Biochemical changes during chilled storage of *Macrobrachium rosenbergii* (De Man 1879) and evaluation of freshness

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Abstract

Chilled storage characteristics of farmed *Macrobrachium rosenbergii* are discussed. Fresh whole samples could be kept in iced storage in acceptable condition for a period of 12 days. The prime quality of the prawns was retained for a period of 6 days. TVBN values showed an increase from 11.2 mg % to 28 mg % by 12 days of iced storage. The bacterial load was in the order 10^4 to 10^6 cfu g^{-1} throughout the chilled storage. Sensory evaluation of the whole prawns and cooked meat was also carried out. Nucleotide degradation in chilled storage was studied. Hypoxanthine levels increased significantly after six days of iced storage. The utility of K value as an indicator of freshness in the chilled storage of *M. rosenbergii* was also investigated.

Keywords: *Macrobrachium rosenbergii,* chilled storage, quality, Hypoxanthine, K value.

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, has good demand in export and domestic markets. Its capability to grow in freshwater and brackishwater habitats, maximum growth rate, omnivorous feeding and hardy nature makes it one of the most suitable species among the palaemonid prawns for aquaculture. It is exported in the trade name "scampi" from India. Resurgence in the farming of this species in India has been noticed in recent years due to the ready availability of seeds. In Kerala the fishery of this species is mainly confined to Vembanad Lake and adjoining rivers, where the exploited stock is estimated to be about 112.8 mt per annum.

Changes in the biochemical characteristics of important species of penaeid prawns during chilled storage have been studied in detail. (Velankar & Govindan 1958,1959; Govindan 1962a, 1962b, 1964; Nair *et. al.* 1962; Susamma *et al.* 1962; Mukundan *et al.* 1981; Achuthankutty & Parulaker 1984). Similar studies on palaemonid prawns are comparatively recent, but not extensively covered as in the case of penaeids. Some aspects of biochemical and nutrient composition, handling and processing, chilled and frozen storage of *M. rosenbergii* were

reported. (Angel *et al.* 1981,1985; Joseph *et al.* 1991; Joseph *et al.* 1992; Sherief *et al.* 1992; Rodrigues *et al.* 2000; Jayasekharan & Ayyappan 2002; Selvakumar *et al.* 2002). These studies have indicated that in general the shelf life of this species in ice is around six to eight days followed by a phase of lowered quality acceptable up to 12 to 13 days. The protein content of this species is also moderately high and does not vary significantly between different morphometric groups. The post-harvest handling of this species involves keeping the catch at ambient temperatures or in ice for more than six hours in the case of local sales or processing for export. This may lead to considerable changes in the quality of the product. Hence the objective of the present study is to assess the quality of the ice stored whole *M. rosenbergii* by biochemical and sensory evaluation methods. Nucleotide degradation during iced storage and the utility of K value as index of freshness for this species are also reported.

Materials and methods

Collection of samples

Macrobrachium rosenbergii reared in a farm near Kochi was used for the study. Live samples collected from the farm during harvest were immediately killed by immersing in chilled water and the whole prawns were packed in flake ice in the ratio of 1:1 (w/w) in insulated thermocole boxes and brought to the laboratory within two hours. Re-icing was done every day. Samples were drawn every two days for the analyses.

Biochemical and sensory evaluation

For biochemical analyses, the minced meat of the prawn was used. Moisture, fat, nitrogen (TN & NPN) and ash contents of the samples were estimated according to AOAC (2000) methods. The changes in water-soluble nitrogen (WSN) was determined by the method of Bligh & Dyer (1959). TVBN was determined by the microdiffusion method of Conway (1962) and alpha amino nitrogen by the method of Pope & Stevens (1939). The total plate count was carried out by standard pour plate method (APHA 1976). Sensory evaluation was done by a taste panel of eight members on raw and cooked samples using a 10-point hedonic scale (Hill & Glew 1973) and the statistical mean was accepted as the sensory score. A score of 4 was fixed as the limit for acceptability. The pH was measured on 2:1 water: prawn homogenate using a Cyberscan 510 pH meter.

Determination of K value

K value and the amount of different nucleotide degradation products were determined in HPLC by the method described by Ryder (1985). A Merck-Hitachi Interface D – 7 000 Liquid Chromatograph (Merck Ltd., Germany) with model L – 7 100 quaternary gradient pump, Rheodyne Model 7 125 syringe as loading sample injector & Merck-Hitachi Model L – 7 400 UV detector set at 254 nm was used. A Lichrospher™ 100 C – 18 encapped Reverse phase (5 µm) column was used. Homogenization of the sample was performed in T 25 basic Ultra-Turrax™ homogenizer from IKA Labortechnik, USA. Centrifugation was performed in a REMI cooling Compifuge (model CPR 30).

A sample of 5 g of the prawn muscle was taken and 50 ml of chilled 0.6 M perchloric acid was added to it. It was homogenized at 0 °C for 1 min. The homogenate was centrifuged at 6 000 rpm

for 20 min at 4 °C. Ten ml of the supernatant was taken and neutralized to pH 6.5 – 6.8 with chilled 1 M KOH immediately. After standing at 0 °C for 30 min, it was filtered through a syringe filter of pore size 0.45 µm. This was stored at – 30 °C for subsequent HPLC analysis. The samples were prepared in duplicate for each analysis.

Chromatographic separation was achieved by isocratic elution with phosphate buffer solution prepared by mixing of 0.04 M potassium dihydrogen phosphate (KH_2PO_4) and 0.06 M dipotassium hydrogen phosphate (K_2HPO_4) in deionized water and filtered through 0.45 µm filter paper. The flow rate in HPLC was maintained at 1.5 ml min^{-1} with pressure ranging from 2 800 – 2 900 psi throughout the chromatographic separation. All solutions were passed through 0.45 µm filter paper prior to the injection onto the column.

Twenty µL of the sample was injected into the HPLC and the elute was monitored at 254 nm. The peaks obtained from the sample were identified by comparing with the peak of chromatogram of the standard solutions. The quantification of each nucleotide breakdown products was done by comparing the peak area of the samples with the peak area of the standards corresponding to each sample.

ATP, ADP, AMP, IMP, Inosine and Hypoxanthine standards used for K value estimation were supplied by Sigma Chemical Co., St Louise, USA. KH_2PO_4 and K_2HPO_4 used for phosphate buffer preparation in K value estimation were supplied by B.D.H. Laboratory, England. De-ionized water for using in HPLC was collected from Millipore filter system (cat. no. – QTUM 0001X) supplied by Millipore, Bangalore, India.

Results and discussion

Proximate composition

The length and weight of the samples used for the study were 16.7 ± 1.16 cm and 55.5 ± 10.39 g, respectively. The meat yield was 31.29 ± 5.55 g. Table 1 gives the proximate composition of fresh *M. rosenbergii*. The values were almost comparable to that reported for other species of prawns (Mukundan *et al.* 1981; Joseph *et al.* 1991; Shereif *et al.* 1992).

Table 1 Proximate Composition of *Macrobrachium rosenbergii*

Moisture %	78.25
Protein %	19.44
Non protein nitrogen %	0.63
Fat %	1.01
Ash %	1.11

Sensory evaluation

The results of the sensory evaluation are given in Table 2. The sensory score reached the limit of acceptance by 12 days of iced storage. The samples had high quality shelf life up to two days without any noticeable change in sensory characteristics except resolution of rigor. The samples had no off odour either on raw or cooked condition up to six days, but changes in taste and texture were noticed. However, no off odour or taste could be detected in the cooked sample by the panel up to 12 days. Angel *et al.* (1981) reported that the species could be kept in iced condition for 14 days without significant deterioration in organoleptic quality although biochemical parameters increased significantly during this period. The texture of the meat became

Table 2 Sensory changes during iced storage of *Macrobrachium rosenbergii*

Storage period (days)	Sensory characteristics		Score (mean ± SD)
	Whole prawn	Cooked meat	
0	Pre rigor state, shell firm & glossy, no odour. Few specimens were soft shelled.	White colour, sweet taste, tender and juicy.	9.1 ± 0.37
2	Post rigor state, shell firm and glossy	White colour, sweet taste, tender and juicy.	8.7 ± 0.19
4	Slight discolouration of the shell	White colour, sweet taste, tender and moderately juicy.	8.0 ± 0.20
6	Slightly loosened shell in few samples.	Slight off-white colour, slight rubbery texture, sweetness of the meat reduced	7.7 ± 0.23
8	Loosened shell in majority of the samples, faint off-odour, pink discolouration of the raw meat in about 25% of the samples.	Almost bland taste, rubbery texture, not juicy, off-white colour. No off-odour or flavour	6.8 ± 0.89
10	Most of the samples had loose shells, hanging head and discoloured meat, faint persistent off-odour.	Bland taste, texture slightly soft, off-white colour. No off-odour or flavour.	5.5± 0. 45
12	Meat almost detached from shell in 50% of the samples, hanging head and discoloured meat, faint persistent off-odour.	Pale brown colour, No off-odour or flavour, bland taste, texture is mushy and disintegrating.	4.5 ± 0.28
14	Meat separated from shell in most of the samples, discoloured meat, off-odour.	Slight putrid odour and brown colour. Disintegrates in mouth before chewing	3.2 ± 0.55

soft after 10 days in ice and by 14 days it became mushy. Mushiness of the meat was reported after 9 days of storage by Angel *et al.* (1985). The cooked meat was found acceptable up to 12 days in ice although there were marked changes in the appearance of the specimens from 8th day onwards indicated by loose shell, discoloured meat and hanging head. Rodrigues *et al.* (2000) has reported that in the case of headless, shell on samples the prime quality could be retained for 8 days in iced storage.

Biochemical analysis

Table 3 shows the changes in biochemical and bacteriological parameters during iced storage of the prawns. Generally in ice stored prawns the absorption of moisture by the muscle and leaching out of nitrogenous compounds masks the spoilage. In marine prawns the total nitrogen, water-soluble nitrogen and non-protein nitrogen contents of the muscle show rapid change within 8-10 days of iced storage (Govindan 1962a). In

Table 3 Changes in biochemical parameters and bacterial count during iced storage of *Macrobrachium rosenbergii*

Days	Moisture (%)	TN (%)	WSN (mg%)	NPN (mg%)	AAN (mg%)	TVN (mg%)	pH	Bacterial count/g
0	78.25	3.11	1330	700	150	11.2	7.13	1.25×10^6
2	78.56	3.04	1330	630	147	15.4	6.91	2.8×10^5
4	78.85	3.01	1260	630	119	18.2	7.17	1.4×10^5
6	79.02	2.78	1330	610	110	21.0	7.55	1.6×10^4
8	79.51	2.49	980	462	81	25.2	7.59	3.8×10^4
10	80. 02	2.77	910	462	84	28.0	7.89	2.2×10^5
12	80.25	2.45	812	392	70	28.2	7.95	10.5×10^4
14	80.80	2.55	995	385	62	36.5	7.90	6.5×10^5

the case of *M. rosenbergii* the gain in moisture content after 14 days of iced storage was 3.26% and the corresponding loss in total nitrogen content was 18%. There was a marked loss of water-soluble nitrogen (WSN), non-protein nitrogen (NPN) and alpha amino nitrogen (AAN) contents after six days of iced storage. This could have resulted in the loss of flavour and the bland taste of the meat after six days.

Total volatile nitrogen (TVN) value, which is an indicator of spoilage, rose from 11.2 to 36.5 mg% during the iced storage. TVN value crossed the permissible limit of 30 mg % only by the 14th day of storage. Angel *et al.* (1981) reported an increase of TVN value from 22 – 31 mg % after 14 days of ice storage of *M. rosenbergii.* The bacterial count was highest for the extremely fresh samples (10^6 cfu g^{-1}), which subsequently lowered to the range of 10^4 to 10^5 cfu g^{-1} during storage. The bacterial flora of the freshwater prawn from the tropics may be dominated by mesophiles. The low temperature and washing effect of the melt ice could have reduced the bacterial load during iced storage. The pH was slightly alkaline and did not vary much during the storage except for a slight reduction initially.

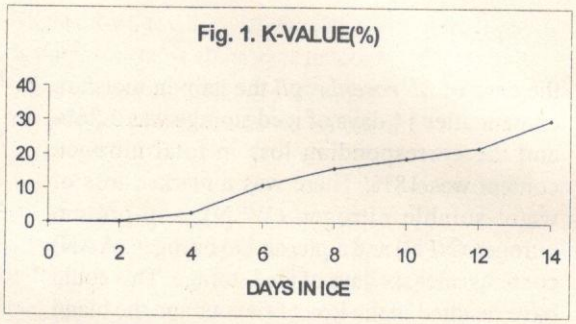
Nucleotide degradation and K value

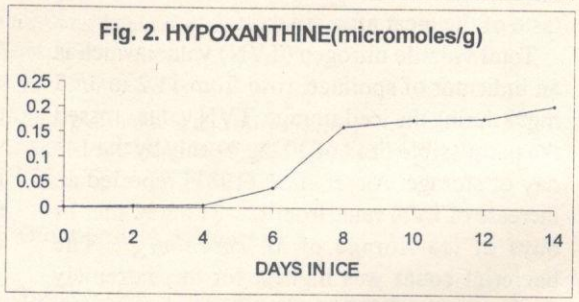
Studies on the nucleotide degradation of marine prawns at different storage conditions have shown that K value is a better indicator of freshness than other biochemical indices (Fatima *et al.* 1981; Matsumoto & Yamanaka 1990; Lakshmanan *et al.* 1998a, 1998b). The K value was below 3% for prime quality fresh prawns and the spoilage was indicated by a K value above 35 – 45%. Hence a value of 20 –30 % is usually considered as good quality prawns. The storage period for many marine species of prawns in ice is 12 – 14 days with K value of 44 – 58% at the limit of acceptance (Lakshmanan & Gopakumar 1999).

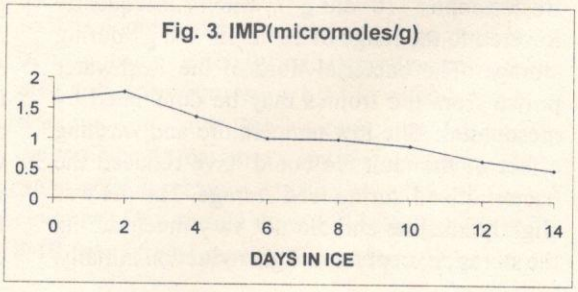
The changes in the nucleotide degradation products and the K value during iced storage of *M. rosenbergii* are given in Table 4 and Figs. 1, 2 & 3. The K value was found to be gradually increasing, and by the time the sample was rejected it reached 30%. The prime quality sample, which was stored in ice up to six days, had a K value of 10.9% and it reached 22% by 12 days. Inosine monophosphate (IMP) values were high during the initial period of storage after which

Table 4 Changes in the nucleotide degradation products and K value during iced storage of *Macrobrachium rosenbergii*

Days	0	2	4	6	8	10	12	14
K-value (%)	0.0302	0.2426	2.5954	10.9233	15.9862	18.9548	22.1498	30.1870
Hx (μ mol g^{-1})	0.0002	0.0026	0.0043	0.0398	0.1623	0.1768	0.1789	0.2063
HxR (μ mol g^{-1})	0.0003	0.0318	0.0391	0.1251	0.0623	0.0601	0.0509	0.0432
IMP (μ mol g^{-1})	1.6326	1.7678	1.3607	1.0095	0.9879	0.8958	0.6524	0.5039
AMP (μ mol g^{-1})	0.0120	0.0064	0.0617	0.0405	0.0293	0.0146	0.0001	0.0001
ADP (μ mol g^{-1})	0.0047	0.0050	0.0390	0.0567	0.0389	0.0313	0.0577	0.0250
ATP (μ mol g^{-1})	0.0086	0.2886	0.1618	0.2374	0.1245	0.0713	0.0976	0.0478







Figs. 1, 2 & 3 Changes in hypoxanthine, IMP and K value during iced storage of *Macrobrachium rosenbergii*

there was gradual decline. It was observed that in this species, adenosine monophosphate (AMP) and IMP levels were less than other species of prawns at the early stages of storage. These give the characteristic flavour for the meat and their low level could be the reason for the absence of any pronounced flavour for the meat of this species even in very fresh condition. The hypoxanthine (Hx) level showed a marked increase after six days of storage; however, at the point of rejection its concentration was only 0.2 μ mol g^{-1}.

Conclusions

The sensory and biochemical evaluation of farmed *M. rosenbergii* stored in ideal iced conditions as whole samples indicated that the prime quality could be retained for a period of 6 days. The samples were in acceptable condition up to 12 days in ice. The quantity of nucleotide degradation products in iced storage was low when compared to penaeid prawns. The K value showed a gradual increase during iced storage and reached 30% at the point of rejection of the samples. The hypoxanthine (Hx) level showed a marked increase after six days of storage; however, its concentration reached only 0.2 μ mol g^{-1} after 14 days in ice. Thus in ice stored *M. rosenbergii*, K value could also be considered along with other biochemical and sensory indices to assess the quality.

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