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Studies on Lipid in the Muscle of Skipjack (*Katsuwonus Pelamis*)—I

Distribution of lipid in skeletal muscle

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Abstract

The total lipid (TL) contents of dorsal muscle, dorsal internal muscle and ordinary muscle were smaller than those of ventral muscle, dorsal external muscle and dark muscle, respectively. The proportion of phospholipid (PL) content of the internal muscle to TL was found to be higher than that of the external in the dorsal or ventral muscle.

The major constituent fatty acids of TL, neutral lipid and PL were 16: 0, 18: 1, and 22: 6 acid, and a similarlity of fatty acid constitution was observed between the external and internal ordinary muscle. Accordingly, it was concluded that there was no characteristic component in the skipjack muscle lipids and the component of fatty acids of each lipid class was the same as that of other marine fish species, and their composition was merely different in the relative ratio. It was assumed that such epipelagic fishes as skipjack had large amount of polyunsaturated acids *i. e.* docosahexaenoic acid.

In spite of the importance of the Pacific skipjack, *Katsuwonus pelamis*, as a food-fish, a search of the literature indicates a paucity of detailed information on the chemical composition of its muscle except special substances (that is myoglobin[1-3] and glucose-6-phosphate[4]). In recent years, many investigators[5] have discussed in detail the biology and habitate of this fish, but there appears to be few informations published on its chemical composition or accetability as fresh food-fish. In regard to the content of lipid, TAKAHASHI[6] examined the relationship between the fat content of skipjack meat and the weight of fatty tissue on the heart, and YAMADA and NAKAMURA[7] reported the results of histochemical observations on the lipids of muscle in several species of fish including skipjack. In this paper, the distribution and some properties of the lipids of skipjack muscle are described.

Materials and methods

Samples of fish were obtained from the south sea of Kyushu (South Japan) in 1973–1975. Four fish (average body length 52.8 cm, average body weight 3300 g) were obtained from off Gaja Is. (about 30°N, 129°E) as A group; 3 fish (average body length 41.5 cm, average body weight 1900 g) from the South Pacific ocean (about

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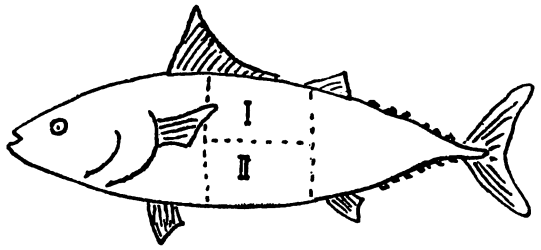
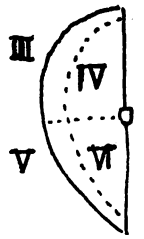


Side view
I Dorsal
II Ventral



Sectional view
III External dorsal
IV Internal //
V External ventral
VI Internal //

Fig. 1. Sampling of fish muscle.

5°N, 149–160°E) as B; and 6 fish (average body length 37.9 cm, average weight 800 g) from Kagoshima bay (about 31°30′N, 131°40′E) as C.

The samples of all these fish were kept at −25°C for several days until required. One round fish of each group was filleted and skinned, and then dorsal muscle was separated into ordinary and dark muscles. Lipid analyses were carried out on the ordinary muscle and on some dark muscle (B and C groups). The rest fish were treated as shown in Fig. 1. Samples of each muscle were minced and mixed thoroughly.

Lipids were extracted from the muscle by the procedure of BLIGH and DYER[8] and then by the method of HANSON and OLLEY[9] were fractionated into two lipid classes, phospholipid (PL) and non-PL. Free fatty acid (FFA) was then removed from the non-PL fraction by McCATHY and DUTHIE's method[10] and neutral lipid (NL) so obtained was fractionated on silica gel G[11]. PL was qualitatively identified on thin layer chromatography (TLC) plates by OWEN's method[12].

The fatty acid composition was determined by gas liquid chromotography (GLC) on 2 m×2 mm glass column which was packed with 10% diethylenglycol succinate (DEGS) on 60/80 mesh, Shimalite W, and was held at column temperature of 185°C. Methylesters prepared by transesterification[11] with sulphuric acid/methanol containing 0.5% benzen. Peak identies were determined from the known standards for each fatty acid reported, and quantitative accuracy was determined by using the internal standardization, respectively. Computation of percentage composition was made by the 'peak height×width at half height formula'[13].

The histochemical observation of muscle tissue was performed by the techniques of YAMADA[7].

Results and discussion

Lipid content

1) Ordinary muscle of dorsal and ventral muscles

Table 1, Plates 1 and 2 as well known[14] showed the ventral muscle of skipjack containing more lipid than in the dorsal muscle. In the external part of dorsal and ventral muscles, the contents of total lipid (TL) and NL were higher than those of the internal part. NL-content value was over 50 per cent of TL-content and there was a positive relationship between NL-content and TL-content. The proportion of PL-content to TL-content in the internal part of various muscles was shown to be higher than that of the external muscle.

Table 1. Lipid content of dorsal and ventral muscles of skipjack. (g/100 g muscle; % of total lipid in parenthesis).

Samples	A			B		
Part of muscle	I	II	III	IV	V	VI
TL	1.66	1.75	1.34	0.53	1.59	0.62
NL	1.03 (62.1)	1.18 (67.3)	0.77 (57.5)	0.17 (32.1)	1.04 (65.4)	0.12 (19.4)
PL	0.55 (33.1)	0.51 (29.3)	0.34 (25.4)	0.28 (52.8)	0.28 (17.6)	0.41 (66.1)
FFA	0.08 (4.8)	0.06 (3.4)	0.23 (17.1)	0.08 (15.1)	0.27 (17.0)	0.09 (14.5)

I–VI: showed in Fig. 1. TL: Total lipid, NL: Neutral lipid, PL: Phospholipid, FFA: Free fatty acid.

Table 2. Lipid content of ordinary and dark muscles of skipjack (indicated by values in g per 100 g of muscle, in % of total lipid in parenthesis).

Samples	A		B	
Muscle	OM	DM	OM	DM
TL	1.92	3.90	0.56	1.79
NL	1.20 (62.5)	2.63 (67.4)	0.27 (48.2)	1.05 (58.7)
PL	0.63 (32.8)	1.14 (29.2)	0.24 (42.8)	0.7 (39.1)
FFA	0.09 (4.7)	0.13 (3.3)	0.05 (8.9)	0.04 (2.2)

OM: Ordinary muscle, DM: Dark muscle.

2) Dark muscle

As already shown by many investigators[15-19] on the other species, in skipjack the dark muscle had a much higher lipid content than that which the ordinary muscle had (Table 2). The proportion of NL-content to TL-content in the dark muscle was higher than the corresponding value in ordinary muscle. It was already described that the cells of dark muscle were narrower than those of white muscle[19]. In this experiments, the above indication was recognized as shown in Plate 3.

These data showed that the lipid content of ventral muscle was higher than that of dorsal muscle, and it was recognized that the proportions of each lipid class content to TL-content in various parts of muscle showed almost the same level, except that external muscle contained more NL than internal muscle.

Component of non-PL fraction

As shown in Table 3, components of non-PL fraction in lipid of fresh dorsal ordinary muscle were mainly tri-glycerides, while lipid of stale muscle contained much di-glycerides and mono-glycerides. In author's another analysis, sterol content level almost agreed with the results obtained in other fish muscles by SHIMMA et al.[20], though the separation of sterol from di-glycerides fraction was not sufficiently. The determined FFA values showed broad variations in sample B and C. It was assumed that the variation of FFA value might be caused by keeping-condition after fish-catching. Namely, the high value of FFA or mono-glycerides shows the result brought forth by the process in which tri-glycerides or phospholipids were hydrolysed progressively by enzymes.

Table 3. Non-phospholipid composition of dorsal (ordinaly) muscle lipid. (Expressed as % of total non-phospholipid).

Sample	TL*	Hydro-carbon	TG	DG+ sterol	MG	FFA	sterol ester
B	1.66	tr.	86.3	8.6	tr.	1.9	3.2
C	0.56	tr.	26.0	43.4	16.0	14.6	tr.

* Expressed as % of wet weight

TL: Total lipid, TG: Triglycerides, DG: Diglycerides, MG: Monoglycerides, FFA: Free fatty acid.

Component of PL fraction

Figure 2 is an example of TLC separation pattern of PL in the dorsal ordinary muscle in sample B. Phosphatidylcholine spot was much coloured but phosphatidyl-inositol and sphingomyelin spots were less coloured, accordingly it was suggested that phophatidycholine would be main component. This pattern was the same as that of 'great silver smelt' body lipid[11]. The TLC pattern of PL was not characteristic of PL in skipjack muscle.

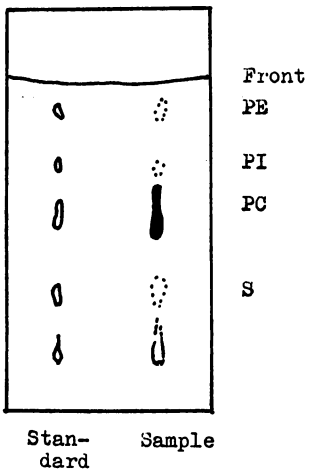


Fig. 2. Thin-layer chromatogram illustrating the separation of phospholipid in the dorsal muscle of sample B.

Solvent system:	Chloroform	65
	Methanol	43
	Water	3
	Acetic acid	1

PE: Phosphatidylethanolamine,
PI: Phosphatidylinositol,
PC: Phosphatidylcholine,
S: Sphingomyelin.

Fatty acid composition of each lipid class

The fatty acid compositions of TL, NL, PL and FFA of dorsal muscle lipid in sample A are given in Table 4. It was found that there was no characteristic component in skipjack muscle lipid, and that the component of fatty acids of each lipid class was the same as that of other marine fish species and that their composition was merely different in the relative ratio. In the case of skipjack, the characteristic of high hexaenoic acid content is evident, while ITO et al.[21] reported that in unsaturated acids tunny oil, swordfish oil and other fish oil contained much monoenoic acid. Hexaenoic acid content of the lipid classes was relatively high, compared with thoes of 'barraude' and 'longnose lancetfish'[22], the fish from the south sea (the Coral Sea). Further reports, HAYASHI and YAMADA[23] described that the component of fatty acids of NL in deep-sea fish consisted of large amounts of monoenoic acids, such as 18: 1 acid, and small amounts of polyenoic acids, such as 20: 5 and 22: 6 acids; and also the polyenoic acid contents showed a tendency to decrease with the increasing of habitat depth, while the monoenoic acids contents tended to increase. Accordingly, it was considered by them that the epipelagic fishes had large amounts of polyenoic acids, whereas mesopelagic fishes had large amounts of monoenoic acids. The lipid of skipjack muscle showed the components of fatty acid made of large amounts of

Table 4. Fatty acid composition of total lipid, neutral lipid, phospholipid and free fatty acid in external and internal dorsal muscles in skipjack muscle (sample B). (% of total detector response).

Number of carbon atoms and double bond	External dorsal muscle				Internal dorsal muscle			
	TL	NL	PL	FFA	TL	NL	PL	FFA
14: 0	3.3	3.1	7.2	1.1	1.0	0.7	0.4	2.0
15: 0	1.6	1.2	0.9	0.8	3.4	0.2	9.7	0.6
16: 0	22.0	20.1	8.7	43.7	20.5	40.2	8.4	32.0
16: 1	4.6	9.0	8.1	—	—	—	—	—
17: 0 (?)	1.9	—	2.4	—	—	—	—	—
18: 0	7.8	6.4	2.7	11.2	9.2	5.9	4.9	16.8
18: 1	10.5	10.8	14.0	5.2	12.4	7.0	19.0	10.1
20: 4	3.2	2.6	5.7	2.9	5.8	4.7	7.0	4.7
20: 5	4.6	4.8	3.8	2.7	4.9	5.6	4.0	—
22: 4 (?)	—	2.6	0.8	—	—	0.5	—	—
22: 5	4.1	5.0	5.5	2.8	5.3	4.0	6.9	3.4
22: 6	36.3	34.4	40.2	29.6	37.5	31.1	39.8	28.0

TL: Total lipid, NL: neutral lipid, PL: Phospholipid, FFA: Free fatty acid.

polyenoic acids such as docosahexaenoic acid. Basing on this fact we may justly say that skipjack is epipelagic fish.

The concentration of 16: 0 acid in the external muscle-NL was relatively lower than the corresponding value in the internal muscle-NL. However, FFA concentration of the external muscle showed higher value than of the internal muscle, and also the total concentration of 16: 0 acid (in NL+in FFA) of external muscle was almost the same with the corresponding value in the internal muscle. This fact shows that NL-fraction was hydrolysed by enzymatic action or some others. Therefore, it is concluded that there was no difference in the composition of fatty acid between external and internal muscle lipids.

Acknowledgment

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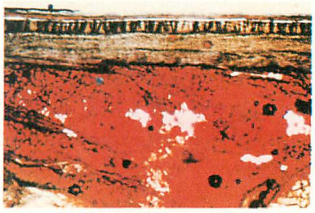
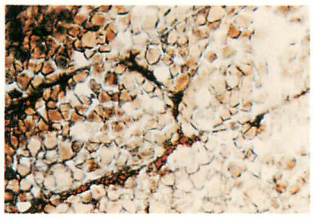
Exteral





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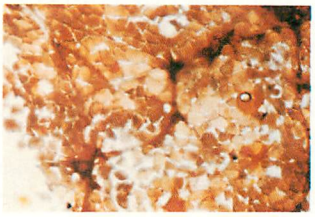
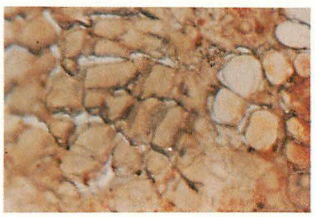


Internal

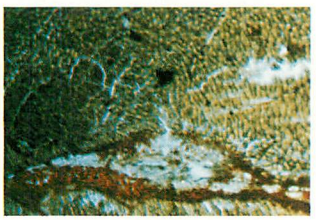


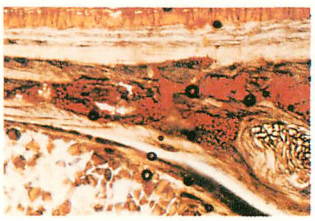
Dorsal

Plate 1. Cross section of dorsal muscle stained with Sudan III. (×40)

Ventral

Plate 2. Cross section of ventral muscle stained with Sudan III. (×40)





Dark

Connective tissue

Plate 3. Cross section of dark muscle and connective tissue stained with Sudan III. (×40)