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Histamine and Other Biogenic Amines Formation in Canned Tuna Fish Inoculated with *Morganella Morganii* or *Proteus Mirabilis* in Determining Food Safety During Temperature Abuse

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Abstract

Histamine and other amines are present in high levels in foods is due to bacterial decarboxylation activity of amino acids. Histamine is considered the most toxic biogenic amine responsible for almost all food amines-intoxication. Histamine production and other biogenic amines including tyramine, cadaverine, and tryptamine were investigated in canned tuna samples that were uninoculated, inoculated with *Morganella morganii*, and inoculated with *Proteus mirabilis*. This study showed that high levels of histamine sufficient to cause histamine poisoning were detected in the uninoculated tuna fish samples kept at room temperature (25°C) for 10d (53.9 ± 7.1 ppm) , or at 37°C for 6 d (126.7 ± 20.8 ppm), and 10 d (266.7 ± 15.3 ppm). Elevated levels of histamine, tyramine, cadaverine, and tryptamine in tuna inoculated with *M. morganii* were reported in samples stored at 25°C for 10 d as follow: 1921.7 ± 27.5, 2002.0 ± 7.2, 2809.2 ± 25.0, and 31.9 ± 1.6 ppm, respectively. While, storage tuna inoculated with *P. mirabilis* at 37°C for 10d in was associated with the highest levels of histamine (296.7 ± 15.3 ppm), tyramine (94.3 ± 9.0 ppm), cadaverine (400.0 ± 10.0 ppm), and tryptamine (161.8 ± 3.7 ppm). In general, Histamine and biogenic amines produced by *M. morganaii* was significantly ($P < 0.05$) more than that produced by *P. mirabilis* under the same conditions, although there were no differences ($P > 0.05$) among bacterial count. Opened canned fish should be stored at 4°C or below and be consumed as soon as possible. It is also advised not to store or display the tuna at room temperature (25-37°C).

Keywords: *Amines intoxication; Histamine; Tyramine; Cadaverine; Tryptamine; Morganella morganii; Proteus mirabilis*

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Introduction

Biogenic amines are natural compounds that can be found in many foods including fish. Biogenic amines play important biological roles in living cells, however, elevated amount of biogenic amine may result in food poisoning. Histamine is considered the most toxic biogenic amine responsible for almost all food amines-intoxication (Zaman., *et al.* 2010). Presence of high levels of histamine and other

amines in foods is due to bacterial decarboxylation of amino acids especially in scombroid fish (Lerke & Bell, 1976; Önal, 2007; Chong., *et al*. 2011). Scombroid fish family includes tuna, mackerel, and saury; biogenic amines poisoning also was associated with and non-scombroid fish such as bluefish, sardine, anchovy, and herring (Flick., *el al*. 2001). Histamine is very stable in food against processing techniques, and is considered as an allergen characterized by difficulty in breathing, itching, rash, and hypertension.

Biogenic amines are produced at very low levels in fresh fish that is not contaminated or spoiled (Özogul & Özogul, 2006). Presence of other biogenic amines produces synergistic effect and enhances the toxicity of histamine through the inhibition of histamine oxidizing enzymes (Taylor 1986; Emborg and Dalgaard 2006). Cadaverine is a formed by bacterial decarboxylation of lysine, while tyramine it often is produced by the decarboxylation of tyrosine. Tyramine reported to induce migraine, increase blood pressure and cause hypertension (Shalaby 1996). Til., *et al*. (1997) reported the estimated level to induce acute toxicity for tyramine and cadaverine is > 2000 ppm. Tryptamines synthesized by decarboxylation of the amino acid tryptophan, and it toxicity is known for hallucinogenic properties (Tittarelli., *et al.* 2015). European Community has suggested that total biogenic amines should not exceed 300 mg/kg in fish and fish products (EC, 1991).

Different concentrations have been reported for the 'safe for consumption' guidelines for different fish species (Arnold& Brown, 1978). The United States Food and Drug Administration established the permissible limit of histamine at 50 mg/kg in edible fish (USFDA, 2002). European Community (EC) set the guideline for histamine content not to exceed 200 mg/kg for acceptance of tuna and other fish belonging to the Scomberesocidae families (EC, 1991). Other countries have also set the upper levels of histamine concentrations for edible fish including South Africa, 100 mg/kg and Australia, 200 mg/kg (Auerswald., *et al*. 2006). The toxic dose is variable; Lehane and Olley (2000) concluded the oral toxicity of histamine to humans is slight at a total dose of 8– 40 mg/kg, moderate at 440 mg/kg and severe at 4100 mg/kg.

Histamine forming bacteria are usually the common post-harvest contaminants of fish. Enterobacteriaceae including Morganella morganii, *Enterobacter cloacae, Citrobacter freundii*, *Escherichia coli, Hafnia alvei, Klebsiella pneumoniae*, and Proteus spp. have been associated with histamine poisoning in fish (Ababouch., *et al*. 1991). Other bacterial species such as *Clostridium* spp., *Vibrio alginolyticus, Acinetobacter lowffii* and Plesiomonas shigelloides are capable of producing histamine have also been identified in fish (Yoshinaga and Frank 1982). Morganella morganii, and Proteus spp. have been reported to be the most prolific histamine producers (Novak, 1998; Economou., *et al*. 2007; Hongpattarakere., *et al*. 2016).

Proteus mirabilis can be identified as a Gram-negative rod that is motile, urease-positive, lactose-negative, indole-negative, and produces hydrogen sulfide, belong to enterobacteriacae family (Schaffer and Pearson, 2015). It is a pathogen of the urinary tract, particularly with long-term catheterization patiants (O'Hara., *et al*. 2000). Morganella morganii is a gram negative, facultative anaerobic bacilli belongs to Enterobacteriaceae family (tribe Proteeae). It is an opportunistic pathogen causes post operation wound and urinary tract infections (Liu., *et al*. 2016). Most histamine-producing bacteria are mesophiles, therefore, temperature is considered a critical factor to support their growth and histamine formation compared to other environmental factors (Kim., *et al*. 2000).

In kitchens (either professional or household), the environmental conditions are far from ideal concerning environmental temperature, preparation time, and hygiene. Therefore, there is a crucial lag phase from taking a tuna out to preparing and consume it. Room temperatures (25-37°C) accelerate bacterial growth, and since most prolific histamine producers are mesophiles and are the common food contaminants, high storage temperatures and inadequate handling procedures can result in the production of histamine and biogenic amines at toxic levels (Tittarelli., *et al*. 2015).

Temperature abuse not only enhances the multiplication of bacteria, but also histidine decarboxylase activity. The enzyme activity is favored by the exposure to temperatures higher than 15°C (Lehane and Olley, 2000). Studies concluded that histamine produced by temperatures higher than 5°C with an optimum of 20–30°C (Kim., *et al*. 2000; Lehane and Olley 2000). Histamine cannot inactivated by

the usual thermal processes such as freezing, chilling, cooking, canning, etc. The sole prevention is to prevent its production by food storage at temperatures below 4°C and avoid prolonged handling at ambient temperatures. The amount of histamine produced is a function of fish species, time, temperature, and type and number of bacteria species present (Herna´ndez-Herrero., *et al*. 1999).

The objectives of this study were: 1) to assess the effect of different temperature regimens (4, 25, 37°C) for different storage durations (2, 6, and 10 d), simulating refrigerated storage and room temperature abuse, on histamine formation together with the development of histamine-forming bacteria in tuna fish. 2) to determine the histamine forming capability of Morganella morganii, versus Proteus mirabilis under previous mentioned storage conditions.

Materials and Methods

Sampling

Canned tuna in water were bought from retail stores and were brought to the laboratory. Tuna fish, skipjack tuna (Katsuwonus pelamis), chunks (30 g) were placed into sterile polyethylene Whirl-pak bags and analyzed immediately.

Preparation of tested bacteria

Morganella morganii, Proteus mirabilis strains were obtained from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University. Stock cultures (10^8 CFU/mL) stored at -70°C in 15% (w/v) glycerol was inoculated into 10mL of Brain Heart Infusion broth (BHI, Lab M, Neogen Company, UK). Each culture of *Morganella morganii, Proteus mirabilis* was sub-cultured twice at 37°C for 24 hr. in BHI, and then was streaked on to Tryptic Soy Agar (TSA, Lab M, Neogen Company, UK) plates. TSA plates were incubated at 37°C for 24 hr. and colonies were confirmed biochemically using API 20E diagnostic strips (Biomérieu, France) in table 1.

		Morganella morganii	***Proteus mirabilis***
ONPG		-	-
Arginine dihydrolase		-	-
Lysine decarboxylase		-	-
Ornithine decarboxylase		+	+
Citrate		-	+
H2S		-	+
Urease		+	+
TDA		+	+
Indole		+	-
Voges-Proskauer		+	-
Gelatinase		-	+
Acid from:	glucose	+	+
	Mannitol	+	-
	Inositol	-	-
	Sorbitol	-	-
	Rhamnose	-	-
	sucrose	-	-
	Melibiose	-	-
	Amylose	-	-
	arabinose	-	-

Table 1: *Biochemical test results of Morganella morganii, and Proteus mirabilis using API 20E diagnostic strips.*

Bacterial preparation and sample inoculation.

Colonies of Morganella morganii, Proteus mirabilis were grown separately in Tryptic soy broths (TSB, Lab M, Neogen Company, UK) and incubated at 37°C for 24 hr. One-milliliter from each culture were dispensed into sterile 0.5-ml polypropylene microcentrifuge tubes (Greiner, Merck Germany) and centrifuged 3 min (Eppendorf, Model 5418, Maximum speed/RCF: 14,000 rpm) to pellet cells. The supernatant was aseptically removed, and the remaining pellet was washed one time with sterile trypticase soy broth, then recentrifuged to pellet. The pellet was resuspended in 25 ml trypticase soy broth and grown aerobically for 4 h at 37°C to form a log-phase culture. Aliquots for direct-surface inoculation of Tuna fish were taken from this culture stocks (Lorca., *et al*. 2001).

A total of 81 tuna fish samples (10 g each) were divided into 3 equal numbers groups (27 in each group): first group samples were uninoculated control group, second group inoculated with 1 ml of M. morganii in log phase (106 CFU/ml, in tryptic soy broth) with a sterile pipette. The third group inoculated with The Proteus mirabilis in log phase (106 CFU/ml, in tryptic soy broth). Inoculum was dripped and evenly distributed over the surface, and the tuna fish was placed into a sterile polyethylene Whirl-pak bag. Samples were stored at 4, 25, and 37°C to simulate mild to moderate temperature abuse conditions, which may occur during preparation of tuna at kitchen or professional restaurants. Samples from each group were stored for 2, 6, and 10 d and then examined for total aerobic bacterial count and quantity of biogenic amines including histamine, tyramine, cadverine, and tryptamine.

Quantitative determination of biogenic amines by Reverse-Phase high performance liquid chromatography (HPLC)

Tuna fish samples (20g) were homogenized using a blender, and a homogenated sample (10g) was extracted with a total volume of 60 ml 5% trichloroacetic acid (T9151; Merck KGaA, Darmstadt, Germany). The extracts were combined and filtered through glass wool following 10 min of centrifugation (6,000 3 g, 108C). The clear supernatant (8 ml) were mixed with 2 ml of 2 M NaOH followed by 2% benzyol chloride (dissolved in acentonitrile). The derivatization mixture was mixed in a vortex for 1 min, left for 5 min at room temperature and centrifuged at 4000 g for 10 min. Saturated sodium chloride (2 ml) was added to stop the reaction. Extraction of amines derivatives was carried out with 2 ml of diethyl ether, and was repeated twice. The organic layer was evapo¬rated to dryness at 40°C in the stream of nitrogen. Dry residue was resuspeneded in 1 ml of acetonitrile and filtered into HPLC vial through 0.45 mm nylon syringe filter. Filtrate (10 µl) was injected into HPLC system. The minimum level of detection was 1 ppm.

The HPLC system consisted of Waters Alliance 2695 separa¬tion module, Waters dual l 2487 UV detector recording chromatograms at 254 nm. Phenomenex Luna C18, 150 × 4.6 mm, 5 mm column was used heated at 35°C. The mobile phase consisted of acetonitrile (A) and water (B). Gradient elution was applied in order to separate different biogenic amines.

Microbiological evaluation.

A 10g samples were placed in sterile Whirl-pak bags and 90 ml of sterile 0.1% peptone solution (Becton Dickinson) were added. Mixture were homogenized for 2 min using a stomacher (Stomacher® 400 Circulator, Seward UK), and then serially diluted. Dilutions were then spread-plated onto plate count agar (lab M, UK) and incubated at 35°C overnight. Colonies count was reported as logs of colony forming units (CFU/g). Experiments were repeated and 3 measurements were conducted for each treatment. The results were represented as means ± standard deviations. Randomly selected colonies were transferred onto trypticase soy agar slants (TSA, Lab M, Neogen Company, UK) and incubated at 35°C for 24h. colonies were screened for histidine decarboxylase activity using Niven's agar (lab M, UK), which were then incubated for 24 h at 37°C. Purple colonies (surrounded by a purple halo on a yellowish background) were identified as histamine forming bacteria (Bakar., *et al*. 2010). Selected colonies were furthered identified using API 20E diagnostic strips (Biomérieu, France) in table 1.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed using SPSS Version 22.0 (Armonk, NY: IBM Crop.). Duncan multiple range test with a 95% confidence level was used to compare means value of biogenic amines (ppm) and bacterial count (Log CFU/g) at different storage temperature and times.

Results and Discussion

Histamines analysis

Uninoculated tuna fish

Histamine level in uninoculated tuna fish was ranged from not detected to 4.4 ± 0.5 ppm when fish stored at 4°C for 10 d (table 2). Several studies reported the rise of histamine content in fresh fish stored at refrigeration temperature. Histamine content was negligible in big-eye tuna (<0.1 mg/100g) at the start of storage, but notable amounts were detected after 6 d at 4°C, exceeding the limit of 50 mg/kg (Silva., et al. 1998). Histamines formation in barramundi (Lates calcarifer) slices kept at 4°C were up to 275 mg/kg at the end of 15 d storage (Bakar., *et al.* 2010). In blue fish stored at 5°C, histamine levels ranged from 18 to 100 ppm during 7 d storage (Lorca., *et al.* 2001). The natural flora of fresh fish in the previous studies might assist in the formation of histamine in the uninoculated fish stored at refrigeration temperatures.

Temperature (°C)	Storage time (d)	Biogenic amines (ppm)				Standard plate count Log (CFU/g)
		Histamine	Tyramine	Cadverine	Tryptamine	
4	2	Nd a	Nd	Nd	Nd	<1 Estimated a
	6	Nd a	Nd	Nd	Nd	<1 Estimated a
	10	4.4 ± 0.5 b	Nd	Nd	Nd	1.1 ± 0.1 b
25	2	Nd a	Nd	Nd a	Nd a	1.1 ± 0.7 a
	6	3.6 ± 0.6 a	Nd	Nd a	Nd a	1.2 ± 0.3 a
	10	53.9 ± 7.1 b	Nd	22.2 ± 2.5 b	5.8 ± 0.2 b	4.7 ± 0.4 b
37	2	21.1 ± 2.6a	Nd a	Nd a	Nd a	2.0 ± 0.6a
	6	126.7 ± 20.8b	5.2 ± 0.3b	100.5 ± 0.5 b	29.8 ± 0.2b	5.8 ± 0.5b
	10	266.7 ± 15.3c	19.6 ± 0.5b	397.0 ± 6.1c	29.9 ± 0.2b	6.8 ± 0.2c

Nd: not detected. The minimum level of detection was 1 ppm.

Table 2: *Histamine, other biogenic amines, and standard plate count during controlled storage of uninoculated tuna fish.*

Studies performed on sterile canned tuna by Ferencik showed that sterile uninoculated tuna fish samples produced no detectable histamine, even after 8 d of storage. Kan., *et al*. (2005) indicated that no histamine could be detected in 63 canned tuna fish samples in Japan. Still histamine can be detected in canned tuna products, as histamine might be formed in fish before processing. Similarly to this study findings, histamine was investigated in canned (290) fish samples of scombroid species of Oman using a high performance liquid chromatography with a fluorescence detector. Histamine was estimated with a detection rate of about 78.9% of canned fish and the mean histamine levels were 3.1 (Yesudhason., *et al*. 2013). A study in Iran reported histamine contents in 44% of canned tuna fish products (88 samples) was analyzed using ELISA method, had higher histamine contents than FDA caution level (Hosseini., *et al*. 2009). In Turkey, Er., *et al*. (2014) found that 10% of canned tuna fish samples (80 samples) had histamine levels of 200-400 mg/kg range. Quality manufacturing is required to prevent histamine contamination in canned fish.

Levels of histamine content in uninoculated tuna samples as it shown in table 2 rose to 53.9 ± 7.1 ppm at 25°C reaching health hazardous levels (>50 mg/kg; FDA, 2002) after 10 d storage. Histamine increased significantly ($p < 0.05$) with elevated temperature at 37°C to reach 126.7 ± 20.8, 266.7 ± 15.3 ppm after 6, and 10 d of storage, respectively. FDA (2011) reported that growth of histamine is more rapid at temperatures of 21.1°C or higher, than at 7.2°C, and growth is optimum at temperatures near 32.2°C. Histamine is the result of high temperature abuse spoilage than low temperature long time spoilage. Histamine levels increased in uninoculated samples and in the same time were associated with the increase in total bacterial count. Total bacterial count estimated to be 4.7 ± 0.4

logs CFU/g at 25°C after 10 d, and increased at 37°C to 5.8 ± 0.5, and 6.8 ± 0.2 logs CFU/g after 6, and 10d, respectively (table 2). The presence of natural histidine-decarboxylating microorganisms would most likely account for the formation and rise of histamine in the uninoculated samples. Bacterial growth and histamine formation in mackerel during storage at 25°C were monitored by Jiang., *et al*. (2013), results were agreed with this study. The highest level of histamine (2,080 mg/kg) was detected at 25°C after 2d of storage. The mixing of the bacteria in a product with high surface area such as minced tuna can result in elevated level of histamine formation if time and temperature abuse occurs (FDA, 2011). In this study, results showed that histamine amounts sufficient to cause histamine poisoning were detected in the tuna fish samples that were kept at room temperature such as 25°C for 10d, or at 37°C for 6, and 10d. Opened canned fish should be stored at 4°C or below and be consumed as soon as possible. It is also advised not to store/display the tuna at room temperature (25-37°C).

Morganella morganii inoculated tuna fish

Histamine levels in M. morganii-inoculated tuna samples were significantly higher ($P < 0.05$) than in the uninoculated samples as data were shown in table 3. The organism has been frequently isolated from fish implicated in scombrotoxicoses, and its ability to decarboxylate histidine to form histamine has been documented (Lorca., *et al*. 2001; Jiang., *et al*. 2013; Hongpattarakere., *et al*. 2016). Histamine increased passing health hazardous levels (>50 mg/kg; FDA, 2002) after 2 d at 25°C (50.3 ± 1.5 ppm) and at 37°C (228.7 ± 8.1), but it was slower at 4°C (table 3). This result might be attributed to bacterial generation time.

Temperature (°C)	Storage time (d)	Biogenic amines (ppm)				Standard plate count Log (CFU/g)
		Histamine	Tyramine	Cadverine	Tryptamine	
4	2	2.1 ± 0.7 a	4.0 ± 1.0 a	24.0 ± 5.3 a	Nd a	5.3 ± 0.2 a
	6	28.3 ± 62.5 b	42 ± 2.6 b	63.8 ± 5.4 b	21.3 ± 3.2 b	4.5 ± 0.7 b
	10	30.7 ± 90.0 b	60.7 ± 5.1 b	84.3 ± 3.8 c	21.2 ± 1.1 b	4.8 ± 0.7 b
25	2	50.3 ± 1.5 a	10.3 ± 1.5 a	58.3 ± 2.9 a	Nd a	5.7 ± 0.1 a
	6	440 ± 52.9 b	58.7 ± 3.2 b	307.7 ± 6.7 b	Nd a	8.1 ± 0.2 b
	10	1921.7 ± 27.5 c	2002.0 ± 7.2 c	2809.2 ± 25.0 c	31.9 ± 1.6 b	8.5 ± 0.2 b
37	2	228.7 ± 8.1 a	67.0 ± 2.6 a	51.7 ± 7.6 a	Nd a	6.5 ± 0.2 a
	6	421.7 ± 60.5 b	54.7 ± 4.5 a	365 ± 5.0 b	Nd a	8.7 ± 0.2 b
	10	746.7 ± 45.1 c	1006.3 ± 5.5 b	1013.3 ± 32.1 c	96.7 ± 2.1 b	9.6 ± 0.3 c

Nd: not detected. The minimum level of detection was 1 ppm.

Table 3: *Histamine, other biogenic amines, and standard plate count during controlled storage of tuna fish inoculated with Morganella morganii.*

The generation time for Morganella morganii in trypticase soy broth at 15, 25°C was rapid (2.6h, and 1.08h, respectively), but was greatly reduced when compared at the lower temperatures of 0–10°C (Klausen and Huss, 1987). At cold storage temperature (4°C), it did not exceed the safety limit after 10 d of storage (30.7 ± 90.0) (table 3). Similarly, a temperature of 4°C showed a bacteriostatic effect on the growth of *M. morganii*, with a negligible amount of histamine was detected after 2 weeks of incubation in tuna fish infusion broth medium (kim., *et al*. 2000). This effect is induced by decreasing metabolic activity, such as enzyme activity and membrane fluidity for transportation, in mesophilic bacteria at refrigeration temperature (Jay, 1996). Low storage temperature of tuna is considered as a safe level to control histamine produced by *M. morganii*. However, other reports conducted on the whole fish reported elevated levels of histamine when stored at 4°C, which might be due to other bacteria than *M. morganii. Mahusain., et al*. (2017) reported the formation of histamine at (4°C) gradually increased throughout the storage period. It exceeded the safety level in ungutted longtail tuna whole fish at

192 hrs (8d). A study conducted by Rossano., *et al.* (2006) on European anchovy, also estimated that histamine level increase with time of storage when stored at 4°C.

The highest level of histamine was found in the inoculated tuna samples with Morganella morganii stored for 10 d at 25°C (1921.7 ± 27.5 ppm). This high level of histamine was associated with the high aerobic bacterial count (APC) at 25°C (8.8 ± 0.2 log CFU/g) (table 3). It is likely the microorganism not only reached elevated numbers in the tuna samples incubated at 25°C but also that the amount and activity of histidine decarboxylase produced by *M. morganii* accounted for the observed high level of histamine. Increases in the histamine concentrations in tuna fish were similarly reported in other studies. During storage at 25°C, histamine reaching higher levels of 4176 mg/kg and 5307 mg/kg after only 3d and 7d, respectively (Nei, 2014). Histamine level > 4000 mg/kg was reported in tuna inoculated with M. morganii after 2 d storage at 25°C (Tao., *et al*. 2009).

Temperature of 25°C was reported to be associated with high microbial growth and histamine formation in tuna fish infusion broth medium inoculated with M. morganii (Kim., *et al.* 2000). At 25°C, the strain produced the highest histamine level, 5,253 ppm, after 48h of incubation. Morganella morganii was reported to produce histamine level up to 100 ppm inoculated in canned tuna at 7, 20, and 30°C after 17d, 1d, and 16h., respectively (Yamani., *et al*. 1981). And also, time taken of histamine production (1d, and 16h) in inoculated canned tuna associated with 5 logs CFU/g of M. morganii bacterial count was faster than this study. It produced histamine of 500 ppm after 3 d. at 20°C, and 19 h. at 30°C, respectively. It took 2d and 30h. to produce histamine up to 2000 ppm at 20, and 30°C, respectively.

The highest level of histamine at 37°C in this study was reported after 10d (746.7 ± 45.1 ppm), it is considered low compared with histamine production at 25°C (1921.7 ± 27.5 ppm). On the other hand, APC after 10d at 37°C (9.6 ± 0.3 log CFU/g) was higher than that at 25°C (8.5 ± 0.2 log CFU/g). The elevated APC at 37°C was not associated with higher levels of histamine, which might be due to presence of other spoilage microorganisms. However, the production rate at 37°C was faster, as it passed the safety level of 50 mg/kg after 2d (228.7 ± 8.1 ppm). Similarly, the histamine level produced from M. morganaii inoculated infusion broth medium fish at 37°C (1,949 ppm at 48h)was much lower than at 25°C, although the strain reached the stationary phase earlier than at 25°C (kim., *et al*. 2000). The highest level of histamine was reported in different fish by kim., *et al*. (2002) as 342 mg/100 g in mackerel; 301 mg/100 g in albacore; and 297 mg/100 g in mahi-mahi stored at 37°C for 24 h.

Proteus mirabilis inoculated tuna fish

Histamine production (296.7 ± 15.3 ppm) of tuna samples inoculated with Proteus mirabilis passed the safety limit (>50 mg/kg; FDA, 2002) after 10 d storage at 37°C. Storage tuna at 37°C was optimal for both microbial growth (9.2 ± 0.2 logs CFU/g) and histamine formation for P. mirabilis. In the rest of other storage times and at different temperatures was neglected or not detected (< 1 ppm estimated) as data were shown in table 4. P. mirabilis was identified as one of histamine producing bacteria in decomposing skipjack tuna, which produced 1340 mg/kg histamine at 38°C for 48h (Yoshinaga and Frank, 1982). Histamine level increased from 5.86 ± 3.64 ppm to 8.46 ± 2.46 ppm when canned tonggol tuna fish were exposed to temperature of 27.6–28.6°C for 1.5h. P. mirabilis was one of histamine producing bacteria associated with elevated histamine level (Hongpattarakere., *et al*. 2016).

Histamine produced by P. mirabilis in general was significantly less than that produced by M. morganaii under the same conditions, although there were no differences ($P > 0.05$) among bacterial count. Enterobactreiacae such as Morganella morganii, and Proteus spp. have been reported to be the most prolific histamine producers (Novak, 1998; Economou., *et al*. 2007; Hongpattarakere., *et al*. 2016; and Jiang., *et al*. 2013). However, according to the finding of this study, Morganella morganii was proven to be significantly ($p < 0.05$) more in histamine production than Proteus mirabilis under the same temperature and storage conditions.

Other Biogenic amines

Histamine is not the only amine responsible for scombroid fish poisoning as reported by Taylor in 1986. Histamine acting with other biogenic amines present in the fish to produce scombroid fish poisoning, primarily cadaverine. Histamine toxicity increased in the

presence of cadaverine and or tyramine by inhibiting diamine oxidase enzyme and histamine N-methyltransferase which are considered as histamine-detoxifying enzymes (Hui and Taylor, 1985; Stratton., *et al*. 1991).

The concentrations of the 3 other biogenic amines including tyramine, cadverine, and tryptamine present in tuna samples inoculated with M. morganii stored at 4, 25, and 37°C for 2, 6, and 10 d are given in table 3. The temperature of 25°C was the most optimum for the biogenic amine formation, as it was for histamine. Tyramine and cadaverine contents reached the maximum levels, 2002.0 ± 7.2, and 2809.2 ± 25.0 ppm, respectively after 10 d at 25°C. Insignificant levels of tryptamine produced in M. morganii inoculated samples when compared with other amines produced. Tryptamine levels were 21.2 ± 1.1, 31.9 ± 1.6, and 96.7 ± 2.1 ppm at 4, 25, and 37°C for 10d; otherwise it was estimated to be < 1ppm (table 3). These results indicated that histamine accumulation by M. morganii was accompanied by other biogenic amines, and all biogenic amines detected showed the highest level of accumulation at 25°C. Kim., *et al*. (2000) reported same trend of elevated cadaverine content, it reached the maximum level (26.7 ppm) at day 3 at 25°C in tuna infusion broth inoculated with M. morganii, while production tryptamine and tyramine was negligible. Cadaverine was reported as 147 ppm when yellowfin tuna fillet was stored at 22°C for 3d (Due., *et al*. 002).

Biogenic amines associated with tuna inoculated with P. mirabilis were detected with highest levels at 37°C after 10 d. of storage. Tyramine, cadaverine, and tryptamine levels were 94.3 ± 9.0, 400.0 ± 10.0, and 161.8 ± 3.7, respectively. This condition (37°C for 10 d) was also associated with the highest levels of histamine produced by P. mirabilis. Highest levels of Tyramine, cadaverine, tryptamine and histamine production were all associated with highest bacterial count of 9.2 ± 0.2 logs CFU/g (table 4). Elevated level of histamine and other amines in fish stored at 37°C might be due to the proliferation of mesophilic bacteria under optimum growth condition as compared to refrigeration storage (4°C). Emmanuelle., *et al*. (2012) found that strong correlation existed between the aerobic plate count and histamine production. In order to maintain the freshness of fish, proper storage is necessary. Low storage temperature can delay the formation of biogenic amines meanwhile high storage temperature provide accumulation of high levels of histamine and other synergistic biogenic amines which result in histamine fish poisoning.

Temperature (°C)	Storage time (d)	Biogenic amines (ppm)				Standard plate count Log (CFU/g)
		Histamine	Tyramine	Cadverine	Tryptamine	
4	2	Nd	Nd	Nd	Nd	4.1 ± 0.1 ab
	6	Nd	Nd	Nd	Nd	4.4 ± 0.2 b
	10	Nd	Nd	Nd	Nd	4.0 ± 0.1 a
25	2	Nd a	Nd a	Nd	Nd	5.6 ± 0.3 a
	6	Nd a	7.2 ± 0.2 b	Nd	Nd	7.6 ± 0.9 b
	10	8.5 ± 0.5b	55.7 ± 4.0 c	Nd	Nd	7.1 ± 0.3 b
37	2	Nd a	Nd a	Nd a	Nd a	6.4 ± 0.4 a
	6	9.8 ± 3.3 a	19.5 ± 1.4b	320.7 ± 4.0b	82.9 ± 6.2b	8.6 ± 0.1b
	10	296.7 ± 15.3b	94.3 ± 9.0c	400.0 ± 10.0b	161.8 ± 3.7c	9.2 ± 0.2c

Nd: not detected. The minimum level of detection was 1 ppm.

Table 4: *Histamine, other biogenic amines, and standard plate count during controlled storage of tuna fish inoculated with Proteus mirabilis.*

Conclusion

This study showed that high levels of histamine sufficient to cause histamine poisoning were detected in the uninoculated tuna fish samples that were kept at room temperature (25°C) for 10 d, or at 37°C for 6, and 10d. Opened canned fish should be stored at 4°C or below and be consumed as soon as possible. It is also advised not to store or display the tuna at room temperature (25-37°C). Histamine accumulation by M. morganii was accompanied by tyramine, cadaverine, and tryptamine detected showed the highest level of accumulation at 25°C. Storage at 37°C for 10 d was associated with the highest levels of histamine and other mines produced by P. mirabilis. In general, Histamine and biogenic amines produced by P. mirabilis was significantly less than that produced by M. morganaii under the same conditions, although there were no differences ($P > 0.05$) among bacterial count.

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