



Assessment of Temperature and Microbiological Quality of Fresh Sardine, Bouge, Saury and Mackerel Marketed in Tripoli City, Libya

Tawfik Mehdi HASSAN 🗅, Amal Abubaker ALHALLUG 🗅, Nuri Sahli MADI 🕩

Department of Food Science and Technology, University of Tripoli, Faculty of Agriculture, Libya

Cite this article as: Hassan, T.M., Alhallug, A.A., Madi, N.S., 2018. Assessment of Temperature and Microbiological Quality of Fresh Sardine, Bouge, Saury and Mackerel Marketed in Tripoli City, Libya. Acta Vet Eurasia 44: 93-100.

Abstract

The aim of this study was to assess the temperature, total aerobic plate count (TAPC), and histamine-producing bacterial count (HPBC) of four types of fish, viz., sardines (Sardinella aurita), bouge (Boops boops), saury (Trachurus mediterraneus), and mackerel (Scomber scombrus), that are sold in three major fish markets in Tripoli's city center. A total of 113 samples of these fish types were collected, both in the morning and in the evening, from July to December in the fishing season. Results showed that the temperature of the collected fish samples ranged from <5°C to 22°C. Of the total 113 fish samples, 5.0%, 52.0%, and 43.0% had temperatures of <5°C, 5°C–14°C, and 15°C–22°C, respectively. The TAPC of all the fish samples ranged from 3.0×10^3 to 3.5×10^7 colony-forming unit/g(cfu/g) of meat (with skin), with a mean of 1.1×10^6 cfu/g. The HPBC ranged from an estimated 5.0×10^2 to 2.7×10^6 cfu/g, with a mean of 1.8×10^5 cfu/g. Statistical analysis of the data showed a weak correlation (r = 0.05) between TAPC and HPBC of all the fish samples collected from the three major markets. The TAPC results revealed that 50%, 46%, 38%, and 17% of

the saury, bouge, mackerel, and sardine fish samples, respectively, did not comply with the standard specification limit (10⁶ cfu/g) prescribed by the Libyan authorities. A total of 26 isolates of histamine-producing bacteria were identified in this study. The majority of them belonged to the Enterobacteriaceae family and were not indigenous to the marine environment. There was a variation in the distribution of these bacterial isolates among all the fish samples during the course of the study. However, Vibrio fluvialis, Erwinia spp., and Klebsiella planticola were detected in all the fish samples throughout the study period. The high TAPC and HPBC recorded in this study could be attributed to cross-contamination due to the poor quality of the surrounding environment and the poor hygienic practices. Therefore, there is an urgent need for proper control of product handling conditions in the fish markets monitored in this study.

Keywords: Bouge, HPBC, mackerel, sardine, saury, TAPC, Tripoli city

Introduction

Sardine (Sardinella aurita), Bouge (Boops boops), Saury (Trachurus mediterrangus), and Mackerel (Scomber scombrus) are among the most popular fish choices for the Libyan consumer because of their reasonable prices and availability during the fishing season. They represent 50% of the total annual fish catch, which amounts to around 38000 tons (Anonymous,

Address for Correspondence: Tawfik M. HASSAN • E-mail: thassan@uot.edu.ly Received Date: 24 April 2018 • Accepted Date: 27 July 2018 • DOI: 10.26650/actavet.2018.417973 © Copyright 2018 by Official Acta Veterinaria Eurasia. Available online at actaveteurasia@istanbul.edu.tr 2014). These fish species have a high nutritive value and they are a source of good quality protein and polyunsaturated fatty acids – in particular, omega 3 and 6 fatty acids. Hassan et al. (2006, 2011), reported that the total lipids of Libyan sardine, bouge and mackerel contain ample quantities of omega 3 fatty acids - 33.11, 28.03 and 20.03% of total fatty acids respectively. In comparison with red meat, fish meat is highly perishable mainly because of its high non-protein nitrogen compound



content, which represents ideal nutrients for spoilage bacteria. In addition, these fish species are particularly sensitive to histamine formation from histamine producing bacteria if they become exposed to poor temperature control when handled, stored or displayed.

Histamine poisoning (also known as scombroid poisoning) occurs following consumption of certain fish species which contain histamine. Symptoms of histamine poisoning appear within anything from a few minutes to 3 hours after consuming these fish whether they are fresh, frozen, canned or dried. Studies and statistical surveys on scombroid poisoning showed that most of the histamine poisoning cases resulted from the consumption of fish belonging to the *scombridae* family - which includes mackerel, *clubeidae* such as sardines (Kim et al., 2004; Moreno et al., 2001) and carangidae -which includes Saury (Brillants et al., 2001; Lokuruka et al., 2004). This is because their muscles are rich in free histidine, the precursor to histamine formation by histamine producing bacteria (Kim et al., 2004; Moreno et al., 2001; Rawles et al., 1996).

Niven et al. (1981) reported that Most of the histamine-producing bacteria isolated from fish muscles belong to mesophilic enteric bacteria, which are not the natural flora of fresh fish. Other studies found the histamine decarboxylase enzyme in some species of bacteria: *Vibrio harveyii, Vibrio alginolyticus, Photobacterium phosphoreum* and *leiognathi spp* which are all indigenous to the marine environment. While they are not potent histamine producers, they were responsible for most of the documented histamine poisoning cases reported (Kanki et al., 2004; Ramesh et al., 1989; Takahashi et al., 2003; Yoshinagu and Frank, 1982).

Due to the lack of local studies on the effect of display conditions in Tripoli fish markets on fish sensitive to histamine production, this study was carried out to determine temperature, total aerobic plate count and histamine producing bacterial count in samples of sardines, saury, bouge and mackerel displayed for sale in three fish markets in Tripoli city during the period July – December of the fishing season.

Materials and Methods

Sample collection

One hundred thirteen samples from fresh sardines, Bouge, Saury and mackerel were collected directly from fish on sale in three main fish markets - A, B and C in Tripoli City, Libya. The samples were collected between 7:00 and 8:00 A.M. and between 12.00 and 13.00 P.M., during the period from July to December of the fishing season. The samples were kept in sterile polyethylene bags and transferred in an icebox, within 15 minutes, to the Microbiology and fish disease laboratory at the marine research center in Tajoura, Libya. The temperature of the displayed fish was taken when the samples were collected.

Sample preparation for bacteriological analysis

From each sample, 5-6 pieces were randomly collected. Meat

muscles were cut from the back and sides of each fish body using a sterile knife and then homogenized in a sterile blender. Twenty five grams of the homogenate was then used for bacteriological analysis.

Determination of Total Aerobic Plate Count (TAPC) and 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 a sterile electric blender for 1 minute. Then, serial dilutions of 10^{-2} , 10^{-4} and 10^{-5} were prepared from the homogenate for TAPC on plate count agar (Oxide Ltd.,Hamsphire, UK), while dilutions of 10^{-4} and 10^{-5} were used for HPBC determination on Niven's medium, according to Swanson et al. (2001). The Niven's medium was prepared according to the procedure of Niven et al. (1981). All plates were incubated inverted at 25°C for 48 ± 2 hours. Plates were incubated at 25°C, as recommended by Nickelson et al. (2001) for the routine assessment of quality of fresh and frozen seafood products.

Colonies with a purple halo grown on Niven agar were counted, aseptically isolated and then purified with the streaking technique on trypticase soy agar plates (Oxide Ltd. Hamsphire, UK). The plates were incubated at 25°C for 24 hours to obtain isolates. Theses isolates were then restreaked on Niven agar medium plates to confirm that they produced purple halo colonies. Pure isolates were gram stained, and microscopically examined under oil immersion, before identification using analytical profile index 20 E (API 20 E kits) for identification and differentiation of member of the family *Enterobacteriaceae* (Biomerieux Inc Boston MA USA) in accordance with Korashy et al. (2005).

Statistical analysis

The results of the TAPC and HPBC were analyzed with the statistical package Minitab 16 (Minitab Inc. State college Pa USA) using descriptive statistics such as minimum, maximum and mean value. The Correlation coefficient test (r) was performed between the TAPC and HPBC data. Significance was considered where p<0.05.

Results and Discussion

Samples temperature

The temperature of fish samples ranged from <5 to 22°C. The percentage of samples that had temperatures <5, 5-14, and 15-22°C were 5.0, 52.0 and 43.0 % respectively out of the total 113 samples (Table 1). It is clear from the results that 95% of the samples collected had a temperature between 5 and 22 °C. This temperature range is suitable for growth of HPB (Economou et al., 2007; Kim et al., 2009). Therefore, the presence of such bacteria might place the fish samples at risk of histamine formation when displayed for sale at temperatures higher than 5°C.

When the fish samples were classified according to the markets included in this study, the results showed that samples taken from fish market B were the best in terms of temperature. Sev-

enty-two percent (72%) of these samples had temperatures between 5 and 14°C, while the rest of the samples collected from the other two markets had temperatures between 15 and 22°C (Table 2).

The results shown in Table 3 reflect the poor refrigeration conditions of the samples, since the percentage of samples that had a temperature of <5°C did not exceed 5% in the morning and 8% in the afternoon at fish market B. Meanwhile, the highest percentage (60%) of samples that had temperatures between 15 to 22°C was recorded in samples collected at noon. This is probably due to poor refrigeration methods applied in these markets, especially for those who depend solely on ice as it melts by the end of the day. These conditions make fish samples more susceptible to histamine formation.

Total aerobic plate count (TAPC) and Histamine producing bacterial count (HPBC)

The TAPC for samples of Sardines, Bouge, Saury and Mackerel ranged from 5×10^3 to 8.9×10^6 , 1.5×10^4 to 5.3×10^6 , 3.2×10^4 to 3.5×10^7 and 5×10^2 to 3×10^6 colony forming unit/gram (cfu/g) meat (with skin) respectively as shown in Table 4. Meanwhile, the range of HPBC for the same samples were from 5×10^2 to 5×10^6 , 6×10^2 to 2.6×10^6 , 3×10^3 to 2.6×10^6 and 5×10^2 to 2.7×10^6 cfu/g meat (with skin) respectively as shown in Table 5. It is noted from the results in Table 4 and 5 that the highest average TAPC and HPBC were recorded in the saury fish samples, followed by bouge and the lowest counts were recorded in samples of mackerel.

When comparing the results for TAPC obtained in this study with the standard specification limit - 10^6 cfu/g fish (GSO 1016: 2015) as prescribed by the Libyan authorities, it was found that 50, 46, 38 and 17% of the Saury, Bouge, Mackerel and sardine

Table 1. The temperature ranges of fish samples collected from three fish markets in Tripoli, Libya, and the percentage of each range

Temperature range (°C)	Numbers of samples	The percentage of each range	
< 5	6	5.0%	
5 – 14	59	52.0%	
15 – 22	48	43.0%	

fish samples did not comply with this limit as shown in Table 6. Remarkably, the total percentage of samples collected from market A that did not comply with this standard did not exceed 11%, whereas the figures for market B and market C were, 52% and 41%, respectively (although market B was equipped with new facilities and utensils). Moreover, the average TAPC in fish market A for all fish types was lower than that recorded in market B and market C.

WHO (2007) indicated that the TAPC rarely reflects the overall quality of fish, but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health conditions of the consumer of such contaminated fish. The results of TAPC and HPBC obtained from the present study reflect the variations in handling conditions that these fish were exposed to from the time of harvesting until delivered to the fish markets.

The range of averages for TAPC of sardine samples found in this study (from 2.8 X 10^5 to 6.4 x 10^5 cfu/g fish meat) was higher than that reported in fresh Libyan sardines (*Sardinella aurita*) in previous studies carried out by Abuzghia (1990) where the TAPCs were between 7.9 x 10^3 and 8.2 x 10^3 and by Hassan et al. (2008) – between 1.0×10^2 and 1.0×10^3 cfu/g fish meat. The variations in TAPC reported in these studies could be attributed to the variations in handling conditions these fish were exposed to from the time of fishing until they reached the laboratories.

The range of TAPC recorded in this study for sardine and mackerel samples was from 5.0×10^3 to 8.9×10^5 and 9.0×10^3 to 1.0×10^6 cfu/g respectively, which is lower than that reported by Korashy et al. (2005) in samples of sardines (*Sardinella gibbosa*), European sardines (*Sardinella pilchards*) and Atlantic mackerel (*Trachurustrachurus*), where the average counts were, 8.6×10^7 , 6.5×10^6 and 7.0×10^7 cfu/g respectively. Furthermore, the results for the same samples showed that HPBCs (2.5×10^3 , 2.1×10^3 and 2.2×10^3 cfu/g, respectively) were lower than the results obtained in this study for sardine and mackerel. The range of HPBCs recorded in this study for sardine and mackerel samples was between 5.0×10^2 (estimated) and 1.4×10^5 and between 5.0×10^2 (estimated) and 6.2×10^5 cfu/g fish, respectively.

The results from this study were also higher than the results reported by Okuzumi et al. (1982), where the range of TAPCs for fresh sardine (*Sardinella melanostic*), saury (*Coloabissaira*) and

Table 2. Classification of fish samples according to their temperatures and the markets included in the study

Fish samples temperature (°C)	Fish market (A)		Fish market (B)		Fish market (C)	
	Number of samples	% samples	Number of samples	% samples	Number of samples	% samples
< 5	-	-	6	12.0	-	-
5 – 14	6	23.0	36	72.0	18	49.0
15 – 22	20	77.0	8	16.0	19	51.0
(-) No samples recorded terr	operatures within this range.					

mackerel (*Scomber japonicas*) in Japan were between 1.1×10^4 and 3.0×10^4 , 1.0×10^2 (estimated) and between 4.9×10^4 and 2.9×105 cfu/g respectively, while the HPBC ranges were, 1.0 to 1.0×10^4 , 5.7×10^3 to 2.1×10^5 and 1.0 to 1.0×10^2 (estimated) cfu/g. Additionally, Lopez – Sabater et al. (1996) found that the HPBC in mackerel was 3.1×10^2 cfu/g fish in Spain, which is lower than the counts recorded in this study.

Statistical analysis of the results showed weak correlation (r=0.05) between TAPC and HPBC in all samples collected from the three markets. This might be related to the randomness of collected samples and the unknown variations in handling conditions that these fish species were exposed to from harvest to delivery to the fish markets.

Identification of HPB isolated from fish samples

The results revealed that twenty-six (26) bacterial types were isolated from the fish samples and identified as HPB. Most of these isolates belong to the family *Enterobacteriaceae*, which are not indigenous to the marine environment, and some be-

Table 3. Classification of fish samples according to their temperatures and the time of sampling

Sampling time	Мо	rning	Noon		
Temperature Range (°C)	Number of samples	% of samples	Number of samples	% of samples	
< 5	4	5.0	2	8.0	
5 – 14	52	59.0	8	32.0	
15 – 22	32	36.0	15	60.0	
Total	88	78.0	25	22.0	

long to *Vibrionaceae* (Table 7). According to these results, the prevalence percentages of *V. fluvialis, Erwinia spp, S. putrefaciens* and *K. planticola* were 18.3, 12.2, 11.9, and 10.0% respectively, while the prevalence percentages of *M. morganii, P. aeruginosa* and *A. baumaii* were almost equal - 6.40, 5.90, and 5.50 respectively. The prevalence percentages of other isolates were lower and ranged between 0.45 and 3.20%.

The prevalence percentages of *S. putrefaciens* and *P. fluorescens* in the sardine samples were 11.9 and 3.2% out of the total isolates, respectively. These percentages are close to those reported by Ababouch et al. (1991) in sardines (*Sardinella pilchardus*) caught off the Atlantic coast (10 and 20%).

The results of this study were compared with a study (Economou et al., 2007) that isolated 77 types of HPB, which accounted for 53% of the total number of bacteria in 30 samples of fresh and frozen albacore tuna (*Thunnus alalonga*) collected from Brazil, Srilanka, The Maldives, Indonesia and Yemen. There was a similarity in types of bacteria isolated from the samples, among which were *P.fluoroscens, P. aeruginosa, E. coli*, and *B. capacia*. However, the differences were in the percent prevalence where their proportion in tuna samples was higher than in the fish samples of this study.

Variations were observed in the prevalence percentages of most types of HPB isolates during the period of the study and even in 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 *Erwinia spp* were 29.0, 8.0, 21.0, and 9.0 respectively. However, the occurrence of *S. putrefaciens* and *P. aeruginosa* was only recorded

Table 4. Total aerobic plate counts (cfu/g) for fish samples collected from three fish markets located within Tripoli city center, Libya

	1					. ,
	Fish market (A)		Fish market (B)		Fish market (C)	
Fish market	Range	Average	Range	Average	Range	Average
Fish type						
Sardine	3.2 x 10⁴ - 9.7 x 10⁵	2.8 x 10⁵	1.1 x 10 ⁴ - 8.9 x 10 ⁶	8.7 x 10⁵	5.0 x 10 ³ - 2.8 x 10 ⁶	6.4 x 10⁵
Bouge	5.3 x 10 ⁴ - 1.8 x 10 ⁶	4.3 x 10⁵	1.5 x 10 ⁴ - 5.3 x 10 ⁶	1.1 x 10 ⁶	5.8 x 10 ⁴ - 2.7 x 10 ⁶	1.1 x 10 ⁶
Saury	1.2 x 10 ⁵ - 2.9 x 10 ⁵	2.1 x 10⁵	3.2 x 10 ⁴ - 3.5 x 10 ⁷	4.0 x 10 ⁶	6.4 x 10 ⁴ - 4.6 x 10 ⁶	1.6 x 10 ⁶

Table 5. Histamine producing bacteria counts (cfu/g) for fish samples collected from three fish markets located within Tripoli city, Libya

	Fish market (A)		Fish market (B)		Fish market (C)	
Fish market	Range	Average	Range	Average	Range	Average
Fish type						
Sardine	3.0 x 10 ³ - 5.0 x10 ⁶	9.8 x 10 ⁴	$5.0 \times 10^2 - 8.2 \times 10^4$	1.9 x 10 ⁴	10 x 10 ² – 2.1 x 10 ⁵	5.1 x 10 ⁴
Bouge	6.0 x10 ² - 1.1 x 10 ⁶	2.0 x 10⁵	2.0 x 10 ³ - 2.8 x 10 ⁵	7.2 x 10 ⁴	8.0 x 10 ³ – 2.6 x 10 ⁶	5.7 x 10⁵
Saury	10 x 10 ³ – 1.5 x 10 ⁴	3.9 x 10⁴	3.0 x 10 ³ – 1.3 x 10 ⁶	1.7 x 10⁵	1.1 x 10 ⁴ - 2.6 x 10 ⁶	6.0 x 10 ⁶
Mackerel	5.0 x 10 ² - 5.7x 10 ⁴	1.6 x 10 ⁴	5.0 x 10 ² - 2.6 x 10 ⁵	4.2 x 10 ⁴	5.0 x 10 ² - 2.7 x 10 ⁶	3.8 x 10⁵

in samples collected during November and December with the percentages being 12.0 and 13.0%, respectively (Figure 1).

Table 6. Numbers and percent of samples (%) having total aerobic plate counts higher than the maximum limit (10⁶ cfu/g fish meat) referred to by the standard specification adopted by the Libyan authority

Fish type	Fish market (A)	Fish market (B)	Fish market (C)	Total number of samples (%) > 10 ⁶ cfu /g
Sardine	2 (7)*	1 (12)	2 (10)	5 (17%)
Bouge	1 (6)	9 (14)	3 (8)	13 (46%)
Saury	0 (6)	8 (13)	7 (11)	15 (50%)
Mackerel	0 (9)	7 (9)	3 (8)	10 (38%)

* Numbers between brackets indicate total number of sample examined.

It is clear from the results that the period of fishing has an important effect on the type of bacteria found in fish samples, as confirmed by Yoshinaga et al. (1982) and Kim et al. (2009). The results from this study are also in agreement with the results of the study conducted by Yagoub (2009) in Khartoum, Sudan on fresh fish, which showed that 53.3 of isolated bacteria belong to *Enterobacteriaceae* and the incidence percentages of species belonging to this family during Summer, Autumn and Winter were 60, 33 and 20%, respectively.

Correlation between temperature of fish samples and prevalence percentages (percentage) of HPB isolates

The results illustrated in Figure 2 indicate the incidence of *V. fluvialis, Erwinia spp, S. putrefaciens, P. aeruginosa, P. fluorscens and A. Baumanii* in all samples irrespective of their temperature. The prevalence percentages of these species were 23.0, 26.0, 12.0, 8.0, 4.0, and 8.0% of total isolates for fish samples recorded at

Table 7. The prevalence percentages (%) of histamine producing bacteria (HPB) in fish samples collected from three fish markets located within Tripoli city, Libya

	Prevalence p				
Type of bacteria	Fish market (A)	Fish market (B)	Fish market (c)	% from total isolates 18.30	
V. fluvialis	23.70	17.70	24.30		
Erwinia spp	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	
Pantoea spp	-	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. anthropi	-	2.65	1.45	1.71	
K. oxytoca	-	2.65	-	1.75	
B. cepacia	-	2.65	-	1.30	
S. plymuthica	8.10	-	-	0.90	
Brucella spp	-	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	



<5°C; 21.0, 8.0, 18.0, 9.0, 2.0, and 5.0% for fish samples recorded between 5 and 14°C; and 30.0, 15.0, 3.0, 2.0, 3.0, and 6.0% for fish samples which had a temperature range between 15 and 22°C, respectively. The Incidence of *M. morganii* was recorded only in fish samples recorded at 5-15 and 15-22°C with prevalence percentages of 8.0 and 11% respectively. The results also showed that *Erwinia spp* made up the highest percentage of isolates from fish samples that had a temperature of <5°C. *V. fluvialis* represented the highest percentage of isolates from fish samples that had a temperature range between 5 and 14°C and 15 and 22°C with prevalence percentages of 20.7 and 30.11% respectively. Furthermore, the prevalence percentages of *Erwinia spp* were 8.49 and 15.12% for fish samples recorded at 5-14 and 15-22°C, respectively.

Conclusion

The results from this study showed that the samples of fish collected from fish markets in Tripoli city were displayed in poor refrigeration conditions. The higher percentage of samples not complying with the standard specification limit prescribed by



the Libyan authorities, and the higher HPBC recorded in this study could be attributed to cross-contamination from the surrounding environment and poor hygiene during handling. Most of the histamine-producing bacteria isolated belong to the family *Enterobacteriaceae* and some belong to *Vibrionaceae*.

These findings represent additional evidence to encourage proper control of handling conditions in those fish markets considered in this study. Since histamine cannot be destroyed by cooking, drying, smoking or freezing, good hygienic practices are the proper way to prevent histamine producing bacteria from growing in fish. Ethics Committee Approval: This research has been planned and implemented taking into account the contents of the ethics of scientific research document that was issued by University of Tripoli under international number 9799959531551 and it was deposited at The national book house in Libya under a legal number 2017/155.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – T.M.H.; Design – T.M.H., A.A.A.; Supervision – T.M.H., N.S.M.; Resources – A.A.A.; Materials - N.S.M., T.M.H.; Data Collection and/or Processing – A.A.A., T.M.H.; Analysis and/or Interpretation – A.A.A., T.M.H., N.S.M.; Literature Search – A.A.A.; Writing Manuscript –T.M.H.; Critical Review – N.S.M.

Acknowledgements: The authors of this work wish to express their profound gratitude to the staff of the Microbiology and fish Disease Laboratory at the Marine Research Center in Tajoura –Libya for their cooperation during the study. The support and encouragement of Mr. Abdul Kareem Ben Issa and MS. Enass Al-Shebani are also duly acknowledged.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

References

- Ababouch, L; Afilal, M. E; Rhafiri, S; Busta, F. F. 1991. Identification of Histamine – producing bacteria isolated from sardine (Sardina pilchardus) stored in ice and at ambient temperature (250C). Food Microbiology 8, 127-136. [CrossRef]
- Abuzghia, M. 1990. Study of spoilage index in cold stored Libyan sardine (*S aurita*). MSc thesis, Faculty of Agriculture, University of Tripoli, Libya.
- **Anonymous. 2014.** Marine products industry status (obstacles and Solution) prepared by marine products committee. Ministry of Industry. Tripoli, State of Libya.
- Brillantes, S; Samosorn, W. 2001. Determination of histamine in fish sauce from Thailand using a solid phase extraction and high performance liquid Chromatography. Fisheries Science 67, 1136 1168. [CrossRef]
- Economou, V; Brett, M; Papadopoulou, C; Frillingos, S; Nichols, T. 2007. Changes in histamine and microbiological analysis in fresh and frozen tuna Muscle during temperature abuse. Food additives and Contaminants August 24, 820-832.
- **GSO 1016: 2015.** Microbiological criteria for foodstuffs. GCC standardization organization.
- Hassan, T. M.; Shaktour, F. G.; Ahmed, A. A.; Melah, M. A. 2006. Monthly Changes in fat composition of Sardine (*Sardinella aurita*) caught From Libyan Coast. Libyan Journal of Marine Science 11, 5-24.
- Hassan, T. M.; Shaktour, F. G.; Ahmed, A. A.; Melah, M. A. 2011. Monthly Changes in fat composition of Libyan Bouge (*Boops boops*) during fishing Season. The Libyan Journal of Agriculture 16, 36-44
- Hassan, T. M.; Abuhlega, T. A.; Ahmed, A. A.; Kalph, M. K. 2008. Shelf life of Libyan frozen sardine. Libyan Journal of Marine Science 12, 5-23.
- Kanki, M; Yoda, T; Ishibashi, M; Tsukamoto, T. 2004. Photobacterium Phosphoreum caused a poisoning incident. International Journal of Food Microbiology 92, 79-87. [CrossRef]
- Kim, M. K; Moh, J. H; Hwang, H. J. 2009. Biogenic amine formation and bacterial contribution in fish, squid and shellfish. Food Chemistry 1, 1-9. [CrossRef]
- Kim, S. H; Wei, C. I; Clemens, R. A; An, H. 2004. Histamine accumulation in seafood and control to prevent outbreaks of scombroid poisoning. Journal of Aquatic Food Product Technology 13, 81-100. [CrossRef]

Korashy, N. T. and Hassan, M. F. 2005. Histamine and histamine producing bacteria in some local and imported fish and their public health significance. Research Journal of Agriculture and Biological Science 1, 329-336.

- Lokuruka, M. N; Regenstein, J. M. 2004. Biogenic amines in iced and temperature - abused tropical fish: A comparative study with temperate Atlantic mackerel. Journal of Aquatic Food Product Technology 13, 87-99. [CrossRef]
- Lopez Sabater, I. E; Rodriguez Jerez, J. J; Hernadez Herrero, M; Mora – Venture, T. M. 1996. Incidence of histamine formation bacteria and Histamine content in scombroid fish species from retail markets in the Barcelona area. International Journal Food Microbiology 28, 411-418. [CrossRef]
- Moreno, B. R; Torres, A. E. 2001. Histamine level in fresh fish, a quality Index. Seafood Technology: Safety, Processing Nutrition - Faculty of Public Health. University of Sao Paulo, Av. Br. Arnaldo, 715 HNT, Sao Paulo, sp 01246.
- Nickelson, R.; McCarthy, S.; Finne, G. 2001. Fish crustaceans, and precooked seafood. In: Downes, F. P. and Ito, K. (eds). 4th eds., "Compendium of methods for the microbiological examination of foods", American public health association, p. 497. [CrossRef]
- Niven, C. F; Jeffrey, M. B; Corlett, D. A. 1981. Differential plating medium for quantitative detection of histamine – producing bacteria. Applied and Environmental Microbiology 41, 321 322.
- Okuzumi, M; Okuda, S; Awano, M. 1982. Occurrence of psychrophilic and halophilic histamine – forming bacteria (N – group bacteria) on / in red med fish. Bulletin of Japanese Society of Scientific Fisheries 48, 799-804. [CrossRef]
- Ramesh, A; Ananthalakshmy, V. K; Venugopalan, V. K. 1989. Histamine Production in Indian oil sardine and Indian mackerel by luminous bacteria. Journal of Applied Microbiology and Biotechnology 5, 105-108. [CrossRef]
- Rawles, D. D; flick, G. J; Martin, R. F. 1996. Biogenic amines in fish and shellfish. Advances in Food Nutrition and Research 39, 329-365. [CrossRef]
- Swanson, K. M; Petran, R. L; Hanlin, J. H. 2001. Culture methods for Enumeration of microorganisms. In: Downes, F. P. and Ito, K. (eds). 4th eds., "Compendium of methods for the microbiological examination of foods", American public health association, pp. 53-61. [CrossRef]
- Takahashi, H; Kimura, B; Yoshikawa, M; Fujii, T. 2003. Coloning and Sequencing of histidine decarboxylase genes of Gram – negative histamine Producing bacteria and their application in detection and identification of these organisms in fish. Applied Environmental Microbiology. 68, 2568-2579. [CrossRef]
- WHO (World Health Organization). 2007. The world health report, a safer future. Global public health security in the 21st century. Geneva, Switzer-land. http://www.who.int/whr/2007/en/ (Accessed 20.02.2018).
- Yagoub, O. S. 2009. Isolation of Enterobacteria and Pseudomonas spp from raw fish sold in Khartoum state. Journal of Bacteriology Research 1, 85-88.
- Yoshinaga, D. H; Frank, H. A. 1982. Histamine producing bacteria in Decomposing Skipjack tuna (Katsuwonus pelamis). Applied and Environmental Microbiology 44, 447 – 452.