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Post mortem variation in Total Volatile Base Nitrogen and Trimethylamine Nitrogen between Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*)

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Abstract: Total Volatile Base Nitrgen (TVB-N) and Trimethylamine Nitrogen (TMA-N) of Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*) at ambient temperature was compared at Fish Quality Control Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University, Bangladesh. The TVB-N and TMA-N were determined by using Conway's Micro-diffusion Technique. The nature of biochemical changes was studied up to complete spoilage. The TVB-N contents of Galda and Bagda ranged between 6.65±0.12 mg-N/100g to 8475±0.22 mg-N/100g and 6.72±0.18 mg-N/100g to 91.43±0.49 mg-N/100g. The TMA-N contents of Galda and Bagda ranged between 6.64±0.12 mg-N/100g to 39.38±0.33 mg-N/100g and 6.81±0.17 mg-N/100g to 71.41±0.35 mg-N/100g. A slight higher amount of TVB-N was observed at all stages in Galda as compared to Bagda. TMA-N contents significantly varied between Galda and Bagda (P≤0.05). At all stages of spoilage Bagda showed higher amount of TMA-N.

Keywords: Post mortem, TVB-N, TMA-N, Galda and Bagda.

Introduction

Spoilage is the result of whole series of complicated deteriorative changes brought about in dead fish tissue 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 (Jones, 1954). After death of fish, the oxygen supply in the tissue ceases due to disruption of the circulatory system. In short time of post-mortem, the mitochondrial system ceases to function. Adenosine triphosphate (ATP) is gradually depleted through the action of various ATPase. After residual supplies of creatine phosphate have been depleted, anaerobic glycolysis continue to regenerate some ATP with the end product, lactate accumulation (Foegeding *et al.,* 1996).

Due to formation of lactic acid from glycogen by a series of enzymatic reaction in the tissue which decrease pH value. When the pH reaches a critical value, the ultimate pH, certain critical enzymes, especially phosphofructokinase, are inhibited and glycolysis ceases A drop in the pH of the muscle triggers the release of proteolytic enzymes such as *cathepsin*. Enzymes from spoilage microorganisms can metabolize the amino acids of the fish muscle producing a wide variety of volatile compounds resulting off-flavors and odors. The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage. Total volatile nitrogen has been widely used as an index for freshness of fish (Stansby *et al.,* 1944). The increase in the amount of TVB parallel with the increase in TMA during spoilage. As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA. In Bangladesh Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*) are more important for their fast growth, lucrative size, good taste and high market demand etc. *M. rosenbergii* is locally called Galda. Galda is the largest among all prawns inhabiting fresh or slightly saline water.

Bagda plays a vital role in the national economy of Bangladesh and its contribution to export earning is rapidly increasing. Bangladesh produces 2.5% of the global production of Bagda. The Bagda contributes 92.74% among all exportable fish and fishery product. So far, information on TVB-N and TMA-N contents of Bagda and Galda is scanty in Bangladesh. This leads to the present study. The study was aimed at observing the changes in TVB–N and TMA-N contents of Galda and Bagda.

Materials and Methods

Sample collection and preparation

Bagda and Galda were collected at live condition with an average weight of 90-100g from Gher near Botiaghata Thana and Dumuria thana under Khulna district of Bangladesh, from November to

December, 2008. Total 6 individuals (three of each species) were collected and immediately brought to the Quality Control Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University and kept at ambient temperature (21-23ºC) in plastic tray. Two gram pooled sample was used for biochemical assessment. Experiments were carried out in triplicate for each species.

Technique of analysis

TVB-N and TMA-N were determined according to the procedure of Siang & Kim (1992) by using Conway's Microdiffusion Unit.

The extract was prepared by mixing 2g of the minced/grinded fish muscle with 8ml of 4% TCA in a 50ml beaker/Mackertny bottle and was homogenized properly. It was left for 30 minutes at ambient temperature with occasional grinding. After then it was filtered through filter paper. Then the filtered solution was kept in Mackertny bottle and was labeled.

Three Conway's units were taken which had been thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer ring of each unit was applied sealing agent (Vaseline). Using a micropipette, 1ml of the solution was pipetted into the inner ring of each unit. In to the outer ring of each unit, 1 ml of the sample extract was pipetted. One ml of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit carefully, to prevent any entering the inner ring, and immediately the units were covered and closed with clip. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. The units were placed in an incubator at 37°C for 60 minutes. After that, the units' covers were removed and the inner ring solution, now a green color, was titrated with 0.02N HCl using a burette (50ml) until green colored solution turned to pink. An average titrate volume of HCl was found from the results of three titrations for each fish species muscle sample. For each value the TVB-N values were calculated. A blank test was also carried out using 1ml of 1% TCA, instead of sample extract.

Trimethylamine in species muscle was determined by the Conway technique, which is same as TVB-N determination but prior to addition of potassium carbonate (K_2CO_3), 1ml of 10% neutralized formalin was pipetted to the extract to react with ammonia and thus allow only the TMA-N to diffuse over the unit.

The overall acceptability was measured by a slightly modified score sheet developed by Shewan & Ehrenberg (1977).

Data analysis

Data were analysed by SPSS and Microsoft Excel. ANOVA test were done to observe the variation in TVB-N and TMA-N between Galda and Bagda.

Results

Total Volatile Base Nitrogen (TVB–N) in Galda

The TVB-N contents of Galda and the relation between overall quality acceptance and TVB-N is given in Table 1. The TVB–N value obtained over 60 hours of storage of Galda at ambient temperature ranged between 6.65±0.12 to 84.75±0.22 mg-N/100g. The fish was 'Moderately Acceptable (MA)' up to 35th hour of storage with 39.48±.42 mg-N/100g and at the 40th hour it was considered as 'Just Unacceptable (JU) the value was 46.11±0.09 mg-N/100g. The fish was unacceptable up to the end of the experiment and the last value was 84.75±0.22 mg-N/100g (Table 1). TVB-N content in Galda changes positively over storage period (Table 3).

Table 1: TVB-N contents of Galda (*M. rosenbergii*) and Bagda (*P. monodon*) with time intervals.

Galda (*M. rosenbergii*)			Bagda (*P. monodon*)		
Hours	TVB-N (mg-N/100g)	Accept-ability	Hours	TVB-N (mg-N/100g)	Accept-ability
0	6.65±0.12	HA	0	6.72±.0.18	HA
5	6.81±0.17	A	5	6.81±.0.17	A
10	13.25±0.21	A	10	13.58±0.36	A
15	13.57±0.37	A	15	20.03±0.02	MA
20	19.66±0.57	MA	20	26.77±0.19	MA
25	26.40±0.34	MA	25	33.50±0.44	MA
30	33.45±0.47	MA	30	39.55±0..47	JU
35	39.48±0..42	MA	35	46.57±0.37	U
40	46.11±0.09	JU	40	52.57±0.49	U
44	52.83±0.15	U	45	65.63±0.54	U
50	65.86±0.12	U	50	72.58±0.36	U
55	71.95±0.05	U	55	78.75±0.22	U
60	84.75±0.22	U	60	91.43±0.49	U

Trimethylamine Nitrogen (TMA-N) in Galda

Trymethylamine Nitrogen contents of Galda are presented in Table 2 which ranged between 6.64±0.12 to 45.38±0.33mg-N/100g. Initially TMA-N content was 6.64±0.12mg-N/100g and it was in highly acceptable (HA). The fish was 'Moderately Acceptable (MA)' up to 35th hour of storage (19.95±0.08mg-N/100g) and at 40th hour

it was 'Just Unacceptable (26.12±0.19 mg-N/100g).The fish was unacceptable up to the end of the experiment and the last value of the experiment (45.38±0.33 mg-N/100g). TMA-N content in Galda changes positively over storage period (Table 2).

Table 2: TMA-N contents of Galda (*M. rosenbergii*) and Bagda (*P. monodon*) with time intervals.

Galda *(M. rosenbergii)*			Bagda (*P. monodon*)		
Hours	TVB-N (mg-N/100g)	Accept-ability	Hours	TVB-N (mg-N/100g)	Accept-ability
0	6.64±0.12	HA	0	6.81±.0.17	HA
5	6.74±0.12	A	5	10.81±.0.17	A
10	6.79±0.17	A	10	13.25±0.21	A
15	13.24±0.21	A	15	13.57±0.37	MA
20	13.55±0.39	MA	20	19.66±0.57	MA
25	19.64±0.55	MA	25	26.40±0.34	MA
30	19.94±0.05	MA	30	33.12±0.11	JU
35	19.95±0.08	MA	35	33.41±0.52	U
40	26.12±0.19	JU	40	39.37±0.33	U
44	26.79±0.36	U	45	46.44±0.48	U
50	32.6±0.51	U	50	59.15±0.13	U
55	39.48±0.45	U	55	65.35±0.	U
60	45.38±0.33	U	60	71.41±0.35	U

Total Volatile Base Nitrogen (TVB–N) in Bagda

The TVB-N contents of Bagda at ambient temperature ranged between 6.72±0.18 to 91.43±0.49mg-N/100g (Table 1). The fish was 'Moderately Acceptable (MA)' up to 25th hour of storage (33.50±0.44 mg-N/100g) and at the 30th hour it was considered as 'Just Unacceptable (39.55±0.47mg-N/100g).The fish was unacceptable up to the end of the experiment and the last value of the experiment was 91.43±0.49mg-N/100g.

Table 4 shows that TVB-N content in Bagda changes positively over storage period. There was an excellent positive correlation (r =.993) between storage time and TVB-N

Trimethyl amine Nitrogen (TMA-N) in Bagda

TMA-N contents of Bagda are at ambient temperature ranged between 6.81±0.17 to 71.41±0.35 mg-N/100g (Table 2). Initially TMA-N content was 6.81±0.17mg-N/100g and it was in highly acceptable (HA). The fish was 'Moderately Acceptable (MA)' up to 25th hour of storage (26.40±0034mg-N/100g) and at 30th hour it was considered as 'Just Unacceptable (33.12±0.11 mg-N/100g). The fish was unacceptable up to the end of the experiment (71.41±0.35 mg-N/100g). TMA-N content in Bagda changes positively over storage period (Table 4).

Table 3: Correlation between different parameters in Galda (*M. rosenbergii*).

		Hour	TMA	TVB
Hour	Pearson Correlation	1	0.980**	0.982**
	Sig. (2-tailed)	.	0.000	0.000
	N	13	13	13
TMA	Pearson Correlation	0.980**	1	0.978**
	Sig. (2-tailed)	0.000	.	0.000
	N	13	13	13
TVB	Pearson Correlation	0.982**	0.978**	1
	Sig. (2-tailed)	0.000	0.000	.
	N	13	13	13

** Correlation is significant at the 0.01 level.

Table 4: Correlation between different parameters in Bagda (*P. monodon).*

		Hour	TMA	TVB
Hour	Pearson Correlation	1	0.989**	0.993**
	Sig. (2-tailed)	.	0.000	0.000
	N	13	13	13
TMA	Pearson Correlation	0.989**	1	0.992**
	Sig. (2-tailed)	0.000	.	0.000
	N	13	13	13
TVB	Pearson Correlation	0.993**	0.992**	1
	Sig. (2-tailed)	0.000	0.000	.
	N	13	13	13

** Correlation is significant at the 0.01 level.

Discussion

The volatile bases, in determination method, are aerated or distilled off from a midley alkaline fish extract, collected in standard acid and measured by titration (Stansby & Olcott, 1963)**.** Kimura and Kiamakura (1934) recommended TVBN levels of 10 mg/100g or less for fresh fish, 20–30mg/100g for beginning of spoilage and over 30mg/100g for spoiled fish. Reilly *et al.* (1985) stated that TVB-N are not reliable as indices of quality. Boee *et al.* (1982) working on the storage of shrimp has observed on the storage of shrimp has observed that TVB-N increased evenly. In the present study, the Galda had initial TVB-N value as 6.65±0.12 mg-N/100g and the Bagda had 6.72±0.18 mg-N/100g at ambient temperature. The TVB value of the present study agrees with the recommendation of Kimura & Kiamakura (1934), that means Galda spoiled at 40 hours and at Bagda 30 hours. TVB-N of whiting (*Sillago maculates)* showed slow increase during the first half of storage, and levels increases more rapidly during the later stage at ambient temperature (Estrada *et al.* 1985). At ambient temperature, TVB-N was found to increase in sardine (*Sardina pilchardus*) to reach 57.3 mg/100g of fish at spoilage. The results in the present study were similar to Whiting. Under the local conditions TMA was found to be a good indicator of freshness for

white Pomfret, Chinese, Pomfret & Grouper (Siang and Kim, 1992). The use of TMA-N, as an index of fish freshness, was proposed by Beatty & Gibbons (1936) first. This was based on the observation that the production of TMA was dependent on the bacterial activity as well as from endogenous enzyme. In the present study the acceptable value was 19.95±0.08mg/100g in Galda at 35th hour and 26.40±0.34 mg/100g in Bagda at 25th hour. Connell (1975) recommended 10–15mg/100g for human consumption. There is also wide variation in critical values suggested for individual species, like 5–7 mg/100g for herring and 1–5mg/100g for haddock (Castell & Triggs, 1955). The accepted limit of TMA-N is 12-15 mgN/100g (Monotgomery *et al.*, 1970). The results was found in the present investigation indicated much higher value during the storage period. Horie & Sekine (1956) 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 for several days (Connell, 1975). Quite often, the TMA-N test is inadequate in determining the onset of spoilage for this reason (Laycock & Regier, 1971).

Conclusion

There was no significant variation ($p<0.5$) in TVB-N contents between Galda and Bagda but a slight higher TVB-N was observed at all stages of Galda compared to Bagda. TMA-N contents varied significantly ($p<0.5$) between Galda and Bagda. In all cases Bagda showed higher amounts of TMA-N than the Galda.

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