Extractive Nitrogenous Components of Cultured Milkfish and Tilapia

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The nitrogenous components extracted from the homogeneous fillets of cultured milkfish and tilapia were analyzed and compared. No difference in the profile of extractive components between dorsal and ventral part of milkfish was found, but the latter contained a higher quantity of these compounds than the former. There was a significant difference in the profile of free amino acids (FAA) between milkfish and tilapia. Histidine was the most prominent and accounted for about 80% of the total FAA in milkfish. On the other hand, tilapia was rich in taurine and glycine, but contained a small amount of histidine. A similar profile of nucleotides and related components was found in both fish, and IMP was the most abundant compound. Both fish also contained a significant amount of creatine and certain levels of quaternary ammonium bases, such as trimethylamine oxide (TMAO), glycinebetaine and trigonelline. In general, milkfish extracts contained more FAA, creatinine and trigonelline, but had less nucleotides, creatine, TMAO, TMA and glycinebetaine than those of tilapia. The pattern of the extractive components of milkfish was found similar to that of red-fleshed fish, however, tilapia seemed to be analogous to white-fleshed fish.

Milkfish and tilapia are two major tropically/subtropically cultured fish. Tilapia is the most highly produced aquacultural species in the world and milkfish is widely cultured in Southeast Asia. In Taiwan alone the production of milkfish and tilapia was 40,000 and 55,000 metric tons, respectively, in 1988.¹⁾ In this island, both fish are intensively cultured and fed with artificial diets. Mostly, tilapia is reared in fresh water and milkfish in brackish water.

Due to its delicious and unique taste, milkfish is a highly demanded species. In particular, its ventral meat is more palatable than other parts of the fish. However, tilapia is considered a low grade food fish. The market price of milkfish is 3 to 5 times as high as tilapia. Milkfish is consumed primarily fresh in accordance with consumer preference. So far, the percentage of canned and dried milkfish production is relatively low. Some are used as bait for tuna fishery. Like milkfish, tilapia is consumed primarily fresh.

The extractive nitrogenous components, such as free amino acids, nucleotides, quaternary ammonium bases, etc., have been widely used as indicators to evaluate the taste of fish and shellfish.²⁻⁴⁾ Little information has been reported

on the extractive components of cultured milkfish and tilapia. It is an interesting subject to investigate what chemical composition may possibly be associated with the taste preferences of cultured fish. This study was undertaken to analyze the extractive nitrogenous components and the differences between two major cultured fish, milkfish and tilapia in Taiwan.

Materials and Methods

Samples

Milkfish *Chanos chanos* and hybridized tilapia *Oreochromis aureus* × *O. niloticus* were purchased in February and March 1988 from a local seafood market in Keelung, Taiwan. Tilapias obtained were monocultured all-male fish, but the sex of milkfish could not be decided. Milkfish were in the stage of rigor mortis and tilapias were still alive at the time of purchase. Their body weight and length were 610-820 g (average 680 g) and 20-26 cm (average 24 cm) for milkfish, and 480-670 g (average 620 g) and 13-17 cm (average 15 cm) for tilapia. On arrival at the laboratory, within 1 h after purchase, the fish were immediately decapitated and filleted. Milkfish fillets were then

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divided into dorsal and ventral parts across the lateral line. Three different samples were homogenized separately for extraction.

Preparation of Extracts

Extraction of homogenized fish flesh was carried out with 80% ethanol by the method described by Konosu *et al.*⁵⁾ with the exception that nucleotides and related compounds were determined using a 5% perchloric acid extraction according to the method of Nakajima *et al.*⁶⁾

Chemical Analyses

1. Moisture, lipid and ash were determined as usual. Protein was estimated from the total nitrogen by multiplying 6.25. The total nitrogen was measured by the micro-Kjeldahl method. All analyses were performed in triplicate.

2. Free amino acids were analyzed using a LKB 4150 automatic amino acid analyzer.

3. Nucleotides and related compounds were determined by high performance liquid chromatography (HPLC) according to the analytical procedure described by Chiou and Konosu.⁷⁾

4. Trimethylamine (TMA) was determined by the colorimetric method of Bullard and Collins.⁸⁾ Trimethylamine oxide (TMAO) was measured in the same way as TMA after reduction with titanous chloride by the method of Bystedt *et al.*⁹⁾

5. Creatine and creatinine were analyzed by the colorimetric method described by Niiyama¹⁰⁾ and Yatzidis.¹¹⁾

6. Quaternary ammonium bases including glycinebetaine, homarine and trigonelline were analyzed by HPLC with a little modification of the method of Gorham.¹²⁾ In brief, the extract was previously passed through a glass column packed with Amberlite CG-400 (chloride form) and Amberlite CG-50 (hydrogen form). The column was eluted with water and the unadsorbed fraction was collected, evaporated and dissolved in the mobile phase. The chromatographic analysis was performed on a Whatman Partisil 10-SCX (4.6 mm i.d. $\times$ 25 cm) using 50 mM KH_2PO_4 containing 1.5% (v/v) of methanol as eluent. The flow rate was 1 ml/min and the detection was monitored at 205 nm and 270 nm.

Results

Proximate Composition

The proximate compositions of the flesh of milkfish and tilapia are listed in Table 1. As for

Table 1. Proximate composition of milkfish and tilapia and tilapia

	Milkfish		Tilapia
	Dorsal meat	Ventral meat	
Moisture	77.0	76.6	77.8
Protein	18.4	18.0	17.9
Lipid	2.5	3.8	1.9
Ash	1.1	1.0	0.9

Table 2. Free amino acids of milkfish and tilapia (mg/100 g)

	Milkfish		Tilapia
	Dorsal meat	Ventral meat	
Taurine	39	44	207
Aspartic acid	1	2	4
Threonine	5	8	9
Serine	3	5	1
Asparagine	—	4	14
Glutamic acid	5	5	7
Glutamine	4	8	11
Proline	2	3	6
Glycine	23	34	104
Alanine	15	21	21
Valine	2	2	—
Methionine	—	—	—
Isoleucine	1	4	—
Leucine	2	4	—
Tyrosine	1	4	—
Phenylalanine	tr	—	—
Tryptophan	—	—	—
Lysine	9	17	10
Histidine	441	534	18
Arginine	—	—	—
Total	554	699	412
Ammonia	5	8	4

—, not detected; tr, trace.

milkfish, the ventral meat contained higher amount of lipid, and had lower amounts of moisture and protein than those of its dorsal meat. In tilapia, the content of moisture was higher, but protein and lipid were lower when compared to those of milkfish. The differences in the proximate composition between the two species, however, were not substantial.

Free Amino Acids

The composition of free amino acids (FAA) in the ethanol extracts from the flesh of milkfish and tilapia is shown in Table 2. The total content of FAA in milkfish samples was higher than that in

tilapias (Unless otherwise stated, the amounts of extractive nitrogenous components were expressed in terms of mg in 100 g meat). Regardless of similar FAA profile, the total amount of FAA in ventral meat was 699 mg which indicated a 26% more FAA than 554 mg found in dorsal meat of milkfish. Histidine, taurine, glycine, alanine and lysine were the predominant FAA, of which histidine was the most prominent one and accounted for about 80% of the total FAA in dorsal meat, and 76% in ventral meat, respectively. As compared with the composition of FAA in the extracts from 60 species of commercially important fish and shellfish,²⁾ milkfish had a similar FAA pattern to migratory fish, such as mackerel, tuna and skipjack, which also possessed a very high level of histidine. The second most abundant FAA found in the muscle of milkfish was taurine, which amounted to 7 and 6% of the total FAA in dorsal and ventral meat, respectively. The other FAA were found only in small amounts.

There were differences in FAA composition between the extracts from milkfish and tilapia. The total content of FAA in tilapia samples was 412 mg, which was lower than that of milkfish. The predominant FAA of tilapia were taurine, glycine, alanine, histidine and asparagine. Taurine and glycine accounted for about 50% and 25% of the total FAA, respectively, which were much higher than those in milkfish. The histidine level (18 mg), however, was much lower than 441 mg found in milkfish. Asparagine was a major amino acid in tilapia, while this amino acid was detected only in a very small amount in milkfish. Unlike milkfish, tilapia had a FAA profile similar to white-fleshed fish, such as flounder, sea bream and rockfish; they were rich in taurine, glycine and alanine.^{2,13)}

Nucleotides and Related Compounds

The levels of 14 nucleotides and related compounds in the perchloric acid extracts are shown in Table 3. IMP, inosine, adenine and hypoxanthine were the common compounds found in milkfish samples. The total content of these compounds in the dorsal meat of milkfish was about half of that in ventral meat, but both had a similar profile of these compounds. IMP was the most predominant compound which accounted for 88% and 92% of the total compounds in dorsal and ventral meat, respectively. The other nucleotides and related compounds were detected only in a very small amount. The profile of these

Table 3. Nucleotides and related compounds of milkfish and tilapia

	(mg/100 g)		
	Milkfish		Tilapia
	Dorsal meat	Ventral meat	
ATP	—	—	3
ADP	—	—	—
AMP	+	+	6
IMP	36	75	109
CMP	+	+	1
GMP	+	+	2
UMP	—	—	—
Isnosine	3	4	8
Adenosine	—	—	+
Guanosine	+	+	1
Cytidine	—	—	—
Hypoxanthine	+	1	1
Adenine	1	1	1
Cytosine	tr	+	—
Total	41	82	132

—, not detected; tr, trace; +, less than 0.4 mg/100 g.

Table 4. Other nitrogenous components of milkfish and tilapia

	(mg/100 g)		
	Milkfish		Tilapia
	Dorsal meat	Ventral meat	
Creatine	104	154	192
Creatinine	3	2	—
TMAO	2	11	54
TMA	+	tr	3
Glycinebetaine	13	15	28
Homarine	+	+	—
Trigonelline	10	11	7
Total	132	193	284

—, not detected; tr, trace; +, less than 0.4 mg/100 g.

compounds in tilapia was similar to that of milkfish. However, tilapia had for greater levels of nucleotides and related compounds. IMP (109 mg) was also the main compound found in tilapia samples. The other compounds were detected in small amounts; and their levels were all less than 9 mg.

Other Nitrogenous Constituents

As shown in Table 4, creatine was another important extractive component with 104 mg and 154 mg in dorsal and ventral meat of milkfish as well as 192 mg in tilapia samples. Creatinine was present in a very low level in all samples. These findings agreed with those of many other

species of fish which contained high levels of creatine and small amounts of creatinine.²⁾ Quaternary ammonium bases, trimethylamine oxide (TMAO), TMA, glycinebetaine and trigonelline found at certain levels in both fish (Table 4). Only a very small amount of homarine was found in milkfish samples. In tilapia, the levels of TMAO and glycinebetaine were higher, but trigonelline was less than those of milkfish. Homarine could not be found in the tilapia sample.

Discussion

There were differences between the extractive components prepared from milkfish and tilapia. Milkfish extracts contained more free amino acids, creatinine and trigonelline, but had less nucleotides, creatine, TMAO, TMA and glycinebetaine than those of tilapia. These differences might be related to the flavor and physiology of both fish.

The total of extractive nitrogenous components amounted to 727 mg, 974 mg and 828 mg in the extract of dorsal and ventral meat of milkfish, and tilapia, respectively. Although milkfish was considered more palatable, the taste of the samples was difficult to be compared by the total amount of extractive components without organoleptic tests of reconstructed extract, using an omission test method. With respect to individual compounds, histidine was the most significantly different compound between these two fish. The fact that free histidine contributed to the taste of fish has been reported by Simidu *et al.*¹⁴⁾ and Endo *et al.*¹⁵⁾ However, Konosu *et al.*¹⁶⁾ and Hughes¹⁷⁾ indicated that it did not contribute appreciably to the taste. The role of histidine in making up the taste of milkfish is interesting and should be a subject for future study. Some nucleotides, such as IMP and GMP are now well known as the taste-active components.^{2,3)} The high level of IMP in milkfish and tilapia was very specific and contribute to the taste of these two fish.

As mentioned above, milkfish had a similar FAA pattern to red-fleshed migratory fish. There was no obvious relationship between the behavior of fish and the profile of the extracted components, but the active red-fleshed fish usually contained a very high level of histidine and low level of taurine as compared with white-fleshed fish.^{2,13,18)} It has been indicated by Gordon and Hong¹⁸⁾ that milkfish was a relatively short-distance migratory fish and probably carried out long-distance movement. Milkfish seemed to be an analogue of

migratory fish.

On the contrary to milkfish, tilapia possessed a similar FAA pattern to that of white-fleshed fish. The high content of taurine and TMAO in tilapia was significantly different from those of milkfish. Generally, the contents of both compounds found in white-fleshed fish were also higher than those in red-fleshed fish.^{2,13,18)} The contribution of these two compounds to the seafood taste was not clear, but they have been reported functioning for osmoregulation in aquatic animals.^{2,20)} Tilapias were euryhaline and were able to survive, grow and some species even reproduce in sea water up to 4% salinity.²¹⁾ Taurine and TMAO might be related to the wide tolerance of salinity and play an important role of osmoregulation for tilapias.

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