

Histamine and Histamine Producing Bacteria in Some Local and Imported Fish and Their Public Health Significance

¹Nahla, T. Korashy and ²Hassan El-S. M. Farag

¹Microbiology Department, Port Said Lab., Animal Health Research Institute, Dokki, Giza, Egypt.

²Food Hygiene Department, Port Said Lab., Animal Health Research Institute, Dokki, Giza, Egypt.

Abstract: One hundred eighty of apparently healthy samples of some species of local and imported fish were randomly purchased from Port-Said markets. The samples were examined for counting, isolation, and identification of total aerobic and histamine producing bacteria and for determination of the histamine levels in each sample. The mean values of total aerobic and histamine producing bacterial counts of local fish samples were higher than that of imported fish samples while the mean values of histamine levels in the muscle tissues of local fish samples were lower than that of imported fish samples. The counts of total aerobic and histamine producing bacteria and the levels of histamine in the muscle tissues samples of some species of local fish were ranged from 8.5×10^2 to 8.5×10^8 , from 1.5×10^1 to 9.5×10^3 CFU/g fish and from 7.00 to 26.00 mg/100 g fish respectively, while that of imported one were ranged from 2.5×10^4 to 6.0×10^8 , from 2.6×10^1 to 9.5×10^3 CFU/g fish and from 18.00 to 50.00 mg/100 g fish respectively. The number of total aerobic and histamine producing bacterial isolates of local fish were 71 and 11 while that of imported fish were 98 and 25 isolates respectively. The bacterial isolates in both local and imported fish samples were identified as *Micrococcus* spp., *Planococcus* spp., *Morganella morganii* (*P. morganii*), *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Proteus vulgaris* and *Aeromonas* spp. All isolates had different incidences at each species of fish and some isolates were histamine producing positive. The frequency distribution and the relationship between the total aerobic and histamine producing bacterial counts and histamine levels were discussed.

Key words: Histamine, fish, bacteria

INTRODUCTION

Fish is an important and good quality source of protein; vitamins and minerals but it deteriorate fastly when decarboxylase enzyme can be formed by bacterial growth causing generation of bioactive amine including histamine^[1].

Histamine is heterocyclic biologically active primary amine formed post-mortem in the muscle of scombroid and non-scombroid fish rich in free histidine by the action of certain bacteria in addition to the histamine released during decomposition and spoilage by proteolysis^[2]. The level of histamine produced in fish mainly exogenous and serves as indicators of spoilage^[3].

The problem of histamine intoxication is due to consumers is unlikely to identify the histamine levels before eating the fish as fish appearance and smell appear normal. Also histamine is heat stable and once formed not destroyed by cooking, canning, smoking, and freezing while enzyme is affected by cooking^[4,5].

Histamine poisoning results from consumption of spoiled fish contain high levels of histamine results from growth and action of certain bacteria aided by the incorrect storage^[6].

The consumption of fish with high levels of histamine can cause food born disease and intoxication^[1] and the toxicity occur from several minutes to several hours after ingestion of toxic fish and the illness typically lasts few hours but may continue for several days which include cutaneous rash, urticaria, burning itching, edema, gastrointestinal inflammation, nausea, vomiting, diarrhea, haemodynamic hypotension and neurological headache^[7,8]. Also cardiovascular shock associated with subendocardial, myocardial infarction or acute pulmonary edema with myocardial ischemia were recorded^[9].

Histamine consumed in spoiled fish is more toxic than pure histamine taken orally due to a missing factor as imidazole compound and histamine like compound derived from histidine consequently increase the histamine poisoning problem^[10,11].

Therefore, the present study was carried out to evaluate some species of local and imported fish by the counting of the total aerobic and histamine-producing bacteria and determination of histamine levels in muscle tissues. The relationships between the different measured parameters and their public health significance were done.

MATERIALS AND METHODS

Samples collection: A total of 180 of apparently healthy samples of various species of local fish (30 each of *Euthynnus affinis* “Kawakaw”, *Sardinella gibbosa* and *Mugil cephalus* “Mullet”) and imported fish (30 each of *Trachurus Trachurus* “Atlantic Horse Mackerel”, *Orcynopsis unicolor* “Plain bonito” and *Sardina pilchardus* “European pilchard”) were purchased from Port-Said markets. Each individual sample was placed separately into plastic bag on ice, thoroughly identified and delivered to the Lab.

Preparation of the samples for bacteriological examination: A representative 25 g of fish meat sample was taken aseptically and homogenized^[12].

Enumeration of bacterial flora: One ml from each dilution was spread thoroughly and uniformly onto marked petri plates of Trypticase Soy agar. The aerobic plate count was expressed as number of organisms/g of food^[13,14].

Biochemical and morphological identification: The isolates were identified by Gram stain, catalase test, oxidase test, carbohydrates fermentation, TSI slant, citrate test and urease test and others biochemical tests, Lechowich^[15] and Sneath, *et al*^[16].

Screening for histidine decarboxylating activity of all isolates: The isolates were tested with modified Niven’s medium in broth cultures (composed of modified Niven’s medium without agar) containing inverted Durham’s tubes as carbon dioxide gas traps or in agar surface streak, Yoshinaga and Frank^[12].

Enumeration of histamine producing bacteria: One ml from each dilution was spread thoroughly and uniformly onto marked petri plates of Niven’s agar medium using pour plate technique for counts of histamine producing bacteria, according to Niven, *et al*^[13], Yoshinaga and Frank^[12] and Fletcher, *et al*^[14].

Histamine level estimation: Estimation of histamine in the different collected fish muscle samples was conducted by thin layer chromatography (TLC) methods according to Schutz, *et al*^[17], Voigt and Eitenmiller^[18] and A.O.A.C.^[19].

Statistical Analysis: Minimum, maximum, mean, standard deviation and standard error of mean and frequency distribution were used to describe data. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 10.0 (SPSS Inc., Chicago, IL, and USA).

RESULTS AND DISCUSSIONS

Fresh fish do not contain free histamine but contain amino acid L-Histidine. Additional histidine may be released during the decomposition and spoilage by proteolysis whereby the protein structure was degraded and amino acid histidine liberated^[2]. The contamination of fish after caught with histamine producing bacteria and the releases of microbial contents of the intestine due to postmortem disintegration results in contamination of muscle tissue and accumulation of histamine^[20].

The summarized results given in table (1-2) showed that the mean values of total aerobic and histamine producing bacterial counts in local fish samples were 6.7×10^6 and 2.2×10^3 , 8.6×10^7 and 2.5×10^3 , and 2.3×10^6 and 2.5×10^3 CFU/g fish for *Euthynnus affinis* “Kawakaw”, *Sardinella gibbosa* and *Mugil cephalus* “Mullet” respectively while that of imported fish samples were 7.0×10^7 and 2.2×10^3 , 4.2×10^6 and 1.3×10^3 and 6.5×10^6 and 2.1×10^3 CFU/g fish for *Trachurus Trachurus* “Atlantic Horse Mackerel”, *Orcynopsis unicolor* “Plain bonito” and *Sardina pilchardus* “European pilchard” respectively. The obtained results were higher than that reported by^[21,5] and this may be attributed to the defect in time/temperature abuse of the fish chilling immediately after caught. The mean values of total aerobic and histamine producing bacterial counts in local fish were higher than that of imported one and this may be due to the freezing state of the imported fish, which reduced the bacterial floral counts more than the cold storage state of local fish^[22-24]. The wide range between histamine producing bacteria counts and total aerobic bacterial counts was due to histamine producing bacteria comprise a minor count in freshly caught fish and easily killed by freezing^[13].

The histamine levels in tables (1-2) revealed that the mean values of histamine levels in muscle tissues of the local fish samples were 22.10, 16.40 and 11.00 mg/100 g fish for *Euthynnus affinis* “Kawakaw”, *Sardinella gibbosa* and *Mugil cephalus* “Mullet” respectively, while that of imported fish samples were 45.00, 27.80 and 22.40 mg/100 g fish for *Trachurus Trachurus* “Atlantic Horse Mackerel”, *Orcynopsis unicolor* “Plain bonito” and *Sardina pilchardus* “European pilchard” respectively. These results were lower than that reported by Yoshinaga and Frank^[12], Su, *et al*^[25] and Kim, *et al*^[5] and higher than

Table 1: Statistical analytical results of total aerobic and histamine producing bacterial counts (CFU/g fish) and histamine levels (mg/100 g fish) in some species of local fish.

Statistics	Local fish								
	Euthynnus affinis "Kawakaw"			Sardinella gibbosa			Mugil cephalus "Mullet"		
	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels
Min.	2.0 X 10 ³	7.5 X 10 ¹	18.00	3.5 X 10 ³	1.5 X 10 ¹	13.00	8.5 X 10 ²	7.0 X 10 ¹	7.00
Max.	2.8 X 10 ⁷	7.5 X 10 ³	26.00	8.5 X 10 ⁸	9.5 X 10 ³	19.00	8.0 X 10 ⁶	8.5 X 10 ³	15.00
Mean	6.7 X 10 ⁶	2.2 X 10 ³	22.10	8.6 X 10 ⁷	2.5 X 10 ³	16.40	2.3 X 10 ⁶	2.5 X 10 ³	11.00
SE	1.9 X 10 ⁶	4.5 X 10 ²	00.44	4.7 X 10 ⁷	6.6 X 10 ²	00.33	6.1 X 10 ⁵	5.4 X 10 ²	0.42
SD	1.0 X 10 ⁷	2.5 X 10 ³	2.40	2.6 X 10 ⁸	3.6 X 10 ³	1.83	3.4 X 10 ⁶	3.00X 10 ³	2.32

Min. = Minimum, Max. = Maximum, SE = Standard Error, SD = Standard Deviation.

Table 2: Statistical analytical results of total aerobic and histamine producing bacterial counts (CFU/g fish) and histamine levels (mg/100 g fish) in some species of imported fish.

Statistics	Imported fish								
	Trachurus Trachurus "Atlantic Horse Mackerel"			Orcynopsis unicolor "Plain bonito"			Sardina pilchardus "European pilchard"		
	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels	Total aerobic bacterial counts	Histamine producing bacterial counts	Histamine levels
Min.	2.5 X 10 ⁴	2.6 X 10 ¹	39.00	4.0X 10 ⁴	8.5 X 10 ¹	22.00	7.5 X 10 ⁴	8.5 X 10 ¹	18.00
Max.	6.0 X 10 ⁸	7.5X 10 ³	50.00	3.0 X 10 ⁷	4.5 X 10 ³	39.00	4.6 X 10 ⁷	9.5 X 10 ³	27.00
Mean	7.0 X 10 ⁷	2.2 X 10 ³	45.00	4.2 X 10 ⁶	1.3 X 10 ³	27.80	6.5 X 10 ⁶	2.1 X 10 ³	22.40
SE	3.3 X 10 ⁷	5.3 X 10 ²	0.70	1.6 X 10 ⁶	2.5 X 10 ²	0.81	2.5 X 10 ⁶	5.2 X 10 ²	0.50
SD	1.8 X 10 ⁸	2.9 X 10 ³	3.70	8.9 X 10 ⁶	1.4 X 10 ³	4.50	1.4 X 10 ⁷	2.9 X 10 ³	2.70

The relationship between the total aerobic and histamine producing bacterial counts and histamine levels are non significant at P < 0.05 and P < 0.01 using t-test in all samples

Table 3: Frequency distribution of total aerobic and histamine producing bacterial counts in some species of local fish.

Count range	Local fish											
	Euthynnus affinis "Kawakaw"				Sardinella gibbosa				Mugil cephalus "Mullet"			
	Total aerobic bacterial counts		Histamine producing bacterial counts		Total aerobic bacterial counts		Histamine producing bacterial counts		Total aerobic bacterial counts		Histamine producing bacterial counts	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0.0 - < 10 ²	0.00	0.00	3.00	10.00	0.00	0.00	9.00	30.00	0.00	0.00	3.00	10.00
10 ² - < 10 ³	0.00	0.00	15.00	50.00	0.00	0.00	12.00	40.00	3.00	10.00	15.00	50.00
10 ³ - < 10 ⁴	12.00	40.00	12.00	40.00	18.00	60.00	9.00	30.00	12.00	40.00	12.00	40.00
10 ⁴ - < 10 ⁵	6.00	20.00	0.00	0.00	6.00	20.00	0.00	0.00	6.00	20.00	0.00	0.00
10 ⁵ - < 10 ⁶	6.00	20.00	0.00	0.00	3.00	10.00	0.00	0.00	6.00	20.00	0.00	0.00
10 ⁶ - < 10 ⁷	6.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	10.00	0.00	0.00
10 ⁷ - < 10 ⁸	0.00	0.00	0.00	0.00	3.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
≥10 ⁸	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00

Table 4: Frequency distribution of total aerobic and histamine producing bacterial counts in some species of imported fish.

Count range	Imported fish											
	Trachurus Trachurus Atlantic Horse Mackerel				Orcynopsis unicolor "Plain bonito"				Sardina pilchardus "European pilchard"			
	Total aerobic bacterial counts		Histamine producing bacterial counts		Total aerobic bacterial counts		Histamine producing bacterial counts		Total aerobic bacterial counts		Histamine producing bacterial counts	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0.0 - < 10 ²	0.00	0.00	3.00	10.00	0.00	0.00	3.00	10.00	0.00	0.00	3.00	10.00
10 ² - < 10 ³	0.00	0.00	18.00	60.00	0.00	0.00	18.00	60.00	0.00	0.00	15.00	50.00
10 ³ - < 10 ⁴	0.00	0.00	9.00	30.00	0.00	0.00	9.00	30.00	0.00	0.00	12.00	40.00
10 ⁴ - < 10 ⁵	9.00	30.00	0.00	0.00	9.00	30.00	0.00	0.00	3.00	10.00	0.00	0.00
10 ⁵ - < 10 ⁶	6.00	20.00	0.00	0.00	9.00	30.00	0.00	0.00	15.00	50.00	0.00	0.00
10 ⁶ - < 10 ⁷	9.00	30.00	0.00	0.00	9.00	30.00	0.00	0.00	9.00	30.00	0.00	0.00
10 ⁷ - < 10 ⁸	3.00	10.00	0.00	0.00	3.00	10.00	0.00	0.00	3.00	10.00	0.00	0.00
≥10 ⁸	3.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00

Table 5: Frequency distribution of histamine level in some species of local and imported fish.

Count range	Local fish						Imported fish					
	Euthynnus affinis "Kawakaw"		Sardinella gibbosa		Mugil cephalus "Mullet"		Trachurus Trachurus "Atlantic Horse Mackerel"		Orcynopsis unicolor "Plain bonito"		Sardina pilchardus "European pilchard"	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0.0 - < 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5- < 10	0.00	0.00	0.00	0.00	6.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00
10- < 15	0.00	0.00	3.00	10.00	21.00	70.00	0.00	0.00	0.00	0.00	0.00	0.00
15 - < 20	3.00	10.00	27.00	90.00	3.00	10.00	0.00	0.00	0.00	0.00	3.00	10.00
20 - < 25	21.00	70.00	0.00	0.00	0.00	0.00	0.00	0.00	6.00	20.00	21.00	70.00
25- < 30	6.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	18.00	60.00	6.00	20.00
30- < 35	0.00	0.00	0.00	0.00	0.00	0.00	3.00	10.00	3.00	10.00	0.00	0.00
35- < 40	0.00	0.00	0.00	0.00	0.00	0.00	9.00	30.00	3.00	10.00	0.00	0.00
40- < 50	0.00	0.00	0.00	0.00	0.00	0.00	15.00	50.00	0.00	0.00	0.00	0.00
= 50	0.00	0.00	0.00	0.00	0.00	0.00	3.00	10.00	0.00	0.00	0.00	0.00
Total	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00

that reported by Gajewska and Ganowiak^[26]. Also these results were exceeded the defect action levels stated by FDA/CSAN^[3] and the levels stated by E.O.S.Q.C.^[27, 28]. The histamine levels of the imported samples were higher than that of the local samples and this may be attributed to poor handling and contamination of fish by histamine producing bacteria immediately after fish caught and

before freezing storage Leuschner and Hammes^[29]. Also repeated thawing and freezing and defect in the storage temperature with high levels of free L-histidine results in activation of histidine decarboxylase enzymes consequently accumulation of histamine, Gingerich, *et al*^[21] and Kerr, *et al*^[30] besides the stability of histamine in the frozen state Becker *et al*^[4].

Table 6: Frequency distribution of bacterial isolates of some species of local and imported fish.

Bacterial isolates	Gram stain reaction	Local fish				Imported fish			
		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
I-Obligatory aerobic									
1- Micrococcus spp.	+ve	8	11.27	0	0.00	7	7.14	0	0.00
2- Planococcus spp.	+ve	3	4.23	0	0.00	4	4.08	0	0.00
II-Facultative anaerobic									
1-Morganella morganii (P. morganii)	-ve	3	4.23	3	27.27	7	7.14	7	28.00
2-Enterobacter aerogenes	-ve	1	1.41	1	9.09	3	3.06	3	12.00
3-Enterobacter cloacae	-ve	5	7.04	1	9.09	7	7.14	1	4.00
4-Klebsiella pneumoniae	-ve	1	1.41	1	9.09	2	2.04	2	8.00
5-Vibrio parahaemolyticus	-ve	10	14.08	1	9.09	15	15.31	3	12.00
6-Vibrio alginolyticus	-ve	6	8.45	1	9.09	8	8.16	2	8.00
7-Vibrio anguillarum	-ve	12	16.90	2	18.18	20	20.41	4	16.00
8-Proteus vulgaris	-ve	7	9.86	1	9.09	13	13.27	3	12.00
9-Aeromonas spp.	-ve	15	21.13	0	0.00	12	12.24	0	0.00
Total		71	100.00	11	100.00	98	100.00	25	100.00

Table 7: Frequency distribution of bacterial isolates of some species of local fish .

Bacterial isolates	Gram stain reaction	Local fish													
		Euthynnus affinis "Kawakaw"				Sardinella gibbosa				Mugil cephalus "Mullet"					
		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates			
No.		%		No.		%		No.		%		No.		%	
I-Obligatory aerobic															
1- Micrococcus spp.	+ve	3	9.09	0	0.00	3	13.04	0	0.00	2	13.33	0	0.00		
2- Planococcus spp.	+ve	1	3.03	0	0.00	1	4.35	0	0.00	1	6.67	0	0.00		
II-Facultative anaerobic															
1-Morganella morganii	-ve	1	3.03	1	16.67	1	4.35	1	33.33	1	6.67	1	50.00		
2-Enterobacter aerogenes	-ve	1	3.03	1	16.67	0	0.00	0	0.00	0	0.00	0	0.00		
3-Enterobacter cloacae	-ve	2	6.06	1	16.67	2	8.70	0	0.00	1	6.67	0	0.00		
4-Klebsiella pneumoniae	-ve	1	3.03	1	16.67	0	0.00	0	0.00	0	0.00	0	0.00		
5-Vibrio parahaemolyticus	-ve	5	15.15	0	0.00	3	13.04	1	33.33	2	13.33	0	0.00		
6-Vibrio alginolyticus	-ve	3	9.09	1	16.67	2	8.70	0	0.00	1	6.67	0	0.00		
7-Vibrio anguillarum	-ve	5	15.15	1	16.67	4	17.39	1	33.33	3	20.00	0	0.00		
8-Proteus vulgaris	-ve	3	9.09	0	0.00	3	13.04	0	0.00	1	6.67	1	50.00		
9-Aeromonas spp.	-ve	8	24.24	0	0.00	4	17.39	0	0.00	3	20.00	0	0.00		
Total isolates		33	100.00	6	100.00	23	100.00	3	100.00	15	100.00	2	100.00		

Table 8: Frequency distribution of bacterial isolates of some species of imported fish.

Bacterial isolates	Gram stain reaction	Imported fish											
		Trachurus Trachurus "Atlantic Horse Mackerel"				Orcynopsis unicolor "Plain bonito"				Sardina pilchardus "European pilchard"			
		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates		Incidence of aerobic bacterial isolates		Incidence of histidine decarboxylase isolates	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I-Obligatory aerobic													
1- Micrococcus spp.	+ve	3	6.38	0	0.00	2	7.14	0	0.00	2	8.70	0	0.00
2- Planococcus spp.	+ve	2	4.26	0	0.00	1	3.57	0	0.00	1	4.35	0	0.00
II-Facultative anaerobic													
1-Morganella morganii	-ve	4	8.51	4	28.57	2	7.14	2	33.33	1	4.35	1	20.00
2-Enterobacter aerogenes	-ve	1	2.13	1	7.14	1	3.57	1	16.67	1	4.35	1	20.00
3-Enterobacter cloacae	-ve	3	6.38	1	7.14	2	7.14	0	0.00	2	8.70	0	0.00
4-Klebsiella pneumoniae	-ve	1	2.13	1	7.14	0	0.00	0	0.00	1	4.35	1	20.00
5-Vibrio parahaemolyticus	-ve	9	19.15	2	14.29	4	14.29	0	0.00	2	8.70	1	20.00
6-Vibrio alginolyticus	-ve	3	6.38	1	7.14	3	10.71	1	16.67	2	8.70	0	0.00
7-Vibrio anguillarum	-ve	10	21.28	2	14.29	6	21.43	1	16.67	4	17.39	1	20.00
8-Proteus vulgaris	-ve	6	12.77	2	14.29	3	10.71	1	16.67	4	17.39	0	0.00
9-Aeromonas spp.	-ve	5	10.64	0	0.00	4	14.29	0	0.00	3	13.04	0	0.00
Total isolates		47	100.00	14	100.00	28	100.00	6	100.00	23	100.00	5	100.00

The results given in table (3-4) showed that the total aerobic bacterial counts of local fish samples were ranged from 10^3 to $< 10^7$ CFU/g fish for 100% of *Euthynnus affinis* "Kawakaw" samples, from 10^3 to $< 10^6$ and from 10^7 to $< 10^8$ CFU/g fish for 90% and 10% of *Sardinella gibbosa* samples respectively while 100% of *Mugil cephalus* "Mullet" samples were ranged from 10^2 to $< 10^7$ CFU/g fish. The total aerobic bacterial counts of imported fish sample were ranged from 10^4 to $\geq 10^8$ CFU/g fish for 100% of *Trachurus Trachurus* "Atlantic Horse Mackerel", and from 10^4 to $< 10^8$ CFU/g fish for 100% of each of *Orcynopsis unicolor* "Plain bonito" and *Sardina pilchardus* "European pilchard" samples. The histamine producing bacteria were ranged from 0.00 to $< 10^4$ CFU/g fish for 100% of the all local and imported fish samples.

The frequency distribution in table (5) showed that the histamine levels of local fish were ranged from 15 to < 30 , from 10 to < 20 and from 5 to < 20 mg/100 g fish for *Euthynnus affinis* "Kawakaw", *Sardinella gibbosa* and *Mugil cephalus* "Mullet" samples respectively, while that of imported fish were ranged from 30 to 50, from 20 to < 40 and from 15 to < 30 mg/100 g fish for *Trachurus*

Trachurus "Atlantic Horse Mackerel", *Orcynopsis unicolor* "Plain bonito" and *Sardina pilchardus* "European pilchard" samples respectively. The wide range and the variation between the total aerobic counts, histamine producing bacterial counts and histamine levels in all types of local and imported fish samples indicate a non-significant relationship between total aerobic and histamine producing bacterial counts and histamine levels and this result agreed with that reported by Fletcher *et al*^[14] and Gingerich *et al*^[21].

Our results in table (6-8) showed that the incidence of aerobic and histamine producing bacterial isolates in local fish samples were 71 and 11 isolates respectively, while that of imported samples were 98 and 25 isolates respectively. The high incidence of aerobic and histamine producing bacterial isolates in local fish samples were recorded in *Euthynnus affinis* "Kawakaw" and representative by 33 and 6 isolates respectively while the low incidence were recorded in *Mugil cephalus* "Mullet" and representative by 15 and 2 isolates respectively.

The high incidence of aerobic and histamine producing bacterial isolates in imported fish samples were

recorded in *Trachurus Trachurus* “Atlantic Horse Mackerel” and representative by 47 and 14 isolates respectively, while the low incidence were recorded in *Sardina pilchardus* “European pilchard” and representative by 23 and 5 isolates respectively.

Also our result in table (6-8) showed that the identified non histamine producing bacterial isolates were *Micrococcus* spp., *Planococcus* spp. and *Aeromonas* spp. while the identified histamine producing bacterial isolates were *Morganella morganii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum* and *Proteus vulgaris*. The main histamine producing bacterial isolates which found in all types of local and imported fish samples were *Morganella morganii*. These results agreed with the results recorded by Frank *et al*^[20], Niven *et al*^[13] and Yoshinaga and Frank^[12] whose reported that the histamine producing bacteria were exclusively mesophilic bacteria and belonging to family Enterobacteriaceae.

Despite to histamine is heat stable toxin, not destroyed by cooking and no available methods of preparation, including freezing, canning, and smoking will destroy the causative toxins Kim, *et al*^[5], thus rapid chilling of fish post harvested and storage below refrigeration temperature are commonly used in controlling histamine formation Behling and Taylor^[31]. In conclusion, significant decomposition and histamine formation can be avoided by good fish handling practices including icing or rapid immersion of the catch in water chilled to (-1 °C) followed by uninterrupted frozen storage

REFERENCES

1. Moreno, R.B. and E.A. Torres, 2001. Histamine levels in fresh fish, a quality index. Session 42, Seafood Technology, Safety, Processing 2001, IFT Annual Meeting. New Orleans, Louisiana.
2. Eitenmiller, R. and S. Desouza, 1984. “Enzymatic Mechanisms for amine formation in Fish” in seafood toxins, edited by E.P. Ragelis, American chemical Society, Washington, D.C. pp 443-442
3. Food and Drug Administration “FDA/CFSAN”, 1995. Decomposition and Histamine-Raw, frozen Tuna and Mahi-Mahi, Canned Tuna and Related Species. Revised compliance Policy Guide, Availability. Department of Health and Human Services Food and Drug administration.
4. Becker, K., K. Southwick, J. Reardon, R. Berg and N. MacCormack, 2001. Histamine poisoning associated with eating tuna burgers. JAMA. 285: 1327-1330.
5. Kim, S.H., K.G. Field, D.S. Chang, C.I. Wei and H. An, 2001. Identification of bacteria crucial to histamine accumulation in pacific mackerel during storage. J. Food Prot. 64 (10): 1556-1564.
6. Sabroe, R. and B. Kobza, 1998. Scombrototoxic fish poisoning. Clin. Exp. Dermatol. 23 (6): 258-259
7. Hall, M., 2003. Something fishy: six patients with an unusual cause of food poisoning! Emerg. Med (Fremantle) 15 (3): 293-295.
8. Predy, G., L. Honish, W. Hohn and S. Jones, 2003. Was it something she ate? Case report and discussion of scombroid poisoning. CMAJ 168 (5) 587-588.
9. Specht, D., 1998. Scombroid fish poisoning. Journal of Emergency Nursing 24 (2): 118-119.
10. Mitchell, J.W., 1993. Scombrototoxic fish poisoning. A report prepared for the Ministry of Health. Institute of Environmental Health and Forensic Sciences Limited, Mt Albert Science center, NZ, 18pp.
11. Lehane, L. and J. Olley, 2000. Histamine fish poisoning revisited. Int. J. Food Microbiol. 58 (1-2) 1-37.
12. Yoshinaga, D. and H. Frank, 1982. Histamine-Producing bacteria in decomposing Skipjack tuna (*Katsuwonus pelamis*). Applied and Environmental Microbiology 44 (2): 447-452.
13. Niven, C., M. Jeffrey and D. Corlett, 1981. Differential plating Medium for Quantitative Detection of Histamine producing Bacteria. Applied and Environmental Microbiology 41(1), 321-322
14. Fletcher, G., G. Summers and P. Van Veghel, 1998. Levels of Histamine and Histamine-Producing Bacteria in Smoked Fish from New Zealand Markets. J. Food Prot. 61 (8): 1064-1070.
15. Lechowich, R., 1978. Microbiology of meat. p 230-286. In. J. F. Price and B. F. Schweigert (ed.), The science of meat and meat products. W. H. Freeman and Co., San Francisco.
16. Sneath, P.A., N.S. Mair, M.E. Sharpe and J.C. Holt, 1985. Bergey’s Manual of Systematic Bacteriology. 8th ed. Williams & Wilkins, Baltimore, London, Los Angeles, Sydney.
17. Schutz, D., G. Chang and I. Bjeldanes, 1976. Rapid thin layer chromatographic methods for the detection of histamine in fish products. J. A.O.A.C., Vol. 58 (6) 1224.
18. Voigt, M. and R. Eitenmiller, 1977. An evaluation of extraction and thin layer chromatography procedures for quantification of biogenic amines in foods. Lebeusm. Wiss. U. Techhnol, 10, 26.
19. Association of Official Analytical Chemists “AOAC”, 1990. Histamine in seafood. Flurometric method, pp. 876-877. In K. Helrich (ed.), Official Methods of Analysis, 15th ed., vol. 2. Method 977.13. Association of Official Analytical Chemists, Inc., Arlington, Va.
20. Frank, H., D. Yoshinaga and W. Nip, 1981. Histamine formation and honey combing during decomposition of Skipjack tuna, *Katsuwonus pelamis*, at elevated temperature. Mar. Fish Rev.10: 9-12

21. Gingerich, T., T. Lorca, G. Flick, M. Pierson and H. McNair, 1999. Biogenic Amine Survey and Organoleptic Changes in Fresh, Stored, and Temperature-Abused Bluefish (*Pomatomus saltatrix*). *J. Food Prot.* 62 (9): 1033-1037.
22. Food and Drug Administration "FDA", 1999. Food code U.S. Public Health Service, U.S. Dep. of Health and Human Services. Pub. No. PB99-115925. Washington, D.C.
23. Moore, J. and R. Madden, 2001. Survival of *Campylobacter coli* in porcine liver. *Food Microbiology.* 18:1-10.
24. Farag, H. and T. Nahla, Korashy, 2005. Effect of freezing on psychrophilic, mesophilic and thermophilic bacteria in fresh meat. *Egypt. J. Comp. Path. & Clinic. Path.* 18 (2): 185-199.
25. Su, S., S. Chou, P. Chang and D. Hwang, 2000. Determination of biogenic amines in fish implicated in food poisoning by micellar electrokinetic capillary chromatography. *J. Chromatogr. B. Biomed. Sci. Appl.* 1; 749 (2): 163-169.
26. Gajewska, R. and Z. Ganowiak, 1992. Evaluation of degree of freshness and value of fish and fish product intake based on histamine and trimethylamine analysis. *Rocz. Panstw. Zakl. Hig.* 43(3-4): 245-251.
27. Egyptian Organization for Standardization and Quality Control, 2000. Egyptian Standard for cold fish. 3494/2000.
28. Egyptian Organization for Standardization and Quality Control, 2003. Egyptian Standard for frozen fish. 0889-01/2003.
29. Leuschner, R. and W. Hammes, 1999. Formation of biogenic amine in mayonnaise, herring and tuna fish salad by lactobacilli. *International Journal of Food Sciences and Nutrition*, 50(3),159-164.
30. Kerr, M., P. Lawicki, S. Aguirre and C. Rayner 2002. Effect of Storage Conditions on Histamine Formation in Fresh and Canned Tuna. Public Health Division, (1st ed) Victorian Government Department of Human Services.
31. Behling, A. and S. Taylor, 1982. Bacterial histamine production as a function of temperature and time of incubation. *J. Food Sci.* 47:1311-1314 and 1317.