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RESEARCH ARTICLE

Comparative Study of Basic Characteristics of Ordinary and Dark Muscle in Skipjack Tuna (*Katsuwonus pelamis*)

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Abstract Differences between ordinary and dark muscle of skipjack tuna (*Katsuwonus pelamis*) including proximate composition, flavor, color, texture, and freshness were investigated. Ordinary muscle had a higher crude protein content, but a lower crude lipid content than dark muscle. Alcohols (30.41%) and aldehydes (25.56%) were the prominent flavor compounds present in ordinary muscle, whereas hydrocarbons (39.51%) and ketones (21.81%) were more abundant in dark muscle. Different L*, a*, and b* values were also observed. Texture profile analysis (TPA) showed that dark muscle had higher values for adhesiveness, and lower values for cohensiveness, chewiness, and resilience. After mechanical breaking, large myofibril fragments were observed in ordinary muscle under phase contrast microscopy, but not in dark muscle. Freshness indices, including K values, total volatile basic nitrogen (TVB-N), and thiobarbituric acid (TBA) values of dark muscle were higher than for ordinary muscle.

Keywords: basic characteristic, comparison, dark muscle, ordinary muscle, skipjack tuna

Introduction

Skipjack tuna (*Katsuwonus pelamis*) is an important tuna species that holds a dominant position in the world seafood market (1). Skipjack tuna is the main species caught, and catches have doubled during the past 15 years (2). Skipjack tuna contains ordinary muscle and dark muscle and the dark muscle, located deep within the tuna body, makes up 13-16% of a headless skipjack tuna (3). This proportion of dark muscle in skipjack tuna is much higher than in other fish species, and allows skipjack tuna to swim at high speeds for long periods of time without fatigue (2).

A new use for dark muscle is needed to achieve better use as a high quality protein source and to increase the economic value of dark muscle (2). Efficient recovery and use of fish by-products have already been explored and by-products have been used to produce fish oil, fishmeal, fertilizer, pet food, and fish silage. However, most of these products have a low economic value (4). Therefore, room for improvement in dark muscle applications is large. The processing of fish is closely associated with the basic characteristics of fish muscle because consumer acceptance of fishery products depends on several attributes of food quality, including nutrition, flavor, texture, color, freshness, and suitability for processing and preservation (5).

Previous studies have focused on biochemical changes in ordinary and dark muscle during storage. Sohn and Ohshima (6) observed lipid oxidation of ordinary and dark muscle in skipjack tuna during ice storage, and found that the lipid hydroperoxide content in dark muscle was significantly ($p<0.05$) higher than in ordinary muscle. Similar results were also reported for the ordinary and dark muscle of cultured yellowtail during ice storage (7). Changes in the oxymoglobin and metmyoglobin contents of the dark muscle of Eastern little tuna stored under different atmospheric systems has been reported (8). In order to systemically reveal the different characteristics of ordinary and dark muscle, which can act as a guide for processing of dark muscle, this study focused on different basic characteristics, including the proximate composition, flavor, color, texture, and freshness of both the ordinary and dark muscle of skipjack tuna.

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Materials and Methods

Sample preparation Skipjack tuna (*Katsuwonus pelamis*) with an average fish weight of 1±0.1 kg were obtained from the western Pacific Ocean (near the East China Sea). Tuna were stored in ice after they were caught and were transported to the laboratory within 24 h. After arrival, fish were washed, headed, gutted, skinned, and filleted on 2 sides. These processes were carried out in a cold room at a temperature of 4°C. Analysis of ordinary and dark muscle was used to determine the proximate composition, flavor, color, texture, and freshness.

Proximate composition analysis Moisture, crude protein, crude lipid, and ash contents were measured using procedures reported by Li *et al*. (9). Results were expressed as g/100 g of muscle (wet weight basis).

Flavor analysis The volatile compounds of ordinary and dark muscle were determined using a solid-phase micro-extraction-gas chromatography-mass spectrometer (SPME-GC-MS; Thermo Finnigan ThermoQuest, San Jose, CA, USA) following the modified method of Iglesias and Medina (10). Fish samples were homogenized with a homogenizer (HR2850; Philips Ltd., Amsterdam, Netherlands) for 30 s and a 3 g sample was placed into a 15 mL headspace vial (Thermo Finnigan ThermoQuest). Then, 75 μm of Car/Polydimethylsiloxane (PDMS) extraction fiber (Supelco, Inc., Bellefonte, PA, USA) was used to absorb gas in the headspace vial (Thermo Finnigan ThermoQuest) for 30 min. The volatile compounds were then desorbed in the GC injector port for 3 min at 250°C. Afterwards, GC-MS analyses were carried out on a gas chromatograph equipped with a split/splitless injector and coupled with a mass detector (Thermo Finnigan ThermoQuest). Compounds were separated on a 30 m×0.25 mm×0.25 μm fused silica DB-Wax capillary column (Alltech, Deerfield, IL, USA). The GC oven temperature program was an initial value of 40°C for 3 min, followed by an increase of 5°C/min to 80°C, then an increase of 10°C/min to a final temperature of 230°C followed by holding for 7 min. Helium was used as carrier gas at a constant flow of 0.8 mL/min. The mass spectrometer (Thermo Finnigan ThermoQuest) was operated in electron impact mode and the source temperature was set at 200°C. Acquisition was performed in a 33-450 atomic mass unit (amu) range with a scan rate of 0.25 s/scan. All analyses were performed at an ionization energy at 70 eV, a filament emission current of 200 microamps (μA), and an electron multiplier voltage of 350 V. Identities of volatile compounds of ordinary and dark muscle were determined using the Xcalibur data system (version 1.0; Thermo Electron Co., San Jose, CA, USA), together with the National Institute of Standards and Technology (NIST) mass spectra search library. Pure standards (analytical grade; Merck, Darmstadt, Germany) were injected to confirm identifications. The relative contents of volatile compounds were obtained by comparing the GC peak area of each identified component integrated from the total peak area of all volatiles.

Color analysis The color of both ordinary and dark muscle of skipjack tuna was measured using an automatic Color Difference Meter colorimeter (HunterLab ColorQuest.XE, Reston, VA, USA). The values of L*, a*, and b* were used to evaluate the meat color.

Texture analysis Texture profile analysis (TPA) was performed using a TA.XT texture analyzer (Stable Micro Systems Ltd., Surrey, UK). A flat-ended cylinder (P/36R) with a diameter of 35 mm was used and the thickness of the fillets was 10 mm. The flat-ended cylinder approached samples at a speed of 1 mm/s and penetrated into fillets to a sample depth of 50% of the sample thickness. Then, the force was reduced and the fillet was allowed to rebound 10 sec with the cylinder just touching the surface. After this, the cylinder was pressed on the fillets a second time, and the adhesiveness, cohensiveness, chewiness, and resilience of the fish muscle were obtained.

The microstructures of ordinary and dark muscle described as the morphology of myofibril fragmentation were determined using a phase contrast microscope (Olympus BX41; Olympus Ltd., Tokyo, Japan). A total of 10 g of sample was homogenized with a homogenizer (HR2850; Philips Ltd.) for 2 min in 30 mL of a buffer solution (0.1 M KCl, analytical grade; Shenbo Chemical Ltd., Shanghai, China), 0.02 M potassium phosphate (analytical grade; Hushi Chemical Ltd., Huzhou, China), 0.001 M EDTA (analytical grade; Fuding Chemical Ltd., Tianjin, China), 0.001 M $MgCl_2$ (analytical grade; Shenbo Chemical Ltd.), and 0.001 M NaN3 (pH 7.0, analytical grade; Sigma-Aldrich Ltd., Steinheim, Germany). The homogenate was centrifuged with a centrifuge (CR21 GII; Hitachi Ltd., Tokyo, Japan) at 1,006×*g* for 15 min at 2°C. Then, the supernatant was discarded, and the pellet was homogenized with a homogenizer (HR2850; Philips Ltd.) for 2 min in 30 mL of the initial buffer solution and centrifuged with a centrifuge (CR21 GII; Hitachi Ltd.) again at 1,006×*g* for 15 min at 2°C. Afterwards, the supernatant was discarded, and the pellet was suspended in 30 mL of the initial buffer solution. The myofibril suspension was poured through cotton gauze (two layers; Hengtai Ltd., Taizhou, China) to remove connective tissue and the morphology of myofibril fragmentation was observed.

Freshness analysis Measurement of K value, an index of adenosine-triphosphate (ATP) and its breakdown products,

was performed according to the modified procedure of Li *et al*. (9) using HPLC (Waters Co., Milford, MA, USA) equipped with an XDB C18 column (4.6×250 mm, 5 μm). The histamine content was determined following the modified method of Vinci and Antonelli (11). Total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) values were measured following the method of Li *et al*. (9).

Statistical analysis Data were expressed as mean±standard deviation (SD) of measurements in triplicate. All data were subjected to analysis of variance (ANOVA). Differences in mean values were determined based on the least significant difference (LSD, $p<0.05$) procedure of the statistical analysis system software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Proximate composition analysis The proximate composition of ordinary and dark muscle samples of skipjack tuna, including moisture, crude protein, crude lipid, and ash are shown in Table 1. The proximate composition of skipjack tuna can be influenced by many factors, including species, growth stage, season, and catch location (12). Crude protein was the major constituent of both ordinary and dark muscle, indicating that skipjack tuna is a good resource of amino acids (12). The ash contents of both ordinary and dark muscle samples were not significantly different ($p>0.05$) between the 2 muscle types. A significant difference ($p<0.05$) was observed in the moisture, crude protein, and lipid contents between ordinary and dark muscle. The crude protein content of ordinary muscle was higher than for dark muscle. However, the crude lipid content of ordinary muscle was lower.

Table 1. The proximate composition of ordinary and dark muscle in skipjack tuna

g/100 Muscle	Ordinary muscle	Dark muscle	*p*-value[1)]
Moisture	73.62±0.21	74.94±0.20	*
Crude protein	23.85±1.11[a2)3)]	21.63±1.58[b]	*
Crude lipid	0.53±0.18[b]	1.52±0.65[a]	*
Ash	1.45±0.18	1.46±0.13	NS

[1)]*p*-values were derived from ANOVA and LSD testing. Significant at *$p<0.05$; NS, not significant
[2)]Each value represents a mean±SD ($n=3$).
[3)a,b]Values with dierent superscripts within a row are signicantly different.

Flavor analysis GC-MS chromatograms of volatile compounds in the ordinary and dark muscle of skipjack tuna are shown in Fig. 1 and the volatile compounds identified and quantified from ordinary and dark muscle samples are listed in Table 2. There were 60 volatile compounds identified in skipjack tuna, of which 41 and 43 compounds were detected in ordinary and dark muscle, respectively. These compounds were divided into 9 groups including alcohols, aldehydes, hydrocarbons, ketones, acids, esters, aromatics, furans, and sulfur-compounds. The total contents of these different chemical families in ordinary and dark muscle (sum of the relative volatile contents in the same families, %) are shown in Fig. 2.

Different flavor characteristics between ordinary and dark muscle were observed. Alcohols and aldehydes were the dominant compounds in ordinary muscle with respective total contents of 30.41 and 25.56%. In contrast, the main volatile compounds detected in dark muscle were hydrocarbons and ketones with respective totals of 39.51 and 21.81%.

Totals of 11 and 10 kinds of alcohols were identified in ordinary and dark muscle, respectively. Alcohols in ordinary muscle were the most abundant compounds, with

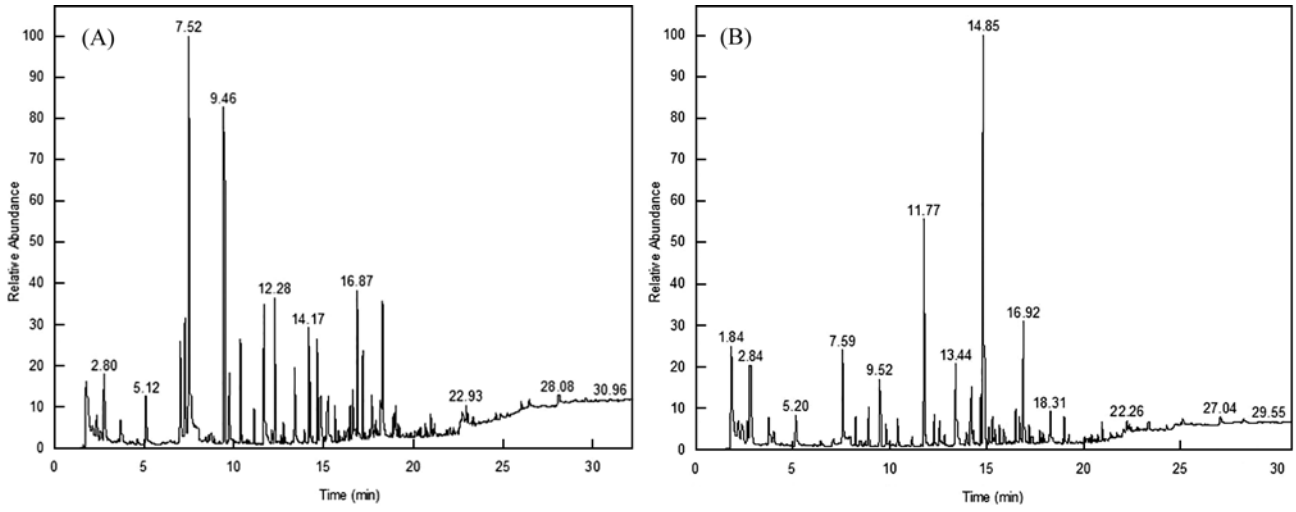

Fig. 1. GC-MS chromatograms of the volatile compounds in ordinary and dark muscle of skipjack tuna. (A) and (B) show GC-MS chromatograms of ordinary and dark muscle samples, respectively.

Table 2. GC–MS analysis of the volatile components of ordinary and dark muscle in skipjack tuna

No.	Retention time (min)		Compound name	Relative content (%)		
	Ordinary sample	Dark sample		Ordinary sample	Dark sample	*p*-value[1)]
Alcohols						
1		7.93	2-Hexadecanol	ND[2)]	0.49±0.02[a3)4)]	*
2	8.73		3-methyl-2-Butanol	0.36±0.03[a]	ND	*
3	9.46	9.52	1-Penten-3-ol	9.21±0.52[a]	2.87±0.12[b]	*
4	10.37	10.43	3-methyl-1-Butanol	2.97±0.03[a]	1.35±0.05[b]	*
5	11.13		1-Pentanol	0.93±0.05[a]	ND	*
6	11.72		3-methyl-2-Heptanol	4.83±0.23[a]	ND	*
7	12.15		(E)-2-Penten-1-ol	0.29±0.04[a]	ND	*
8	12.28	12.33	(Z)-2-Penten-1-ol	3.53±0.05[a]	1.40±0.02[b]	*
9		12.59	1-Tridecanol	ND	1.14±0.12[a]	*
10	12.79	12.84	1-Hexanol	0.49±0.02	0.42±0.06	NS
11	13.38	13.44	2-Nonen-1-ol	3.08±0.14[b]	5.15±0.11[a]	*
12	14.17		1-Octen-3-ol	2.72±0.13[a]	ND	*
13		14.31	1-Heptanol	ND	0.83±0.04[a]	*
14		15.45	2,3-Butanediol	ND	0.75±0.02[a]	*
15	17.16	17.22	3-Cyclohexene-1-ethanol	2.00±0.03[a]	0.68±0.05[b]	*
Aldehydes						
16	2.60		Propanal	0.43±0.02[a]	ND	*
17		4.05	3-methyl-Butanal	ND	1.06±0.08[a]	*
18	7.52	7.59	Hexanal	18.89±0.90[a]	5.90±0.23[b]	*
19	9.78	9.85	Heptanal	2.80±0.32[a]	1.29±0.12[b]	*
20	11.90		Octanal	0.31±0.02[a]	ND	*
21		13.61	(Z)-2-Nonenal	ND	0.44±0.07[a]	*
22	15.24	15.29	Benzaldehyde	1.37±0.04	1.35±0.04	NS
23	16.75		(Z)-2-Decenal	0.41±0.05[a]	ND	*
24	17.69		(D)-Lilac aldehyde	1.35±0.10[a]	ND	*
Hydrocarbons						
25	2.17		Heptane	0.78±0.09[a]	ND	*
26		2.70	Octane	ND	1.55±0.12[a]	*
27		11.19	2-Methylpropyl-oxirane,	ND	0.48±0.03[a]	*
28		11.85	Dodecane	ND	0.79±0.04[a]	*
29		13.95	2,6,10,14-Tetramethyl-Heptadecane	ND	0.53±0.02[a]	*
30	14.81	14.85	Hexadecane	2.58±0.32[b]	22.32±0.98[a]	*
31		15.15	Cyclopentadecane	ND	0.93±0.07[a]	*
32	15.61	15.66	Pentyl-Cyclopropane	0.80±0.18	0.64±0.12	NS
33	16.64	16.69	2-Chloro-2-nitro-Propane	1.47±0.32	1.70±0.27	NS
34	16.87	16.92	2,6,10,14-Tetramethyl-Pentadecane	4.02±0.03[b]	4.95±0.10[a]	*
35		8.26	5,5-Dimethyl-1,3-Octadiene	ND	1.27±0.03[a]	*
36		8.92	2,7-Dimethyl-3,5-Octadiene	ND	1.96±0.05[a]	*
37		10.01	(D)-Limonene	ND	0.56±0.01[a]	*
38	14.66	14.71	3,5,5-Trimethyl-2-Hexene	2.24±0.03[a]	1.83±0.05[b]	*
Ketones						
39	2.80	2.84	Acetone	3.87±0.34[b]	6.74±0.40[a]	*
40	3.74	3.80	2-Butanone	1.35±0.05[b]	2.38±0.19[a]	*
41	5.12	5.20	2-Pentanone	2.82±0.33	2.67±0.39	NS
42	7.03		2,3-Pentanedione	4.92±0.19[a]	ND	*
43		11.77	3-Hydroxy-2-Butanone	ND	10.02±0.23[a]	*
44	19.11		1-(3-butyloxiranyl)-Ethanone	0.35±0.05[a]	ND	*

Table 2. Continued

No.	Retention time (min)		Compound name	Relative content (%)		
	Ordinary sample	Dark sample		Ordinary sample	Dark sample	*p*-value[1)]
Acids						
45		14.22	Acetic formic anhydride	ND[2)]	2.74±0.67[a3)4)]	*
46	16.47	16.52	Butanoic acid	0.91±0.06[b]	1.57±0.05[a]	*
47	18.98	19.02	Hexanoic acid	1.05±0.30	1.32±0.27	NS
48	21.20	20.94	Octanoic acid	0.40±0.02[b]	0.78±0.08[a]	*
49	22.66		2-Hydroxypropanoic acid	0.90±0.07[a]	ND	*
50		27.04	Tetradecanoic acid	ND	0.77±0.05[a]	*
Esters						
51	14.26		Formic acid, heptyl ester	1.50±0.14[a]	ND	*
52	22.73	22.33	Hexadecanoic acid, methyl ester	0.90±0.16[a]	0.53±0.02[b]	*
Aromatics						
53	17.87	17.92	Methoxy-phenyl-Oxime	0.44±0.09	0.53±0.12	NS
54	18.26	18.31	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-Benzene	5.34±0.78[a]	2.24±0.47[b]	*
55		22.06	1-Methyl-2-[(3-methylphenyl)methyl]-Benzene	ND	0.47±0.08[a]	*
56		22.26	1,4-Dimethyl-7-(1-methylethyl)-Azulene	ND	0.78±0.11[a]	*
57	22.93		1,6-Dimethyl-4-(1-methylethyl)-Naphthalene	0.83±0.10[a]	ND	*
Furans						
58	4.63		2-Ethyl-furan	0.33±0.06[a]	ND	*
Sulfur-compounds						
59	2.38	2.41	Borane-methyl sulfide complex	1.06±0.02[b]	1.83±0.05[a]	*
60	7.27		Dimethyl disulfide	5.17±0.12[a]	ND	*

[1)]*p*-values were derived from ANOVA and LSD testing. Significant at **p*<0.05; NS, not significant
[2)]ND, not detected
[3)]Each value represents a mean±SD (*n*=3).
[4)a,b]values with dierent superscripts within a row are signicantly different.

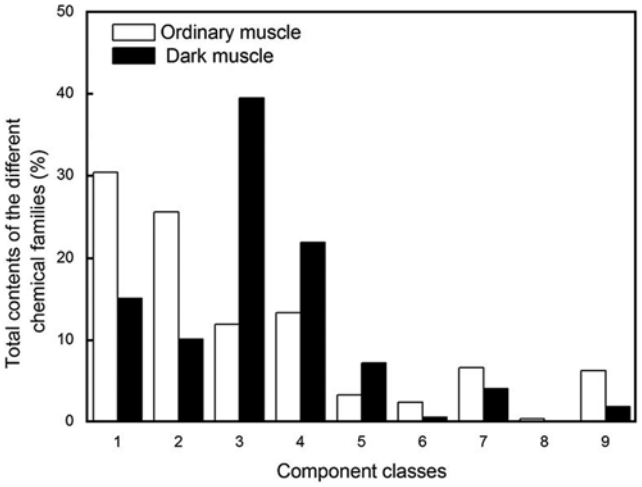

Fig. 2. The total contents of the different chemical families of ordinary and dark muscle in skipjack tuna. Component classes: 1, alcohols; 2, aldehydes; 3, hydrocarbons; 4, ketones; 5, acids; 6, esters; 7, aromatics; 8, furans; 9, sulfur-compounds

amounts significantly higher (p<0.05) than in dark muscle. The presence of alcohols might be due to the action of lipoxygenase on fatty acids, or to reduction of carbonyls to alcohols. Saturated alcohols, such as 1-pentanol, were not important contributions to the flavor of ordinary and dark muscle because of relatively high odor threshold values (13). However, the unsaturated alcohols present in ordinary muscle, including 1-penten-3-ol, 2-penten-1-ol, 2-nonten-1-ol, 1-octen-3-ol, and 3-cyclohexene-1-ethanol played important roles in the meat flavor due to low threshold values, which could explain the different flavor characteristics of ordinary and dark muscle. 1-penten-3-ol and 1-octen-3-ol, derived from auto-oxidation of unsaturated fatty acids, are related to a fresh sweet flavor (14). Therefore, 1-penten-3-ol and 1-octen-3-ol have been regarded as useful quality markers for fish. In addition, 3-cyclohexene-1-ethanol, previously described in the leaves of plants, was associated with fresh and mint flavor attributes (15).

Aldehydes are recognized as the main fresh flavor in fish meat. Ordinary muscle contained more aldehydes. In particular, the relative content of hexanal was the highest (18.89%) among the identified volatile compounds in ordinary muscle. The flavor of hexanal has been described as oxidized fatty, green, grassy, powerful, and penetrating (15). The high level of this compound can be linked to the fatty acid composition of ordinary muscle since hexanal is formed by oxidation of linoleic acid, an abundant fatty acid in fish muscle (15). Other aldehydes, such as heptanal,

benzaldehyde, and lilac aldehyde, mainly obtained from ordinary muscle, were also detected. Heptanal is generated from n-6 polyunsaturated fatty acid oxidation (16). Benzaldehyde arises from amino acid degradation and might have a desirable effect on fish aroma because of a pleasant almond nutty and stone fruit aroma (13). Lilac aldehyde, identified only in ordinary muscle, has a floral lilac flavor (15). In comparison, 3-methylbutanal, present only in dark muscle, is derived from both Strecker degradation and microbial activity on leucine (16). Therefore, 3-methylbutanal can be used as an index of microbial growth in skipjack tuna.

Hydrocarbons were mostly present in dark muscle and the major hydrocarbon detected in dark muscle was hexadecane, which constituted 22.32% of the total peak area. A total of 13 hydrocarbons were identified in dark muscle, with only 6 hydrocarbons detected in ordinary muscle. Hydrocarbons can be formed from auto-oxidation processes through alkyl radicals, or from carotenoid decomposition (13). In general, hydrocarbons, such as hexadecane, are not important contributions to the flavor of fish meat due to high odor threshold values. However, some branched chain hydrocarbons, mainly obtained from dark muscle, can affect the flavor of fish meat.

Several ketones were observed in the volatile compounds of both ordinary and dark muscle. The relative content of ketones in ordinary muscle was less than in dark muscle. Thermal degradation, lipid oxidation, amino acid degradation, and Maillard reactions were the possible mechanisms for ketone formation (17). Therefore, more ketones detected in dark muscle than in ordinary muscle could indicate more biochemical changes in dark muscle. Ketones can contribute to floral and fruity sweet flavors and, with extension of the carbon chain, the strength of the flavor gradually increases (17).

Other volatile compounds, including acids, esters, aromatics, furans, and sulfur-compounds, were also detected in both ordinary and dark muscle. No significant (p>0.05) differences between ordinary and dark muscle samples were observed for the amounts of volatile compounds, including acids, esters, and aromatics. However, furans and sulfur-compounds were mainly found in ordinary muscle. A small quantity of 2-ethylfuran was detected in ordinary muscle, which could be caused by a series of biochemical reactions (18). Other fish species, such as Atlantic horse mackerel, also contain furan related compounds (10). Sulfur-compounds were reported to affect the overall fish flavor, even when present in low amounts. Dimethyl sulfide, identified in ordinary muscle with a relatively high content, might be formed from enzymatic or thermal degradation of dimethyl-β-propiothetin (19).

Color analysis Meat color contributes substantially to the fish image. Instrumental color results, including L*, a*, and b* values of ordinary and dark muscle are shown in Table 3. Significantly (p<0.05) higher L* and b* values were observed in ordinary muscle than in dark muscle, in agreement with differences between ordinary and dark muscle reported from Eastern little tuna (8). These color distinctions might be caused by a higher myoglobin content in dark muscle, which contributes to the reddish brown color of fish muscle (7). In fact, when fish is cut up, oxygen comes into contact with myoglobin on exposed muscle surfaces. The oxygen is absorbed and reacts with the myoglobin to form a bright red pigment (oxy-myoglobin), which causes the attractive red color of some fish meat (20).

Table 3. Color, texture, and freshness analyses of ordinary and dark muscle in skipjack tuna

Indices	Ordinary muscle	Dark muscle	p-value[1)]
Color analysis			
L*	46.90±1.27[a2)3)]	31.32±2.34[b]	*
a*	2.36±0.75[b]	4.89±0.68[a]	*
b*	5.53±1.67[a]	1.74±0.29[b]	*
Texture (TPA) analysis			
Adhesiveness (g)	85.02±2.68[b]	126.67±10.93[a]	*
Cohesiveness	0.34±0.02[a]	0.25±0.03[b]	*
Chewiness	334.48±9.48[a]	109.80±5.43[b]	*
Resilience	0.12±0.03[a]	0.06±0.01[b]	*
Freshness analysis			
K-value (%)	6.15±0.81[b]	19.43±1.18[a]	*
TVB-N (mg/100 g)	12.30±0.28[b]	17.36±0.39[a]	*
Histamine (mg/100 g)	0.56±0.10	0.74±0.12	NS
TBA (mg MDA/kg)	0.11±0.01[b]	0.20±0.07[a]	*

[1)] p-values were derived from ANOVA and LSD testing. Significant at *p<0.05; NS, not significant
[2)] Each value represents a mean±SD (n=3).
[3)a,b] Values with dierent superscripts within a row are signicantly dierent.

Texture analysis Differences in texture between ordinary and dark muscle were evaluated based on a texture profile analysis (TPA), parameters of which are shown in Table 3. Although TPA results from different samples were highly variable, the texture properties of ordinary and dark muscle directly indicated the texture differences between the 2 muscle types. Values of cohesiveness, chewiness, and resilience of ordinary muscle were significantly (p<0.05) higher than corresponding values for dark muscle. Conversely, dark muscle had significantly (p<0.05) higher values for adhesiveness.

The texture of fish meat is associated with the muscle fiber density and depends on a number of intrinsic biological factors (21). Higher values (p<0.05) of cohesiveness and resilience probably indicate better textural attributes of

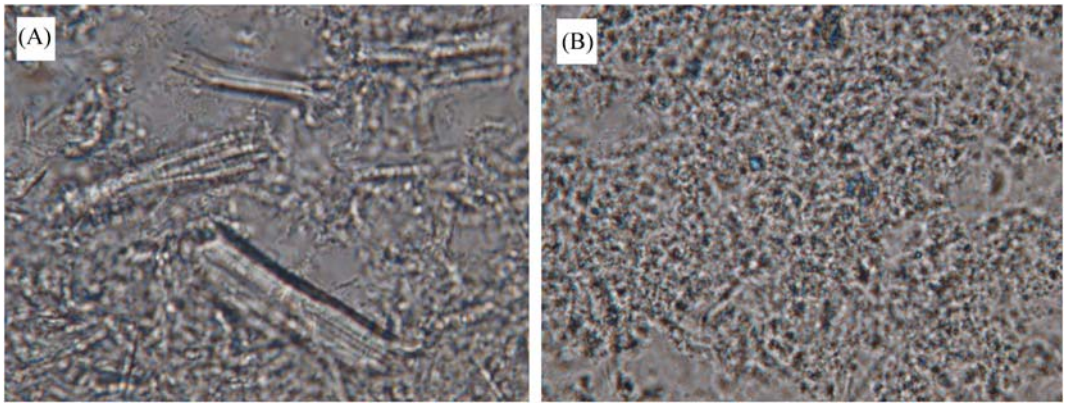

Fig. 3. Optical photomicrographs of myofibril fragmentation of ordinary and dark muscle samples after application of mechanical force. (A) and (B) show photomicrographs of ordinary and dark muscle samples, respectively at a magnification of ×1,000.

ordinary muscle because cohesiveness and resilience are both measures of the elasticity of muscle. They both describe the ability of muscle to recover from deformation and to offer resistance to subsequent deformation (22).

Microstructures of ordinary and dark muscles can be described by the morphology of myofibril fragmentation, which is highly correlated with sensory and Warner-Bratzler measures of muscle tenderness (23). Mechanical disruption is required to demonstrate the fragility of muscle myofibers (24). Optical myofibril photomicrographs of ordinary and dark muscle samples after application of mechanical force are shown in Fig. 3. Different myofibril morphologies of ordinary and dark muscles were present. Several distinct, large myofibril fragments were observed in ordinary muscle after application of mechanical force. In contrast, no large myofibril fragments were observed in optical photomicrographs of dark muscle. These myofibril morphology differences can be ascribed to the different activities of several enzymes in ordinary and dark muscle, including calpain, trypsin, protease, lipase, and DNase, all of which contribute to the shortening of separated myofibrils of fish muscle (25).

Freshness analysis Freshness is regarded as an objective attribute of sensory, chemical, microbial, and physical parameters. The freshness values of ordinary and dark muscle were determined based on K values of total volatile basic nitrogen (TVB-N), histamine production, and TBA (Table 3). Higher K values determined in dark muscle might be due to a faster catabolic ATP rate in dark muscle, compared with ordinary muscle (26). Higher TVB-N levels in dark muscle, mainly due to ammonia and amines, might result from degradation of proteins and non-protein nitrogenous compounds (27). The histamine content was not significantly (p>0.05) different between ordinary and dark muscle, which was not consistent with results reported for big-eye tuna (28). The process of catching skipjack tuna may be responsible for this difference because the histamine content is easily influenced by variations in temperature and unstable environments (29). Higher TBA values for dark muscle might be due to a higher lipid content in dark muscle (Table 1).

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