LIPID HYDROLYSIS IN MACKEREL *(RASTRELLIGER KANAGURTA)* DURING FROZEN STORAGE

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The hydrolytic changes in the lipids of mackerel *(Rastrelliger kanagurta)* during storage at -18°C was studied with a view to understand the factors involved in the formation of free fatty acids. Only the phosphorylated fraction did undergo hydrolysis at an appreciable rate. It was found that the free fatty acid production was mainly associated with the phospholipid hydrolysis. As regards the triglycerides and unsaponifiable matter, there was no significant change in levels during frozen storage.

INTRODUCTION

Hydrolytic cleavage of lipids during frozen storage of fish is an important factor contributing to the quality deterioration of the products. Free fatty acids produced during lipid hydrolysis are known to be the cause of deterioration in the quality of frozen products. Dyer and Morton (1956), Dyer *et al.* (1956), and Dyer and Fraser (1959) have postulated the relationship between lipid hydrolysis and protein denaturation. The free fatty acid production in cod (Olley and Lovern, 1960) and in many other species of fish (Olley *et al.*, 1962) is known to be caused mainly by the phospholipid break down. The extent of the hydrolytic changes in the major lipid fractions of mackerel *(Rastrelliger kanagurta)* during frozen storage and their relationship to the production of free fatty acids is the subject of the present investigation.

MATERIALS AND METHODS

Fresh mackerel *(Rastrelliger kanagurta)* collected from landing sites were frozen in contact plate freezer at -40°C and stored at -18°C for a period of five months. The total lipids of the skeletal muscle were extracted by the method of Bligh and Dyer (1959) and purified by the method of Folch *et al.* (1957). Iodine value (Wijs) and the content of unsaponifiable matter were determined by the A. O. C. S. (1957) procedures. Free fatty acids were separated by column chromatography on silicic acid and estimated by titration with alkali (Olley and Lovern,

TABLE I

Changes in the lipid fractions during frozen storage

	Storage time in days						
	0	15	30	45	60	105	150
Total lipids (g./100g. muscle)	1.49	1.48	1.48	1.46	1.45	1.42	1.43
Non-phosphorylated fraction (g./100g. muscle)	0.722	0.741	0.772	0.867	0.98	0.975	1.03
Triglycerides (% of muscle)	0.285	0.288	0.277	0.386	0.31	0.307	0.292
Phosphorus (% of lipids)	2.11	1.98	1.89	1.53	1.28	1.22	1.18
N. S. matter (% of lipids)	3.27	3.07	2.91	2.90	2.86	2.59	2.89
Iodine value (Wijs)	170.1	164.7	150.6	140.6	129.7	128.0	130.2

1960). Phosphorus was determined by the method of King (1932).

The lipid samples were separated into non-phosphorylated and phosphorylated fractions by column chromatography on silicic acid (Silica for column chromatography, according to Ramsey and Patterson, Fluka, A. G.). The sample was applied as chloroform solution. The fractionation was carried out by stepwise elution with chloroform for non-phosphorylated lipids (monitored by thin-layer chromatography), and with increasing concentrations of methanol in chloroform and finally with pure methanol. An aliquot of the total phospholipids was hydrolysed by the metod of Lovern *et al* (1959). Ethanolamine and serine in the hydrolysate were separated by paper chromatography (Olley, 1956). Quantitation was done spectrophotometrically after eluting out the separated spots from the paper. Choline in the hydrolysate was determined by the method of Hawk (1947).

Separation and estimation of the triglycerides in the non-phosphorylated fraction were carried out by chromatography on florisil column (Litchfield *et al*.,) 1964).

RESULTS AND DISCUSSION

The lipid content of the muscle was about 1.5% and this remained fairly constant during storage (Table I). The phosphorus content of the lipid was found to decrease progressively, indicating loss of phospholipids. The level of unspaonifiable fraction of the lipid was very little affected by the freezing and storage. As

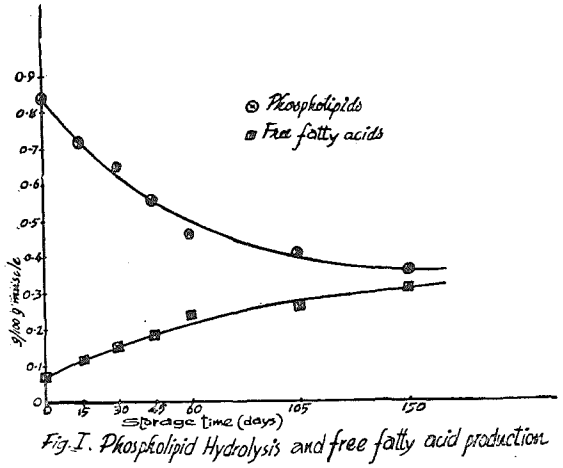

Fig. 1

regards the other major component of the non-phosphorylated fraction, *viz.*, the triglycerides, there was no significant variation in its content throughout the period of storage.

As can be seen from Fig. 1, the phospholipid hydrolysis was rapid in the initial stages and slowed down as the storage progressed. Similar observations have been reported for the lipids of several other species (Olley and Lovern, 1960; Olley *et al*, 1962). After three months of storage, the phospholipid content of the muscle decreased by about 53% of the original level and then the rate of hydrolysis slowed down markedly. Lovern and Olley (1962) have shown that phospholipid hydrolysis in cod muscle during frozen storage continued till about 70-80% of the original phospholipids have been used up. In the present study, however, at the end of the five months period 58% of the total phospholipids had undergone hydrolysis and the process was apparently still continuing.

The levels of phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl serine were almost the same as that already reported for the same species (Gopakumar and Rajendranathan Nair, 1971). Phosphatidyl choline and phosphatidyl ethanolamine were the two major constituents of the phosphorylated fraction that underwent rapid hydrolysis during storage while phosphatidyl serine remained almost unaffected throughout. The rates of hydrolysis of the two fractions were almost identical (Fig. 2). Similar patterns of lipid hydrolysis were encountered in frozen cod (Bligh and Scott, 1966) and in iced cod (Lovern, *et al.*, 1959). Bligh and Scott *(op. cit)* found that the hydrolysis of phosphatidyl ethanolamine appeared to cease after four months while phosphatidyl choline continued to undergo hydrolysis. But in the present case both phosphatidyl choline and phosphatidyl ethanolamine were undergoing hydrolysis even after five months of frozen storage, though at a much reduced rate.

The decrease in the phospholipid con-

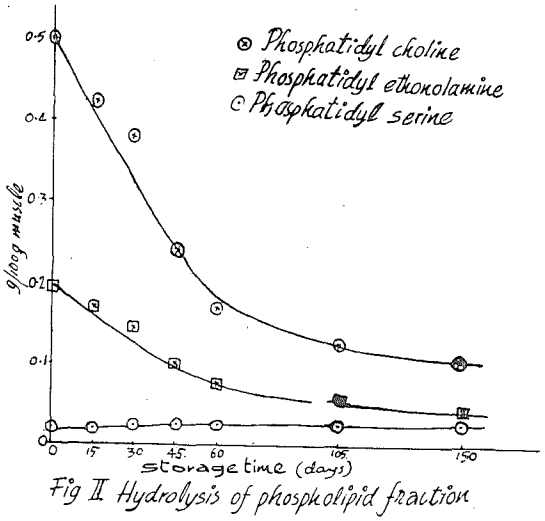

Fig. 2

tent of the muscle was accompanied by a corresponding increase in the free fatty acid concentration, indicating that the free fatty acid production is mainly associated with the phospholipid breakdown (Fig. 1). This is in agreement with the findings of Olley, *et al.* (1962) for a number of species of fish. The free fatty acid level continued to rise throughout the period of storage.

The study clearly indicate that there exists a close relationship between phospholipid breakdown and free fatty acid production during storage under frozen conditions. Almost the whole of the free fatty acids formed in the muscle is coming from this source. And among the various constituents of the phospholipid fraction, it is phosphatidyl choline and phosphatidyl ethanolamine that contribute mainly to the formation of free fatty acids. Other lipid fractions remain almost unaffected under the conditions of the investigation.

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