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Effect of Defatting Treatment on the Protein Recovery of Coastal Pelagic and Deep-Sea Fish

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Abstract

Fish muscle lipid of three kinds of Mesopelagic fish, three kinds of Benthic fish and two kinds of Epipelagic fish were investigated. In general, the lipid content and the amount of highly unsatulated fatty acid were in decreasing order: Epipelagic fish > Benthic fish > Mesopelagic fish. But Skilfish which belongs to Mesopelagic fish was found to be an exception, and it conteined lipid of more than 22%. The amount of its highly unsatulated fatty acid was a trace. Three different defatting treatments, i.e. leaching, n-hexane treatment and directed succinylation of fish flesh were carried out. There was a tendency in that the larger the lipid content was, the more protein and lipid, especially simple lipid, flowed out. The leached and n-hexane treated fish flesh were also succinylated. And based on the initial results, it was concluded that succinylation might be an effective way to recover protein from these unexploited fish even though their protein had been denatured.

Introduction

In accordance with the discovery of new fishing ground in the open sea, a considerable amount of unexploited fish have been captured recently. In order to utilize these fish, specificity and functional properties of their flesh has to be examined. And also, in order to utilize those protein resources thoroughly, an effective way of recovering the protein has to be found in regards to the defatting effect during the treatment. In this study, special regards to deep-sea (mesopelagic) fish, bottom-dwelling (benthic) and coastal pelagic (epipelagic) fish were investigated from the view point of lipid, protein, moisture content and their behaviors during several kinds of defatting processes.

Materials and Methods

Materials As shown in Table 1, three kinds of mesopelagic fish, and also three kinds of benthic fish, and two kinds of epipelagic fish (dark-fleshed fish) were used as samples.

Methods The fish flesh were comminuted into small pieses and were subjected to moisture, crude portein and lipid content determination. Moisture content was measured by using a Kett infrared moisture meter Model FD-310. Crude protein content was determined by multiplying 6.25 to the total nitrogen content which had been determined by Micro Kjeldahl method. Total lipid content was

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Table 1 *Biological characteristics of mesopelagic, benthic and epipelagic fish.*

	Species	Mean body length and weight. Nos. of individual used. Locality of catch.	Depth captured.	Date of catch.
A	"Munedara" *Nematonurus pectoralis*	69 cm, 1930 g 1 sample. The offing of Tokachi, Hokkaido.	800-1000 m	Apr. 1978
	"Shirogenge" *Lycogramma zesta*	49 cm, 740 g 2 samples. The bank of Kitami-Yamato, Hokkaido	400-600 m	June 1978
	Skilfish *Erilepis zonifer*	84 cm, 13 kg 1 sample. The Emperor seamount.	800 m	July 1978
B	Pacific black halibut *Reinhardtius matsuurai*	50 cm, 1320 g 1 sample. The bank of Kitami-Yamato Hokkaido.	400-600 m	June 1978
	"Urocomegarei" *Acanthopsetta nadeshnyi*	33 cm, 310 g 2 samples. The bank of Rebun, Hokkaido.	400-600 m	June 1978
	Arrow-toothed halibut *Atheresthes evermanni*	40 cm, 550 g 2 samples. The offing of Tokachi, Hokkaido.	400-600 m	Apr. 1978
C	Mackerel (lean) *Scomber japonicus*	18 cm, 75 g 4 samples. The coast of Kamiiso, Hokkaido.	~ 10 m	Nov. 1978
	Sardine (lean) *Sardinops melanosticta*	18 cm, 80 g 4 samples. The coast of Kamiiso, Hokkaido.	~ 10 m	Nov. 1978
	Sardine (fatty) *Sardinops melanosticta*	17 cm, 80 g 4 samples. The coast of Kamiiso, Hokkaido.	~ 10 m	July 1979

A: Mesopelagic fish, B: Benthic fish, C: Epipelagic fish

determined by extracting the lipid according to the method of Bligh-Dyer, and phospholipid content was determined by multiplying 25 to the phosphorus content of the lipid which had been determined by the Fiske-Sabbarow method. Fatty acid composition of the total lipid was determined by Gas Liquid Chromatography (Column: 10% DEGS, 3 mm×1 m, Column temp.: 205°C, N_2: 40 ml/min.). Methyl esterification for this analysis was carried out by adding 10% HCl-methanol to the lipid. In order to analyze the effect of leaching from the view point of defatting amount as well as component of lipid and protein recovery, the analysis mentioned above was carried out on the leached flesh. Leaching was done in the following manner.

(1) *Defatting the Fish Flesh by Leaching*

Comminuted samples were leached twice with 5 volumes of water, and then rinsed with 5 volumes of 0.5 M NaCl aqueous solution, centrifuged at 11000 g for 20 min. Residue was collected. In addition to leaching, the following treatments

were done as well, and the defatting effect as well as protein recovery were compared.

(2) *Defatting the Fish Flesh by n-Hexane Treatment*

Comminuted samples were stirred at 200 rpm with 5 volumes of n-hexane for 2.5 hrs., and were filtered under reduced pressure. This stirring and filtering process were repeated twice.

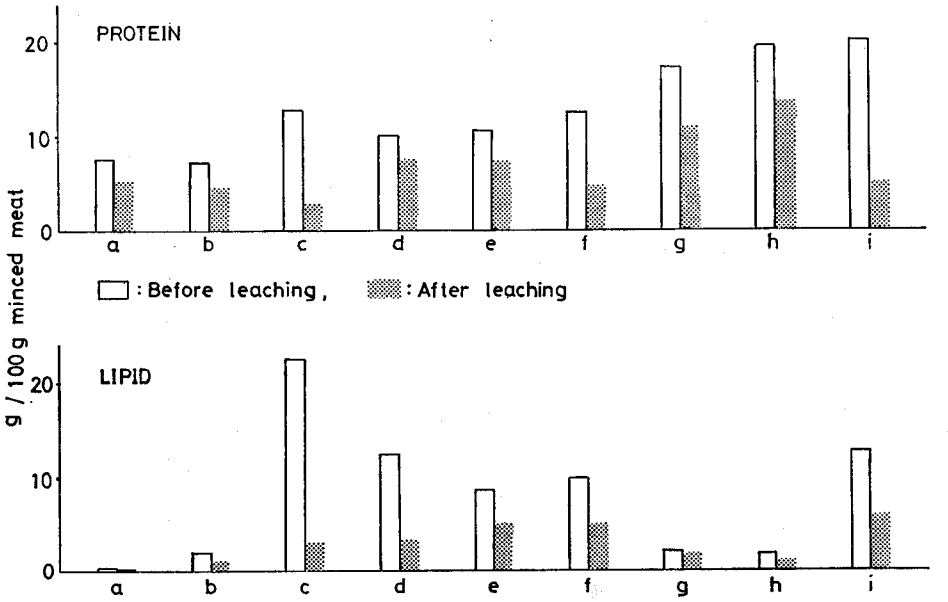
(3) *Defatting the Fish Flesh by Directed Succinylation*

Comminuted samples were suspended in 15 volumes of 5% NaCl aqueous solution. Succinylation[1)2)] was carried out at 2°C by adding 10 times the amount of succinic anhydride to the protein suspention and by maintaining the pH around 8 during the reaction by adding 4N NaOH. After the completion of the reaction, 5N HCl was added to adjust the pH from 3.5 to 4 in order to precipitate the succinylated protein around its isoelectric point. The precipitate was then washed two to three times with water and then neutralized with NaOH. Afterwards, it was dialyzed against water and the succinylated protein was prepared. Leached flesh and n-hexane treated flesh were also succinylated in order to compared the protein recovery between succinylation and 5% NaCl extration. In this case, the biuret method was used to determine the protein content.

Results and Discussion

(1) *Lipid content and composition*

As shown in Fig. 1 and Table 2, mesopelagic fish such as "munedara"



Abbrev. a:"Munedara", b:"Shirogenge", c: Skilfish, d: Pacific black halibut, e:"Urocomegarei", f: Arrow-toothed halibut, g: Mackerel (lean), h: Sardine (lean), i: Sardine (fatty)

Fig. 1. Changes in protein content and lipid content, before and after leaching.

Table 2 *Lipid, protein and moisture content of the fish examined.*

	Species	Moisture	Crude protein	Total lipid	Simple lipid	Conjugated lipid	Polyenoic acids/Total fatty acids
A	"Munedara"	91.6	7.5	0.4	0.3	0.1	39.9
	"Shirogenge"	90.2	7.2	2.1	1.9	0.2	16.5
	Skilfish	63.8	12.8	22.5	22.0	0.5	1.3
B	Pacific black halibut	74.3	10.0	12.3	12.1	0.2	8.0
	"Urokomegarei"	80.7	10.5	8.6	8.1	0.5	14.6
	Aarrow-toothed halibut	61.2	12.3	9.8	9.5	0.3	15.7
C	Mackerel	74.1	17.2	2.1	1.8	0.3	38.2
	Sardine (lean)	77.7	19.5	1.8	1.5	0.3	50.2
	Sardine (fatty)	66.4	20.0	12.6	11.8	0.8	29.9

g/100 g flesh
A: Mesopelagic fish, B: Benthic fish, C: Epipelagic fish

Table 3 *Changes in protein and lipid, before and after leaching.*

	Species	Crude protein	(Recovery)	Total lipid	(Ratio of defatting)	Simple lipid	Conjugated lipid	Polyenoic acids/Total fatty acids
A	"Munedara"	7.5		0.4		0.3	0.1	39.9%
		5.2	(69%)	0.2	(50%)	0.1	0.1	47.6%
	"Shirogenge"	7.2		2.1		1.9	0.2	16.5%
		4.5	(63%)	1.1	(48%)	1.1	0.1	16.7%
	Skilfish	12.8		22.5		22.0	0.5	1.3%
		2.8	(22%)	2.9	(87%)	2.7	0.2	2.5%
B	Pacific black halibut	10.0		12.3		12.1	0.2	8.0%
		7.5	(75%)	3.3	(73%)	3.2	0.1	10.7%
	"Urokomegarei"	10.5		8.6		8.1	0.5	14.6%
		7.3	(69%)	5.1	(41%)	4.8	0.3	16.0%
	Arrow-toothed halibut	12.3		9.8		9.5	0.3	15.7%
		4.8	(39%)	5.1	(48%)	4.9	0.2	20.2%
C	Mackerel	17.2		2.1		1.8	0.3	38.2%
		8.8	(51%)	1.9	(10%)	1.7	0.2	34.4%
	Sardine (lean)	19.5		1.8		1.5	0.3	50.2%
		13.7	(70%)	1.3	(28%)	1.0	0.3	50.5%
	Sardine (fatty)	20.0		12.6		11.8	0.8	29.9%
		4.8	(24%)	6.6	(52%)	5.2	0.8	30.5%

g/100 g flesh (minced meat)
Upper: Prior to leaching, Lower: After leaching
A: Mesopelagic fish, B: Benthic fish, C: Epipelagic fish

and "shirogenge" contained very small amounts of lipids. But amoung the mesopelagic fish, skilfish was found to be an exception in that it contained lipids of more than 22% and the content of its poly unsatulated fatty acids, which are

characteristic of marine fish lipid, was very small. Lipid content of the epipelagic fish such as sardine changed drastically depending on the season they were captured. The content of their poly unsaturated fatty acids were relatively high. Benthic fish such as pacific black halibut, "urokomegarei" and arrow-toothed halibut had the medium lipid and poly unsaturated fatty acid contents when compared with mesopelagic fish and epipelagic fish. Conjugated lipid content was found to be low in all the fish examined.

(2) *Defatting effect of leaching and protein recovery*

In the case of fatty fish, leaching was found to be a very effective defatting method (Fig. 1). And from Table 3, the largely removed lipid was simple lipid as Takahashi et al.[3] and Tagawa et al.[4] pointed out. But there was no correlation between the defatted lipids and their fatty acid composition. As illustrated in Fig. 1 and Table 3, there was a tendency for the recovery of protein to be significantly low in fatty fish, probably due to the coelution of protein with lipids during the leaching process[5].

(3) *Comparison of defatting effect and protein recovery among several kinds of treatment of fatty fish*

Several treatments, i.e. leaching, n-hexane treatment and directed succinylation were carried out and the amounts of 5% NaCl exractable protein were compared. As illustrated in Table 4, the recovery of 5% NaCl extractable protein was low among the fatty fish examined, especially the one treated by n-hexane.

Table 4 *Defatting effect and protein recovery after leaching, n-hexane treatment and directed succinylation.*

Species	Defatting treatment	Total lipid	Simple lipid	Conjugated lipid	Untreated protein content	Protein recovery after defatting	
						5% NaCl ext. protein	Succinylated protein
Sardine (fatty)	Untreated	12.6	11.8	0.8	20.0	8.2 (41%)**	—
	Aftern h-exane extraction	3.8 (70%)*	3.0	0.8		5.9 (30%)**	—
	After leaching	6.0 (52%)*	5.2	0.8		6.4 (32%)**	11.4 (57%)**
	Directed succinylation	3.0 (76%)*	3.0	0		— **	14.0 (70%)**
Arrow-toothed halibut	Untreated	9.8	9.5	0.3	12.3	10.3 (84%)**	—
	After n-hexane extraction	3.4 (65%)*	3.2	0.2		8.5 (69%)**	12.6 (102%)**
	After leaching	5.1 (48%)*	4.9	0.2		4.8 (39%)**	—
	Directed succinylation	—	—	—		—	10.4 (85%)**
Skilfish	Untreated	22.5	22.0	0.5	12.8	6.7 (53%)**	—
	After n-hexane extraction	2.0 (91%)*	1.5	0.5		2.5 (20%)**	8.5 (67%)**
	After leaching	2.9 (87%)*	2.7	0.2		2.8 (22%)**	8.5 (67%)**
	Directed succinylation	7.7 (66%)*	7.5	0.2		—	7.4 (58%)**

g/100 g untreated flesh * Defatting ratio (%) ** Protein recovery (%)

This was thought to be due to the denaturation of protein by the solvent treatment which caused the insolubilization of protein. But in the case of directed succinylation of untreated flesh, protein recovery in the form of succinylated protein was satisfactory in addition to its effective defatting effect. Furthermore, the protein recovery of the treated (leached or n-hexane treated) flesh in the form of succinylated protein was higher than that of 5% NaCl extracted protein. And it was concluded that succinylation is a very effective way of recovering denaturated protein from the defatted flesh. Recently, a case of food poisoning causing diarrhea occured in Japan. The poisoning was linked to the injestion of Skilfish. But by applying these defatting techniques, Skilfish becomes edible and might even be expected to serve as a new material for fabricated foods stuffs.

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