Effect of Storage Temperature on Histamine Formation and Bacterial Growth in Whole Three Fish Species (*Rastrelliger kanagurta, Sardinella gibbosa and Lethrinus lentjan*)

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Abstract: Histamine formation and bacterial growth in three fish species, Indian mackerel (*Rastrelliger kanagurta*), Goldstripe sardinella (*Sardinella gibbosa*) and Pink ear emperor (*Lethrinus lentjan*) were monitored during storage at 4°C for 144 hours and 25°C for 24 hours. Throughout the storage periods, Pink ear emperor had the lowest levels of histamine, 1.3±0.2ppm and 3.4±0.4 ppm respectively, while in Indian mackerel and Goldstripe sardinella reached to13.0±2.6 ppm, 7.0±0.2 ppm at 4°C, and 175.0±2.6 ppm, 122.9±3.5 ppm at 25°C, respectively. Indian mackerel and Goldstripe sardinella were more susceptible to histamine formation, at 25°C they exceeded the admissible maximum levels of histamine 50 ppm that established by Food and Drug Administration. Histamine formation were controlled and bacterial growth were slowed by 4°C. Bacteria were grown in all fish species above the microbial spoilage limit of fish 7 log cfu/g that limited by International Commission on Microbiological Specifications for Foods, and reached to about, 7.33±0.31 and 7.23±0.26 log cfu/g in Pink ear emperor, 7.83±0.23 and 8.25±.51 log cfu/g in Indian mackerel, 7.61±0.06 and 8.29±0.62 log cfu/g in Goldstripe sardinella, at 4°C and 25°C, respectively. In this study, histamine levels were varied significantly among fish species. On the other hand, no significant differences were found in bacterial growth among fish species that stored under the same storage temperature.
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Key words: Histamine; bacterial growth; Indian mackerel; Goldstripe sardinella; Pink ear emperor; storage temperature.

1. Introduction

Annually, there is an increasing demand for seafood, representing a steady global increase in seafood consumption [1]. Seafood is a good source for a digestible animal protein, healthful polyunsaturated fatty acids, minerals and vitamins that support the biochemical processes of the human body [2]. However, seafood-borne disease have increased and there is a serious interest in seafood safety worldwide to decrease the health risks and diseases caused by seafood consumption [3, 4]. A group of diseases which associated with spoiled seafood consumption have been reported. These diseases occur due to the presence of some toxins in seafood. Histamine (Scombrotoxin) fish poisoning (HFP) has been reporting for many years as the most outbreak seafood poisoning in many countries [5, 6, 7].

HFP as a result from consumption of seafood that already contaminated by toxin called histamine or scombrotoxin formed by certain bacteria growth in seafood [4]. HFP is usually a mild illness with a different symptoms, symptoms of HFP occurring a few minutes to several hours following after consumption. The most frequently symptoms are headache, dizziness, skin itching, pain, swelling of the tongue, oral burning, edema, blistering and local inflammation, nausea, vomiting, abdominal cramps and diarrhea, hypotension and heart palpitation. The severity of the symptoms might be different depending on concentration of histamine and the sensitivity of the person. Also symptoms might be severe for the children, pregnant, elderly [4, 5, 6, 8-12] and for those taking medicines such as isoniazid [13].

Fish is the most seafood susceptible for HFP due to the great content of histidine in their muscles [9] especially, pelagic fish that belong to families Scombridae and Scomberosocidae such as, tuna (yellowfin, big-eye, bluefin, skipjack, and albacore), skipjack, saury,bonito and mackerel, and several species of non-scombroid such as sardines, pilchards, anchovies, marlin, bluefish and mahi-mahi [4, 6, 8, 14].

Formation of histamine in fish occurs by certain bacteria, which can produce enzymes called histidine-decarboxylase that convert the histidine to histamine, this process called histidine decarboxylation. Gram negative bacteria are most likely to form histamine [7, 8, 12]. A number of the histamine-forming bacteria are facultative that can grow in low levels of oxygen and some of them are halotolerant or halophilic. Histidine-decarboxylase can be produced from Enterobacteriaceae, Clostridium, Lactobacillus, Vibrio, Pseudomonas, Raoultella and Photobacterium [4, 5, 6, 10, 12, 16,

17]. Despite of many different species of bacteria can produce Histidine-decarboxylase, but their histamine-forming abilities are significantly different, e.g. *Morganella morganii* capable to form >1000ppm of histamine in culture broth, while *Entrobacter cloacae* which belong to the same family of *Morganella morganii* might to form about 125ppm [9, 15]. These bacteria naturally occurring in the aquatic environment, skin, gills and viscera of the fish and may be grow and expand fast to other places in the fish during poor handling [18]. The bacterial action might begin rapidly after fish died even before fish autolysis occurs [6], and their ability to grow and produce decarboxylase in a wide range of temperatures [19]. Decarboxylases might form histamine and be more active at pH ranged between 2.5-6.5 [4, 20].

Histamine poisoning occurs in all parts of the world, and can be the most common form of poisoning caused by consuming fish. Good statistics on the occurrence does not exist because the mild nature of the disease and misdiagnosis of histamine poisoning where physicians diagnose histamine poisoning as food allergy [10, 12]. Japan and the United States and the United Kingdom are the countries with the largest number of reported histamine poisoning incidents, this may mean they are better reporting of histamine poisoning incidents. The incidents were reported but less in other places in Europe, Asia, Africa, Canada, New Zealand and Australia [8, 10].

Histamine forms due to expose fish to elevated temperature after catch, and the levels of histamine increase by temperature and time abuse. Kim *et al*. [21]. showed that the histamine levels of Saury, Mackerel, Spanish mackerel and Amberjack stored at 25°C for 24hours, were 2123.9ppm, 1776.7ppm, 189.9ppm and 36.6ppm, respectively. while at storage condition 4–10°C histamine levels increased gradually after 2–3 days. The histamine formation in big eye fish and skipjack fish during storage at 4°C, 10°C and 22°C, histamine level was >500 ppm at 22°C in 24 hours for skipjack and 48 hours for big eye tuna, the increasing of histamine level was slowed at 10°C and 4°C, after 3 days at 10°C and 6 days at 4°C significant levels of histamine were formed [31]. Histamine formation in fish does not stop at 4°C or below, histamine formation was stopped at frozen storage only [23]. Many studies showed the effect of storage temperature on histamine formation in different fish species, all the studies agreed that histamine is not formed in fish stored at 0°C or below [4].

Newly caught fish contains negligible levels of histamine [24]. However, inappropriate handling, processing or distribution of fish may cause histamine formation rapidly. Hygienic conditions and propitiate temperatures (≤ 4.4°C) during all times of fish handling can be affective to prevent or minimize the levels of histamine [4]. Histamine can be useful to determine the fish freshness since histamine formation is related to bacterial activities. The importance of histamine determination in fish due to its harmful effects on human health and fish quality. FDA established the level of 50ppm to control histamine formation in fish. Fish containing histamine more than this level considered spoiled and unfit for human consumption [6]. Our objective was to study the histamine formation and bacterial growth in three commercial fish species from Red Sea under controlled storage temperatures. In this study, the histamine formation and bacterial growth were determined during storage at 4°C for 144 hours and 25°C for 24 hours.

2. Materials and methods

2.1. Raw material and experimental design

Indian mackerel (*Rastrelliger kanagurta*), Goldstripe sardinella (*Sardinella gibbosa*) and Pink ear emperor (*Lethrinus lentjan*) were obtained from Jeddah Fish Landing Center. They were placed into a portable iced cool boxes separately and delivered to the Faculty of Marine Science Laboratories of the King Abdulaziz University in Abhor-Jeddah within approximately 1-2 hours. Each species was divided into three groups and stored at storage temperatures 4°C and 25°C. As soon as samples were delivered to the laboratories, they were immediately examined (time zero). For the analyses, samples stored at 4°C were examined every 48 hours, while those stored at 25°C were examined every 6 hours.

2.2. Microbiological analysis

Total bacterial count (TBC) was determined in triplicate by direct viable count (DVC) method [25] from each of three fish species stored at 4°C and 25°C under sterile conditions. Aseptically, 1 g of tissue from dorsal side of each sample was homogenized in 9ml of sterilized sea water. 1 ml of each homogenized liquid was serially diluted to six dilutions. Sterilized micropipette was used to transfer 0.1ml of each dilution onto the surface of Marine agar plates and by sterilized spreader the sample were spread gently on the surface of culture medium. Then, the plates were incubated at 25°C for 48 hours.

2.2. Histamine analysis

Histamine content in fish samples was determined in triplicate by following the protocol of Veratox for histamine test kit, competitive direct enzyme-linked immunosorbent assay (Neogen Corporation). Sample were prepared by adding 10 g of the homogeneous mixture to a clean bottle containing 90 ml of distilled water. About 15 minutes

to allow the suspensions to settle. Approximately 5 ml of supernatant was collected and filtered through Whatman no.1 folded filter paper into a clean tube. The filtered samples were processed as described in the protocol of Veratox for histamine test kit using a pre-programmed histamine determination test on the STAT-FAX 303 plus Microstrip Reader at a wavelength 650 nm.

2.3. Statistical analysis

Minimum, maximum, mean, standard deviation and standard error of mean were used to describe data. The values were expressed as mean ±SD. Data obtained were analyzed by using independent measures One-way Analysis of Variance (ANOVA) and Post-hoc multiple comparisons conducted by using Least Significant Difference (LSD), $P < 0.05$ were considered as significant values. Statistical analysis was performed by using statistical package IBM SPSS software, version 21, and Microsoft Excel, version 2007.

3. Results

3.1. Microbiological analysis

The initial TBC (time zero) of Indian mackerel, Goldstripe sardinella and Pink ear emperor were 2.41±0.23 log cfu/g, 2.8±0.14 log cfu/g and 2.20±0.02 log cfu/g, respectively. During storage of 4°C TBC in fish samples after 48 hours were 4.88±0.21 log cfu/g in Indian mackerel, 4.88±0.12 log cfu/g in Goldstripe sardinella and 4.97±0.13 log cfu/g in Pink ear emperor, after 96 TBC were 6.14±0.19 log cfu/g in Indian mackerel, 6.08±0.20 log cfu/g in Goldstripe sardinella and 6.14±0.06 in Pink ear emperor, and in 144 hours of storage TBC were 7.83±0.24 log cfu/g in Indian mackerel, 7.61±0.06 log cfu/g in Goldstripe sardinella and 7.33±0.31 in Pink ear emperor (table 1, fig. 1). The numbers of bacterial colonies that proliferated in the each period of storage were as followed; in the first 48 hours were about 2.47±0.26 log cfu/g in Indian mackerel, 2.08±0.08 log cfu/g in Goldstripe sardinella and 2.74±0.09 in Pink ear emperor, in the second 48 hours were 1.26±0.28 log cfu/g, 1.20±0.16 log cfu/g and 1.17±0.08 log cfu/g respectively, and in the third 48 hours were 1.69±0.26 log cfu/g, 1.53±0.26 log cfu/g and 1.19±0.29 log cfu/g, respectively (Table 2, Fig. 2).

At 25°C, TBC in fish samples in 6 hours of storage increased greatly to 5.11±0.63 log cfu/g in Indian mackerel, 5.13±0.35 log cfu/g in Goldstripe sardinella and 5.28±0.02 log cfu/g in Pink ear emperor, after 12 hours of storage at TBC reached to 7.44±0.09 log cfu/g in Indian mackerel, 7.22±0.09 log cfu/g in Goldstripe sardinella and 6.66±0.15 log cfu/g in Pink ear emperor, TBC of samples in 24 hours of storage were 8.25±0.51 log cfu/g in Indian mackerel, 8.29±0.06 log cfu/g in Goldstripe sardinella and 7.23±0.02 log cfu/g in Pink ear emperor (Table 3, Fig. 3). The numbers of bacterial colonies that proliferated in the each period of storage were as followed; in the first 6 hours were about 2.7±0.73 log cfu/g in Indian mackerel, 2.33±0.43 log cfu/g in Goldstripe sardinella and 3.08±0.01 log cfu/g in Pink ear emperor, in the second 6 hours were 2.33±0.53 log cfu/g, 2.09±0.40 log cfu/g and 1.38±0.50 log cfu/g respectively, and in the period between 12-24 hours were 0.81±0.50 log cfu/g, 1.07±4.25 log cfu/g and 0.25±0.13 log cfu/g, respectively (Table 4, Fig. 4).

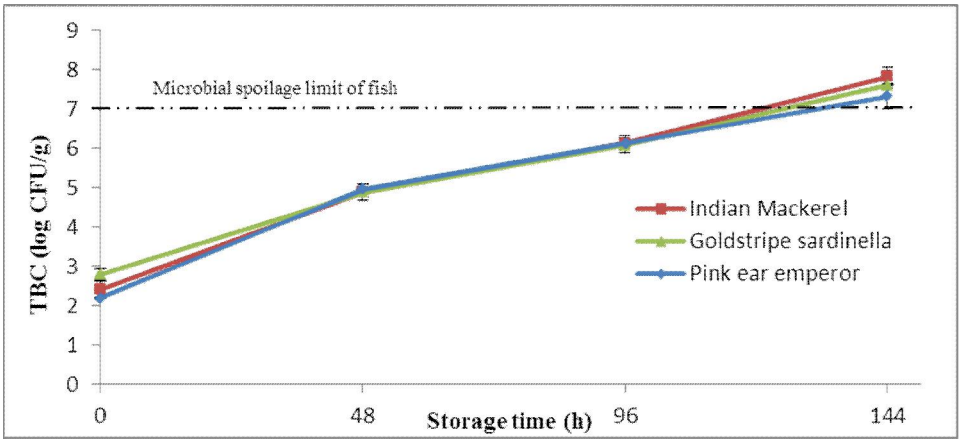

Figure 1. TBC values (log cfu/g) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 4°C. Each point shown is the mean value of three determinations of each sampling. Error bars represent the standard deviation.

Table 1. Changes in TBC (log CFU/g) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 4°C.

Time (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0	2.41±0.23[a]*	2.80±0.14[a]	2.20±0.02[a]
48	4.88±0.21[b]	4.88±0.12[b]	4.97±0.13[b]
96	6.14±0.19[c]	6.08±0.20[c]	6.14±0.06[c]
144	7.83±0.24[d]	7.61±0.06[d]	7.33±0.31[d]
192	ND**	ND	ND
Total	5.32±2.07	5.34±1.84	5.17±1.98

*. Values are means ± standard deviation (SD) of three determinations of each sampling. Means followed by same letter (s) are not significantly different (P>0.05). **. Not data found.

Table 2. Bacterial proliferation (log CFU/g) in Indian mackerel, Goldstripe sardinella and Pink ear emperor during each period of storage at 4°C.

Storage period (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0-48	2.47±0.26	2.08±0.08	2.74±0.09
48-96	1.26±0.28	1.20±0.16	1.17±0.08
96-144	1.69±0.26	1.53±0.26	1.19±0.29

*. Values are means ± standard deviation (SD) of three determinations of each sampling.

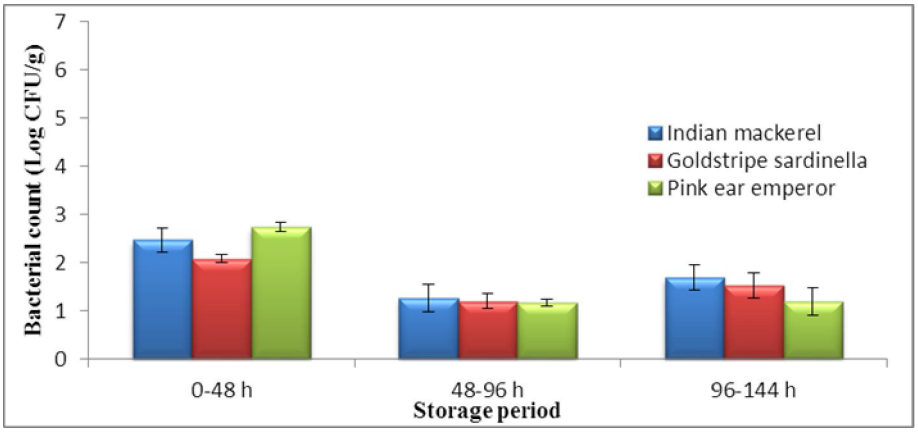

Figure 2. TBC proliferation (log cfu/g) in Indian Mackerel, Goldstripe sardinella and Pink ear emperor during storage period of 4°C. Each point shown is the mean value of three determinations of each sampling. Error bars represent the standard deviation.

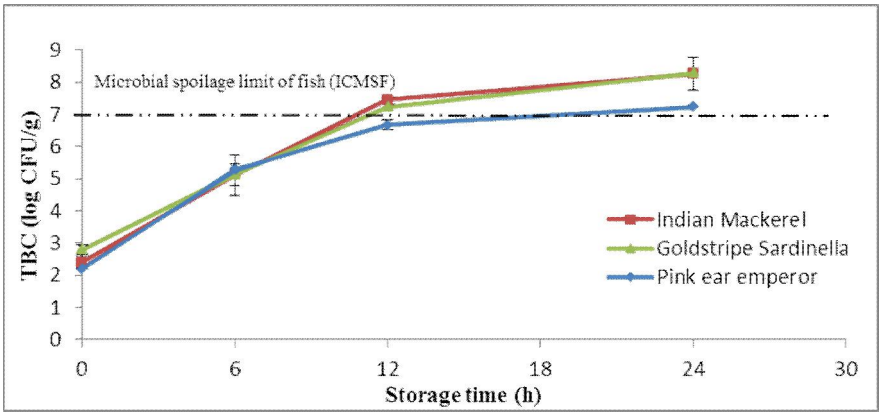

Figure 3. TBC values (log cfu/g) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 25°C. Each point shown is the mean value of three determinations of each sampling. Error bars represent the standard deviation.

Table 3. Changes in TBC (log CFU/g) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 25°C.

Time (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0	2.41±0.22[a]*	2.80±0.14[a]	2.20±0.02[a]
48	5.11±0.63[b]	5.13±0.35[b]	5.28±0.02[b]
96	7.44±0.09[c]	7.22±0.09[c]	6.66±0.15[c]
144	ND**	ND	ND
192	8.25±0.5[d]	8.29±0.06[d]	7.23±0.03[d]
Total	**5.80±2.40**	**5.86±2.20**	**5.34±2.04**

*. Values are means ± standard deviation (SD) of three determinations of each sampling. Means followed by same letter (s) are not significantly different (P>0.05). **. Not data found.

Table 4. Bacterial proliferation (log CFU/g) in Indian mackerel, Goldstripe sardinella and Pink ear emperor during each period of storage at 25°C.

Storage period (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0-6	2.7±0.7336	2.33±0.431	3.08±0.01
6-12	2.33±0.532	2.09±0.4	1.38±0.5
12-24	0.81±0.5	1.07±4.25	0.25±0.125

*. Values are means ± standard deviation (SD) of three determinations of each sampling.
**. Data of period (12-18 h) not found.

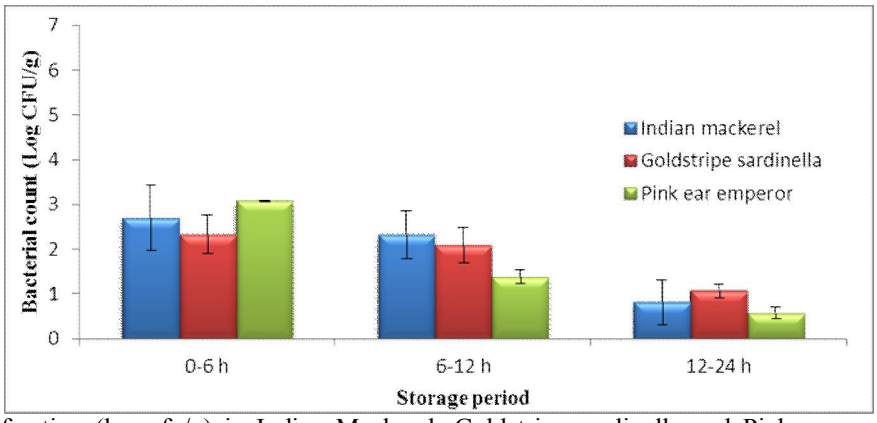

Figure 4. TBC proliferation (log cfu/g) in Indian Mackerel, Goldstripe sardinella and Pink ear emperor during storage period of 25°C. Each point shown is the mean value of three determinations of each sampling. Error bars represent the standard deviation.

3.2 *Histamine analysis*

The initial level of histamine in Indian mackerel, Goldstripe sardinella and Pink ear emperor were below the levels 1ppm. During storage of 4°C TBC in fish samples after 48 hours were 2.17±1.02ppm in Indian mackerel, 1.50±0.32ppm in Goldstripe sardinella and 0.03±0.02ppm in Pink ear emperor, after 96 histamine levels were 5.00±0.50ppm in Indian mackerel, 4.00±0.32ppm in Goldstripe sardinella and 0.26±0.05 in Pink ear emperor, and in 144 hours of storage histamine levels were 13.00±1.65ppm in Indian mackerel, 7.00±0.20ppm in Goldstripe sardinella and 1.32±0.23ppm in Pink ear emperor (table 5, fig. 5). The histamine contents that formed in the each period of storage were as followed; in the first 48 hours were about 2.13±1.03ppm in Indian mackerel, 1.47±0.35ppm in Goldstripe sardinella and 0.01±0.03ppm in Pink ear emperor, in the second 48 hours were 2.83±1.18ppm, 2.50±0.46ppm and 0.23±0.06ppm respectively, and in the third 48 hours were 8.00±2.59ppm, 3.00±0.28ppm and 1.06±0.23ppm, respectively (Table 6, Fig. 6).

At 25°C, histamine levels in fish samples in 6 hours of storage were 5.50±1.80ppm in Indian mackerel, 2.37±0.32ppm in Goldstripe sardinella and 0.02±0.00ppm in Pink ear emperor, after 12 hours of storage at histamine levels were 122.50±2.29ppm in Indian mackerel, 94.00±1.73ppm in Goldstripe sardinella and 0.04±0.02ppm in Pink ear emperor, histamine levels in samples stored for 24 hours reached to 175.00±2.65ppm in Indian mackerel,

122.90±3.57ppm in Goldstripe sardinella and 3.40±0.49ppm in Pink ear emperor (Table 7, Fig. 7). The histamine content that formed in the each period of storage were as followed; in the first 6 hours were about 5.46±1.81ppm in Indian mackerel, 2.34±0.29ppm in Goldstripe sardinella and 0.00±0.01ppm in Pink ear emperor, in the second 6 hours were 117.00±3.24ppm, 91.63±2.02ppm and 0.02±0.02ppm respectively, and in the period between 12-24 hours were 52.50±0.50ppm, 28.90±4.25ppm and 3.36±0.48ppm, respectively (Table 8, Fig.8).

Table 5. Changes in histamine content (ppm) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 4°C.

Time (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0	0.04±0.02[a]*	0.03±0.03[a]	0.02±0.01[a]
48	2.17±1.02[a]	1.50±0.32[a]	0.03±0.02[b]
96	5.00±0.50[a]	4.00±0.32[b]	0.26±0.05[c]
144	13.00±1.65[a]	7.00±0.20[b]	1.32±0.23[c]
192	ND**	ND	ND
Total	**5.05±5.28**	**3.13±2.78**	**0.41±0.57**

*. Values are means ± standard deviation (SD) of three determinations of each sampling. Means followed by same letter (s) are not significantly different (P>0.05).
**. Not data found.

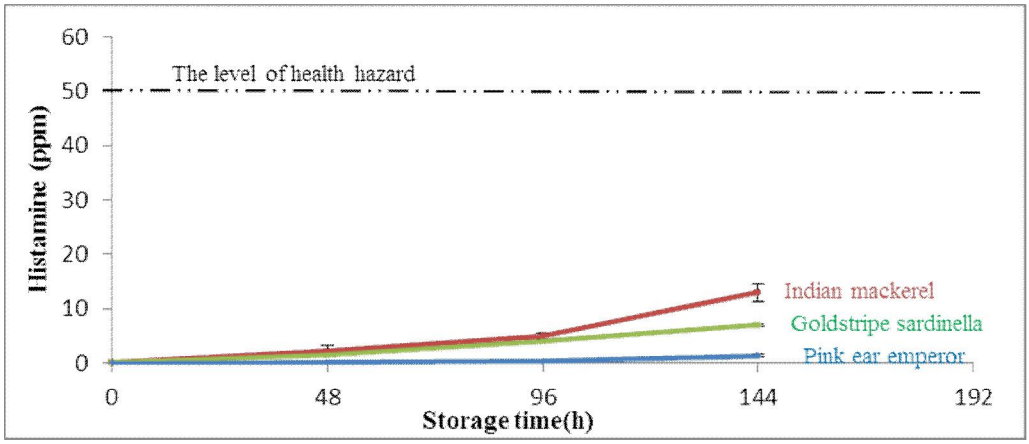

Figure 5. Changes of histamine levels (ppm) in Indian mackerel, Goldstripe sardinella and Pink ear emperor at storage 4°C. Each point shown is the mean value of three determinations for each sampling. Error bars represent the standard deviation (SD).

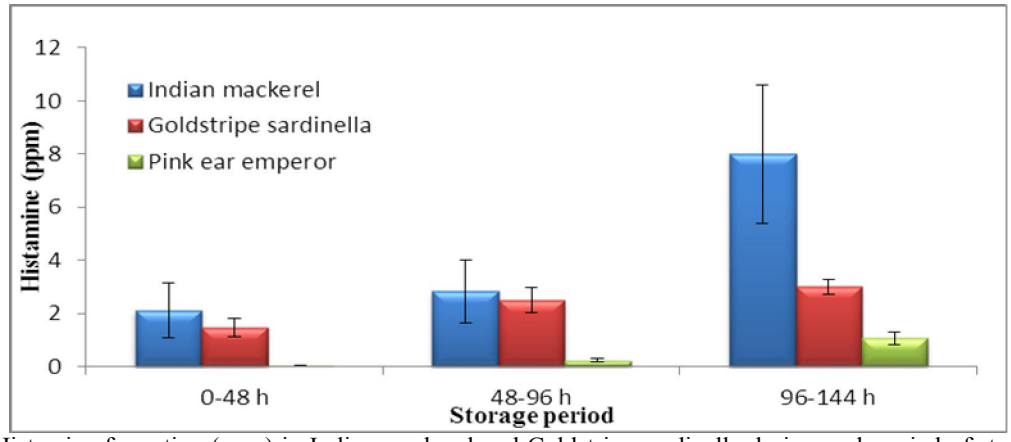

Figure 6. Histamine formation (ppm) in Indian mackerel and Goldstripe sardinella during each period of storage at 4°C. Each point shown is the mean value of three determinations for each sampling. Error bars represent the standard deviation (SD).

Table 6. Histamine formation (ppm) in Indian mackerel, Goldstripe sardinella and Pink ear emperor during each period of storage at 4°C.

Storage period (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0-48	2.47±0.26	2.08±0.08	2.74±0.09
48-96	1.26±0.28	1.20±0.16	1.17±0.08
96-144	1.69±0.26	1.53±0.26	1.19±0.29

*. Values are means ± standard deviation (SD) of three determinations of each sampling.

Table 7. Changes in histamine content (ppm) of Indian mackerel, Goldstripe sardinella and Pink ear emperor stored at 25°C.

Time (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0	0.04±0.02[a]*	0.03±0.03[a]	0.017±0.01[a]
6	5.50±1.80[a]	2.37±0.32[b]	0.02±0.00[c]
12	122.50±2.29[a]	94.00±1.73[b]	0.04±0.02[c]
18	ND**	ND	ND
24	175.00±2.65[a]	122.90±3.57[b]	3.40±0.49[c]
Total	**75.76±78.71**	**54.83±57.05**	**0.87±1.54**

*. Values are means ± standard deviation (SD) of three determinations of each sampling. Means followed by same letter (s) are not significantly different (P>0.05).
**. Not data found.

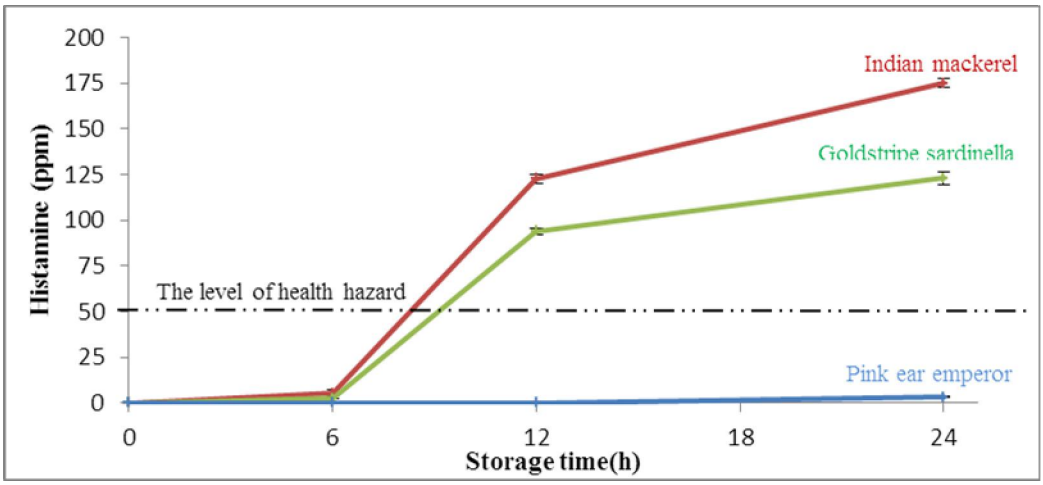

Figure 7. Changes of histamine levels in Indian mackerel, Goldstripe sardinella and Pink ear emperor at storage 25°C. Each point shown is the mean value of three determinations for each sampling. Error bars represent the standard deviation (SD).

Table 8. Histamine formation (ppm) in Indian mackerel, Goldstripe sardinella and Pink ear emperor during each period of storage at 25°C.

Storage period (h)	Indian mackerel	Goldstripe sardinella	Pink ear emperor
0-6	5.46±1.81	2.34±0.29	0.00±0.01
6-12	117.00±3.24	91.63±2.02	0.02±0.02
12-24	52.50±0.50	28.90±4.25	3.36±0.48

*. Values are means ± standard deviation (SD) of three determinations of each sampling.
**. Data of period (12-18 h) not found.

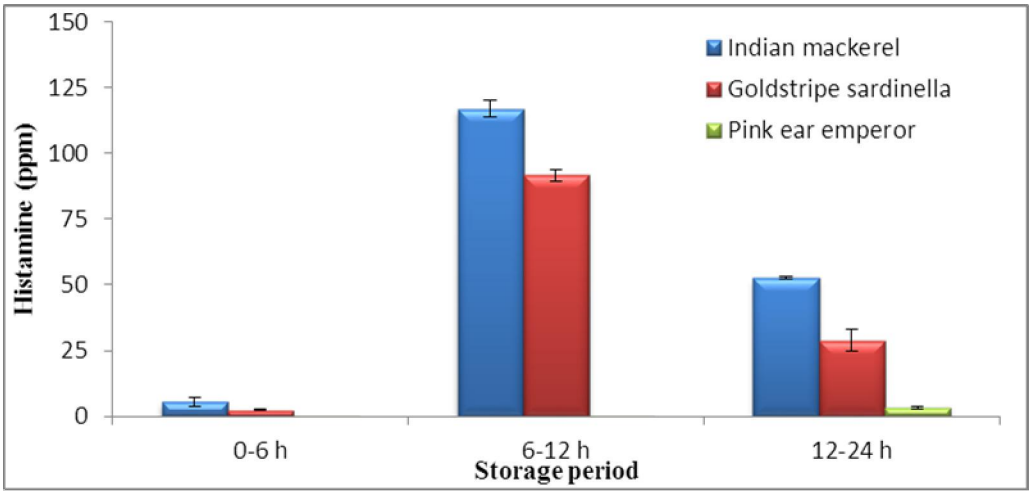

Figure 8. Histamine formation (ppm) in Indian mackerel and Goldstripe sardinella during each period of storage at 25°C. Each point shown is the mean value of three determinations for each sampling. Error bars represent the standard deviation (SD).

4. Discussion

4.1. Microbiological analysis

In this study, bacterial growth was increased dramatically in all sampled fish species during storage at both temperatures (4°C and 25°C). However, bacterial growth in fish species exceeded the microbial spoilage limit of fish, 7 log cfu/g (according to ICMSF) rapidly during storage at 25°C than at 4°C (figs. 1 and 3). In the same way, the rate of bacterial growth was not significantly different among the fish species during storage at the similar conditions (Tables 1 and 3). Bacteria grew very slowly in fish stored at 4°C compared to those stored at 25°C; therefore, the bacterial spoilage delayed in fish stored at 4°C. Fish bacteria showed a very sensitive to temperature, higher temperatures stimulated the fish bacteria to growth and multiply rapidly using the organic compounds of fish as food to obtain the energy, while lower temperatures did not totally stop the growth of bacteria but they might inhibit the bacterial growth and activities [26, 27]. bacteria have an ability to grow and produce enzymes in a wide range of temperatures [19]. The tropical regions which have a temperature ranged of 25-40°C are suitable for bacterial growth in fish [23]. The bacterial action could begins rapidly after fish died at suitable temperature even before fish autolysis occurs [6]. Elevated temperature lead to accelerate the bacterial growth [19]. Many studies demonstrated that the load of bacterial growth almost similar in the case of different fish species in the same environment, especially when fish exposed to similar treatments [9, 23, 29, 30]. Kim *et al*. [9] concluded that the trend of bacterial growth was not significantly different among fish species, when they exposed a group of different fish species (Mackerel, Albacore, Mahi-mahi and Salmon) separately to the same storage conditions.

4.2. Histamine analysis

Fig. 5 show the changes of histamine levels in fish species at storage 4°C. The histamine formation was increased very slowly at 4°C. Despite histamine levels were insignificant and did not exceeded the health hazard level of histamine; 50ppm [4], significant differences were found among fish species (Table 5). On the other hand, histamine levels of fish at 25°C were increased rapidly and exceeded the limit of 50ppm in Indian mackerel and Goldstripe sardinella only. elevated storage temperature lead to form high levels of histamine rapidly, histamine levels rate in Indian mackerel and Goldstripe sardinella was rising up greatly during storage at elevated temperature and lower temperatures did not stop histamine formation totally but allow it increase gradually slower. Similar effects demonstrated by Kim *et al*., [9]; Silva *et al*., [22]; Yamanaka *et al*., [31]; Visciano *et al*., [32].

Histamine levels of Pink ear emperor were negligible compared to Indian mackerel and Goldstripe sardinella at both storage temperatures (Fig. 5 and 7) possibly due to the significant differences in the presence of the histidine content in their tissues. In this study, Indian mackerel was the most susceptible to form histamine followed by Goldstripe sardinella. These suggestions agreed with the earlier reports by FDA, [4]; FAO/WHO, [6]; Kim *et al*., [9]; Pan and James, [19]; Afilal *et al*., [20]; Arnold and Brwon, [33]; Shakila *et al*., [34]. Silva *et al*. [22] resulted that histamine levels were >500ppm at 22°C for 24 hours in Skipjack (13400-20000ppm of histidine) and for 48 hours in Big-eye tuna (7450ppm of histidine). Kim *et al*. [29] found the

histamine levels in Mackerel at 4°C were insignificant for 144 hours, thereafter increased to up 574 ppm in 336 hours. Tsai *et al*. [35] noted that histamine did not stop at 4°C or below, histamine possibly stop at frozen storage only. FDA [4] reported, histamine formation at high temperatures is more rapid than moderate temperatures, histamine also possibly form at low temperatures. Shakila *et al*. [34] observed that histamine increased greatly on storage at 32±2°C, but the rate of change varied among fish species.

Relationship between bacterial counts and histamine formation

In the present study, the microbial fish spoilage limit is 7 log cfu/g according to ICMSF and the health hazard limit of histamine level of fish is 50ppm according to FDA. The results obtained from histamine determinations and TBC in histamine-fish samples (Indian mackerel and Goldstrpe sardinella) at storage temperatures 4°C and 25°C showed that at 4°C, TBC of Indian mackerel were 7 log cfu/g within about 118.16 hours and of Goldstripe sardinella within about 122.76 hours. By the time of the bacteriologically rejection occurrence in Indian mackerel and Goldstripe sardinella, histamine levels were 9.06ppm and 5.61ppm respectively, these levels of histamine were significantly lower than the health hazard level of histamine (50ppm). And at 25°C, TBC of Indian mackerel were 7 log cfu/g within about 15.58 hours and of Goldstripe sardinella within about 15.61 hours. By the time of the bacteriologically rejection occurrence in Indian mackerel and Goldstripe sardinella, histamine levels of Indian mackerel were 50ppm within about 7.28 hours and of Goldstripe sardinella within about 9.65 hours. The results in this study suggest that TBC appeared to be unreliable indicator to detect hazard levels of histamine, despite of histamine formation typically follows bacterial growth [9]. In this study, statistically no significant correlation between histamine formation and bacterial growth (r = -0.27 at 4°C, r = -0.09 at 25°C). this might be due to the ability of bacteria to form histamine varies from species to another, and many of them maybe inactive in fish tissue. Similar suggestions previously achieved by Basavakumar *et al*. [36]; Gingerich *et al.* [37]; Subburaj *et al*. [38]. Many studies reported that Enterobacteriaceae, Clostridium, Lactobacillus, Vibrio, Pseudomonas, Raoultella, Photobacterium most likely to form significant amounts of histamine especillay, *Morganella Morganii, Raoultella planticols, Raoultella ornithinolytica, Entrobacter aerogenes, Entrobacter gergoviae, Photobacterium damselae, Klebsiella oxytoca* [4- 6, 9,10, 12, 16, 17].

5. Conclusions, Recommendations and Future works

Fish has a short shelf-life after catch. Proper fish handling and storage in low temperatures considerably delays spoilage occurrence in fish. In this work, Indian mackerel, Goldstripe sardinella and Pink ear emperor stored in two storage conditions 25°C and 4°C, revealed that storage temperatures directly effect on shelf-life of fish, where the higher storage temperature caused rapid spoilage than the lower storage temperatures. The storage temperature of 4°C creates unfavorable environmental conditions for bacterial growth and delays the spoilage of fish, while storage temperature of 25°C allowed the bacterial to grow and histamine to form which lead to reduce the shelf-life of fish. Finally, all fish species in this study were reached to the microbiological spoilage, while histamine spoilage in fish was found only in Indian mackerel and Goldstripe sardinella. Therefore, the recommendations will be as follows:

- Rapid chilling of fish as soon as fish are caught and keeping fish at low temperatures (≤4°C) to reduce bacterial growth and histamine formation in fish.
- Gutting the fish can help to chill fish rapidly and reduces the numbers of bacteria.
- Histamine is good indicator to determine the freshness of pelagic fish species.
- TBC is good indicator to determine the freshness for demersal fish species.
- Use the standard hazard level of histamine (50ppm) that established by USFDA as the level of rejection for histamine-fish species.

As histamine fish poisoning is the most common toxicity related to seafood consumption which representing the most of all seafood-related food-borne diseases in many countries, seek for further studies to improve the quality and safety of seafood is needed.

Suggested needed studies are as follows:

- Further studies on the relationship between histamine toxicity and other biogenic amines.
- Identify histamine-forming bacteria in marine fish in Red Sea environments and in fish processing environments to determine the ability of these bacteria that exist in such environments to contaminate fish.
- Study the effect of storage at Vacuum and modified atmospheres on histamine formation in fish.

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