PRELIMINARY OBSERVATIONS ON CHANGES IN NUCLEOTIDES IN OIL SARDINE AND CERTAIN PENAEID PRAWNS DURING CHILL STORAGE

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Preliminary study has been made of the changes in common 5' nucleotides in oil sardine (*Sardinella longiceps*) and two penaeid prawns of Indian waters during chill storage. The course of nucleotide degradation has been followed in the fresh fish and shell fish during ice storage. The level of inosine monophosphate (IMP) in prawns showed significant but steady decrease during ice storage and this appears to serve as useful indication of length of storage. Comparison has been made on the pattern of nucleotide changes in block frozen fish and individually quick frozen fish stored at –23°C.

INTRODUCTION

Recent work on nucleotides and their degradation products has shown their importance in the technology of flesh foods mainly in connection with flavour. Contributions made by nucleotides to fish quality during early post mortem storage period are reviewed by Jones (1961), Burt (1965) and Tarr (1966) which provide sufficient evidence to show that 5′ nucleotides possess properties of enhancing desirable flavour and of suppressing undesirable ones to a certain extent. In fish muscle, inosine 5′ monophosphate (IMP) which is produced by the deamination and partial dephosphorylation of adenosine 5′ triphosphate during death struggle is the major constituent possessing this property. Subsequent enzymatic degradation of this compound is suggested as the cause for the loss of flavour during storage (Jones and Murray, 1964). Hypoxanthine formed from inosine, the dephosphorylated product of IMP is believed to account for the development of off flavour in staling fish.

The changes in nucleotides during chill storage of several important fishes of Atlantic and Pacific waters have been exhaustively studied (Saito 1961, Jones and Murray 1962, Kassemsarn *et al* 1963, Guardia and Doller 1965, Dyer *et al* 1966, Fujii *et al* 1966, Tetuo *et al* 1966, Spinelli 1967). No systematic studies are however undertaken on the above lines in the case

of important fishes of Indian waters where the surface temperatures may reach 25 - 30°C. The present paper gives an account of the preliminary investigations made on the general nucleotide pattern in on of the commercially important teleost fishes viz. oil sardine and in two species of penaeid prawns of this region during ice and frozen storage.

Materials and methods

Oil sardine (*Sardinella longiceps*) and two species of prawns (*P. indicus and M. dobsoni*) obtained in rigor state were used for the study. Sardines were procured at Manassery, a fish landing centre in Cochin and prawns from departmental trawler 'FISH TECH'. Sardines were held in ungutted condition and prawns immediately beheaded and stored as 'headless' They were washed free of slime and put in polythene bags to prevent loss of catabolites by leaching and maintained at the temperature of melting ice. For frozen storage studies, the samples were quick frozen in plate freezer (plate temperature: -40° C) for 2½ hours and stored at-23°C. Frozen blocks of sardines and prawns were prepared with 150 ml and 240 ml respectively of glazing water for 450 g of the material. A batch of sardines from the same lot was also individually quick frozen and stored at the same temperature for comparative study.

Preparation of extract

The method of Jones and Murray (1962) has been employed for the preparation of muscle extracts. 40 g muscle was homogenised with 80 ml of 0.6 N perchloric acid at 0°C and filtered. The filtrate was adjusted rapidly to pH 6.5 with 5 N KOH at 0°C and maintained at that temperature for 30 minutes. It was then filtered and made up to a final volume of 250 ml. Neutralised extracts were stored in frozen condition pending analysis.

Total nucleotides were estimated by the method of Lento *et al* (1964) which consisted in eluting the nucleotides absorbed on a column of Dowex 1 x 8 (formate) maintained at 2°C with 4 N HCl. For the estimation of the individual nucleotides, the neutralised extract was chromatographed on a refrigerated column of Dowex 1 x 8 (200–400 mesh, 18.2x1.2 cm). The column was washed with water until the O.D of effluent at 260 μ fell to 0.01. Nucleotides retained on the column were eluted with a gradient of formic acid and water, the fractions scanned in U. V. light to fix the peaks and the fractions corresponding to the peaks pooled and evaluated spectrophotometrically at their U. V. absorption maxima. After separation of IMP the column was eluted with 4 N. formic acid containing 1.2 N sodium formate and combined value of ADP and ATP determined.

Results and discussion

Nucleotide levels in fresh fish.

Analysis of several samples of fresh sardines and prawns have shown that the total nucleotide contcentration varied within very wide limits. The levels found in fresh samples of oil sardine, *Sardinella longiceps* varied between 9.0 & 14.0μM/g expressed as IMP equivalent. In both the species of prawns, *P.indicus* and *M. dobsoni* sampled as soon as they were landed total nucleotides were in the range 6.0-14.0μ M/g. The levels of IMP recorded 1–4μ M/g in both the groups. Although the initial conditions upon which the specific differences in nucleotide concentration in different species depended are not clearly understood, it has been suggested (Partmann, 1964) that besides individual variations due to physiological state, changes in magnitude of initial stores of creatine phosphate and glycogen might influence the levels of total nucleotides. According to Jones *et al*

(1964), samples of fresh cod in different physiological conditions prior to death showed nucleotide concentration varying more than 20 fold and even in fishes of same nutritional condition, ATP values varied 10 fold at the time of entry into rigor. In the case of oil sardine and species of prawn studied, it appears more reasonable to assume that factors like post mortem holding temperature and relative concentration and activity of glycolytic enzymes might be playing more significant role in determining the initial concentration of nucleotides.

Nucleotide concentration in ice stored fish.

The changes in total nucleotide concentration observed during ice storage in the case of oil sardine and two species of prawns are represented graphically (Fig. I). It has been found that the fall in nucleotide concentrations occurred in the samples at a slow rate upto 2 days of ice storage and thereafter it proceeded at a much faster rate. Curiously, the rate of nucleotide degradation during ice storage followed similar pattern in the 2 groups of fish examined. The pattern of change of nucleotides in ice storage has been shown by several workers to be related to species (Creelman & Tomlinson 1960, Ehira & Anakawa 1966, Spinelli 1967). However the trend of results obtained in this series tends to indicate that rather than species antemortem activity between landing and storage in ice which would have proceeded to the same degree might be the most important factor involved. This is also corroborated by the results on the changes of individual nucleotides ATP+ ADP, AMP & IMP obtained with prawns *P. indicus.* As shown in Fig. II, high level of IMP has been registered in the fresh sample and the peak of abrupt increase of

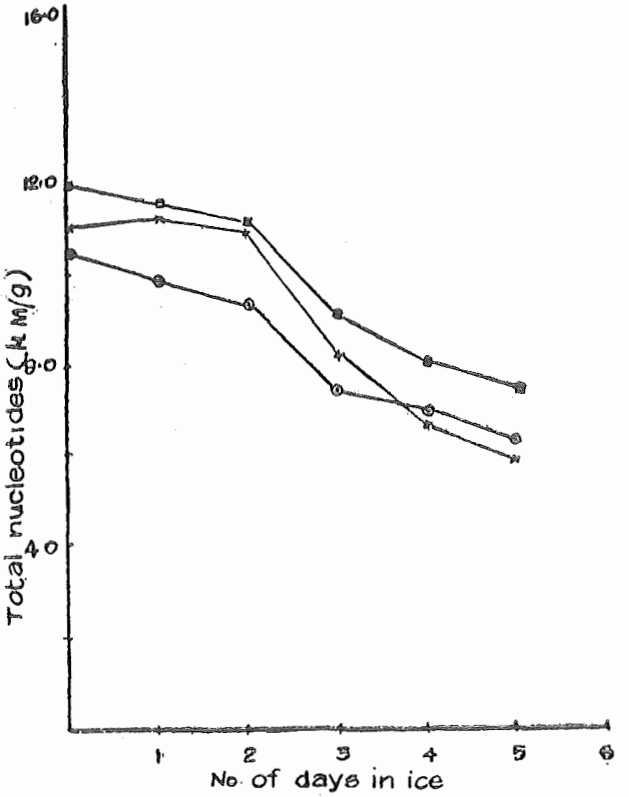

Fig. 1. Changes in total nucleotides during ice storage. ×—× oil Sardine; ⊙—⊙ M. dobsoni □—□ P. indicus.

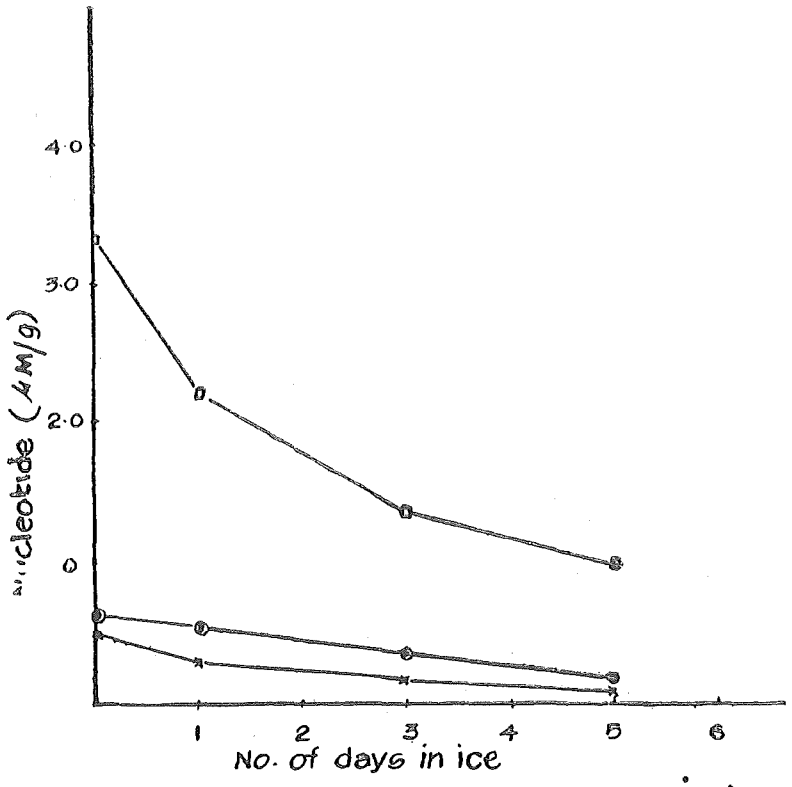

Fig. II. Changes in individual nucleotides in P. indicus during ice storage.
O—O AMP; X—X ADP+ATP; □—□ IMP.

IMP during early period of holding in ice, as was expected, was found to be absent. Working with plaice, Fujii *et al* (1966) found that the decrease of ATP & ADP corresponded very well with rapid increase of IMP during first four days of ice storage. The levels of IMP in headless prawns steadily decreased during the storage period and more markedly than the levels of AMP and ADP + ATP and this change might give useful indication of the length of ice storage. However Arai Ken-ichi (1966) who worked with isolated systems hae suggested that ths route passing through IMP is not considered likely in the pathway of degradation of ATP in prawns

Nucleotide changes in frozen fish.

The changes in the levels of total nucleotides in samples of oil sardine and headless *P. indicus* held at -23°C after quick freezing are given in Tables I and II. The pattern of nucleotide degradation differed considerably in frozen prawns from those held in ice storage and this is attributable to higher autolytic activity in the latter. Although quick freezing and storage amound the temperature of -23°C are known to inhibit the activity of enzymes involved in nucleotide cleavage, the changes in the levels of nucleotides (Fig. III) would show that as suggested by Jones (1964) they are sufficiently active during frozen storage. In the case of quick frozen headless prawns, the total nucleotide concentration underwent small change in four months at -23°C. Individual nucleotides AMP, ADP+ATP did not change significantly during the storage period. The level of IMP decreased steadily but much less rapidly than in ice storage. Results of frozen sardines on the

TABLE I CHANGES IN TOTAL NUCLEOTIDES IN FROZEN OIL SARDINE (*Sardinella longiceps*) DURING STORAGE AT–23°C.

INDIVIDUAL QUICK FROZEN			BLOCK FROZEN			
Series	Immediately after freezing	Stored $4\frac{1}{2}$ months	Series	Imm diately after faeezing.	Stored 3 months	Stored 6 months
			(Total nucleotides μ M/g)			
1	13.05	9.17	1	9.77	8.64	7.86
2	12.17	7.04	2	7.46	7.46	6.17
3	12.26	4.56	3	8.65	8.41	7.66

TABLE II CHANGES IN TOTAL NUCLEOTIDES IN BLOCK FROZEN HEADLESS PRAWNS (*P. indicus*) DURING STORAGE AT –23°C.

Series	Immediately after freezing	Frozen storage at –23°C 2 months	4 months	6 months
		(total nuclotides μ M/g)		
1	12 53	11.93	11.79	11.67
2	10.89	10.72	10 47	9.25
3	8.95	8.74	8.67	6.74
4	7.87	7.80	7.47	6.90

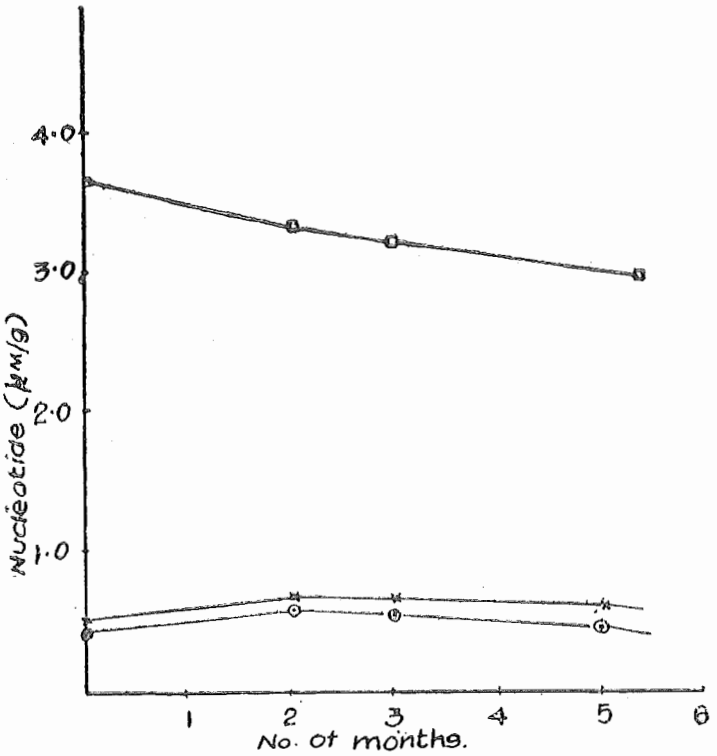

Fig. III. Changes in individual nucleotides in P indicus during frozen storage at –23°C. ⊙—⊙ AMP; x—x ADP+ATP, □—□ IMP

other hand indicate that cleavage occurred much faster compared to parwns under the same conditions. Dyer *et al* (1966) in their studies with frozen sword fish have observed rapid sequence of nucleotide changes in red muscle than in ordinary muscle. It is possible that besides other factors, higher enzymic activity leading to comparatively rapid nucleotide cleavage in the case of oil sardine might be attributable to the characteristic red meat of the fish in contrast to white meat of prawns.

As seen from Table I, sardines obtained by individual quick freezing (IQF) and block freezing behaved differently at the same temperature with regard to nucleotide degradation. In block frozen sardines total nucleotides changed significantly at slower rate than those in IQF samples. The possible reasons for the difference in behaviour are not clearly understood. However it is likely that this may be due to difference in extractability of bound nucleotides in comparatively more denatured muscle from IQF sardines held without glaze. According to Tomlinson and Geiger (1963) such bound nucleotides also appear to be protected from those enzymes which destroy free adenine nucleotides *post mortem.*

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Abbreviations used.

AMP — Adenosine 5′ Monophosphate
ADP — Adenosine 5′ diphosphate
ATP — Adenosine 5′ triphosphate
IMP — Inosine 5′ monophosphate
IAF — Individual Quick Frozen

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