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Biogenic and volatile amine-related qualities of three popular fish species sold at Kuwait fish markets

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Abstract

Three fish species commonly consumed in Kuwait were studied for their total volatile base nitrogen (TVBN) and biogenic amine composition during normal commercial activities at the fish markets. Samples of the fish species Zubaidi or silver pomfret (*Pampus argenteus*), Hammour or orange–spotted grouper (*Epinephelus coioides*), and Negrule or grunt (*Pomadasys kaakan*) were analyzed using steam distillation and high performance liquid chromatography (HPLC) procedures. Fish samples collected in the morning had lower levels of TVBN, ranging from 25.9 mg/100 g to 30.2 mg/100 g compared with the range of 31.2 mg/100 g to 40.9 mg/100 g for samples collected in the evening. Among all three fish species, Zubaidi (*Pampus argenteus*) showed the lowest TVBN content while Negrule (*Pomadasys kaakan*) had the highest. Biogenic amine levels were also low in the fish species. The data suggest that fish handling practices at the fish markets in Kuwait do not result in significant abuse of fish temperatures, and that the amounts of volatile and biogenic amines produced during normal activities did not rise to levels indicating fish decomposition.

Keywords: Volatile amines; Biogenic amines; Histamine; Putrescine; Cadaverine; Trimethylamine

1. Introduction

Biogenic amines are non-volatile amines which are produced in fish and shellfish products after death. The class includes cadaverine, putrescine, spermidine, spermine, tyramine, tryptamine, and histamine, and these are reported to originate from the decarboxylation of specific free amino acids in fish and shellfish tissue (Rawles, Flick, & Martin, 1996). When held for excessive periods of time, the microflora of fish convert free amino acids to biogenic amines that produce characteristic symptoms in certain humans after fish consumption. Low levels of biogenic amines in food are not considered a serious risk. However, if the amount consumed is high enough, or normal pathways of amine catabolism are inhibited, various physiological effects, such as hypotension or hypertension, nausea, headache, rash, dizziness, cardiac palpitation and emesis, and even death may occur (Rawles et al., 1996). Biogenic amines are also considered precursors of carcinogens, such as *N*-nitrosamines, and they are also an indicator of food quality (Mietz & Karmas, 1977).

In view of the importance of biogenic amines for human health and food safety, it is important to monitor their levels in foodstuffs and beverages. Histamine is produced and metabolized by humans during normal metabolism. It is mainly found in foods which have been subjected to microbiological and biochemical deterioration due to processing, ripening or storage. Higher concentrations of biogenic amines may be found in foods containing protein produced in microbiotic processes, as well as in microbiotically deteriorated proteins, e.g. in fish. The presence of histamine is, therefore, a criterion when controlling the quality of fish. Secondary amines, such as putrescine and cadaverine, are good indices of spoilage of marine fish (Mietz & Karmas, 1977), and they can potentiate the toxicity of histamine and react with nitrites to form nitrosamines (Cinquina et al., 2004). Dawood, Karkalas, Roy, and Williams

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(1988) reported that the concentration of putrescine and cadaverine in the flesh of chilled-stored rainbow trout (*Salmo irideus*) exceeded 1.10 ppm by the second day of whole fish storage at 0 °C. These workers indicated that the determination of these two diamines could be used to assess fish freshness.

The volatile amines trimethylamine (TMA) and dimethylamine (DMA) have been widely used as indicators of freshness of marine fish (Castell, Smith, & Neal, 1971). The formation of trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVBN) has also been related to microbial growth, and several authors have described a positive relationship between the formation of these volatile amines and the formation of biogenic amines in different fish species (Baixas-Nogueras, Bover–Cid, Vidal–Carou, & Veciana–Nogues, 2001; Ruiz-Capillas & Moral, 2001).

Despite the fact that fish is an important source of good quality protein, vitamin, and minerals, its deterioration is very fast when enzymes are formed by bacterial growth which then generate biogenic amines, including histamine. Therefore, the quantification of amines in fish is used as a quality index, and can be used as an important tool for sanitary surveillance (Moreno & Torres, 2001).

Factors involved in the formation of histamine have been widely studied in several different fish species. Tuna and other fish from the families Scomberesocidae and Scombridae, and a non-scombroid fish, mahi-mahi or dolphin, are known to contain high levels of histamine when spoiled (Rawles et al., 1996). A good correlation has been found in Spanish mackerel (*Scomberomorus maculatus*) between the levels of histamine, cadaverine, and putrescine and the time and temperature of decomposition (Middlebrooks, Toom, Douglas, Harrison, & McDowell, 1988). Several studies, conducted to ascertain histamine contents of foods, have also concluded that temperature and time of storage influence microbial growth and hence the spoilage of fish products (Baixas-Nogueras et al., 2001; Flick, Oria, & Douglas, 2001; López-Sabater, Rodrıguez-Jerez, Hernández-Herrero, & Mora-Ventura, 1996).

Information on the incidents of scombroid fish poisoning due to amine formation in fish available from commercial outlets in Kuwait remains scarce. The objective of this study was to investigate the formation of biogenic and volatile amines in fish sold commercially on the Kuwait fish markets, and the effect of amine formation on fish quality.

2. Materials and methods

2.1. Materials

Standard amines, including histamine dihydrochloride, cadaverine dihydrochloride, putrescine dihydrochloride, and trimethylamine hydrochloride, were purchased from Sigma-Aldrich (Buchs, Switzerland). Other reagents were obtained from Sigma-Aldrich or VWR Scientific (Buffalo Grove, IL). Deionized water was produced from the Millipore Milli-Q water purification system.

2.2. Methods

2.2.1. Fish sample collection

Three fish species commonly consumed in Kuwait – Zubaidi or silver pomfret (*Pampus argenteus*), Hammour or orange–spotted grouper (*Epinephelus coioides*), and Negrule or grunt (*Pomadasys kaakan*) – were used in this study. Representative samples of the fish species are shown in Fig. 1. A total of 54 samples of the three fish species were purchased from one fish market over three non-consecutive days in a one month period during normal commercial activity at intervals of 4 h, namely 8 a.m., 12 noon and 4 p.m. Samples were cleaned and degutted at the fish market and were immediately transported under ice to the laboratory.

At the laboratory, the skin of fish samples was removed and muscle portions were carefully excised and ground with a mixer grinder model RI 112 (Rockwell, Mumbai, India) to produce a sufficiently homogeneous fish mince. Ground fish samples were placed in individual quart size ziploc bags and frozen for analysis within a forty-eight hour period.

2.2.2. Total volatile basic nitrogen (TVBN)

The total volatile basic nitrogen (TVBN) content of fish samples was determined in boric acid solution (1%, w/v) after steam distillation. Approximately 10 g of the ground fish sample were mixed with 90 ml of 0.6 M perchloric acid solution, homogenized for 2 min (Yellowline homogenizer model DI 25 Basic, Ika-Werke, Staufen, Germany) at a speed of 10,000*g* and then filtered through a No. 2 Whatman filter paper. Fifty millilitres of the extract were subjected to steam distillation, following alkalinization with 0.2 M NaOH. The distillate was collected in 100 ml of 1% (w/v) boric acid solution containing Tashiro's indicator. The volatile base nitrogen contained in the distillate solution was determined by titration with 0.01 N HCl and calculated from the formula:

$$\text{TVBN(mg/100 gsample)} = (V_1 - V_0) \times 0.14 \times 2 \times 100/\text{W}$$

where

V_0 volume of hydrochloric acid used in blank titration,

V_1 volume of hydrochloric acid used in sample titration,

W weight of fish sample in grams.

All analyses were performed in duplicate.

2.2.3. Biogenic (non-volatile) amines

2.2.3.1. Preparation and derivatization of standard amine solution. Stock solution of standard biogenic amines was prepared by dissolving histamine dihydrochloride

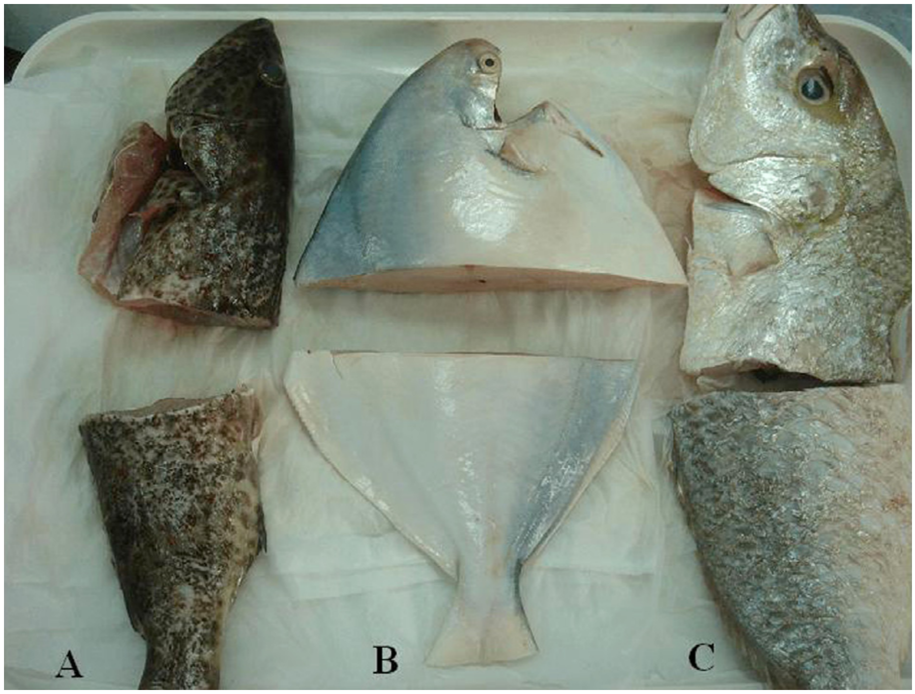

Fig. 1. Representative samples of fish species collected at the fish market and used in the study. (*A*) Hammour, (*B*) Zubaidi, and (*C*) Negrule.

(165.7 mg), cadaverine dihydrochloride (171.4 mg), putrescine dihydrochloride (182.9 mg), and trimethylamine hydrochloride (161.7 mg) in 10 ml of deionized water to obtain a final concentration of 10 mg/ml free amine of each standard (Yen & Hsieh, 1991). Derivatization to convert standard amines into hydrophobic compounds was achieved using the procedure described by Yen and Hsieh (1991). The benzoyl derivatives of the amines were prepared by adding 1 ml of 2 M NaOH to 50 μl of the standard amine solution, followed by 10 μl of benzoyl chloride. The mixture was mixed on a vortex mixer and allowed to stand for 20 min at room temperature. Saturated sodium chloride solution (2 ml) was added to stop benzoylation, followed by extraction of amines with 4 ml of diethyl ether. The mixture was centrifuged at 10,000*g* for 10 min at 4 °C, after which the upper organic layer was transferred into a tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500 μl of methanol and 20 μl aliquots were injected for HPLC analysis.

2.2.3.2. Extraction and derivatization of fish amines. Five grams of each ground fish sample were homogenized for 3 min with 20 ml of 6% (w/v) trichloroacetic acid (TCA) in a Yellowline homogenizer model DI 25 Basic (Ika-Werke, Staufen, Germany) at 10,000*g*. The homogenate was centrifuged (Sigma Laboratory Centrifuge, Model 3K30, Germany) at 10,000*g* for 10 min at 4 °C and then filtered through Whatman No. 2 filter paper. The filtrate was placed in a volumetric flask and enough 6% TCA was added to make up to a total volume of 25 ml. Two ml from each extract were derivatized with benzoyl chloride, as previously described in preparation for HPLC.

2.2.3.3. Separation and quantification of biogenic amines by HPLC. The levels of putrescine (PUT), cadaverine (CAD), histamine (HIS), and trimethylamine (TMA) were determined by high performance liquid chromatography procedures. The HPLC system consisted of a Waters Model 600 Liquid chromatograph, consisting of a pump, gradient former, and an integrator. A Photodiode Array Detector Model 996 was used at a wavelength of 254 nm, and a Symmetry C18 reverse-phase column (4.6 × 250 mm), model WAT 054275, (Waters Corporation, Dublin, Ireland) was used for separation.

The gradient elution profile was water:acetonitrile in which acetonitrile was increased from 40% to 80% in 20 min at a flow rate of 1.0 ml/min. The acetonitrile was then increased to 100% in 5 min, and finally decreased to 40% in 5 min for a total run time of 30 min.

To identify peaks, the solution of standard amines was analyzed using the same programme, and the retention times and spectra of standards and samples were compared.

2.3. Statistical analysis

Statistical analysis of the data was performed with SYSTAT 11 (SYSTAT Software, Inc. Richmond, CA, USA). The data were subjected to analysis of variance (ANOVA) to study the variation in the means of the analyzed amines as a function of time of fish collection.

Significant differences among means was determined using Tukey's pairwise comparison test at the 5% level.

3. Results and discussion

3.1. Volatile amines

The levels of total volatile basic nitrogen (TVBN) in the three raw fish species studied are shown in Table 1. TVBN levels in all fish species increased throughout the day during normal commercial activity on each day that sampling was done. Fish samples collected in the morning had lower levels of TVBN, ranging from 25.9 ± 0.20 mg/100 g to 30.2 ± 0.58 mg/100 g for all three species compared with the range of 31.2 ± 0.39 mg/100 g to 40.9 ± 0.19 mg/100 g for samples collected in the evening. Among all three fish species, Zubaidi (*Pampus argenteus*) showed the lowest TVBN content, ranging from 25.9 ± 0.20 mg/100 g to 31.2 ± 0.39 mg/100 g, while Negrule (*Pomadasys kaakan*) had the highest TVBN (33.0 ± 0.79 mg/100 g to 40.9 ± 0.19 mg/100 g) at all three sampling times on each sampling day.

The significant increases ($p \leqslant 0.05$) in TVBN levels during the day may be due to handling practices, resulting in bacterial growth. Hernández-Herrero, Roig-Sagués, López-Sabater, Rodríguez-Jerez, and Mora-Ventura (1999) reported a significant increase in TBVN levels during the ripening of salted anchovies and attributed such increases to bacterial and enzymatic action.

With the exception of Zubaidi samples collected at 8 a.m. and 12 p.m., which showed TVBN levels of 25.9 ± 0.20 mg/100 g and 28.7 ± 0.59 mg/100 g, respectively, all other samples showed TVBN levels close to or higher than 30 mg/100 g, the level legally permitted by some organizations for raw fish (Periago, Rodrigo, Ros, Rodriguez-Jerez, & Hernandez-Herrero, 2003). The levels found also suggest that, apart from Zubaidi fish collected in the morning, all other samples collected might be at the initial stage of decomposition, since TVBN values were greater than 25.0 mg/100 g. Tsai, Kung, Lee, Lin, and Hwang (2004) reported middle freshness quality in sailfish fillets found to contain levels of TVBN between 4.6 to 25.0 mg/100 g, and an initial decomposition level for sailfish samples with TVBN values between 25.0 and 36.5 mg/100 g. Similarly, an average concentration of 22.9 mg/100 g TVBN was obtained in fresh albacore specimens which were consequently determined to be of high quality (Ben–Gigirey, De Sousa, Villa, & Barros–Velazquez, 1998).

The levels of TVBN values found in all the fish samples were generally high compared to those found in some fresh fish species. Periago et al. (2003) found the TVBN values in fresh tuna roe to be 6.25 ± 0.10 mg/100 g, while values ranging from 3.79 ± 0.30 mg/100 g to 13.7 ± 0.46 mg/100 g have been reported for fresh anchovies (Pons-Sánchez-Cascado, Veciana-Nogués, Bover-Cid, Mariné-Font, & Vidal-Carou, 2005). The higher values of TVBN in fish species observed in the current study compared to the reported safe level in other fresh fish species may, therefore, be indicative of the initial microbial load present in the fish species. The practice usually adopted by fish sellers at the Kuwait fish market involves keeping the fish catch that arrives from sea late in the evening and not sold before the end of the day under ice until the following morning when commercial activities begin. During normal commercial activity, fishes are displayed on concrete benches under ice and covered with ice shavings which are changed periodically by the vendors. It therefore, may appear, as indicated by the levels of TVBN, that samples collected in the morning were not fresh but were from the previous day's catch which was kept under ice. If not properly handled, left-over fish from a previous day's commercial activity may develop enough of a bacterial population to initiate decomposition before the fish is stored under ice for marketing the following day. However, visual observation of all the fish species collected for the study did not show any obvious signs normally associated with fish decomposition.

Various components contribute to the spoiling parameter of fish. First is the microbiological degradation of trimethyl amine oxide (TMAO), which serves to regulate the osmotic pressure in the fish, to trimethylamine (TMA) and ammonia. Further degradation products, collectively termed biogenic amines, include histamine, putrescine and cadaverine, are produced.

Table 1
Levels of total volatile basic nitrogen (TVBN) found in raw Hammour, Negrule, and Zubaidi fish species[A]

Sampling time	No. of samples[B]	TVBN (mg/100 g)		
		Hammour	Negrule	Zubaidi
8 a.m.	18	30.2 ± 0.58^{b}	33.0 ± 0.79^{b}	25.9 ± 0.20^{c}
12 p.m.	18	30.9 ± 0.69^{b}	39.9 ± 0.20^{a}	28.7 ± 0.59^{b}
4 p.m.	18	34.9 ± 0.29^{a}	40.9 ± 0.19^{a}	31.2 ± 0.39^{a}

Means in the same column followed by different superscripts are significantly different ($p \leqslant 0.05$).
[A] Values are means ± standard deviations of samples taken over three non-consecutive day period.
[B] Total number of fish samples collected at each time over the three-day sampling period.

3.2. Biogenic (non-volatile) amines

Fig. 2 shows the HPLC profiles of the standard amines. In general, all the standard amines were separated in 20 min of run time with good peak resolution, sharpness, and symmetry.

The amine levels found in all the fish species are summarized in Table 2. Biogenic amines were found in low or moderate concentrations in all fish species throughout the day. Most of the fish samples taken in the morning did not show any detectable biogenic amine content. Histamine, putrescine, and cadaverine were not detected in Hammour samples collected in the morning or in the afternoon, while values of 3.3 ± 1.2 mg/100 g, 5.2 ± 0.9 mg/

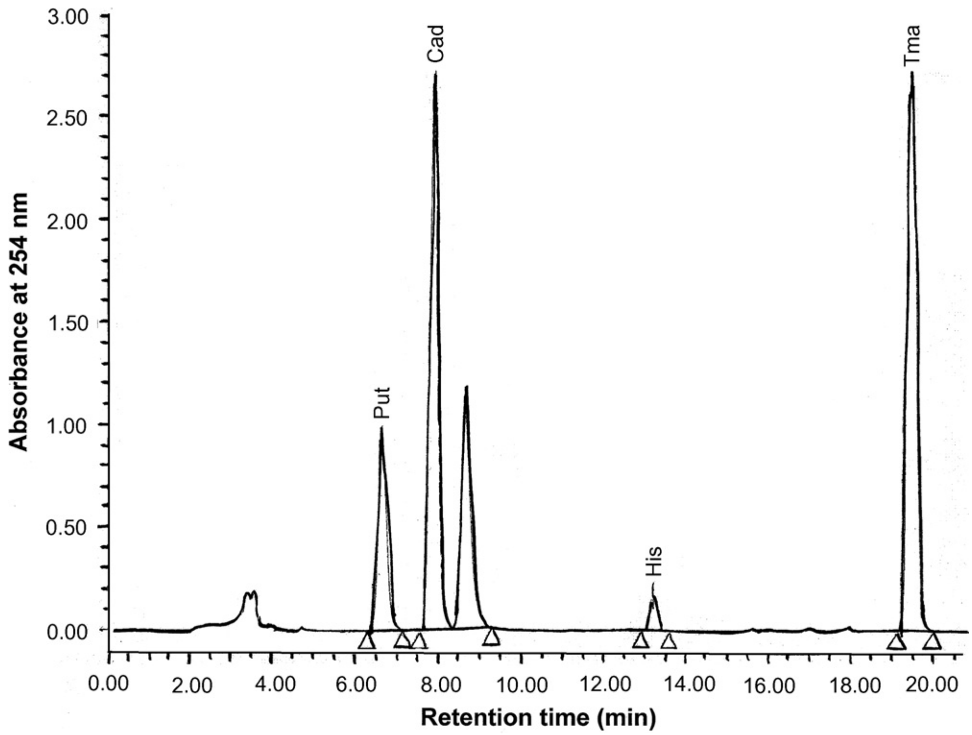

Fig. 2. Chromatographic separation of benzoyl derivatives of standard biogenic amines and trimethylamine.

100 g, and 3.5 ± 0.7 mg/100 g, respectively, were detected in samples collected in the evening. No histamine was detectable in Zubaidi fish samples collected in the morning and afternoon, but 10.4 ± 0.3 mg/100 g was determined in the evening samples. On the other hand, putrescine and cadaverine were present at detectable levels in Zubaidi at all times during the day. Negrule did not show the presence of any biogenic amine with the exception of samples collected at 12 p.m. and 4 p.m., which showed low levels of histamine at 12.3 ± 1.9 mg/100 g and 12.9 ± 0.3 mg/100 g, respectively. Putrescine and cadaverine were also present in moderate amounts in samples collected in the evening.

Similar findings were reported in a study by Ben-Gigirey et al. (1998), in which cadaverine and histamine did not increase significantly, even after 9 months of frozen storage. Staruszkiewicz et al. (2004) found no significant increases in histamine levels in samples of fresh fish held for short incubation periods, and neither were significant increases in cadaverine or putrescine observed. Moreover, histamine, the causative agent for fish poisoning, is not present in the flesh of fish when it is caught, but occurs when histamine-producing bacteria decarboxylate histidine to histamine during the spoilage process (Klausen & Huss, 1987; Morrow, Margoiles, Rowland, & Roberts, 1991). The data in the current study, therefore, indicate that histamine-producing bacteria were not present, or present at very low levels in the fish samples, and that the fish samples collected were generally of average hygienic quality with respect to histamine production, since all showed levels of histamine and other biogenic amines below the recommended hazard level of 50 mg/100 g (Fletcher, Summers, & Van Veghel, 1998; Hwang, Chang, Shiau, & Cheng, 1995).

The predominant amine found in all fish species throughout all sampling days and times was the volatile amine trimethylamine (TMA). The levels of TMA increased in all fish samples throughout the day, with the greatest increase occurring in Zubaidi, which increased from 11.3 ± 0.1 mg/100 g in the morning to 17.3 ± 2.5 mg/100 g in the evening, an increase of 53%. With the exception of Hammour samples collected in the evening, TMA values were generally near the maximum of 15 mg/100 g permitted for raw fish (Periago et al., 2003).

TMA is the most commonly used volatile amine in the fish industry for evaluating freshness and spoilage in marine fish, since it is produced during chilled storage of fish from bacterial utilization of trimethylamine oxide (TMAO), a naturally occurring osmoregulatory substance found in most marine fish species (Koutsoumanis, Lampropoulou, & Nychas, 1999). The amount of TMA produced is, therefore, a measure of the activity of spoilage bacteria in the fish and so is an indicator of the degree of spoilage. The absence (or extremely low levels) of production of TMA in some fish species during storage has been reported (Koutsoumanis et al., 1999), and was attributed to the fact that *S. putrefaciens* population never reached levels of 10^8–10^9 CFU/g, a population considered crucial to the formation of TMA (Dalgaard, Gram, & Huss, 1993).

Table 2
Levels of biogenic amines and trimethylamine in raw Hammour, Negrule, and Zubaidi

Time	No. of Samples[A]	Amine concentration (mg/100 g)											
		Hammour				Negrule				Zubaidi			
		HIS	PUT	CAD	TMA	HIS	PUT	CAD	TMA	HIS	PUT	CAD	TMA
8 a.m.	18	nd	nd	nd	16.3 ± 0.6^b	nd	nd	nd	12.2 ± 1.6^c	nd	11.2 ± 0.8^b	22.6 ± 0.8^c	11.3 ± 0.1^c
12 p.m.	18	nd	nd	nd	17.5 ± 0.4^b	12.3 ± 1.9^a	nd	nd	15.4 ± 0.4^b	nd	12.9 ± 0.2^{ab}	25.9 ± 0.3^b	13.4 ± 0.3^b
4 p.m.	18	3.3 ± 1.2	5.2 ± 0.9	3.5 ± 0.7	19.6 ± 2.3^a	12.9 ± 0.3^a	7.5 ± 1.8	7.9 ± 0.2	17.7 ± 1.0^a	10.4 ± 0.3	13.5 ± 1.9^a	29.9 ± 1.2^a	17.3 ± 1.5^a

HIS – Histamine; PUT – Putrescine; CAD – Cadaverine; TMA – Trimethylamine.
Values are mean ± standard deviation of samples taken over three non-consecutive days.
Means in the same column followed by different letters are significantly different ($p \leqslant 0.05$).
nd: Not detected (amine level is less than 0.1 mg/100 g).
[A] Number of samples for each fish specie collected over three-day period.

4. Conclusion

Biogenic and volatile amine levels determined in three commonly consumed fish species in Kuwait were low compared to maximum values recommended by some international organizations. In all the fish species studied, total volatile base nitrogen (TVBN) content of fish species increased during the day, possibly as a result of handling practices in the fish market. However, the levels of TVBN found were mostly at the border of the levels legally permitted for raw fish. On the other hand, the biogenic amines histidine, which is mainly responsible for scombroid fish poisoning, putrescine, and cadaverine were found in low concentrations in all the fish species studied, indicating an average or good hygienic quality. Most of the fish samples taken in the morning did not show any detectable biogenic amine content, and that microbial activity in the fish species during the day did not rise to the levels necessary to decompose the fish samples to render them unsafe for human consumption.

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