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Lipids of Milkfish (*Chanos chanos*, Forskall)

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Abstract

There is an urgent need for thorough and reliable information on the lipid content and fatty acid composition of milkfish. The lipid content and fatty acid composition varies with anatomical location. The ventral muscle of milkfish contained more lipids than the dorsal muscle. Palmitic (16:0), Palmitoleic (16:1) stearic (18:0), oleic (18:1), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids were found to be the most abundant fatty acids. Also, marked variations in the concentration of the fatty acid composition between dorsal and ventral muscles were observed. Dorsal muscle contained more phospholipid than the ventral muscle. This is substantiated by the presence of higher concentrations of 20:5, 22:5 and 22:6 acids in the dorsal muscle. Also included is the fatty acid content of milkfish which can be a useful information for the nutrient data bank.

Introduction

The introduction of modern techniques of fish cultivation in the Philippines increased in 1973. Demands for milkfish remained strong but its limited supply caused fluctuations in prices from time to time. The development and exploitation of non-conventional methods of fish cultivation was encouraged by the government. In the Philippines, interest in milkfish productions was stimulated by the successful introduction of fish pens in the Laguna Lake area.

However, the utilization of these cultured fish must accompany the concomitant need for information concerning the chemical composition and nutrient content of this fish. Such data are needed by food processors and technologists to devise optimum processing and storage conditions for this fish as milkfish production increases.

Milkfish (*Chanos chanos*, Forskall) is a very popular fish in the Philippines, but few studies have been done about its lipid and fatty acid contents. The authors examined the lipids and fatty acids of milkfish in an attempt to provide useful information for better processing and storage conditions for this fish. Actual content of each fatty acid in milkfish as g fatty acid per 100 g muscle was determined from the fatty acid composition of milkfish.

Materials and Methods

Fish:

Milkfish (*Chanos chanos*, Forskall) (Fig. 1) was caught in Laguna Lake, off the shore of Jala-Jala, Rizal during the month of December, 1980. Prior to freezing,

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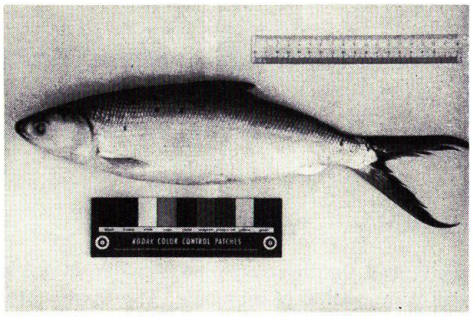

Fig. 1. Milkfish (*Chanos chanos*, Forskall).

the head, tail, fins and viscera were removed. The fish was frozen and stored at –40°C until analyzed. The total length of the fish was 42 cm and the weight, 650 g. After thawing overnight in a cold room (4°C), fish fillets were obtained by cutting the fish lengthwise along the backbone. To check if variations in lipid content were present, different sections of the muscle fillets were cut and divided into dorsal, ventral and whole muscle portions. The fillets were cut into small portions and mixed thoroughly before taking samples for analysis.

Lipid extraction:

Fish lipids were extracted by using the method of Bligh and Dyer with slight modifications.[1] Representative samples of the muscle portion (25 g) were homogenized in a Waring blendor for 2 min with 75 ml mixture of chloroform and methanol (2:1, by vol). Twenty-five ml of chloroform was added to the mixture and after blending for an additional 30 sec, distilled water (25 ml) was added and blended again for 30 sec. The homogenate was centrifuged at 3,000 rpm for 1 hr and the chloroform layer (lower phase) taken using a 25 ml pipet and transferred to a previously weighed flask and concentrated to constant weight under vacuum and then the lipid content was determined.

The qualitative and quantitative analyses of the lipid were carried out by thin layer chromatography (TLC). The fractionation of neutral lipid and phospholipid was accomplished on a preparative plate. The development solvent systems used were n-hexane-diethyl ether-acetic acid (70:30:1, by vol) for neutral lipid and n-hexane-acetone (8:2, by vol) followed by chloroform-methanol-ammonia-water (70:30:2:3, by vol) or chloroform-methanol-acetic acid-water (25:15:4:2, by vol) for phospholipid. The individual lipid components were quantitated by densitometer (Ozumor–82, Asuka Kgōyō K.K.) after spraying the plate with copper acetate-phosphoric acid reagent and heating for 15–20 min in an oven of 150°C.

Methylation:

Methyl esters of fatty acids were prepared according to the method of Prevot and Mordret.[2] Analysis of the methyl esters was done using a Hitachi 063 gas chromatograph equipped with a 3 m (i.d. 3 mm) glass column packed with Unisole 3000 on a Uniport C (80–100 mesh). The column, injection port and detector temperatures were 230°C, 265°C and 252°C, respectively. The flow rate of the carrier gas (nitrogen) was 40 ml/min.

Phospholipid content was determined using the colorimetric method of Stewart.[3)]

Calculations:

The method of calculating g fatty acid (FA)/100 g muscle or g fatty acid (FA)/100 g total lipid (TL) was done first by calculating the lipid factor of triglyceride (TG) and phospholipid (PL). Then the conversion factor (F) which is defined as the weight of fatty acids in 1 g of fat were calculated. In this experiment, the derivation of the factors resulted in the following data.

For 1) Dorsal muscle

$$TG \times 0.95 + PL \times 0.72 = F$$

2) Ventral muscle

$$TG \times 0.95 + PL \times 0.71 = F$$

3) Whole muscle

$$TG \times 0.95 + PL \times 0.72 = F$$

The factor is then converted to fatty acid methyl ester data to values suitable for food composition tables. The calculations then proceed as follows:

$$F \times FA \times TL \text{ (decimal)} = \text{g FA/100 g muscle}$$

Results

The results of the lipid analysis are given in Table 1. The lipid content of the milkfish was found to vary according to the location of the muscle portion. The ventral muscle was found to be richer in lipids than the dorsal muscle. As shown in Table 2, 92.13% of these lipids were found to be neutral lipid and only 7.87% were phospholipid. On the other hand, the dorsal muscle was found to contain higher phospholipid (18.11%) and lower neutral lipid (81.89%) contents.

Table 1. *Total lipid content of different muscle portions of milkfish (Chanos chanos,* Forskall).

Lipid g/100 g Muscle

	Dorsal Muscle	Ventral Muscle	Whole Muscle
Neutral Lipid	2.94	4.92	3.74
Phospholipid	0.65	0.42	0.54
Total Lipid	3.59	5.34	4.28

Triglyceride was found to be the main component of the neutral lipid while phosphatidylethanolamine and phosphatidylcholine were found to be the main components of the phospholipid (Table 2).

Table 3 shows the individual fatty acid composition of the different muscle portions of the milkfish. Palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids were the major fatty acids in this fish. The concentration of the fatty acid compositions of the different muscle portions varied markedly. Palmitic (16:0) acid was higher in the ventral muscle. However, docosahexaenoic (22:6) acid tends to be higher in the

Table 2. *The lipid classes of milkfish muscle lipid.*
Lipid g/100 g Total Lipid

	Dorsal Muscle	Ventral Muscle	Whole Muscle
NPL	81.89	92.13	87.39
TG	66.57	79.02	70.56
FFA	5.57	2.81	5.84
DG	1.11	0.94	1.40
MG	1.39	0.94	1.17
ST	2.51	2.43	2.34
DAGE	1.67	3.74	2.34
SE+HC	3.07	2.25	3.74
PL	18.11	7.87	12.61
PC	7.24	3.56	5.14
PE	5.29	2.25	3.74
PI	1.67	0.37	1.17
PS	0.58	0.19	0.47
SPM	1.39	0.75	0.93
LPC	0.56	0.19	0.23
UK-1	0.28	0.19	0.23
UK-2	1.10	0.37	0.70

NPL – Non-phospholipid
TG – Triglyceride
FFA – Free fatty acid
DG – Diglyceride
MG – Monoglyceride
ST – Sterol
DAGE – Diacylglyceryl ether
SE+HC – Sterol ester+ Hydrocarbon
PL – Phospholipid
PC – Phosphatidylcholine
PE – Phosphatidylethanolamine
PI – Phosphatidylinositol
PS – Phosphatidylserine
SPM – Sphingomyelin
LPC – Lyso-phosphatidylcholine
UK – Unknown

dorsal muscle. Other fatty acids were consistent in their distribution throughout the various muscle portion.

Table 4 shows the comparison of the fatty acid composition between the triglyceride, phosphatidylethanolamine and phosphatidylcholine fractions of the whole muscle lipid of milkfish. Palmitic (16:0) and oleic (18:1) acids were found to be the main fatty acids of the triglyceride fraction. However, in the phosphatidylcholine fraction, palmitic (16:0), oleic (18:1), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids were the main components. The phosphatidylethanolamine fraction was found to be rich in stearic (18:0), oleic (18:1), and docosahexaenoic (22:6) acids.

Discussion

Lipids are not evenly distributed in the body of fish. The results are in agreement with that of Kinsella et al.[4] in that generally, the ventral muscle portion of fish is much richer in lipid than the dorsal muscle portion of the fish.

As shown in Table 1, the non-phospholipid content of the ventral muscle was about 1.7 times that of the dorsal muscle. This fact was also reflected in the amount of triglyceride in the ventral muscle, which was 1.2 times that of the dorsal muscle (Table 2). On the other hand, the amount of phospholipid in the dorsal

Table 3. *Fatty acid composition of milkfish muscle lipid.*

Fatty acid	Dorsal Muscle			Ventral Muscle			Whole Muscle		
	TL	NL	PL	TL	NL	PL	TL	NL	PL
UK	0.30	—	—	0.30	—	0.14	0.30	—	—
14:0	3.26	3.68	0.99	3.40	3.66	0.66	3.34	3.84	0.76
UK	0.23	0.26	—	0.26	0.23	—	0.23	0.26	—
15:0	0.27	0.29	0.13	0.28	0.28	—	0.28	0.30	0.10
UK	0.06	0.12	0.82	—	0.10	0.76	0.05	0.11	1.02
16:0	28.44	30.22	21.44	30.24	31.54	23.39	29.64	31.06	23.30
16:1	6.53	7.05	0.78	7.59	8.00	0.67	6.76	7.54	0.80
17:0	1.90	2.08	0.40	1.93	2.10	0.33	1.85	2.12	0.36
17:1	0.62	0.71	0.33	0.75	0.80	0.20	0.62	0.74	0.29
18:0	6.25	6.47	6.33	5.61	5.78	5.25	6.16	6.35	5.70
18:1	19.64	20.95	14.97	19.86	20.70	13.96	19.56	20.78	14.83
18:2	2.31	2.39	1.56	2.38	2.42	1.52	2.36	2.42	1.52
19:1	3.24	3.40	0.89	3.87	4.01	0.77	3.37	3.70	0.75
18:4	0.99	0.93	0.29	1.04	1.03	0.37	1.00	0.99	0.30
20:1	2.34	3.00	0.26	2.09	2.08	0.23	2.20	2.48	0.24
20:2ω6	0.44	0.33	0.18	0.29	0.38	0.19	0.37	0.36	0.16
20:2ω5	0.70	0.58	0.83	0.63	0.60	0.80	0.64	0.65	0.72
20:3	2.62	2.19	6.69	2.19	1.86	6.81	2.51	2.11	6.42
20:4	2.60	2.50	0.51	2.74	2.67	0.55	2.40	2.17	0.52
20:5	3.44	2.29	10.08	3.38	2.83	11.15	3.26	2.08	10.67
22:3	0.38	0.20	—	0.32	0.38	—	0.30	0.31	0.12
UK	0.60	0.48	0.57	0.49	0.37	0.30	0.58	0.43	0.34
22:4	1.44	1.07	3.14	1.19	0.84	2.68	1.34	1.00	2.62
22:5	2.99	2.69	2.25	2.44	2.10	2.30	2.82	2.50	2.08
22:6	8.41	6.12	26.56	6.73	5.24	26.97	8.06	5.70	26.38
Saturated	40.12	42.74	29.29	41.46	43.36	29.63	41.27	43.67	30.22
Monoenoic	32.37	35.11	17.23	34.16	35.59	15.83	32.51	35.24	16.91
Polyenoic	26.32	21.29	52.09	23.33	20.35	53.34	25.06	20.29	51.51

muscle was 1.5 times that of the ventral muscle (Table 1). This was evident in the phosphatidylethanolamine and phosphatidylcholine content of the dorsal muscle (Table 2) which was about 2.3 times and 2.0 times, respectively, of that contained in the ventral muscle portion.

In general, phospholipid content is inversely proportional to the lipid content of fish and shows the least variation in fish muscle. In addition, phospholipid also contains high amount of unsaturated fatty acids such as 20:5 and 22:6 acids. The concentration of docosahexaenoic (22:6) acid in the total lipid (Table 3) increased as the lipid content decreased. This high amount of 22:6 acid reflects the amount of phospholipid in the lipid of the dorsal muscle of milkfish. Dorsal muscle had a higher concentration of 22:6 acid (8.41%) than the whole muscle and ventral muscles (8.06% and 6.73%) respectively. This observation was also consistent with the observation of Kinsella et al.[4] and Saddler et al.[5]

The concentration of 20:5, 22:5 and 22:6 acids was higher in the dorsal muscle (14.84%, Table 3) than in the ventral muscle (12.55%). Whole muscle concentration of 20:5, 22:5 and 22:6 acids was found to be the average of the dorsal and ventral values.

Table 4. *Comparison of the fatty acid composition of neutral lipid and phospholipid in whole muscle of milkfish.*

Fatty acid	Neutral Lipid	Phospholipid	
	Triglyceride	Phosphatidyl-choline	Phosphatidyl-ethanolamine
UK	—	—	0.70
14:0	5.56	2.11	4.31
16:0	35.83	31.21	7.79
16:1	9.50	1.36	1.95
17:0	2.63	—	0.94
17:1	0.58	—	—
18:0	5.21	5.17	22.35
18:1	22.29	20.88	11.76
18:2	—	1.12	—
18:3	1.81	—	1.22
19:1	3.75	—	0.67
19:2	1.05	—	0.52
20:1	1.84	—	—
20:2	—	0.72	1.09
20:3	1.50	6.90	9.10
20:4	1.82	—	—
20:5	1.86	11.09	6.27
22:4	0.42	2.44	3.47
22:5	1.61	2.08	2.78
22:6	2.74	14.92	25.08

Comparison between neutral lipid and phospholipid fractions showed neutral lipid (TG) to have significantly low 20:5 and 22:6 acids when compared to phospholipids (PC and PE), which abundantly contained 16:0, 16:1, 18:1, 20:5 and 22:6 (Tables 3 and 4).

Knowledge of the fatty acid composition of this fish can be very helpful not

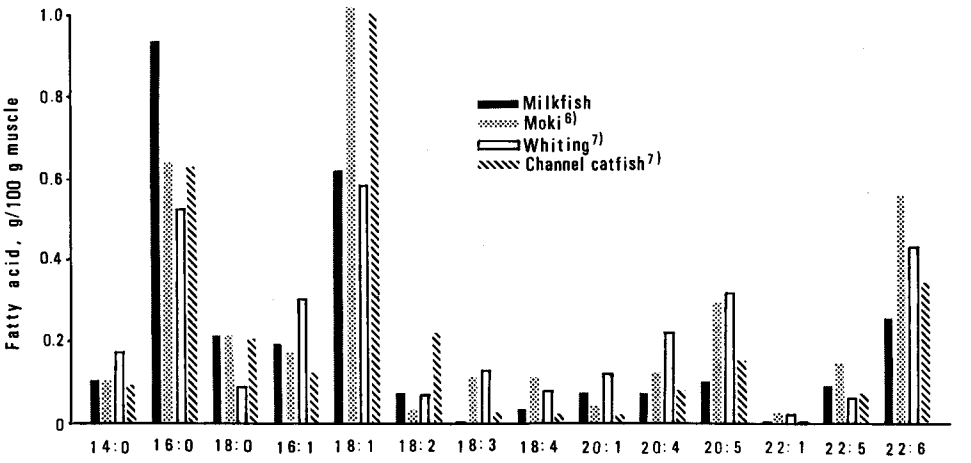

Fig. 2. Actual amount of nutritionally important fatty acid.

only in the processing and storage aspect but in the nutritional evaluation of this very popular fish in the Philippines as well.

The method of calculating the actual amount of fatty acid as g fatty acid (FA)/ 100 g muscle necessitates the derivation of reasonable factor (F). The factor (F) can be easily calculated when the lipid class is given. The factor (F) is then converted to fatty acid methyl ester data to values suitable for food composition tables.

Fig. 2 shows the actual amount of fatty acid composition of milkfish as g fatty acid/100 g fish muscle, using factors derived from the data of Table 3. Milkfish was found to contain the following nutritionally important fatty acids such as 14:0, 16:0, 16:1, 18:1, 18:2, 22:5, and 22:6. Also included in the table are published data taken from other literatures concerning the following fishes: moki (*Latridopsis ciliaris*)[6], whiting (*Mercluccius bilinearis*)[7] and channel catfish (*Ictalarus punctatus*).[7]

The actual fatty acid composition of milkfish compared well with the published data of fishes like moki in 14:0, 18:0, 16:1, 18:2, 20:1, 20:4 and 20:5; whiting in 18:2, 18:4, 20:1 and 22:5 and channel catfish in 14:0, 18:0, 18:3, 18:4, 20:1, 20:4, 20:5 and 22:5 acids. Milkfish showed an average fatty acid distribution in its muscle among these 3 fish species.

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