Lipid Breakdown in Oil Sardine *(Sardinella longiceps)* During Frozen Storage*

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The problem of hydrolysis of lipids and consequent accumulation of free fatty acids and development of rancidity due to oxidation of the lipids are major problems in frozen storage of oil sardine *(Sardinella longiceps)*. The course of the phospholipid breakdown, production of free fatty acids and the changes taking place in the major unsaturated fatty acids during frozen storage are described in this paper. The rate of free fatty acid production is faster in the fish, with the higher fat content. Unlike in lean fish, the neutral lipids are found to contribute substantially to the free fatty acid production. The fatty acids most affected during storage are $C_{20:5}$ and $C_{22:6}$. The polyene indices were found to decrease during storage. These effects are more pronounced in the fish with the higher fat content.

Sardine and mackerel together form the major portion of the fish landed on the Kerala and Karnataka coasts. The high lipid contents of these two fishes render processing and storage difficult. Deterioration of the lipid fraction is, probably, the most serious problem in the frozen storage of these fatty fish. Free fatty acids, produced during the hydrolysis of the lipids on frozen storage are known to cause denaturation of proteins in fish (Dyer & Morton, 1956; Dyer *et al.*, 1956; Dyer & Frazer, 1959). The major source of these free fatty acids is found to be the phospholipid fraction in many species of fish, especially in those with low lipid contents (Olley & Lovern, 1960; Olley *et al.*, 1962; Nair *et al.*, 1976). Development of rancidity is the other serious problem adversely affecting the acceptability of frozen products. Lipids of mackerel and oil sardine are known to be very rich in polyunsaturated fatty acids. Oxidation of these polyunsaturated acids during frozen storage leads to the development of rancidity. The present paper reports the results of a study of the hydrolytic changes in the lipids of oil sardine of two different seasons and consequent development of free fatty acids. It also elucidates the changes that the major classes of fatty acids undergo during storage.

Materials and Methods

Fish samples were collected from the landing sites around Cochin, one in November and the other in January. They were frozen at –40°C. The blocks were subsequently stored at –18°C, wrapped in polythene sheets. The extraction of lipids, determination of free fatty acids and the fractionation of the lipids into phosphorylated and non-phosphorylated fractions were carried out as described previously (Nair, *et al.*, 1976). Separation and estimation of the components of the phospholipid fraction were carried out by thin-layer chromatography on silica gel G (Parker & Peterson, 1965). Methyl esters of the fatty acids prepared by the method of AOAC. (1975) were analysed on a column (S.S., 2m x 6mm o.d.) of 15% DEGS on chromosorb W (60–80 mesh), using a 'Toshniwal' gas chromatograph equipped with flame ionization detector and strip chart recorder. Operating conditions were: oven temperature, 192°C; injection port temperature, 250°C; detector temperature, 250°C and carrier gas (nitrogen) flow rate, 40ml/min. Identification of peaks was done by comparison with standards. The peak areas were calculated by triangulation and the results expressed as uncorrected area percentages.

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Results and Discussion

The fish collected in November had a lipid content of 6% and that in January 10%. The patterns of hydrolysis of phospholipids and development of free fatty acids are somewhat different from that reported by Olley & Lovern (1960) and Olley *et al.* (1962) for other types of fish. Hydrolysis proceeded almost at the same rate in both the samples of fish (Fig. 1), but it was much slower than that reported for lean fish. The breakdown of phospholipids almost stopped after about 90 days. Compared to previous reports, the proportion of phospholipids remaining unhydrolysed was very much higher in both the cases (84.6% in the leaner sample and 82.6% in the fatter sample).

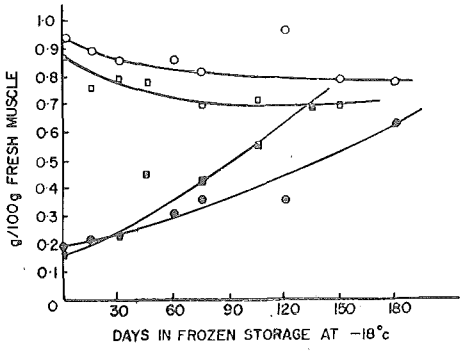

Fig. 1. Phospholipid breakdown and free fatty acid production in oil sardine, of two different fat contents during storage at —18° C.

- ○ Phospholipids in sample with 6% lipids
- □ Phospholipids in sample with 10% lipids
- ● Free fatty acids in sample with 6% lipids
- ▩ Free fatty acids in sample with 10% lipids

The development of free fatty acids in the muscle did not follow a course corresponding to the decrease in the phospholipid content. The rate of formation of free fatty acids was faster than that of phospholipid decomposition. The free fatty acid concentration went on increasing even after the phospholipid hydrolysis practically ceased. Moreover, production of free fatty acids is faster in the fish with the higher fat content, although its phospholipid content is slightly lower. These observations suggest that in the fatty fish, unlike in the lean fish, the non-phosphorylated fraction also undergoes considerable hydrolysis. The early cessation of the phospholipid hydrolysis may be due to the inhibitory effect of the increasing concentration of free fatty acids on the phospholipase.

Fig. 2 indicates that the major components of the phospholipid fraction undergo the same pattern of changes as reported by Viswanathan Nair *et al.* (1976). The fatty

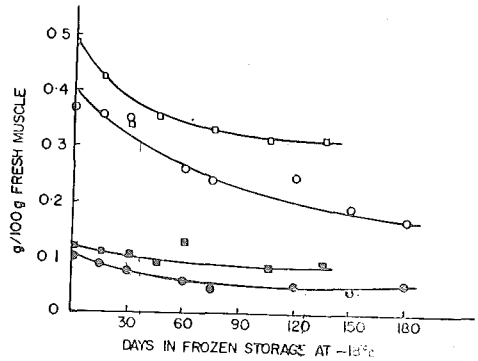

Fig. 2. Changes in the levels of phosphatidyl choline and phosphatidyl ethanolamine in the lipids of oil sardine during storage at — 18 °C

- ○ Phosphatidyl choline in fish with 6% lipids
- □ Phosphatidyl ethanolamine in fish with 6% lipids
- ● Phosphatidyl choline in fish with 10% lipids
- ▩ Phosphatidyl ethanolamine in fish with 10% lipids

acid composition of the lipids is simlar to that reported earlier for the same species (Gopakumar & Nair, 1966). The proportions of saturated and monoenoic fractions remain almost constant during storage (Table 1). The major components in the polyunsaturated group are $C_{20:5}$ and $C_{22:6}$. These two were the most affected during storage, as indicated by the gas chromatographic data. The loss of these acids due to oxidative degradation is comparatively more severe in the fish with the higher lipid content.

The importance of polyene index as a measure of rancidity in frozen mackerel has been established by Ke *et al.* (1976). Good correlation between the polyene index and formation of 2, 4, 7–decatrienals and other

Table 1. *Changes in the major groups of fatty acids in sardine during frozen storage*

		Storage in days								
		0	15	30	60	75	105	120	150	180
Sample I 6%lipids	Total saturated acids %	46.84	46.11	42.81	44.92	43.67	—	45.05	—	41.04
	Total monoenoic acids %	26.80	25.93	29.4	31.12	30.82	31.04	28.12	—	30.57
	$C_{20:5}$ %	9.107	11.06	9.286	8.844	9.419	8.603	8.312	—	7.065
	$C_{22:6}$ %	4.597	4.323	4.088	4.934	3.896	3.879	4.047	—	3.784
Sample II 10%lipids	Total saturated acids	41.86	40.57	44.08	43.06	43.53	44.87	—	46.14	—
	Total monoenoic acids %	29.35	31.69	27.13	32.64	29.83	26.11	—	32.03	—
	$C_{20:5}$ %	9.919	10.65	9.679	8.037	8.262	7.597	—	6.950	—
	$C_{22:6}$ %	5.109	5.399	4.765	4.407	3.290	3.082	—	2.840	—

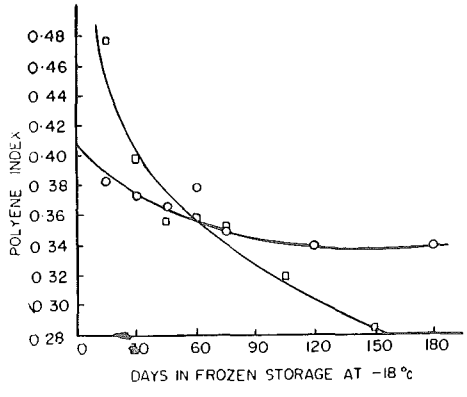

Fig. 3. Changes in the polyene indices in the lipids of oil sardine during storage at — 18°C.
○ 6% lipids □ 10% lipids

unsaturated aldehydes which are suspected to be responsible for the rancid flavour has also been reported by the same authors earlier (Ke *et al.*, 1974). The polyene indices for the two samples of fish under investigation are found to decrease with storage (Fig. 3), the fall being more rapid in fish having higher lipid content. This increased susceptibility to rancidity cannot be attributed to higher levels of $C_{20:5}$ or $C_{22:6}$ acids because both these acids are present almost to the same extent in both the samples. The difference in the rates of oxidation in the two samples may, therefore, be attributed to other factors as pointed by Ke & Ackman (1976) for mackerel meat and skin lipids. Since the free fatty acids are more prone to oxidation than the intact glycerides, the increased rate of hydrolysis of the glycerides in the fatter sample may also contribute to the higher rate of oxidation.

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