

HISTAMINE LEVEL AND HISTAMINE-PRODUCING BACTERIA ISOLATED FROM SALTED AND FREEZE SARDINE FISH (*Sardina* spp.)

Maged Mostafa Mahmoud^{1,2,3}, Ahmed Mahmoud Al-Hejin^{1,4}, Ali Meawad Ahmed⁵, Ahmed M. Elazzazy^{6,7*}

¹King Fahd Medical Research Center, King Abdulaziz University, P.O. Box 80216, Jeddah, 21589, Saudi Arabia, ²Department of Medical Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, ³Molecular Genetics and Enzymology Department, Human Genetics Division and Genome Research, National Research Centre, Cairo, Egypt, ⁴Department of Biological Sciences, Faculty of Science, P.O. Box 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia, ⁵Departments of food Hygiene, Faculty of Veterinary Medicine, Suez Canal University, Egypt, ⁶Department of Biological Sciences, Faculty of Science, University, of Jeddah, Jeddah, Saudi Arabia, ⁷Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Institute, National Research Centre, Dokki, Giza 12622, Egypt

*Corresponding author, E-mail: ahmedazazy8@hotmail.com, amelazzazy@uj.edu.sa

Abstract: Histamine is the biogenic amine responsible for scombroid poisoning in various types of fish and fish products. The study evaluated histamine content and bacteriological quality of salted and freeze sardine samples. All fish samples were cultured on Reinforced Closterdial medium; mannitol salt (MS) agar and violet red bile glucose (VRBG) agar, the positive colonies were sub-cultured on histamine-forming bacterium agar, followed by biochemical and PCR identification. Production of histamine in the fish muscles was measured by high performance liquid chromatography. The mean histamine values were about; 8.25 mg/100g. The mean of total aerobic counts was; $4 \times 10^4 \pm 2.1 \times 10^4$ while, the total anaerobic count; $5 \times 10^4 \pm 1.2 \times 10^3$ Log CFU/g. *Enterobacteriaceae spp.* about; $5 \times 10^4 \pm 2.5 \times 10^4$ Log CFU/g while, the mean value of *Staphylococcus spp.* about; $3 \times 10^4 \pm 3.7 \times 10^4$ Log CFU/g. The mean value of histamine-forming bacterial were; 3.3×10^6 Log CFU/g. Molecular Identification For Enterobacteriaceae were *Klebsiella pneumonia*, *Staphylococcus xylosum*, *Escherichia coli* and *Enterobacter cloacae*. We concluded that during storage, there was a positive relationship between the histamine levels and the histamine-forming bacteria (HFB) numbers, while, histamine and the bacteria forming histamine were recorded in all examined sardine samples.

Key words: histamine; *Escherichia coli*; *Klebsiella pneumonia*; molecular identification; scombroid; *Staphylococcus xylosum*

Introduction

Sardine, *Sardina pilchardus* is one of small pelagic oily fish, which is characterized by dark-colored flesh and strong flavors and affordable price. Fish spoilage changes occur as soon as fish dies, and they take place in a successive process, its consumption might result in some foodborne illnesses and outbreaks (1). Scombroid and other dark muscle fish such as tuna, mackerel, blue fish, sardine, carangids, herring, and anchovies

are prone to form histamine, as their muscles are histidine-rich (2). Histamine ($C_5H_9N_3$) is one of the biogenic amines which leading to food-borne intoxication which is named as histamine poisoning. Histamine has pharmacological effects e.g. inflammation & vasodilation by allergic reaction mediation (2). The symptoms of scombroid poisoning appear within a few minutes after eating fish of Scombridae family and related species. The first symptoms are cutaneous, with flush, pruritus, and erythema of the face and trunk having an urticarial appearance, together with faintness. Gastrointestinal symptoms include nausea, vomiting, abdominal cramps and occasionally diarrhea (3). The

major bacterial species reported in fish, which can form histamine, are: *Klebsiella pneumonia*, *Staphylococcus xylosum*, *Escherichia coli*, *Morganella morganii*, *Hafnia alvei*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Proteus mirabilis*, *Serratia liquefaciens*, *Serratia fonticola*, *Citrobacter freundii* and *Raoultella ornithinolytica* (4). These types of bacteria naturally present on the gills, external surfaces and in the gut of live saltwater fish with no harm to the fish. Up on death, the defense mechanism of the fish no longer inhibits bacterial growth in the muscle tissue and histamine forming bacteria may start to grow resulting in the formation of biogenic amines (5). Inadequate cooling following harvest promotes bacterial histamine production and can result in outbreaks of scombroid poisoning (6). Specific conditions are needed for preservation of fish and avoid the chemical intoxication hazardous (7). Freshly caught fish does not contain histamine however, once fish starts to decay or is subjected to temperature abuse; histamine can start to accumulate. The production of histamine in fish is generally believed to result from the growth of Gram negative, rod shaped bacteria capable of producing pyridoxal phosphate dependent decarboxylase enzymes (8). This process can start as soon as the fish dies and kept at temperature above 4°C for an extended period (FAO/WHO, 2012). Histamine forming bacteria produce histidine decarboxylase (HDC) enzyme, once the HDC enzyme is synthesized, it continues to produce histamine even if the bacteria get inactivated (EFSA, 2011; FDA, 2011). Cooking can inhibit the HPB and the HDC, while histamine cannot be degraded by cooking, smoking, or canning of fish, indicating that both raw and cooked fish might cause HPB (2). Fish is very important source of protein especially in Egypt. Fish and fish products are one of the most important food stuffs as they are one of the cheapest sources of animal protein (9). So far, little information is available about HPB in Egypt. Therefore, this study was conducted for evaluation of the histamine content in addition to bacteriological quality of sardine fish samples.

Materials and methods

Samples collection: A total of 50 sardine samples were collected from various commercial fish retailers and markets at Ismailia city, Egypt. Samples were kept in icebox during transportation

to the laboratory and stored at -20 °C until further analysis. bacteriological evaluation was determined in the all samples.

Determination of pH value, salt content, water activity and total volatile nitrogenous compound (TVN): The muscles of sardine fish samples (15 g) were blended in mixer for 10 min at 6000 rpm with 30 ml distilled water to make thick slurry. The salt content of the fish muscle was determined by the method of AOAC procedures (1995)(10. 10 ml of the filtered extract (D: dilution coefficient) were pipetted into 100ml Erlenmeyer and 1ml of K_2CrO_4 (IN) was added. The samples were titrated with 0.1N $AgNO_3$ standard solution (f: factor) until the first perceptible pale red brown appeared (T: titration volume).

Calculation: Concentration of sodium chloride based on sodium can calculated by the formula: $NaCl (\%) = 0.00585 \times T \times D \times \dots \times F$. Water content was detected by Hygrometer electric instrument at 27 °C. The pH was measured using pH meter. The (TVN) was measured by dissolving 100g of the sardine fish sample in 200ml (7.5%) aqueous solution of trichloro-acetic acid, then filter the mixture and mixing 25ml of the extract in a distillation flask with 6ml 10%NaOH. The TVN condensed is mixed with 10ml (4%) boric acid and 0.04ml of methyl-red and bromocresol green indicator which turns green when alkalinized by the TVN (11).

Calculation: The TVN content was calculated by the formula: $TVN = (14 \times a \times b \times 300) / 25$ (mg/100g). Where: (a) is a volume of sulphuric acid (ml) and (b) is the normality of sulphuric acid (%).

Microbiological analysis: The following Bacterial counts and Isolation of histamine-forming bacteria were carried out according to (12), the flesh of fish sample transferred to a homogenizer to mince the sample using Moulinex Food Processor (La Kiloulinette, type D56, Paris, France), 25g of homogenized sample was added to 225 mL of 0.1% peptone water using plastic bag, put in a Seward stomacher (4001R/LIK) for 2 min. prepared serial dilutions a final dilution of (10⁻⁷) by peptone water.

Total aerobic bacterial count: Standard plate count agar by spread plate method were used for the determination of aerobic plate counts, and then incubated at 30±1°C/72±3 hrs.

Total anaerobic colony counts: The same procedures as for the previous sample preparation were incorporated using Reinforced Closterdial

medium agar (Oxoid; CM151), incubated in an anaerobic jar (Gaspak plus anaerobic system) at 37°C/48 hours.

Staphylococci spp.: Staphylococci spp. were determined on Baird Parker Agar (Oxoid CM 275) supplemented by egg yolk tellurite at 37 °C for 24–48 h. Typical black colonies with zones around and atypical black colonies were considered as *Staphylococcus* spp.

Total Enterobacteriaceae Counts: *Enterobacteriaceae* were detected using violet red bile glucose agar (VRBGA) which were incubated at an inverted position at 30–35°C/24 hrs.

Measurement of Biogenic amine content in fish muscles using HPLC: Fish samples were subjected to HPLC to analyses the contents of histamine, putrescine, cadaverine, serotonin, phenylethylamine, spermidine and tyramine. In brief, 5g muscle from each sample was homogenized separately using 10mL trichloroacetic acid extracting solution (Thermo Fischer Scientific, Waltham, MA) and centrifugation. The supernatant was filtered, mixed with 1mL of 1M NaOH (Thermo Fischer Scientific), and incubated at RT for 5 min. One milliliter of ophthalaldehyde (Acros Organics, Geel, Belgium) and 3mL ethyl acetate (Thermo Fischer Scientific) were then added, and the homogenate was precipitated by centrifugation. The pellets were dried on a rotatory evaporator, then re-suspended in 1 ml acetonitrile (Thermo Fischer Scientific). A histamine dihydrochloride standard solution (Sigma- Aldrich, St. Louis, MO) was serially diluted in 0.1M HCl (Thermo Fischer Scientific) to obtain the following concentrations: 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/L. The standard and the processed samples were injected into intersil C18 columns (Agilent Technologies, Waldbron, Germany); each determination was injected twice. The chromatographic separation was performed using 1050 HPLC (Agilent Technologies) according to (13).

Determination of total histamine-forming bacterial counts: Spread 0.1ml aliquot of diluted sample on histamine-forming bacterium isolation agar (HBI agar) mixed with L-histidine. Incubation of agar plates for 4 days/35°C, counted the purple and blue colonies, picked on trypticase soy agar (Difco) to identification by pure cultures (14).

Identification histamine-forming bacteria: Bacterial isolates were identified using the Vitek instrument (bio-Merieux Vitek Inc., Hazelwood, Mich.,

U.S.A.). Additional biochemical tests were carried out for definitive species identification by screening on (TSI) triple sugar iron agar, Vogts-proskauer (VP) test, Oxidase test, Fermentation test, Methyl-Red test Citrate-utilization test, Urease test, Indole production test (15, 16).

PCR Identification: DNA extraction was done using marketed kit for DNA extraction (Presto Mini-DNA Bacteria Kit. Ltd. USA). Preparation of DNA: using 0.05 N NaOH and 0.25% sodium dodecyl sulfate. PCR product analysis performed using a 1% agarose substance by electrophoresis apparatus and observed under the UV trans-illuminator. Amplification and sequence determination of 16S rDNA.

Statistical analysis; Data are presented as mean±SEM. Analysis was performed using GraphPad Prism 7.03 software (GraphPad Software, San Diego, CA, USA). Statistical differences over two groups were verified by Student's *t*-test

Results

The obtained results in Table 1 represented the values of the salt content, pH, total volatile nitrogen (TVN), water activity. The salt content, pH value, TVN and water activity in all samples ranged from 0.01 to 9.6%, 6.1 to 7.6, 6 to 7.8 and 21.4 to 35.7 mg/100 g respectively. The average TVN level (24.3 mg/100 g) of frozen samples were significantly ($P < 0.05$) lower than those of salted samples. The levels of TVN in most fish samples were below the Egyptian regulatory standard of 25 mg/100 g, 6 (Table 1).

TVN, including NH₃, dimethylamine (DMA) and trimethylamine (TMA) is used as indicators for the fish spoilage and quality. In this study, the TVN values in salted sardines were significantly higher than those obtained in frozen samples. Bacteriological profiles of total coliforms, Aerobic, anaerobic, *Enterobacteriaceae* spp. *E. coli* and *Staphylococcus* spp. counts were detected in table 2. Statistical analysis for Different Types of Microorganisms (Log CFU/g) in the Sardine samples were; the total aerobic counts reported about; 8×10^3 , 6×10^4 , $4 \times 10^4 \pm 2.1 \times 10^4$ as minimum, maximum, mean ±SE values respectively while, about; 5×10^3 , 6×10^4 , $5 \times 10^4 \pm 1.2 \times 10^3$ Log CFU/g were detected as minimum, maximum, mean ±SE values respectively in case of total anaerobic count. *Enterobacteriaceae* spp. showed about; 4×10^3 ,

6.0×10^5 , $5 \times 10^4 \pm 2.5 \times 10^4$ Log CFU/g as minimum, maximum, mean \pm SE values respectively while, about; 3×10^3 , 4×10^5 , $3 \times 10^4 \pm 3.7 \times 10^4$ Log CFU/g were detected as minimum, maximum, mean \pm SE values respectively in case of *Staphylococcus spp.* counts. The lower water content (<0.92) and higher salt concentration (>4.0%) in these salted sardine samples apparently had some inhibitory effect on coliform bacterial growth.

Table 3 reported the contents of biogenic amines in the frozen and salted sardine fish. The average content of each of the seven biogenic amines in all samples was less than 10 mg/100 g. Based on the results, it is cleared that higher levels of histamine were found in salted and frozen sardine fish was 8.25 and 0.64 mg/100g. Among them, higher level of biogenic amines in salted sardine samples as follow, the average cadaverine (2.82 mg/100 g), tyramine (4.17 mg/100 g), putrescine (4.72 mg/100 g) and agmentine (4.57 mg/100 g) this results of salted sardine samples were significantly ($p < 0.05$) higher than those of frozen samples.

Figure 1 and 2 show the identity and Frequency distribution of histamine-forming bacterial isolates of frozen sardines' samples as determined by 16S rDNA sequences. They were identified as *Citrobacter freundii* (thirteen strain), *Enterobacter aerogenes* (ten strain), *Enterobacter cloacae* (twelve strain), *Klebsiella pneumoniae* (eleven strain), *Micrococcus spp* (fourteen strain), *Proteus vulgaris* (thirty strain), *Pseudomonas aeruginosa* (seventeen strain), *Staphylococcus xylosum* (sixteen strain), In this study, that the highest incidence was recorded by *Proteus vulgaris* spp. which represented 12 (20%) and 18 (29%) in both frozen and salted samples respectively. On the other side, the lowest incidence in frozen samples was recorded by *Micrococcus spp.* and *Staphylococcus xylosum* spp. which represented 4 (7%). Although, the lowest incidence in salted samples was recorded by *Enterobacter aerogenes* spp. which represented 4 (5%) then *Citrobacter freundii* and *Enterobacter cloacae* which represented 4 (6%).

Table 1: Values of the salt content, pH, water content, total volatile nitrogen (TVN) in Frozen and salted Sardine sam-ples

Samples	Salt content (%)	pH	Water content	TVN(mg/100 g)
Frozen Sardine	0.01-0.12 (0.05 \pm 0.01) A	6.2-7.2(6.6 \pm 0.1) A	48-78 (63.4 \pm 2,9)A	21.4-30.4 (24.3 \pm 0.93)B
Salted Sardine	4.5-9.6(7.6 \pm 0.5)A	6.1-7.6 (6.75 \pm 0.2)A	6-17 (13.6 \pm 1.3)A	23.1-35.7 29.9 \pm 1,59)A

Mean \pm SD. Values in the same column with different letters are statistically different ($P < 0.05$).

Table 2: Microbial content and percentage of evidence of aerobic and Anaerobic plate count (APC), total coliform (TC), *Escherichia coli* (*E. coli*), *Enter. cloacae* and *Staph. xylosum* in Frozen and salted Sardine samples

Sam-ples	APC		TC		E. coli(Anaerobic plate count		Enter. cloacae		Staph. xylosum	
	Percent %	(log CFU/g)	Percent %	(MPN/g)	Percent %	(MPN/g)	Percent %	(log CFU/g)	Percent %	(log CFU/g)	Percent %	(log CFU/g)
Frozen Sardine	25	$2 \times 10^3 \pm 0.9$ B	5	<3	25	<2	75	$2 \times 10^2 \pm 0.9$ B	50	$3 \times 10^3 \pm 1.2$ B	25	$3 \times 10^3 \pm 2.2$ B
Salted Sardine	100	$8 \times 10^3 - 6 \times 10^4 (4 \times 10^4 \pm 1.8)$ A	20	<3	50	<3	100	$5 \times 10^3 - 6 \times 10^4 (5 \times 10^4 \pm 1.2)$ A	100	$4 \times 10^3 - 6 \times 10^5 (5 \times 10^4 \pm 2.5)$ A	50	$3 \times 10^3 - 4 \times 10^5 (3 \times 10^4 \pm 3.7)$ A

Table 3: Contents of biogenic amines in in Frozen and salted Sardine samples

Samples	No. of samples	Cad	Tyr	His	Agm	Put	Phe	Spd
Frozen Sardine	50	0.26 (0.01 \pm 0.05)A	0.97 (0.37 \pm 0.22)A	1.64 (0.13 \pm 0.1)A	ND-0.15 (0.03 \pm 0.01)A	0.52 (0.05 \pm 0.14)A	ND	ND
Salted Sardine	50	2.82 (0.69 \pm 0.48)A	4.17 (1.72 \pm 1.21)B	8.25 (0.89 \pm 1.62)A	4.57 (5.13 \pm 4.79)B	4.72 (2.18 \pm 2.00)B	ND	ND

Cad: cadaverine; Tyr: tyramine; His: histamine; Agm: agmatine; Put: putrescine; Phe: 2-phenylethylamine; Spd: spermidine.

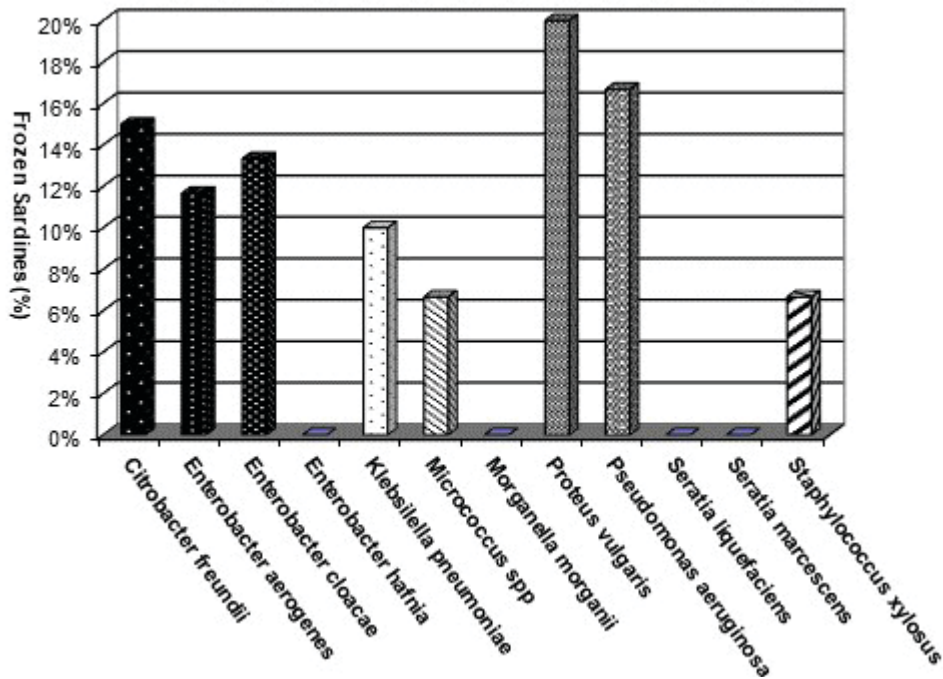


Figure 1: Frequency distribution of histamine-forming bacterial isolates of frozen sardines' samples

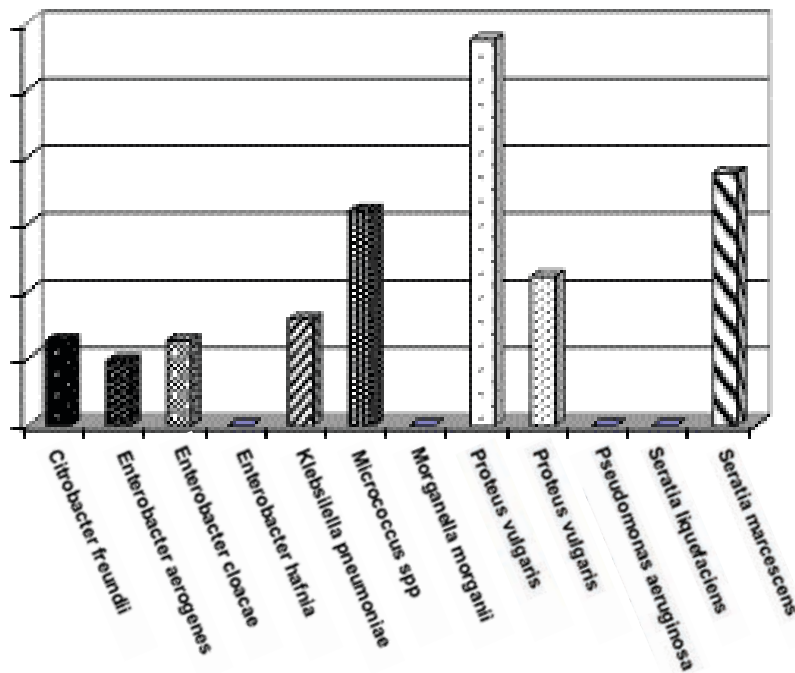


Figure 2: Frequency distribution of histamine-forming bacterial isolates of salted sardines' samples

Discussion

Scombroid is a type of food poisoning experienced around the world, with a higher reported prevalence in Japan, England and the United States. This could be attributed to the high consumption of scombroid fish (17). Histamine

presence in food items reflect the food quality, bad storage conditions one of the major inducers of histamine formation and aggregation as the histamine-forming bacteria can produce the enzyme "histamine-decarboxylase" which converts histidine amino acid in fish tissues in histamine even at low temperatures as 5°C. formation of

histamine not degraded by freezing or heating (18). Based on the finding that the higher levels of TVN noticed in dried salted fish samples, we explained that these samples have been seriously contaminated during food preparation and processing (19). The obtained results of TVN in frozen sardine samples were nearly like those were submitted by (19). Although, lower TVN results were obtained by (20) confirmed that the TVN values of frozen sardine samples were ranged from 15 to 17mg/100g. El-Dengawy et al. (21) studied that mean TVN values of frozen sardine samples were 13.34 ± 0.00 and 16.23 ± 0.00 mg/100g. (22) reported that the TVN values of frozen sardines were ranged from 11.2 to 19.9 mg/100g. Similar results with TVN (43 mg/100 g) and higher salt concentration (5.6%) in salted fish product were reported (23). The total aerobic, anaerobic and *Enterobacteriaceae spp.* were approximately 100% of the total sample count, while only 50% of tested samples had *Staphylococcus spp.* Similar results in the total aerobic count were detected by (24,25) during their assessment of the bacteriological quality of the bacteriological quality of some meat products in the Egyptian retail markets. Also, nearly similar results observed by (26) during their bacteriological evaluation of different fish samples of Dharan markets as; (54%) in case of *staphylococcus sp.* Lower results reported by (27) during their microbiological quality surveillance in Istanbul as (96.67%). While, only about (24%) of *Enterobacteraceae sp.* and (8%) of *staphylococcus sp.* was estimated by (28). Similar results found by Salem, et al. (28) who recorded about 5.61×10^5 Log CFU/g of total aerobic counts. Ragab, et al. (24) detected about (6.6×10^8 Log CFU/g) of total aerobic counts. While, (29) also recorded about (4.27×10^3 Log CFU/g) of *Enterobacteraceae sp.* and 5.6×10^5 Log CFU/g of *staphylococcus sp.* was detected by (30). Lower result observed by Shaltout, et al. (20) who obtained about 8.03×10^4 Log CFU/g of total aerobic count while, in case of *Enterobacteraceae sp.* they recorded about (2.02×10^2 Log CFU/g) and isolated about (2.67×10^2 Log CFU/g) of *staphylococcus*. Most aerobic of these bacteria would die or at least stop growing as the salt content of the fish was increased to 6–8%. (26) also reported similar findings with the unclean market and storage temperatures could have contributed to the high contents of TVN in samples (26). The observed results of histamine in frozen sardine samples were parallel to those

were pointed out by Lee et al. (31). Biji et al. (32) confirmed that histamine levels in sardines were ranged from 1.0 to 2.6 mg/100g. Also, Martin et al. (33) studied that mean values of histamine were ranged from 0.21 ± 0.37 to 1.04 ± 0.82 mg/100g in samples. On the other hand, higher histamine results were reported by El Hariri, et al. (34) estimated the level of histamine in the *S. pilchardus* muscle at the rejection time (12 days) preserved in ice was 16.2 mg/100g. Li et al. (35) reported that mean histamine content of samples was 16.4 ± 2.6 mg/100g. Aruna et al. (36) reported that histamine level of frozen sardines were ranged from 12 to 32 mg/100g. The strong evidence exists that the toxic effects of histamine affected and increased by the biogenic amines in fish tissue by inhibiting intestinal histamine-metabolizing enzymes such as diamine oxidase (13). Histamine-forming bacteria species which isolated in this study have been previously recorded by different investigators (37-39). However, the *Enterobacter* strains were isolated from many scombroid fish species (40). This increment in histamine concentration for sardine samples caused by consumptions of poor-quality raw food and bad handling techniques, improper storage and defective processing methods (17). Salting techniques can remove the bacterial contamination but unable to destroy histamine (scombroid) toxin of fish poisoning (41). If histamine levels in fish and its products are more than 50mg/100g, it is reported as the hazard action level of histamine (42). According to, U.S. Food and Drug Administration (U.S.FDA) histamine should be found at 50ppm of histamine to be enough to cause the scombroid poisoning signs, While, the Egyptian Organization for Standardization "EOS" (2005); permits histamine to be found at 20mg/100g (9, 43). The higher levels of fish histamine affected by certain factors such as natural microorganisms which depend on habitat, storage temperature after caught and improper handling on board the harvest vessel. Naturally, live fish contains different bacterial species on; external surfaces, gills, and in the intestinal content but after the death of fish, the defense mechanisms of fish can't inhibit the growth of bacteria which allowing the replications of microorganisms. Sabry et al., (44) showed the occurrence of histamine producing bacteria associated with histamine production in muscles of retail sardine and mackerel in Egypt. The sardine samples showed a relatively high level of histamine than the legal limit, possibly

due to temperature abuse. The habitats in marine water differ and can be affected by the origin of fish contamination. The main approach after fish catching depend on the melting temperature in addition to the presence of some conditions which controlling the speed of reaching these temperatures such as fish size, fish initial temperature and the ice content of fish (45-47).

Conclusion

Results in this study indicated to that histamine and the bacteria forming histamine were recorded in all examined sardine samples. Temperature, salt concentration and water activity greatly influenced the histamine production in Sardine samples. The rate of histamine accumulation was rapid in salted fish. Increase of bacterial counts and histamine content slowed down with the decrease of storage temperature. It is referred that hygienic conditions and storage time/temperature can significantly affect the frozen fish quality. bacteriological quality of anaerobic, *Enterobacteriaceae* spp. *E. coli* and *Staphylococcus* spp. counts with no coliforms, was 8×10^3 , 5×10^4 , 5×10^4 · 3×10^4 log CFU/g, respectively. The histamine content in all samples was less than 10 mg/100 g USFDA guideline value. Molecular Identification of Sardines samples declared as following; 25/50 (50%), 10/50 (20%), 10/50 (20%), 5/50 (10%) for *Klebsiella pneumonia*, *Staphylococcus xylosum*, *Escherichia coli* and *Enterobacter cloacae* respectively. The isolated histamine-forming bacterial species which have been demonstrated in the examined sardine samples. There must be an attention to the fishing process which should be carried out under good hygiene to decrease the initial bacterial load.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. (G: 344-141-1441). The authors, therefore, acknowledge with thanks DSR technical and financial support.

Reference

1. Simat V, Hamed I, Petričević S, et al. Seasonal Changes in Free Amino Acid and Fatty Acid

Compositions of Sardines, *Sardina pilchardus*. *Foods* 2020; 9: 1–12.

2. Soldo B, Šimat V, Vlahović J, et al. High quality oil extracted from sardine by products as an alternative to whole sardines: Production and refining. *European Journal Lipid Science Technology* 2019; 121–30.

3. Harmelin Y, Hubiche T, Pharaon M, Del Giudice P. Three cases of scombroid poisoning. *Annales de Dermatologie et de Venereologie Journal* 2018; 145(1): 29–32.

4. Satomi M. Effect of Histamine-producing bacteria on fermented fishery products. *Food Science and Technology Research* 2016; 22(1): 1–21.

5. Hassan M, Shaltout M, Saqur N. Histamine in Some Fish Products. *Archives of Animal Husbandry & Dairy Science* 2020; 2(1):1–3.

6. Hungerford JM (2010) Scombroid poisoning: a review. *Toxicology Journal* 56(2): 231–43.

7. Bandarra NM, Marçalo A, Cordeiro A, et al. Sardine (*Sardina pilchardus*) lipid composition: Does it change after one year in captivity. *Food Chemistry* 2018; 244: 408–13.

8. Yesudhasan P, Al-Zidjali M, Al-Zidjali A, Al-Busaidi M, et al. Histamine levels in commercially important fresh and processed fish of Oman with reference to international standards. *Food Chem* 2013; 140(4):777–83.

9. Elshafey WS, Ibrahim HM, Hassan MA, Maarouf AA. Assessment of histamine and putrescine residues in fish and shellfish. *Benha Vet Med J* 2018; 34:178–87, <https://doi.org/10.21608/bvmj.2018.44744>

10. A.O.A.C. (1995): Official methods of analysis of the Association of Official Analytical Chemists. Fifteenth ed. Published by Association of Official Analytical chemists. Arlington, Virginia, USA.

11. Malle P, Poumeyrol M. A new chemical criterion for the quality control of fish: trimethylamine/total volatile basic nitrogen (%). *Journal of Food Protocol* 1989; 52: 419–23.

12. Fletcher GC, Summers G, van-Veghel PWC. Levels of histamine and histamine-producing bacteria in smoked fish from New Zealand markets. *J Food Protect* 1998; 61:1064–70.

13. Tahmouzi S, Khaksar R, Ghasemlou M. Development and validation of an HPLC-FLD method for rapid determination of histamine in skipjack tuna fish (*Katsuwonus pelamis*)” *Food Chem* 2011; 126:756–76.

14. Patange SB, Mukundan MK, Ashok Kumar K, A simple and rapid method for colorimet-

ric determination of histamine in fish flesh. *Food Control* 2005; 16: 465–72.

15. Kung H, Lee Y, Huang Y, et al. Histamine formation in flying fish contaminated with *Staphylococcus xylosum*. *International Aquatic Research* 2016; 8: 217–26.

16. Syahri Y, Baharuddin F, Fachruddin A. Biochemical tests and identification of potential indigenous bacteria from nickel post-mining land in Pomalaa. *Earth and Environmental Science* 2019; 1–7.

17. Shakila RJ, Lakshmanan R, Jeyasekaran G. Incidence of amine forming bacteria in the commercial fish samples of Tuticorin region. *Indian Journal of Microbiology* 2002; 42: 147–50.

18. Ruiz-Capillas C, Herrero A, Impact of biogenic amines on food quality and safety. *Foods* 2019; 8(62): 1–6.

19. Calanche J, Samayoa S, Alonso, et al. Assessing the effectiveness of a cold chain for fresh fish salmon (*Salmo salar*) and sardine (*Sardina pilchardus*) in a food processing plant *Food Control* 2011; 33: 126–35.

20. Özogul F, Polat, A. and Özogul, Y. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). *Food Chemistry* 2004; 85: 49–57.

21. El-Dengawy RA, El-Shehawey SM, Kassem AE et al. Chemical and microbiological evaluation of some fish products samples. *Journal of Agriculture, Chemistry and Biotechnology* 2012; 3 (8): 247–59.

22. Farag MA. Estimation of biogenic amine concentration in fresh and processed sardine fish products during different storage conditions. *World Journal of Fish and Marine Sciences* 2013; 5 (6): 628–36.

23. Chen HC, Huang YR, Hsu HH, et al. Determination of histamine and biogenic amines in fish cubes (*Tetrapturus angustirostris*) implicated in a foodborne poisoning. *Food Control* 2010; 21: 13–8.

24. Ragab WS, Hassan E, Al-Geddawy M, Albie A. Bacteriological Quality of some Meat Products in the Egyptian Retail Markets. *Assiut Journal of Agricultural Science* 2016; 47: 422–9.

25. Shaltout FA, Salem A, Khater D, Lela R. Impact of some natural preservatives on bacterial profile of pinched peat in Egypt. *Benha Veterinary Medical Journal* 2016; 31 (1): 35–42.

26. Bantawa K, Rai K, Limbu D, Khanal H. Food-borne bacterial pathogens in marketed raw

meat of Dharan, eastern Nepal. *BMC research notes* 2018; 11 (1): 1–5.

27. Erdem K, Saglam D, Ozer D, Ozcelk E. Microbiological quality of minced meat samples marketed in Istanbul. *Van Veterinary Journal* 2014; 25, (3): 67–70.

28. Salem A, Shawky N, Abo-Hussein L. Microbiological profile of some meat products menofia markets. *Benha Veterinary Medical Journal* 2018; 34 (2): 1–7.

29. Hassanien F, Shaltout F, Mohamed H, Hatem E. Quality assurance of some locally processed meat products. *Benha Veterinary Medical Journal* 2018; 34, (1): 41–7.

30. Haileselassie M, Taddele H, Adhana K, Kallayou S. Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pacific journal of tropical biomedicine* 2013; 5; 407–12.

31. Lee YC, Kung H, Wu C et al. Determination of histamine in milkfish stick implicated foodborne poisoning. *Journal of Food and Drug Analysis* 2016; 24: 63–71.

32. Biji KB, Ravishankar C, Venkateswarlu R et al. Biogenic amines in seafood: A review. *Journal of Food Science and Technology* 2016; 53: 2210–8.

33. Martin N, Trmčić A, Hsieh T, et al. The Evolving Role of Coliforms as Indicators of Unhygienic Processing Conditions in Dairy Foods. *Frontiers in Microbiology* 2016; 7, 1549. <https://doi.org/10.3389/fmicb.2016.01549>.

34. El Hariri O, Bouchriti N, Bengueddour R. Risk assessment of histamine in chilled, frozen, canned and semipreserved fish in Morocco; Implementation of risk ranger and recommendations to risk managers. *Foods* 2018; 7 (10): 157–67.

35. Li C, Vrdoljak G, Moezzi B. Sampling and analysis of histamine in fish products from local Northern California markets. *Food & Nutrition Journal* 2018; 3: 189–200.

36. Aruna V, Amruthavalli V, Gayathri R. Was histamine toxicity known to Ayurveda Siddha 5000 years ago?. *World News of Natural Science* 2019; 23: 166–72.

37. Torido Y, Takahashi T, Kuda T, Kimura B. Analysis of the growth of histamine-producing bacteria and histamine accumulation in fish during storage at low temperatures. *Food Control* 2012; 26: 174–7.

38. Kung FH, Huang YC, Lin MC, et al. The histamine content of dried flying fish products

in Taiwan and the isolation of halotolerant histamine-forming bacteria. *Journal of food and drug analysis* 2015; 23: 335–42.

39. Joshi PA, Bhoir V. Fish spoilage by amine forming bacteria in commercial fish samples. *International Journal of Research and Scientific Innovation* 2016; 3: 2321–705.

40. Kim SH, Velazquez BJ, Gigirey BB, et al. Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science Biotechnology* 2003; 12: 451–60.

41. Benkerroum N. Biogenic amines in dairy products: Origin, incidence, and control means” *Comprehensive Reviews in Food Science and Food Safety* 2016; 15: 801–26.

42. Simora R, Peralta E. Occurrence of histamine and histamine-forming bacteria in Philippine traditional dried-salted fish products. *Asian Fisheries Science* 2018; 31: 73–88.

43. Food and drug administration, (FDA). Fish and fishery products. Hazards and controls guid-

ance, 4th Edition. Chapter 27: Scombrototoxin (histamine) formation 2011; 240: 402–10.

44. Sabry MA, Mansour HAA, Ashour RM, Hamza E. Histamine-producing bacteria and histamine induction in retail sardine and mackerel from fish markets in Egypt. *Foodborne Pathog Dis* 2019;16 (9): 597–603.

45. Refai M, El-Hariri M, Ahmed Z, El Jakee J. Histamine producing bacteria in fish. *Egyptian Journal of Aquatic Biology & Fisheries* 2020; 24 (7): 1–11.

46. Koral S., Kose S. The effect of using frozen raw material and different salt ratios on the quality changes of dry salted atlantic bonito (*Lakerda*) at two storage conditions,” *Food and Health* 2018; 4, (4): 213–30.

47. Cicero A, Cammilleri G, Galluzzo F et al. Histamine in fish products randomly collected in southern Italy: A 6-year study. *Journal of Food Protection* 2020; 16: 241–8.