Lipid Oxidations in Ordinary and Dark Muscles of Fish: Influences on Rancid Off-odor Development and Color Darkening of Yellowtail Flesh During Ice Storage

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ABSTRACT: We investigated the role of lipid oxidation in the development of a rancid off-odor in the early stage of ice storage in both ordinary and dark muscles of cultured yellowtail (*Seriola quinqueradiata*). The total lipid hydroperoxide content and thiobarbituric acid-reactive substances (TBARS) of the dark muscle were significantly higher ($P < 0.01$) than those of the ordinary muscle during 2 d of ice storage. The increase in the metmyoglobin content of the dark muscle was accompanied by gradual darkening of the muscle color during ice storage. To distinguish the changes in odor characteristics of the muscles, sensory evaluation was carried out. Slight changes in the intensity of 7 different odors were noted in the ordinary muscles, and there was a significant increase in the overall, fishy, spoiled, and rancid smells of the dark muscle during 2 d of ice storage. No correlation was found between the total content of lipid hydroperoxide and the odor intensities in ordinary muscle; however, there was a significant correlation between the total lipid hydroperoxide content and rancid off-odor and overall smell intensities in the dark muscle. The rate of lipid oxidation of the yellowtail dark muscle was significantly faster than that of the ordinary muscle. Lipid oxidation of the dark muscle was closely related to meat darkening and development of the rancid off-odor during the early stage of ice storage.

Keywords: dark muscle, hydroperoxide, lipid oxidation, metmyoglobin, rancid off-odor

Introduction

Fish oils are rich in n-3 polyunsaturated fatty acids (PUFA) such as eicosapentenoic acid (EPA) and docosahexenoic acid (DHA). A variety of research has been devoted to the biological importance of PUFA in terms of physiological function (Suzuki 1999; Connor 2000; Laidlaw and Holub 2003). Partially purified EPA and DHA from fish oils are used as ingredients in both functional food (Ohshima 1998) and pharmaceuticals (Hata and Makuta 1990; Hata and others 1992a, 1992b). The PUFAs in fish muscle are usually oxidized in the early stage of storage, even at low temperature. The color and odor of raw fish are the most important freshness indicators for consumers; this is especially important when consuming "sashimi," a Japanese dish of thinly sliced and garnished raw fish. The color tone of fish muscle depends on the ratio of myoglobin/hemoglobin content in the muscle (Matsuura and Hashimoto 1959; Richards and Hultin 2002). Moreover, methemoglobin and metmyoglobin, the oxidized analogues of hemoglobin and myoglobin respectively, usually contribute to lipid oxidation in fish flesh (Koizumi and others 1987; Chan and others 1997).

The higher fraction PUFAs in fish flesh oils are oxidized to form hydroperoxide as a primary oxidation product during processing and subsequent storage. Hydroperoxides are readily decomposed to produce a variety of volatile compounds, including aldehydes, ketones, and alcohols (Frankel and others 1984). The formation of the secondary lipid oxidation products will be one of the main causes of the development of undesirable odors in fish flesh, and human olfactory receptors usually have remarkably low organoleptic thresholds to most of these volatile compounds (Ke and others 1975; McGill and others 1977; Swoboda and Peers 1977; Josephson and others 1984). Therefore, it is important to monitor the progress of lipid oxidation in fish muscles in the early stage when evaluating the development of rancid off-odor in raw fish flesh due to lipid oxidation. There have been few systematic studies that elucidate the relationship between lipid peroxidation and rancid off-odor development in fresh fish flesh. Neither has there been an adequate and reproducible analytical procedure that measures a trace amount of hydroperoxide as a primary oxidation product. Recently, we developed a flow injection analysis (FIA) system coupled with a fluorescence detection system that enabled us to determine hydroperoxides in fish muscle at pmole levels during the early stages of lipid oxidation (Sohn and others 2005).

This study was conducted not only to ascertain the different trends in lipid oxidation in dark and ordinary muscles of raw fish during early stages of storage, but also to determine the degree of correlation between the intensities of developed odors and the amount of lipid hydroperoxide in the fish muscle. The effect of lipid peroxidation on the meat color was also evaluated in the muscles of several fish species.

Materials and Methods

Fish sample preparation

Japanese butterfish (*Hyperoglyphe japonica*), Pacific saury (*Cololabis*

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saira), Japanese Spanish mackerel (*Scomberomorus niphonius*), and chub mackerel (*Scomber japonicus*) were purchased from a local retailer for preliminary storage experiments (Trial I). Fish were all purchased within 24 h after harvest. Live cultured yellowtail (*Seriola quinqueradiata*) and amberjack (*Seriola purpurascens*) were landed at the Misaki fishing port in Kanagawa prefecture, Japan. The sacrificed fish were kept on ice and transported to the Kanagawa Fisheries Research Inst. laboratory for inclusion in Trial I and a further named Trial II. Characteristics of sample fish are summarized in Table 1.

Table 1—Body size of sample fish species

Fish species (scientific names)	Nr of fish	Fork length (cm)	Body weight (kg)
Yellowtail (*Seriola quinqueradiata*)	10	51 to 53	3.52 to 3.87
Amberjack (*Seriola purpurascens*)	3	50 to 54	3.16 to 3.42
Japanese butterfish (*Hyperoglyphe japonica*)	2	42 to 44	1.92 to 2.34
Pacific saury (*Cololabis saira*)	20	29 to 32	0.17 to 0.21
Japanese Spanish mackerel (*Scomberomorus niphonius*)	6	39 to 43	0.57 to 0.62
Chub mackerel (*Scomber japonicus*)	4	37 to 40	0.75 to 0.83

Ice storage of fish muscles

Ordinary and dark muscles were minced individually with a food processor (model MK-K50, Natl., Tokyo, Japan) and kept on ice in polyethylene bags for 4 d (Trial I).

Both dark and ordinary muscles isolated from live yellowtail specimens were subjected to iced storage for 2 d in a similar manner to Trial I samples. Lipid oxidation analyses and sensory evaluation were performed on these muscles (Trial II).

Determination of total lipid hydroperoxides

Total lipid hydroperoxides were analyzed by the flow injection analysis (FIA) system equipped with a fluorescent detection system using diphenyl-1-pyrenylphosphine (DPPP) as previously reported (Sohn and others 2005). Briefly, a 1-mL solution of 1-myristoyl-2(12-((7-nitro-2-1,3-benzoxadiazol-4-yl)amino)dedecanoic acid (NBD)-labeled *sn*-glyero-3-phosphocholine (PC) (4.82 nmole/mL in methanol) was added to 5 g of minced muscle as an internal standard and lipid was extracted and purified according to the Bligh and Dyer procedure (1959). The homogenate was centrifuged at 1700 × *g* for 8 min at 4 °C. The lower layer was dehydrated by anhydrous sodium sulfate and filtered through a membrane filter (PTFE 0.20 μm, Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate obtained was made up to 10 mL total volume with chloroform. A 20-μL portion of the sample solution was injected into the FIA system for determination of total lipid hydroperoxide content.

Measurement of thiobarbituric acid-reactive substances

The thiobarbituric acid-reactive substances (TBARS) were measured according to the protocol of Uchiyama and Mihara (1978). Briefly, 0.5 g of minced muscle was homogenized with 4.5 mL of 1.15% (wt/vol) KCl solution. A 0.5-mL portion of the homogenate was transferred into a screw-capped test tube and 0.3 mL of 1% (w/v) phosphoric acid and 1.0 mL of 0.67% (w/v) 2-thiobarbituric acid solution were added. The mixture was vortexed and subsequently heated for 45 min with a heating block at 95 °C. After cooling to room temperature, 4.0 mL of *n*-butanol was added to the test tube; the tube was vortexed and then centrifuged at 1700 × *g* for 10 min. The butanol phase was separated and absorbance was read at 535 nm and 520 nm using a Shimadzu spectrophotometer (model UV-160A, Kyoto, Japan).

Metmyoglobin analysis

The percentage of metmyoglobin in total myoglobin contained in the dark muscle was measured according to a procedure of Bito (1965). Briefly, 3 g of minced muscle was transferred into a 38-mL polypropylene tube. Ten milliliters of ice-cold distilled water was added to the tube, and the contents were mixed with a Teflon®-coated magnetic stirring bar. The mixtures were held at 4 °C for 10 min and centrifuged at 1700 × *g* for 5 min. The supernatant was filtered through an Advantec nr 1 filter paper (Advantec Toyo Roshi Kaisha, Ltd.). The filtrate was adjusted to pH 6.8 to 7.0 by 1 *M* NaOH. The centrifugal separation was repeated and the absorbance of the supernatant was read at 540 nm (E_{540}) and 503 nm (E_{503}). The percentage of metmyoglobin was calculated using an absorption ratio (E_{540}/E_{503}) according to a formula for tuna myoglobin as reported by Bito (1965).

Sensory evaluation

For Trial II, 2 live yellowtails were sacrificed and the dark and ordinary muscles were separated on the 1st d and stored on ice for 2 d (2-d stored sample). On the 2nd day, 2 additional live fish were sacrificed and the muscles stored for 1 d in the same way (1-day stored sample). On the 3rd day, 2 more live fish were sacrificed and the fresh muscles were analyzed immediately (0-d stored sample). The 3 groups of ordinary and dark muscles (0-d, 1-d stored, and 2-d stored) were subjected to sensory evaluation as described subsequently.

A trained panel of people affiliated with Nippon Suisan Kaisha Central Research Laboratory and who had extensive experience in the evaluation of off-odors and off-flavors of raw fish muscles and fish oils carried out the sensory evaluation in individually partitioned sensory testing booths. The group consisted of 8 males and 3 females who ranged in age from 28 y to 45 y. Ten grams of ordinary or dark muscle were put in a 100-mL brown glass Erlenmeyer flask and mixed thoroughly with a spatula. All samples were kept on ice until subjected to sensory analysis. The flasks were placed at room temperature 10 min before evaluation and the sensory analysis was completed within 30 min. First, 3 groups of the ordinary muscles were presented simultaneously to evaluate the odor using a paired comparison for 2 of 3 samples. For the evaluation of 6 odor categories (except overall smell), all 3 ordinary muscle samples of different storage periods were tested. Subsequently, the other 3 samples of the dark muscles were evaluated in a similar manner.

The intensities of overall, fishy, spoiled, rancid, grassy, bloody, and milky smell of the ordinary and dark muscles were evaluated following the Scheffé's paired comparison (Scheffé 1952). For the evaluation of overall smell, the panelists were asked whether a proposed sample was comparatively stronger or weaker in odor intensity using the other sample as a reference. A 7-point scale was used: scoring ranged from very weak (score = –3) to very strong (score = +3) with the center point (score = 0) representing the "same intensity as the reference sample." For each of the other categories, the intensities of odors were evaluated using a 5-point scale ranging from none (score = 0) to strong (score = 4). The maximum and minimum scores within the 11 panelists were removed for statistical analysis.

Fatty acid analysis

Tricosanoic acid methyl ester was added to the accurately weighed lipid (about 50 mg) as an internal standard, and the mix-

Table 2—Fatty acid compositions of ordinary and dark muscles of various fish species

	mg/g muscle											
	Yellowtail		Amberjack		Japanese butterfish		Pacific saury Japanese		Spanish mackerel		Chub mackerel	
Fatty acid	Ordinary	Dark	Ordinary	Dark	Ordinary	Dark	Ordinary	Dark	Ordinary	Dark	Ordinary	Dark
14:0	4.44	8.38	4.56	11.16	1.25	3.39	0.99	11.41	2.12	3.12	2.05	2.68
15:0	1.09	1.68	0.41	1.25	0.39	0.93	0.10	1.14	0.21	0.30	0.52	0.78
16:0	20.93	35.74	9.94	38.35	8.66	23.40	2.57	24.66	11.68	16.82	13.42	19.36
17:0	0.77	1.16	ND	ND[a]	0.42	1.05	0.08	0.80	0.22	0.32	0.57	0.97
18:0	4.29	8.03	2.00	10.79	3.24	8.11	0.53	4.03	2.50	3.62	3.31	5.08
Saturated	31.52	54.99	16.91	61.55	13.96	36.88	4.27	42.04	16.73	24.18	19.87	28.87
16:1n-7	6.73	10.57	4.72	15.40	2.15	5.86	0.47	5.33	3.67	5.64	2.28	3.39
18:1n-9	16.22	30.51	8.07	46.49	13.61	35.52	0.78	8.08	13.32	17.71	10.81	16.11
18:1n-7	2.71	5.15	1.43	ND	1.55	4.00	0.18	2.10	2.18	3.23	1.60	ND
20:1n-9	ND	ND	ND	ND	1.49	3.80	1.90	24.03	0.18	1.14	1.04	1.69
20:1n-7	1.19	2.51	1.39	8.60	0.39	1.12	0.59	6.33	0.13	0.19	0.27	0.43
22:1n-11	0.35	0.29	0.74	6.27	0.34	1.13	3.05	35.99	0.53	0.92	0.21	0.42
22:1n-9	0.95	1.23	ND	ND	0.60	1.98	0.17	1.76	0.15	0.24	0.46	0.82
Monoenoic	28.15	50.26	16.35	76.76	20.13	53.41	7.14	83.62	20.16	29.07	16.67	22.86
16:2n-4	0.89	1.56	0.23	1.18	0.37	0.86	ND	ND	0.29	0.42	0.25	0.67
16:3n-4	ND	ND	0.54	0.93	0.17	0.45	ND	ND	0.28	0.48	0.29	0.48
18:2n-6	2.42	3.61	2.08	8.38	0.21	0.60	0.25	2.69	0.47	0.72	0.53	0.81
18:3n-3	1.01	1.54	0.54	1.93	0.12	0.34	0.21	1.94	0.25	0.40	0.36	0.41
18:4n-3	1.08	1.54	0.76	2.36	ND	0.23	0.68	5.64	0.51	0.76	0.40	0.43
20:4n-6	1.78	2.55	0.62	2.60	1.32	3.17	0.10	0.95	0.55	0.79	1.07	1.64
20:4n-3	0.51	0.74	0.34	1.41	0.16	0.44	0.18	1.71	0.22	0.33	0.23	0.28
20:5n-3	6.09	9.02	4.44	16.25	1.70	4.60	1.07	8.88	4.24	6.10	3.03	3.90
22:3n-6	0.31	0.63	0.26	0.61	ND	ND	0.07	0.54	0.19	0.28	ND	0.16
22:5n-6	1.00	1.59	ND	ND	0.70	1.75	ND	0.49	0.20	0.35	0.58	1.04
22:5n-3	2.36	3.71	1.08	5.67	0.91	2.57	0.26	3.11	0.91	1.47	0.74	1.17
22:6n-3	18.19	25.77	7.91	40.23	3.60	10.26	3.55	26.91	8.19	13.82	8.95	15.19
Polyenoic	35.64	52.26	18.80	81.55	9.26	25.27	6.37	52.86	16.30	25.92	16.43	26.18
Total	95.31	157.51	52.06	219.86	43.35	115.56	17.78	178.52	53.19	79.17	52.97	77.91

[a]ND = not detected.

ture was saponified by 1 *M* KOH in methanol and subsequently methylated with 14% BF_3-CH_3OH to obtain fatty acid methyl esters (FAME). The FAME were analyzed with a Shimadzu GC-15A gas chromatograph (Kyoto, Japan) equipped with an open tubular capillary column (0.25-mm inner dia × 30 m, 0.25-μm film thickness, Supelco, Bellefonte, Pa., U.S.A.). The oven temperature was kept at 140 °C for 1 min and programmed to a final temperature of 240 °C at a rate of 1 °C/min. The temperature of both the injector and detector was kept at 250 °C. Helium was used as carrier gas at the column inlet pressure of 2 kg/cm^2.

Statistical analysis

The chemical and instrumental analyses were carried out in triplicate and represented as mean value ± standard deviation. Application of The Student *t*-test (Box and others 1978) determined the significant differences among the mean values. As statistically significant difference between 2 mean values was declared at $P < 0.05$ and $P < 0.01$. The data from the sensory evaluation was analyzed by 1-way analysis of variance (ANOVA).

Results and Discussion

Fatty acid compositions (Trial I)

The fatty acid composition of both the ordinary and dark muscles of various fish species during ice storage is summarized in Table 2. The predominant fatty acids were 16:0, 16:1n-7, 18:1n-9, 20:5n-3 (EPA), and 22:6n-3 (DHA) in both of ordinary and dark muscles. The contents of PUFAs, including EPA and DHA were markedly higher in the dark muscle compared with the ordinary muscles among all fish species. For amberjack, Japanese butter-

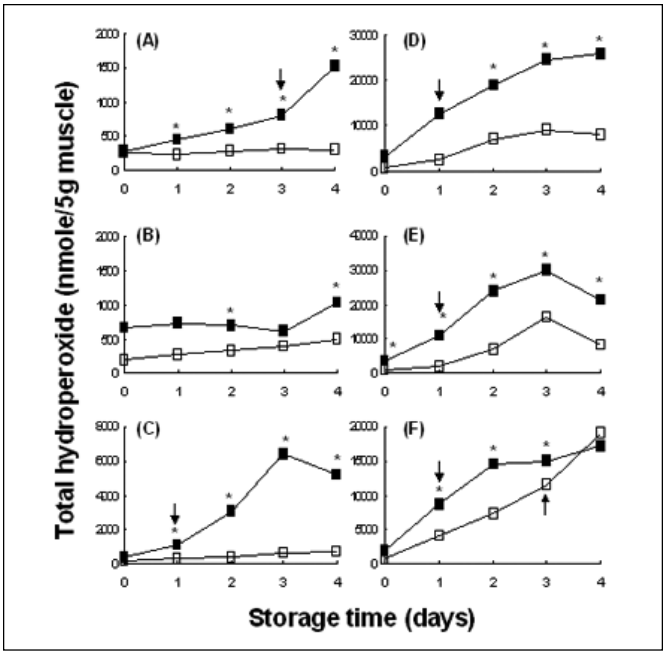


Figure 1—Changes in total hydroperoxide in ordinary and dark muscles during 4 d of ice storage. (a) Yellowtail; (b) amberjack; (c) Japanese butterfish; (d) Pacific saury; (e) Japanese Spanish mackerel; and (f) chub mackerel. Significant differences ($P < 0.01$) between ordinary (□) and dark (■) muscles are indicated by asterisks (*). Arrows indicate the 1st recognition of rancid off-odor.

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fish, and Pacific saury, the EPA and DHA contents of the dark muscles were 3 to 8 times higher than that of the ordinary muscles. The percentage of saturated fatty acids in the ordinary and dark muscles of the chub mackerel were the highest of all species examined and were measured to be 37.5% and 37.1%, respectively. The percentage of unsaturated fatty acids in the total fatty acids of Pacific saury ordinary and dark muscles were the highest of all species examined and found to be 76.0% and 76.5%, respectively.

Changes in total lipid hydroperoxide in fish muscles (Trial I)

Changes in total lipid hydroperoxide in the muscles of yellowtail, amberjack, Japanese butterfish, Pacific saury, Japanese Spanish mackerel, and chub mackerel during ice storage are shown in Figure 1. The total lipid hydroperoxide content in the ordinary muscle of yellowtail was low, amounting to 297 nmole/5 g muscle and remained unchanged during 4 d of ice storage. On the other hand, total lipid hydroperoxide content in the dark muscle was significantly higher ($P < 0.01$) than ordinary muscle throughout the storage time, even though the initial amount in the dark muscle was close to that of the ordinary muscle prior to storage (Figure 1a). The total lipid hydroperoxide content in the ordinary muscles of amberjack and Japanese butterfish increased at a slower rate than the dark muscle in which the hydroperoxide content increased rapidly throughout the storage time (Figure 1b and 1c). The total lipid hydroperoxide content of Pacific saury, Japanese Spanish mackerel, and chub mackerel tended to increase in both dark muscle and ordinary muscle throughout the storage time. Interestingly, the total lipid hydroperoxide content in the ordinary muscle of chub mackerel increased rapidly and exceeded that in the dark muscle after 4 d of ice storage (Figure 1d, 1e, and 1f).

Lipid content and total lipid hydroperoxide content in ordinary and dark muscles are shown in Table 3. The dark muscle contained a higher lipid level than ordinary muscle. The initial ratio of total lipid content between the dark and ordinary muscles (dark muscle/ordinary muscle) of yellowtail, amberjack, Japanese butterfish, Pacific saury, Japanese Spanish mackerel, and chub mackerel were 2.0, 4.5, 2.3, 9.2, 1.7, and 1.1, respectively. However, the initial ratios of total lipid hydroperoxide between the dark and ordinary muscles of yellowtail, amberjack, Japanese butterfish, Pacific saury, Japanese Spanish mackerel, and chub mackerel were 0.5, 0.8, 1.3, 0.5, 3.9, and 2.6, respectively. The total lipid content of the dark muscle of Pacific saury was the highest of all, and the total lipid hydroperoxide amounted to 2.62 μmole/g lipid at 0 d of storage time. Contrary to this, the total lipid content of the dark muscle of chub mackerel was the lowest of all, and the total lipid hydroperoxide content was remarkably higher than most of the other fish dark muscles. Thus, there was no correlation between the ratio of lipid content and total lipid hydroperoxide between ordinary and dark muscle of among fish species.

Development of rancid off-odor in fish muscles (Trial I)

In the yellowtail muscles, lipid oxidation caused the off-odor to develop in the dark muscle after 3 d of ice storage. The only species to develop an off-odor in the ordinary muscle throughout 4 d of ice storage was the chub mackerel in which the rancid off-odor was noted after 3 d (Figure 1a and 1f). On the contrary, the rancid off-odor developed in the dark muscles of Japanese butterfish, Pacific saury, Japanese Spanish mackerel, and chub mackerel after 1 d of ice storage.

Changes in color of fish muscles (Trial I)

Changes in color tones of the ordinary and dark muscles during 4 d of ice storage are shown in Figure 2. No discoloration of the ordinary muscle of Japanese butterfish occurred after 4 d of ice storage. The color of ordinary muscle in the Japanese Spanish mackerel changed slightly from white to pink after 4 d of ice storage. The ordinary muscles of both Pacific saury and chub mackerel changed from light pink to dark brown after 4 d of ice storage. A drastic color change from reddish brown to dark brown in the dark muscles of Japanese butterfish, Pacific saury, Japanese Spanish mackerel, and chub mackerel was seen after 4 d of ice storage.

Table 3—Lipid contents and total lipid hydroperoxide contents of fish muscles stored on ice

	Lipid contens g/100 g muscle		Total lipid hydroperoxides μ mole/g lipid	
Fish muscles	**0 d**	**4 d**	**0 d**	**4 d**
Yellowtail				
Ordinary muscle	8.0	7.4	0.63	0.80
Dark muscle	16.2	12.2	0.33	2.49
Amberjack				
Ordinary muscle	3.4	4.1	1.14	2.43
Dark muscle	15.2	14.3	0.87	1.56
Japanese butterfish				
Ordinary muscle	5.6	3.7	0.41	3.79
Dark muscle	12.6	11.2	0.52	9.26
Pacific saury				
Ordinary muscle	2.7	2.5	4.96	64.61
Dark muscle	24.8	23.5	2.62	21.98
Japanese Spanish mackerel				
Ordinary muscle	6.6	7.1	1.65	22.97
Dark muscle	11.0	12.8	6.39	33.16
Chub mackerel				
Ordinary muscle	8.0	6.7	1.61	56.97
Dark muscle	8.5	8.8	4.20	39.02

Changes in hydroperoxide, TBARS, and metmyoglobin contents in yellowtail muscles (Trial II)

Changes in total hydroperoxide content in yellowtail muscles are shown in Figure 3a. The total hydroperoxide content remained unchanged during 2 d of ice storage. The hydroperoxide content in the dark muscle, however, increased markedly after 1 d of ice storage and was significantly higher in the dark muscle than in the ordinary muscle throughout the storage time. These results concur with those obtained from another yellowtail specimen in Trial I (Figure 1a).

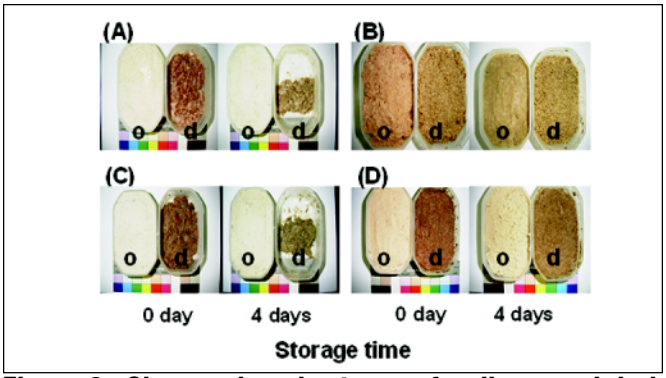


Figure 2—Changes in color tones of ordinary and dark muscles of several species of fish during 4 d of ice storage. (a) Japanese butterfish; (b) Pacific saury; (c) Japanese Spanish mackerel; and (d) chub mackerel. (d) = dark muscle; (o) = ordinary.

Changes in TBARS of ordinary and dark muscles of yellowtail during ice storage are shown in Figure 3b. The TBARS of the ordinary muscle slightly decreased throughout storage time and were lower than the dark muscle during 2 d of ice storage. The changes observed in TBARS suggest that the decomposition rate of hydroperoxide was slower in the ordinary muscle during the early stages of ice storage. On the other hand, the TBARS of the dark muscle increased significantly on day 2, suggesting that hydroperoxide decomposition was faster in the dark muscle than in the ordinary muscle during the early stages of ice storage.

Changes in the ratios of metmyoglobin in total myoglobin are shown in Figure 3c. The myoglobin content in the ordinary muscle of yellowtail was below the detectable amount of about 1 mg/100 g muscle. Metmyoglobin accounted for 12% of total myoglobin in the initial stage of storage and increased rapidly to 70% after 2 d of ice storage.

Table 4—Changes in *L, *a**, and *b** values of ordinary and dark muscles of yellowtail during ice storage**

	Storage time (d)[a]		
MuscleColor	0	1	2
Ordinary			
*L**	55.11 ± 0.46a	57.29 ± 0.97b	56.08 ± 0.2b
*a**	-0.41 ± 0.13a	0.65 ± 0.01b	1.22 ± 0.04c
*b**	12.92 ± 0.09a	14.29 ± 0.47b	15.01 ± 0.46b
Dark			
*L**	35.44 ± 0.74a	35.04 ± 0.53a	33.56 ± 0.39b
*a**	19.28 ± 0.82a	17.01 ± 0.86b	13.9 ± 1.14c
*b**	15.01 ± 0.77a	16.09 ± 0.44ab	16.74 ± 0.63b

[a]Means with different letters in the same rows are significantly different ($P < 0.05$).

Color tones of yellowtail muscles (Trial II)

Changes in *L**, *a**, and *b** measured by the CIELAB method values during ice storage are shown in Table 4. The *a** value (index of redness) was significantly higher for the dark muscle than the ordinary muscle at 0 d of storage and subsequently decreased significantly during 2 d of ice storage. Changes in the *a** values of the dark muscle coincided with the changes in the metmyoglobin level shown in Figure 3c. The *b** value (index of yellowness) tended to increase in both ordinary and dark muscles throughout the storage time. The *L** value (index of luminance) changed only slightly during 2 d of ice storage.

Changes in odor intensities (Trial II)

Changes in the odor intensities of yellowtail ordinary and dark muscles during ice storage are shown in Figure 4. The ordinary muscle scored close to 1 for overall smell (weak smell) on day 0 of ice storage, and as storage progressed, the intensity declined. The dark muscle on the other hand, was scored negatively on day 0 of ice storage, suggesting that the panel detected no smell. On day 1, a weak smell was detected, and by day 2, the odor intensity had increased markedly. No significant differences were found in the fishy, spoiled, and rancid off-odor intensities between the ordinary and the dark muscles on day 0. However, the intensities of these smells increased significantly after 1 d of ice storage in the dark muscle. The ordinary muscle differed since no significant differences in the intensities of smells, except for rancid off-odor, were noticed throughout the 2 d of ice storage. No significant difference was found between the ordinary and dark muscles for grassy and bloody smells. The milky smell was distinguished only in the ordinary muscle. There were no significant changes over time in the intensity of the milky smell in the ordinary muscle. The overall smell and rancid off-odor increased significantly throughout storage time in the dark muscle (Figure 4a and 4d).

A linear regression analysis was carried out on the sensory evaluation results and total hydroperoxide content for ordinary and dark muscles. The correlation coefficients are summarized in Table 5. No significant correlation between the intensities of smell and hydroperoxide content was found in ordinary muscle. A significant correlation ($P < 0.05$) between the intensity of rancid off-odor and total lipid hydroperoxide content with a determination coefficient

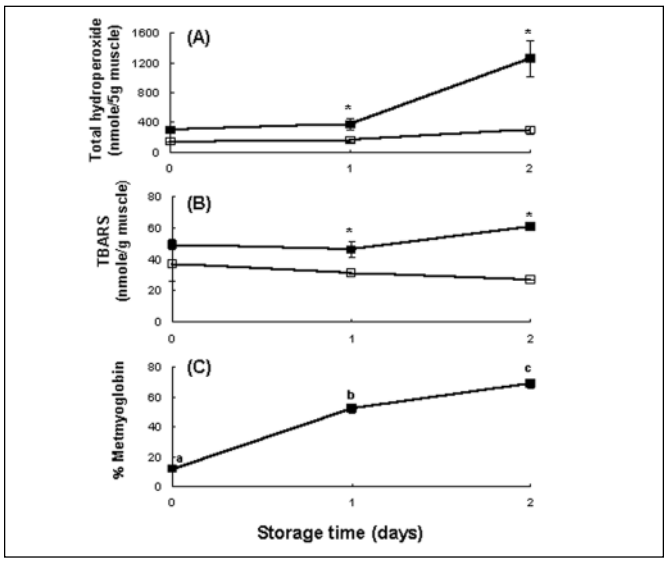


Figure 3—Changes in hydroperoxide (a), thiobarbituric acid-reactive substances (TBARS) (b), and % metmyoglobin (c) of yellowtail muscle during 4 d of ice storage. Significant differences ($P < 0.01$) between ordinary (□) and dark (■) muscles are indicated by asterisks (*). For the dark muscle, values with different letters (a-c) significantly differ ($P < 0.01$).

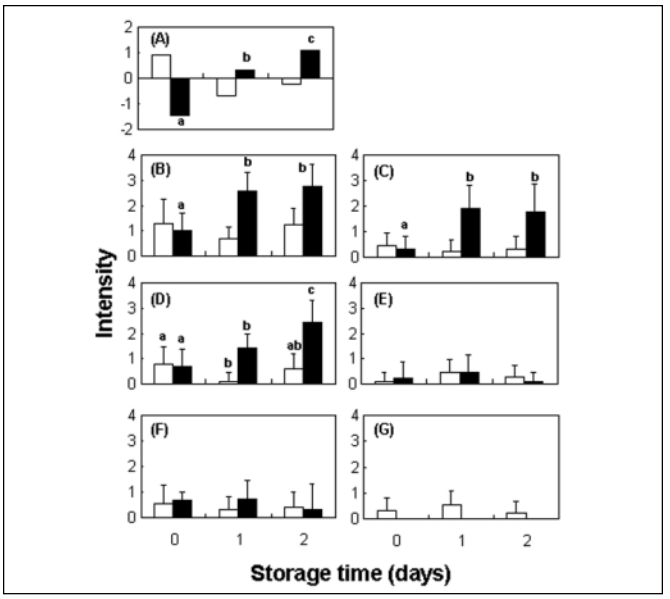


Figure 4—Changes in odor intensities in ordinary and dark muscles of yellowtail during 2 d of ice storage. (a) overall; (b) fishy; (c) spoiled; (d) rancid off-odor; (e) grassy; (f) bloody; and (g) milky smell. For each muscle for a given storage period, bars with different letters (a-c) are significantly differ ($P < 0.05$). ordinary (□); dark muscle (■).

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Table 5—Linear regression analyses between total hydroperoxide contents and odor intensities in ordinary and dark muscles of yellowtail during 2-d of ice storage

	Correlation coefficient (r^2)	
Smell	Ordinary muscle	Dark muscle
Overall	0.0170	0.6670
Fishy	0.0981	0.4215
Spoiled	0.0133	0.2554
Rancid off-odor	0.0258	0.8629
Grassy	0.0177	0.5023
Bloody	0.1412	- 0.9570
Milky	0.4451	—

$P < 0.05$

of 0.8629 was found in dark muscle. A significant ($P < 0.05$) relationship between the intensity of overall smell and total lipid hydroperoxide content with a coefficient of 0.6670 was also found.

Discussion

This study showed that the total hydroperoxide content in dark muscles was higher than in ordinary muscles in all fish species studied. These results concur with those obtained by Nakamura and others (1998) who reported that the levels of lipid peroxides were higher in the physiologically active tissues such as liver and dark muscle compared with ordinary muscle just after sacrifice. The present study also revealed that both the accumulating rates and the actual amount of lipid hydroperoxide in the ordinary muscles of fish varied greatly from species to species. These results indicate that the lipid hydroperoxide accumulation in the early stage of lipid oxidation differs not only between fish species but also between ordinary and dark muscle.

The most important indices by which consumers evaluate the freshness and quality of raw muscle foods are color and flavor (Risvik 1994). Hemoglobin and myoglobin are the most abundant heme proteins in blood and dark muscle, respectively. Moreover, the differences in the color tone of ordinary muscles among fish species are mainly due to different ratios of oxymyoglobin and metmyoglobin. Indeed, our study revealed variation in the redness of ordinary muscles between species. The bright red color of oxymyoglobin changes to brown or dark brown because of the oxidation of myoglobin. Myoglobin is the predominant pigment in most fish muscles and it is well known that the high myoglobin content in dark muscle contributes to the reddish brown color of the flesh. The rate of muscle discoloration is closely related to the rate of oxymyoglobin oxidation (Haard 1992; Kannan and others 2001).

The highly unsaturated fatty acids of fish muscle are readily decomposed and produce secondary oxidation products such as aldehydes, ketones, alcohols, and other carbonyl compounds. Propanal and several other volatile compounds are generated in the dark muscles of sardines and mackerel at a higher rate than in ordinary muscles (Tokunaga and others 1981; Hirokawa and Kisaki 1990). The contribution of heme proteins to lipid peroxidation has been suggested (Koizumi and others 1987; Chan and others 1997; Richards and Hultin 2003; Undeland and others 2004). Ohshima and others (1988) reported that the lipid oxidation in fish muscle was promoted by autoxidation of myoglobin, suggesting a close relationship between lipid oxidation and myoglobin oxidation. Moreover, O'Grady and others (2001) reported a relationship between oxymyoglobin oxidation and lipid oxidation in bovine muscle. It is believed that formation of metmyoglobin by the oxidation of myoglobin predominantly in dark muscle, accelerates lipid oxidation and leads to the generation of greater amounts of hydroperoxide. Thus, the lipid oxidation associated with metmyoglobin formation may have caused the development of the rancid off-odor and fishy smell in dark muscle. The ordinary muscle of yellowtail, amberjack, and other white muscle fish, contain a low level of metmyoglobin and the influence of myoglobin oxidation on the development of rancid off-odor appears to be insignificant. On the other hand, the ordinary muscles of chub mackerel and skipjack contain high levels of hydroperoxide and the decomposition of this hydroperoxide may contribute to the development of rancid off-odor.

Conclusions

During ice storage, the changes in total lipid hydroperoxide levels in ordinary and dark muscles differed among species. The total lipid hydroperoxide content in yellowtail dark muscle was 5 times higher than in ordinary muscle on day 4 and suggests, therefore, that the rate of lipid oxidation in whole muscle is probably reflected in the high lipid oxidation rate of dark muscle. The lipid content did not, however, influence the accumulation of lipid hydroperoxides. The metmyoglobin percentage in yellowtail dark muscle increased significantly with extended ice storage, indicating that the oxidation of myoglobin preceded lipid oxidation. Thus, the oxidation of myoglobin plays an important role in the generation of hydroperoxide. A higher content of PUFA and myoglobin in dark muscle is possibly another important factor when considering the reasons associated with susceptibility to lipid oxidation and generation of rancid off-odor under refrigerated conditions.

In conclusion, the oxidation of myoglobin is the main cause in the development of the unpleasant color and undesirable odor during ice storage of fish muscle. Maintaining fish at high quality during ice storage will require suppression of myoglobin oxidation, which will in turn decrease lipid oxidation and off-odor development.

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