Lipid Components and Enzymatic Hydrolysis of Lipids in Muscle of Chinese Freshwater Fish

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ABSTRACT: The lipid and fatty acid composition of muscle of 10 species of freshwater fish obtained from a market of Shanghai City was examined. Total lipids (TL) ranged over 0.9–4.7% of muscle for all samples. The content of triacylglycerol (TG) in muscle ranged over 0.2–3.4% and that of polar lipids (PL) was 0.5–1.3%. Differences of TL content were dependent on TG contents. The predominant important fatty acids (>10% of the total fatty acids in TL) were 16:0 and 18:1n-9 with some 16:1n-7, 18:2n-6, and 22:6n-3. The polyunsaturated fatty acids (PUFA) content was 10.2–43.4%, and especially Chinese sea bass contained above 20% of 22:6n-3 in the total fatty acids. There were higher levels of PUFA such as 20:5n-3 and 22:6n-3 in PL than in neutral lipids. Muscle of the silver carp was stored at 20°C, and changes of lipid classes during storage were examined. Free fatty acids increased, and PL decreased during storage. This phenomenon was inhibited by heating the muscle, suggesting that lipid hydrolysis by phospholipase occurred in silver carp muscle.

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KEY WORDS: Chinese freshwater fish, enzymatic hydrolysis, fatty acid, muscle lipid.

Fishery production in the People's Republic of China (China) in 1995 was about 29 million tons, and now China is the world's leading fishery producer (1). Regarding fishery products of China, production of freshwater fish was about 11 million tons and occupied about 10% of world fisheries. The silver carpis a major freshwater fish in China and is the fourth species in world production following Peruvian anchovy, South pacific jack mackerel (jurel), and Alaska pollack (1). Most of the freshwater fish in China are cultured fish. They are widely consumed in large cities near culture ponds as fresh fish, but are not available as processed foods. It is expected that the production of Chinese freshwater fish will be increased in the future by the application of new technology in aquaculture. Further development of new processing technology for Chinese freshwater fish is also necessary for stabilizing this valuable food supplement not only in China but also in the world.

For successful processing, it is necessary to determine the properties of the materials. Lipid components in the fish affect the nutritional value and preservation period of the fish products. Nutritionists and food scientists need lipid and fatty acid composition data to aid them in dietary formulation, nutrient labeling, processing, and product development (2).

Several studies have been conducted on the lipid components of Chinese freshwater fish (3–5), but the samples examined in these studies were mostly fish of the Cyprinidae family. We have now examined the lipid and fatty acid compositions of four species of fish of the families Channidae, Percichthyidae and Synbranchidae, in addition to six species of Cyprinidae obtained from a market in Shanghai City. Formerly, Chinese sea bass was confused with Japanese sea bass, *Lateolabrax japonicus*. Yokogawa and Seki (6) elucidated morphological and genetic differences between Japanese and Chinese sea bass. Although, Chinese sea bass is a marine species, it has a strong tolerance of fresh water (6) and is cultivated in fresh water or brackish water in China. Furthermore, Chinese sea bass is sold as living fish in a freshwater tank at Chinese markets, similar to the other freshwater fish. Therefore, we treated Chinese sea bass as freshwater fish in the present study.

In previous research (7–19), enzymatic hydrolysis of lipids in fish muscle was reported in some lean and fatty fish such as cod, skipjack, carp, sardine, and rainbow trout. Free fatty acids (FFA) accumulate in muscle lipids from enzymatic hydrolysis of lipids, and they degrade the quality of fish muscle (20–22). We therefore also examined the enzymatic hydrolysis that occurs in the muscle of silver carp.

EXPERIMENTAL PROCEDURES

The samples of Chinese freshwater fish examined for lipid

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components in this study and the lipid content in their muscle are shown in Table 1. These samples were purchased in December 1997 at a market in Shanghai City. The muscle, including white and dark tissue, was minced. Total lipids (TL) were extracted from the minced muscle according to the procedure of Bligh and Dyer (23).

The lipid compositions were analyzed by thin-layer chromatography with flame-ionization detection (TLC–FID) method (24) using the Chromarod S-III and the Iatroscan MK-5 (Iatron Laboratories, Tokyo, Japan) with *n*-hexane/diethylether/acetic acid (70:30:1,vol/vol/vol) as the developing solvent. Peak area percentages were obtained with an Iatrocorder TC-11 (Iatron Laboratories).

Fractionation of TL into neutral (NL) and polar (PL) lipids was carried out by column chromatography using silicic acid (Silica Gel 60; Merck, Darmstadt, Germany) with chloroform and methanol as the developing solvents. Fractionation of TL into triacylglycerol (TG), FFA, and PL was carried out by TLC on Silica Gel 60 plates (Merck) with the same solvent system of TLC–FID method.

The lipids were converted into fatty acid methyl esters using 5% HCl/methanol. The methyl esters obtained were purified by a Sep-Pak Plus silica cartridge (Waters Co., Milford, MA) and eluted with dichloromethane.

The gas chromatographic analysis of the methyl esters was conducted with a Shimadzu GC14A instrument (Shimadzu Seisakusho Co., Kyoto, Japan), with a FID on a fused-silica capillary column coated with Omegawax 320 (30 m × 0.32 mm i.d.). The carrier gas was helium. The column temperature was 210°C, and the injector and detector temperatures were 230°C. Peak area percentages were obtained with a Shimadzu integrator C-R6A (Shimadzu Seisakusho Co.). The fatty acid component of each peak of the gas chromatogram was identified on the basis of the agreement of retention time data with those of the reference specimen.

Methyl esters different in degrees of unsaturation were fractionated by preparative TLC on $AgNO_3$-impregnated Silica Gel 60 plates with *n*-hexane/ethyl acetate (85:15, vol/vol).

The fatty acid pyrrolidides prepared by the method of Anderson *et al.* (25) were subjected to gas chromatography–mass spectrometry (GC–MS). GC–MS analyses were carried out with a Shimadzu QP-5000 instrument (Shimadzu Seisakusho Co.) equipped with a capillary column (25 m × 0.22 mm i.d.) coated with CPB-20M. All spectra were obtained at an ionization energy of 70eV and at a source temperature of 260°C.

To investigate the enzymatic hydrolysis of lipids in muscle of Chinese freshwater fish during storage, the muscle of silver carp purchased in November 1997 was minced and stored at 20°C. To prevent putrefaction and lipid oxidation, 9 g of NaCl and 1 mL of a solution of antibiotics (0.6% of penicillin, streptomycin, and amphotericin B) were added per 60 g of minced muscle according to the method of Fujii *et al.* (26), and about 10 g of each sample was packed with an iron oxygen absorbant (Mitsubishi Gas Chemical Co., Tokyo, Japan) in a multilayer bag (polyvinylidene chloride coated oriented nylon–polyethylene film) and sealed. To deactivate enzymes in muscle, a portion of minced muscle was heated in boiling water for 10 min, and after cooling, NaCl and the antibiotics solution were added to the heating muscle. The heated muscle was packed and stored as unheated samples.

RESULTS AND DISCUSSION

The content and composition of lipids from muscle of the Chinese freshwater fish examined in this study are shown in Table 1. The TL, TG, and PL contents of muscle were 0.9–4.7%, 0.2–3.4%, and 0.5–1.3%, respectively. Both sterols and FFA content in muscle were less than 0.1%, and the major components of TL were TG and PL. Variations of PL contents among fish species were less than those of TG contents. Therefore, in the Chinese freshwater fish examined, differences in TL content of the muscle may be due to differences in the amount of TG. In other fish, the lipid content of fish is influenced by the content of NL (27). In the present study, the major component of NL in Chinese freshwater fish muscle was TG.

The fatty acid composition of TL from the Chinese freshwater fish is shown in Table 2. The predominant fatty acids (>10% of the total fatty acids in TL) in one or more samples

TABLE 1
Lipid Contents of Muscle of Chinese Freshwater Fish

Family	Scientific name	English name	Body length (cm)	Body weight (kg)	Lipid contents of muscle (%)				
					TL[a]	TG	FFA	ST	PL
Cyprinidae	*Carassius auratus auratus*	Crucian carp	22.0	0.24	1.19	0.26	ND	0.02	0.92
	Cyprinus carpio	Common carp	42.2	1.54	1.86	0.81	ND	0.04	1.01
	Aristichthys nobilis	Big-head carp	39.0	1.03	0.94	0.17	0.01	0.03	0.73
	Hypophthalmichthys molitrix	Silver carp	37.7	0.83	1.37	0.56	0.04	0.04	0.74
	Ctenopharyngodon idellus	Glass carp	47.0	1.43	3.06	1.84	ND	0.02	1.20
	Megalobrama amblycephala	Bluntnose black bream	24.0	0.50	4.73	3.40	ND	0.02	1.31
Channidae	*Ophicephalus argus*	Snake-head fish	35.0	0.35	1.06	0.24	ND	0.03	0.78
Percichthyidae	*Lateolabrax* sp.	Chinese sea bass	28.0	0.38	3.04	2.04	ND	0.03	0.96
	Siniperca chuatsi	Chinese bass	32.0	0.58	3.76	3.00	ND	0.03	0.73
Synbranchidae	*Monopterus albus*	Swamp eel	58.0	0.20	1.06	0.55	ND	0.03	0.48

[a]TL, total lipids; TG, triacylglycerols; FFA, free fatty acids; ST, sterols; PL, polar lipids; ND, not detected.

TABLE 2
Fatty Acid Composition[a] of Total Lipids from Muscle of Chinese Freshwater Fish (%)

	Crucian carp	Common carp	Big-head carp	Silver carp	Grass carp	Bluntnose black bream	Snake-head fish	Chinese sea bass	Chinese bass	Swamp eel
14:0	1.0	1.6	0.9	2.5	1.5	1.9	2.3	3.9	1.7	2.0
15:0	0.4	0.4	0.7	1.1	0.2	0.2	0.8	0.7	1.1	1.2
16:0	18.6	16.9	12.5	16.4	21.9	19.7	18.2	19.4	16.2	20.5
17:0	0.4	0.4	0.6	0.6	0.1	0.1	0.6	0.6	1.0	2.5
18:0	3.8	4.7	2.7	2.7	3.6	3.9	3.7	4.5	3.3	8.2
Total sat.	24.2	24.0	17.4	23.3	27.3	25.8	25.6	29.1	23.3	34.4
16:1n-7	2.5	7.2	3.7	7.7	9.7	10.9	7.8	8.0	3.3	8.8
18:1n-9	18.3	27.3	19.8	10.9	33.3	40.6	12.2	14.0	32.2	14.5
18:1n-7	4.2	4.4	4.6	3.1	2.7	3.8	4.0	2.9	1.9	4.5
20:1n-9	2.6	1.1	4.4	0.9	1.5	2.6	1.2	0.7	1.1	0.5
20:1n-7	0.9	0.1	1.7	0.1	0.2	0.5	0.2	0.2	ND	0.1
22:1n-11	1.8	0.1	2.8	0.1	0.4	1.4	0.2	0.1	0.1	ND
Total mono.	30.3	40.2	37.0	22.8	47.8	59.8	25.6	25.9	38.6	28.4
18:2n-6	12.5	9.0	11.8	2.9	8.8	5.1	3.9	1.7	21.1	5.0
18:3n-3	1.7	2.3	3.3	7.0	4.5	0.7	3.4	0.9	2.7	2.6
18:4n-3	0.1	0.6	0.2	1.7	0.1	0.1	0.5	0.6	0.5	0.2
20:4n-6	6.5	3.8	3.5	4.2	2.2	1.4	4.9	2.0	2.0	6.3
20:3n-3	0.2	0.1	0.3	1.0	0.4	0.1	0.5	0.2	0.2	0.5
20:4n-3	0.3	0.6	1.0	2.4	0.3	0.1	0.7	0.7	0.3	0.5
20:5n-3	1.8	5.2	4.1	8.3	0.7	0.2	2.1	3.9	0.7	1.0
22:4n-6	1.0	0.6	0.7	0.8	0.2	0.3	1.4	0.8	0.7	2.4
22:5n-6	2.5	0.5	3.5	2.6	0.9	1.1	1.9	1.7	1.0	1.1
22:5n-3	1.5	2.1	2.4	2.6	0.6	0.1	3.9	5.6	0.7	2.5
22:6n-3	9.9	5.3	7.3	10.5	2.8	1.4	14.8	23.4	2.9	4.7
Total PUFA	38.0	30.1	38.1	44.0	21.5	10.6	38.0	41.5	32.8	26.8
Others	7.4	5.8	7.5	9.6	3.5	3.8	10.9	3.6	5.2	10.4

[a]Sat., Saturated fatty acids; mono., monoenoic fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

were 16:0, 16:1n-7, 18:1n-9, 18:2n-6, and 22:6n-3. The total of these five fatty acids made up 48.5–77.7% of the total fatty acids.

Total saturated fatty acids accounted for 17.3–34.5% of the total fatty acids in all samples. The most predominant saturated fatty acid was 16:0, followed by 14:0 and 18:0; 16:0 accounted for 12.5–21.9% of the total fatty acids.

Total monounsaturated fatty acids exceeded 22% of the total fatty acids in all samples, and the major monounsaturated fatty acids were 16:1n-7, 18:1n-9, and 18:1n-7. The C_{20} and C_{22} monounsaturated fatty acids, which are important in some marine fish such as sardine, herring, salmon, capelin and sand lance (27–34), were found at low levels in these freshwater fish. In some previous papers (32–34), it was suggested that C_{20} and C_{22} monounsaturated fatty acids were incorporated in lipids of marine fish from Copepoda in their diets. Furthermore, Ackman *et al.* (35) pointed out that in the freshwater milieu there are no organisms comparable with the marine Copepoda, and the major source of 22:1 acids in freshwater fish is simple chain elongation from 18:1n-9. All the samples used in this study were cultured fish, and the low levels of C_{20} and C_{22} monounsaturated fatty acids in them suggest that their diets did not contain materials, such as marine fish meal from the Clupeid family of fish, which enhance accumulation of C_{20} and C_{22} monounsaturated fatty acids (36,37).

In this study, the content of polyunsaturated fatty acids (PUFA) ranged from 10.5 to 44.2%. Recently, effects of fish consumption for human health were elucidated, and n-3 PUFA in fish, such as 20:5n-3 and 22:6n-3, have been noted as useful substances (38–42). The content of 20:5n-3 and 22:6n-3 in the fatty acids of four species of Chinese freshwater fish (bluntnose black bream, grass carp, Chinese bass, and swamp eel) muscle was 0.2–1.0 and 1.4–4.7%, respectively. These levels of 20:5n-3 and 22:6n-3 were lower than those of marine fish (27–34). But in the other six species (crucian carp, common carp, big-head carp, silver carp, snake-head fish, and Chinese sea bass), 20:5n-3 and 22:6n-3 contents were 1.8–8.3 and 5.3–23.4%, respectively. The contents of 22:6n-3 above 5% of the total fatty acids are similar to those of marine fish (27–34), especially, Chinese sea bass which contained 23.4% of 22:6n-3. The content of longer-chain PUFA such as 20:4n-6, 20:5n-3, and 22:6n-3 was high in PL, but 18:2n-6 was higher in NL as shown in Table 3.

Kojima *et al.* (43,44) determined the fatty acid composition of some freshwater fish in Lake Biwa in Japan. In these studies, the content of 18:2n-6 of freshwater fish was found to be higher than in marine fish. In the present study, the content of 18:2n-6 ranged from 1.7 to 21.1% of the total fatty acids, and except for Chinese sea bass (the content of 18:2n-6

TABLE 3
Major Fatty Acids of Neutral and Polar Lipids[a] from Muscle of Chinese Freshwater Fish (%)

	Crucian carp		Common carp		Big-head carp		Silver carp		Grass carp		Bluntnose black bream		Snake-head fish		Chinese sea bass		Chinese bass		Swamp eel	
	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
16:0	18.1	18.5	17.7	13.9	12.6	11.6	16.4	15.8	24.5	15.6	20.1	13.1	17.1	16.3	21.4	19.8	16.3	18.7	21.5	14.8
18:0	2.9	4.7	3.5	7.2	1.8	3.5	1.7	4.4	3.1	4.6	3.6	4.5	2.9	3.8	3.8	6.8	2.9	5.1	7.8	5.8
16:1	5.9	1.1	10.0	3.3	5.2	2.8	10.1	3.9	11.9	5.1	11.9	5.8	15.5	5.1	10.7	3.0	3.8	1.2	11.2	2.4
18:1	31.2	12.2	36.7	15.8	28.3	18.3	14.7	10.7	36.9	14.9	44.8	21.5	19.7	10.7	17.1	9.5	33.4	17.2	19.4	11.7
18:2n-6	13.7	9.7	10.1	5.5	12.9	11.0	3.2	2.0	8.4	7.6	5.0	5.6	4.3	2.9	1.7	0.8	21.0	11.0	5.2	3.9
18:3n-3	2.5	1.0	2.8	1.0	4.5	2.7	8.3	3.4	4.6	2.6	0.7	0.6	4.6	1.5	1.0	0.4	2.8	0.6	3.0	0.5
20:4n-6	1.4	11.3	1.3	8.6	1.1	6.3	2.5	6.3	0.6	10.3	0.6	8.7	2.4	7.5	1.4	4.4	1.2	9.4	2.2	19.4
20:5n-3	0.7	3.5	2.6	9.9	1.2	6.7	6.8	9.9	0.3	3.0	0.3	1.3	1.8	2.2	3.1	5.4	0.7	1.7	0.5	1.0
22:5n-3	0.4	2.3	0.6	4.8	0.5	2.5	1.7	3.2	0.3	2.4	0.1	0.9	1.9	4.8	4.5	3.0	0.6	1.8	2.1	2.6
22:6n-3	1.8	16.6	0.8	14.7	1.1	10.8	5.4	16.2	0.6	13.2	0.4	8.7	3.3	23.3	16.9	32.8	1.5	15.0	2.2	11.5
Others	21.4	19.1	13.9	15.3	30.8	23.8	29.2	24.2	8.8	20.7	12.5	29.3	26.5	21.9	18.4	14.1	15.8	18.3	24.9	26.4

[a]NL, neutral lipids; PL, polar lipids.

was 1.7%), these values were higher than those of marine fish but similar to the data of Japanese freshwater fish shown by Kojima *et al*. (43,44) and most of Ethiopian freshwater fish shown by Zenebe *et al*. (45). Chinese bass contained 21.1% of 18:2n-6, mostly in place of 20:5n-3 (0.7%) and 22:6n-3 (2.9%). Similarly, Mississippi farm-raised channel catfish contained a high level of 18:2n-6 (12%) and a low level of 20:5n-3 (0.4%) and 22:6n-3 (1.2%) in their total fatty acids (46). Chinese sea bass is originally a marine species, containing high contents of 20:5n-3 and 22:6n-3 and a low content of 18:2n-6, similar to Japanese sea bass (47). The contents of 18:2n-6, 20:5n-3, and 22:6n-3 of Indian freshwater fish (*Callichrous pabda*) (48) were similar to Chinese sea bass, but the content of 20:4n-6 was higher than for all Chinese freshwater fish examined in this study.

Liu (3) examined the fatty acid composition of five species of Chinese freshwater fish belonging to the Cyprinidae family, and reported high contents of 18:3n-3 in the muscle of grass carp (32.2–34.6%). However in the present study, the content of 18:3n-3 was modest at 0.7–7.0% of total fatty acids, with the highest content of 18:3n-3 in silver carp lipids (7.0%).

Diet has a major effect on the fatty acid composition of fish lipids (49). Varieties of fatty acid composition of freshwater

TABLE 4
Fatty Acid Composition of the Lipids of Silver Carp Muscle Before[a] and After Storage (%)

	B-TL	A-TL	B-FFA	A-FFA	B-TG	A-TG	B-PL	A-PL
14:0	1.2	1.2	1.3	0.7	4.3	4.4	0.5	0.4
16:0	15.5	15.8	18.0	16.0	18.5	17.8	15.3	13.7
16:1n-7	5.0	5.2	3.8	4.6	10.2	9.7	4.1	3.5
18:0	5.1	5.2	7.3	5.1	2.4	2.5	6.0	7.1
18:1n-9	8.4	8.7	7.1	9.1	9.8	10.4	8.2	7.1
18:1n-7	4.2	4.4	3.3	3.8	5.2	5.1	4.3	5.7
18:2n-6	3.3	3.5	2.6	3.5	3.7	4.5	3.6	3.2
18:3n-6	0.1	0.2	0.1	0.1	0.3	0.4	0.1	0.1
18:3n-3	3.8	3.9	3.4	3.8	6.2	6.3	3.1	2.3
18:4n-3	0.6	0.5	0.5	0.5	1.2	1.3	0.4	0.2
20:1n-11	0.7	0.8	0.6	0.7	1.0	1.0	0.6	0.7
20:1n-9	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
20:2n-6	0.6	0.6	0.4	0.5	0.5	0.5	0.6	0.8
20:3n-6	1.0	1.0	0.7	1.1	0.5	0.5	1.0	0.8
20:4n-6	6.9	6.8	4.9	7.9	1.8	1.9	7.6	6.6
20:3n-3	0.9	0.9	0.7	0.8	0.9	0.8	0.9	1.0
20:4n-3	1.9	1.8	1.6	2.1	1.8	1.8	1.6	1.1
20:5n-3	8.8	8.5	7.7	10.5	3.8	3.9	8.9	5.5
22:1n-11	ND	0.1	0.3	ND	0.1	0.1	0.1	0.4
22:4n-6	0.9	0.8	0.8	1.0	0.2	0.2	0.8	0.6
22:5n-6	3.9	3.6	2.3	3.6	0.8	0.7	3.9	4.9
22:5n-3	3.3	3.1	3.5	3.8	1.2	1.2	3.1	2.1
22:6n-3	10.8	10.1	6.1	9.6	2.8	2.7	10.8	13.3
Others	13.0	13.2	22.9	11.1	22.5	22.0	14.4	18.8

[a]B, before storage; A, after storage for 8 d at 20°C; TL, total lipids; FFA, free fatty acids; TG, triacylglycerols; PL, polar lipids; ND, not detected.

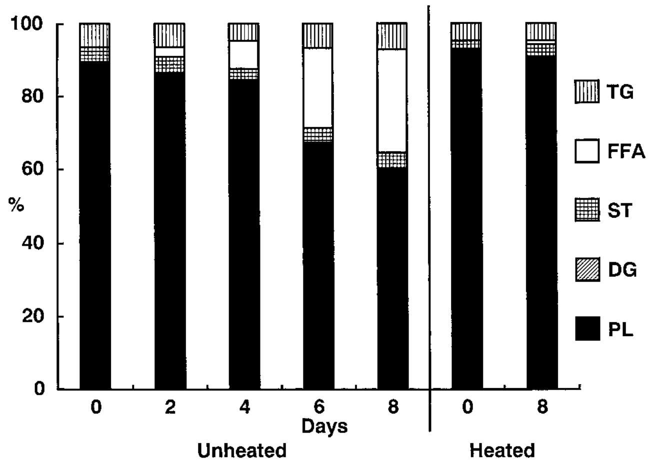

FIG. 1. Changes in the lipid class of silver carp muscle during storage at 20°C (heated: heated in boiling water for 10 min). TG, Triacylglycerols; FFA, free fatty acids; ST, Sterols; DG, diacylglycerols; PL, polar lipids.

fish in this study would be influenced by their diet. Most Chinese freshwater fish are cultured fish; therefore, it will be possible to control fatty acid profiles of enormous Chinese freshwater fishery resources by their diet.

Changes of the lipid classes of silver carp muscle during storage at 20°C for 8 d are shown in Figure 1. The lipid content of muscle of silver carp purchased in November 1997 was 0.9%, and the PL and TG content of the TL was 89.4 and 6.4%, respectively. The lipid content and composition were different from those of the same species purchased in December 1997. During storage for 8 d, FFA increased from 0 to 28%, PL decreased from 89 to 60%, but TG contents did not change. These phenomena were inhibited by heating muscle as shown in Figure 1. Thus, it is suggested that hydrolysis of lipids by phospholipase had taken place in silver carp muscle and phospholipases in heated muscle were deactivated. Enzymatic hydrolysis of fish muscle lipids during storage at low temperature is known in some fish (cod, skipjack, carp, sardine, rainbow trout, etc.) (7–19). In this study, we determined that the enzymatic hydrolysis of lipids in silver carp muscle occurred during short-term storage at 20°C, and this suggests that enzymatic hydrolysis would occur in frozen silver carp muscle during long-term storage. Changes of fatty acid composition during storage are shown in Table 4. The fatty acid composition of TL and TG did not change during storage, and the fatty acid composition was not influenced by lipid oxidation. However some change of PL fatty acid composition was observed. Levels of 20:5n-3 decreased and 22:6n-3 increased during storage. The 20:5n-3 was evidently more prone to hydrolysis than the 22:6n-3.

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