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Nutritional, textural and quality attributes of white and dark muscles of little tuna (*Euthynnus affinis*)

Sathish Kumar Kannaiyan[1]*, Chrisolite Bagthasingh[2], Vijayarahavan Vetri[3], Shanmugam Seerappalli Aran[4], & Kaliyamurthi Venkatachalam[2]

[1]Fish Processing Division, Central Institute of Fisheries Technology (CIFT), Cochin

[2]Institute of Fisheries Post Graduate Studies (IFPGS), Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Chennai

[3]Fisheries College and Research Institute (FC&RI), Thoothukudi

[4]Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Nagapatinam

*[E-Mail: sathishcife@gmail.com]

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The proximate composition, fatty acid profile, texture, colour and freshness of white and dark muscles of little tuna (*Euthynnus affinis*) were investigated. The moisture content was higher in white muscle (75.52±0.13%) compared to that in dark muscle (74.85±0.10%). Both white and dark muscle had higher levels of protein, 23.12± 0.13% and 23.15± 0.02%, respectively. Analysis of fatty acid profile by gas chromatography showed that the dark muscle had high levels of eicosapentaenoie acid (C20:5) and docosahexaenoic acid (C22:6) compared to white muscle. Texture profile analysis showed that dark muscle had higher values for hardness (3.74±0.15kgF), whereas adhesiveness, cohesiveness, springiness and chewiness values were greater for white muscle. Colour analysis revealed dark muscle had lower L* value than that of white muscle. The total volatile base nitrogen, tri - methyl amine, texture profile analysis and histamine contents were higher in dark muscle. Overall nutritional quality of dark muscle was superior to that of white muscle.

[**Keywords:** Little tuna, Muscle type, Proximate composition, Fatty acid composition, Colour analysis, Freshness analysis]

Introduction

Fish is consumed globally because of its unique source of nutrients, high protein content and low saturated fatty acid. Fish is a rich sources of omega-3 (n-3) polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), which play an important role in the prevention of heart disease and can be used to treat diseases like hypertension, arthrosclerosis, cancer and enhance the development of mammalian brain[1-2].

The muscle of fish is generally white in colour; however, some fishes contain a certain amount of dark tissue of red or brown colour called the dark muscle. The dark colour is due to the large amount of myoglobin content which renders the reddish brown colour to the meat[3]. Fish with dark muscle constitute 40-50% of the total catch in the world[4]. The proportion of dark muscle may vary based on the activity of the fish. Pelagic fish that swim continuously have 48% of the body weight made of dark muscle[5]. The dark muscle is located directly under the skin, which and is used for continuous swimming at low speed, while the white muscle is used for short bursts of swimming[6]. Active fish such as tuna, herring, mackerel etc have more dark muscle than bottom dwelling fishes such as flounder and cod[7]. The dark muscle makes up 13-16% of headless skipjack tuna[8] and help this species of fish to swim at high speed for longer periods of time without fatigue[9]. The white meat contains less lipids as compared to dark meat and the amount of lipid can vary based on the species and time of harvesting[10] and dark muscle which contains more lipid is prone to lipid oxidation more than white muscle[11].

Euthynnus affinis (Cantor, 1849) popularly known as 'little tunny' or 'kawakawa', and belongs to the family scombridae, fetch high value in the global market[12]. Little tuna (*E. affinis*) is available in the Indian Ocean and the Gulf of Thailand and form huge landing with the volume of 45600 metric tons and a value of 37 million US dollars in 2007 and provide a high economic value for canning and sashimi industries[13]. The sashimi market which was exclusively centered in Japan, has recently expanded worldwide[14]; therefore, this fish fetches a high price in the raw seafood market, representing a very interesting species from both nutritional and

economic point of view. Along the Indian coast, *E. affinis* is exploited throughout the coastal states and islands and forms the bulk of the tuna landings of the country[15-16]. There is no study on the nutritional qualities of dark and white muscle of this species. Therefore, the objective of this study is to compare the proximate composition, fatty acid profile, colour, texture and freshness of dark and white muscle of little tunny which can serve as a baseline data for selecting suitable processing methods and also development of potential nutraceuticals.

Materials and Methods

Materials

Samples of little tuna (*E. affinis*) were procured from the landing centre of Thoothukudi coast of South India. The samples were placed in sterile polythene bags, kept in ice and brought to the laboratory within 30 min. In the laboratory, the fish was washed and length (cm) and weight (g) data were recorded. Then fishes were dissected to remove skin, gills, fins and viscera and the muscle portions (both white and dark) were collected for analysis.

Proximate Composition Analysis

Moisture content was determined by hot air oven method[17]. Nitrogen content was determined by Kjeldhal method[17] using KEL PLUS – Elite ExVA – digestion and distillation apparatus (Pelican Equipment's, Chennai, India), and the protein content was calculated by multiplying the nitrogen content with a factor of 6.25. The crude fat was determined by Soxhlet method17 using petroleum ether (60-80 °C) as solvent in a SOCS PLUS-SCS 08R system (Pelican Equipment's, Chennai, India). Ash content was determined in a Muffle furnace at 500-550 °C for 16 hrs[17].

Fatty acid Analysis

For determination of fatty acid composition by gas chromatography, total lipid was extracted from fish using chloroform and methanol[18], methylation of fatty acids by BF3-methanol and subsequent analysis of fatty acids methyl esters (FAME) by capillary gas chromatography[19]. Fatty acid profile was analysed using gas chromatography (Clarus 580, Perkin Elmer, USA) with a flame ionization detector and capillary column. The oven temperature was 50 °C, held 3 min, raised to 150 °C held 5 min and raised to 250 °C. The injector and the detector temperature were set at 220 °C and 280 °C respectively. The sample volume used was 1 µl and the carrier gas was controlled at 16 psi. The split was 1:10. Fatty acid was identified by comparing the retention times of FAME with a standard component FAME mixture (Supelco, USA). GC analysis was performed (n=3) and the results were expressed in area % as a mean value ± standard error.

Texture Analysis

Texture profile analyses (TPA) was performed using a TA plus texture analyser (Lloyd Instruments, Hampshire, UK)[20]. A flat-ended cylinder with a diameter of 35 mm was used and the thickness of the fillets was 7 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 secs with the cylinder just touching the surface. After this, the cylinder was pressed on the fillets second time and the hardness, cohesiveness, springiness, chewiness and adhesiveness of the white and dark muscles were obtained. The results were expressed as mean value ± standard error.

Colour Analysis

The colour of both white and dark muscles was measured using Hunter Lab Colorimeter model no. D/8-S (Miniscan XE Plus, Plate 3.5, USA) with geometry of diffuse /8°C (sphere 8 mm view) and illuminant of D 65/10°C[21]. The tuna muscle was made into 7 mm thickness fillets and the colour differences of white and dark fillets were measured. The values of L*, a* and b* were used to evaluate the meat colour and the results were expressed as mean ± standard error (n=3).

Freshness Analysis

The histamine content was determined by the modified method of Cinquina et al. (2004)[22] using HPLC (Shimadzu Co., Milford, MA, USA). Tuna sample was homogenised with perchloric acid. The extracted solution was injected into HPLC system equipped with UV-VIS detector and was resolved in C18 column (ODS, 4.6×250 mm, 5 µm) using ion-pair chromatography method. The absorbance area of histamine peak at 214 nm in sample was compared with the absorbance of standard histamine solutions and the concentration was calculated.

Thiobarbituaric acid-reactive substances (TBARS) of fish sample was estimated according to the method of Tarladgis et al. (1960)[23]. The amount of TBARS

was expressed as mg malonaldehyde (MDA)/kg sample. Trimethylamine-nitrogen (TMA-N) and total volatile base-nitrogen (TVB-N) was determined by micro diffusion method[24]. TMA-N and TVB-N contents were expressed as mg/100 g of muscle.

Statistical Analysis

Data were expressed as mean±standard error (SE) 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 (Turky HSD, $P<0.05$) procedure of the statistical analysis system software (SPSS Inc., Chicago, IL, USA)[25].

Results and Discussion

Proximate Composition Analysis

The proximate composition including moisture, protein, lipid and ash in the dark and white muscles is given in Table 1. Proximate composition is generally a good indicator of the physiological condition of the fish. Tuna is considered as an excellent source of high quality protein. The moisture content was higher in white muscle (75.52±0.13 g/100 g muscle) compared to dark muscle, while lipid and ash were higher in dark muscle (0.44±0.003 & 1.29±0.008 g/100 g of muscle, respectively). Both white and dark muscles had higher level of protein (23.12% and 23.15%, respectively).

Earlier studies by Undeland et al. (1998)[26] on herring (*Clupea harengus*) have shown that lipid content in dark muscle was three times higher compared to white muscle. The white muscle had higher moisture, ash content and lower amount fat content in chub and blue mackerel[27] as well as yellow tail (*Seriola quinqueradiata*)[28]. In skipjack tuna (*Katsuwonus pelamis*), moisture content, lipid and ash content were higher in dark muscle[29]. The dark colour in dark muscle is due to the higher myoglobin and haemoglobin content which is five times higher in dark muscle compared to white muscle[30]. In general, the proximate composition of fishes varies with feed intake, size of fish, season, sex and other environmental conditions[31].

Fatty Acid Analysis

The fatty acid composition of dark and white muscles of tuna studied is listed in Table 2. The major fatty acids found were C16:0, C18:0, C18:1, C20:4, C20:5, and C22:6. The major fatty acid class was PUFA followed by SFA and MUFA; similar result was observed in little tuna (*Euthunnus alletteratus*) from the Mediterranean[32]. Palmitic acid was the dominant saturated fatty acid in both dark and white muscles, contributing approximately 57.50% (white muscle) and 48.89% (dark muscle) of the total saturated fatty acids. Among the SFA, white muscle had high amount of palmitic acid (20.12%), while dark muscle had high amount of stearic acid (13.73%) which is similar to earlier studies in fish[19,33-36]. Palmitic acid occurs naturally in fish, being a source of metabolic energy for their growth[37]. The MUFA content in white and dark muscle was 13.14% and 11.52%, respectively. Among the monounsaturated fatty acids, oleic and palmitoleic acids were higher in white muscle (11.08% and 1.45% respectively) than that in the dark muscle. The result showed that tuna muscles had higher percentage of PUFA's than the SFA and MUFA, which is contrast to studies in yellow tail (*Seriola quinqueradiata*)[28] where, the dark muscle contained lower percentage of PUFA and higher percentage of SFA and MUFA. PUFA content in white and dark muscles in the tuna studied was 51.86% and 55.87%, respectively. Among PUFA's, DHA was 42.70% and EPA was 5.27% in dark muscle (Table 2). Similar result was observed in yellow fin tuna studied by Karunarathna & Attygalle[38]and contradictory to other studies[27,39], where the levels of PUFA including EPA (20:5) and DHA (22:6) were higher in the dark muscle compared to light muscle in chub mackerel and skipjack tuna. The fatty acid composition of fish varies with species, sex, age, water temperature, degree of pollution, nutritional condition, and season[40-41]. The general recommended level for daily intake of DHA/EPA is 0.5 g for infants and 1 g for adults[42]. Therefore, consumption of *E. affinis* can be considered as a good source of these fatty acids. Fishes are excellent source of EPA and DHA as the major constituent of PUFA. Although the muscle tissue of the tuna species

Table 1 — Proximate composition of white and dark muscles in little tuna

Parameter (g/100 g of muscle)	White muscle	Dark muscle
Moisture	75.52±0.13[b]	74.85±0.10[a]
Crude protein	23.15±0.02[a]	23.12±0.13[a]
Crude lipid	0.07±0.002[a]	0.44±0.003[b]
Ash	1.23±0.01[a]	1.29±0.008[b]

Results are mean ± standard error (n=3); values with different letters within a row are significantly different ($p<0.05$) in one-way ANOVA followed by Tukey HSD test.

Table 2 — Fatty acid profile of white and dark muscles of little tuna

S. No.	Fatty acids	Name	Tuna white (Area %)	Tuna dark (Area %)
		Saturated fatty acid (SFA)		
1	C12:0	Lauric	0.28	0.08
2	C14:0	Myristic	0.66	0.63
3	C15:0	Pentadecanoic	0.33	0.34
4	C16:0	Palmitic	20.12	15.97
5	C17:0	Heptadecanoic	0.86	1.23
6	C18:0	Stearic	12.43	13.73
7	C20:0	Arachidic	0.18	0.34
8	C21:0	Henicosanoic	0.00	0.06
9	C22:0	Behenic	0.13	0.22
10	C23:0	Tricosanoic	0.00	0.06
		Monounsaturated fatty acid (MUFA)		
11	C16:1	Palmitoleic	1.45	1.17
12	C17:1	cis-10- Heptadecanoic	0.21	0.08
13	C18:1 trans 9	Elaidic	0.00	0.00
14	C18:1 cis 9	Oleic	11.08	9.54
15	C20:1 cis 11	cis -11- Eicosenoic	0.25	0.34
16	C22:1 cis 13	Erucic	0.15	0.28
17	C24:1 cis 15	Nervonic	0.00	0.05
		Polyunsaturated fatty acid (PUFA)		
18	C18:2 cis 9,12	Linoleic	1.39	2.23
19	C18:3 cis 6,9,12 gamma	Linolenic	0.17	0.18
20	C18:3 cis 6,9,12,15 alpha	Linolenic	0.09	0.00
21	C20:2 cis 11,14	cis-11,14-Eicosadienoic	0.26	0.45
22	C20:3 cis 8,11,14	Eicosatrienoic	0.18	0.23
23	C20:4 cis 5,8,11,14	Arachidonic	7.48	5.35
24	C22:2 cis 13,16	Docosadienoic	0.14	0.22
25	C20:5 cis 5,8,11,14,17 EPA	Eicosapentanoic	5.27	4.51
26	C22:6 cis 4,7,10,13,16,19 DHA	Docosahexaenoic	36.88	42.70
	ΣSFA		34.99	32.66
	ΣMUFA		13.14	11.52
	ΣPUFA		51.86	55.87
	n3		42.41	47.39
	n6		8.87	7.58
	n3/n6		4.78	6.25
	PUFA/SFA		1.48	1.71

Results are mean ± standard error (n=3)

contains low levels of fat (2%) and has desirable fatty acid profile and can contribute to good health[43].

The quality of fat is determined by the balance between PUFA and SFA consumed in the diet and is an important feature that influences the risk of cardiovascular disease. PUFA/SFA ratio gives a good indication of the fatty acid composition of fish. A minimum of PUFA/SFA ratio (0.45) is recommended by HMSO, (1994)[44]. PUFA/SFA ratio in the white and dark muscles of tuna studied was 1.48 and 1.71, respectively, which is above the recommended level. Pigott and Tucker (1990)[45] suggested that the omega-3/ omega-6 ratio is a useful indicator for comparing relative nutritional value of fish. The n3/n6 ratio in the white and dark muscles was 4.78 and 6.25, respectively. The n3/n6 ratio was much higher than the little tuna studied from Srilanka where the n3/n6 ratio was only 0.4 in white muscle and 0.95 in dark muscle[39]. Higher n3/n6 ratio of fatty acid has a positive influence on the lipid content. An increase in

Table 3 — Colour, texture and freshness analysis of white and dark muscles of little tuna

Indices	White muscle	Dark muscle
Colour analysis		
L*	38.72±2.47[b]	26.68±0.86[a]
a*	6.80±0.72[a]	7.29±0.55[a]
b*	13.16±0.27[b]	10.77±0.33[a]
Texture profile analysis (TPA)		
Hardness (kgf)	3.40±0.23	3.74±0.15
Adhesiveness (kgf)	0.12±0.03	0.10±0.02
Cohesiveness	0.13±0.01	0.08±0.01
Springiness (mm)	1.17±0.07	1.01±0.04
Chewiness (kgf)	0.57±0.12	0.34±0.06
Freshness analysis		
TVB-N (mg/100 g)	13.02±0.17[a]	18.6±0.34[b]
TMA (mg/100 g)	3.45±0.03[a]	5.53±0.14[b]
TBA (mg MDA/kg)	0.10±0.005[a]	0.18±0.005[b]
Histamine (ppm)	--	2.82±0.11

Results are mean ± standard error (n=3); values with different letters within a row are significantly different ($p<0.05$) in one way ANOVA followed by Tukey HSD test.

the human dietary ω-3/ω- 6 fatty acid ratio helps in prevention of coronary heart disease by reducing plasma lipids and reduce the risk of cancer[46-47]. It was suggested that a ratio of 1:1–1:5 would constitute a healthy human diet[48].

Texure Analysis

The textural difference of white and dark muscles was evaluated based on TPA parameters, including hardness, adhesiveness, cohesiveness, springiness and chewiness as shown in Table 3. The high variability of the results from different samples directly indicated the textural difference between the two muscle types. The values of adhesiveness, cohesiveness, springiness and chewiness were higher than corresponding values of dark muscle. Conversely, dark muscle had higher values of hardness (3.74±0.15 kgf) due to differences in chemical composition, fiber diameter and muscle heterogeneity[20,49,50] and similar result was observed by Shulai et al. (2014)[29] in skipjack tuna (*K. pelamis*). The texture of fish muscle is related with the muscle fiber density and depends on a number of intrinsic biological fators[51]. The higher value of cohesiveness in white muscle (0.12±0.03 kgf) indicates better textural attributes because it is a measure of the elasticity of muscle. The fluctuations in water, protein and fat content, which are caused by cycles in the reproduction process, have a significant impact on the texture of fish meat[52].

Colour Analysis

Colour is an important quality attribute of meat and seafood, especially for tuna where the market value is based on muscle appearance and colour[53]. Colour of tuna meat depends on the amount of myoglobin and haemoglobin content in the muscles[54]. The results of the colour measurement of white and dark muscle are shown in Table 3. The white muscle had higher L* and b* when compared to dark muscle and similar result was observed in little tuna (*E. affinis*)[55] and skipjack tuna (*K. pelamis*)[29]. The dark muscle had higher value of a* (redness) due to high amount of myoglobin content in dark muscle which contribute to the reddish brown colour of fish muscle[56] and similar result was reported in dark muscle of yellow fin tuna (*Thunnus albacarus*)[57]. The concentration of myoglobin in the muscle of tuna is 3-4 times higher than the same in mammal muscle[58]. The dark muscle also contains large amount of haemeproteins, low molecular weight metal and microbial enzymes[26].

Freshness Analysis

The white and dark muscle freshness values were determined based on TVB-N, TMA, histamine content and TBARS (Table 3). The dark muscle had higher TVB-N value than the white muscle, which is similar to studies by Shulai et al. (2014)[29], mainly due to ammonia and amines which might result from protein degradation and non-protein nitrogenous compounds[59]. The TMA value was higher in dark muscle than the white muscle. The histamine content was observed in dark muscle which clearly revealed that the dark muscle is prone to quick spoilage than the white muscle, similar to earlier studies reported

for big-eye tuna. The TBARS value was higher in dark muscle which might be due to a higher lipid content in dark muscle, similar to the report in skipjack tuna (*K. pelamis*)[29].

Conclusion

The study has shown that little tuna is an excellent source of nutrients and fatty acid. The data from the present study could serve as baseline data for further studies. These nutritive, textural and quality characteristics of white and dark muscles of tuna indicate that this species is very much suitable as raw material for food industry. The dark muscle has a high nutritive value with respect to its total lipid content and that it is mainly in the form of PUFA's. Little tuna is a good source of protein which is similar to the protein content of other commercially important tuna species such as skipjack, yellow fin and big eye.

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