Free Amino Acids and Nucleotide-Related Compounds in Milkfish (*Chanos chanos*) Muscles and Viscera

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Free amino acids (FAAs) and nucleotide-related compounds in white muscle, dark muscle, and the viscera of milkfish (*Chanos chanos*) were analyzed by an automatic amino acid analyzer and high-performance liquid chromatography. Histidine was the most prominent FAA in milkfish white muscle, accounting for 63% of the total FAAs. In dark muscle, histidine accounted for 33%, and taurine, 38%. The most abundant FAA in the viscera was taurine followed by glutamic acid, alanine, and histidine. The viscera contained a much lower amount of histidine than both muscles. Inosine 5′-monophosphate (IMP) was the major nucleotide compound in white muscle and its level was about 3 times higher than that in dark muscle. The IMP level in the viscera was far less than those in muscles; however, the viscera contained more hypoxanthine and xanthine.

Keywords: *Milkfish; free amino acids; histidine; nucleotides; muscles; viscera*

INTRODUCTION

Milkfish is a major tropically/subtropically cultured fish. It is an important food fish in Southeast Asia, particularly in the Philippines, Indonesia, and Taiwan (Chen, 1990). Due to its palatability, milkfish is a valued species in Taiwan. The production of cultured milkfish in this island was about 67 000 metric tons in 1994 (TFB, 1995). It is consumed primarily fresh in accordance with consumer preference. In addition to muscle tissue, the viscera of milkfish are consumed as delicacies, particularly in the southern part of Taiwan. A small amount of fish is processed for canned and dried products.

Non-protein nitrogenous compounds, such as free amino acids (FAAs) and nucleotide-related compounds, have been widely used as indicators for evaluation of the taste of fish and shellfish (Konosu and Yamaguchi, 1982; Nishimura and Kato, 1988; Komata, 1990; Fuke, 1994). In addition to taste contribution, some specific compounds play important roles in physiological functions such as osmoregulation and buffering capacity in the tissues of aquatic animals (Schoffeniels and Gilles, 1972; Castellini and Somero, 1981; Suyama et al., 1986; Wright et al., 1986; Van Waarde, 1988). Many studies have been done on the constituents of non-protein nitrogenous compounds in the muscles of fish and shellfish, but little information has been reported on these compounds in fish viscera.

Milkfish contains a considerable amount of superficial dark muscle lying under the skin along the lateral line. The chemical composition of the dark muscle in many species of fish has been reported (Love, 1980; Suzuki et al., 1987; 1990), but information regarding the difference in chemical composition between dark and white muscles in milkfish is not available. The objective of this study is to establish basic data of proximate composition, FAAs, and nucleotide-related compounds in white muscle, dark muscle, and the viscera of milkfish.

MATERIALS AND METHODS

Samples. Fresh milkfish (*Chanos chanos*) were purchased from June to December 1994 from seafood markets in Keelung and Taipei, Taiwan. Six groups totaling 30 fish were collected with body weight and length ranging from 240 to 1363 g and 25 to 40 cm, respectively. The fish, still in the rigor mortis stage at the time of purchase, were stored on ice during transportation to the laboratory in Keelung. On arrival at the laboratory within 2 h after collection, at least two fish from each group were immediately decapitated and filleted. White muscle (ordinary muscle), dark muscle (red muscle), liver, intestines, kidney, and the mixture of the other parts of viscera were separated, weighed, and then combined and homogenized for chemical analyses.

Proximate Composition. Moisture, crude protein, lipid, and ash were determined according to the AOAC (1990) method. Protein was estimated from the total nitrogen multiplied by 6.25.

Free Amino Acids. The extract of FAAs was prepared according to the method described by Konosu et al. (1974). A 10 g tissue sample was homogenized in 20 mL of 7% cold trichloroacetic acid (TCA) using a Polytron homogenizer for 2 min. The homogenate was centrifuged at 4000*g* (4 °C) for 20 min. The precipitate was extracted twice by the same method. The supernatants were combined and made up to 100 mL with 7% TCA. A 20 mL TCA-extracted supernatant was mixed with an equal amount of ether to remove the TCA. This procedure was repeated successively 5 times for complete removal of TCA. The aqueous solution was evaporated to dryness in a vacuum evaporator at a temperature below 40 °C. The dried matter was diluted with water and made up to 25 mL for free amino acids analysis.

Free amino acids were separated by ion exchange chromatography and analyzed by a Hitachi L-8500 high-speed amino acid analyzer with a Hitachi 2622 SC packed column (4.6 mm × 60 mm). The buffers used were the standard lithium citrate buffers. Postcolumn derivatization with ninhydrin yielded amino acid derivatives which were measured the absorbance at 570 and 440 nm. Analytical conditions and guaranteed operations including reproducibility of peak area and separation rate were performed according to the manual provided by the manufacturer (Hitachi, Ltd., Tokyo, Japan). FAA levels were estimated on the basis of peak areas of known concentrations of the standards (Wako, Ltd., Osaka, Japan) by using a Hitachi D-2850 Chromato data processor. All analyses were done in six replicates.

Nucleotides and Related Compounds. A 5 g sample was homogenized in 25 mL of 6% cold perchloric acid (PCA) for 2 min, followed by centrifugation at 1600*g* (4 °C) for 20 min. The precipitate was extracted by the same method, and the procedure was repeated twice. The supernatants were combined and adjusted to pH 6.5 with KOH and then incubated

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Table 1. Weight Ratio (%)[a] and Proximate Composition (%)[b] of Various Tissues in Milkfish

tissue	ratio	moisture	crude fat	crude protein	ash
white muscle	43.97 ± 3.73	74.97 ± 2.74[b]	2.62 ± 0.52[d]	20.82 ± 1.62[a]	1.34 ± 0.13[a]
dark muscle	4.65 ± 0.94	65.39 ± 1.83[c]	14.59 ± 1.74[c]	18.76 ± 0.41[b]	1.18 ± 0.04[c]
liver	1.02 ± 0.23	63.98 ± 1.05[c]	20.56 ± 1.93[b]	13.45 ± 1.05[c]	1.28 ± 0.08[ab]
intestines	0.97 ± 0.19	76.05 ± 0.80[b]	4.55 ± 0.32[d]	18.71 ± 0.72[b]	1.25 ± 0.05[abc]
kidney	0.61 ± 0.12	82.66 ± 0.92[a]	2.34 ± 0.27[d]	13.19 ± 0.95[c]	1.20 ± 0.02[bc]
others[c]	1.79 ± 0.26	53.81 ± 6.36[d]	25.72 ± 4.07[a]	18.39 ± 1.12[b]	1.23 ± 0.08[bc]

[a] Expressed as mean ± standard deviation of 12 fish. [b] Expressed as mean ± standard deviation ($n = 6$). Means followed by the same letter within each column are not significantly different at $P = 0.05$. [c] Others: all viscera except liver, intestines, and kidney.

for 30 min at 0 °C to precipitate potassium perchlorate. After filtration, the supernatant was made up to 100 mL with PCA (pH 6.5) for nucleotide compound analysis. Nucleotides (adenosine 5′-triphosphate, ATP; adenosine 5′-diphosphate, ADP; adenosine 5′-monophosphate, AMP; inosine 5′-monophosphate, IMP; cytidine 5′-monophosphate, CMP; guanosine 5′-monophsophate, GMP; uridine 5′-monophosphate, UMP), nucleosides (inosine, adenosine, guanosine, cytidine, uridine), and bases (hypoxanthine, xanthine, guanine) were determined by high-performance liquid chromatography (HPLC, Shimadzu LC-10A) with some modifications of the method described by Suwetja et al. (1989). In brief, a 25 μL portion of the PCA extract, previously filtered through a 0.22 μm membrane, was injected into a Cosmosil packed column (4.6 mm × 250 mm). Two mobile phases used for the separation of nucleotide compounds consisted of eluent A, 50 mM KH_2PO_4−K_2HPO_4 (pH 6.5), and eluent B, a mixture of eluent A solution and methanol (9:1, v/v). Eluent A was applied for 14 min, followed by a linear gradient with an increase in eluent B up to 100% in 11 min and then by eluent B for 25 min. The flow rate was set to 0.7 mL/min, and the column temperature was held at 25 °C. The elute was monitored by UV absorption at 254 nm.

The nucleotide-related compounds were identified by comparing the retention times of peaks in HPLC between samples and authentic compounds (Sigma Chemical Co., St. Louis, MO). For quantitation, calibration curves were constructed by using the HPLC peak areas of authentic compounds in concentrations from 0.05 to 0.8 nmol. Various tissues each with six replicates were extracted and analyzed for nucleotide-related compounds.

Statistical Analyses. Data were analyzed using analysis of variance (ANOVA) through the General Linear Model Program (SAS, 1988). Duncan's multiple range test was applied to determine the significance of differences between means.

RESULTS AND DISCUSSION

Tissue Ratio. The tissue weight expressed as the percentage of whole fish weight is presented in Table 1. The ratios of muscles and viscera were 48.62 and 4.39%, respectively. Other tissues including the head, fins, scales, skin, gill, and bones together accounted for 46.99%. Fish muscle is comprised of both white and dark muscles. Dark muscle can normally be divided into two types, superficial and deep-seated. Only superficial dark muscle was found in milkfish. The proportion of dark muscle to white varies among species, increasing with the activity of the fish (Love, 1970). Pelagic fish usually contained more dark muscle than ground fish (Nonaka, 1987). The proportion of dark muscle to white in milkfish was 10.57%, which was lower than that in mackerel, anchovy, saury, and herring, but higher than that in Spanish mackerel, shark, and snakehead fish (Nonaka, 1987). Gordon and Hong (1986) indicated that the milkfish is a relatively short-distance migratory fish and probably capable of long-distance movement. A considerable amount of dark muscle might be associated with high activity in the milkfish.

Proximate Composition. The proximate composition of the tissues is shown in Table 1. White muscle contained higher amounts of moisture, protein, and ash than dark muscle ($p < 0.05$); however, the latter had about 5 times more lipids than the former. This result generally agreed with those previously reported for the muscles of migratory marine fish (Suzuki et al., 1987) and freshwater fish (Suzuki et al., 1990). According to the classification described by Stansby (1963), milkfish seemed to be a lean fish, that is, low in fat (<5%) from the viewpoint of white muscle. This lean fish showed little fluctuation in proximate composition of the white muscle through the whole year cycle (Chiou et al., 1995). There was a significant difference in proximate composition between muscles and viscera. The fat content in the viscera, with the exception of kidney, was significantly higher than that in white muscle. The high fat content in the viscera was assumed to be one of the reasons why the viscera were customarily consumed as delicacies in Taiwan.

Free Amino Acids. Table 2 shows the constituents of FAA (expressed as μmol/g of wet wt) of the various tissues in milkfish. There was no significant difference ($p > 0.05$) in the total FAA content among tissues except for intestines. The individual FAA level was different for each tissue. The predominant FAA in white muscle were histidine, taurine, and glycine, of which histidine accounted for 63% of the total FAAs. Dark muscle also contained a large amount of histidine and taurine, but the histidine level was only about half in white muscle. Taurine was the most dominant FAA in dark muscle and accounted for 38% of the total FAAs. In addition to histidine and taurine, the other FAAs were found only in small amounts in both muscles. As compared with the composition of FAAs in 60 species of fish and shellfish (Konosu and Yamaguchi, 1982), milkfish had a FAA pattern similar to migratory fish, such as mackerel, tuna, and skipjack, which also possessed a very high level of histidine. The living environment, swimming activity, and feeding diet for pond-reared milkfish are apparently different from those for migratory marine fish. The reason why milkfish muscle has a FAA profile similar to that of migratory fish is an interesting subject for future study.

Histidine has been reported to function as a chemical buffer in migratory red-fleshed fish muscle when fish move vigorously, resulting in accumulation of acidic end products during the period of anaerobic metabolism (Castellini and Somero, 1981; Suyama et al., 1986; Van Waarde, 1988). Milkfish is an active fish (Gordon and Hong, 1986), and the high level of histidine in its white muscle may play a role in buffering capacity. We found that the concentration of histidine in milkfish white muscle tended to increase with the animal size (data not shown). Larger fish might need more histidine for buffering function as the movement activity increased with the fish size. A subsequent study on the effect of growth on the contents of histidine and other FAAs will be conducted in this laboratory. Histidine can give rise to toxic levels of histamine under conditions leading to

Table 2. Free Amino Acids (μmol/g of wet wt)[a] of Various Tissues in Milkfish

	muscle		viscera			
	white	dark	liver	intestines	kidney	others[b]
phosphoserine	0.06 ± 0.01[c]	0.09 ± 0.03[c]	0.32 ± 0.13[b]	0.51 ± 0.26[ab]	0.54 ± 0.25[a]	0.35 ± 0.15[ab]
taurine	7.65 ± 4.78[b]	24.67 ± 7.88[a]	24.95 ± 2.27[a]	25.18 ± 4.36[a]	21.30 ± 4.19[a]	22.84 ± 2.02[a]
aspartic acid	0.21 ± 0.14[b]	0.15 ± 0.09[b]	2.48 ± 1.79[a]	3.52 ± 1.84[a]	2.64 ± 1.14[a]	2.20 ± 1.17[a]
threonine	0.87 ± 0.25[c]	0.76 ± 0.34[c]	2.47 ± 1.36[b]	4.09 ± 1.92[a]	2.76 ± 1.31[ab]	2.26 ± 1.33[bc]
serine	0.78 ± 0.32[c]	0.77 ± 0.41[c]	3.16 ± 2.06[b]	5.41 ± 2.29[a]	3.44 ± 1.62[b]	3.12 ± 1.96[b]
asparagine	0.48 ± 0.10[b]	0.37 ± 0.12[b]	0.50 ± 0.09[b]	3.20 ± 1.36[a]	0.92 ± 0.81[b]	0.99 ± 0.86[b]
glutamic acid	1.20 ± 0.51[c]	1.29 ± 0.44[c]	5.88 ± 1.77[b]	8.20 ± 3.31[a]	5.05 ± 1.50[b]	4.91 ± 2.24[b]
glutamine	1.13 ± 0.10[e]	3.36 ± 0.45[c]	4.58 ± 0.34[a]	3.95 ± 0.13[b]	2.93 ± 0.10[d]	4.52 ± 0.25[a]
proline	1.01 ± 0.73[c]	0.67 ± 0.52[bc]	2.20 ± 1.39[abc]	3.76 ± 1.82[a]	2.59 ± 1.35[ab]	2.23 ± 1.35[abc]
glycine	6.67 ± 4.25[a]	4.34 ± 3.51[a]	3.19 ± 2.80[a]	7.18 ± 3.44[a]	6.50 ± 5.41[a]	4.40 ± 2.64[a]
alanine	2.33 ± 0.88[d]	3.20 ± 0.27[cd]	7.12 ± 2.07[ab]	8.45 ± 3.72[a]	5.84 ± 2.42[abc]	5.37 ± 2.34[bc]
valine	0.58 ± 0.15[c]	0.54 ± 0.15[c]	2.49 ± 1.72[b]	4.34 ± 2.17[a]	2.53 ± 1.18[b]	2.37 ± 1.47[b]
methionine	0.12 ± 0.06[c]	0.11 ± 0.04[c]	0.98 ± 0.78[b]	1.90 ± 0.96[a]	1.16 ± 0.45[ab]	1.13 ± 0.76[ab]
isoleucine	0.23 ± 0.10[c]	0.24 ± 0.10[c]	1.59 ± 1.24[b]	3.09 ± 1.72[a]	1.96 ± 1.01[ab]	1.70 ± 1.17[b]
leucine	0.36 ± 0.16[c]	0.42 ± 0.17[c]	3.31 ± 2.58[b]	6.03 ± 3.34[a]	4.18 ± 1.95[ab]	3.34 ± 2.29[b]
tyrosine	0.18 ± 0.07[c]	0.18 ± 0.08[c]	1.24 ± 0.98[bc]	2.73 ± 1.56[a]	1.61 ± 0.80[b]	1.36 ± 0.97[b]
phenylalanine	0.11 ± 0.05[c]	0.15 ± 0.07[c]	1.27 ± 1.04[b]	2.40 ± 1.34[a]	1.53 ± 0.77[ab]	1.34 ± 0.97[b]
β-alanine	0.07 ± 0.02[d]	0.08 ± 0.02[d]	0.60 ± 0.14[a]	0.50 ± 0.28[ab]	0.31 ± 0.23[bc]	0.27 ± 0.16[cd]
ornithine	0.17 ± 0.09[b]	0.11 ± 0.04[b]	1.67 ± 1.20[a]	0.37 ± 0.27[b]	0.52 ± 0.30[b]	0.47 ± 0.34[b]
lysine	2.33 ± 1.47[b]	1.90 ± 1.02[b]	3.39 ± 2.11[ab]	6.13 ± 3.30[a]	5.18 ± 2.56[a]	3.35 ± 2.13[ab]
histidine	45.49 ± 14.20[a]	21.17 ± 6.52[b]	3.33 ± 1.96[c]	5.49 ± 3.00[c]	12.09 ± 6.71[c]	3.10 ± 1.82[c]
arginine	0.48 ± 0.36[d]	0.32 ± 0.18[d]	0.79 ± 0.60[cd]	4.24 ± 2.27[a]	2.82 ± 1.41[ab]	2.16 ± 1.82[bc]
total	72.51 ± 5.55[b]	64.92 ± 4.74[b]	77.50 ± 22.51[b]	110.67 ± 36.60[a]	88.41 ± 27.43[ab]	62.87 ± 32.04[b]

[a] Expressed as mean ± standard deviation ($n = 6$). Means followed by the same letter within each row are not significantly different at $P = 0.05$. [b] Others: see Table 1.

Table 3. Nucleotides and Related Compounds (μmol/g of wet wt)[a] of Various Tissues in Milkfish

	muscle		viscera			
	white	dark	liver	intestines	kidney	others[b]
CMP	–[c]	–	0.16 ± 0.16	0.15 ± 0.07	0.06 ± 0.09	0.10 ± 0.05
UMP	0.04 ± 0.03	–	0.35 ± 0.33	0.45 ± 0.43	0.07 ± 0.06	0.24 ± 0.37
GMP	0.09 ± 0.05[bc]	0.04 ± 0.02[c]	0.29 ± 0.24[abc]	0.55 ± 0.38[a]	0.35 ± 0.12[ab]	0.33 ± 0.30[ab]
IMP	11.28 ± 3.07[a]	3.85 ± 0.86[b]	0.39 ± 0.18[c]	0.58 ± 0.46[c]	1.37 ± 0.92[c]	0.61 ± 0.34[c]
ATP	0.11 ± 0.05	0.07 ± 0.03	–	–	0.07 ± 0.05	0.05 ± 0.04
ADP	0.27 ± 0.07[a]	0.18 ± 0.06[abc]	0.13 ± 0.12[bc]	0.24 ± 0.17[ab]	0.09 ± 0.05[c]	0.16 ± 0.11[abc]
cytidine	–	–	–	0.27 ± 0.19	0.06 ± 0.04	0.12 ± 0.11
guanine	–	–	0.48 ± 0.72	0.94 ± 0.98	0.23 ± 0.30	0.54 ± 0.68
hypoxanthine	0.54 ± 0.68[b]	1.03 ± 0.46[ab]	1.15 ± 0.35[ab]	1.57 ± 0.67[a]	1.63 ± 0.79[a]	1.14 ± 0.68[ab]
xanthine	0.03 ± 0.02[c]	0.07 ± 0.03[bc]	2.33 ± 0.89[a]	1.72 ± 0.94[a]	0.75 ± 0.45[b]	0.52 ± 0.36[b]
AMP	0.15 ± 0.07[a]	0.09 ± 0.03[ab]	0.07 ± 0.05[ab]	0.05 ± 0.03[b]	0.04 ± 0.03[b]	0.09 ± 0.06[ab]
uridine	–	–	0.02 ± 0.05	0.05 ± 0.04	0.04 ± 0.03	0.02 ± 0.03
inosine	1.24 ± 0.96[ab]	1.90 ± 0.60[a]	0.42 ± 0.19[c]	1.05 ± 0.50[bc]	0.83 ± 0.15[bc]	1.11 ± 0.67[bc]
guanosine	0.02 ± 0.01[c]	0.05 ± 0.02[bc]	0.10 ± 0.09[ab]	0.16 ± 0.05[a]	0.10 ± 0.09[ab]	0.15 ± 0.05[a]
total	13.77 ± 2.54[a]	7.30 ± 1.05[b]	5.93 ± 1.87[b]	7.98 ± 4.61[b]	5.65 ± 1.87[b]	5.17 ± 3.47[b]

[a] Expressed as mean ± standard deviation ($n = 6$). Means followed by the same letter within each row are not significantly different at $P = 0.05$. [b] Others: see Table 1. [c] –, undetectable or trace (<0.01 μmol/g).

histidine decarboxylation (Russell and Maretic, 1986; Taylor, 1989). Because the formation of histamine in milkfish relates to food safety, this demands further investigation.

The FAA constituents of the viscera differed from those in the two types of muscles. All the viscera characteristically contained a much lower level of histidine than muscles. However, the taurine levels in the viscera, ranging from 21 to 25 μmol/g, were significantly higher than the 8 μmol/g found in white muscle. In addition to histidine and taurine, the viscera contained considerable amounts of other FAAs, significantly higher than those in muscles.

Nucleotides and Related Compounds. Results of the determination of 14 nucleotides and related compounds in the various tissues of milkfish are shown in Table 3. The total content of these compounds in white muscle was significantly higher than that in dark muscle and the viscera ($P < 0.05$). IMP, inosine, and hypoxanthine were the major compounds in white muscle, of which IMP was the most prominent one and accounted for 82% of the total content. The amounts of ATP, ADP, and AMP were relatively small. The very low concentration of ATP and the high concentration of IMP indicated that the breakdown of ATP to IMP proceeded rapidly in milkfish muscle. Fish allowed to struggle prior to death undergo a faster rate of ATP depletion and a corresponding shorter period for onset of rigor (Hultin, 1985). The average rate of ATP decline during ice storage was about 6 times faster for sturgeon allowed to struggle than for anesthetized fish (Izquierdo-Pulido et al., 1992). The IMP/ATP ratio in stressed blue tilapia was distinctly higher than that in the non-stressed fish (Korhonen et al., 1990). Milkfish is an active fish (Gordon and Hong, 1986). The harvesting process might frighten and exhaust the fish, thus resulting in a rapid depletion of ATP and accumulation of IMP.

IMP was also the major nucleotide compound in dark muscle, but its level was only about one-third of that in white muscle. The levels of inosine and hypoxanthine in dark muscle were higher than those in white muscle. In addition to IMP, inosine, and hypoxanthine, the other compounds in both white and dark muscles were

detected in small amounts. The profile of nucleotide-related compounds in milkfish and its difference in both white and dark muscles were in agreement with those in migratory marine fish (Suzuki et al., 1987) and freshwater fish (Suzuki et al., 1990).

The profiles of the nucleotides and related compounds in the visceral tissues were similar, but they were significantly different from those in both muscles. The IMP levels in the viscera were far less than those in muscles; however, the viscera contained more hypoxanthine and xanthine. Hypoxanthine and xanthine were the products in the progress of IMP breakdown (Jones and Murray, 1964; Surette et al., 1988; Mulchandani et al., 1990). Dephosphorylation of IMP to inosine in fish muscle by enzyme 5′-nucleotidase was found to be correlated with lowered fish quality (Surette et al., 1988; Marseno et al., 1992). The accumulation of hypoxanthine and xanthine in the viscera indicated that the viscera might possess high activities of 5′-nucleotidase resulting in a rapid degradation of IMP.

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