Salequzzaman, M.; Mahmud, A.T.M.T. and Azam, K. 2006. An assessment of bio-chemical quality of ice-stored tiger shrimp (*Penaeus monodon*) from coastal Bangladesh. *Khulna University Studies*, 7(2): 97-103.

Khulna University Studies, 7(2): 97-103, 2006

RESEARCH ARTICLE LIFE SCIENCE

AN ASSESSMENT OF BIO-CHEMICAL QUALITY OF ICE-STORED TIGER SHRIMP (*PENAEUS MONODON*) FROM COASTAL BANGLADESH

Md. Salequzzaman[1*], A.T.M. Toufiq Mahmud[2] and Khairul Azam[2]

[1]*Environmental Science Discipline, Khulna University, Khulna 9208, Bangladesh*

[2]*Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9208, Bangladesh*

KUS 05/41-271105

Submitted: November 27, 2005; Accepted: Deccember 27, 2006

Abstract: An investigation focused on bio-chemical evaluation of Tiger Shrimp, Bagda (*Penaeus monodon)* of different ranges of marketable size collected in fresh condition from Koiya Bazar that stored in ice at a ratio of 1:2 of shrimp: ice at Fisheries and Marine Resources Technology (FMRT) Discipline of Khulna University, Bangladesh. Iced storage trial was carried out over 14 days and analyzed TVB-N, TMA, and pH. The range of TVB-N, TMA, and pH are between 2.692 ± 0.172 mg/100g to 16.118 ± 0.032 mg/100g, 5.385±0.026458 mg/100g to 10.764±0.036056 mg/100g, and 6.6 ± 0.1 to 7.2 ± 0.152 respectively. Highly positive relationship was observed between storage days and TVB-N values (r^2 = 0.7722), TMA values (r^2 = 0.7095), and pH value (r^2 = 0.9007). The overall results of TVB-N and TMA-N contents of *P. monodon* stored in ice rose as spoilage advanced. pH also increased with the increase in spoilage cause by bacteria. There are some discrepancies that should be that need for further investigation, particularly for daily de-gassing, prevention of the formation of bubbles in the reagent line.

Key words: Preservation, ice storage, tiger shrimp, *Penaeus monodon*, bio-chemical quality

Introduction

The black tiger shrimp (*Penaeus monodon*), locally known as ‘Bagda’, has a number of advantages over other crustaceans for polyculture. For example it adapts to wide range of temperature (20-32 ºC), fast growing individuals reach marketable size in about 4 to 5 months, has high nutritional value and have omnivorous feeding habits (Anon, 1997). This shrimp has a high demand in domestic and international market. But there are many non-satisfactory returns are occurring in the trading activities from the catch of this shrimp mainly due to its quantitative and qualitative loss in Bangladesh. Qualitative losses consist of losses in commercial value, in comparison to fresh shrimp through loss of quality (Rasel, 2002). Fresh shrimp is one, which is newly produced not stored or preserved and has its original qualities unimpaired in anyway (Dassow, 1963). During peak season, a price of shrimp drops sharply whereas the prices are generally high in lean season. Moreover, shrimp spoils rapidly because it is highly perishable commodity. Therefore, it needs post harvest processing like ice-stored processing to hold its qualitative value.

[*] Corresponding author: Phone: +880 41 813239, Cell: 01716 111 075 ;
E-mail: <salek_uz@yahoo.com>, <msalequzzaman@hotmail.com>;

DOI: https://doi.org/10.53808/KUS.2006.7.2.0541-L

Alike other animals, just after death of shrimp, spoilage begins through a series of changes in the condition of toxic accompanied by alteration in taste, smell, appearance or texture (Singleton and Sainsbury, 1978) in dead shrimp muscle by its own enzyme, by bacteria and by chemical action (Shewan, 1976). The early reaction of spoilage is autolytic and bacterial enzymes become progressively the more active in the later stages (Singleton and Sainsbury, 1978). To minimize these biochemical changes, which cause deterioration; ice-storage is an important technique.

The combined total amount of ammonia (NH_3), di-methylamine (DMA) and tri-methylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the shrimp and is commonly used as an estimate of spoilage. Total volatile nitrogen has been widely used as an index for freshness of shrimp or fish (Singleton and Sainsbury, 1978).

However, some of the bio-chemical changes is not possible to control such as the amount of TVB increases parallel with the increase in TMA during spoilage's the bacterial activity as well as spoilage increases after the death of a shrimp, a subsequent increase in the reduction of trimethyl amino oxide (TMAO) to TMA (Wood *et al.*, 1942).

From the above perspectives, the overall objective of the paper is to analyze the biochemical changes in shrimp after death that stored in ice and how the changes affected the quality of the shrimp. The specific objectives are to determine the total volatile base nitrogen (TVB-N), tri-methylamine-nitrogen (TMA-N) and pH along with other characteristics of ice-stored shrimp and these characteristics help to define a fresh shrimp. Finally the paper recommends how these changes can be minimized to keep well the quality of the shrimp.

Materials and Methods

The experiment is initiated by shrimp stored in ice (1:2) that undergone through changes with storage time and storage temperature. In this situation, the biochemical changes of shrimp depends on the species, catching methods, size, season (Singleton and Sainsbury, 1978) and fishing grounds, storage temperature, storage time and various conditions influencing the action of microorganisms (Ranke, 1959). However, the experiment was not maintained a definite regular pattern.

As the spoilage of shrimp held in ice is a bacteriological phenomenon, and the chemical changes that take place are mainly due to bacterial enzymes (Liston, 1980). Thus the experiment has been focused on the substances that accumulate in large amount in shrimp tissues bring spoilage and can be readily measured. This is because of a dominant interest in practical methods for determining the degree of spoilage so that shrimp caught can be graded. The marker compounds measured routinely for the determination of shrimp quality are TMA, TVB, and Volatile Acids (VA) (Liston, 1980). The following processes were followed for this experiment.

Collection of test specimen: The shrimp sample (*Penaeus monodon*) in iced condition was collected in the month of December with an average weight of 50-75 g, purchased from the Koiya Bazar of Khulna. The condition of the sample was extremely fresh at the purchase time. After collection of sample, it was stored in the insulator box with ice at the ratio of 1:2 (shrimp : ice) and brought immediately to the Quality Control Laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University for the further storage.

Experimental design: All necessary facilities i.e. insulation box, cold storage, electric balance, blender, pH meter, water bath, drying oven, Conway apparatus, incubator and all other laboratory facilities for chemical analysis were ensured to be suitable and available in the laboratory. In order to find out the distinct organoleptic changes with day in iced storage, at first the sample were assessed according to the procedure specified by Shewan and Ehrenbreg (1977) (Table 1) and overall acceptable limit was scored according to the same procedures, but modified by Rasel (2002) (Table 1). In this procedure, the shrimp was stored in an insulation box and kept in ice at a ratio of 1:1 (ice: shrimp). Storage environment was maintained by draining the insulated boxes for melted ice intermittently and more ice was added to keep the temperature at 0^oC throughout the entire storage period.

After evaluating the distinct organoleptic changes with day until spoilage occurred, the shrimp obtained for the second time in iced storage that kept in the same insulation box. Ice was changed every day and drainage of melted ice was ensured. The samples from the box were withdrawn to determine the overall quality in terms of bio-chemical parameter i.e. pH, TVB–N and TMA–N, in accordance with the distinct organoleptic changes at the interval of 3-4 days. All of the experiments were conducted in the microbiology laboratory of FMRT Discipline of Khulna University, Bangladesh.

Table 1. Overall acceptability scores by different scientists of the tiger shrimp, (*Penaeus monodon)***.**

Acceptability characteristics	Score (Shewan & Ehrenberg, 1977)	Modified score (Rasel, 2002)
Highly acceptable (HA)	10	9.5 – 10
Acceptable (A)	9	8.5 - 9.4
Moderately acceptable (MA)	8	6.5 - 8.4
Just acceptable (JA)	6	5.5 - 6.4
Just Unacceptable (JU)	5	3.5 - 5.4
Unacceptable (U)	3	1.5 - 3.4
More unacceptable (MU)	1	0.5 - 1.4
Extremely unacceptable	0	0.0 - 0.4

Bio-chemical analyses protocol: pH, TVB-N and TMA-N were determined in according to the procedure stated in the laboratory manual for fish and fish products edited by Siang and Kim (1992).

Results

Total volatile base nitrogen (TVB - N)**:** The amount of TVB-N obtained over 14 days storage of *P. monodon* in ice ranged between 2.692 ± 0.172 mg/100g to 16.118 ±0 .032 mg/100g. It was exception incident that TVB-N content was reduced showing a value of 8.068 ± 0.020 mg/100g on the 10th day of storage period as the value was 10.763 ± 0.058 mg/100g on the 5th day of storage (Fig. 1). In the later period, the TVB-N value increased sharply. The value decreased abruptly from 10.763 ± 0.058 to 8.0682 ±0.020 mg/100g between 5th and 10th days of storage. On the 11th day of storage period, TVB-N value was 10.764 ± 0.040 mg/100g when the shrimp was 'Just Acceptable' (JA). However, the value was 13.452 ± 0.0321 mg/100g when the shrimp was rated as 'Just Unacceptable' (JU) on the 12th day of storage. Again the TVB-N content increased markedly after the shrimp had undergone just spoilage on the 14th day of storage and the value was 16.118 ± 0.0321 mg/100g. Regression analysis (Fig. 4) illustrates that TVB-N content in *P. monodon* changes positively over storage period. There is excellent positive correlation (r^2 = 0.7722) between storage days and TVB-N.

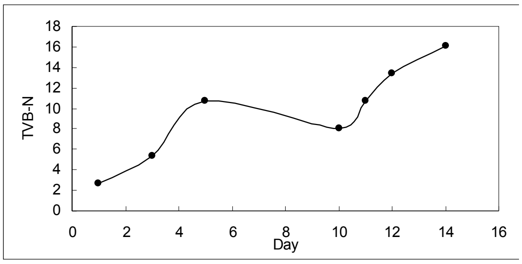

Fig. 1. TVB–N content of *Penaeus monodon* during ice-storage.

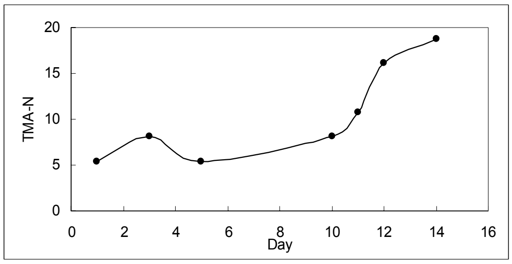

Fig. 2. TMA–N content *Penaeus monodon* of during ice-storage.

Tri-Methylamine-Nitrogen (TMA-N)**:** On the 1st day of storage period when the fish was fresh, TMA-N content in *P. monodon* was low (5.385 ± 0.026 mg/100 g). There was an exception change in TMA-N content with the progress of storage period. On the 5th day of storage, the TMA-N value was come down slightly (Fig. 2). From the 10th day onward until the end of the trial, the value increased slowly. However, the TMA-N value was 10.764 ± 0.035 mg/100g on the 11th day when the shrimp quality rating was (JA) while the TMA-N content showed a value of

16.143 ± 0.02 mg/100g on the 12th day when the shrimp was treated as JU. The TMA-N content increased markedly after the shrimp had undergone just spoilage on the 14th day of storage and the value was 18.805 ± 0.0321 mg/100g. Regression analysis (Fig. 5) showed significant correlation ($r^2 = 0.7095$) between storage period and TMA-N indicating a highly positive relationship.

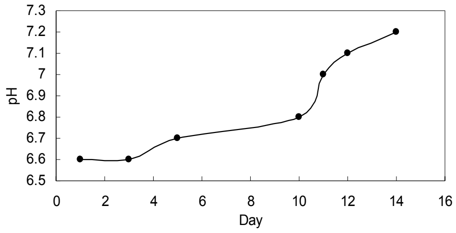

Fig. 3. pH content *Penaeus monodon* of during ice-storage.

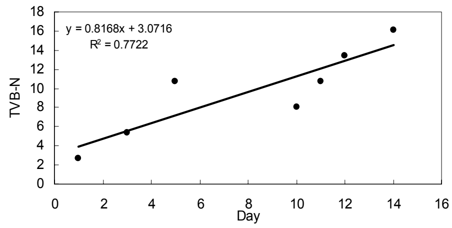

Fig. 4. Correlation between Storage days and TVB-N.

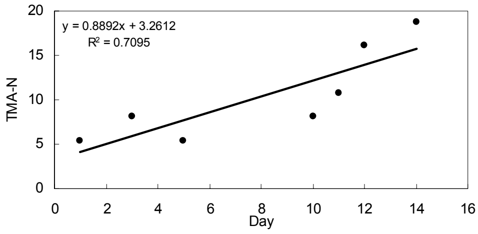

Fig. 5. Correlation between Storage days and TMA-N.

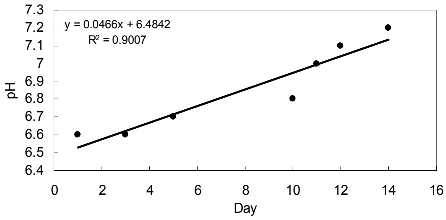

Fig. 6. Correlation between Storage days and pH.

pH: Over a range of 14 days of storage trial, pH in *P. monodon* varied between 6.6 ± 0.1 to 7.2 ± 0.152. It was observed that pH value shows upward movement with the progress of storage days. On the 11th day of storage when the shrimp was JA, pH value was 7.0 ± 0.115, whereas pH was 7.1 ± 0.152 on 12th day when the shrimp was JU (Fig. 3). There is a positive correlation between storage period and pH ($r^2 = 0.9007$) indicating (Fig. 6). However there is a good relationship of pH with TVB-N and TMA-N that shows in Fig. 7.

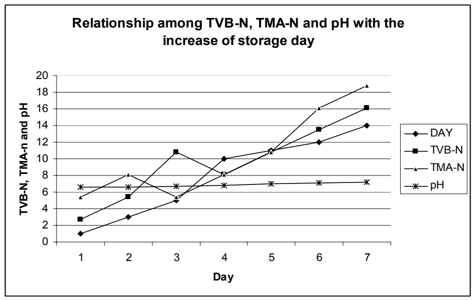

Fig. 7. Relationships among TVB-N, TMA-N and pH with the increase of storage day.

Discussion

Total volatile base-nitrogen (TVB-N)**:** The level of total volatile nitrogenous base increases after initiated the spoilage, both enzymically and bacterially, and thus can be used as an index of spoilage. Using TVB-N as such an index of spoilage does not distinguish the origin or component of these volatile compounds, hence it use in more general. The use of TVB-N as an index of spoilage was first proposed by Shewan (1976). Based of the nature of the spoilage, it is assumed that the low value of TVB-N is an indication of quality of fresh shrimp initially while the high value may be due to action of autolytic enzymes and spoilage bacteria which might have passed their lag pH.

In the present study, the shrimp had initial TVB-N value in the range of 2.692± 0.172 mg N/100g during iced storage; on the other hand the TVB-N content in Bele (*Glossogobius giuris*) and puti (*Puntius stigma*) were 11.45 mg/100g and 17.84 mg/100g respectively as fish were treated as highly acceptable (Rubbi *et al.*, 1985). Dassow (1963) recommended that TVB-N levels of 10 mg/100g or less for fresh fish, 20 – 30 mg/100g for beginning of spoilage and over 30 mg/100g for spoiled fish. Azam *et al.* (1997) recorded a very high TVB-N value (17.03 mg-N) initially for tilapia. Similarly Mlay *et al.* (1985) also observed very high value of TVB-N (51.8 mg). However, the TVB value of the present study agrees with the findings of Dassow (1963).

After 10^{th} day of storage, the TVB-N value becomes reduced. It might be due to the presence of ammonia constituent in TVB-N that was leached out by melting ice. During iced storage experiment with Roundfish, TVB-N contents stayed typically on the level met in freshly caught shrimp during the first week of iced storage that decreased somewhat due to its soluble constituents alike ammonia which is being leached out with melting ice (Oehlenschlager, 1996). Nonetheless, the TVB-N value increased progressively with increased storage period in later storage (Adebona, 1982). However, TVB-N increases slowly during the chilled storage of most freshwater fish (Nair *et al.*, 1971), principally because of their low negligible content of Tri-Methyl-Amine oxide.

Tri-methyl-amine nitrogen (TMA - N)**:** TMA-N is an excellent indicator for the onset of spoilage and for the different stages of spoilage, because of its universal production in all shrimp and fish species. The fishy odor is produced when TMA-N reacts with fat in the muscle of shrimp/ fish (Oehlenschlager, 1996). In the course of spoilage, many off-odors are produced by bacteria, indicating the onset and development of spoilage (Reineccius, 1979). More TMA-N is produced from TMAO by bacterial action than by fish tissue enzymes. Both these two actions are responsible for the 'fish odor' during spoilage (Singleton and Sainsbury, 1978). The participation of cytochromes as election carriers together with the enzyme TMAO reductase is responsible for the bacterial reduction of TMAO to TMA-N (Sakaguchi and Kawai, 1982). Beatty (1938) found that at least 94% of TMA-N in spoiling fish originates from TMAO. The result obtained (Fig. 2) in this experiment, support that there is an increase in the TMA-N content of *P. monodon* after 10^{th} day of storage up to the complete spoilage of the sample and the value of TMA-N at the end of 14^{th} day of storage is 18.143 mg/100g. The increase of this value in relation to the storage days is statistically significant and shows positive correlation (Fig. 5).

Beatty (1938) suggested a TMA-N content of 4–6 mg/100g as the critical value for the edibility of fish, while Liston (1980) recommended 10–15 mg/100g for human consumption. There is a wide variation in critical values suggested for individual species, like 5–7 mg/100g for herring (Sigurdisson, 1955) and 1–5 mg/100g for haddock (Love, 1980). Though, on the rejection, the level of TMA-N studied in the present investigation was bellowing the limits suggested by Liston (1980) and the suggestion of Sigurdisson (1955) appears to be applicable to fish and shrimp studied. However, the TMA-N value determined does not seem solely to be useful as an index of freshness.

As definite bacteria produce TMA-N and this bacterial activity is directly affected by temperature, therefore temperature gradients in individual shrimp can affect TMA-N levels (Azam *et al.*, 1997). Foegeding *et al.* (1996) also found a sudden increase in TMA-N (> 10 mg %) to be concurrent with the onset of bacterial purification. Although bacterial growth begins immediately after the resolution of rigor, TMA-N formation does not exist for several days (Love, 1980). Quite often, the TMA-N test is inadequate in determining the onset of spoilage for this reason (Azam *et al.*, 1997; Laycock and Regier, 1971).

pH: The muscle of living fish, in general, are approximately neutral i.e. its pH is near 7.0. Due to the post-mortem anaerobic formation of lactic acid from glycogen by series of reaction, usually the pH value decreases (Tarr, 1954). As spoilage advances, the pH value rises first slowly and later quite rapidly. The increase in pH values can be attributed to the higher levels of volatile compounds produced by microbial and enzymes in fish (Reilly *et al.,* 1985). The initial post-mortem pH varies with species, catching ground and season (Love, 1980). Over a range of 14 days of storage trial, pH in *P. monodon* varied between 6.6 ± 0.1 to 7.2 ± 0.152. It was observed that pH value shows upward movement with the progress of storage days.

Reilly *et al.* (1985) observed that the pH value of the *P. monodon* ranged from 7.1-8.1 stored in ice. pH in sardine (*Sardine pilchards*) increase between 6.35-6.77 during iced storage situation (Ababoueb *et al*., 1976). pH increased rapidly from 6.2 to 7.5 at day 14 in gold line sea bream during ice storage condition (Adebona, 1982). However, the pH value reached 7.2 in *P. monodon* at end of 14 days storage in ice situation in this experiment. It may be due to alkalinity of ammonia and amino compounds formed during bacterial portends formed of storage period (Poulter and Nicolaides, 1985). Ababoueb *et al.* (1976) reported that pH had little influence on the textural deterioration of chub mackerel under storage in ice.

Conclusion

The bio-chemical characteristics of ice-stored shrimp are important for economically viable entrepreneurship in Bangladesh, particularly for exporting this product to foreign countries to earn the foreign currencies. This experiment has found out some important bio-chemical factors in ice stored shrimp i.e. which conditions are spoilage situations and which situations is good enough to export the products. For example, the shrimp in iced storage ranged in terms of TVB-N between 2.692 ± 0.172 to 16.118 ±0 .032 mg/100g. This result is highly positive relationship (r^2 = 0.7722) between storage days and TVB-N values. For TMA content in *P. monodon* stored in ice, the acceptable limit was recorded in range of 5.385±0.026458 to 10.764±0.036056 mg/100g, in which the statistical analysis indicated significant differences ($p \leq 0.05$) between storage time and TMA-N values during the storage period in ice. The overall results indicate that the TVB-N and TMA-N contents in *P. monodon* stored in ice rose as spoilage advanced. pH also increased with the increase in spoilage cause by bacteria. Therefore the experiment recommend the use of adequate ice for storage the shrimp by ice just after catching from the pond or gher to keep the quality good up to reach the processing plant for further processing and preservation. It is recommended to use double ice of shrimp weight that means 2:1. Because without using the ice, blackening of shrimp carapace, softening the shell, reddish color of the shrimp, increase the water activity, trigger the microbial activity which causes spoilage of the product, loss of product due to spoilage, decrease the demand of the product in both domestic and international markets, and causing lower price of the product have been occurred.

Acknowledgement: The authors wish to thank to the head of the FMRT Discipline of Khulna University for giving us the permission to use the Microbiology Laboratory for this work.

References

Ababoueb, L.H.; Souribri, L.; Rhaliby, K.; Ouahdi, O.; Bttal, M. and Busta, F.F. 1996. Quality changes in sardines (*Sardina pilchardes*) stored in ice and at ambient temperature. *Food Microbiology,* 13: 123-132.

Adebona, M.B. 1982. *Studies on thePreservation of Fish by Ice*. FAO Fisheries Report, Rome, Italy, pp. 27-31.

Anon. 1997. *Coastal Shrimp Aquaculture in Asia*. FAO Fisheries Report, Rome, Italy.

Azam, K.; Rahman, M.S.; Miah, M.A.H. and Smith, J. 1997. Quality assessment of tilapia *(Oreochromis niltlica)* during iced storage. *Progressive Agriculture*, 8 (1 & 2): 175-80.

Beatty, S.A. 1938. Studies of fish spoilage: its origin of tri-methyl-amine product during the spoilage of cod muscles press juice. *Journal of Fisheries Research Board of Canada,* 4: 63-68.

Dassow, J.W. 1963. *Handling of fresh fish in industrial technology*. Reinhold Publishing Corp, New York, pp. 276.

Foegeding, F.; Cassidy, P. and Horner, W. 1996. Characteristics of edible muscle tissue. In: *Food Chemistry*. 3rd edn., Blackwell Scientific Publication, Oxford.

Laycock, R.A. and Regier, L.W. 1971. Trimethylamine producing bacteria on Haddock *(Melanogrammus aeglefinus)* fillets during refrigerated storage. *Journal of Fisheries Research Board of Canada,* 28 (3): 306-309.

Liston, J. 1980. Microbiology in fishery science. p. 138. In: Connell, J.J. and Staff of the Torry Research Station (eds.), *Advances in Fisheries Science and Technology*. Fishing News Books, London.

Love, R.M. 1980. *The Chemical Biology of Fishes*.Vol. 2, Academic Press, London.

Mlay, M.L.; Kikreh, S.M.; Kirenga, J.; Nichodemus, F.L.A.; Karnicki, Z.S.; Santos, C.; Dos, A.M.L.; Nyagambi, J. and Buga, M. 1985. Effect of planting practices on the keeping quality of fish caught in lake Victoria. p. 8. In: Reilly, A. (ed.), *Spoilage of Tropical Fish and Product Development*. Food and Agricultural Organization, Rome, Italy.

Nair, R.B.; Tharamni, P.K. and Loburg, N.N. 1971. Studies on chilled storage of freshwater fish. *Journal of Food Science and Technology*, Mysore, 8: 53-56.

Oehlenschlagler, J. 1996. Suitability of ammnia -N, dimethylamine -N, trimethylamine -N, trimethylamine Oxide -N and volatile basic nitrogen -N as freshness indicator in sea food. pp. 93-101. In: *Methods to Determine the Freshness of Fish in Research and Industry*. United Conference, November 12-14, 1997.

Poulter, N.H. and Nicolaides, L. 1985. Quality changes in Bolivian freshwater fish species during storage in ice. p. 13. In: Reilly, A. (ed.), *Spoilage of Tropical Fish and Product Development*. Food and Agricultural Organization, Rome.

Ranke, B. 1959. Microbiology in fishery science. *Architecture of Fish Conservation,* 1: 117.

Rasel, A.S.M. 2002. Shelflife of tilapia *(Tilapia nilotica)* and bhetki *(Lates calcarifer)* stored in ice and ambient temperature. MSc. Thesis, Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh.

Reilly, A.; Bernate, M.A. and Dangla, E. 1985. Quality changes in brackish water prawns *(Penaeus monodon*) during storage at ambient temperature in ice and after delays in icing. p. 72. In: Reilly, A. (ed.), *Spoilage of Tropical Fish and Product Development*. Food and Agricultural Organization, Rome.

Reineccius, G.A. 1979. Off-flavors in meat and fish: A review. *Journal of Food Science*, 44 (l): 12-24.

Rubbi, S.F.; Rahaman, M.M.; Khan, A.R.; Jahan, S.S. and Begum, M. 1985. Proximate composition and quality of some commercial species of freshwater fish. *Bangladesh Journal of Science,* 5 (1): 1-20.

Sakaguchi, M. and Kawai, A. 1982. Changes in free amino acids and creatine contents in yellowtail *(Seriola quinqueradiata)* muscle during ice storage. *Journal of Food Science*, 47 (3): 1662-1666.

Shewan, J.M. 1976. The bacteriology of fresh and spoilage fish and biochemical changes induced by bacterial action. pp. 377-94. In: *Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish*. Tropical Products Institute, London.

Shewan, J.M. and Ehrenberg, R.T. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. pp. 51-66. In: *Handling, Processing and Marketing of Tropical Fish*. Tropical Products Institute, London.

Siang, N.C. and Kim, L.L. 1992. Determination of trimethylamine oxide, trimethylamine and total volatile basic nitrogen by Conway's Micro-Diffusion Method. In: Miwa, Ji. (eds.), *Laboratory Manual on Analytical Methods and Procedures for Fish and Fisheries Products*, Southeast Asia Fisheries Development Center, B3.1-B3.6.

Sigurdsson, G.J. 1955. Comparison of chemical tests of the quality of fish. *Anal. Chem.,* 19: 892-902.

Singleton, P. and Sainsbury, D. 1978. *Dictionary of Microbiology*. John Wiley and Sons, New York.

Tarr, H.L.A. 1954. Microbiological deterioration of fish post mortem: its detection and control. *Bacteriology Review,* 12: 1-15.

Wood, A.J.; Sigurdission, G.J. and Dyer, W.J. 1942. The surface concept in measurement of fish spoilage. *Journal of Fisheries Research,* 6 (1): 53-60.