrio vulnificus* (1.9%) (Table 2). *V. vulnificus* inhabits warm brackish seawater. It was recently reported in six patients in the Ningbo, China eating raw or underdone seafood (Wang *et al*., 2023). Recent studies reported the elevated surface water temperatures because of global warming boosted

the spreading of *V. vulnificus* (Lin *et al*., 2021). This was in coordination with our results, as *V. vulnificus* was identified in smoked fish roe in the summer season (Fig. 1). Increased seawater temperature backs the growth and distribution of different bacterial populations to cause illnesses (Matanza and Osorio, 2018).

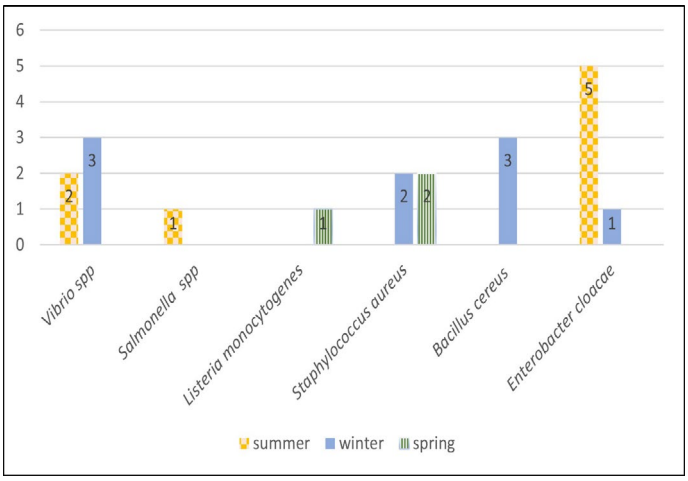

Fig. 1. Seasonal pattern of isolated bacteria from cold-smoked herring roe.

Salmonella is the subsequent utmost public bacterial agent that causes foodborne diseases and multi-state outbreaks in the U.S. food system (CDC, 2017). Seafood-borne illnesses have directed food authorities to apply a zero-tolerance for *Salmonella*. In this work, *Salmonella* spp. has been identified in one sample (1.9%) (Table 2). Like the finding of one *Salmonella* spp. in grey mullet salted roe by Voidarou *et al*. (2011). While was not detected in other salted and dried products of tuna roe (bottarga) (Scano *et al*., 2013). In our study, one *Salmonella* spp was detected in the summer season (Fig. 1). In agreement with Bassal *et al*. (2023), the nontyphoidal salmonellosis incidence rates were consistently high from June to September, while decreased between December and February winter months (Bassal *et al*., 2023). As high temperatures prosper *Salmonella* growth, and people's attitudes in summer vacation with eating outside might increase the risk of foodborne illnesses. The relation between salmonellosis and the air temperature was documented by Kynčl *et al*. (2021) in the Czech Republic. Recording that each 1°C increase in air-temperature led to a 6.2% elevated salmonellosis level.

Bacillus cereus group is associated with diarrheal and emetic food intoxication. In this work, three (5.8%) *B. cereus* were confirmed by 16S rRNA gene sequencing (Genbank accession numbers: ON332031, ON332032, and ON3320330) (Table 2). Previously, in Taiwan fishing ports, *B. cereus* was recorded in the seawater samples (7.9%) and shellfish products (0.68%) (Hsu *et al*., 2021). Suggesting seawater as a source of contamination. In addition, herbs and spices might considered as a source of *Bacillus* spp. As *Bacillus* spp was reported as the main pathogen in red pepper powder (Bi Jeon *et al*., 2020). *B. cereus* can endure cooking temperatures, grow and multiply, and consequently cause food intoxication (Van Doren *et al*., 2013). Plus, it can survive harsh environments such as cold weather, and that explains the occurrence of *B. cereus* in the winter season (Fig. 1).

Clostridium botulinum was not detected in this study from smoked herring roe samples. In contrast, *C. botulinum* (type E) was identified in whole fish and roe ranged from 4-14% in Finland's commercial importance (Hyytiä *et al*., 1998). *Clostriduim perfringens* (vegetative forms) have been identified in salted grey mullet roe and salted gray *Mugil cephalus* "bottarga"(Voidarou *et al*., 2011; Brandas *et al*., 2015). *Enterobacter cloacae* were also identified in this work (Table 2). *E. cloacae* is a common bacterium in healthy human gut microflora (Leong *et al*., 2017), however, it also causes nosocomial infections (Galetti *et al*., 2022). Plus, it is associated with seafood contamination, especially with unhygienic measures (Almeida *et al*., 2018).

Foodborne pathogens might exist in the final products due to poor handling, inadequate processing, unsanitary practices, and cross-contamination. In this research, we applied extra thermal processing of RTE cold smoked herring roes at 85°C for 1 and 5 minutes. Aerobic and anaerobic plate count results and counts of previous pathogens all indicated the best result of the complete absence of bacteria at 85°C for 5 minutes (Table 3). Aerobic plate counts previously recorded extended from less than 1.0 to 7.1 log CFU/g in salted mullet roe in Taiwan (Kung *et al*., 2008). The same recommendation of using mild heat (60°C/20min) with other combined treatments recorded effectively inactivated *B. cereus* without incurring quality deterioration in the food processing

Table 3. Counts of different bacteria (Log CFU/g) of cold-smoked herring fish roes after thermal processing at 85 °C for 1 and 5 minutes.

Bacterial count	*Control*	*Bacterial count at 85 °C for 1 min*	*Bacterial count at 85 °C for 5 min*
Aerobic plate count	2.3 ± 0.32	1.1 ± 0.21	nt
Enterobacteriaceae	2.05 ± 0.43	1.09 ± 1.03	nt
Vibrio	1.79 ± 0.43	< 1 (0.86 ± 1.21)	nt
Salmonella	1.55 ± 0.13	nt*	nt
Listeria	1.55 ± 0.13	< 1 (0.54 ± 0.78)	nt
Staphylococcus	2.31 ± 0.54	1.03 ± 0.99	nt
Anaerobic bacteria	2.36 ± 0.05	< 1 (0.89 ± 0.85)	nt

*nt: not detected

Table 4. Biogenic amines content (mg/100g) in cold-smoked herring roes including fresh smoked, heat processed at 85°C/5 min, and stored at 0°C/6 months.

Biogenic amines	Fresh cold smoked roe (Control)	Fresh and heat processed at 85°C for 5 min.	Stored at 0°C for 6 Mon.
Spermine	0.117 ± 0.21	nt	0.25
Putrescine	nt *	nt	nt
Cadaverine	0.820 ±11.59	nt	nt
Histamine	nt	nt	2.77
Tyramine	nt	nt	0.34
Spermidine	nt	nt	nt

*nt: not detected

plants (Bi Jeon *et al*., 2020). Likewise, the application of mild heat ranged from 58, 61, and 62.5°C for salmon samples combined with salting achieved at least 3 log-reduction in *L. monocytogenes* contamination (Hansen *et al*., 2021). Based on our results (Table 3), heat application at 85°C for 5 minutes is recommended to confirm the safety of RTE cold smoked herring fish roes.

The levels of biogenic amines (BA) in seafood are considered as an indicator of spoilage during storage and a quality index (Baixas-Nogueras *et al*., 2005). The average content of BA in all samples was less than 3 mg/100g (Table 4), which is less than the permitted limit suggested by the US Food and Drug Administration (5.0 mg/100g). Only spermine (0.117 mg/100g) and cadaverine (0.820 mg/100g) were detected in control samples, while histamine and the other amines were not found (Table 4). Likewise, Atmaca *et al*. (2023) reported that spermine levels exhibited fluctuations and cadaverine was inconsistent in farmed rainbow trout during cold storage (0, 2 and 4°C) and both were not considered effective markers of freshness. Heating roes at 85°C for 5 min eliminated the bacteria as explained before in Table 3, and that could explain the absence of all 6 BA after heating. Because the creation of BA depends on the bacterial contamination and bacterial decarboxylation of free amino acids (Kočar *et al*., 2021).

After cold storage of roes at 0°C for 6 mon, spermine increased to 0.25 mg/100g, and histamine and tyramine appeared (2.774, 0.344 mg/100g, respectively) as shown in Table 4. Histamine poisoning is the utmost common food intoxication related to BA. In this study, histamine was within the safe level (<5 mg/100g). Other amines can raise the toxicity of histamine or react with nitrites to form carcinogenic nitrosamines (Kočar *et al*., 2021). Parallel results were recorded by Restuccia *et al*. (2015), as histamine level in mullet bottarga after 6 months of storage at 4-8°C was 2.7 mg/100g. Kung *et al*. (2008) analyzed 16 salted mullet bottarga in Taiwan, only one sample had a higher histamine content (8.18 mg/100g) than the permissible limit.

CONCLUSION

Microbiological evaluation of smoked roes extracted from cold smoked herrings revealed multiple foodborne pathogens, and according to Egyptian standards, it should be free from foodborne pathogens. Heating at 85°C for 5 min. eliminated all bacteria. Biogenic amines and histamine levels were within safety permits. Additional safety limits should be applied during the handling and processing of fish roe to minimize potential risks to public health.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Ahmed, O.M., 2019. Histamine and Other Biogenic Amines Formation in Canned Tuna Fish Inoculated with *Morganella morganii* or Proteus mirabilis in Determining Food Safety during Temperature Abuse. Nutrition and Food Toxicology 3, 690-700.

Almeida, M.V.A., Brito, I.L.P., Carvalho, A.L.S., Costa, R.A., 2018. In vitro resistance of *Enterobacter cloacae* isolated from fresh seafood to colistin. Rev. Soc. Bras. Med. Trop. 51, 674-675. doi: 10.1590/0037-8682-0287-2017. PMID: 30304276.

Amin, H.F., Carne, A., Bekhit, A.E.D.A., 2022. Fish roe products: middle east perspective. In: Fish Roe. Academic Press. pp. 283-310.

AOAC, 2007. Association of Official Analytical Chemists Official Methods of Analysis, 18th ed., Gaithersburg.

Atmaca, E., Alkan, A., Das, Y.K., Aksoy, A., 2023. Evaluating biogenic amines and pH levels in farmed rainbow trout (*Oncorhynchus mykiss*) for freshness assessment during storage. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 40, 770-780. doi: 10.1080/19440049.2023.2218941

Autio, T.S., Hielm, M., Miettinen, A.M., Sjöberg, K. Aarnisalo, J., Björkroth, T., Sandholm, M., Korkeala, H., 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl. Environ. Microbiol. 65, 150-155.

Azwai, S.M., Alfallani, E.A., Abolghait, S.K., Garbaj, A.M., Naas, H.T., Moawad, A.A., Gammoudi, F.T., Rayes, H.M., Barbieri, I., El-daghayes, I.M. 2016. Isolation and molecular identification of *Vibrio*s by sequencing of 16S rDNA from seafood, meat, and meat products in Libya. Open Vet. J. 6, 36-43. https://doi.org/10.17795/ajcmi-23233.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M.T. Mariné-Font, A., Vidal-Carou, M.C., 2005. Biogenic Amine Index for Freshness Evaluation in Iced Mediterranean Hake (*Merluccius merluccius*). J. Food Prot. 68, 2433–2438. doi: 10.4315/0362-028X-68.11.2433.

Bassal, R., Davidovich-Cohen, M., Yakunin, E., Rokney, A., Ken-Dror, S., Strauss, M., Wolf, T., Sagi, O., Amit, S., Moran-Gilad, J., Treygerman, O., Karyo, R., Keinan-Boker L., Cohen, D., 2023. Trends in the Epidemiology of Non-Typhoidal Salmonellosis in Israel. Int. J. Environ. Res. Public Health 20, 5626. doi: 10.3390/ijerph20095626.

Bekhit, A.E.D., 2010. Fermentation of Fish Roe. In: The encyclopedia of biotechnology in agriculture and food. Heldman, D.R., Hoover, D.G., Wheeler, M.B., Eds. Taylor Francis Publishing. pp. 251-255.

Bi Jeon, E., Choi, M.S., Kim, J.Y., Park, S.Y., 2020. Synergistic Effects of Mild Heating and Dielectric Barrier Discharge Plasma on the Reduction of *Bacillus cereus* in Red Pepper Powder. Foods 11, 171. doi: 10.3390/foods9020171.

Bledsoe, G.E., Bledsoe, C.D., Rasco, B., 2003. Caviars and fish roe products. Critical Reviews in Food Science and Nutrition 43, 317-356.

Boyer R.F. 1993. Modern Experimental Biochemistry, 2nd ed.; Benjamin Cummings Series in the Life Sciences and Chemistry, California, pp. 51 – 55.

Brandas, V., Orrù, G., Carraro, V., Sanna, A., Brajon, G., Salati, F., Sanna, C., Ciusa, M.L., Meloni, M.and Coroneo, V., 2015. Recovery of *Staphylococcus aureus* in Gray *Mugil cephalus* Roe (Bottarga): Investigation by an Integrated Cultural/Molecular Approach. J. Food Sci. 80, 1285-90. doi: 10.1111/1750-3841.12891.

Bunga, S.J., Ahmmed, M.K., Lawley, B., Carne, A., Bekhit, A.E.A., 2022. Physicochemical, biochemical and microbiological changes of jeotgal-like fermented Chinook salmon (*Oncorhynchus tshawytscha*) roe. Food Chem. 398, 133880. doi: 10.1016/j.foodchem.2022.133880.

Caredda, M., Addis, M., Pes, M., Fois, N., Sanna, G., Piredda, G., 2018. Physico-chemical, colorimetric, rheological parameters and chemometric discrination of the origin of *Mugil cephalus* roes during the manufacture process of bottarga. Food Research International 108,128-135.

CDC (Centers for Disease Control and Prevention), 2017. Reports of *Salmonella* Outbreak Investigations from 2017. Available online: https://www.cdc.gov/foodsafety/pdfs/2015foodborneoutbreaks_508.pdf

Chiţu, M., Ciufecu, C., Năcescu, N., 1977. The isolation and characterization of some *Vibrio parahaemolyticus* strains isolated from salted herring and roe. Zentralbl Bakteriol Orig A. 238, 59-65.

Deabes, M.M., Naguib, K.H., Ayesh, A.M., El-Damaty, E.M., Rowayshed, G.H., 2018. Comparison of the HPLC and the TLC technique for the determination of biogenic amines spiked to sausage and smoked herring samples. Enliven: Toxicol. Allied Clin. Pharmacol. 5, 001.

Elbashir, S., Jahncke, M., DePaola, A., Bowers, J., Schwarz, J., Punchihewage-Don, A.J., Min, B., Rippen, T., Parveen, S., 2023 Prevalence and Abundance of Bacterial Pathogens of Concern in Shrimp, Catfish and Tilapia Obtained at Retail Stores in Maryland, USA. Pathogens. Pathogens 12,187. doi: 10.3390/pathogens12020187.

EOS, 2005. Egyptian Standard of Specifications of Smoked Fish, Ministry of Industry and Technological Development, Cairo, Egypt, 2005/288

EOS,1996. Egyptian Standard of Specifications of fish roes and caviar, Ministry of Industry and Technological Development, Cairo, Egypt.

Maturin, L., J. Peeler, T., 2001, Chapter 3. Aerobic Plate Count. In: Food and Drug Administration (FDA), Bacteriological Analytical Manual Online, 8th Edition, Silver Spring, Berlin, 1998.

Solomon, H.M., Lilly, T., 2001. Bacteriological analytical manual. chapter 17, *Clostridium botulinum*. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-17-*Clostridium*-botulinum

Kaysner, C.A., DePaola, A., Jones, J., 2004. Bacteriological Analytical Manual (BAM) Main Page. 2004. Available online: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-*Vibrio*

Hitchins, A.D., Jinneman, K., Chen, Y., 2022. FDA, Bacteriological analytical manual. BAM Chapter 10. Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. US Food and Drug Adminstration.

Galetti R, Andrade, L.N., Sazinas, P., Jelsbak, L., Darini, A.L.C., 2022. pST15-IncHI2 plasmids co-harboring mcr-9 and several other antibiotic resistance genes in heavy metal tolerant *Enterobacter cloacae* complex isolates from hospital infections. J. Glob. Antimicrob Resist. 31,187-188. doi: 10.1016/j.jgar.2022.09.002.

González-Rodríguez, M.N., Sanz, J.J., Santos, J.A., Otero, A., García-López, M.L., 2002. Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the retail level. Int. J. Food Microbiol. 77, 161-168. doi: 10.1016/s0168-1605(02)00048-x.

Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M., Jellouli, K., 2014. Structural differences between chitin and chitosan extracted from three different marine sources. Int. J. Biol. Macromol. 65, 298-306. https://doi.org/10.1016/j.ijbiomac.2014.01.045.

Handa, S., Kimura, B., Takahashi, H., Koda, T., Hisa, K., Fujii, T., 2005. Incidence of *Listeria monocytogenes* in raw seafood products in Japanese retail stores. J. Food Prot. 68, 411-415.

Hansen, T.B., Abdalas, S., Al-Hilali, I., Hansen, L.T., 2021. Predicting the effect of salt on heat tolerance of *Listeria monocytogenes* in meat and fish products. Int. J. Food Microbiol. 352, 109265. doi: 10.1016/j.ijfoodmicro.2021.109265.

Hsu, T.K., Tsai, H.C., Hsu, B.M., Yang, Y.Y., Chen, J.S., 2021. Prevalence, enterotoxin-gene profiles, antimicrobial resistance, and genetic diversity of *Bacillus cereus* group in aquatic environments and shellfish. Sci. Total Environ. 758,143665. doi: 10.1016/j.scitotenv.2020.143665.

Hyytiä, E., Hielm, S., Korkeala, H., 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products. Epidemiol Infect.120, 245-50. doi: 10.1017/s0950268898008693.

Iwamoto, M., Ayers, T.B., Mahon, E., Swerdlow, D.I., 2010. Epidemiology of seafood-associated infections in the United States. Clin. Microbiol. Rev. 23, 399–411. doi:10.1128/CMR.00059-09.

Iwatsuki ,T., Kanazawa, T., Ogasawara, T., Hosotani, K., Tsuchiya ,K., Watanabe, S., Suzuki ,T., Moriuchi, R., Kanesaki, Y., Dohra, H., 2021. 16S rRNA Gene Amplicon Sequencing of Gut Microbiota in Three Species of Deep-Sea Fish in Suruga Bay, Japan. Microbiol Resour Announc. 10, e01260-20. doi: 10.1128/MRA.01260-20.

Kočar, D., Köse, S., Tufan, B., Ščavničar, A., Pompe, M., 2021. Determination of Biogenic Amines in Fresh Fish and Processed Fish Products Using IC-MS/MS. Foods. 10, 1746. doi: 10.3390/foods10081746.

Kung, H.F., Chien, L.T., Liao, H.J., Lin, C.S., Liaw, E.T., Chen, W.C., Tsai, Y.H., 2008. Chemical characterization and histamine-forming bacteria in salted mullet roe products. Food Chem. 110, 480-485. doi: 10.1016/j.foodchem.2008.02.029.

Kynčl, J., Špačková, M., Fialová, A., Kyselý, J., Malý, M., 2021. Influence of air temperature and implemented veterinary measures on the incidence of human salmonellosis in the Czech Republic. BMC Public Health 21, 55. doi: 10.1186/s12889-020-10122-8.

Lapa-Guimarães, J., Trattner, S., Pickova, J., 2011. Effect of processing on amine formation and the lipid profile of cod (*Gadus morhua*) roe. Food Chem. 129, 716-723. doi: 10.1016/j.foodchem.2011.05.010.

Lehane, L., Olley, J., 2000. Histamine fish poisoning revisited. International Journal of Systematic Microbiology 58, 1–37.

Leong, L.E.X., Shaw, D., Papanicolas, L., Lagana, D., Bastian, I., Rogers, G.B., 2017. Draft genome sequences of two *Enterobacter cloacae* subsp. cloacae strains isolated from Australian hematology patients with bacteremia. Genome Announc. 5, e00756-17.

Lin, I.C., Hussain, B., Hsu, B.M., Chen, J.S., Hsu, Y.L., Chiu, Y.C., 2021. Prevalence, genetic diversity, antimicrobial resistance, and toxigenic profile of *Vibrio vulnificus* isolated from aquatic environments in Taiwan. Antibiotics (Basel) 10, 50505. doi: 10.3390/antibiotics10050505, PMID

Matanza, X.M., Osorio, C.R., 2018. Transcriptome changes in response to temperature in the fish pathogen *Photobacterium damselae* subsp. damselae: Clues to understand the emergence of disease outbreaks at increased seawater temperatures. PLoS One 13, e0210118. doi: 10.1371/journal.pone.0210118.

Miya, S., Takahashi, H., Ishikawa, T., Fujii, T., Kimura, B., 2010. Risk of *Listeria monocytogenes* contamination of raw ready-to-eat seafood products available at retail outlets in Japan. Appl. Environ. Microbiol. 76, 3383-3386. doi: 10.1128/AEM.01456-09. Epub 2010 Mar 26.

Piras, C., Scano, P., Locci, E., Sanna, R., Marincola, F.C., 2014. Analysing the effects of frozen storage and processing on the metabolite profile of raw mullet roes using (1)H NMR spectroscopy. Food Chem. 159,71–79.

Rawles, D.D., Flick, G.I., Martin, E.E., 1996. Biogenic amines in fish and shellfish. Advances in Food and Nutrition Research 39, 329–364.

Restuccia, D.U., Gianfranco, S., Marco, B., Rosa, T., Francesco, M., Nevio, P., Monica, R.L., 2015. Evaluation of fatty acids and biogenic amines profiles in mullet and tuna during six months of storage at 4-8°C. J. of Food Composition and Analysis 40, 52-60.

Rosa, A., Scano, P., Atzeri, A., Deiana, M., Falchi, A.M., 2013. Potential anti-tumor effects of *Mugil cephalus* processed roe extracts on colon cancer cells. Food Chem Toxicol. 60, 471–478.

Scano, P., Rosa, A., Pisano, M.B., Piras, C., Cosentino, S., Dessi', M.A., 2013. Lipid components and water soluble metabolites in salted and dried tuna (Thunnus thynnus L.) roes. Food Chem. 138, 2115-2121. doi: 10.1016/j.foodchem.2012.11.095.

Shimizu, A., Ichiba, T., Kawano, J., Igimi, S., 2007. Contamination of *Staphylococcus aureus* in ready-to-eat foods. Shokuhin Eiseigaku Zasshi. 48, J341- 344.

Sidhu, K.S., 2003. Health benefits and potential risks related to consumption of fish or fish oil. Regul. Toxicol. Pharmacol. 38, 336–344.

Taylor, S.L., 1986. Histamine food poisoning: toxicology and clinical aspects. Critical Reviews in Toxicology. 17, 91–128.

Van Doren, J.M., Neil, K.P., Parish, M., Gieraltowski, L., Gould, L.H., Gombas, K.L., 2013. Foodborne illness outbreaks from microbial contaminants in spices, 1973–2010. Food Microbiol. 36,456–464. doi: 10.1016/j.fm.2013.04.014.

Voidarou, C., Alexopoulos, A., Plessas, S., Noussias, H., Stavropoulou, E., Fotou, K., Tzora, A., Skoufos, I., Bezirtzoglou, E., Demertzi-Akrida, K., 2011. Microbiological quality of grey-mullet roe. Anaerobe 17, 273-275. https://doi.org/10.1016/j.anaerobe.2011.03.008.

V'uorela, R., Kaitaranta, J., Linko, R.R., 1979. Proximate Composition of Fish Roe in Relation to Maturity. Canadian Institute of Food Science and Technology Journal 12, 186-188. https://doi.org/10.1016/S0315-5463(79)73133-6.

Wang, J., Weng, X., Weng, Y. Xu, Q., Lu, Y., Mo, Y., 2023.Clinical features and treatment outcomes of *Vibrio vulnificus* infection in the coastal city of Ningbo, China. Front Microbiol.14, 1220526. doi: 10.3389/fmicb.2023.1220526.

Yu, Y., Wang P., Bian L., Hong S., 2018. Rare Death via Histamine Poisoning Following Crab Consumption: A Case Report. J. Forensic Sci. 63, 980–982. doi: 10.1111/1556-4029.13611.

Zhernov, Y.V., Simanduyev, M.Y., Zaostrovtseva, O.K., Semeniako, E.E., Kolykhalova, K.I., Fadeeva, I.A., Kashutina, M.I., Vysochanskaya, S.O., Belova, E.V., Shcherbakov, D.V., Sukhov, V.A., Sidorova, E.A., Mitrokhin, O.V., 2023. Molecular Mechanisms of Scombroid Food Poisoning. Int. J. Mol. Sci. 24, 809. doi: 10.3390/ijms24010809.