Food Chemistry **26** (1987) 85–96

Phospholipid Changes in Muscles of Plathead Grey Mullet *(Mugil cephalus)* During Frozen Storage

L. A. El-Sebaiy, S. M. Metwalli & M. E. Khalil

Food Science and Technology Department, Kafr El-Sheikh,
Faculty of Agriculture, Tanta University, Tanta, Egypt

(Received 22 August 1986; revised version received 15 October 1986;
accepted 19 December 1986)

ABSTRACT

*Small and large size whole and eviscerated plathead grey mullet fish (*Mugil cephalus*) were stored at −14°C in polyethylene bags for 6 months. Samples were taken at intervals for 6 weeks, lipids extracted and analyzed for total lipid content, free fatty acids (FFA), peroxide value, total phospholipid phosphorus and individual phospholipids. The apparent total lipid content increased during frozen storage. The amount of FFA increased four times in the small fish and six times in the large fish. Viscera stimulated the FFA development. Peroxide values showed marked increases in all fish treatments, indicating that fatty acids were considerably oxidized. Eviscerated small fish showed much higher increases in peroxide values. The phospholipid content decreased by 94·3% and 96·8% of the original content for whole and eviscerated small fish, respectively, and by 80·3% and 84·3% for whole and eviscerated large fish, respectively.*

The relationship between FFA content and phospholipid phosphorus of total lipid indicated a suitable storage period to be 6 weeks. Eight phospholipid fractions were identified in both sizes of fish. In small mullet, phosphatidyl choline (PC) was the major fraction followed by phosphatidyl serine (PS), lysophosphatidyl choline (LPC), phosphatidyl inositol (PI), sphingolipid (SL), phosphatidyl ethanolamine (PE) and phosphatidyl acid (PA). In large mullet, PS, PC and SL were the major phospholipids followed by PI, LPC, PE and PA. After the storage period, the major fractions declined while PA and LPC showed very high increases, indicating that phospholipases

were present and able to degrade the phospholipids. The degradation of phospholipid fractions was much higher in eviscerated fish.

INTRODUCTION

During prolonged storage of fish at frozen temperature, phospholipids in the flesh are degraded due to hydrolysis catalyzed by phospholipases in the muscle and release of the free fatty acids (FFA). The FFA could then cause denaturation of proteins in the flesh (Lovern *et al.*, 1959; Olley & Lovern, 1960). Phospholipid hydrolysis occurs in cod flesh stored at temperatures ranging from +20 to −29°C. The hydrolysis is promoted entirely by tissue enzymes, with non-enzymic reactions on bacteria playing negligible roles. Freezing appears to activate the system, eliminating the initial lag at 0°C and permitting nearly as rapid degradation at −14°C as at 0°C. The temperature coefficient is about eight times greater in frozen than in unfrozen fish, over the ranges −22 to −14°C and 0 to +20°C, respectively. In all cases there was a virtually simultaneous loss of both fatty acids from the phospholipid molecule.

Phospholipid hydrolysis was demonstrated in dorsal muscle from whole frozen stored salmon as shown by losses of specific phospholipids. There appeared to be a preferential hydrolysis of phosphatidyl ethanolamine (PE) (Braddock & Dugan, 1972). El-Bastavizi & Smirnova (1972) studied changes in the phospholipid of frozen pike during storage. After 9 months of storage of pike at −15°C, the amount of FFA increased twelve times. The phospholipid content decreased by a factor of 3. Phosphatidyl choline (PC) and PE decreased by 23% and 26%, respectively. Castell (1974) pointed out that changes in lipids in marine products are almost always accompanied by changes in proteins and other non-lipid components of muscle. He found that not only do these changes take place simultaneously but that they are often inter-related and that oxidation of lipids is only one part of an integrated system of reactions that occur as fish muscle deteriorates on storage. These changes of lipids may well affect flavour and texture during processing. Shoeb *et al.* (1973) studied the phospholipid composition of the head, viscera and muscles of marine mullet (*Mugil cephalus*). Phosphatidyl serine (PS), PC, PE, SL and an unidentified phospholipid were demonstrated. PC and PE were the major phospholipids present.

The work proposed here was designed to provide information on the phospholipids of mullet obtained from El-Zawia fish farm at Kafr El-Sheikh, either in small or large sizes, which are commercially produced. Data were also obtained on the changes of phospholipids in flesh of mullet stored at frozen temperature (−14°C).

MATERIALS AND METHODS

Materials

Plathead grey mullets (*Mugil cephalus*), commonly named Bouri fish, were obtained from the El-Zawia fresh water fish farm at Kafr El-Sheikh during the season of September–December, 1985. The average length and weight of the fish used were 22·4 cm and 107 g, 26·5 cm and 220 g for small and large sizes, respectively. After fishing, all fish were washed with fresh water, packed in crushed ice, and then transported to the laboratory within 1 h. The average length and weight were taken. Eviscerated fish were prepared and re-washed with fresh water. The time from death to commencement of the experiment never exceeded about 2 h. The experiment was planned to store whole and eviscerated small and large size fish at $-14°C$ over a storage period of 24 weeks. Three fish were placed in each polyethylene bag; the bags were sealed and stored at $-14°C$.

Preparation of samples for analysis

Whole and eviscerated small and large fish were withdrawn after 0, 6, 12, 18 and 24 weeks. The three fish in each bag were used as one sample. They were skinned and the flesh was minced, well mixed and immediately taken for analysis. In the case of the whole fish, the three fish were previously eviscerated before the rest of the preparation.

Methods

Moisture content of the flesh was determined by the AOAC (1975) method using a vacuum oven at 98–100°C for *ca.* 5 h having pressure equivalent to ≤ 25 mm Hg.

Total lipid extract was prepared using the procedure described by Kates (1972) for extraction of animal tissues which is a modification of the method of Blight & Dyer (1959). The procedure is as follows: to 6–7 g of minced fish is added 3 ml of water and 30 ml of methanol:chloroform (2:1, v/v) and the mixture is blended in an homogenizer (type 324 'Mechanika precyzyjna' cooperative company, Warszawa, Boremlowska 6, Poland) for 2 min at room temperature. The homogenate is centrifuged, the supernatant is decanted, and the residue is re-extracted with 38 ml of methanol:chloroform:water (2:1:0·8) by homogenization for 2 min. After centrifugation, the combined supernatants are diluted with 20 ml each of chloroform and water and the phases separated by centrifugation or in a separatory funnel. The lower chloroform layer is withdrawn and

concentrated in a rotary evaporator at 30–35°C (benzene is added to aid the removal of traces of water), and the residue is dissolved in a suitable volume (e.g. 10 ml) of chloroform. Aliquots were taken for the determination of total lipids by the procedure described by Kates (1972) for the dry weight determination.

FFA and peroxide values were determined in aliquots of total lipid extract using the methods of the AOCS (1957). A mean equivalent of 300 has been assumed for the FFA. Total phospholipid phosphorus was determined in aliquots of the total lipid extract by the method of Rouser *et al.* (1970).

Separation of phospholipid class

Phospholipids were separated from the total lipid extract using preparative TLC plates (1 mm thick) coated with silica gel G. The solvent system used was petroleum ether:diethyl ether:acetic acid (70:30:2). The phospholipids remaining at the origin of the plates were scraped and eluted by the method of Braddock & Dugan (1972) which was modified by El-Sebaiy *et al.* (1980) using an eluent composed of methanol:acetic acid:water (94:1:5), evaporated on a rotary film evaporator and redissolved in chloroform. Total phospholipid phosphorus was determined by the method of Rouser *et al.* (1970).

Separation and quantification of individual phospholipids

Individual phospholipids were fractionated and quantified using the method described by Dunn *et al.* (1969). Borated silica gel plates and a solvent system composed of chloroform:methanol:water (65:25:4) were used. Identifications of individual phospholipids were made by specific spray reagents and a lipid extract prepared from albino rat which contained the known fractions of phospholipids as described by El-Sebaiy *et al.* (1980).

RESULTS AND DISCUSSION

Total lipid extract

The muscles of the small-size mullet contained higher total lipid than those of the large size (Table 1). The apparent increase in the total lipid content during frozen storage is attributed to the increased extractability of lipids liberated after denaturation of lipoprotein of the fish muscles during cold storage (Kinumaki *et al.*, 1970; Castell, 1974).

TABLE 1
Lipid Hydrolysis in Muscles of Plathead Grey Mullet Stored at −14°C

Samples	*Storage period (weeks)*	*Moisture (%)*	*Total lipid (%)*	*Free fatty acids (g/100 g lipid)*	*Peroxide value*	*Phospho-lipid phosphorus (% in lipid)*	*Phospho-lipid phosphorus (% of dry muscle)*
Small fish with viscera and eviscerated	0	77·3	12·6	4·1	4·3	1·038	0·131
Large fish with viscera and eviscerated		76·6	7·5	4·5	4·9	3·550	0·265
Small fish with viscera	6	75·1	13·6	7·3	9·8	0·086	0·012
Eviscerated small fish		75·7	13·4	5·8	16·3	0·120	0·016
Large fish with viscera		78·2	7·3	11·6	7·8	1·040	0·076
Eviscerated large fish		78·2	9·9	9·1	9·4	1·020	0·101
Small fish with viscera	12	75·8	17·3	10·9	11·6	0·082	0·014
Eviscerated small fish		76·0	13·6	7·5	32·2	0·085	0·012
Large fish with viscera		78·4	7·5	17·5	19·6	0·820	0·062
Eviscerated large fish		76·2	9·1	12·7	24·8	0·880	0·080
Small fish with viscera	18	74·5	18·7	11·7	14·1	0·064	0·012
Eviscerated small fish		74·6	13·7	9·5	36·5	0·047	0·007
Large fish with viscera		77·3	7·9	21·3	23·6	0·750	0·059
Eviscerated large fish		76·4	9·7	18·0	30·6	0·770	0·075
Small fish with viscera	24	75·7	16·6	16·5	32·8	0·059	0·010
Eviscerated small fish		74·2	13·9	16·0	41·5	0·033	0·005
Large fish with viscera		77·3	7·9	24·4	31·5	0·700	0·055
Eviscerated large fish		78·0	8·5	22·3	37·2	0·560	0·048

FFA and peroxide value

Development of FFA as g/100 g lipid calculated at mean equivalent 300 is clearly observed from Table 1 and Fig. 1. During the period of cold storage, the amount of FFA in small-size mullet increased about four times, while the large sizes increased about six times after the end of the storage period. Similar results were mentioned by El-Bastavizi & Smirnova (1972) who found that FFA in pike increased twelve times after 9 months' storage at −15°C. Viscera, either in small or large sizes of mullet, stimulated the development of FFA; this may be due to a higher content of hydrolytic enzymes in viscera than in other tissues.

Table 1 and Fig. 1 show that peroxide value increased gradually. The increase was much greater in eviscerated fish; this may be due to the greater content of oxygen with the opening fish tissue. This increase indicated that fatty acids were considerably oxidized during the storage period. It was observed that rancid odour developed as the peroxide value increased. Takama (1974) found that the main carbonyl compounds formed during autoxidation were acetaldehyde, propionaldehyde and acetone; the main volatile fatty acids formed were caproic and propionic acids. Some of these oxidized products react with salt-soluble proteins.

The lipid extract was of a brownish colour and of high viscosity which may be due to the lipid browning reactions involving phospholipids, free amino groups and aldehyde groups of sugars. Castell (1974) reported that

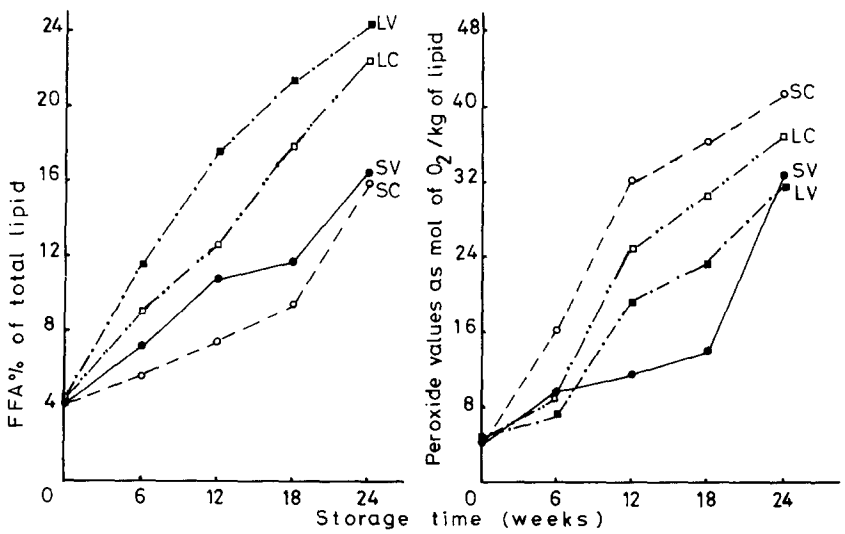

Fig. 1. Development of free fatty acids (FFA) and peroxide values in lipids of frozen fish. SV: Small fish with viscera; SC: eviscerated small fish; LV: large fish with viscera; LC: eviscerated large fish.

changes in lipids of marine products are almost as great as in proteins and other non-lipid components of muscle.

Total phospholipids

Table 1 shows that total phospholipid phosphorus decreased markedly during the storage period. Most of the decrease was observed within the first 6 weeks of storage which amounted to 91·7% and 88·4% for small fish with viscera and eviscerated, respectively, and to 70·7% and 71·3% for large fish with viscera and eviscerated, respectively. At the end of the storage period the decreases amounted to 94·3% and 96·8% for small fish with viscera and eviscerated, respectively, and to 80·3% and 84·2% for large fish with viscera and eviscerated, respectively. These results show that the loss of phospholipids was greater in eviscerated than in whole fish, indicating that the phospholipid hydrolysis was promoted entirely by tissue enzymes, which correlates with the work of Olley & Lovern (1960). Evisceration may lead to contact between viscera enzymes and fish tissue.

The relationship between FFA content and phospholipid phosphorus content of the lipid (Fig. 2) showed that, at a given phospholipid percentage, eviscerated fish had lower FFA contents than fish with viscera. Phospholipid phosphorus of total lipid decreased gradually with the increase of FFA to a particular point, after which the increase of the FFA was very fast, accompanied with quality losses in the organoleptic properties, colour,

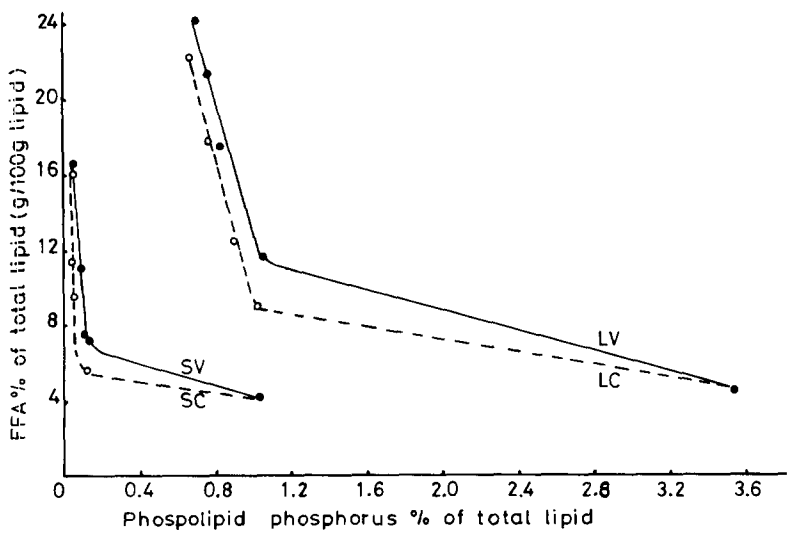

Fig. 2. Fall in phospholipid phosphorus with development of free fatty acids (FFA). SV: small fish with viscera; SC: eviscerated small fish; LV: large fish with viscera; LC: eviscerated large fish.

texture and odour. This particular point could be considered the optimum period which was equal to 6 weeks' storage. These results indicate that phospholipids were hydrolyzed to fatty acids during 6 weeks' storage, after which the hydrolysis of triglycerides took place, showing the sharp increase of FFA. The FFA production during frozen storage through the hydrolysis of triglycerides was mentioned by Awad *et al.* (1969) and Bosund & Ganrot (1969). The aforementioned results correlate with those of El-Bastavizi & Smirnova (1972) who found that, after 9 months' storage of frozen pike at $-15°C$, the total phospholipid content decreased by a factor of 3 and triglycerides by 20%. Kimumaki *et al.* (1970) found that, during frozen storage of fish, phospholipids were hydrolyzed to fatty acids through mono- or diglycerides and FFA were derived from phospholipids but not from triglycerides.

Individual phospholipid fractions

Seven phospholipids were identified and quantified in the muscles of small and large mullet fish with viscera and eviscerated; namely, PS, lysophosphatidyl choline (LPC), phosphatidyl inositol (PI), sphingolipid (SL), PC, PE and phosphatidic acid (PA). Trace amounts of phosphatidyl glycerol (PG) were found in all examined samples. In small mullet (Tables 2 and 3), PC was the major phospholipid (35·5%) followed by PS, PI, LPC, SL, PE and PA. Phospholipid analysis of large-size mullet (Tables 4 and 5) showed that PS (26·6%), SL (18·5%) and PC (18·5%) were the major phospholipid fractions followed by PI, LPC, PE and PA. As shown in Table 2, after 24 weeks' storage of small-size mullet with viscera PS, PI, PC and PE decreased by 7·76%, 52·0%, 26·2% and 43·6%, respectively, while LPC, SL and PA increased by 73·2%, 49·9% and 158%, respectively. Table 3 shows that, after the same storage period of eviscerated small-size mullet, the decreases of PS, PI, PC and PE were 34·2%, 7·55%, 32·4 and 17·5%, respectively, while the increases of LPC, SL and PA were 46·0%, 20·1% and 315%, respectively. Table 4 shows the individual phospholipid of large-size mullet with viscera. After the storage period, the major phospholipid fractions PS, PI, SL and PC decreased by 54·8%, 9·12%, 57·8% and 20·7%, respectively. LPC, PE and PA increased by 158, 9·97% and 210%. Table 5 shows that, in eviscerated large mullet, PS, PI, SL and PC decreased by 42·8%, 21·6, 38·4% and 12·7%, respectively. LPC, PE and PA increased by 123%, 2·75% and 170%, respectively.

The data presented imply a preferential hydrolysis for PC and PE in small fish and PS, SL and PC in large fish; this may be related to the fatty acids present in the α and β positions of the phospholipids. Olley *et al.* (1969) reported that $C_{16:0}$, $C_{18:1}$ and $C_{20:6}$ acids became a greater proportion of the

TABLE 2

Individual Phospholipids of Plathead Grey Mullet (Small Size with Viscera) Stored at −14°C

Storage time (weeks)		*Phospholipid classes**							*Total recovered*
		PS	*LPC*	*PI*	*SL*	*PC*	*PE*	*PA*	
0	*a*	4·25	3·51	3·53	3·12	9·66	2·18	0·94	27·2
	b	15·6	12·9	13·0	11·5	35·5	8·02	3·46	
6	*a*	2·92	2·70	2·39	2·39	5·82	1·42	1·18	18·8
	b	15·5	14·2	12·7	12·7	30·9	7·56	6·32	
12	*a*	5·15	5·29	3·88	4·84	10·3	2·50	2·45	34·4
	b	15·0	15·4	11·3	14·1	29·7	7·29	7·10	
18	*a*	5·25	6·40	3·06	5·66	9·95	2·13	2·84	35·3
	b	14·8	18·0	8·67	16·0	28·2	6·01	8·05	
24	*a*	3·85	6·03	1·67	4·61	7·02	1·21	2·39	26·8
	b	14·4	22·5	6·23	17·2	26·1	4·52	8·91	

* The sequence of the classes in the Table as separated on the chromatographic plate.
a Phospholipid phosphorus in μg P: 20 μl containing 28·2, 19·8, 37·7, 38·7 and 29·2 μg were applied on the chromatographic plates. Total recoveries were 96·3, 95·2, 91·2, 91·2 and 91·8%, respectively.
b Phospholipid as per cent of the total phosphorus recovered.

TABLE 3

Individual Phospholipids of Plathead Grey Mullet (Eviscerated Small Size) Stored at −14°C

Storage time (weeks)		*Phospholipid classes**							*Total recovered*
		PS	*LPC*	*PI*	*SL*	*PC*	*PE*	*PA*	
0	*a*	4·25	3·51	3·53	3·12	9·66	2·18	0·94	27·2
	b	15·6	12·9	13·0	11·5	35·5	8·02	3·46	
6	*a*	3·32	3·46	2·82	2·73	7·00	1·73	1·43	22·5
	b	14·8	15·4	12·5	12·1	31·0	7·71	6·33	
12	*a*	3·41	4·52	3·37	3·35	8·25	1·90	2·42	27·2
	b	12·5	16·6	12·4	12·3	30·2	6·96	8·90	
18	*a*	3·89	5·92	4·18	4·39	9·59	2·14	4·14	34·2
	b	11·3	17·3	12·2	12·8	27·9	6·25	12·1	
24	*a*	3·53	6·51	4·13	4·74	8·24	2·28	4·93	34·4
	b	10·3	19·0	12·0	13·8	23·9	6·62	14·3	

* The sequence of the classes in the Table as separated on the chromatographic plate.
a Phospholipid phosphorus in μg P: 20 μl containing 28·2, 24·9, 29·2, 37·7 and 37·7 were applied on the TLC plate. Total recoveries were 96·3, 90·3, 93·3, 90·9 and 91·2%.
b Phospholipid as percentage of total recovered.

TABLE 4
Individual Phospholipids of Plathead Grey Mullet (Large Size with Viscera) Stored at −14°C

Storage time (weeks)		*Phospholipid classes**							*Total recovered*
		PS	*LPC*	*PI*	*SL*	*PC*	*PE*	*PA*	
0	*a*	4·57	2·00	2·30	3·19	3·19	1·00	0·94	17·2
	b	26·6	11·6	13·4	18·5	18·5	5·82	5·47	
6	*a*	4·46	5·38	3·37	4·07	4·39	1·48	2·23	25·4
	b	17·5	21·2	13·3	16·0	17·3	5·81	8·80	
12	*a*	5·40	9·15	4·91	5·11	5·83	2·25	4·80	37·8
	b	14·4	24·4	13·1	13·7	15·6	6·00	12·8	
18	*a*	4·94	10·1	4·77	5·12	5·59	2·35	5·00	37·8
	b	13·1	26·6	12·6	13·5	14·8	6·20	13·2	
24	*a*	3·67	9·18	3·72	2·39	4·49	1·96	5·20	30·6
	b	12·0	30·0	12·2	7·81	14·7	6·40	17·0	

* The sequence of the classes in the Table as separated on the chromatographic plates.
a Phospholipid phosphorus; 20 μl containing 18·3, 26·3, 40·5, 39·5 and 33·0 were applied on the TLC plates. Total recoveries were 94·1, 96·7, 92·5, 95·7 and 92·9%, respectively.
b Phospholipid as percentage of the total recovered.

TABLE 5
Individual Phospholipids of Plathead Grey Mullet (Eviscerated Large Size) Stored at −14°C

Storage time (weeks)		*Phospholipid classes**							*Total recovered*
		PS	*LPC*	*PI*	*SL*	*PC*	*PE*	*PA*	
0	*a*	4·57	2·00	2·30	3·19	3·19	1·00	0·94	17·2
	b	26·6	11·6	13·4	18·5	18·5	5·82	5·47	
6	*a*	4·21	3·55	2·68	3·17	3·70	1·06	1·71	20·1
	b	20·9	17·7	13·3	15·8	18·4	5·27	8·52	
12	*a*	6·13	7·12	4·52	4·66	6·33	1·86	4·30	34·9
	b	17·6	20·4	13·0	13·4	18·1	5·32	12·3	
18	*a*	5·77	8·31	4·20	4·38	6·06	1·87	4·83	35·4
	b	16·3	23·5	11·9	12·4	17·1	5·27	13·6	
24	*a*	5·20	8·90	3·59	3·90	5·53	2·05	5·06	34·2
	b	15·2	26·0	10·5	11·4	16·2	5·98	14·8	

* The sequence of the classes in the Table as separated on the chromatographic plate.
a Phospholipid phosphorus in μg P: 20 μl containing 18·3, 22·1, 39·5, 37·7 and 36·7 were applied on the TLC plates. Total recoveries were 94·1, 90·7, 88·3, 94·0 and 93·2%, respectively.
b Phospholipid as percentage of the total recovered.

FFA and the $C_{18:0}$ and $C_{22:6}$ acids were involved in the remaining phospholipid. Braddock and Dugan (1972) reported that the hydrolytic enzymes could be affected by many factors such as the synthesis of substrates, oxidative reactions resulting in inhibition and concentration of solutes during freezing and frozen storage. These factors may affect the mode of attack or the orientation of substrates which, in turn, affect the selectivity and rate of hydrolysis.

REFERENCES

AOAC (1975). *Methods of analysis of the Association of Official Analytical Chemists.* (12th edn), Washington, DC, USA.

AOCS (1957). *Official and tentative methods.* The American Oil Chemists Society. 35 East Wacker Drive, Chicago I, Illinois.

Awad, A., Powrie, W. D. & Fennema, O. (1969). Chemical deterioration of frozen bovine muscle at −4°C. *J. Food Sci.*, **33**, 227.

Blight, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, 1959, **37**, 911.

Bosund, I. & Ganrot, B. (1969). Lipid hydrolysis in frozen Baltic herring. *J. Food Sci.*, **34**, 13.

Braddock, R. J. & Dugan, L. K. (1972). Phospholipid changes in muscle from frozen stored Lake Michigan Coho salmon. *J. Food Sci.*, **37**(3), 426–9.

Castell, C. H. (1974). Interaction of lipids with non-lipid components of fish muscle during storage. Abstracts of Papers, American Chemical Society, 167, AGFD 38.

Dunn, C. R., Keere, B. B. & Elkan, J. G. H. (1969). A technique for improved thin layer chromatography of phospholipids. *J. Chromatog.*, **45**, 326–8.

El-Bastavizi, M. A. & Smirnova, G. A. (1972). Change in the phospholipid of frozen pike during storage. *Ryb. Khoz (Moscow)* (3), 57–9.

El-Sebaiy, L. A., El-Mahdy, A. R., Mostafa, E. I. & Mohamed, M.S. (1980). A modified method for the isolation and quantification of oil seed phospholipids. *Food Chemistry*, **5**, 217–29.

Kates, M. (1972). *Techniques of lipidology: Isolation, analysis and identification of lipids.* North Holland Publishing Company, Amsterdam, London, American Elsevier Publishing Co. Inc., New York.

Kimumaki, T., Iida, H. & Shimma, E. (1970). Changes in lipid components during frozen storage of fish. I. Extraction and separation of lipids. *Bulletin of the Tokai Regional Fisheries Research Laboratory* No. 61, 27–44. c.f. *P.S.T.A.* **5**(12) (1973).

Lovern, J. A., Olley, J. A. & Watson, H. A. (1959). Changes in the lipids of cod during storage in ice. *J. Sci. Food Agric.*, **10**, 327–37.

Olley, J. & Lovern, J. A. (1960). Phospholipid hydrolysis in cod fish stored at various temperatures. *J. Sci. Food Agric.*, **11**, 644–52.

Olley, J., Farmer, J. & Stephan, E. (1969). The rate of phospholipid hydrolysis in frozen fish. *J. Food Technol.*, **4**, 27.

Rouser, G., Fieskher, S. & Yamamoto, A. (1970). Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids*, **5**(5), 494–6.

Shoeb, Z. E., Salama, F. M. & El-Nockrashy, A. S. (1973). Investigation on fish oils. V. Phospholipid composition of *Anginlla vulgaris* and *Mugil cephalus*. *Nahrung*, **17**(1), 31–40.

Takama, K. (1974). Changes in the flesh lipids of fish during frozen storage. IV. Autoxidation of triglycerides and lecithin during cold storage. *Bulletin of the Faculty of Fisheries. Hokkaido University*, **25**(2), 154–61. *C.F.F.S.T.A.*, 7(8)8R 468 (1975).