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LIPID COMPOSITION OF COMMON BIVAIVE MOLLUSCAN SHELLFISH IN EGYPT.

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ABSTRACT

Little is known about the composition of flesh lipids of bivalve molluscan shellfish Om-Elkholoul (Vedge shell, Donax trunculus) and Gandoufly (Butter fish, Tapes spp.) which brought from Edco Lake (Behera) and El-Max region (Alexandria), respectively.

Lipid classes and fatty acid compositions of flesh lipids were studied. Increased proportions of polar lipids in Om- Elkhouloul were mainly due to increased percentage of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and plasmalogen.

In total polar lipids, saturated fatty acids generally decreased and mono-unsaturated fatty acids (derived from PE - plasmalogen) were found to be higher in Om-Elkholoul than in the Gandoufly. However, the proportions of polyunsaturated fatty acids in individual phosphoglycerides were generally higher in Om-Elkholoul, due to mainly increased docosahexaenoic acid (22:6 n-3), and were lowest in Gandoufly. Relatively high percentage of oleic acid (18:1 n-9) was found in all the phosphoglycerides, but primarily PC were higher in Om-Elkholoul than in Gandoufly.

Fatty acids were distributted within individual phosphoglycerides with a characteristic pattern that did not change with species, although the relative amounts of individual fatty acids were altered.

The variations and roles of the different lipid components of bivalve molluscans tissue were discussed with respect to lipid compositions and functions in the same tissue of other fishes and vertebrates.

INTRODUCTION

Bivalve molluscan shellfish have great importance all over the world, this is attributed to three reasons, the first is a delicious taste as a sea food commonly consumed in the coastal cities either in fresh state or cooked with a sauce named hebach. Secondly they contain high calories, proteins, and minerals, especially phosphorous which make them distinguished from other kinds of marine food or any other source of protein (WHO, 1972). The third reason is sanitary, this is due to their filter feeding habits (Anon, 1979, Evison, 1987).

The nutritional properties of long chain n-3 fatty acids, especially eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) acids have been under intensive study in the last decade. These fatty acids seem to be able to modify lipid metabolism, membrane properties, platelet behavior etc. (Kinsella, 1987). Aquatic animals are the superior source of EPA and DHA in human nutrition. The fatty acid compositions of common species in the northern hemisphere are all well known, and they are also the main sources for the fish oil industry (Young and Chem, 1989). At the moment, however, the inclusion of fish in the diet is the only certain safe way to increase the intake of these fatty acids (Goodnight ***et al.***, 1989).

In addition to n-3 fatty acids, the amounts of n-6 fatty acids in species from different climates are of interest. In contrast to cold sea water species, it has been shown that many aquatic animals from warm and temperate waters contain considerable amounts of n-6 fatty acids (Ackman, 1989).

Phospholipids are considered as an important constituent of the cell membranes, their compositions more or less differing with species, tissue, age and diet (Levy and Joncourt, 1973; Medwadowski and Lyman, 1973). Therefore, the present study was under taken to compare polar and neutral lipids fractions of two common used bivalve molluscans species from coastal

parts of Egypt. The distribution of fatty acids in the polar lipid fractions was also investigated to compare the deposition pattern of different fatty acids.

MATERIALS AND METHODS

MATERIALS: Twenty-five random samples of both bivalve molluscan shellfish Om-Elkholoul (wedge shell, Donax trunculus) and Gandoufly (Butter fish, Tapes spp.) were harvested from Lake Edco (Behaira) and El- Max region (Alexandria) Egypt respectively. All samples were found at sea food markets in Alexandria Governorate. They were directly transferred to the laboratory with a minimal delay, where they were divided into five groups, each group contains five samples and subjected to lipid analysis.

Preparation of molluscan samples; was carried out by scrubbing the animal shell with a stiff bristle brush under cold potable water to remove mud and placed on clean paper to drain. To open the clam, it washed in the hand while the edge of the knife was placed at the junction of the bills and forced between the shells with a queezing motion. The body of the animal after cutting of the adductor muscles were transferred to the sample container (West and Coleman, 1986).

METHODS

Extraction of total lipid :

Flesh lipids were extracted by homogenization in a teflon pestle glass homogenizer in chloroform/methanol (2:1 v/v) containing 0.01 % butylated hydroxytoluene (BHT) (Folch ***at al.***, 1957).

Separation of phospholipid subfractions :

Phospholipids were subfractionated by thin layer chromatography (TLC) according to fine and Sprecher (1982) on pre-coated G-60 silica gel -TLC Plates (Merck, Darmstadt Germany) with a mixture of chloroform: methanol: water 65:25:4 (v/v) as mobile phase. The spots on the chromatogram were identified using authentic standards (Serdary Res. Lab. Bellefonte, Cal, USA). After development, the plates were dried in a stream of CO_2 and the spots were visualized with the phosphorus specific reagent 8- anilinonaphthaline sulphate

(ANS) in 5% methanol. In other case the air-dried chromatogram was exposed to iodine vapour for visualization of the different phospholipid subfractions such as, Lysophosphotidyl choline (LPC), Sphingomyelin (SM), Phosphotidytcholine (PC), Phosphatidyl-Serine (PS), Phosphatidyinositol (PI), Phosphatidylethanolamine (PE), Phosphotidic acid (PA) and Cardiolipin (CL), respectively. The spots were removed for quantitative determination of phosphorus according to Rouser et al. (1970).

Separation of neutral lipid content :

The neutral lipids were analyzed according to Malins and Mangold (1960), on pre-coated G-60 Silica gel TLC plates (Merk, Darmstadt, Germany). The development was done in petroleum ether : dieehyl ether : acetic acid (85 : 15 : 1 V/V) as solvent, Identification of the spots was done by comparing the RF values to those known standards (Supelco, Bellefonte, Cal, USA). The spots were detected by spraying the plates with iodine vapour. The neutral lipid fractions were evaluated semiquantitively with a Telechrom OE 976 densitometer (Chinoin, Budapest, Hungary).

Fatty acid Compositions :

Total or individual phospholipids were transmethylated in the presence of 5% HCL in absolute methanol at 80°C in sealed vials for 2.5 hrs.

Gas chromatography of fatty acid methylesters:

Methylesters were separated using a Hewlett-Packard 5890 II equipped with a capillary column coated with SP 2330 of 0.25m thickness (0.25 mm 1.D. x 30 ml CPS-Li Quadrex, New Haven, CT. USA). High purity nitrogen was applied as carrier gas with a flow rate of 230 KPa. Hydrogen was used at 100 KPa and 280 KPo. The dual column system was programmed from 160°C to 200°C to give partial separation of (18 : 4n-3) and 20:1 n-9) at a rate of 1°C/min. The detector temperature and injector temperature were 250°C and 230°C respectively. The peaks were identified by means of primary and secondary standards and by plotting Log. relative elution temperature versus the number of carbon atoms (Schmit and Wynner, 1966). The percentage composition was calculated as a weight percentage (w/w %) using a Hewlett-Packard 3396A

integrator. All peaks between myristic acid (14.0) and docosahexaenoic acid DHA (22: 6n-3) were included in the calculations
All solvents contained 0.01% (w/v) BHT as antioxidant.

Statistical analysis :

The results are presented as means ± SD of five samples Snedcor and Cochran 1982).

RESULTS

In molluscans tissues, polar lipids content as a percentage of total lipids increased in Om-Elkholoul than in Gandoufly with a conconmitant decrease in the percentage of neutral lipids (Table 1). The major polar lipid fractions were phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and the their percentage showed significant increases ($P \leq 0.05$) in Om-Elkholoul than in Gandoufly. In contrast, the proportions of phosphatidylserine (PS) and phosphatidylinositol (PI) remained relatively constant in both types of molluscan shellfish. The percentages of the shigomyelin and sulphatides increased significantly in Om-Elkholoul. The major neutral lipid classes in molluscan species were cholesterol, triacyglycerol (TAG) and sterol esters (SE). The proportions of cholesterol was almost constant within the both studies types, but the proportions of TAG and SE decreased significantly in Om-Elkholoul.

Regarding total lipid, the proportions of total saturates (mainly 16:0 and 18:0) and total monoenes (mainly 18:1 n-9) were significantly greater in Om-Elkholoul than in Gandoufly when determined in the total lipids. In contrast the polar lipid, the proportion of total saturates and mono-unsaturates were significantly greater in Gandoufly than in respectively in Om-Elkholoul (Table 2). The percentages of total n-3 polyunsaturated (PUFAs), predominantly 20:5 n-3 and 22:6 n-3, were much lesser in Gandoufly, while the amount of total n-6 PUFAs were found to be lesser in Om-Elkholoul than in Gandoufly.

Polar lipid contained total monoenes greater than the total lipids Table (2). Om-Elkhouloul contained total saturates, monoenes and polyenoic amounted to 34.6; 15.4 and 50.0% respectively, while Gandoufly involved 32.3; 22.5 and 42.2% consequently, concerning the total lipids. The polar lipids contained

different percentages of these fatty acid groups are presented in Table (2). However, the fatty acid composition of individual polar lipid fractions was found to be quite different for both Om-Elkhouloul and Gandoufly (Table 3).

DISCUSSION

Polar lipid fractions were found to be predominant and cholesterol accounted for over 75 % of total neutral lipids. The percentages of total polar lipids were very similar to those of trout and cod (Tocher and Sargent 1990) and those of sea bass that had been fed on cod-liver oil diet (Pagliarani ***et al.***, 1986).

Compared to cell membranes in general, there is obvious excess of PC compared to PE of both species and the PC:PE ratio reverts to a normal value as development proceeds. This is compatible with a marked selective catabolism of PC, but there is some possibility for choline released during hydrolysis of PC to convert to betaine for use as an intracellular osmolytes before potassium becomes available from outside the cell by sodium pumping (Falk-Peterson, et al. 1989).

The most predominant feature of the fatty acid composition in flesh lipids was the increase in 20:5 n-3, which related entirely to PE than other phosphoglycerides. This may be due to a preferential selectivity of the enzymes to the diglyceride substrates (Weiss ***et al.***, 1960). The polyunsaturated fatty acids were observed in all the phosphoglycerides fractions, although there was an increase in 18:1 n-9 the most predominant in PC. The increase in 20:5 n-3 led to an increment in the synthesis and accumulation of PE plasmalogens. PE-plasmalogens are implicated in myelin membrane composition (Sastry 1985).

The present study confirms the presence of relatively high levels of 18:1 n-9 in phosphoglycerides, especially PC, from Om-Elkholoul tissues, previously reported for carp (Natarajan ***et al.***, 1985), and marine fish (Tocher and Sargent 1984; Bell and Tocher 1989). However, the distribution of the individual PUFA and fatty acids in general between different phosphoglycerides in molluscans is similar to that in the fish and mammals. Specially, 18:0 and enriched of C_{22} PUFA in PS, high 16:0 and lower PUFA in PC, intermediate levels of 22:6 n-6 in PE and high 18:0 and C_{20} PUFA (primarily 20:4 n-6 and 20:5 n-3) in PI found in the present study.

Table (1): Lipid composition (as % total lipid) of edible portion from bivalve molluscans shellfish (Om-Elkholoul and Gandoufly).

Lipid fractions	*Om- Elkholoul*	*Gandoufly*
(1) Neutral Lipids	-	-
Sterolester (SE)	2.2 ± 0.08	11.0 ± 1.00
Triacylglycerol (TAG)	7.04 ± 0.30	15.20 ± 1.30
Free Fattyacids (FFA)	1.23 ± 0.50	1.80 ± 0.40
1,2 Diacylglycerol (DAG)	0.41 ± 0.10	N.D.
Cholestrol (Ch)	19.00 ± 1.20	20.50 ± 1.90
Polar lipids	70.0 ± 2.75	51.50 ± 2.30
2- Polar Lipids	-	-
Neutrol Lipid (NL)	3.11 ± 0.32	6.50 ± 0.50
Sterol ester (SE)	11.50 ± 1.7	16.17 ± 1.40
Phosphatidic acid (PA)	2.91 ± 0.30	2.13 ± 0.60
Cardiolipin (CL)	1.40 ± 0.10	3.60 ± 0.10
Lysophosphatidyl ethanolamine (LPE)	1.6 ± 0.10	2.75 ± 0.20
Phosphatidylethanolamine (PE)	28.80 ± 1.85	17.96 ± 1.05
Phosphatidyl serine (PS)	3.65 ± 0.70	3.15 ± 0.45
Phosphatidyl choline (PC)	35.20 ± 0.40	29.80 ± 1.90
Sphingomyelin (SM)	13.45 ± 2.40	8.17 ± 0.25
Phosphatidyl inositol (PI)	6.45 ± 0.20	6.70 ± 0.30
lysophophatidyl choline (LPC)	1.83 ± 0.10	2.57 ± 0.10

N.D : not detected
Data are the means ± SD of five samples.

Table (2): Fatty acid composition (percentage weight of total and polar) lipids of bivalve molluscans shellfish) Om-Elkholoul & Gandoufly.

	Total lipids		Polar Lipids	
Fatty acid	Om-Elkholoul	Gandoufly	Om-Elkholoul	Gandoufly
14:0	1.6	4.0	0.3	2.4
16:0	21.8	21.3	13.6	17.1
18:0	9.2	5.1	6.3	2.4
20:0	2.0	1.9	0.3	1.3
Total saturates	34.6	32.3	20.5	23.3
14:1	0.6	0.1	1.3	0.9
16:1 n-7	3.9	9.3	3.3	14.9
18:1 n-9	9.3	8.4	16.9	5.6
20:1	1.0	1.8	0.6	1.7
22:1	0.6	2.9	0.6	2.3
Total monoenes	15.4	22.5	22.7	25.4
18:2 n-6	4.4	8.4	3.3	8.1
18:3 n-3	1.0	1.5	0.6	2.2
18:4 n-4	0.1	1.2	0.7	1.4
20:3 n-6	0.9	0.7	1.4	0.3
20:4 n-6	1.4	5.6	5.1	4.8
20:4 n-3	0.1	0.7	0.1	0.5
20:5 n-3	15.8	11.2	17.8	14.2
22:4 n-6	0.4	0.6	1.4	2.0
22:5 n-6	1.6	1.4	0.5	2.8
22:5 n-3	1.8	2.5	2.8	2.3
22;6 n-3	22.5	11.6	25.9	12.8
total polyenoic	50.0	45.2	59.6	51.4
n-6 series	8.7	16.7	11.7	18.3
n-3 series	41.3	28.5	47.9	33.1

Values are averages of five measurements.

Table (3): Fatty acid composition (percentage weight) of individual polar lipids of bivalve molluscans shellfish Om-Elkholoul and Gandoufly.

Fatty acid	*PC*		*PE*		*PS*		*PI*	
	Om-El	Gand	Om-El	Gand	Om-El	Gand	Om-El	Gand
14:0	0.3	1.2	trace	2.0	trace	0.3	0.4	1.0
16:0	20.1	18.1	10.1	18.0	8.9	15.9	22.8	19.4
18:0	5.1	3.6	5.3	0.8	7.1	2.0	9.4	22.2
20:0	0.2	1.0	0.4	1.0	0.8	0.5	0.6	1.8
Total saturates	25.7	23.8	15.8	21.8	16.8	18.7	33.2	44.4
14:1	trace	trace	0.7	0.6	0.6	0.5	0.6	0.8
16:1 n-7	4.6	9.7	5.9	12.9	4.1	3.2	2.6	7.0
18:1 n-9	18.4	9.5	17.1	6.6	15.6	18.5	7.9	12.1
20:1	0.4	2.0	0.5	0.9	0.6	0.8	1.1	0.5
22:1	--	3.1	0.3	1.3	0.8	1.6	0.7	0.6
Total monoenes	23.5	24.3	21.5	22.3	21.7	24.0	12.9	21.0
18:2 n-6	2.9	9.4	4.9	9.1	3.5	7.2	12.4	4.5
18:3 n-3	0.2	3.5	1.4	0.8	0.3	0.2	0.3	1.6
18:4 n-3	0.5	1.6	0.3	0.2	0.4	0.8	0.1	1.0
20:3 n-6	1.0	1.4	1.8	0.5	0.9	1.1	1.1	0.5
20:4 n-6	4.5	6.4	3.3	2.4	2.5	trace	5.1	4.8
20:4 n-3	0.5	0.3	0.3	0.9	0.1	5.9	1.1	0.7
20:5 n-3	15.2	11.0	18.5	11.5	13.5	13.0	12.7	5.9
22:4 n-6	0.4	1.2	0.5	1.2	0.5	1.0	2.7	1.2
22:5 n-6	1.3	3.4	0.4	2.1	0.4	1.3	0.7	2.9
22:5 n-3	1.5	2.8	1.8	3.9	3.4	2.7	1.6	0.9
22;6 n-3	22.9	12.9	28.5	20,7	36.2	23.7	16.1	14.8
total polyenoic	50.7	51.9	59.7	55.9	61.5	57.3	53.9	34.6
n-6 series	9.7	21.8	10.9	15.3	7.8	10.6	22.0	9.9
n-3 series	40.9	30.1	48.8	40.6	53.7	46.7	31.9	20.7

Values are averages of five measurements.
Om-El- = Om-Elkholoul Gand = Gandoufly
PC = Phosphotidylcholine
PE = Phosphotidylethanolamine
PS = Phosphotidyl-serine
PI = Phosphotidylinositol

Not with standing, the relative percentages of individual fatty acids in molluscans phosphoglycerides are very variable, depending upon species. For instance, the percentage of 20:4 n-6 in PI from molluscans was lower than that found in carp (Natarjan et al., 1985) and in rainbow trout (Tocher and Sargent 1984), but higher than that in cod (Bell and Dick 1990). Docosahexaenoic acid (22:6 n-3) is generally the major PUFA in all vertebrate tissues, but its level varies (Sastry 1985). The proportion of 22:6 n-3 in phosphoglyceride fractions from molluscans were comparable but, in general more than those found in tissue from marine fish (Tocher and Sargent 1984), carp (Natarjan ***et al.***, 1985) and various species of marine teleosts (Kreps, 1981). It is known that large amounts of 22:6 n-3 required for biogenesis of membranes (Bazan, 1990). Possible role of 22:6 n-3 in muscular tissues may include effects on the biophysical properties of membrane, modulation of lipid - protein interactions and membrane bound enzymes and a role as precursor for functionally important lipoxygenase products (Neuringer ***et al.***, 1988; Bazan 1990). In the phosphoglycerides from aquatic animals some accumulation of 22:6 n-3 was observed. This is consistent with the accumulation of 22:6 n-3 in avians (Anderson ***et al.***, 1989) and mammals (Neuringer ***et al.***, 1986; Neuringer ***et al.***, 1988; Bazan 1990).

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