

Selective Release of Fatty Acids During Lipid Hydrolysis in Frozen-stored Milk Fish (*Chanos chanos*)

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Lipid hydrolysis and the nature of fatty acids lost as a result of lipid hydrolysis in milk fish (*Chanos chanos*) during frozen storage at -20°C is discussed in this paper. There was a preferential loss of saturated acids during the first three weeks of storage. This was followed by loss of polyunsaturated acids during the next seven weeks. Sharp decrease in the levels of monounsaturated acids was observed from the 10th week of frozen storage. These observations are due to the preferential hydrolysis of phospholipids with relatively high proportion of saturated acids during the first three weeks, followed by the hydrolysis of phospholipids with high proportions of polyunsaturated fatty acids from the 3rd to the 10th week, and finally, predominant hydrolysis of neutral lipids from the 10th week onwards. Storage of fish in the ice prior to freezing was found to accelerate lipid hydrolysis, especially that of neutral lipids, during frozen storage.

Lipid hydrolysis during chilled and frozen storage of fish results in accumulation of free fatty acids (FFA) in fish muscle. Phospholipids (PL) are the major lipid constituent that undergo hydrolysis during the early stages of frozen storage of fish (Dyer & Fraser, 1959; Olley & Lovern, 1960; Olley *et al.*, 1962; Nair *et al.*, 1976; Nair *et al.*, 1978). Since the fatty acid composition of PL and neutral lipids (NL) are different (Addison *et al.*, 1968; Froines *et al.*, 1965; Ackman & Burgher, 1964; Silk & de Koning, 1964), composition of FFA formed during hydrolysis will depend on the composition of lipid which had undergone hydrolysis. High levels of $\text{C}_{20:1}$ and $\text{C}_{22:1}$ acids in FFA fraction is an indication of preferential hydrolysis of triglycerides (Takama *et al.*, 1967). Ackman *et al.*, (1969) had found that FFA formed during frozen storage of Newfoundland capelin were richer in $\text{C}_{20:1}$, $\text{C}_{22:1}$ and $\text{C}_{22:6}$ acids than the whole lipids. Such data are helpful to get an insight into the mechanism involved in lipid hydrolysis in frozen fish. Fatty acid pattern of fish from Indian coastal waters appears to be different from that of fish from temperate or cold water regions (Nair, 1981) and no information on the type

of FFA released from these fish during frozen storage is available. This paper reports the course of lipid hydrolysis during frozen storage and the change in the fatty acid composition of the unhydrolysed lipids remaining in milk fish (*Chanos chanos*).

Materials and Methods

Milk fish, caught from a nearby culture farm was iced and brought to the laboratory immediately. A portion of it was frozen at -40°C (Sample 1) and the rest was stored in ice. Portions of ice-stored fish were frozen on the 3rd and 6th days (Samples 2 and 3 respectively). Frozen samples were wrapped in high density polyethylene sheets and stored at -20°C . Samples were withdrawn at regular intervals for analysis. Muscle from edible portions of 3 or 4 fish were collected and lipids were extracted from 100 g portions of the minced muscle with chloroform methanol (2:1 v/v) mixture (Bligh & Dyer, 1959). Chloroform layer was washed to remove non-lipid impurities (Radin, 1969) and evaporated under vacuum at 40°C . Evaporated samples were stored at -20°C , under nitrogen, pending analysis. Lipid phosphorus was determined by sulphuric acid-perchloric acid digestion followed by spectro-photometric measurement (Dittmer & Wells, 1969) and the phospholipid content

*Formed part of the Ph.D. thesis (University of Kerala) of the first author.

was calculated from this. NL and PL were separated on silicic acid column. NL were eluted with chloroform and PL, with chloroform methanol mixtures and finally, with methanol. Identification and quantitative determination of PL components were carried out by thin-layer chromatography (Parkar & Peterson, 1965). FFA was determined by fractionation on silicic acid column and titration of FFA fraction (Olley & Lovern, 1960). Lipid samples were washed with 0.2 N sodium bicarbonate to remove FFA, saponified, unsaponifiables removed, and the liberated fatty acids were converted to their methyl esters using boron trifluoride-methanol (AOAC, 1975). Gas chromatographic analysis of the methyl esters was carried out as described in a previous report (Nair & Gopakumar, 1978).

Results and Discussion

All analyses were carried out in triplicate and the values reported are the mean. Changes in the levels of FFA and PL are shown in Fig. 1. PL hydrolysis in all the

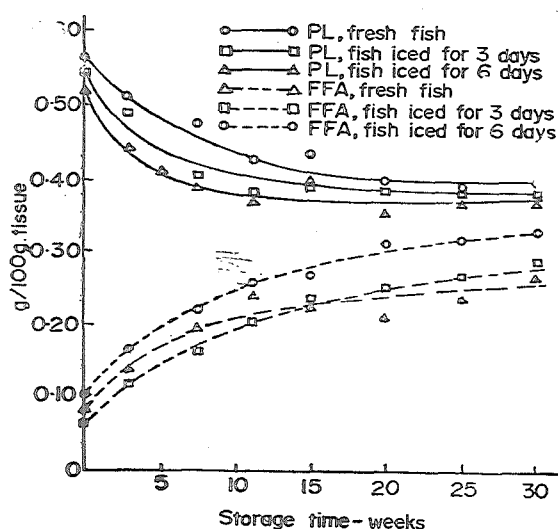


Fig. 1 Changes in the levels of phospholipids (PL) and free fatty acids (FFA) in milk fish during frozen storage

samples almost stopped after about 10 weeks of frozen storage. About 55% of the total PL still remained unhydrolysed at this stage. Formation of FFA also levelled off by this time in sample 1. The slow increase in the levels of FFA in samples 2 and 3 even after the cessation of PL hydrolysis may be due to the hydrolysis of NL in

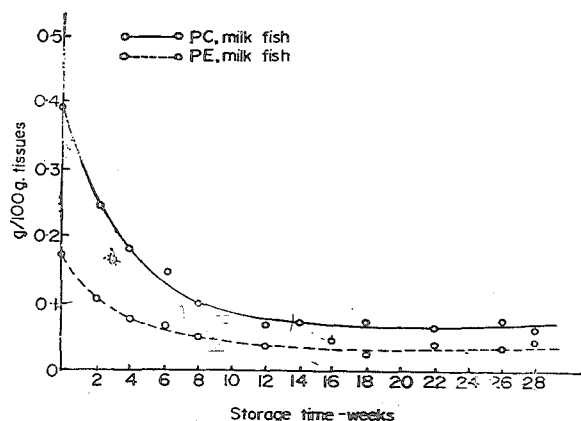


Fig. 2 Changes in the levels of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in milk fish during frozen storage

these samples. Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were the major components of PL fraction (Table 1). Rate of hydrolysis of PC was higher than that of PE (Fig. 2), resembling the pattern that had been reported earlier (Nair *et al.*, 1976; Nair *et al.*, 1978).

Table 1. Phospholipid composition of milk fish* (% of fresh tissue)

Total phospholipids	0.58
Phosphatidyl choline (PC)	0.38
Phosphatidyl ethanolamine (PE)	0.17

*Phospholipids other than PC and PE were not determined

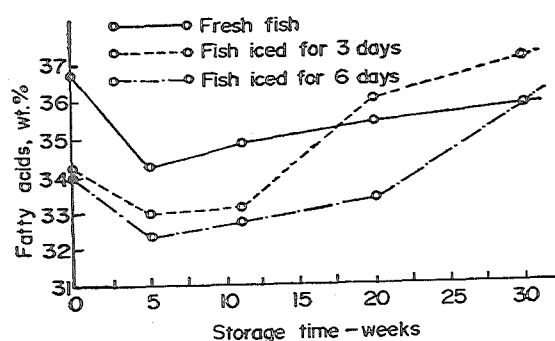


Fig. 3 Changes in the levels of saturated acids in milk fish during frozen storage

Proportion of total saturated acids in the unhydrolysed lipids decreased sharply during the first three weeks (Fig. 3). This period corresponds to the initial fast decrease in the level of PL. No significant change in

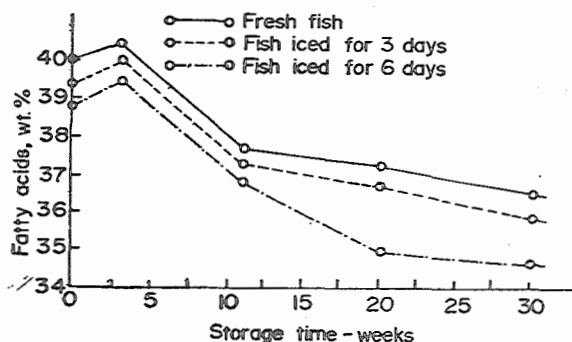


Fig. 4 Changes in the levels of poly unsaturated acids in milk fish during frozen storage

the proportion of these acids was observed from the third to the 10th week. Thereafter there was a small increase in samples 2 and 3. Increase in the level of total polyunsaturated fatty acids (PUFA) was only marginal during the first three weeks (Fig. 4). Although this increase was small, it was evident in all the three samples. Proportion of mono-unsaturated acids went on increasing until the 10th week of frozen storage and thereafter decreased (Fig. 5).

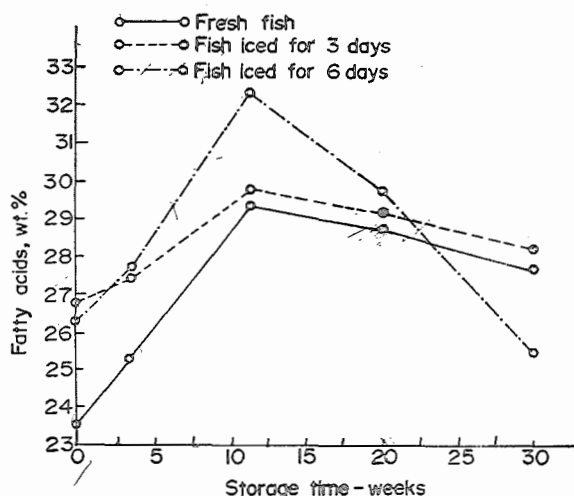


Fig. 5 Changes in the levels of mono unsaturated acids in milk fish during frozen storage

The above pattern of release of fatty acids shows that lipid hydrolysis in frozen milk fish takes place in three stages: (i) the first three weeks when there was a preferential loss of saturated acids accompanied by increase in the level of mono unsaturated acids and a marginal increase of the level of PUFA, (ii) the next seven weeks when levels of PUFA decreased with increase in the proportion of

mono unsaturated acids and (iii) the period after 10 weeks when proportion of mono unsaturated acids started to come down and rate of loss of PUFA was slowed down.

PL in milk fish is richer in PUFA than the NL. Therefore, rapid hydrolysis of PL during the first phase of frozen storage should have resulted in the initial loss of PUFA. But the behaviour observed during the first three weeks was a preferential loss of saturated acids. Loss of PUFA was observed from the 3rd week of frozen storage. Thus it appears that hydrolysis of PL proceeds in stages. Available evidences are against a step-wise hydrolysis (i.e. PL lyso derivative fatty acids) of PL molecules in fish muscle during frozen storage (Lovern & Olley, 1962; Olley *et al.*, 1962; Bligh & Scott, 1966). Therefore, it may be concluded that phospholipid classes with relatively high proportions of saturated acids were hydrolysed initially (first three weeks) followed by those with relatively high proportions of PUFA (3rd to 10th week). Release of specific fatty acids during frozen storage of fish had been reported earlier also. Braddock & Dugan (1972) had observed that $C_{20:4}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$ acids were more concentrated while, $C_{16:0}$ and $C_{18:1}$ acids were less concentrated in the PL remaining after frozen storage of coho salmon muscle at -20°C for one year. They had concluded that this was due to losses of specific fatty acids from the PL fraction. Oshima *et al.* (1984) have recently presented evidence for selective hydrolysis of PL species. They had found that PL species with certain fatty acid combinations only were hydrolysed during storage of cod flesh in ice.

NL in comparison with PL are known to be richer in mono unsaturated acids (Ackman & Burgher, 1964; Silk & de Koning, 1964). Proportions of total saturated acids in the NL and PL of milk fish were found to be 39.8% and 39.0% respectively of the total fatty acids, those of monounsaturated acids were 31.2% and 27.7% and PUFA, 27.2% and 33.2% (Nair, 1981). PL are mainly affected during the early stages of lipid hydrolysis and hence the lipids remaining unhydrolysed may get progressively rich in NL and hence, in mono unsaturated

fatty acids. A decrease in the proportion of these acids was observed after 10 weeks of frozen storage indicating that, as discussed earlier, NL also hydrolysed at this stage. This decrease was more sharp in sample 3 than in the other two samples. It may be noted that it was in this sample that accumulation of FFA continued at the highest rate even after the cessation of hydrolysis of PL, indicating comparatively fast hydrolysis of NL.

Storage of milk fish in ice, prior to freezing, was found to exercise some influence on lipid hydrolysis during frozen storage. Rate of formation of FFA was higher in sample 3 than that in samples 1 and 2. Deng (1978) had observed similar behaviour in mullet. Storage of fish in ice prior to freezing appears to accelerate lipid hydrolysis, especially that of NL, during frozen storage.

The authors are grateful to Shri M. R. Nair, Director, Central Institute of Fisheries Technology for the kind permission to publish this paper.

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