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ORIGINAL ARTICLE

Changes on biogenic, volatile amines and microbial quality of the blue swimmer crab (*Portunus pelagicus*) muscle during storage

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Abstract Biogenic amines (BAs) are a group of substances with low molecular weight organic compounds such as aliphatic, aromatic or heterocyclic structures that are naturally present in animal tissues. The aim of this study was to investigate the changes on the formation of biogenic amine, bacterial load and biochemical characteristics in blue swimmer crab (*Portunus pelagicus*) at different storage temperatures (4 and 20 °C) up to 96 h. From seven BAs only four biogenic amines (tryptamine, putrescine, histamine, and tyramine) were detected while, the cadaverine, spermidine and spermine were absent in all investigated samples. Histamine was the major biogenic amine formed during the storage times and reached the highest concentration of 7.55 ± 0.46 mg/100 g and 17.68 ± 1.30 mg/100 g after 96 h at 4 and 20 °C, respectively. This level of histamine exceeded the maximum tolerance level of 5 mg/100 g. However, the proper icing procedure retarded the histamine effects, resulting only 7.55 mg/100 g after 96 h of ice storage. Spoilage indicator putrescine was only detected after 24–96 h of storage at 4 and 20 °C, respectively. The total volatile base nitrogen and the trimethylamine-nitrogen concentrations were considered to be reliable indicators of freshness index in blue swimmer crab. An aerobic mesophilic plate count of 6.68 and 7.31 log CFU/g were noted for crab stored in ice and ambient temperature after 96 h storage, respectively. It could be concluded that the biogenic amine forming bacteria and other susceptible perishing factors responsible for the biogenic amine formation could be prevented by continuous storage of *P. pelagicus* at low temperature.

Keywords Histamine · *Portunus pelagicus* · TMA-N · TVB-N · Tyramine

Introduction

There is strong evidence that consumption of fish containing high levels of amino acids and fatty acids is favorable for human health. The protein presented in shell fish has a great biological value with high conversion ratio giving nutritional benefits to humans. The protein content was reported to be high in hard shell crab (32.6%) than those with soft crab (17.17%) of *P. sanguinolentus* (Sudhakar et al. 2009). Since food safety is a key factor for the seafood processing industry, the ingredients and substances present in foods may be harmful to human health that should be considered precise (Yongjin et al. 2009; Bita et al. 2013).

Blue swimmer crab, *Portunus pelagicus* Linneaus, 1758 belongs to Portunidae family, also known as flower and sand crab is known for its culinary value. The production is expanding rapidly Indo-Pacific to meet domestic and export demands, from 156,628 tons in 2000 to 212,571 tons in 2014 (FAO 2016).

Biogenic amines (BAs) are low molecular weight organic bases with biological activity that are formed in food by microbial decarboxylase of the corresponding

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amino acid and/or transamination of aldehydes and ketones by amino acid transaminases (Zhai et al. 2012). Moreover, biogenic amine might be produced following post mortem changes through the decarboxylation of species free amine acids either by autolysis and enzymatic changes (Ruiz-Capillas and Moral 2002). Histamine, putrescine, cadaverine, tyramine, tryptamine, spermine and spermidine are considered to be the most common biogenic amines found in seafood associated with food spoilage (Lehane and Olley 2000).

From a microbiological point of view, different genera are contributory in toxicity, such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, *Photobacterium* and the *Lactic acid bacteria* (*Lactobacillus*, *Pediococcus*, and *Streptococcus*). These bacteria are able to generating hazardous amounts of BAs chiefly histamine in a very short period of time when the fishes are kept at elevated temperatures (Omura et al. 1978).

The main clinical manifestations include rash, oedema and localized inflation of the skin, as well as headache, heart palpitations, nausea and diarrhoea in human (Lehane and Olley 2000). Putrescine, cadaverine and tyramine have the potential to cause illness, even in the absence of histamine, whilst tyramine is reported to induce migraine, increase blood pressure and hypertension (Shalaby 1996). Since biogenic amines might affect the quality and safety of fresh fish, its permissible amount of the products should be regulated. Recently, the FDA has issued industry guidelines aiming at establishing procedures for the safe processing and importing of fish and fishery products based on the hazard analysis and critical control points (HACCP) approach (FDA 2011). A total biogenic amines level of 10 mg/100 g in foodstuffs was considered as dangerous to human beings and animal health (Shalaby 1996). Additionally, recent studies accounted for the biogenic amine formation in various temperature storage of fishes including Indian mackerel (Abu Bakar et al. 2014), mackerel (Jiang et al. 2013), Orange spotter grouper (Bita et al. 2013), Tiger toothed croaker (Moini et al. 2012), carangid fish (Arulkumar and Paramasivam 2014), deep queen fish (Arulkumar and Paramasivam 2015), milk fish and Indian whiting (Arulkumar et al. 2017) and Chinese mitten crab (Kim et al. 2009a, b). The United States Food and Drug Administration (USFDA) has set 5 mg/100 g of histamine in fish and other fishery products as decomposition level and 50 mg/100 g as a hazard action level (USFDA 2001). Mietz and Karmas (1977); Bakar et al. (2010) claimed a strong dependence of histamine content upon the fish species, and accordingly proposed a biogenic amine index (BAI), calculated as (C putrescine + C cadaverine + C histamine + C tyramine) where C denotes concentration expressed in mg kg^{-1}; they suggested that the biogenic amine index (BAI) values exceeding 10 are an indication of excessive quality loss, and said statement was corroborated by Mendes (1999) after examining histamine formation in sardines and mackerel. Krizek et al. (2004) have suggested that putrescine and cadaverine and the sum of both amines are useful quality indicators for common carp flesh, In addition, putrescine values lower than 10 mg/kg can represent the good quality of the common carp flesh, 10–20 mg/kg as acceptable quality and the value over than 20 can indicate the poor quality established on sensory evaluation (Hernandez et al. 2009). Moreover, high levels of putrescine and cadaverine appear to potentiate the toxicity of histamine, as well as tyramine (Taylor 1985). Although a reliable dose–response data are not available pertaining to human consumption, the limited number of animal studies published so far have suggested an oral toxicity of 180 mg/kg body weight/day in Wistar rats (EFSA 2011).

Marine fishes are highly susceptible to rapid spoilage at ambient temperature. Moreover, preservation in ice is one of the most efficient way of retarding seafood spoilage (Abu Bakar et al. 2014). The rate of deterioration during ice storage of fish varies with species and depends on the concentrations of substrates and metabolites in the tissue, microbial contamination and conditions of storage after catch (Pacheco-Aguilar et al. 2000). In this respect, the formation of biogenic amines, as well as volatile base nitrogen (TVB-N) compounds, lipid oxidation, bacterial concentration/type and ATP hydrolysis could be an indicator of spoilage in fish and shell fishes having economic and health implications (Ozogul and Ozogul 2006).

The blue swimmer crab is usually marketed in fresh form, stored in ice, and therefore it is important to determine the amount of biogenic amines in that muscle and leg meat to predict its quality during different time of consumptions. However, this work intends to fill the gap of knowledge about freshness, quality and post-mortem biochemical changes in blue swimmer crab muscle during storage. Hence, the present study was undertaken on the formation of biogenic amines in store meat of blue swimmer crab (*P. pelagicus*) at different temperatures (4 and 20 °C) in order to ensure the quality and safety of crab products.

Materials and methods

Sample collection and storage

Blue swimmer crabs (*P. pelagicus*) were collected from Thondi fish landing center (Latitude: 9° 44′10.69″ N and

Longitude: 79° 01′ 11.83E) Tamil Nadu, situated in the Southeast coast of India in the month of March, 2016. The crab was transported to the laboratory in an ice box under ice condition after 5 h of capture with the ration of crab to ice was approximately 1:3 (w/w). Completely fresh crabs (weight of 67.4–82.5 g) were divided into two lots (each lot contained 50 crabs). The first lot was stored at ambient temperature (20 °C) for 24–96 h. The second lot was stored in an ice cooler box. Water in cooler box was drained off and the ice was replaced to maintain the 4 °C temperature. The crab shells were removed and muscles were extracted from the cavities of the crab body using the tweezers. The obtained muscles sample was taken for analysis for 24, 48, 72 and 96 h during ambient and each 4 days during ice storage. Each experiment was performed in triplicate.

Preparation of standard amine solution

The standards (tryptamine hydrochloride, putrescine hydrochloride, cadaverine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, histamine dihydrochloride, tyramine hydrochloride obtained from Sigma Aldrich St. Louis, MO, USA) were dissolved in 50 ml of a 0.1 M hydrochloric acid and used as the working solution. The concentration of standard solution 1.0 mg/ml for each amine was prepared according to Eerola et al. (1993). Other HPLC grade chemicals were obtained from Merck, Germany.

Sample preparation for biogenic amine analysis

Crab muscle samples were ground in a warring blender for 3 min, five grams of resultant samples were transferred to a 50 ml centrifuge tube. The homogenization was made with 20 ml of 6% trichloro acetic acid (TCA) for 3 min. The collected samples were centrifuged at 10,000 rpm for 10 min at 4 °C then were passed through Whatman filter paper No.2 (Whatman, UK). The obtained filtrates were then placed in volumetric flasks, and TCA was added to bring to a final volume of 50 ml. One ml aliquot of extracted crab sample, 0.2 ml of 2.0 M sodium hydroxide and 0.3 ml of saturated sodium bicarbonate were transferred into the test tube. The solution was added to 2.0 ml of (1.0 g) dansyl chloride solution and dissolved in 100 ml of acetone by using a vortex mixture, and allowed to stand at 40 °C for 45 min. After the reaction, 0.1 ml (25%) ammonia solution was mixed and allowed to stand for 30 min. Acetonitrile was added to make up a final volume of 5 ml and the solution was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was filtrated through a 0.45 μm filter, and then used for HPLC analysis.

Determination of biogenic amines

The level of biogenic amines were determined using a Hitachi Liquid Chromatography (Hitachi, Tokyo, Japan) consisting of model L-7100 pump, a Rheodyne model 7125 syringe loading sample injector and a 2500 chromato-integrator. A Lichrospher 100 RP- 18 reversed phase column (5 μm, 125 × 4.6 mm, Merck, Damstadt, an elution programe began in 50:50 V/V) acetonitrile: water at a flow rate of 1.0 ml/min for 19 min was used followed by a linear increase in medium to 90:10 acetonitrile: water (1.0 ml/min) during the next 1.0 min. The acetonitrile: water mix decreased to 50:50 (1.0 ml/min) for 10 min.

Biochemical analysis

pH

Ten grams of crab muscle samples were homogenized in sterile blender with 10 ml of deionized water to make a thick slurry. The pH of this slurry was measured using a pH meter (Cyberscan, Eutech, Malaysia).

Total volatile base nitrogen (TVB-N) and trimethylamine-nitrogen (TMA-N)

TVB-N and TMA-N were determined using Conway's micro diffusion unit (Siang and Kim 1992). Two grams of the minced crab muscle was mixed with 8 ml of 4% TCA in a 50 ml centrifuge tube and was vortexed for 10 min then was left for 30 min at room temperature with occasional mixing. The sample was centrifuged at 1000 rpm for 10 min. The supernatant was filtered through a filter paper (Whatman No.1) for the analysis. TVB-N was released with the addition of saturated K_2CO_3 and was absorbed by a boric acid solution and the titration was done with 0.02 N HCl. The TVB-N content was calculated and expressed as mg/100 g of crab muscle.

The level of trimethylamine in a crab muscle sample was determined by the Conway technique, which is same as TVB-N determination. One ml of 10% potassium carbonate was added and 1 ml neutralized formalin was pipetted into the extract to react with ammonia and thereby allowing only the TMA-N to diffuse over the unit. The TMA-N content was calculated and expressed as mg/100 g of crab muscle.

Microbiological analysis

Twenty-five grams of crab muscle from each replicate of shell removed sample of three was transferred aseptically to 225 ml of sterile peptone water (0.1% peptone water and 0.85% saline). The resultant was blended in sterile

stomacher bags for 60 s Rodtong et al. (2005). The homogenated sample was serially diluted with 9 ml peptone water and 0.1 ml were spread on aerobic plate count agar (APC) for enumeration of total viable count. The incubation of plate was done at 37 °C for 48 h, then bacterial colonies were counted. Histamine, cadaverine and putrescine forming bacteria were detected using modified Niven's media supplemented with 0.25% L-histidine, L-ornithine, L-lysine according to Joosten and Northolt (1989). Bacterial colonies showing that the purple halo or slight purple color were counted as biogenic amines forming bacteria at 37 °C for 48 h.

Statistical analysis

The effect of storage time on biogenic amine contents were analyzed using one-way analysis of variances (ANOVA). The mean was compared using Duncan's multiple range tests. The significant difference defined at $P < 0.05$ performed using the OriginPro 8 version (OriginLab Corporation, Northampton, MA, USA). The mean was compared in triplicate for biochemical analysis and in duplicate for microbial analysis.

Results and discussion

Changes in biogenic amine content

Biogenic amine index (BAI) has been calculated using the equation as mentioned in materials and methods. Figure 1a, b shows the BAI in each storage period (4 and 20 °C) for crab. The obtained results revealed an increase pattern similar to tryptamine and tyramine, as a dominated formed biogenic amines during this present study. The 4 and 20 °C of storage temperature showed maximum BAI index (30.93 and 259.1) respectively. Bakar et al. (2010) reported increased BAI in association with the length of storage as shown in *Lates calcarifer*, and is in agreement with the present study. Similarly, Bita et al. (2013) reported that, the BAI was 98.25 founded in *Epinephelus coioides* during storage periods.

Biogenic amine content was increased twofold during storage time at 4 and 20 °C as shown in Fig. 1a, b. Histamine was the major biogenic amine formed, followed by tryptamine, putrescine, tyramine. Tryptamine was present at the highest levels of 11.15 ± 0.79 mg/100 g after 96 h at 4 °C while, tryptamine was recorded at level 105 ± 0.61 mg/100 g after 96 h at 20 °C. The obtained showed in Fig. 1a, b, elevated levels of biogenic amines at the different storage times. Similarly, Kim et al. (2009a, b) reported a level of 0.927 mg/100 g and 0.896 mg/100 g tryptamine in chinese mitten crab muscle during 4 and

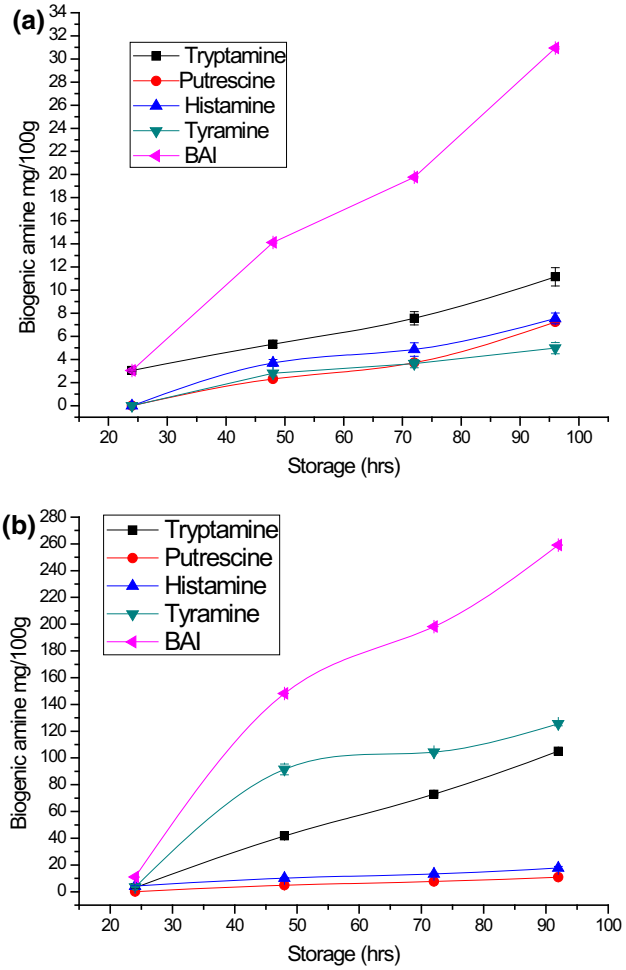

Fig. 1 **a** Biogenic amines content of blue swimmer crab *P. pelagicus* stored at 4 °C. Data are expressed as mean ± SD. **b** Biogenic amines content of blue swimmer crab *P. pelagicus* stored at 20 °C. Data are expressed as mean ± SD

20 °C at 72 and 24 h of storage respectively, which was comparatively lower than the results of the present study. Hu et al. (2012) reported that, the level of tryptamine was 0.59 mg/100 g in saury and 0.37 mg/100 g in blue scad muscle during 4 °C. Kim et al. (2009a, b) reported similar increases of maximum levels of tryptamine (0.89 mg/100 g) from the swimming crab (*Portunus trituberculatus*) at 20 °C in the storage time of 24 h.

In this study, the initial concentration of putrescine in crab was reached maximum levels 7.25 ± 0.92 mg/100 g at 4 °C for 96 h and 10.82 ± 0.23 mg/100 g at 96 h of storage at 20 °C respectively, due to the increased bacterial load 6.7 log CFU/g (Fig. 3a, b). Putrescine is mainly produced by bacterial decarboxylation of ornithine (Ozogul et al. 2006). It has been documented that the type and concentration of biogenic amines formed will depend on the biogenic amine formed bacteria and their relative abundances (Rezaei et al. 2007). Although putrescine have

no adverse effects on human health, they play an important role in seafoods poisoning as they can potentiate the toxicity of histamine by inhibiting histamine metabolising enzymes of diamine oxidase and histamine methyl transferase (Lehane and Olley 2000). The threshold limits for human and animal consumption of putrescine from 0.3 to 0.7 mg/100 g were reported in *Penaeid* shrimp and Norway lobster respectively. But, levels of 19.87 mg/100 g by putrescine were reported from orange spotted grouper after 18 days storage in ice at 0 °C (Bita et al. 2013). Kim et al. (2009a, b) reported similar increased levels of putrescine (0.72 mg/100 g) at 20 °C in the storage time of 24 h. In another study Hu et al. (2012) was observed low content 9.8 mg/100g of putrescine in blue scad fish during storage at 4 °C. The lowest level of 1.73 mg/100g of putrescine was earlier reported from the swimming crab (*Portunus trituberculatus*) Kim et al. (2009a, b).

Cadaverine, spermidine and spermine were not detected at both storage periods. Absence of these amines indicated that the respective precursor amino acids and/or decarboxylating bacteria were not sufficient for the formation of these biogenic amines (Fig. 1a, b). Spermidine and spermine were detected at low concentrations and ranged from 0.3 to 0.4 mg/100 g in salted sardine to 3.0 and 4.0 mg/100 g in feseekh, respectively (Rabie et al. 2011). In another study, low contents of spermidine and spermine were recorded in smoked fish were 1.286 and 2.228 mg/100 g, by 120 days; 0.805 and 0.644 mg/100 g in sardine, by 120 days; and 0. 366 and 0. 871 mg/100 g in anchovy, also by 120 days (Rabie et al. 2014). Similarly, Kim et al. (2009a, b) reported that, 0.66 and 0.89 mg/100 g of cadaverine was observed in Chinese mitten crab during 4 and 20 °C at 12 and 24 h of storage respectively. In this study, there is no amount of cadaverine; spermine and spermidine were obtained in crab during storage. In agreement with our findings, (Bita et al. 2013; Ozogul et al. 2008) documented that there is no detectable of cadaverine found in grouper fish muscle during storage times of 0 °C. The lowest concentration was 1.06 mg/100 g of cadaverine were earlier reported from the swimming crab (*Portunus trituberculatus*) Kim et al. (2009a, b). Hu et al. (2012) reported that, 1.33, 0.21 and 0.29 mg/100 g cadaverine, spermidine and spermine were observed in blue scad during storage at 4 °C. In addition, Krizek et al. (2004) reported that, the more amount of cadaverine 0.51 mg/100 g found in *Cyprinus carpio* stored at 3 °C for 14 days. Kim et al. (2009a, b) reported similar increases of cadaverine (0.35 mg/100g) at 20 °C in the storage time of 24 h.

Histamine is a toxic biogenic amine and the causative agent of histamine fish poisoning (Lehane and Olley 2000). Histamine was major biogenic amine formed in blue swimmer crab at short duration storage of 7.55 ± 0.46 mg/100 g after 96 h at 4 °C and 17.68 ± 1.30 mg/100 g after 96 h at 20 °C showed in Fig. 1a, b. Unfortunately, values as high as 21.1 mg/100 g have been found by Rabie et al. (2009) in salted fermented fish Feseekh after 60 days of storage, which constitutes a health hazard for consumption at large. Hu et al. (2012) reported that, the histamine was 6.81 mg/100 g for blue scad fish, which was comparatively much lower than the results of the present study. Ozogul et al. (2006); Bita et al. (2013) reported increases in levels of histamine with an increase in the storage temperature. Histamine and other biogenic amine are mainly formed during bacterial spoilage owing to decarboxylation of free amino acids by bacterial decarboxylase activity (Ozogul et al. 2006). Chen et al. (2007) reported that Chinese mitten crab appeared more susceptible to histamine formation, probably because of the high content of free histidine in its meat. Similarly, it became obvious that blue swimmer crab is also more susceptible to histamine formation, because of the presence of free histidine in its meat. This was consistent with the findings of Moini et al. (2012), Rezaei et al. (2007) and Ozogul et al. (2008). They suggested that there is a small concentration of histidine, the histamine precursor; in live fish and that the interaction with mesophilic bacteria i.e. histamine decarboxylase is relatively inactive in ice storage. The HACCP system, histamine can be used as in indicator of the quality of fresh aquatic fish for some species. Kim et al. (2009a, b) reported similar increases in maximum levels of histamine (1.81 mg/100 g) at 20 °C in the storage time of 24 h. Histamine levels in *P. pelagicus* were 4.23 ± 0.21 and 17.68 ± 1.30 mg/100 g respectively, at 24–96 h of storage at 20 °C. The large increase of biogenic amines was observed when aerobic mesophilic bacterial count reached 6.7–7 log CFU/g (Fig. 3a, b). For the storage of crab at 20 °C temperature, the concentration of histamine was below the level of 4.23 ± 0.21 mg/100 g after 24 h. This level increased to 17.68 ± 1.30 mg/100 g after 96 h exceeded 5 mg/100 g the legal level set by FDA (2011) for marine fish.

Above the maximum permissible level of 10–80 mg/100 g of tyramine can cause hypertension both in human and animals (Shalaby 1996). It was observed that fresh marine fish contain trace amounts or no tyramine, but a large quantity can be found in spoiled and/or fermented fishery products (Prester 2011). In our present study, tyramine was present at level of 4.98 ± 0.49 mg/100 g after 96 h at 4 °C and 125.6 ± 1.49 mg/100 g after 48 h at 20 °C. Tyramine content was increased beyond toxic levels from 91.5 ± 4.03 mg/100 g 48 h at 125.6 ± 1.49 mg/100 g after 96 h at 20 °C (Fig. 1a, b). Storage of the blue swimmer crabs refrigerated at 4 °C lowered formation of tyramine significantly ($P < 0.05$) compared with that at room temperature. Similarly, Kim et al. (2009a, b) reported that, the tyramine content in Chinese mitten crab was 3.28 mg/kg after 72 h at 4 °C and

2.48 mg/kg after 24 h at 20 °C storage respectively. Moini et al. (2012) stated that the low concentration of tyramine was 14.7 mg/100 g muscle of tiger toothed croaker during ice storage. Similar change of tyramine was 4.9 mg/kg observed in blue scad during ice storage. But, levels of 14.37 mg/100 g tyramine was reported from orange spotted grouper after 18 days storage in ice at 0 °C (Bita et al. 2013). Maximum permissible level of 10–80 mg/100 g of tyramine can cause hypertension both in human and animals (Shalaby 1996). Kim et al. (2009a, b) reported that, the high level of tyramine ranged from 101 to 222 mg kg in mackerel, saury, Spanish mackerel and amberjack during ice storage.

pH

The pH level of blue swimmer crab (*P. pelagicus*) increased gradually with increasing storage time at 4 °C and 20 °C. The initial pH level in blue swimmer crab muscles were 6.74 ± 0.07 after 24 h storage, respectively, at 4 °C and 7.55 ± 0.32 after 24 h storage at 20 °C, and it gradually increased after the end of storage (Fig. 2a, b). Storage of the crabs at 4 °C lowered the levels of pH significantly ($P > 0.05$) compared with that at room temperature (20 °C). The level of pH in Meder's mangrove crab was 7.2 ± 0.11 on day one at 2 °C (Noojuy and Boonprab 2008). The level of pH can induce accumulation of amino acid and promote the formation of biogenic amines (Yongjin et al. 2009). Due to lowering of pH after death, the lysosomal membranes get ruptured, releasing the enzyme hydrolysis resulting in the rapid degradation of meat constituents. The degradation of protein results in raising the water holding capacity and increased hydrogen ion concentration (pH). Biogenic amines were mainly produced by endogenous and/or exogenous decarboxylation in raw and processed fish and the content of free amino acid in the fish muscle. In addition, a bacterium present is capable of decarboxylase activity and environmental conditions such as pH and temperature are responsible for biogenic amines production (Ozogul et al. 2008).

Changes in total volatile base nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N)

The level of TVB-N contents were 8.16 ± 0.80 mg/100 g, at 4 °C and 33.86 ± 1.46 mg/100 g at 20 °C after 24 h and TVB-N gradually increased in the blue swimmer crab (Fig. 2a, b) after 96 h storage at 4 °C and 20 °C respectively. Significant differences ($P < 0.05$) were found in TVB-N contents between blue swimmer crabs stored at 4

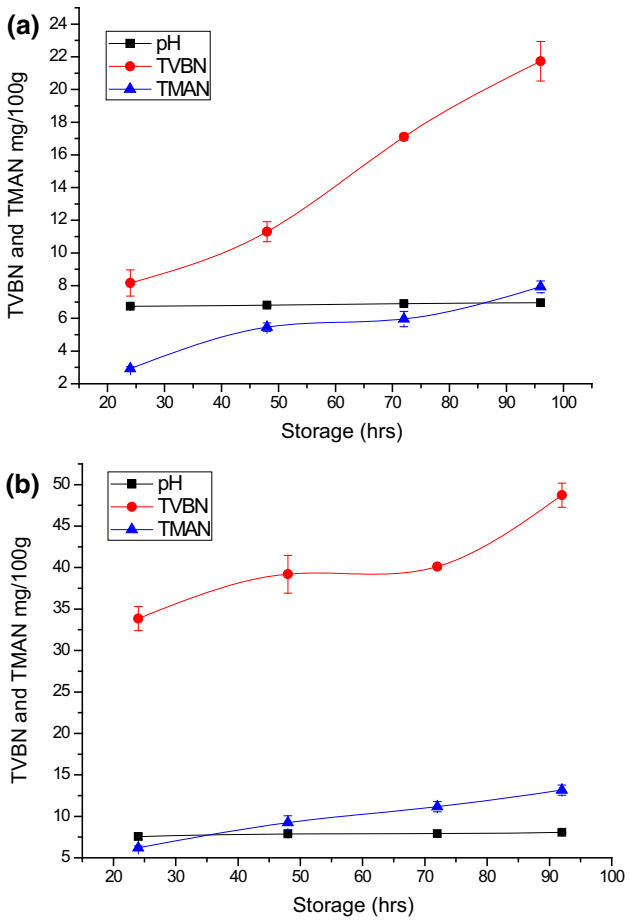

Fig. 2 **a** pH, TVB-N and TMA-N of blue swimmer crab (*P. pelagicus*) stored at 4 °C. Data are expressed as mean ± SD. **b** pH, TVB-N and TMA-N of blue swimmer crab (*P. pelagicus*) stored at 20 °C. Data are expressed as mean ± SD

and 20 °C during storage periods. This level was greater than the acceptable limit of 35 mg/100 g (Ruiz-Capillas and Moral 2001). The Meder's mangrove crab had the low level (30.02 ± 0.74 mg/100 g) of TVB-N after 15 days of storage at 2 °C (Noojuy and Boonprab 2008). The content of TVB-N increased to 56.44 mg/100 g at 20 ± 1 °C after 12 h of storage in Chinese mitten crab (Kim et al. 2009a, b). The value of TVB-N content was 3.64 ± 0.66 mg N/100 g and 3.73 ± 0.58 mg N/100 g in ice stored *P. monodon* after 12 h in bamboo basket and plastic basket at 28 °C (Mahmud et al. 2007). The storage temperature and pH are most important in overprotective TVB-N content of crab muscle. In the present study, blue swimmer crab shells were removed only at the time of analysis and the meat samples were not exposed to additional bacterial contamination. Therefore, it appears that the microflora *Gammaproteobacterium* group originally present in the blue swimmer crab meat might have contributed to increase in the level of TVB-N significantly

after 12 h storage when exponential growth began (Data not shown).

In the present study, TMA-N content of 2.93 ± 0.12 mg/100 g and 6.2 ± 0.24 mg/100 g was noticed, respectively, at 4 °C and 20 °C after 24 h and the level increased gradually after 48 h storage (Fig. 2a, b). When the crabs stored at 4 °C, levels of TMA-N initially remained at low levels but increased sharply ($P < 0.05$) after 72 h, finally reached a concentration of 7.93 ± 0.36 mg/100 g by the end of 96 h of storage. This indicated that TMA-N is not a good indicator of spoilage in blue summer crab as only 13.16 ± 0.62 mg/100 g of it was produced over 96 h of storage at 20 °C. In contrast, the highest level 12 mg/100 g in TMA-N content was reported in hake during the ice storage (Ruiz-Capillas and Moral 2001). It has been reported that TMA-N is associated with fish spoilage in many fishes (Ozogul et al. 2006). Kim et al. (2009a, b) reported the lowest TMA-N content of 0.35 ± 0.05 mg/100 g after 24 h of storage and 2.57 ± 0.23 mg/100 g after 48 h of storage in Chinese mitten crab muscle at 20 °C. Our result is comparable with that Mahmud et al. (2007), who reported the low TMA-N content of 5.02 ± 0.41 mg/100 g and 5.16 ± 0.47 mg/100 g in ice stored *P. monodon* after 24 h in bamboo basket and plastic basket respectively at 28 °C.

Changes in microbial quality

Changes of bacterial count in blue swimmer crab stored in ice and ambient temperature are demonstrated in Fig. 3a, b. The initial aerobic plate count of blue swimmer crab in this study was 3.72 log CFU/g. The bacterial counts increased significantly during the storage except for putrescine and cadaverine forming bacteria in crab stored at 4 °C. Crab sample stored at ambient temperature showed a faster increase rate of bacterial counts (Fig. 3a). The initial aerobic mesophilic bacteria count was 5.02 log CFU/g at 24 h and end of the storage the count was rose into 7.32 log CFU/g at 96 h of storage at 20 °C. This value was reached 7 log CFU/g, the upper limit established by The International Commission on Microbiological Specification for Foods (ICMSF 1986) for rejection marine fish. For ice storage (4 °C), the aerobic mesophilic plate count increased slowly and reached 6.68 log CFU/g at the end of the storage.

Putrescine, cadaverine and histamine forming bacterial counts were lower than average of 0.52 log CFU/g aerobic mesophilic plate count in crab stored for 24 h at 4 °C. At the end of the storage, the number of putrescine, cadaverine and histamine forming bacteria increased and tended to be similar to that found in aerobic mesophilic bacteria at ice (4 °C) temperature (Fig. 3a, b). It seemed likely putrescine, cadaverine and histamine forming bacteria were mostly

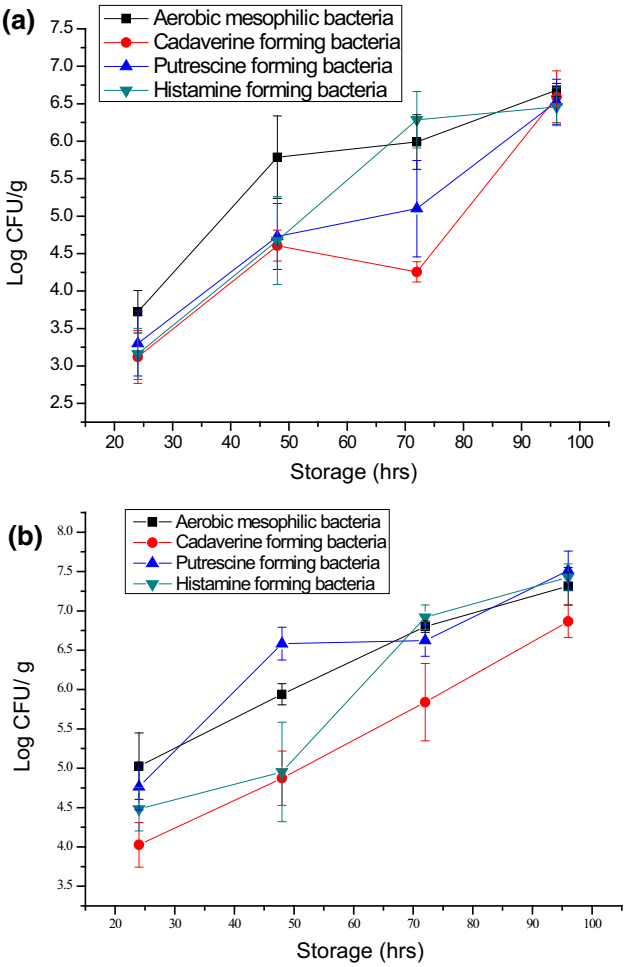

Fig. 3 **a** Microbiological changes in crab during storage (**a**) at ice 4 °C. *Error bars* represent SD. **b** Microbiological changes in crab during storage at ambient temperature (20 °C). *Error bars* represent SD

mesophiles in this experiment. In this study, putrescine, cadaverine and histamine forming bacteria remained constant throughout the storage period. The results pertaining specifically to putrescine and histamine forming bacteria were able to survive and decarboxylate amino acids at 4 °C. Similarly, Abu Bakar et al. (2014) reported putrescine and cadaverine forming bacteria were able to live and decarboxylate amino acids at 0 °C and 68.3% of aerobic mesophilic cadaverine forming bacteria and 73.5% putrescine forming bacteria in Indian mackerel fish.

In agreement with our finding, TPC increased to 6.7 log CFU/g by day one and to 8.0 log CFU/g on day two stored mackerel at 25 °C (Jiang et al. 2013). Moini et al. (2012) reported increased mesophilic bacterial load during 18 days of ice storage at 0 °C in *Otolithes ruber* as in the present study. The lowest TPC recorded was 5.18 ± 0.24 log CFU/g in Meder's mangrove crab on 0 day of storage at 2 °C (Noojuy and Boonprab 2008). Likewise, orange

spotted grouper muscle had 4.61 CFU/g of TPC on 18 days storage at 0 °C (Bita et al. 2013). In the present study, the increased mesophilic bacteria counts indicated their ability to promote the formation of biogenic amines during two different periods of storage (Fig. 3a, b). Bacterial load of 6–7 log CFU/g of TPC at 4 and 20 °C, is usually responsible for the formation of 50 ppm histamine (i.e. maximum residue limit of histamine for human consumption) (USFDA 2001). In the present study, the bacterial has load increased to 6.68 log CFU/g and 7.31 log CFU/g after 96 h of storage at (4 and 20 °C) and is unacceptable for human consumption. For ambient temperature (20 °C) storage, concentration of histamine tallied with aerobic mesophilic plate count reaching the acceptable limit after 72 h of storage. A significant amount of histamine was only detected when the crab sample raised aerobic mesophilic and histamine forming bacteria count close to 7 log CFU/g.

Conclusion

Shelf life of blue swimmer crab in this study was 96 h stored at 4 and 20 °C predicated to biogenic amines, TPC, TVB-N, TMA-N and pH. Histamine was the predominant amine formed in blue swimmer crab meat during different times of storage. Tryptamine and tyramine can be produced in high level in blue swimmer crab and poses safety hazard if the crab exposed to temperature abuse. Spoilage indicator putrescine was detected after 48–96 h of storage at 4 °C and 24–96 h, respectively, at 20 °C. It could be conducted that on capture *P. pelagicus* must be used for further processing within 24 h after death if stored refrigerated and/or immediately after death when stored at room temperature. This will not only take care of the quality of the crabs, but will also be helpful in prolonging the shelf life.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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