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HISTAMINE LEVELS AND HISTAMINE PRODUCING BACTERIA IN FOUR SELECTED FISH SPECIES DISPLAYED IN THREE FISH MARKETS WITHIN TRIPOLI-CITY LIBYA

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ABSTRACT

The study aimed to assess temperature, histamine level and histamine producing bacteria (HPB) in four species of fresh fish samples i.e, *Sardinellaaurita*, *Boopsboops*, *Trachurusmediterraneus* and *Scomberscombrus* that are collected from three markets (A, B, C) within Tripoli city Libya. The results revealed that 95% of the fish samples had a temperature range between 5-22^{0}C, while 5% had a temperature < 5^0 C. Histamine was recorded in 43% of the samples. The ranges of histamine in sardine, bouge, saury, and mackerel samples were 1.29-5.74; 1.34-29.74;1.31-7.57 and 1.39-2.49 mg/100 g meat, respectively. These levels did not exceed the maximum limit (10 mg/100) adopted by the Libyan authority, except one sample (29.74 mg/100 g meat). A significant difference (P<0.05) in histamine levels was observed among the three markets. However, a non-significant difference (P>0.05) was observed between the fish species. The range for the means of HPBC in sardine, bouge, saury, and mackerel samples were 1.8×10^4-5.4×10^4; 6.4×10^4-2.0×10^5;6.4×10^4-6.910^5; 1.6×10^4-4.1×10^5cfu/g fish meat, respectively. Most of the HPB isolates were belonged to the family *Enterobacteriaceae* and some belong to the family *Vibrionaceae*. *Vibrio fluvialis* recorded the highest prevalence percentage (18%) followed by *Erwiniaspp*, *S. putrrefacienes,* and *K. planticola*, i.e.,12.2, 11.9 and 10.0%, respectively. The results of this study reflect the poor cooling conditions of the samples and poor cooling techniques practiced in these markets. Therefore, the hygienic practices in these markets have to be improved, and preferably the HACCP system has to be implemented.

1.Introduction

Histamine fish poisoning (known as scombroid fish poisoning) is the most common form of fish intoxication caused by seafood products and usually presents an allergic reaction. Many reports of histamine poisoning outbreaks were associated with the consumption of raw, cooked, frozen, salted and canned fish (Feldman et al. 2005; Becker et al. 2001; Lehan and Olley, 2000; Etkind et al. 1987; and Merson et al. 1974) which reflects the fact that histamine is heat stable and is not affected by freezing, salting and/ or drying. In Europe, 56 out of the 71 food borne diseases and outbreaks (78.9%) reported during 2011, were attributed to histamine fish poisoning (EFSA,2013).Under improper refrigeration or icing conditions, certain species of fish are sensitive to histamine formation. Tuna, Sardine, Bouge, Saury, and Mackerel are examples of such species (Alhalluge, 2012). The flesh of these species of fish contains the amino acid histidine, which is the precursor for histamine formation by decarboxylase enzymes via bacterial action, under improper icing or cooling conditions (FDA, 2001). The rate of histamine formation

and accumulation to a toxic level is so fast that it occurs before fish spoilage indices can be detected by sensory evaluation (Kim et al. 2004).

Ayesh et al. (2012) mentioned that the United State, Food and Drug Administration (FDA) had stated that histamine levels must be used as a guideline in Hazard Analysis and Critical Control Point (HACCP) programs for fish and has set the maximum action level of 50 ppm. It has also been reported that the family Enterobacteriaceae is the most important histamine forming bacteria in fish. *Morganellamorganii, Klebsiella pneumonia, Proteus vulgaris, and Hfniaalevi*are known to originate from fish implicated incidents of histamine poisoning (Huss et al. 2000; Lehan and Olley, 2000; and Frank, 1985).

Little is known about the epidemiology of histamine fish poisoning in Libya, particularly regarding the overall risk in regularly consumed fish species sensitive to histamine formation. Therefore, this study aimed to evaluate display conditions in terms of temperature, histamine producing bacteria count, and histamine levels in Sardine, Bouge, Saury, and Mackerel fish species displayed for sale in three main fresh fish markets located within Tripoli city, Libya.

2.Material and methods

2.1.Sample collection

Fresh fish samples (113 samples) of sardine, Bouge, Saury, and mackerel were collected directly from fish quantities displayed for sale in three main fish markets A, B, and C in Tripoli City -Libya. The samples were collected at 7,00 – 8,00 A.M. and at 12 – 1 P.M., during the period from July to December of their fishing season. The samples were kept in sterile polyethylene bags and transferred in icebox within 15 minutes to the Microbiology and fish disease laboratory at the marine research center in Tajoura, Libya. Meanwhile, the temperature of the displayed fish was measured at the time of sample collection.

2.2.Samples preparation for chemical and microbiological analysis.

A sample consists of 5 – 6 pieces from each sample of fish species were randomly withdrawn. Meat muscles with skin were cut from the back and sides of each fish body with a sterile knife and homogenized in a sterile blender, and then the meat homogenate was divided into two 25 grams' parts. One part was used for histamine determination while the other part of the homogenate was used for bacteriological analysis.

2.3.Histamine determination.

2.3.1.Fish samples extract preparation.

Five grams of fish meat homogenate were homogenized with 20 ml of 6% trichloroacetic acid solution (TCA) previously cooled to 4^0C. in electric blender for 3 min. The homogenate was filtered through Whatman No. 2 filter paper. The filtrate was placed in a 50 ml volumetric flask and the volume was completed to the mark with distilled water (Hwang et al, 1997).

2.3.2. Preparation of stock Standard histamine solution.

A stock solution of histamine was prepared by dissolving 0.0828 grams of histamine dichloride (C2H9N3.2HCL) (Acros Organics New Jersey USA) in a small volume of 0.1 M HCl solution in 50 ml volumetric flask and the volume was completed to the mark with 0.1 M HCL. This gives a 1 mg/ml histamine stock solution. Then 1 ml of this stock solution was transferred to a 10 ml volumetric flask and the volume was completed to the mark by 0.1 M HCl solution. This gives 0.1 mg histamine/ml stock solution. Then working standard solutions 0.02 – 4 ug histamine were prepared from the 0.1 mg/ml histamine stock solution (Hwang et al, 1997).

2.3.3. Histamine separation and determination.

The benzyl derivatives of the standard histamine solutions and the fish samples extracts were prepared according to Anderson (2008) and Hwang et al (1997). One ml of 2 M sodium hydroxide (NaOH) solution and 10 ul of Benzyl chloride were added sequentially to 2 ml of standard histamine solution or the fish sample extracts. The resulting solutions were vortex

mixed and allowed to stand at 30° C for 40 min. Then Benzoylation process was stopped by adding 2 ml of saturated sodium chloride solution (NaCl) and the mixed solution was extracted with 3 ml of Diethyl ether to separate the histamine. The solution was then centrifuged at 10,000 g for 10 min at 40° C, and the separated upper organic layer was transferred to a dry clean test tube and evaporated to dryness by purified Nitrogen gas. The dried residue was dissolved in one ml Acetonitrile. Aliquots of 20 ul from the residue acetonitrile solution were injected into the HPLC unit (Perkin Elmer 200 equipped with UV detector) at the Food and Drug Control Center Tripoli branch according to the operation conditions showed in table (1). The gradient elution program began with 50,50 (v/v) acetonitrile, water at a flow rate of 1 ml/min for 19 min, followed by a linear increase to 90,10 acetonitrile, water (1 ml/min) during the next 1.0 min. The acetonitrile, water mix decreased to 50, 50 (1.0 ml/min) for 10 min.

Table 1. Operation conditions for the high-performance liquid chromatography used for Histamine determination in fish samples extracts.

Category	Operation condition
Column type used	C 18 – reversed-phase column
Length and diameter of column	125 X 2.5 mm
Mobile phase	Acetonitrile , water (50 , 50)
Detector	UV
Wavelength	254 nm

2.4.Determination of histamine producing bacterial count (HPBC).

Twenty-five grams of minced homogenized fish meat was mixed with 225 ml of 0.1 % sterile peptone water in sterile electric blender for 1 minute. Then, serial decimaldilutions of 10^{-2}, 10^{-4} , and 10^{-5} were prepared from the homogenate and were used for HPBC determination on duplicate plates containing Niven's medium agar according to Swanson et al. (2001). All plates were incubated inverted at 25° C for 48 $\pm$ 2 hours. Plates were incubated at 25° C as recommended by Nickelson et al (2001) for routinequalityassessment for fresh fish and frozen seafood products.

Colonies with purple halo grown on Niven's medium were counted, aseptically isolated and then purified by streaking technique on trypticase soy agar plates. The plates were incubated at 25° C for 24 hours. Pure isolates were Gram-stained and microscopically examined under oil immersion, before identification using API 20 E kits according to Korashy et al. (2005).

3. Results and discussions

3.1.Fish samples Temperature and their Histamine contents.

The temperature of the fish samples included in this study ranged between < 5 – 22^0 C. Percentage of samples that had temperatures < 5, 5 – 14 and 15 – 22^0 C were 5, 52, and 43% respectively of the total samples examined in this study (Table 2).

Table 2. The temperature ranges of Fish samples collected from the three fish markets in Tripoli city, Libya, and the percentage of each range.

Temperature range (^{0}C)	Numbers of samples	The percentage of each range
‹ 5	6	5.31 %
5 – 14	59	52.20%
15 – 22	48	42.50 %

Histamine wasrecorded in 43% of the fish samples. The histamine content of these samples ranged between 1.29 – 29.74 mg/100 g of meat with an average of 2.9 ± 4.15 mg/ 100 g of meat. The temperature of these samples ranged between< 5 to 22^{0}C, and 53% of them had a temperature range of 5 – 14^{0}C (Table 3). The results also showed that 41 and 52% of the samples collected during the morning and noontime respectively contained histamine (Table 4).

Table 3. The percentages (%) of fish samples containing histamine, the ranges and means of their histamine levels in reference to their temperatures.

Fish temperature (^{0}C)	**Total fish samples**	**Samples containing histamine (%)**	**Histamine Level (mg %)**		
			Min	**Max**	**Mean**
< 5	5	10	1.29	2.35	1.82 ± 0.38
5 – 14	26	53.06	1.30	7.57	2.49 ± 1.47
15 – 22	18	36.73	1.31	29.74	3.84 ± 6.64
Total	49	43.36	1.29	29.74	2.91 ± 4.15

Table 4. The percentages of fish samples containing histamine in reference to their Sampling time.

Sampling time	**Total number of samples**	**Number of samples containing histamine**	**% of samples containing histamine**
Morning	88	36	41
Noon	25	13	52

The percentages of sardine, bouge, saury, and mackerel samples that contained histamine were 31, 46, 53, and 30 % respectively. Their histamine content ranged between 1.29 – 5.74, 1.34 – 29.74, 1.31 – 7.57 and 1.39 – 2.49 mg / 100 g meat respectively (Table5). Statistical analysis of histamine levels according to the types of fish included in this study did not show significant differences ($P > 0.05$) (table 5). Meanwhile, the histamine contents of the fish samples collected from the fish market A were significantly different ($P < 0.05$) from the histamine contents of the fish samples collected from the other two fish markets B and C (table 6). Hence, the highest histamine level (29.74 mg/100 g) was recorded in this market in bouge fish samples.

Table 5. The percentages (%) of fish samples containing histamine, the ranges and means of their histamine levels in reference to the Fish Types studied.

Fish type	**Total samples**	**No. samples containing histamine**	**Samples containing histamine (%)**	**Histamine Level (mg %)**		
				Min	Max	Mean
Sardine	32	10	31.25	1.29	5.74	2.02 ± 1.35
Bouge	28	13	46.42	1.34	29.74	4.51 ± 7.72
Saury	23	17	53.13	1.31	7.57	2.78 ± 1.65
Mackerel	30	9	30.30	1.39	2.49	1.85 ± 0.45

No significant differences between the means at P = 0.05.

Table 6. Percentages of fish samples containing histamine in reference to the fish markets examined

Fish market code	Total samples	No. samples containing histamine	Samples containing histamine (%)	Histamine Level (mg %)		
				Min	Max	Mean
(A)	26	8	30.77	1.39	29.74	$7.14^{b}\pm9.45$
(B)	50	23	46.00	1.29	4.15	$2.13^{ad}\pm0.83$
(C)	37	18	48.65	1.31	5.52	$2.03^{a}\pm1.01$
Total	113	49	43.36	1.29	29.74	2.91 ± 4.15

Means having same superscript letters aren't significant at P = 0.05

3.2.Histamine producing bacterial count (HPBC).

The HPBC of Sardine, Bouge, Saury and Mackerel collected from the three markets ranged between $5 \times 10^2 – 1.4 \times 10^6$, $2 \times 10^3 – 2.6 \times 10^6$, $2 \times 10^3 – 2.6 \times 10^6$ and $5 \times 10^2 – 2.7 \times 10^6$cfu / g meat respectively (table 7). The lowest value for HPBC 5×10^2cfu / g fish meat was observed in muscle tissues of Sardine and Mackerel samples collected from the fish market B While, the highest HPBC value 2.7×10^6cfu / g fish meat was recorded in the muscle tissues of mackerel samples collected from the fish market C. The results of the statistical analysis for the HPBC in Sardine, Bouge, Saury, and Mackerel among the three fish markets were not significantly different (P>0.05).

Table 7. The counts (cfu/gm) of histamine producing bacteria (HPBC) in Sardine, Bouge, Saury and Mackerel collected from the three markets examined

Fish type		Sardine	Bouge	Saury	Mackerel
Fish Market (A)	Min	3.0×10^3	6.0×10^2	1.0×10^4	5.0×10^2
	Max	1.4×10^6	1.1×10^6	1.5×10^6	5.7×10^4
	Mean	2.8×10^5	2.0×10^5	2.6×10^5	1.6×10^4
Fish Market (B)	Min	5.0×10^2	2.0×10^3	2.0×10^3	5.0×10^2
	Max	8.2×10^4	2.8×10^5	2.8×10^5	2.6×10^5
	Mean	1.8×10^4	6.4×10^4	6.4×10^4	4.2×10^4
Fish Market (C)	Min	1.0×10^3	8.0×10^3	1.3×10^4	5.0×10^2
	Max	2.1×10^5	2.6×10^6	2.6×10^6	2.7×10^6
	Mean	5.4×10^4	5.7×10^5	6.9×10^5	4.1×10^5

*Means of HPBC in each fish types are not significantly different between the three markets (P > 0.05).

3.3.Identification of HPB isolated from fish samples

According to the identification tests for the isolates from the fish samples, twenty-six (26) bacterial types were identified as HPB. Most of these isolates belong to the family *Enterobacteriaceae*, which are not indigenous to the marine environment, and some belong to *Vibrionaceae*. The results presented in table 8 indicated that the prevalence percentages of *V. fluvialis, Erwiniaspp, S. putrefaciens,* and *K. planticola* were 18.3, 13.2, 11.9, and 10.0 % respectively, while the prevalence percentages of *M. morganii, P. aeruginosa* and *A. baumaii* were almost equal i.e.6.40, 5.90, and 5.50 %respectively. The prevalence percentages of

other isolates were lower and ranged between 0.45–3.20 %.

Variations were observed in the prevalence percentages of most types of HPB isolates during the period of the study and even for the same type of bacteria, since the prevalence percentages of *V. fluvialis* during the months of July, August, September, October, November, and December were 21.0, 39.0, 30.0, 42.0, 30.0 and 25.0 % respectively, while those of *Erwiniaspp* were 29.0, 8.0, 21.0, and 9.0 respectively. However, the occurrence of *S. putrefaciens* and *P. aeruginosa* was only recorded in samples collected during November and December with the percentages 12.0 and 13.0 %, respectively (figure 1).

Table 8. The prevalence percentages (%) of histamine producing bacteria in fish samples collected from three fish markets located within Tripoli city, Libya.

Type of bacteria	fish market (A)	fish market (B)	fish market (C)	% of total isolates
V. fluvialis	23.70	17.70	24.30	18.30
Erwiniaspp	13.40	9.70	17.40	13.20
S. putrefaciens	-	14.20	14.50	11.90
K. planticola	9.80	7.08	17.40	10.00
M. morganii	4.90	5.30	8.70	6.40
P. aeruginosa	-	7.96	5.79	5.90
A.baumanii	9.80	4.40	5.80	5.50
P. flouorscens	-	5.31	1.45	3.20
Pantoeaspp	-	6.20	-	3.20
K. pneumonia	6.60	0.88	2.90	2.87
E. cloacae	-	1.80	5.80	2.58
A. hydrophila	3.30	3.50	-	2.18
P. mirabilis	-	2.70	1.50	1.78
O. anthropic	-	2.65	1.45	1.71
K. oxytoca	-	2.65	-	1.75
B. cepacia	-	2.65	-	1.30
S. plymuthica	8.10	-	-	0.90
Brucellaspp	-	0.90	1.50	0.86
P. vulgaris	-	1.77	-	0.86
S. liquefaciens	4.30	-	-	0.86
S. maltophilia	-	0.90	1.45	0.86
R. aquatilis	-	1.88	-	0.86
P. alcalifacients	-	-	5.41	0.86
P. rettgeri	-	-	3.31	0.45
E. coli	-	-	3.30	0.45
C. freundii	1.45	-	-	0.45

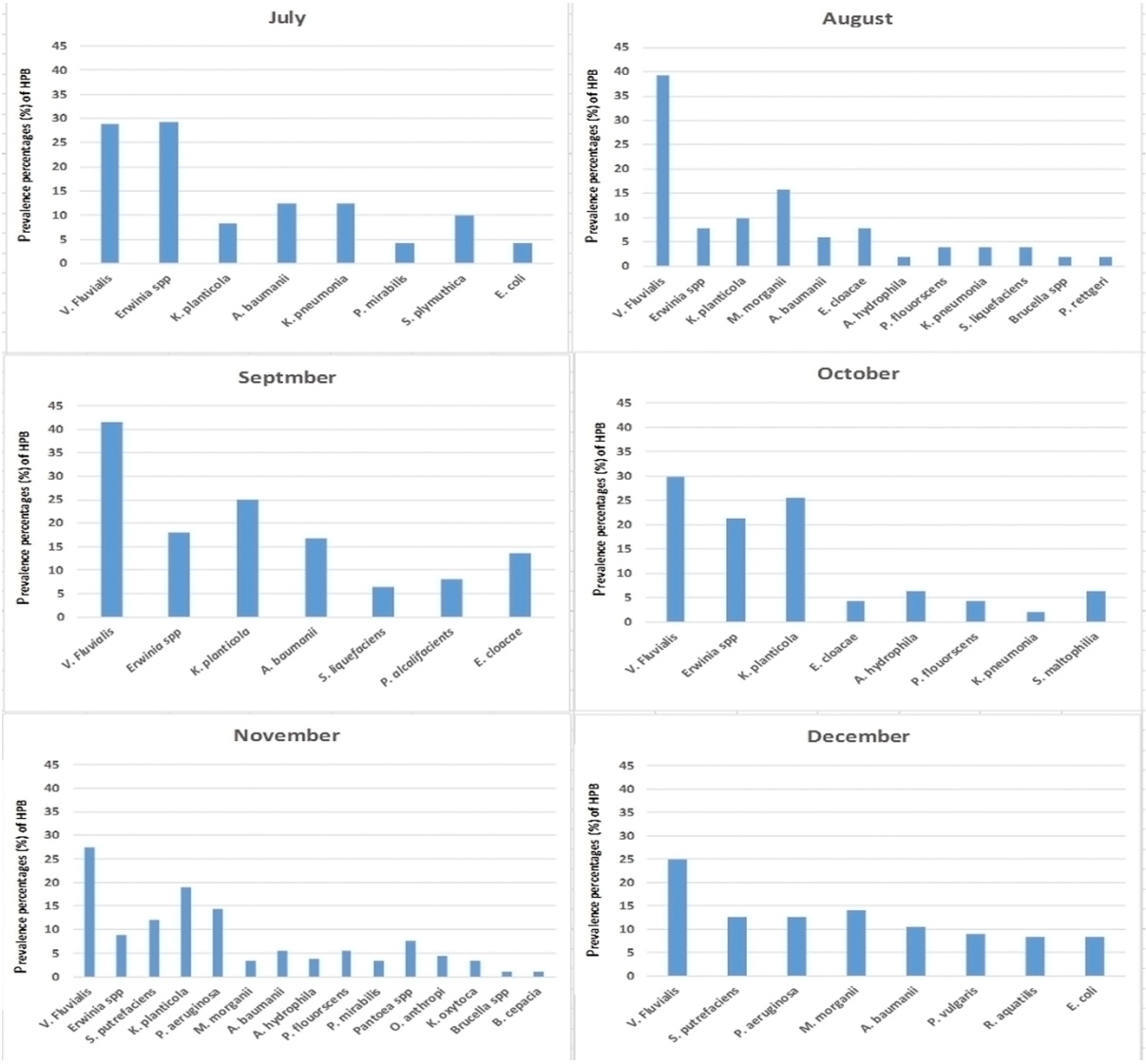

Figure1. Prevalence percentages (%) of HPB in fish samples collected during the period from July to December.

3.4.Discussions

3.4.1. Fish temperature and histamine contents.

Fish is a perishable food, which needs immediate cooling from the moment of catching until received by the consumer (from boat to throat) to assure its safety. The fish types included in this study (Sardine, Bouge, Saury, and Mackerel) are the most popular fish consumed in Libya,because of their reasonable prices and availability during their fishing season, which lasts from May to December. These fish species are susceptible to histamine formation due to their high contents of the free amino acid histidine that is the precursor for histamine formation by the decarboxylase enzyme through bacterial action when exposed to temperature /time abuse (Kim et al. 2004; Moreno et al. 2001 andRawles, 1996).

The results from this study showed that 95% of the collected fish samples (107/113) had a temperature range between 5 to 22^0 C,5% of the fish samples (6/113) had a temperature below 5^0 C (table 2). These results reflect the poor cooling conditions of the fish samples, and the poor cooling techniques practiced in the three markets, especially for those who depend solely on ice because of melting by the end of the day. These conditions will render fish samples more susceptible to histamine formation. The proper

icing and /or cooling practices of these types of fish should be below 5^0 C, and as close as possible to the melting point of ice to keep them in good quality and not suitable for histamine formation.

The results presented in Table 3 showed that 43% of the fish samples collected from the three markets contained histamine. The temperatures of these samples ranged between< 5 to 22^0 C, and more than 50% of these samples had a temperature range between 5 to 14^0 C. Even though histamine was recorded in 43% of all samples examined in this study, the levels did not exceed the maximum limit (10 mg %) adopted by the Libyan authority, except one sample. However, the presence of histamine in the samples indicates the fact that these samples were exposed to temperature-time abuse somewhere during handling. This is supported by the findings that 41 and 52% of the samples collected in the morning and noontime respectively contained histamine. In addition to that, the lowest percentages of samples contained histamine were recorded in fish samples that had a temperature < 5^0 C, while the highest percentage was observed in samples that had temperature range between 5 – 14^0 C, and the highest mean of histamine content(3.84 ± 6.64 mg %) was recorded in the samples that had temperature range between 15 to 22^0 C. Kim et al.(2002) and Lehan et al. (2000) reported that histamine production in fish starts at $\geq 5^0$ C, and the optimum temperature for histamine formation range from 20 to 30^0 C in the presence of HPB that belong to *Enterobacteriaceae.* However, Auerswald et al. (2006) pointed out that > 15^0 C is the optimum temperature for HPB growth and production of decarboxylase enzyme. Meanwhile, Kim et al. (2003) reported that 25^0 C is the optimum temperature for histamine production in fish.

Wide variations in the percentages of histamine levels in Sardine, Bouge, Saury, and Mackerel samples were observed as shown in table 5. However, a non-significant difference was recorded between them. These results reflect the randomness of these samples in terms of handling conditions to which they were exposed from catching until the time of sample collection. This is clear from the high value of standard deviations for the mean of histamine content (2.9 ± 4.15 mg %) in these samples (table 3).

One-way analysis of variance for the histamine content in the fish samples among the three fish markets showed significant differences ($p < 0.05$) (table 6). The mean value of histamine content in the fish samples collected from the fish market A 7.14 mg % was significantly different from the means values for histamine contents in the fish samples collected from the other two markets (table 6). These differences could be related either to variations in icing or cooling practices applied during handling and/ or to the fact that one of the fish samples collected from the fish market showed a high histamine content 29.74 mg % and this might contribute to the overall mean value of these group of samples from the fish market A.

The range of histamine content found in this study for mackerel samples was 1.39 – 2.49 mg %, which is higher than that reported by Lokuruk et al. (2006), in samples of Mackerel (Scomber*scomber*) collected from Brooklyn port in New York city, USA where the range of histamine was 0.20 – 0.21 mg %, and with the study of Fletcher et al. (1995) where the histamine contents in Mackerel *(Scomberaustralasicus*) samples did not exceed 1 mg %. The results of the histamine contents in Mackerel samples in this study were lower than that reported by Okuzumi et al. (1982) in fresh Mackerel (Scomber japonicas) samples collected from markets in Tokyo- Japan where the histamine contents ranged 2 – 87.2 mg %, and by Joshi et al. (2011) 20 – 30 mg % in Indian Mackerel (*Rastrelliger Kamasutra*) samples collected from local markets in Kalyan city-India.

The range of histamine levels (1.31 – 7.57 mg %) reported in this study for Saury fish sample was lower than that recorded in Saury (*Saira cololabis*) fish samples collected from Korea and Japan markets where the histamine levels ranged between 0.01 – 31.43 and 2.0 – 144 mg %respectively (Kim et al. 2009 and Okuzumi et al. 1982).

The variations between the results of histamine contents obtained in fish samples collected from the three local markets and that recorded in fish samples of other regions could be related to the variations in handling practices (time /temperature) that these fish samples were exposed to from catching until sample collection.

3.4.2. Histamine producing bacterial count (HPBC).

The range for the means of HPBC in Sardine and Mackerel samples in this study were 1.8 x 10^4 – 5.4 x 10^4 and 1.6 x 10^4 – 4.1 x 10^5cfu / g fish meat respectively, which are higher than those (2.5 x 10^3, 2.1 x 10^3 and 2.2 x 10^3cfu /g fish meat) reported by Korashy et al. (2005) in samples of Sardine (Sardinella*gibbosa*), European Sardine (Sardinella*pilchardus*) and Atlantic Mackerel (Trachurustra*churus*) respectively. The results of this study were also higher than those reported by Okuzumi et al. (1982) where the range of HPBC for fresh Sardine (*Sardinellamelanostic*), Saury (*Coloabissaira*) and in Japan Mackerel (*Scomber japonicas*) was 1.1 x 10^4 – 3.0 x 10^4, 5.7 x 10^3 – 2.1 x 10^5 and 1.0 – 1.0 x 10^2 (estimated) cfu / g respectively. Additionally, Lopez-Sabater et al. (1996) found that HPBC in Mackerel was 3.1 x 10^2cfu / g fish in Spain, which is lower than the counts recorded in this study.

Statistical analysis of the results for HPBC in each fish species among the three markets (table 7) showed non-significant differences ($P > 0.05$). This might be related to the randomness of the collected samples and the unknown variations in handling conditions that these fish species were exposed to from fishing boats until collected from the three markets.

3.4.3 HPB isolates.

The results from this study showed that most of the HPB isolates from the fish samples belong to the family *Enterobacteriaceae* that are not indigenous to the marine environment and some belong to*Vibrionacea* (table 8). These findings agree with the results of Economou et al. (2007), Emborg et al. (2005), Ababouch et al. (1991) and Taylor (1986), where they found that most of the histamine producing bacteria in fish belong to the family *Enterobacteriacea*e such as *Klebsiella pneumonia, E. coli, Morganellamorganii, Enterobacterclocacae, Serratia marcescens and Hafnia alvei.*

The prevalence percentages of the bacterial isolates presented in table (8) ranged between 3.20 and 18.30%. The highest percentage (18%) was recorded for *V. fluvialis* followed by *Erwiniaspp, S. putrefaciens,* and *K. planticola* where their prevalence percentages were 13.2, 11.9, and 10.0% respectively. The prevalence percentages of *S.putrefaciens* and *P.fluoroscens* in the Sardine samples were 11.9 and 3.2% out of the total isolates respectively. These percentages are resembling those (10 and 20%) reported by Ababouch et al. (1991) in Sardine (*Sardinella pilchardus) caught from Atlantic coast.*

When comparing the results of this study with that of Economou et al. (2007) where, 77 types of HPB were isolated, which account for 53% of the total number of bacteria in 30 samples of fresh and frozen Albacore tuna (*Thunnusalalongua*) collected from five countries. There was a similarity in types of bacteria isolated from the samples among which were*P.fluorescens, P. aeruginosa, E.coli, B. capacia.* The observed differences were in their prevalence percentages, which were higher in the tuna samples compared to the fish samples of this study.

The variations in the prevalence percentages of the most types of HPB isolates during the period of the study and even for the same type of bacteria illustrated in figure (1), reflect the important effect of the month of the year on the type of bacteria found on the fish samples. These findings are in good agreement with those of Yoshinga et al. (1982) and Kim et al. (2009). Furthermore, the results obtained from this study are in agreement with the results of Yagoub (2009) who found that 53.3% of isolated bacteria from fresh fish in Khartoum, Sudan belong to *Enterobacteriaceae* and the incidence percentage of species belong to this family during summer, autumn and winter were 60, 33 and 20% respectively.

4.Conclusions

The obtained results indicated that the fish species included in this study were exposed to time-temperature abuse during handling and display, since only 5% of the samples collected, had a temperature below 5^0 C and 43% out of the total samples contained histamine. Most of the isolated HPB belong to the family *Enterobacteriaceae* and some to *Vibrionaceae*, and their prevalence depended on the month of the year. Therefore, it is strongly recommended to set up a plan to improve the handling practices and display conditions of such fish types to keep them safe from boat to the throat.

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