

Amino Acid and Fatty Acid Composition of the Muscle Tissue of Yellowfin Tuna (*Thunnus Albacares*) and Bigeye Tuna (*Thunnus Obesus*)

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Abstract The nutritional compositions of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*) were investigated. The two species had high protein (23.52 – 23.72%) and low fat content (1.93 – 2.06%). No significant differences were found in the moisture, protein, fat, and ash contents between the two tuna fish species. Eighteen amino acids were identified in both fish species, and glutamic acid was the most predominant (12.45% in yellowfin tuna and 11.28% in bigeye tuna). The total amino acid (TAA) content ranged between 82.66% and 84.49% (dry weight). The ratios of essential amino acids (EAA) to TAA in yellowfin tuna and bigeye tuna were 44.95% and 45.64%, respectively. With the exception of tryptophan, the other EAA scores were >100 %. The major fatty acids were C16:0, C18:1, C22:6 (DHA), and C18:0. Yellowfin tuna had a higher concentration of DHA (20.22%, % of total fatty acids), however, no significant differences in C20:4 (ARA) and C20:5 (EPA) were found between the two tuna fish. The n-3/n-6 ratios of yellowfin tuna and bigeye tuna were 3.29 and 4.56, respectively. This study shows that these tuna species have high nutritional qualities.

Keywords: amino acid, amino acid score, fatty acid, yellowfin tuna, bigeye tuna

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1. Introduction

Eighty percent of the fat- and moisture-free sections of fish consist of protein [1]. No fish can grow or reproduce without a continuous supply of protein. The muscle tissue of fish, which is the most predominant tissue, is the main segment used for human consumption. Fish muscle tissue has an abundance of amino acids and flavors and is highly digestible [2].

Fish muscle tissue also contains n-3 polyunsaturated fatty acids (n-3 PUFA) including eicosapentaenoic acid (EPA; C20:5, n-3) and docosahexaenoic acid (DHA; C22:6, n-3). These fatty acids possess anti-cancer and heart-protective activities, and anti-arteriosclerotic actions [3]. The fatty acid composition of fish muscle is influenced by various factors; e.g., there are differences in the EPA and DHA concentrations among fish species and fishing localities [4,5]. Furthermore, the fatty acid composition in identical fish species is further affected by season, sea area, and maturity [6].

Yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*T. obesus*) are the two most economically valuable species targeted by tuna longline fisheries in the Pacific Ocean. For several decades, distant water longliners from Japan, Korea, and Taiwan have been particularly active in the

Pacific Ocean, where these two species are commonly found. The global demand and subsequent production of tuna has increased exponentially over the past 50 years [7]. The pressure on wild fish populations is now threatening tuna fisheries worldwide; some tuna populations have reached the limits of sustainability as a result of overfishing [7]. The total tuna yield obtained from tuna ranching is also limited by quota restrictions placed on wild-caught fish. To alleviate the pressures on wild tuna stocks, overcome the restrictions placed on tuna ranching, and satisfy the growing demand for tuna, hatchery techniques for tuna must be developed. Major progress has recently been reported regarding the reproduction, larval rearing, and fingerling production of the Pacific bluefin tuna (*Thunnus orientalis*) in Japan [8]. In the case of yellowfin tuna and bigeye tuna; however, commercial scale production has not been achieved as a result of low survival rates.

Several studies have focused on the digestive enzyme activities [9], age and growth [10,11], and habitat utilization of yellowfin tuna and bigeye tuna [12]. However, there is a lack of information on the nutritional quality of yellowfin tuna and bigeye tuna. The aim of this study was to assess the essential amino acid score and the proximate amino acid and fatty acid compositions of the muscle tissue of yellowfin tuna and bigeye tuna.

2. Experimental

2.1. Materials

Wild yellowfin tuna ($n = 3$; mean weight = 35.87 ± 12.79 kg) and bigeye tuna ($n = 3$; mean weight = 33.22 ± 4.27 kg) were caught in the Pacific Ocean near the Marshall Islands on August 21, 2012 (LIANCHENG OVERSEAS FISHERY (SHENGZHEN) CO., LTD.). The tuna fish were stored in polyurethane boxes with plenty of ice until the boat reached port. The dorsal ordinary muscles were removed from the fish at low temperature (4°C) and stored at -70°C for further analyses.

2.2. Proximate Composition Measurement

Proximate analyses to determine crude protein, fat, ash, and moisture were performed in triplicate for each sample using standard Association of Official Analytical Chemists methods [13]. The samples were dried at 105°C to a constant weight to determine moisture content; nitrogen was measured using the Kjeldahl method to determine protein levels ($\text{N} \times 6.25$). Lipids were extracted using a Soxhlet apparatus (Soxtec System HT, 1043 Extraction Unit, Tecator, Sweden) and ash was combusted at 550°C in a SX2-12-10 muffle furnace (Rongfeng Corporation, Shanghai, China).

2.3. Amino Acid Composition Measurement

The amino acid analysis was performed by ion exchange chromatography (IEC) using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, Dublin, Ireland). Acid hydrolysis was used for the analysis of all amino acids with the exception of tryptophan, which was analyzed by alkaline hydrolysis.

2.4. Protein Quality Estimation

The amino acid score of the essential amino acids was calculated using the formula:

$$\text{Amino acid score}(\%) = \frac{\text{sample amino acid (mg/g)}}{\text{reference amino acid (mg/g)}} \times 100, [14]$$

2.5. Fatty Acid Composition Measurement

Total lipids in muscle tissue were extracted using a chloroform-methanol solution (2:1, v/v) according to the method described by Peng et al. 2008 [15]. Capillary gas chromatography (GC) was used to determine the fatty acid profiles using an HP6890 (FID detector) and a SPTM-2380 column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.20 \mu\text{m}$). Nitrogen was the gas carrier and the column temperature was programmed to increase from 140°C to 240°C at a rate of $4^{\circ}\text{C}/\text{min}$ (with holds of 5 and 10 min at 140°C and 240°C , respectively). The fatty acids were detected at 260°C using a split injector (50:1) and identified by comparing their retention time to a chromatographic Sigma standard. The peak areas were determined using Varian software.

2.6. Statistical Analyses

All analyses were carried out in triplicate and the data were expressed as mean \pm standard deviation. The mean

values were subjected to t-tests. Significance was set at $P < 0.05$.

3. Results

Both species consisted of 72.89 – 73.57% moisture, 23.52 – 23.72% protein, 1.93 – 2.06% fat, and 1.54 – 1.77% ash (Table 1). No differences in moisture, protein, fat, and ash contents were observed between the species ($P > 0.05$). In yellowfin tuna, the total fat and moisture contents were 1.93% and 73.57% by weight, respectively. In bigeye tuna, the fat and moisture contents were 2.06% and 72.89% by weight, respectively.

Table 1. Proximate composition of the muscle tissue of yellowfin tuna and bigeye tuna (% wet basis)

Proximate	Yellowfin tuna	Bigeye tuna
Moisture	73.57 ± 0.55	72.89 ± 0.63
Crude protein	23.52 ± 0.61	23.72 ± 0.16
Crude fat	1.93 ± 0.13	2.06 ± 0.57
Crude ash	1.54 ± 0.06	1.77 ± 0.13

Table 2. Amino acid composition of the muscle tissue of yellowfin tuna and bigeye tuna (% dry basis)

Amino acid	Yellowfin tuna	Bigeye tuna
Serine	3.23 ± 0.05	3.12 ± 0.04
Tyrosine ^a	3.19 ± 0.09	3.14 ± 0.07
Proline	3.08 ± 0.08	2.99 ± 0.08
Aspartic acid	8.29 ± 0.17	8.11 ± 0.12
Glutamic acid	$12.45 \pm 0.04^*$	11.28 ± 0.12
Glycine	3.75 ± 0.10	3.66 ± 0.09
Alanine	5.14 ± 0.11	5.02 ± 0.06
Cystine ^a	0.44 ± 0.02	0.49 ± 0.04
Arginine	5.11 ± 0.10	4.96 ± 0.09
Threonine ^a	3.85 ± 0.08	3.75 ± 0.09
Valine ^a	4.54 ± 0.20	4.63 ± 0.13
Methionine ^a	2.55 ± 0.04	$2.76 \pm 0.03^*$
Isoleucine ^a	4.06 ± 0.11	4.01 ± 0.10
Leucine ^a	6.99 ± 0.10	6.86 ± 0.13
Phenylalanine ^a	3.30 ± 0.12	3.32 ± 0.08
Lysine ^a	8.19 ± 0.12	7.93 ± 0.11
Tryptophan ^a	0.88 ± 0.06	0.86 ± 0.10
Histidine ^b	5.49 ± 1.48	5.26 ± 0.29
EAA	37.97 ± 0.90	37.73 ± 0.66
TAA	84.49 ± 3.01	82.66 ± 1.56
EAA/TAA	44.95 ± 0.54	45.64 ± 0.06

EAA, essential amino acids; TAA, total amino acids.

^aEssential amino acids according to [14].

^bIndispensable amino acids according to [14].

*Significant differences between yellowfin tuna and bigeye tuna ($P < 0.05$, t-test).

Eighteen amino acids were identified (Table 2). The most predominant amino acids were glutamic acid, aspartic acid, and lysine (ranging from 7.93% to 12.45%). Of these amino acids, glutamic acid was the most predominant. Glutamic acid represented 12.45% and 11.28% of the amino acid content of the muscle tissues of yellowfin tuna and bigeye tuna, respectively. With the exception of glutamic acid, no significant differences between the two tuna species were found in the other amino acids ($P > 0.05$). The ratios (expressed as percentage) of the essential amino acids (EAAs) to the total amino acids (TAAs) in yellowfin tuna and bigeye tuna were 44.95% and 45.64%, respectively. As shown in Table 3, lysine was the EAA with the highest concentration in yellowfin tuna (92.30 mg/g protein) and bigeye tuna (88.79 mg/g protein). Tryptophan was the

EAA with the lowest concentration. The concentrations of the other EAAs were higher than the reference standard concentration. With the exception of tryptophan, all of the EAA scores were > 100 % (Table 4).

Table 3. Essential amino acid concentrations of yellowfin tuna and bigeye tuna compared to a FAO/WHO standard (mg/g protein)

Amino acid	FAO/WHO standard [14]	Yellowfin tuna	Bigeye tuna
Isoleucine	28	45.73	44.90
Leucine	66	78.83	76.76
Lysine	58	92.30	88.79
Methionine+ Cystine	25	33.72	36.39
Phenylalanine + Tyrosine	63	73.07	72.28
Threonine	34	43.42	41.93
Tryptophan	11	9.92	9.63
Valine	35	51.20	51.84
Histidine	19	61.91	58.84

Table 4. Essential amino acids score (%) for yellowfin tuna and bigeye tuna

Amino acid	Yellowfin tuna	Bigeye tuna
Isoleucine	163.31	160.36
Leucine	119.43	116.30
Lysine	159.14	153.09
Methionine + Cystine	134.88	145.56
Phenylalanine + Tyrosine	115.99	114.73
Threonine	127.70	123.34
Tryptophan	90.18	87.55
Valine	146.28	148.12
Histidine	325.85	309.69

Table 5. Fatty acid composition of the muscle tissue of yellowfin tuna and bigeye tuna (% of total fatty acids)

Fatty acid	Yellowfin tuna	Bigeye tuna
C14:0	1.30 ± 0.40	1.66 ± 0.02
C15:0	0.60 ± 0.13	0.73 ± 0.26
C16:0	26.18 ± 0.30*	24.55 ± 0.14
C17:0	1.47 ± 0.18	1.07 ± 0.20
C18:0	14.50 ± 0.08*	8.43 ± 0.40
C20:0	0.55 ± 0.07	0.37 ± 0.09
C22:0	0.41 ± 0.01*	0.20 ± 0.03
C16:1	3.08 ± 0.02	3.42 ± 0.37
C17:1	0.83 ± 0.23	0.92 ± 0.23
C18:1, n-9	20.32 ± 0.16	24.19 ± 1.24*
C20:1, n-9	1.30 ± 0.16	2.15 ± 0.13*
C22:1, n-9	0.55 ± 0.23	0.85 ± 0.42
C24:1, n-9	0.58 ± 0.11	0.90 ± 0.20
C18:2, n-6	1.31 ± 0.20	0.92 ± 0.08
C20:2, n-6	0.38 ± 0.04	0.49 ± 0.21
C20:3, n-3	1.10 ± 0.36	0.26 ± 0.06
C20:4, n-6 (ARA)	5.04 ± 1.05	4.03 ± 0.35
C20:5, n-3 (EPA)	2.39 ± 0.23	3.27 ± 0.28
C22:5, n-3	1.25 ± 0.35	1.07 ± 0.04
C22:6, n-3 (DHA)	16.91 ± 0.06	20.22 ± 0.89*
SAF	44.99 ± 0.89*	37.00 ± 0.02
MUFA	26.66 ± 0.13	32.40 ± 1.48*
PUFA	28.35 ± 1.02	30.26 ± 1.01
n-3 HUFA	21.64 ± 0.26	24.82 ± 1.07
n-3/n-6 ratio	3.29 ± 0.67	4.56 ± 0.24
PUFA/SAF ratio	0.64 ± 0.04	0.82 ± 0.03*

SAF, total saturated fatty acid; MUFA, total monounsaturated fatty acid; PUFA, total polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid.

*Significant differences between yellowfin tuna and bigeye tuna ($P < 0.05$, t-test).

The most abundant fatty acid in the two tuna species was C16:0 (26.18% in yellowfin tuna and 24.55% in bigeye tuna) (Table 5). The other major fatty acids were C18:1, C22:6 (DHA), and C18:0. The DHA, C18:1, and

total monounsaturated fatty acids (MUFA) concentrations in bigeye tuna were significantly higher than those present in yellowfin tuna ($P < 0.05$). However, yellowfin tuna had a higher concentration of saturated fatty acids (SAF). The total PUFA in yellowfin tuna and bigeye tuna were 28.35% and 30.26%, respectively. No significant differences in C20:4 (ARA) and C20:5 (EPA) were found between yellowfin tuna and bigeye tuna ($P > 0.05$). The n-3/n-6 ratios of yellowfin tuna and bigeye tuna were 3.29 and 4.56, respectively.

4. Discussion

In this study, the total fat contents in yellowfin tuna and bigeye tuna were 1.93% and 2.06% by weight, respectively, and the total moisture contents were 73.57% and 72.89% by weight, respectively. Previous study reported that low-fat fish have a higher moisture content [16]. Similar to the results obtained in our study. Both tuna fish are rich in protein (24%), with a higher protein content than other fish such as *Pampus punctatissimus* [5], and *Channa lucius* [4]. However, compare to the other tuna fish species, such as bluefin tuna *Thunnus orientalis*, the muscle protein contents of yellowfin tuna and bigeye tuna were slightly lower than that of bluefin tuna (26%) [17].

Amino acids are important components that play key roles in many healing processes; amino acid deficiencies will hinder many recovery processes. For example, glycine, a major component of human skin collagen, together with other amino acids (e.g., alanine, proline, arginine, serine, isoleucine, and phenylalanine) form polypeptides that promote re-growth and tissue healing [18]. Glutamic acid is essential for cell proliferation [5]. In this study, glutamic acid was the most predominant among all the amino acids in tuna fish. The two tuna species contain the amino acids required for wound healing. The ratios (expressed as percentage) of the essential amino acids (EAAs) to the total amino acids (TAAs) in yellowfin tuna and bigeye tuna were higher than the reported values for the requirements of different age human (i.e., 39% for infants, 26% for children, and 11% for adults) [14]. Additionally, the total EAA content of the tuna muscle tissue is much higher than that present in a reference protein. Thus, these two tuna fish are good sources of amino acids.

Lysine concentrations in tuna fish were much higher than the concentration in the reference protein (58 mg/g). Therefore, sulfur-containing EAA in tuna fish can supplement the lack of these amino acids in plant proteins. The concentrations of tryptophan in the two species were lower than the reference standard concentration. With the exception of tryptophan, all of the EAA scores were > 100 %. According to the amino acid scores (AAS), tryptophan would be the limiting amino acid in the two tuna species.

Fatty acids are important for human and animal health; some fatty acids are precursors to eicosanoids, which are important bioregulators in many cellular processes [19]. Fish is known to contain certain polyunsaturated fatty acids (PUFA) that regulate prostaglandin synthesis and induce wound healing [20]. In this study, the muscle tissues of the two tuna species were rich in DHA and

C20:5 (EPA). The DHA contents of yellowfin tuna and bigeye tuna were 16.91% and 20.22%, respectively, which was slightly less than that of bluefin tuna (23%) [17]. Previous studies have shown that the DHA content of the muscle tissue of widely migratory fish (e.g., tuna) is higher than non-migratory species [21]. DHA and EPA have been shown to protect against human coronary artery disease [22]. Yellowfin tuna and bigeye tuna also contained arachidonic acid (ARA; C20:4), a precursor to prostaglandins and thromboxanes [23].

The n-3 and n-6 PUFAs have been shown to have positive effects on cardiovascular diseases and certain types of cancer [24]. PUFA composition may vary among fish species, even among freshwater and saltwater fish [25]. The n-3/n-6 ratio is a useful indicator for comparing the relative nutritional values of different fish oils. It has been reported that human diets containing an n-3/n-6 ratio of 1 to 5 are healthy diets [16]. The two tuna species in this study had an n-3/n-6 ratio (3-5) within the recommended ratio. The PUFA/SAF ratios obtained in this study ranged between 0.64 and 0.82, revealing that yellowfin tuna and bigeye tuna are good sources of PUFAs. Previous study had shown that the PUFA/SAF ratio of bluefin tuna was about 1.00 [17], which was higher than those of yellowfin tuna and bigeye tuna.

Although our data for protein, amino acid, and fatty acid concentrations may depend on the season, geographical location, and environmental growth conditions, this study demonstrates that both yellowfin tuna and bigeye tuna are good sources of amino acids and fatty acids and the tryptophan would be the limiting amino acid in the two tuna species.

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