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AMP and IMP Accumulation in *Penaeus indicus* and *Penaeus monodon* During Iced and Frozen Storage

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Nucleotide degradation profile and quality changes in shrimp, *Penaeus indicus* and *Penaeus monodon* stored in ice and at -18°C were studied. Nucleotide breakdown products like AMP, IMP, inosine and hypoxanthine, and K-value were determined and correlated with sensory characteristics. There was high accumulation of AMP and IMP in both iced and frozen shrimp. K-value of iced *P. monodon* reached 63.4% and of *P. indicus* 74.57% in two weeks. K-value around 20% indicated excellent quality in both species. In frozen samples K-value reached 58.6% in *P. monodon* and 60.2% in *P. indicus* in 12 months. Sensory assessment and measurement of texture also were employed to assess the quality.

Key words: AMP, IMP, inosine, hypoxanthine, K-value

Nucleotide based methods have assumed importance in the assessment of freshness of fish (Amu and Disney, 1973; Martin *et. al.*, 1978; Jacober & Rand, 1982). Individual nucleotides and their ratios have been used to indicate quality of many species of fish (Gill *et. al.*, 1987; Green *et. al.*, 1990; Price *et. al.*, 1991, Hattula and Kiesvaara, 1992; Ryder *et. al.*, 1993; Lakshmanan *et. al.*, 1996). However, very little information is available on the nature of nucleotide degradation in shrimp and its effects on quality. Arai (1966) reported that adenosine triphosphate (ATP) degradation in marine shellfish takes place by a different pathway than in finfish. He proposed two pathways for the breakdown of ATP in Japanese shrimp, *Pandalus hypsinotus*. One involves direct deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP), while the other involves the dephosphorylation of AMP to adenosine (AdR) followed by deamination to inosine (HxR).

Nucleotide degradation in *Penaeus indicus* and *Penaeus monodon* during iced and frozen storage was studied and the levels of AMP, IMP and hypoxanthine (Hx) were correlated with sensory characteristics. K-value as a quality index in processed shrimp was also studied. The results are presented in this paper.

Materials and Methods

Farmed shrimp, *P. indicus* and *P. monodon*, weighing 10-15 and 60-70 g respectively, procured live were headed and iced immediately. One lot was frozen and stored at -18°C. The other lot was iced in the ratio 1:2 and re-iced every day.

The nucleotides and breakdown products were determined using HPLC (Ryder, 1985). Muscle extract with 0.6 M perchloric acid was filtered through 0.45 µm syringe

filter for injection on the HPLC. A Hewlett Packard HPLC (Model 1090) equipped with 5 μ ODS Hypersil column (250 x 4.6 mm) was used. Operating conditions were 100 to 110 bar pressure, 30±1°C temperature, flow rate 1 ml/min and a mobile phase of 0.06 M K_2HPO_4 and 0.04 M KH_2PO_4 at pH 6.5 - 6.8. Eluates were monitored at 254 nm. K-value was computed as defined by Saito *et. al.*, (1959). Volatile base nitrogen was determined by the microdiffusion method of Conway (1962). Sensory evaluation of raw and cooked (steaming for 10 min) samples was done by a trained panel of eight members on a 10 point hedonic scale. Cutting and piercing strength were determined using Rheotex (SD 305).

Results and Discussion

Figs 1 and 2 illustrate the pattern of nucleotide degradation in *P. indicus* and *P. monodon* during storage in ice. The initial levels of total nucleotides, ATP and ADP were 13.45, 1.724 and 2.391 μmol/g respectively in *P. indicus* and 16.03, 4.693 and 4.86 μmol/g in *P. monodon*. By one day in ice, both ATP and ADP declined rapidly and ATP reached insignificant level in both species. However, ADP remained around 0.5 μmol/g throughout storage after one day in both species.

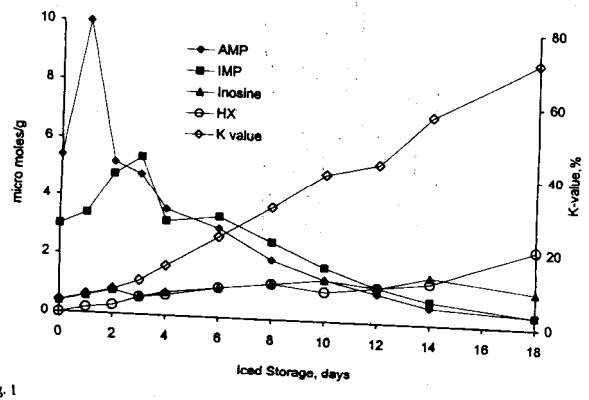

Fig. 1

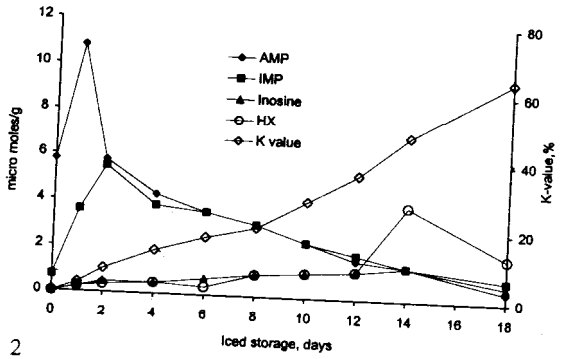

Fig. 2

Pattern of nucleotide degradation in *P. indicus* (Fig. 1) and *P. monodon* (Fig. 2) during iced storage

The initial levels of AMP and IMP were high. AMP reached maximum in 24 h. The high levels of AMP accumulation in the muscle of shrimp is unique in that AMP rapidly deaminates to IMP in fish muscle. After 24 h AMP declined sharply and afterwards only gradually. On 18th day AMP was 1.12 µmol/g in *P. monodon* while it was only 0.45 µmol/g in *P. indicus*. IMP level reached maximum in two days in *P. monodon* and in three days in *P. indicus*. Though it decreased gradually in both species, more than one µmol/g was present in *P. monodon* at the end of 14 days and in *P. indicus* at the end of 12 days.

Hypoxanthine increased slowly during storage and reached 1.73 µmol/g on 14th day in *P. monodon* and 1.28 µmol/g on 12th day in *P. indicus*. It reached the limiting value of 2 µmol/g by 18 days in *P. monodon*. A similar pattern was found in *P. indicus* and the value reached 2.59 µmol/g by 18 days. The data indicate that these are neither inosine nor hypoxanthine accumulating species. The inosine content also increased in the same pattern as Hx up to 14 days and then declined.

Saito *et. al.* (1958) and Suwetja *et. al.* (1989) had studied the accumulation of IMP and AMP in crustaceans. With no or low AMP deaminase activity in marine invertebrates, they concluded that the major pathway of ATP degradation in marine invertebrates is ATP —> ADP —> AMP —> AdR —> Hx R —> Hx. Saito *et. al.* (1958) stated that crustaceans accumulate relatively high amounts of AMP during storage at -5°C. However, in the present study accumulation of both AMP and IMP was observed. Fatima *et. al.* (1981) also observed an accumulation of IMP in *P. merguensis*.

Table 1. Changes in the sensory characteristics of *P. indicus* and *P. monodon* during iced storage

Days	0	2	4	6	8	10	12	14	18
P. indicus									
Raw	Shining fresh sea weedy odour	Shining	Appearance & odour very good	Slightly tough, good odour	No discolo-uration	Slightly faded, no off odour	Slightly bleached appearance	Bleached, slight off odour, few black pieces	Bleached, soft, black-ening
Cooked	Sweet, juicy, soft & firm	Sweet, meaty, soft & firm	Flavour & texture good	Flavour good, soft & firm	Flavour good, firm texture	Flavour good, slightly tough	Less sweet, slightly tough	Bland taste, tough	Bland taste, tough
Overall score	9.4±0.5	9.10±0.6	8.50±0.5	8.0±0.4	7.6±0.5	6.8±0.6	6.1±0.6	5.6	4.4±0.5
P. monodon									
Raw	Shining, fresh sea weedy odour	Shining	Appear-ance & odour very good	Appear-ance & odour good	Appear-ance & odour good	Good appear-ance	Slightly faded appear-ance	Odour & appearance satisfactory	Bleached few black spots, slight off odour
Cooked	Sweet, juicy, soft, firm	Very good flavour/ texture	Sweet, soft & firm	Good flavour/ texture	Sweet, soft & firm	Good flavour & texture	Slightly tough, bland	Bland, no off odour, tough texture	Tough texture, slight off odour
Score	9.6±0.4	9.0±0.5	8.6±0.6	8.2±0.4	7.9±0.5	7.1±0.4	6.5±0.5	6.0±0.6	4.8±0.5

Table 2. Changes in physical, chemical and sensory properties of *P. indicus* during storage at -18°C

Months	0	1	2	3	5	6	8	10	12
TVBN, mg/100 g	16.60±0.8	18.40±1.2	23.60±0.7	28.30±1.04	30.43±0.5	29.6±0.8	30.50±0.7	33.70±0.6	37.85±0.8
pH	6.60	6.94	7.05	7.14	7.20	6.98	7.18	7.3	7.40
Cutting strength, g									
Raw	54±8.6	66±6	48.8±6.5	56.7±5	50±4	46±6	44±11	40±10	36±8
Cooked	365±18.4	460±12	416±6	487±12	501±8	494±7.5	514±16	519±11	526±14
Piercing strength, g									
Raw	54±6.5	46±8	56±4	44±6	52±5	48±6	47±8	41±8	42±6
Cooked	78±5.6	98±5	101±10	93±8	96±6	94±5	97±7	103±14	97±8
Sensory characte-ristics	Very good flavour, soft & firm	Very good flavour & texture	Good flavour, soft & firm	Sweet flavour, soft & firm	Good flavour & texture	Good flavour & texture	Less sweet, slightly tough	Bland, slightly tough	Tough, slightly bitter
Average score	9.4±0.5	8.8±0.6	8.4±0.5	8.0±0.7	7.6±0.8	7.1±0.6	6.8±0.5	6.0±0.6	4.8±0.6

The relatively low initial levels of IMP in the two species seemed to be due to low AMP deaminase activity. From the processing point of view it has a beneficial effect in that AMP would form a continued reserve for IMP and hence retain the characteristic flavour for a longer period. Sakaguchi *et. al.* (1991), while studying the ice storage characteristics of the oyster, observed the accumulation of AMP and IMP in its adductor muscle. This was supported by the finding that the muscle tissue of the bivalves and gastropods have much lower AMP deaminase activities than that of fish and mammals (Fujisawa and Yoshino, 1985; 1987). Sakaguchi *et. al.* (1991) proposed that two path ways are operating in ATP degradation. One involves the direct deamination of AMP to IMP, while the second involves the dephosphorylation of AMP to adenosine, then to inosine and Hx. In the present study it is presumed that both the above pathways are operating in these species as AMP and IMP concentrations ran parallel during ice storage.

K-value increased linearly with time in both species; however, the rate was comparatively faster in *P. indicus* than in *P. monodon.* Sensory assessment showed that both species retained prime quality up to 6 and 8 days in ice when K-values reached 21.72 and 19.68% respectively. After this both species showed significant reduction in freshness, although panelists rated *P. indicus* good upto 12 days and *P. monodon* good upto 14 days (Table 1) with corresponding K-values 44.22 and 46.52%. Prime quality shrimp had a minimum AMP and IMP levels of 3 µmol/g and K-value ~20%. Decline in the levels of AMP and IMP seemed to reduce flavour. Knowledge of the levels of AMP, IMP and K-value of shrimp is helpful in assessing their initial quality. Hx does not appear to be a promising index as it became prominent only after 14 days in ice. For acceptable quality shrimp, the K-value should be in the range 40-45% with AMP+IMP above 2 µmol/g.

Figs 3 and 4 illustrate the average variations in IMP, AMP, Hx and K-value in *P. indicus* and *P. monodon* during frozen storage. There was high AMP and IMP accumulation in both shrimp. The maxima were observed during the first month and thereafter both components started declining, AMP more sharply than IMP. The pattern of nucleotide changes was similar in both species and the K-value was 58-60% after one years storage. AMP and IMP levels remained high in both species. Measurement of texture using Rheotex indicated toughening during storage. The muscle pH increased from 6.6 to 7.6 in both shrimp during this period. TVB-N values are presented in Tables 2 and 3. TVB-N increased steadily and reached 36.75 in *P. monodon* and 38.60 mg/100g in *P. indicus* at the end of 12 months.

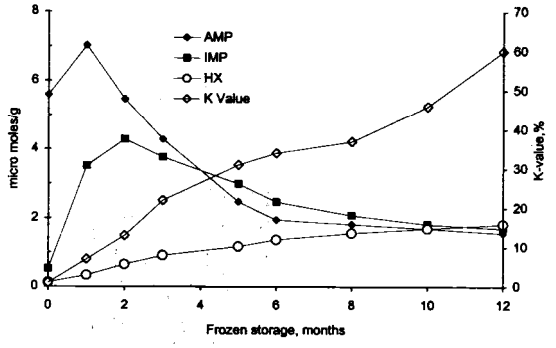

Fig 3

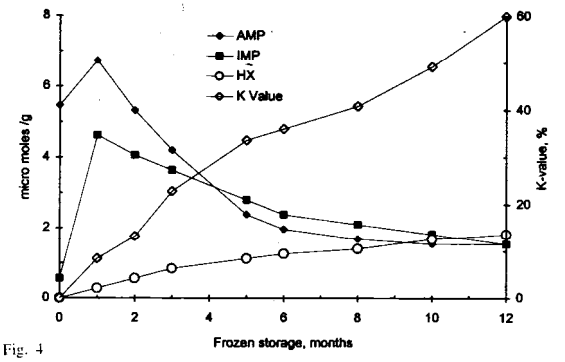

Fig. 4

Pattern of nucleotide degradation in *P. indicus* (Fig. 3) and *P. monodon* (Fig. 4) during frozen storage

Sensory evaluation revealed that samples were good with sweet meaty flavour and soft and firm texture when the IMP level was >2 μmol/g. Both shrimp retained their characteristic flavour and texture upto 8 months when the K-values were 40.3% in *P. monodon* and 46.3% in *P. indicus* and remained acceptable even after 12 months.

Table 3. Changes in physical, chemical and sensory characteristics of *P. monodon* during storage at -18°C

Months	0	1	2	3	5	6	8	10	12
TVBN, mg/100 g	14.59±0.5	21.67±0.7	24.75±0.6	26.67±0.5	28.50±0.46	29.70±0.7	28.60±0.65	32.4±0.7	38.75±0.6
pH	6.68	7.04	7.12	7.16	7.20	7.24	7.30	7.4	7.6
Cutting strength, g									
Raw	58±13.7	56.3±12.5	48.6±4.8	52.6±5	59.7±9.9	64±7	44.7±6.5	49.7±9.8	54.7±16.5
Cooked	320±25	345±18	449±38.6	480±36	477±44	493±23	511±18	523±12	389±17
Piercing strength, g									
Raw	44±3.4	35.4±9.8	40±7.9	39.6±6	40.4±4.1	25.7±5.9	30.5±6	44±7.9	40.4±7.6
Cooked	78±6.3	86.7±21	90.4±15.7	88.4±7	104.2±12	94.2±12	102.45±27	105±6.5	93.4±21
Sensory chracte-ristics	Sweet meaty flavour, soft & firm	Very good flavour & texture	Sweet meaty flavour, soft & firm	Sweet flavour, soft & firm	Good flavour & texture	Good flavour, soft & firm	Less sweet, slightly tough	Slightly tough	Bland, tough
Score	9.6±0.4	9.0±0.6	8.5±0.7	8.2±0.5	8.0±0.55	7.5±0.5	6.8±0.6	6.1±0.4	5.1±0.5

Table 4. Correlation coefficient of storage time, sensory score, K-value and nucleotide degradation products in *P. indicus* and *P. monodon* during iced storage

	Overall score	Storage time
P. indicus		
K-value	-0.9605 [a]	0.9886 [a]
IMP level	0.9220 [a]	-
IMP+AMP	0.9150 [a]	-
Hx	-0.8890 [b]	-
P. monodon		
K-value	-0.9624 [a]	0.9896 [a]
IMP level	0.8276 [b]	
IMP+AMP	0.8535 [b]	
Hx	-0.9549 [a]	

Level of significance a = 0.1%, b = 1.0%

Regression analysis of storage time and overall acceptability score with K-value showed significant relations in both the species (Table 4). K-value showed significant positive correlation with storage time and negative correlation with overall score. A good correlation exists between overall score and IMP concentration as well as the sum of AMP and IMP concentrations. K-value, IMP and AMP appear good indices of shrimp quality. Levels of AMP and IMP in the muscle of frozen shrimp also are indicative of the quality of the raw material before freezing. Kiesvaara *et. al.* (1990) also remarked that K-value and IMP levels can determine the quality of fish. Fatima *et. al.* (1981) found good correlation between IMP and sensory score in shrimp and reported that Hx above 2 µmol/g is indicative of spoilage. The present observations indicate that AMP and IMP correlates with acceptability of the two species studied.

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