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Studies on extractive components of *Channa argus*

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Abstract: In this study, ATP and its related compounds, free amino acids, polyamines and monoamines, glycogen and glycolytic intermediates and organic acid were analyzed in the dorsal, ventral and caudal muscles of snakehead *Channa argus* together with the liver and gonad immediately after death. Total levels of ATP and its related compounds in the muscle ranged from $7.5 \mu\text{mol} \cdot \text{g}^{-1}$ to $8.0 \mu\text{mol} \cdot \text{g}^{-1}$. ATP concentrations were 3.9, 4.1 and $4.7 \mu\text{mol} \cdot \text{g}^{-1}$ in the dorsal, ventral and caudal muscles, respectively. ATP level was high (63%) in the caudal muscle which moves hard. Small amounts of adenosine was detected in the liver together with inosinic acid. It can be considered that there exist two metabolic pathways of ATP degradation in the liver of snakehead. The total amounts of free amino acids were 436.0, 405.0 and $356.3 \text{ mg} \cdot (100 \text{ g})^{-1}$ in the dorsal, ventral and caudal muscles. Taurine and glycine were major free amino acids and accounted for 68% to 73%. Alanine and glutamic acids were detected in fairly large amounts. Spermine and spermidine were detected in fairly high amounts in the muscle. In the liver and gonad putrescine was detected in the highest amounts, followed by spermidine and spermine. Immediately after death glycogen level was about 0.5% and fairly large amounts of glucose and glucose-6-phosphate, and large amounts of lactic acid were detected.

Key words: *Channa argus*; extractive components; ATP and related compounds; free amino acids; polyamines

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1 Introduction

In China, freshwater fishes have been regarded as important. In recent years, the cultivation of freshwater fishes plays an extremely important role in fisheries in China. According to the statistics in 2002, the total production of fishery of China amounting to about 46 million tons is by far the highest in the world, and it is the characteristics of Chinese fishery in that the production of cultured fishes and shellfishes is very high (19 million tons), compared with other countries.

Among edible freshwater fishes in China, snakehead *Channa argus* is one of the most famous fishes. There is the highest production of snakehead along the Yangtze River and especially snakehead is an economically important freshwater fish in the middle and lower reaches of Yangtze. The meat of snakehead is delicious and is consumed mainly by home cooking.

It has been said that to eat snakehead meat hastens the recovery of the wound after an operation; women take this fish after childbirth. Recently a nutritional value has been reconsidered.

Snakehead is a carnivorous fish and grows up fast. Snakehead lives in lake, marsh and river all the year round, and passes the winter at the bottom of the rivers or the deep water places.

There have been many reports on the extractive components in the muscle of marine fishes, but little information has been available for those in the muscle of freshwater fishes.

In Japan, Suyama and Hirano^[1-3] investigated the quality of wild and cultured ayu *Plecoglossus altivelis*. Kojima and Sato^[4] examined fatty acids composition in the muscle of fishes which belong to *Cyprinidae* family. Yamanaka *et al.*^[5] reported the concentrations of polyamines in several freshwater fishes. In China, Chen *et al.*^[6] reported the

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composition and seasonal variation of free amino acids in the meat of some cultured freshwater fishes. Deng and Sato^[7] reported the relation between freshness and changes in ATP related compounds of silver carp meat during storage at different temperatures.

There have been no studies on extractive components of snakehead. In this study, ATP and its related components, free amino acids, polyamines and monoamines, glycogen and glycolytic intermediates, and organic acids were analyzed in the dorsal, ventral and caudal muscles together with the liver and the gonad immediately after death.

2 Materials and Methods

2.1 Samples

Three live specimens of snakehead *Channa argus* cultured in Suzhou were transported to laboratory in August. In Tab. 1 are shown total length and body weight of three live specimens of snakehead. After being spiced at the apinal bulb, each 2.5 g of dorsal, ventral and caudal muscles, liver, and gonad was weighed, homogenized by polytron (PT 10-35 Kinematica) in 20 mL of ice-cold 6% perchloric acid, and centrifuged at $18000 \times g$ for 10 min. Then the precipitation was homogenized again in 6% perchloric acid and centrifuged. These two supernatants were combined and neutralized with potassium hydroxide. After centrifugation, the supernatant was made up to 25 mL with distilled water. The extracts were frozen at $-30\text{ }^{\circ}\text{C}$ and ready for analysis. Analytical results were shown in the mean values of three specimens.

Tab. 1 Total length and body weight of *Channa argus* specimens

specimen no.	total length (cm)	body weight (g)
1	45.0	740
2	40.0	560
3	36.0	450
mean	40.3	583

2.2 Determination of ATP and its related compounds

ATP and its related compounds were determined by high performance liquid chromatography (HPLC)

on an Asahipak GS-320 HQ column with $200\text{ mmol}\cdot\text{L}^{-1}$ sodium dihydrogen phosphate (pH 2.9), and absorbance of the eluate was measured at 260 nm, as reported by Matsumoto and Yamanaka^[8].

2.3 Determination of free amino acids

Free amino acids were analyzed by HPLC on a strong cation exchange Shim-pack ISO-07/S1504 Li column, according to the method reported by Matsumoto and Yamanaka^[9].

2.4 Determination of polyamines and monoamines

Tyramine (Tym), putrescine (Put), cadaverine (Cad), histamine (Him), agmatine (Agm), tryptamine (Tpm), spermidine (Spd) and spermine (Spn) were analyzed by HPLC on a reverse phase Shim-pack CLG-ODS column, as reported by Yamanaka and Matsumoto^[10].

2.5 Determination of glycogen and free glucose

According to the enzymatic method of Keppler and Decker^[11], free glucose was determined with hexokinase and glucose-6-phosphate dehydrogenase on the basis of the increment in optical density of NADPH at 340 nm. Glycogen was hydrolyzed with amyloglucosidase and the resultant glucose was determined in the same manner.

2.6 Determination of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P)

G6P and F6P were determined by the method of Racker^[12] with G6P dehydrogenase on the basis of the increment in optical density of NADPH at 340 nm. F6P was converted to G6P with glucosephosphate isomerase and determined as stated above.

2.7 Determination of organic acids

Citric, malic, succinic, lactic and acetic acids were determined by HPLC on Shim-pack SCR-102H column as reported by Wongso and Yamanaka^[13].

3 Results

3.1 ATP and its related compounds

Contents of ATP and its related compounds in the dorsal muscle, ventral muscle, caudal muscle, liver and gonad are shown in Fig. 1. Total levels of ATP and its related compounds in the muscle ranged from $7.5\text{ }\mu\text{mol}\cdot\text{g}^{-1}$ to $8.0\text{ }\mu\text{mol}\cdot\text{g}^{-1}$.

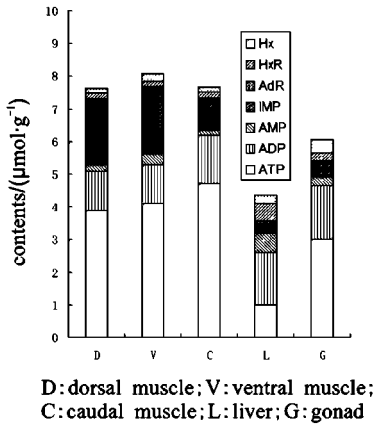


Fig. 1 Contents of ATP and its related compounds in *Channa argus*

Immediately after death ATP concentrations were 3.9, 4.1 and 4.7 $\mu\text{mol}\cdot\text{g}^{-1}$ in the dorsal, ventral and caudal muscles, respectively, and exceeded 50% of total concentrations of ATP and its related compounds. ATP level was high (63%) in the caudal muscle. IMP in the dorsal and ventral muscles was detected as the second high level, being 1.9 and 2.1 $\mu\text{mol}\cdot\text{g}^{-1}$. On the other hand, in the caudal muscle ADP level was the second high which was 1.5 $\mu\text{mol}\cdot\text{g}^{-1}$, while IMP was detected at the low level (0.8 $\mu\text{mol}\cdot\text{g}^{-1}$). Immediately after death, in the liver and gonad the total amounts of ATP and its related compounds were 4.3 and 6.0 $\mu\text{mol}\cdot\text{g}^{-1}$ which were lower than those in the muscle. In the gonad ATP concentration was 50%, followed by ADP (28%), but in the liver ATP was detected at the low level (28%) and ADP was highest (36%). It was very interesting that adenosine (AdR) was detected in a small amount in liver.

3.2 Free amino acids

In Tab. 2 are shown the contents of free amino acids in the parts of the muscle of snakehead immediately after death.

The total amounts of free amino acids were 436.2, 405.0 and 356.3 $\text{mg}\cdot(100\text{g})^{-1}$ in the dorsal, ventral and caudal muscles, respectively. The total amount in the caudal muscle was a little lower than the others. Taurine (Tau) and glycine (Gly) were major free amino acids and accounted for 68% to

73%. Alanine (Ala) and glutamic acid (Glu) were detected in fairly large amounts. The contents of free amino acids in the liver and gonad of snakehead are shown in Tab. 3. The total amounts in the gonad were a little higher and those in the liver were a little lower than those in the caudal muscle. The composition of free amino acids was considerably different from that of muscle. Tau was dominant in the liver and gonad as well as in the muscle, but Glu was the second high. In the liver Ala and lysine (Lys) were detected in large amount. In the gonad phosphoethanolamine was specifically detected in large amounts, followed by proline, Gly and Lys.

Tab. 2 Contents of free amino acids in the muscle of *Channa argus*

amino acids	$\text{mg}\cdot(100\text{g})^{-1}$		
	dorsal muscle	ventral muscle	caudal muscle
taurine	231.1	217.7	162.9
aspartic acid	1.5	1.5	1.6
hydroxyproline	2.3	1.8	2.0
threonine	7.2	6.8	7.7
serine	5.4	5.0	5.0
glutamic acid	10.7	9.8	12.0
α -amino adipic acid	3.7	1.7	1.7
proline	5.7	5.0	5.0
glycine	87.2	77.4	79.4
alanine	21.9	21.8	19.5
citrulline	1.0	0.9	1.1
α -aminobutyric acid	0.4	0.4	0.4
valine	6.8	6.4	6.2
methionine	4.7	5.7	5.6
isoleucine	5.8	5.5	5.2
leucine	9.4	9.0	8.7
cystathionine	0.8	0.5	—
tyrosine	4.2	3.4	3.4
phenylalanine	5.1	4.5	4.3
β -alanine	0.4	0.3	0.4
β -aminoisobutyric acid	0.3	0.2	0.4
γ -aminobutyric acid	0.1	0.1	0.1
histidine	5.1	5.3	7.5
camosine	0.6	0.5	—
omithine	1.4	1.4	1.6
ammonia	1.6	1.1	1.3
lysine	8.7	8.3	9.5
arginine	3.1	3.6	3.8
total amount	436.2	405.0	356.3

Notes: "—" means not detected

Tab. 3 Contents of free amino acids in the liver and gonad of *Channa argus*

amino acids	mg ^a (100 g) ⁻¹	
	liver	gonad
taurine	176.3	186.1
phosphoethanolamine	4.6	28.8
aspartic acid	9.6	6.7
threonine	7.4	5.3
serine	3.7	2.3
glutamic acid	21.7	31.2
α-amino adipic acid	3.9	1.9
proline	14.2	20.5
glycine	15.9	20.2
alanine	20.9	14.2
citrulline	1.5	0.2
α-aminobutyric acid	0.2	0.1
valine	4.3	5.2
methionine	-	0.7
isoleucine	2.7	3.7
leucine	5.0	7.0
cystathionine	1.0	0.6
tyrosine	1.1	2.1
phenylalanine	2.7	4.8
β-alanine	0.4	0.2
β-aminoisobutyric acid	0.2	0.6
γ-aminobutyric acid	0.2	8.9
histidine	2.8	1.0
ornithine	1.6	0.9
ammonia	1.3	1.0
lysine	20.8	18.3
arginine	3.7	1.7
total amount	327.7	374.2

Notes: " - " means not detected

3.3 Polyamines and monoamines

In Tab. 4 are shown the contents of polyamines and monoamines in parts of muscle and in the liver and the gonad of snakehead immediately after death. Though tyramine(Tym), putrescine(Put), cadaverine(Cad), histamine(Him), agmatine(Agm) and tryptamine were not detected in muscle, spermidine(Spd) and spermine(Spn) were detected in large amount in muscle. In liver and the gonad Put was detected with the highest amount, followed by Spd and Spn. In gonad and liver Cad was contained in fairly high amounts and Him, Agm and Tym were detected in small amounts. In the skin of snakehead small amounts of Spd, Spn, Him and Agm were detected.

Tab. 4 Contents of polyamines and monoamines in the *Channa argus*

amines	in the <i>Channa argus</i>			mg ^a (100 g) ⁻¹		
	dorsal muscle	ventral muscle	caudal muscle	liver	gonad	skin
tyramine	-	-	-	0.4	1.6	-
putrescine	-	-	-	18.9	36.7	-
cadaverine	-	-	-	3.3	8.1	-
histamine	-	-	-	1.8	2.4	0.3
agmatine	-	-	-	1.8	2.1	0.2
tryptamine	-	-	-	-	-	-
spermidine	2.8	2.0	5.2	17.1	26.0	1.8
spermine	8.4	5.2	5.6	12.6	13.3	1.8

Notes: " - " means not detected

3.4 Glycogen, glucose, G6P and F6P

In Tab. 5 are shown the contents of glycogen, glucose, G6P and F6P in the dorsal muscle and the ventral muscle of snakehead immediately after death. The level of glycogen is slightly higher in the ventral muscle than that in the dorsal muscle, while the level of glucose was slightly higher and those of G6P and F6P were higher in the dorsal muscle than those in the ventral muscle.

Tab. 5 Contents of glycogen, glucose, G6P and F6P in the dorsal and ventral muscles of *Channa argus*

	mg ^a (100g) ⁻¹	
	dorsal	ventral
Glycogen	459.3	542.7
Glucose	51.9	47.7
G6P	44.3	30.0
F6P	7.5	5.5

3.5 Organic acids and phosphoric acid

In Tab. 6 are shown the contents of organic acids and phosphoric acid in the dorsal muscle and in the ventral muscle of snakehead immediately after death. The content of lactic acid was by far high in the organic acids and higher in the dorsal muscle than that in the ventral muscle. Small amounts of malic acid and acetic acid were detected. Phosphoric acid was in large amount.

4 Discussion

Regarding the reason why ATP level is high in the caudal muscle of snakehead, it can be considered that ATP is necessary to take active exercise in the caudal muscle.

Tab. 6 Contents of organic acids and phosphoric acid in the dorsal and ventral muscles of *Channa argus* mg (100 g)⁻¹

acids	dorsal	ventral
phosphoric acid	29.1	21.6
citric acid	-	-
malic acid	1.4	1.0
succinic acid	-	-
lactic acid	115.2	72.5
acetic acid	1.3	0.9

Notes: “-” means not detected

Small amounts of AdR was detected in the liver together with IMP. Nonratip and Yamanaka^[14] observed small amounts of IMP and AdR in ascidian muscle, and they reported that there are two pathways of ATP degradation in the muscle of ascidian. In the same way it can be generally considered that there exist two metabolic pathways of ATP degradation in the liver of snakehead.

According to the report by Chen and Luo^[6], Tau, Gly and histidine (His) were major free amino acids in the muscle of five cultured freshwater fishes in China (grass carp, bighead, silver carp, crucian carp and blunt snout bream). In the muscle of snakehead Tau and Gly were major free amino acids, but His was detected only in small amounts. The former five freshwater fishes belong to Cyprinidae family, while the latter belongs to Characidae family. Consequently, it can be considered that the difference of fish family influences the concentration of His.

Tau which is a sulphur containing free amino acid is dominant in cultured freshwater fishes in China. Tau plays important roles in depression of blood pressure, lowering of cholesterol, prevention of drunken sickness and so forth. In the liver and the gonad Tau was dominant, and phosphoethanolamine was specifically high in amounts in the gonad.

In the muscle of snakehead, Spd and Spn were only detected, but in the gonad large amounts of Put, Spd and Spn, fairly high amounts of Cad, and small amounts of Him, Agm and Tym were detected. There have been no reports on polyamines and monoamines in the liver of fishes. In the liver of snakehead high levels of Put, Spd and Spn were detected along with

low levels of Cad, Him, Agm and Tym. Yamanaka *et al.*^[5] reported that Spn, Spd and Put were distributed in the muscle and the gonad of carp and rainbow trout cultured in Japan; content of Spn was the largest, followed by Spd, and Put was smallest in quantity. A large amount of Spn was determined in the spermary [103 mg • (100 g)⁻¹] and in the ovary [46 mg • (100 g)⁻¹], and a fairly large amount of Spn was present in the muscle [12 to 14 mg • (100 g)⁻¹], which had an influence on the smell of carp^[5]. Spn, Spd and Put may be concerned with strong smell of snakehead. Mackie *et al.*^[15] reported that Spn, Spd, Put, Agm and Cad were detected in the gonad of herring (*Clupea harengus*), and Spn, Spd, Put, Agm, Cad, Tym and Him were detected in the gonads of mackerel (*Scomber scombrus*) and scallop (*Pecten maximus*).

According to Yamanaka *et al.*^[16], the content of glycogen is about 1000 mg • (100 g)⁻¹ in the muscle of carp immediately after death. Therefore, glycogen content in the muscle of snakehead is nearly the half of carp. G6P is detected in the dorsal muscle of carp in the amounts of about 20 mg • (100 g)⁻¹ immediately after death^[17], while G6P level in the dorsal muscle of snakehead is twice as high as carp.

In organic acids, lactic acid was dominant in the muscle of snakehead, and the level of lactic acid was higher in the dorsal muscle than that in the ventral muscle. Lactic acid is an end product of glycolysis. In the dorsal muscle of carp immediately after death lactic acid is detected at the concentration of 100 mg • (100 g)⁻¹^[16], which is almost the same as snakehead.

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乌鳢抽出物成分的研究

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摘要: 分析了乌鳢 (*Channa argus*) 即杀后背肉、腹肉、尾部肉、肝脏、生殖腺中 ATP 关联化合物、游离氨基酸、多胺、糖元及糖酵解中间代谢物、有机酸等的含量。ATP 关联化合物在肌肉中的总量为 $7.5 \sim 8.0 \mu\text{mol} \cdot \text{g}^{-1}$ 。ATP 在背肉含量为 $3.9 \mu\text{mol} \cdot \text{g}^{-1}$, 腹肉为 $4.1 \mu\text{mol} \cdot \text{g}^{-1}$, 尾部肉为 $4.7 \mu\text{mol} \cdot \text{g}^{-1}$ 。运动激烈的尾部肉 ATP 占 63%, 含量相当高。肝脏中有少量的腺苷与肌苷酸一起被检出, 据此可认为 ATP 的分解存在两个途径。游离氨基酸总量在背肉中为 $436.0 \text{ mg} \cdot (100\text{g})^{-1}$, 腹肉中为 $405.0 \text{ mg} \cdot (100\text{g})^{-1}$, 尾部肉中为 $356.3 \text{ mg} \cdot (100\text{g})^{-1}$ 。牛磺酸和甘氨酸为主要氨基酸, 占 68% ~ 73%。丙氨酸和谷氨酸也相当高的含量检出。肌肉中的多胺检出为精胺和亚精胺, 肝脏和生殖腺中有较高浓度的腐胺及亚精胺和精胺检出。即杀后糖元的量约占肌肉的 0.5%, 还有相当多的葡萄糖和 6 磷酸葡萄糖的糖酵解中间代谢物以及其最终产物乳酸的大量检出。

关键词: 乌鳢; 抽出物成分; ATP 关联化合物; 游离氨基酸; 多胺

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