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Histamine Producing Bacteria in Fish

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ABSTRACT

Production of histamine as one of the biogenic amines (BA) in fish and fish products represents a health risk to human food intoxication. The current survey was conducted to identify the most predominant histamine-producing bacteria and estimation of histamine levels in fish and fish products in Egypt. One hundred and fifty samples (Tilapia, Sardines, Smoked herring, Mackerel, Filet of Carp, Morgan, and Pilchards) were collected from Giza retail shops and investigated bacteriologically by conventional identification methods. Fish specimens were collected for estimation of histamine levels using ELISA. The results indicated that *Enterobacteriaceae* was the predominant histamine producing isolates. The bacterial isolates among the 15 histamines positive fish were cultivated on Niven's agar to detect the histamine producing bacteria. The most frequently isolated species were *Klebsiella* (33.3%), staphylococci (24.7%), *Salmonella* (22.7%), *E.coli* (18.7%), *Pseudomonas* (18%), *Proteus* (16 .7%) and *Vibrio* (6.7%) species. Using ELISA test, 15 out of 150 examined fish and fish product samples (10%) had histamine in their tissues. Fesikh had the highest histamine level. Conclusively, the application of early detection of biogenic amines mainly histamine producing bacteria could reduce the health risk of histamine intoxication associated fish and fish product microbial deterioration. Improvement of a selective medium to detect decarboxylating bacteria may be a valuable tool.

INTRODUCTION

Several non scombroid fish such as sardine, anchovy, herring, marlin, western Australian salmon, sockeye salmon, amberjack, and cape yellow tail and sword fish have been often implicated in incidence of scombroid poisoning **(Price and Melvin, 1994).** The histamine intoxication is called scombroid poisoning since the syndrome occurs as a result of ingesting spoiled fish of Scombridae and Scombresocidae and the fishes include tuna, mahi mahi, blue fish, bonito, skip jack, saury, mackerel and others (**FDA, 2001)**. The biogenic amines (BA) are produced during the end of shelf life and hence their levels can be considered as a spoilage index rather than a quality index **(Ozogul and Ozogul, 2006)**.

Although many have been found in fish, only.The most common BA found in foods and causing histamine poisoning and tyramine toxicity are histamine, cadaverine, tyramine, agmatine, spermine, tryptamine, 2-phenylethylamine, spermidine and putrescine (**Ladero *et al*., 2010; Naila *et al*., 2010; Chong *et al*., 2011; Naila, *et al*., 2012).** Various species of *Enterobacteriaceae* contain histidine decarboxylase and have the ability to produce histamine **Huang *et al.*** (**2010**). Biogenic amines like cadaverine, putrescine, spermidine and spermine do not have any adverse health effect, but sometimes they react with nitrite to form carcinogenic nitrosoamines **Onal *et al.*** (**2013).** Fresh fish do not contain free histamine but contain amino acid L-histidine. Histamine is formed in fish by certain bacteria capable of producing histidine decarboxylase enzyme which can converse the free histidine, naturally present in the muscle of some fish, to histamine (**FAO/WHO, 2013)**. High amounts of BA are produced significantly during processing and storage of seafood as a result of microbial contamination and inadequate storage conditions through decarboxylation of specific free amino acids by exogenous decarboxylase enzymes released by microorganisms associated with seafood. Biogenic amines like histamine, putrescine and cadaverine are considered as indicators of fish spoilage **(Lee *et al*., 2015; Biji *et al.*, 2016).** Histamine levels were detected in 352 (7.6%) samples, with a maximum of 4,110 mg kg^{-1} and mean values of 908.9 ± 1,226.79 and 344.01 ± 451.18 mg kg^{-1} for fresh and processed fish samples, respectively. No histamine levels were found in canned tuna and smoked fish samples in contrast to most of the data reported in the literature **Cicero *et al*.** (**2020)**. The current survey was established to isolate histamine producing bacteria and determine histamine level using ELISA in fish and fish products in Egypt.

MATERIALS AND METHODS

Samples

A total of 150 random samples of fish and fish products Table 1, were obtained from retail shops in Giza Governorates. Twenty fish samples were collected from each of the following: Nile tilapia (*O. niloticus*) , Ringa smoked herring (*Clupea harengus*), fesekh salted (*Mugil cephalus*), fresh Mackerel fish (*Scomberomorus brasiliensis*), and Morgan Red porgy (*Pagrus pagrus*), but 10 samples of fillet carp (*Cyprinus carpio),* and 40 samples of fresh sardine (*Sardina pilchardus*). Samples were transferred to the laboratory in ice box under complete aseptic conditions. The specimens were taken from muscle and internal organs from each fish homogenized by blender and immediately subjected to bacteriological examination for histamine producing bacteria and estimation of histamine levels.

Table 1. Number and types of the examined fish samples.

Non Scombroid fish Types	No.	Scombroid fish Types	N0.
Nile tilapia	20	Ringa (Smoked herring)	20
Fillet carp	10	Fresh sardine	40
Morgan (Red porgy)	20	Fresh Mackerel	20
Fesikh (salted mullet)	20		
Total	70		80

Microbiological Isolation and Identification

Tissue aliquots of 10 g from each fish sample were aseptically homogenized in a sterile homogenizer flask with 90 ml of sterile 0.1% peptone for 2 min. The specific media used were TCBS (Oxoid) for isolation of *Vibrio* species, Salmonella Shigella agar (Hi media) and MacConkey agar (Hi media) for *Enterobacteriaceae*, cetrimide agar (Hi media) for *Pseudomonas* species and mannitol salt agar medium (Oxoid) for *Staphylococcus* species. Identification of bacterial isolates was carried out according to **Quinn *et al*. (2002).**

Detection of histamine in fish tissue*:*

Determination of histamine in the fish samples was performed using ELISA method (**Rahimi *et al*., 2012)** and specified histamine test kit obtained from R-Biophram AG, German.

Histidine decarboxylase activity of the bacteria:

Screening of bacteria producing histamine was applied by using Niven agar **(Niven *et al.*, 1981)** for detection of purple halo around colonies as an indicator of histamine producing bacteria among 15 fish positive for histamine production. Production of histamine by the isolates was established by observing their ability to grow in Niven's medium and Niven's modified medium as recorded by **Niven *et al*. (1981); Joosten and Northolt (1989) and Mavromatis and Quantick (2002)**. The plates were incubated at 37^0C for 48–72 h and examined daily for detection of purple colony, surrounded by a purple halo on a yellowish background.

RESULTS

Occurrence of bacteria among the examined samples:

The bacteriological examination of tested fish and fish product samples was illustrated in Table (2) Data revealed that the most frequently isolated species were *Klebsiella* (33.3%) followed by staphylococci (24.7%), *Salmonella* (22.7%), *E. coli* (18.7%), *Pseudomonas* (18%), *Proteus* (16.7%) and *Vibrio* (6.7%) species.

Table 2. Bacteria isolated from the examined fish and fish products.

Source of isolates	No. of exam. Samples	Staphylococci		Gram negative species											
				Pseudomonas		*Vibrio*		*Klebsiella*		*E. coli*		*Salmonella*		*Proteus*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tilapia	20	4	20	4	20	-	0	5	25	2	10	3	15	2	10
Morgan	20	2	10	1	5	1	5	3	15	3	15	3	15	1	5
Fillet	10	1	10	2	20	1	10	7	70	2	20	6	60	6	60
Mackerel	20	8	40	2	10	2	10	6	30	4	20	4	20	4	20
Sardines	40	11	27.5	5	12.5	2	5	12	30	4	10	8	20	7	17.5
Smoked Herring	20	6	30	7	35	2	10	11	55	8	40	7	35	3	15
Fesikh	20	5	25	6	30	2	10	6	30	5	25	3	15	2	10
Total	150	37	24.7	27	18	10	6.7	50	33.3	28	18.7	34	22.7	25	16.7

Histamine Levels among the examined samples using ELISA:

Using Elisa test, 15 out of 150 examined fish and fish product (10%) had histamine in their tissues. As shown in Table (3) the level of histamine found in Fesekh is 50 - 50.8 mg/100mg, Carp Fillet 22.5, Ringa 8 – 10, and Mackerel 20.6 – 23, Sardine 17.55- 23, Morgan 12.59 and Tilapia 8.97-9.76 mg/100mg. Histidine content was 2.1–2.2 % of total amino acid content in natural and acid assistant fermented fish product**.**

Table 3. Detection of histamine (mg/100mg) in tissues of fish and fish products.

Name	Quantity (mg/100mg)	Type
1. (Ringa)	10	Scombroid
2. (Ringa)	8	Scombroid
3.Mackerel	20.6	Scombroid
4.Mackerel	23	Scombroid
5.Sardine	19.62	Scombroid
6.Sardine	22	Scombroid
7.Fesikh	50	Non scombroid
8.Fesikh	50.8	Non scombroid
9. Tilapia	8.97	Non scombroid
10.Tilapia	9.76	Non scombroid
11. Fillet	22.5	Non scombroid
12.Sardine	17.55	Scombroid
13.Sardine	23	Scombroid
14. Morgan	12.59	Non scombroid
15. Morgan	12.59	Non scombroid

Histamine level among the examined isolates using modified Niven's method:
The bacterial isolates among 6 histamine positive fish were investigated. The isolates were *Klebsiella, Salmonella, E. coli, Proteus,* staphylococci and *Vibrio* (Table 4).

Table 4. Histamine producing isolates among histamine positive fish.

Fish type	Histamine amount (mg/100mg)	Histamine producing bacteria
Ringa	10	*Salmonella, E. coli, Klebsiella* and *Proteus*
Ringa	8	Staphylococci
Fesikh	50.8	*Salmonella, Klebsiella* and *Proteus*
Fesikh	50	*Vibrio*
Mackerel	20.6	*E. coli*
Sardine	22	Staphylococci and *Klebsiella*

DISCUSSION

In the present investigation 150 fish and fish products samples (Tilapia, sardines, herring, mackerel, carp filet, Morgan, and pilchards) collected from Giza retail shops were investigated bacteriologically by conventional identification methods.

The results in Table (2) revealed that the most frequently isolated species were *Klebsiella* (33.3%) followed by staphylococci (24.7%), *Salmonella* (22.7%), *E. coli* (18.7%), *Pseudomonas* (18%), *Proteus* (16.7%) and *Vibrio* (6.7%) species. **Piamsomboon *et al*. (2020)** reported that *Aeromonas* and *Edwardsiella* species and streptococci are commonly found in several important aquaculture species. Although fish were apparently healthy, *Photobacterium*, *Vibrio* and *Streptococcus* species were detected (**Meron *et al*., 2020)**. The presence of bacteria in fish tissues is an indicative of poor standards of process hygiene and mishandling during storage **(Simora and Peralta, 2018).**

Using ELISA test, 15 out of 150 examined fish and fish product (10%) had histamine in their tissues. The level of histamine found in Fesekh was 50 - 50.8 mg/100mg, Carp fillet 22.5, Ringa 8 – 10, and Mackerel 20.6 – 23, Sardine 17.55- 23, Morgan 12.59 and Tilapia 8.97-9.76 mg/100mg. ELISA limit for quantification of histamine levels is 2–5 mg/kg, ranging from 0–500 mg/kg, and its general advantages are easy (kit), fast, low equipment costs and possibility of multiple tests simultaneously **(FAO/WHO, 2013).** However, in Canada, Switzerland, and Brazil the maximum permissible limit of histamine in fish and fishery products is 100 mg/kg. The Australian and New Zealand Food Standards Code states that the level of histamine in fish or fish products must not

exceed 200 mg/kg. As well, the histidine content was 2.1–2.2 % of total amino acid content in natural and acid assistant fermented fish product (**Ezzat *et al*., 2015**).

Data in Table (3), showed that the concentrations of histamine in the examined samples of Mackerel ranged (20.6-23 mg/100mg). These results are higher than that reported by **Joshi and Bhoir (2011) ,** in addition the concentration of histamine in the examined samples recorded in fresh mackerel 20 mg per 100 g, while the level of histamine in examined Fesekh ranged 50- 50.8 mg/100mg, Sardine 17.55-23mg/100mg, Ringa 8-10mg/100mg, Tilapia 8.97-9.76mg/100mg, Carp Fillet 22.5 mg/100mg, and in Morgan 12.59mg /100mg which were higher than the defect action level 5 mg/100 g given by **FDA (2011)** .The obtained high histamine levels indicating potential risk for histamine poisoning. However, scombroid fish poisoning will occur in a healthy individual only when a dose of at least 50 mg histamine is consumed. This generally occurs when the fish is having a histamine level of more than 200 mg/kg. Freshly caught scombrotoxin forming fish typically contain histamine level less than 2 mg/kg **(FAO/WHO, 2013).**

The concentrations of histamine in the examined samples of Fesekh ranged from 50-50.8 mg/ 100gm while Sardine ranged from 17.55-23 mg/ 100gm and smoked herring ranged from 8-10 mg/ 100gm these results are lower than that reported by **Nader *et al*. (2016)** which showed that the concentrations of histamine in the examined samples of Fesekh ranged from 6.2 to 82.7 while salted sardine ranged from 4.6 to 37.2 and smoked herring ranged from 4.1 to 58.7. The consumption of histamine in the range of 8–40 mg, 40– 100 mg or more in one meal may cause slight, intermediate and severe poisoning respectively (**Parente *et al.*, 2001)**. In addition, the maximum acceptable limit for histamine in seafood as per FDA regulation is 100 mg/Kg or 100 ppm. Low level of biogenic amines does not cause a serious risk to human health, since the amine oxidase (mono amine oxidase and diamine oxidase) in human intestine can detoxify these amines (**Biji *et al*., 2016).**

The higher prevalence of Histamine Producing Bacteria (HPB) and levels of histamine among sardine as compared with mackerel is in agreement with current results that the sardine was caught locally in Egypt and sold in chilled as a sign of being fresh, whereas mackerel was imported frozen and sold chilled in the markets and because BA formation by bacteria is influenced by temperature between 20 and 37°C is optimum for the growth of microorganisms containing decarboxylase as recorded by **Maijala *et al*. (1993**). As well, once bacterial multiplication occurred the histidine decarboxylases enzyme activity can continue slowly even after bacterial growth has ceased at refrigeration temperatures (**Lehane and Olley, 2000**). The similar effect was recorded when fish exposed to warm waters or air as for tunas which generate heat in their tissues that enhance continuous formation of histamine **(FDA, 2011).** In this aspect, Biogenic amine formation by bacteria is influenced by temperature. Temperature between 20 and 37 °C is optimum for the growth of microorganisms containing decarboxylase (**Maijala *et al.*, 1993)**. In addition, if temperature conditions are suitable the histidine

decarboxylating bacteria naturally present in the skin, gills and gut of a freshly caught fish can multiply rapidly and form histamine. Once bacterial multiplication has occurred the histidine decarboxylases enzyme activity can continue slowly even after bacterial growth has ceased at refrigeration temperatures **(Lehane and Olley, 2000)**. As well, storage temperature is an important factor contributing to the biogenic amine formation especially for fish that is exposed to warm waters or air and for tunas which generate heat in their tissues (**FDA, 2011)**. In this aspect, it is alleged that the HPB is portion of the fish gut and gill microbiome; once the fish dies, the histamine producing bacteria HPB invade the muscles and begin transformation of histidine to histamine where the dead fish exposure to a temperature >4 for an extended period of time increases the activity of the HPB and fastens histamine accumulation, while keeping the fish chilled immediately after catching prevents this process (**Sabry *et al*., 2019)**.

Screening of bacterial producing histamine was applied by using Niven agar for detection of purple halo around colonies as an indicator of histamine producing bacteria among 15 fish positive for histamine production. All spoilage microorganisms do not decarboxylate free amino acids and even within the same species, not all strains develop the same decarboxylating capacity. Hence a low BA concentration may not represent a product with good microbial quality **(Stadnik and Dolatowski, 2010)**. Meanwhile, **Joshi and Bhoir (2011)** detected decarboxylating bacteria in fresh fish viz. Indian mackerel and sardines as well as salted mackerel and sardines, **Ibrahim *et al*. (2017)** in carp fish samples and **Trevisani *et al*. (2017)** in fish of the species *Mugil cephalus, Liza aurata, L. ramada*, and *L. saliens* in Italia. The Niven's method is considered as a suitable and presumptive method in detection of biogenic amines in environmental samples (**Soliman *et al*., 2017)**.

In the present investigation *Enterobacteriaceae* was predominant histamine producing bacteria among the examined fish and products. **Ekici and Alisarli (2008)** recorded that during handling or processing of fresh *Chalcalburnus tarichi Enterobacteriaceae* and *Pseudomonas* species can proliferate and have considerable capacity for histamine formation. *Enterococcus, Enterobacteriaceae, Lactobacillus, Morganella, Photobacterium, Pseudomonas, Raoultella, Staphylococcus* and *Tetragenococcus* species were reported to be the most common bacteria to produce biogenic amine compounds **(Dalgaard *et al.*, 2008; Satomi *et al.*, 2011)**. Histamine producing bacteria are recorded in *Bacillus, Clostridium, Enterobacteriaceae, Hafnia, Klebsiella, Lactobacillus, Morganella, Proteus, Pseudomonas, Staphylococcus* and *Vibrio* species **(Joshi and Bhoir, 2011).**

CONCLUSION

The application of early detection of biogenic amines mainly histamine producing bacteria could reduce the health risk of histamine intoxication associated fish and fish

product microbial deterioration. Improvement of a selective medium to detect decarboxylating bacteria may be a valuable tool. Improvement of hygienic practices in stores, retails, and markets, and emerging control measures for delaying formation of biogenic amines may reduce the health risk of food poisoning.

REFERENCES

Biji, K.B. ; Ravishankar, C.N. ; Venkateswarlu, R. ; Mohan, C.O. and Srinivasa Gopal, T.K. (2016). Biogenic amines in seafood: a review**.** J Food Sci Technol., 53(5): 2210 – 2218.

Chong, C.Y.; Abu Bakar, F.; Russly, A.R. ; Jamilah, B. and Mahyudin, N. A. (2011). The effects of food processing on biogenic amines formation. Int J Food Res., 18(3): 867 – 876.

Cicero, A. ; Cammilleri , G. ; Galluzzo, F.G. ; Calabrese, I. ;Pulvirenti, A. ; Giangrosso ,G. ; Cicero , N. ; Cumbo , V. ; Vella ,A. ; Macaluso , A. and Ferrantelli , V. (2020). Histamine in Fish Products Randomly Collected in Southern Italy: A 6-Year Study. J Food Prot., 16: 241 - 248.

Dalgaard, P.; Emborg, J.; Kjolby, A. ; Sørensen, N.D and Ballin , N.Z. (2008). Histamine and biogenic amines: Formation and importance in seafood. In: Improving seafood products for the consumer, Cambridge, England, Woodhead Publishing.

Ekici, K. and Alisarli, M. (2008). Histamine formation and microbiological changes in endemic *Chalcalburnus tarichi* Pallas 1811 (Inci Kefali) stored at 4^{o}C. Arch Med Vet., 40: 95 - 98.

Ezzat, M.A.; Zare, D.; Karim, R. and Ghazali, H.M. (2015). Trans- and cisurocanic acid, biogenic amine and amino acid contents in ikanpekasam (fermented fish) produced from Javanese carp (Puntiusgonionotus) and black tilapia (Oreochromismossambicus). Food Chem., 172: 893 – 899.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). (2013). Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products. Meeting report.

Food and Drug Administration (FDA). (2001). Fish and fisheries products hazards and controls guidance, 3rd edn. US FDA Center for Food Safety and Applied Nutrition, Maryland.

Food and Drug Administration (FDA). (2011). Fish and Fishery Products Hazards and Controls Guidance, 4th edn. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC.

Huang, Y.R.; Liu, K.J.; Hsieh, H.S. ; Hsieh, C.H.; Hwang, D.F. and Tsai, Y. (2010). Histamine level and histamine-forming bacteria in dried fish products sold in Penghu Island of Taiwan. Food Control, 21: 234 – 1239.

Ibrahim, H.K.; Almayah, A.A. and Issa, A.H. (2017). Molecular Detection of Environmental *Morganella morganii* as Histamine Producing Bacteria. Donnish Journal of Medicine and Medical Sciences, 4: 008 - 013.

Joosten, L.J. and Northolt, M.D. (1989). Detection, Growth, and Amine-Producing Capacity of Lactobacilli in Cheese. Appl. Environ. Microbiol., 55: 2356 - 2359.

Joshi, P.A. and Bhoir, V.S. (2011). Study of Histamine Forming Bacteria in Commercial fish samples of Kalyan city. Int. J. Cur. Sci. Res., 1(2): 39 – 42.

Ladero, V.; Calles-Enríquez, M.; Fernández, M. and Alvarez M.A. (2010).Toxicological effects of dietary biogenic amines. Curr. Nutr. Food Sci., 6: 145 – 156.

Lee, Y.C.; Lin, C.S.; Liu, F.L. ; Huanga, T.C. andTsaic, Y.H. (2015). Degradation of histamine by *Bacillus polymyxa* isolated from salted fish products. J Food Drug Anal., 23: 836 – 844.

Lehane, L. and Olley, J. (2000). Histamine fish poisoning revisited. Int. J. Food Microbiol., 58: 1 – 37.

Maijala, R.L.; Eerola, S.H.; Aho, M.A. and Hirn, J.A. (1993). The effect of GDLinduced pH decrease on the formation of biogenic amines in meat. J. Food Protect., 56: 125 – 129.

Mavromatis, P. and Quantick, P.C. (2002). Modification of Niven's Medium for the Enumeration of Histamine-Forming Bacteria and Discussion of the Parameters, J. Food Prot., 65: 546 – 551.

Meron, D.; Davidovich, N.; Ofek-Lalzar, M. ; Berzak, R. ; Scheinin, A.; Regev, Y. ; Diga, R. ; Tchernov, D. and Morick, D. (2020). Specific pathogens and microbial abundance within liver and kidney tissues of wild marine fish from the Eastern Mediterranean Sea. Microb. Biotechnol., 13: 770 – 780.

Nader, Y.M.; ElBahy, E.F. and Anter, D.M. (2016). Biogenic Amines in Salted and Smoked Herring Fish ,Global Veterinaria., 16: 525 - 529.

Naila, A.; Flint, S.; Fletcher, G. ; Bremer, P. and Meerdink, G. (2010). Control of Biogenic Amines in Food—Existing and Emerging Approaches. J. Food Sci., 75: R139 – R150.

Naila, A.; Flint, S.; Fletcher, G.C. ; Bremer, P. J. ;Meerdink, G. and Morton, R. H. (2012). Prediction of the amount and rate of histamine degradation by diamines oxidase (DAO). Food Chem., 135: 2650 – 2660.

Niven, C.F.; Jeffrey, M.B. and Corlett, D.A. (1981). Differential Plating Medium for Quantitative Detection of Histamine-Producing Bacteria Appl. Environ. Microbiol., 41: 321 - 322.

Onal, A.; Tekkeli, S.E.K. and Onal, C. (2013). A review of the liquid chromatographic methods for the determination biogenic amines in foods. Food Chem., 138: 509 – 515.

Ozogul, F. and Ozogul, Y. (2006). Biogenic amine content and biogenic amine quality indices of sardines (Sardinapilchardus) stored in modified atmosphere packaging and vacuum packaging. Food Chem., 99: 574 – 578.

Parente, E.; Matuscelli, M.; Gadrini, F. ; Grieco, S. ; Crudele, M.A. and Suzzi, G. (2001). Evolution of microbial populations and biogenic amines production in dry sausages produced in southern Italy. J. Appl. Microbiol., 90 :882 – 891.

Piamsomboon, P.; Jaresitthikunchai, J.; Hung, T.Q. ; Roytrakul, S.and Wongtavatchai, J. (2020). Identification of bacterial pathogens in cultured fish with a custom peptide database constructed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). BMC. Vet. Res., 16: 52.

Price, R.J. and Melvin, E.F. (1994). Recommendations for on Board Handling of Albacore Tuna. Sea Grant Extension Program, Food Science and Technology Extension, University of California, Davis.

Quinn, P.J.; Markey, B.K.; Carter, M.E. ; Hartigan, P.; Fanning, S. and Fitzpatrick ,E.S. (2002). Veterinary Microbiology and microbial disease. 1st Published, Blackwell Science Ltd.

Rahimi, E.F.; Nayebpour, and Alian, F. (2012). Determination of histamine in canned tuna fish using ELISA method. Am. Eurasian. J. Toxicol. Sci., 4: 64 - 66.

Sabry, M.A.; Hayam, A.A.M.; Ashour,R.M. and Hamza, E. (2019). Histamine-Producing Bacteria and Histamine Induction in Retail Sardine and Mackerel from Fish Markets in Egypt. Food borne Pathog. Dis., 16: 597 - 603.

Satomi, M.; Furushita, M.; Oikawa, H. and Yano, Y. (2011). Diversity of plasmids encoding histidine decarboxylase gene in *Tetragenococcus* spp. isolated from Japanese fish sauce. Int. J. Food Microbiol., 148: 60 - 65.

Simora, R.C. and Peralta, E.M. (2018). Occurrence of Histamine and Histamine-forming Bacteria in Philippine Traditional Dried-salted Fish Products. Asian Fish. Sci., 31:73 – 88.

Soliman, W.S; Shaapan, R.M.; Mohamed, L.A.;Younes, A. M. ; Elgendy, M.Y. and Salah El din, D. A. (2017). Laboratory Screening of Biogenic Amines Producing Bacteria Potentially Threatens Human Health in Some Egyptian Fish and Fish Products. J. Fish. Aquat. Sci., 12: 134 - 140.

Stadnik, J. and Dolatowski, Z.J. (2010). Biogenic amines in meat and refrigerated meat products. Acta. Sci. Po.l Technol. Aliment., 9(3): 251 – 263.

Trevisani, M.; Mancusi, R.; Cecchini, M. ; Costanza, l. and Prearo, M. (2017). Detection and Characterization of Histamine-Producing Strains of *Photobacterium damselae* subsp. *damselae* Isolated from Mullets. Vet. Sci., 4: 1 - 11.

الملخص العربي

البكتيريا المنتجة للهستامين في الأسماك

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يمثل أنتاج الأحماض الأمينية الحيوية بالأماك ومنتجاتها خطورة صحية بتسمم غذاء الأنسان . أقيم المسح الميداني للتعرف على أغلب أنواع البكتريا سيادة بإنتاجها الهيستامين ةتحديد مستوياته بالسماك ومنتجاتها في مصر . تم تجميع ١٥٠ عينة من السمك البلطي ، الردين ، الرنجة المدخنه ، الماكريل ،فيليه الكارب ، المرجان ، البيلشارس) من محلات التجزئة بمحافظة الجيزة وتم فحصها بكتريولوجيا والتعرف على انواع البكتريا بالطرق التقليدية وكذلك اخذ عينات من أنسجة السماك لتحديد مستوى الهيتامسن بالعينات الموجبة باستخدام الإيليزا .أوضحت النتائج أن العائلة البكتيرية المعوية أكثر سيادة في العينات وانتاجها للهيستامين.. وكانت الأنواع من هذة العاءلة والمعزولة بنسب تكرار كما يلي : *Klebsiella* (33.3%), staphylococci (24.7%), *Salmonella* (22.7%), *E. coli* (18.7%), *Pseudomonas* (18%), *Proteus* (16.7%) and *Vibrio* (6.7%) species.

تم تنميه البكتريا على الوسط الغذائي أجار النيفا لتحديد البكتريا المنتجة للهيستامين . تم تحديد نسب الهيستامين بخمسة عشر (١٠%) من اصل ١٥٠ عينة من أنسجة الأسماك بها عزلات أيجابية بعد أنماؤها على الوسط الغذاءي بأجار النيفا . وأوضح أختبار الإليزا لتحديد مستويات الهيستامين أن الفسيخ أحتوى على أعلى تركيز والذي تراوح بين (50 - 50.8 mg/100mg) تلاها فيليه الكارب ٢٢.٥ ثم الرنجة المدخنة ٨- ١٠ ، والماكريل ٢٠.٦-٢٣ ، السردين ١٧.٥٥-٢٣ ، المرجان 97-8-76-9، على التتابع . خلص البحث إلى ان تطبيق الكشف المبكر للبكتريا المنتجة للأحماض الأمينية الحيوة خاصة الهيستامين يساعد على خفض خطورة التسمم الغذاءي المصاحب لتلف الأغذية الميكروبي . كما أكد اهمية تطوير وتحسين الأوساط التنموية كوسيلة قيمة لإكتشاف البكتريا التي تحتوي على الأنزيم المحول للهيستيدين إلى الهيستامين للحفاظ على صحة الأفراد.