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Biochemical Changes of Fresh and Preserved Freshwater Prawns (*Macrobrachium rosenbergii*) During Storage

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Abstract: Biochemical analysis was carried out for pH profiles, freshness in terms of K-values, amino acids profiles, total volatile bases (TVB), total volatile acids (TVA) and biogenic amines for fresh and preserved *Macrobrachium rosenbergii*. Results showed that pH profiles of *Macrobrachium rosenbergii* explain the inability of this parameter to be used to evaluate the quality of *Macrobrachium rosenbergii*. Thus changes in pH profiles of *Macrobrachium rosenbergii* should be combined with indicators such as total volatile acids and total volatile bases. Total volatile acids of the shrimps increased steadily in small amounts throughout the storage period. A rapid increase in TVB at 10^0C was detected due to the increase in total aerobic bacteria at elevated temperatures. The microbial activities caused the decrease in the amino acids arginine, lysine and histidine which correlated well with the increase in the corresponding biogenic amines such as putrescine, cadaverine and histamine respectively. Preservatives used in this study controlled the production of these biogenic amines without significantly altering the pH of preserved shrimp.

Keywords: ***Macrobrachium rosenbergii*, biochemical changes, sensory quality, storage, K-values, total volatile bases (TVB), total volatile acids (TVA), biogenic amine**

INTRODUCTION

Prawns catches are great commercial value world wide. The demand for fresh product has been growing in recent years, all over the world, including Malaysia. Freshwater prawns, *Macrobrachium rosenbergii* are produced throughout Asia, the Pacific Islands, Israel, Central America and parts of the Southern United States. However, not much information is available on the biochemical changes during storage at refrigerated temperature. Therefore, assurance of both the quality and safety of freshwater prawns will be a major challenge faced by humankind in this new century. The different storage effects on shrimp in terms of rate of quality deterioration are clearly determined by storage temperatures. Similarly, while relating to their biochemical quality, total volatile bases (TVB) level of 30 mg/100 g of shrimp meat is considered to be a useful indicator of the limit of shrimp acceptability. At the same time, shrimp become unacceptable when pH of the tissue exceeds 7.5 and K-values reaching 60%.

As shrimps are very rich in protein and free amino acids, they are highly perishable. Proper icing, chilling and quick freezing are, therefore, imperative as there can be no compromise on quality. Maintenance of quality or prevention of deterioration in shrimp is one of the major problems facing the industry throughout the world. Chemical and biochemical reactions responsible for quality deterioration during storage will slow down with decrease in storage temperature, and the storage life will be prolonged at lower temperatures (Riaz and Qadri, 1990). The muscle tissue of shrimp, like fish spoils faster than mammalian muscle. The more rapid spoilage of the shellfish are due to higher water content, high free amino acid content, more rapid autolysis by the existing enzymes and less reaction of the flesh that favours microbial growth and the lower content of connective tissue as compared to other flesh foods. The higher ultimate pH and the colder water temperature for at least the temperate water shellfish also facilitate the decrease lag time, and

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the more rapid growth and reproduction of bacteria, even with refrigeration. A series of complex changes occur in shrimp which are caused by both natural processes and by the bacteria. In general, shellfish are subjected to the same types of changes similar to those of fish. Therefore, fresh fish or shellfish spoilage goes through the following stages: rigor mortis, dissolution of rigor mortis, autolysis, and bacterial spoilage (Ehira and Uchiyama, 1986).

In light of this situation, this research was conducted to investigate the biochemical changes involved in the loss of quality undergone by Freshwater prawns, *Macrobrachium rosenbergii* during different storage temperature. In this study, the evaluation of sensory features and changes in the most relevant biochemical components –pH, K-value, total volatile acids (TVA), total volatile bases (TVB), amino acids and biogenic amines— were evaluated during ambient, freeze and ice storage.

MATERIALS AND METHODS

Chemicals

Standard histamine, cadaverine and putrescine hydrochloride were purchased from Sigma-Aldrich.

Apparatus

Analyses were performed with a Shimadzu High Performance Liquid Chromatography (HPLC), Japan, consisting of a Model LC - 6A pump, Model SPD-6A UV detector set at 254 nm, a Model C-R6A chromatopac integrator. A LiChrosopher 100RP - 18 reverse phase column (5 um, 125 x 4 mm i.d., E.Merck) was used. Isocratic elution systems were used for the analyses of these amines. The mobile phase of methanol - water (60:40 v/v) at 1.1 ml per min flow rate gave a clear separation and resolution for all the three biogenic amines tested.

Sampling

Live cultured freshwater shrimp (*Macrobrachium rosenbergii*) were obtained from three different sites in Malaysia: Site 1- Kg. Jumbang, Negri Sembilan; Site 2 - Kg. Cangkat Tin, Perak and Site 3 - Kg. Cenderiang, Perak (Figure 1). These sites were chosen for this study due to their consistency in breeding and availability of samples throughout the season. Four months old live, mature adult prawns measuring between 13.00 cm and 18.00 cm maximum in length and weighing between 25.00 g to 71.00 g were brought back to the laboratory from the three random shrimp ponds in Malaysia with their original pond water in sealed oxygenated bags. Samples from each site were then divided into three groups and immediately placed inside clean styreform boxes. One group of shrimp was kept at ambient temperature (28°C) for 20 hours. The second group of samples were stored at 10°C for 10 days and the remaining shrimp were kept in clean crushed ice (ice was removed and replenished on alternate days throughout the storage period), refrigerated for 16 days. Ambient storage was chosen to emulate the poor handling procedure often overlooked by shrimp processors. Ten (10) degree Celsius was adapted from the normal commercial refrigerated storage temperature currently practiced by food handlers whilst icing was chosen as the normal handling procedure practiced after samples were harvested.

Determination of pH

pH of shrimp was measured separately for the body and head regions. Ten (10) gram samples were homogenized and mixed thoroughly with 100 ml distilled water and pH determined using the Cambridge pH meter on every sampling day for all samples at all storage temperature. pH of the pond water was measured immediately after arrival at the laboratory after sampling.

K-Value

The K-value (a freshness index of fish and shellfish) was measured using a muscle homogenate with an Inosine Monophosphate (IMP) - test paper method (Negishi and Karube, 1989).

$$\text{K-value } (\%) = \frac{(\text{Inosine} + \text{Hypoxanthine})}{(\text{Inosinic acid} + \text{Inosine} + \text{Hypoxanthine})} \times 100$$

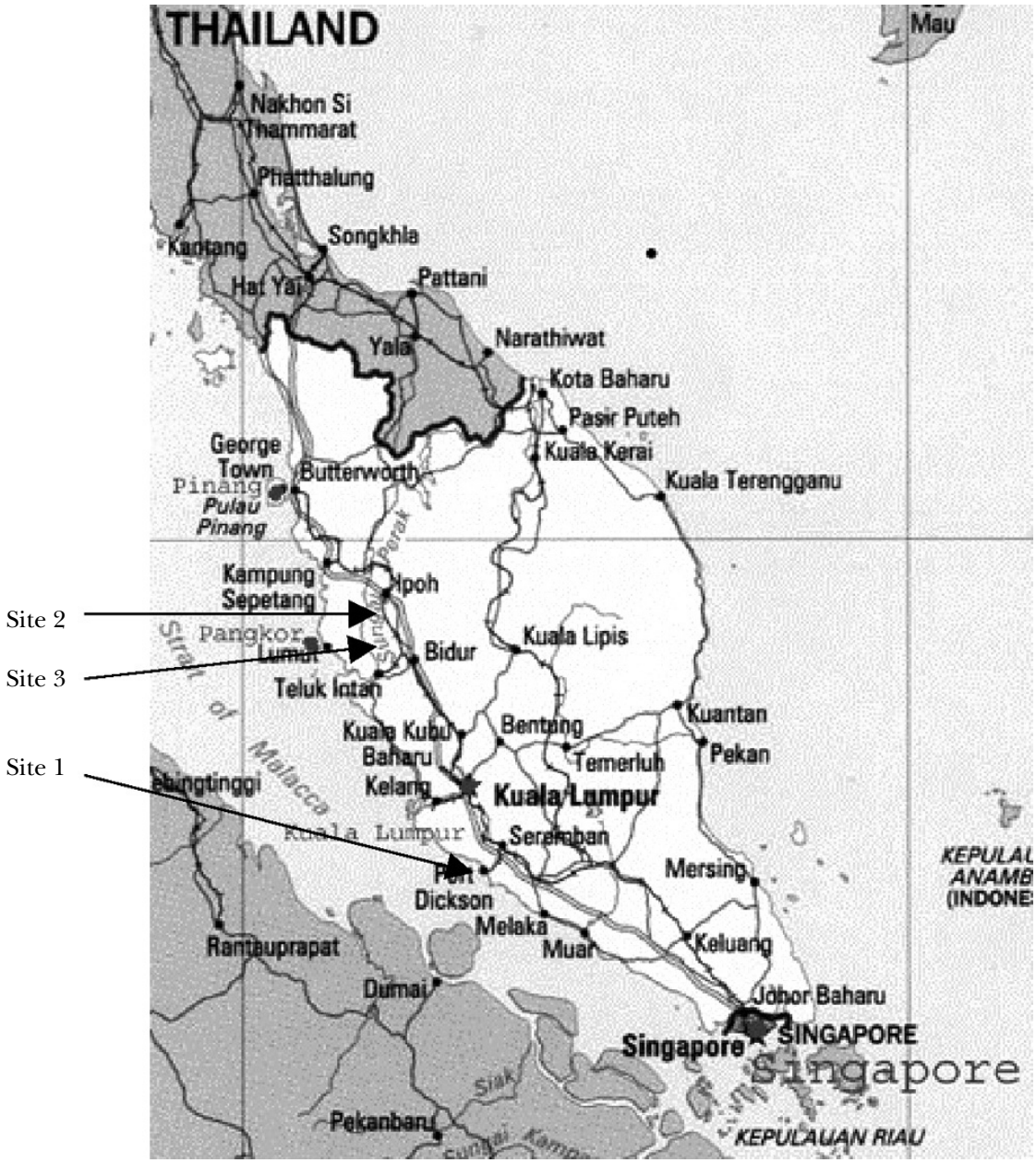

Figure 1: Map of Peninsular Malaysia showing sampling sites for freshwater prawns *Macrobrachium rosenbergii*

Determination of Total Volatile Acids (TVA)

All samples were analyzed for total volatile acids according to the AOAC distillation procedure followed by titration with sodium hydroxide (NaOH) (1984).

Total volatile acids in seafood consist of volatile fatty acids (such as acetic, butyric, propionic and valeric acids) found in shrimp muscle samples. Duplicate pooled samples consisting of three (3) gram shrimp muscle samples was used in 200 ml distilled water collected in distillation flasks. A standard distillation apparatus was set up in the laboratory and a slightly modified procedure according to 18.052 (AOAC, 1984) was used. Steam distillation was carried out using a distillation flask connected to a condenser with the steam inlet tube inserted into the distillation flask, bringing contents of flask to incipient boiling with a burner. The steam inlet tube was connected to steam supply from a boiler and the rate of steam

evolution and the height of burner flame under the distillation flask was regulated so that the volume of liquid in the distillation flask was kept at 150 ml + 10 ml at the end of distillation. The distillate was collected and titrated with 0.1 N NaOH until phenolpthalein end point was reached. Total volatile acids were calculated as follows:

Milliequivalent (meq) NaOH used

$$= \frac{\text{ml NaOH x Normality of NaOH x 60.2}}{\text{dry weight of sample x 1000}}$$

Dry weight of samples was determined by drying the 3 g samples overnight in an oven at 103^{0}C and was calculated asweight of wet samples minus the water (moisture) content.

Determination of Total Volatile Bases (TVB)

Total volatile bases were determined using direct Magnesium Oxide (MgO) method of distillation according to the AOAC method (1984).

A vertical distillation apparatus was set up and determination of total volatile bases was carried out by adding 10 g blended shrimp muscle sample in 300 ml distilled water in 1000 ml round bottom distillation flask. Anti bumping granules and anti foaming silicone preparation were then added together with 2 g magnesium oxide (MgO) and connected to a still. To a 250 ml Erlenmeyer receiving flask, 25 ml of 2% boric acid was added with a few drops of methyl red indicator. The receiving flask was installed such that the receiver tube dipped below the boric acid solution. The distillation flask was heated so that liquid boiled in exactly 10 min. Using the same rate of heating, distillation was carried out for exactly 25 min. After distillation, the solution in the receiver flask was titrated back to the original colour, using a standard 0.05 N H_2SO_4 solution. A blank was titrated which had all the reagents except for the sample. If the blank required more than 0.1 ml titrant new boric acid should be used.

Total volatile bases were expressed as milligrams nitrogen per 100 g samples as follows.

$$\text{mg N per 100g} = \frac{(V4 - V5) \times N2 \times 100 \times 14}{W2}$$

Where:

V4 = volume (ml) H_2SO_4 used for sample
V5 = volume (ml) H_2SO_4 used for blank
N2 = Normality of H_2SO_4
W2 = weight of sample (g)

Determination and Derivatization of Amino Acids

Amino acids profiles in freshwater shrimp were determined using the Amino Acid Analyser. Shrimp samples and standard amino acids solution were subjected to hydrolysis by 6N Hydrochloric acid. Shrimp muscle samples were measured to approximate weight of between 0.5 to 1.0 g and then mixed thoroughly with 15 ml 6N HCl. After which they were bathed ultrasonically for a few minutes to remove dissolved oxygen and later flushed with nitrogen gas. Samples were put in the oven at 110^{0}C for 24 hours. After that, samples were cooled and transferred to 50 ml volumetric flask and water added till a final volume of 50 ml. Sample solution was then filtered through filter paper, rejecting initial filtrate. For standard amino acids solution, 10 ml internal standard (1 ml = 2.5 µmole ± amino butyric acid [AABA]) was added before it was transferred to 50 ml volumetric flask and the volume made up to 50 ml using distilled water. Filtrate could be stored at -20^{0}C for up to 4 weeks.

For HPLC analysis, a small volume of the filtrate was filtered through 0.20 µm aqueous syringe filters, 25 mm diameter, rejecting initial filtrate. 10 µl sample filtrate was pippetted into tubes (measuring 6 x 50 mm). Tubes were placed into reaction vial and vacuumed to 100 millitorr before adding 20 µl fresh redrying solution (consisting of methanol: water: ethylamine at a ratio of 2:2:1 v/v) and mixed thoroughly. Samples were vacuumed to 70 millitorr before adding fresh 20 µl derivatization reagent (consisting of methanol: water: triethylamine: PITC at a ratio of 7: 1: 1: 1), mixed, and the tubes returned to reaction vial and valve closed. They were left to stand at room temperature for 20 min and vacuumed again to 50 millitorr. For immediate analysis of samples, 100 ml sample diluent (consisting of disodium phosphate- Na_2HPO_4) was added and mixed. A limited volume of 20 µl was used as injection volume into HPLC for samples whilst the injection volume for the standard solution was 8 µl. Postponed analysis could also be carried out after vacuum reached 50 torr as done above, seal the vials and stored in a freezer for several weeks.

Determination and Derivatization of Biogenic Amines
Standard amine solutions were prepared by a mix of amines standard solution consisting of putrescine dihydrochloride (182.9 mg), cadaverine dihydrochloride (171.4 mg), and histamine dihydrochloride (165.7 mg) dissolved in 10.0 ml deionised water. The final concentration of each amine (free base) was 10 mg/ml solution.

Sample preparation and amine extraction were carried out for fresh and spoiled samples and derivatised with benzoyl chloride according to the method by Yen and Hsieh (1991). Twenty (20) grams deheaded, peeled shrimp sample was transferred to a 250 ml centrifuge tube and homogenized with 50 ml 6% trichloroacetic acid (TCA) in a Polytron type homogenizer (LH, Ltd England) for 3 min. The homogenate was then centrifuged at 11000 rpm, for 10 min at 4^0C to allow precipitation. The supernatant was filtered through Whatman No. 1 filter paper. The filtrate was placed in a volumetric flask and made up to 100 ml. with 6% trichloroacetic acid.

The benzoyl derivatives of amines were prepared according to Redmond and Tseng (1979) with modifications. One ml 2M sodium hydroxide was added to 50.0 µl of the standard amine solution followed by 10 µl benzoyl chloride, mixed on a vortex mixer and allowed to stand for 20 min. Two ml saturated sodium chloride solution was added, followed by extraction with 4.0 ml diethyl ether, centrifuged at 11000 rpm for 10 min at 4°C and separation was done by washing twice using a separating funnel. The upper organic layer was transferred into a clean tube and evaporated to dryness in a stream of nitrogen gas. The residue was dissolved in 500 µl of methanol and 5 µl aliquots were injected for HPLC analysis.

Biochemical Analysis of Preserved Shrimp
Samples were treated using the following chemical solutions: clean crushed ice (control); 2% sucrose; 2% sodium chloride; 2% sodium metabisulphite; 2% boric acid and 1% lactic acid. After soaking for 30 min in the above solution and the liquid drained, samples were then covered completely in crushed ice and stored in a refrigerator at 10°C for 25 days. Crushed ice was removed and replaced every alternate day until the end of storage period.

RESULTS AND DISCUSSION

The pH of the shrimp's samples from the three sampling sites showed a low initial pH of 6.58. The values however, increased significantly ($p < 0.05$) throughout storage time for all temperatures studied (Figure 2). The increase in pH values could be attributed to the higher levels of total volatile bases produced by the microbial enzymes in shrimps. The relationship between chemicals and microbiological changes in freshwater prawns has been established by studies carried out by Ninan *et al.* (2003). Slower rise in pH from the results at ambient storage explained the higher metabolic rate of activities that might occur due to higher temperature effect as compared to the other lower temperatures. However, there were significant differences ($p < 0.05$) from the initial values compared to the end of storage period for all temperatures studied. The highest pH recorded was 7.5 after 10 days storage at 10°C. A relationship between pH and acceptability of prawns in a study by Shamshad *et al.* (1990) showed that when pH reached above 7.5 the shrimp were rated unacceptable or spoiled. The pH values less than 7.5 recorded in these studies even after the prawns were already spoiled especially at ambient storage could explain the problem using this parameter to evaluate the quality of *Macrobrachium rosenbergii*. Moreover, pH values should be combined with other indicators such as total volatile bases and sensory assessment for them to be indicative of the shrimp quality. Lower pH values might have been caused by the production of lactic acid from glycogen degradation in the prawn meat. The pH of freshwater prawns which showed significant increase during storage until spoilage were in contrast to the studies on black tiger prawns by Middlebrooks *et al.* (1988). They reported a maximum pH of 7.54 after only 4 hours of storage at ambient temperature, but thereafter the pH decreased to 7.2 and then tended to be relatively stable in farmed black tiger prawns when putrefactive odors became evident.

Nucleotide decomposition measured by the freshness index or K-values exceeded 60% after 16 hours storage at ambient and 8 days at 10°C for all the three sites studied. Ninan *et al.* (2003) noticed significant deterioration after 12 days in ice stored cultured *M. rosenbergii*. The K-values did not exceed the 60% level during the ice storage study which may be due to the presence of crushed ice and lower temperature delaying the rate of autolysis of the prawn meat (Figure 3). Ehira and Uchiyama (1987) suggested that K-values in excess of 60% are associated with spoiled fishery products whilst K-values of 20% as freshness limit for similar products. They concluded that many studies on methods to estimate freshness of seafood have been erroneously based on the concept that bacterial action causes deterioration in freshness, whereas in fact freshness declines long before the onset of

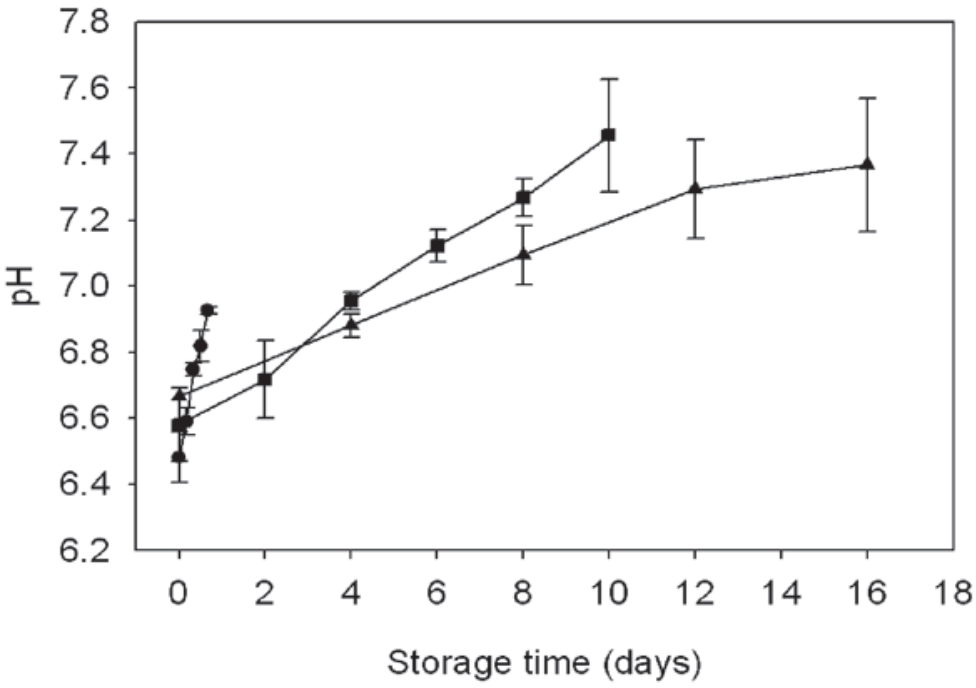

Figure 2: pH of *M. rosenbergii* stored at different temperatures. Bars represent the standard deviation of means from 6 readings (●) ambient storage; (%) 10°C storage; (■) icing storage

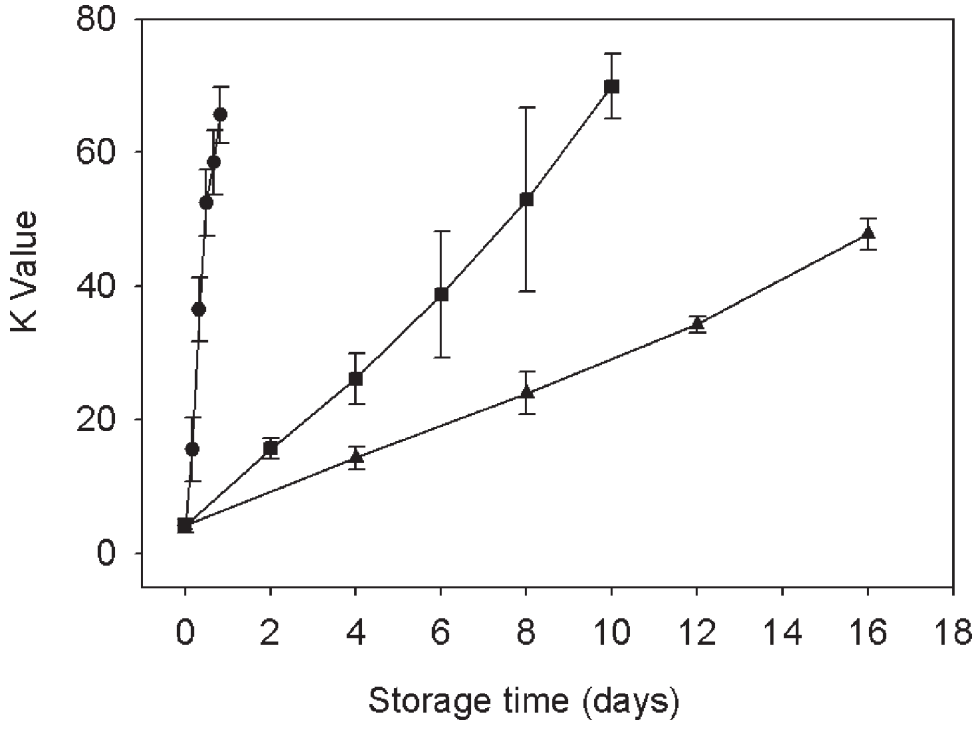

Figure 3: Evolution of K value in *M. rosenbergii* during different storage temperature. Bars represent the standard deviation of means from 6 readings (●) ambient storage; (■) 10°C storage; (▲) icing storage

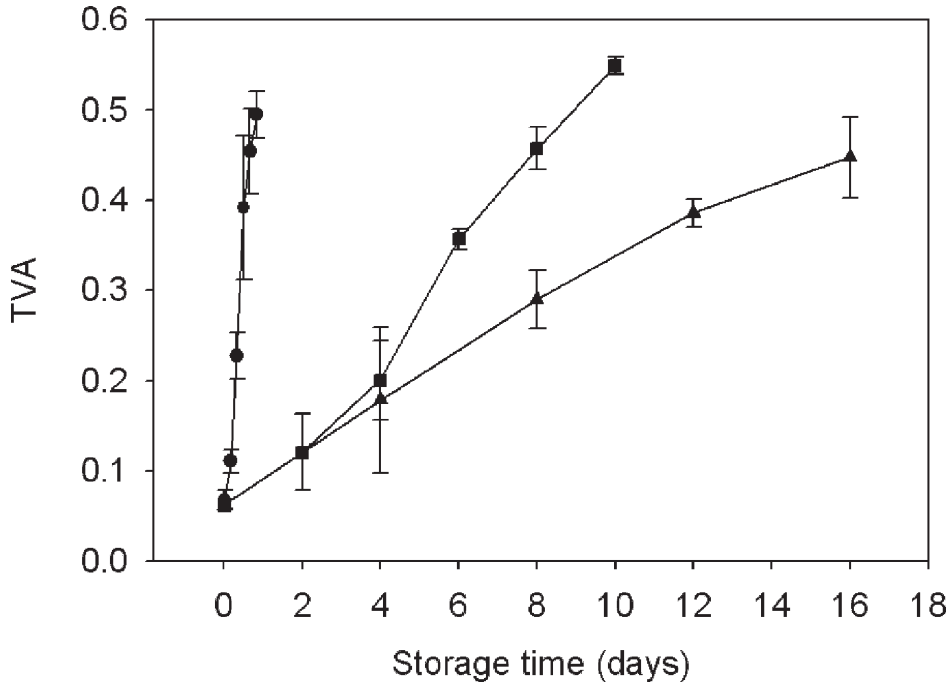

Figure 4: TVA of *M. rosenbergii* during different storage temperature. Bars represent the standard deviation of means from 6 readings. (●) ambient storage; (■) 10°C storage; (▲) icing storage

significant bacterial action. This latter value was observed in the prawn samples after only 4 hours of storage at ambient, 2 days storage at 10°C and 4 days at iced storage for all the sampling sites studied. Since the freshness of shrimp meat deteriorates rapidly, freshness may be considered a synonym of quality. From the results obtained it shows that there is a relationship between freshness of freshwater prawns and K-values where K-value can be used as a means of evaluating the real freshness of shrimp. Moreover, the use of the rapid test paper method containing appropriate enzymes used in this study may be recommended for use at landing sites for freshwater prawns and perhaps also for other related seafood products. Freshness determines the quality of food fish, not only those consumed raw or used in home cooking, but also those to be processed (Gelman *et al.*, 1990).

Total volatile acids of the prawns sample increased steadily but in smaller amounts until the end of the storage periods for all the three sites and storage temperatures (Figure 4). The increase in values of about 6 to 9 fold for all samples from all sites indicated that the levels increased steadily throughout storage period. These values correlated well with quality of the shrimp, indicating that the pattern of decomposition was more along the lines of putrefaction rather than fermentation. Total volatile acids measure approximately volatile short chain fatty acids in the products. This study was designed as a proximate estimation and complementary to the other parameters assessed and therefore caution should be exercised in drawing a more detailed quantitative conclusion from the data obtained. Both TVAs and TVBs were used to evaluate the degree of spoilage for a number of commercially available surimi-based fabricated seafoods (Wekell *et al.*, 1986).

Changes in TVB of freshwater prawns are also presented on Figure 5. The TVBs increased with time and temperature during storage. The initial levels of 17.0 mg/100g prawn sample increased continuously during storage at all temperatures studied. A level of 30 mg/100g of muscle sample has been considered the upper limit above which fishery products are considered unfit for human consumption (Shamshad *et al.*, 1990). Cheuk *et al.* (1979) and Cobb and Vanderzant (1975) also reported 30 mg/100g prawn meat to be a useful indicator of the limit of prawn acceptability. Iced storage samples showed that the upper limit was only reached after between 12 to 16 days of storage. Based on chemical and microbiological analysis, Leitao and Rios (2000) reported a shelf-life of about ten (10) days for pond reared *M. rosenbergii* when stored at 0°C. However, Lalitha and Surendran (2006) found that the limit counts of $10^6 – 10^7$ cfu/g were reached after 16 days storage indicating maximum storage life of 12-16 days

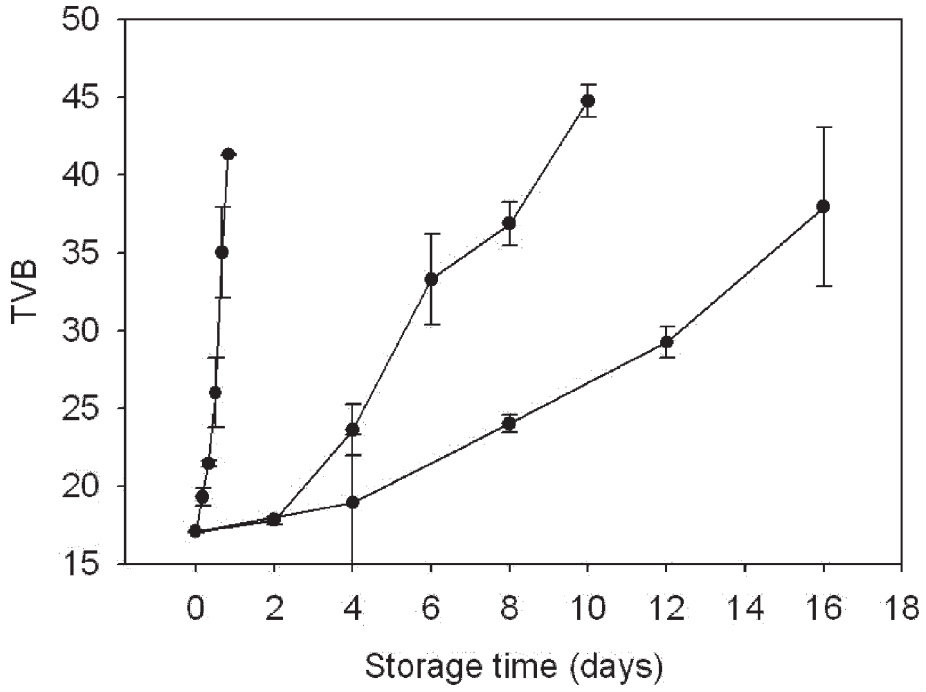

Figure 5: TVB of *M. rosenbergii* during different storage temperature. Bars represent the standard deviation of means from 6 readings. (●) ambient storage; (■) 10°C storage; (▲) icing storage

which was similar to the results of this study. A more rapid increase in TVBs at 10°C and ambient storage was due to increase in total aerobic bacteria at elevated temperatures. Cobb and Vanderzant (1975) found that increased Total volatile bases nitrogen (TVBN) values were related to bacterial spoilage and that some bacterial species produced volatile bases more readily than others. Cheuk *et al.* (1979) found constant TVBN values for brown shrimp during the first 11 days on ice, and during the first 15 days for pink shrimp. Spoilage set in after 16 days for pink shrimp and after 19 days for brown shrimp, where the TVBN values rose to 30 mg/100g. TVB is often used as an indicator for shrimp spoilage. Cobb and Vanderzant (1975) reported that Australia and Japan have been using TVB content of 30 mgN% as limits of acceptability of peeled shrimp but were not good indicators of iced shrimp because of leaching due to the washing effect of melting ice. From the results it shows that in spite of the rejection by the TVB indicator especially for the 10°C stored samples, prawns were still acceptable due to the fact that the putrefactive odour was not detected until kept for more than 12 hours at ambient conditions, 8 d at 10°C and 16 d in iced storage. At this time, the TVB contents were more than 30 mg/100g and slightly fishy odour was detected. This suggests that TVB is related to the fishy odour rather than putrefactive odour. The amount of basic amino acids (arginine, lysine and histidine) showed that in general, there was decrease in values throughout the storage period and temperature studied (Figure 6). The decrease in the three amino acids viz: arginine, lysine and histidine correlated well with the increase in the corresponding biogenic amines they produced (Figure 7).

Microbial activity forms enzymes including decarboxylases that in turn, under favorable conditions breakdown amino acids into their corresponding amines (Farn and Sims, 1987). Arginine will be metabolised into ornithine and/ or putrescine whereby putrescine formation requires the growth of arginine utilizing strains of bacteria. Decarboxylation of lysine by specific microorganisms leads to formation of cadaverine whilst histidine decarboxylases are responsible for the production of histamine. The resulting ability to produce histamine is limited to a rather small number of bacterial species (Dainty *et al.*, 1983). The levels of other amino acids in freshwater prawns were also determined and was found that

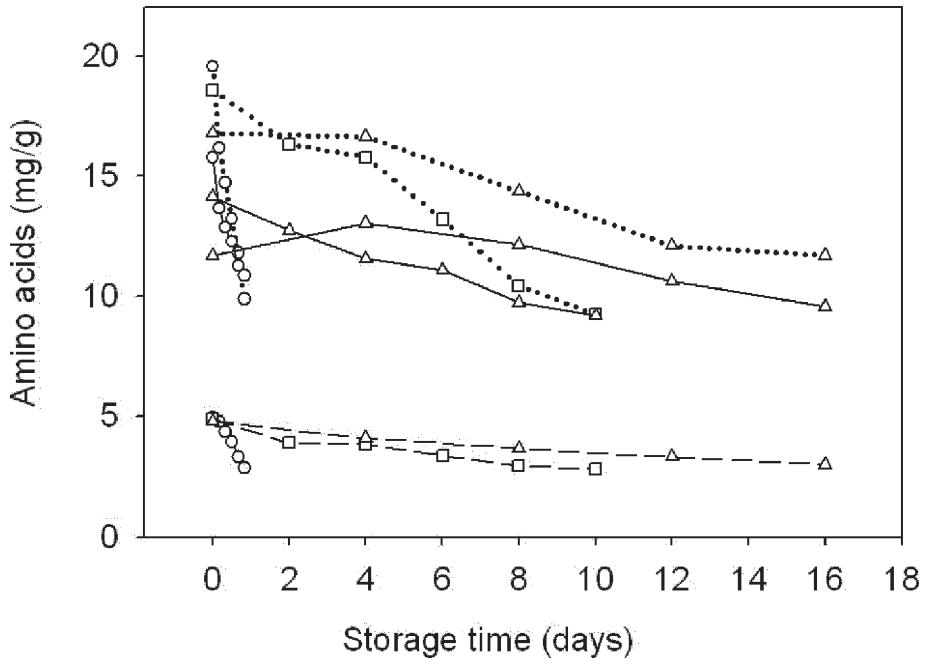

Figure 6: Amino acids profile of *M. rosenbergii* during storage. (···o···) arginine at ambient storage; (—o—) lysine at ambient storage; (—o—) histidine at ambient storage; (··□ ···) arginine at 10°C storage; (—□—) lysine at 10°C storage; (—□—) histidine at 10°C storage; (···Δ···) arginine at icing storage; (—Δ—) lysine at icing storage; (···Δ···) histidine at icing storage

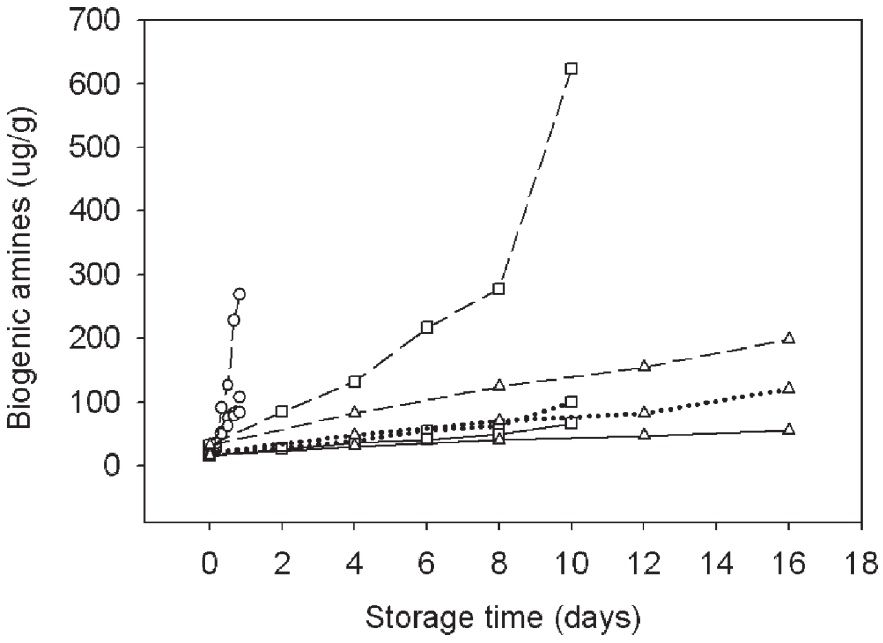

Figure 7: Biogenic amines profile of *M. rosenbergii* during storage. (···o···) putrescine at ambient storage; (—o—) cadaverine at ambient storage; (—o—) histamine at ambient storage; (··□ ···) putrescine at 10°C storage; (—□—) cadaverine at 10°C storage; (—□—) histamine at 10°C storage; (···Δ···) putrescine at icing storage; (—Δ—) cadaverine at icing storage; (···Δ···) histamine at icing storage

levels of basic amino acids (arginine, lysine and histidine) including tyrosine and methionine decreased during storage, whereas other amino acids except for proline, accumulated in the prawn muscle. The decrease in the three amino acids *viz*: arginine, lysine and histidine correlated well with the increase in the corresponding biogenic amines they produced (Figure 7). On the other hand, at all storage temperatures, alanine, valine, leucine and isoleucine accumulated more rapidly than glycine, phenylalanine or serine. These results were similar to that reported by Ababouch *et al.* (1991). Accumulation of some amino acids is indicative of degradation of the prawn muscle protein, by autolytic and/or bacterial enzymes during storage (Hatula *et al.*, 1995).

The levels of histamine, putrescine and cadaverine in spoiled fishery products can vary considerably depending upon the type of products, the spoilage bacteria and the conditions of storage leading to spoilage. A histamine level of 5 mg per 100 g is considered as the limit for product quality or spoilage whilst 50 mg/100g is considered hazardous (Malle *et al.*, 1996). However, because the distribution of histamine producing bacteria is limited and histamine is not always produced during fish spoilage, cadaverine is more frequently found in spoiled fish (Okuzumi *et al.*, 1989). The amount of cadaverine in spoiled fish varies, but levels of 10 to 60 mg per 100 g (100 to 600 mg/g) have been found (Kim and Bjeldanes, 1979; Staruszkiewicz and Bond, 1981). Putrescine levels in spoiled fishery products are usually less than 10 mg/100g (100 mg/g) (Staruszkiewicz and Bond, 1981).

Preservatives tested prevented the production of histamine throughout the storage period. Chemicals such as sodium metabisulphite, boric acid and lactic acid could also inhibit putrescine and cadaverine production during early storage of between 10 and 15 days before the amines start to reappear during storage. The levels of histamine, cadaverine and putrescine in spoiled fishery products may vary considerably with the type of fish, composition of bacteria, and the conditions of storage leading to spoilage. Large amounts of putrescine and cadaverine in fish could form in the absence of histamine. These fish, (with high amounts of putrescine and cadaverine but in the absence of histamine) even though spoiled are not known to be hazardous. However, if large amounts of putrescine and /or cadaverine were formed along with rather modest amounts of histamine, this fish could be hazardous (Hwang *et al.*, 1995).

CONCLUSION

All the preservatives tested did not influence the pH of preserved shrimp comparable to controls. The gradual rise in pH of the samples throughout the storage period shows that when pH reached 7.5 or above this parameter could be used to evaluate the quality of *M. rosenbergii* during iced storage. A relationship between pH and acceptability of shrimp was noted in a study by Shamshad *et al.* (1991), whereby when pH reached 7.5 the shrimp were rated unacceptable or spoiled. Studies also found that pH and TVB increased if stored at ambient temperature. This is because of microbial growth and activities which are reflected by decreases in basic amino acids and increases in biogenic amines. Shelf-life is considered as storage time until spoilage. From the results obtained in this study, the shelf-life limit for *M. rosenbergii* is 16 days and therefore, it is recommended that the prawns be kept at iced conditions for not more than 14 days, 8 days at 10 C and less than 12 hours for ambient storage.

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