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# Comparative analysis of the amino acid composition and proteomic patterns of the muscle proteins from two teleosts, *Siniperca chuatsi* L. and *Hypophthalmichthys molitrix* L.

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**Abstract:** The amino acid composition and proteomic pattern of the muscle proteins from two teleosts, the mandarin fish, *Siniperca chuatsi* and silver carp, *Hypophthalmichthys molitrix* were analyzed by HPLC and two-dimensional (2-D) gel electrophoresis. The total amount of essential amino acids in *S. chuatsi* is 34.7 $mg \cdot g^{-1}$ of the muscle protein, while that in *H. molitrix* is 33.6 $mg \cdot g^{-1}$. The values for the taste-enhancer amino acids (aspartic acid and glutamic acid) are 23.8 $mg \cdot g^{-1}$ in *S. chuatsi* and 15.3 $mg \cdot g^{-1}$ in *H. molitrix*. The muscle proteins from the two types of fish were resolved into a similar number of spots in 2-D gels (about 260 at pI 3-10), although the protein-size distributions are dramatically different between the two teleosts. Our study is the first to demonstrate significant differences in protein composition and patterns between two species of farmed fish, and provides reference values for aquaculture and efforts aimed at fish stock improvement and fish protein utilization.

**Key words:** amino acid; proteomic pattern; 2-D gel electrophoresis; *Siniperca chuatsi*; *Hypophthalmichthys molitrix*
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## 1 Introduction

Aquatic products are one of the most important foods for human being. Fishes, in particular, are highly valued for their unsaturated fatty acids and proteins rich in essential amino acids. Four fish species, black carp (*Mylopharyngodon piceus* L.), grass carp (*Ctenopharyngodon idellus* L.), bighead carp (*Aristichthys nobilis* L.) and silver carp (*H. molitrix*), known colloquially as "the four large family fishes", are major aquacultural species in Asia, especially in China[1]. In recent years, several other fishes, both indigenous and non-native, have been introduced as intensive culture species and they include cod (*Gadus morhua* L.)[2], mullet (*Mugil cephalus* L.)[3], silver crucian carp (*Carassius auratus gilebio* L.)[4], and mandarin fish. (*S. chuatsi*)[5]. Among them, the Mandarin fish has become quite popular in China because of its good meat texture, flavor, and high nutritional value. We hypothesize that such qualitative differences between species of fish may reflect their differences in protein and/or amino acid composition, controlled by specific genes encoding the muscle proteins. In most species of fish, protein constitutes about 15% to 20% of the total mass, while the amount of the essential amino

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acids ranges from 20% – 50% of the total amino acid content[6]. Corser *et al*.[7] analyzed the fatty acid profile, mineral composition, and essential amino acid content of 12 species of fish and found protein contents from 18.7% to 25.5% and essential amino acid amounts of 8.73 to 10.93 mg·g$^{-1}$ of fish. Goodman-Lowe *et al*.[8] demonstrated that the teleosts vary in the amounts of crude protein and fat, and that essential amino acids are about half as abundant (35.8 ± 2.6)% as non-essential amino acids (64.2 ± 2.6)%.

Proteome analysis is a promising technique for characterizing protein expression patterns on a large scale, for example, between different tissues of a fish or between species of fish. Proteome analysis can be used to identify species[9], look at protein induction, monitor changes in fish muscle proteins during ice storage[2], assess changes of fish muscle under various processing conditions[10] and measure the myosin isoenzymes of Arctic charr[11]. Several other studies have reported proteome characteristics of shrimp, salmon[12], sea bass[13], and cod[2]. The objectives of this study were two-folds: (1) to compare the protein contents and amino acid compositions of the muscle proteins between mandrian fish and silver carp; and (2) to initiate a comparative proteomic analysis aimed at identifying biological parameters of importance to fish aquaculture, including differentially expressed muscle-related proteins.

## 2 Materials and methods

### 2.1 Preparation of fish muscle samples

Live mandrian fish and silver carp at an average body mass of about 500 g were purchased from the Changsha Fish Market (Changsha, Hunan, China) and kept at our laboratory in tap water until use. The species of Mandarin fish was identified and confirmed by examining external anatomical characteristics. About 50 g of muscle tissues were dissected from the dorsal regions of each species and the muscle samples were quickly frozen in liquid nitrogen until use or put on ice for immediate experiments.

### 2.2 Analysis of amino acid composition of the muscle proteins

Protocols for muscle protein preparation and amino acid analysis were as described by Chong *et al*.[14] with some modification. Briefly, the muscle samples were cut into very fine pieces, freeze-dried, and then transferred to a constant dryer at 110 ℃ for 24 hours. 5 mg of fully-dried muscle samples were then hydrated in 200 μL of 6 mol·L$^{-1}$ HCl plus 1 g of phenol in a hydration bottle and then vacuumed and were subjected to liquid-nitrogen treatment for three times. The samples were hydrated in nitrogen gas at 105 ℃ for 20 – 24 hours and then vacuum-dried with 10 μL of the sample drying solution (ethanol∶water∶tri-ammonium, 2 vol∶2 vol∶1 vol) until the HCl and phenol were completely removed. Finally, each sample was dissolved in 20 μL newly prepared solution (B: water: tri-ammonium: PITC (Phenylisothiocyanate) = 7∶1∶1∶1) and incubated at room temperature for 20 – 30 min until the samples were fully dried.

The samples were then processed for analysis of amino acid composition by dissolving them in 100 μL of phosphate buffer (700 mg $Na_2HPO_4$ dissolved in 100 mL distilled water, pH 7.4) and applied to an Alliance HPLC System for amino acid analysis using a C18HPLC column (4.6 × 250 mm, Vydac).

### 2.3 Amino acid standards and data analysis

10 μL of amino acid standards (Sigma) were used in the Alliance system analysis at 254 nm and three repeats for the standards were done to establish stable images for the system. Samples were analyzed using the same procedures and parameters as for the standards. For analysis of amino acid composition and concentrations, HPLC spectra obtained for samples and standards were compared to determine both the types (time of appearance) and relative amounts (size of peaks) of each amino acid.

### 2.4 Protein sample preparation for 2-D gel electrophoresis

To prepare fish muscle crude proteins, 10 g of muscle tissues obtained from dorsal regions were ground in a mortar for 30min under liquid nitrogen. The fully ground samples were transferred to a centrifuge tube and lysed in 200 μL of

lysing buffer (0.5 mmol·L$^{-1}$ NaCl, 10 mmol·L$^{-1}$ Tris-HCl (pH 7.5), 1% TritonX-100, 0.1% SDS, 1 mmol·L$^{-1}$ EDTA and 1 mg·mL$^{-1}$ PMSF) for 30 min. After two rounds of centrifugation at 100 g and 12 000 g respectively at 4 ℃ for 30 min, the supernatants were saved and kept at − 80 ℃ for protein concentration determination and 2-D gel electrophoresis. Protein determination was done using the Bradford method according to the manufacturer's instructions (BioRad).

**2-D electrophoresis** 2-D electrophoresis was performed as described by Huang *et al*.[3] with some modifications. Proteins extracted from the muscle tissues of both types of fish were separated in the first dimension according to charge using 18-cm long 1D Immobiline Drystrips (Amersham Biosciences) and a linear pH gradient of 3 − 10. The 1D Immobiline Drystrips were rehydrated overnight in 8 mol·L$^{-1}$ urea, 0.5%(w/v) 3, 3-chlolamidopropyl--dimethylammonio-1-propanesulfonate (CHAPS), 0.25% Pharmalyt 3 − 10, 10 mmol·L$^{-1}$ DTT, and Orange G as a dye. The protein samples (10 μg) were diluted to 0.8 μg of protein/μL in rehydration buffer (8 mol·L$^{-1}$ urea, 0.5% (w/v) CHAPS, 2.0% Pharmalyt 3 − 10, 10 mmol·L$^{-1}$ Tris-HCl, pH 8.3, 0.1% (w/v) SDS), and 30 μg were loaded onto each rehydrated 1D Immobiline Drystrip. Isoelectric focusing was carried out at 20 ℃ using a Multiphor Ⅱ flatbed (Amersham Bioscience). The separation was performed at 30V for 10 − 16 h, followed by 500 V for 1 h, 100 V for 1 h and finally at 800 V for 30 min. After separation of the proteins in the first dimension, the strips were stored at − 80 ℃ until separation in the second dimension. Separation in the second dimension was carried out essentially as for regular SDS-polyacrylmide gel electrophoresis[15]. Prior to SDS-PAGE, the gel strips were first reduced for 30 min in equilibration buffer (6 mol·L$^{-1}$ urea, 50 mmol·L$^{-1}$ Tris-HCl, pH 8.8, 30% (v/v) glycerol, 2% (w/v) SDS, 1% (w/v) DTT), and then subjected to a 10-min alkylation treatment with 4.5% (w/v) iodoacetamide in 10 mL of equilibration buffer. The equilibrated 1D gel strips were placed on the SDS-polyacrylamide gels and sealed with 0.5% (w/v) agarose in running buffer (25 mmol·L$^{-1}$ Tris-base, 192 mmol·L$^{-1}$ glycine and 0.1% (w/v) SDS). Second dimension electrophoresis was carried out at 30 V for 15 min, followed by 100 V until the dye migrated to the bottom of the gel. The gels were stained with either Coomassie blue or Silver as described by Zhang and MacRae[16].

**Gel image analysis** Coomassie blue or silver stained gels from both mandarin fish and silver carp were scanned using a Qinghua ultraviolet scanner in transmission mode, and the protein spots from each gel were also manually counted. The proteome mapping patterns were characterized according to differences in protein distribution and the differential expression of the muscle proteins between the two species. Each gel was repeated four times and an average value was used for the data analysis and typical gel images were chosen.

## 3 Results and discussion

The dry protein content per gram of the muscle from each of the two fish species was determined by the drying ash protocol[14], and such values are often used as a measure of the quality of fish meat. As shown in Fig.1, the amount of dry protein in *S. chuatsi* was found to be 18.5% of the total wet muscle tissue, while in silver carp it was 16.0%. The dry protein contents of both fishes are within the 15% − 25% range typical for vertebrates[14]. However, the protein content of the mandrian fish is about 12.4% higher than that of silver carp, which is evidence that mandrian fish meat may be the superior food, at least by these criteria.

To compare the meat quality of the two fish species, *S. chuatsi* and *H. molitrix* we analyzed their amino acid compositions from the muscle proteins using HPLC system and amino acid standards (Fig.2). The amino acid compositions of the soluble muscle proteins of the two fishes were analyzed and the data are presented in Tab.1. Because tryptophan was destroyed and two other amino acids, asparagine

and glutamine, were converted to aspartic acid and glutamic acid by hydrolysis during sample preparation, only 17 amino acids were analyzed in the study. The total amount of essential amino acids in mandrian is 34.7 mg·g$^{-1}$ muscle protein, while in silver carp it is 32.6 mg·g$^{-1}$, which is about 6.1% lower than the mandarin's value. The total amounts of flavor amino acids (aspartic acid, glutamic acid, glycine and alanine) and taste-enhancer amino acids (aspartic acid and glutamic acid) are 30.4 and 23.8 mg·g$^{-1}$ of the muscle proteins in mandarin fish; the corresponding values for silver carp are significantly lower: 21.3 and 15.3 mg·g$^{-1}$, respectively. It is striking as summarized in Tab. 1 that among the amino acids analyzed, four of the amino acids analyzed, glutamic acid, aspartic acid, leucine, and lysine, are present at very high levels in Mandrian fish (13.8, 9.6, 8.8 and 6.2 mg·g$^{-1}$ of muscle protein, respectively), compared to the silver carp (8.3, 6.9, 7.5 and 1.77 mg·g$^{-1}$ respectively).

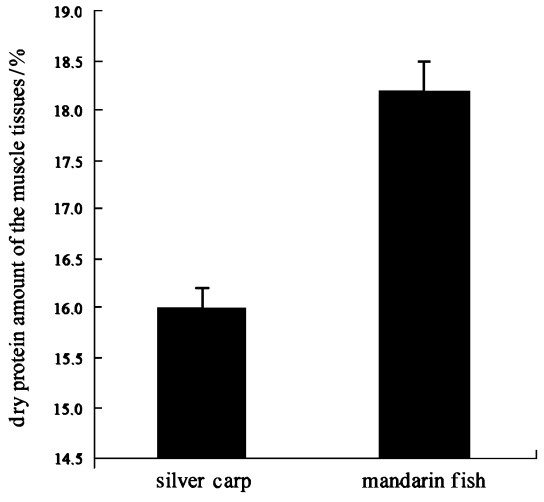


Fig.1 Comparison of the dry protein amounts of the muscle tissues between the mandarin fish and silver carp

The relative amount of dry proteins was calculated by dry protein divided by total wet muscle proteins. The data show an average of four repeats

The quality of a food protein is a function of the types and amounts of amino acids it contains. This is because the essential amino acids must be obtained in the diet and different amino acids have different physiological functions in human metabolism[17]. Consequently, the consumer value of fish is directly related to its nutritional value. Our results reveal the muscle of the madrian fish to be an exceptionally high quality food for human being. Firstly, the essential amino acids make up 34.7% of all amino acids, and the ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) is 1.03. Both these values exceed recommendations of the Food and Agriculture Organization for an "ideal" food protein (EAA > 40% and EAA/NEAA > 0.6)[18]. Secondly, the Mandrian protein has an extremely high content of the flavor-enhancers, glutamic acid and aspartic acid (28.8% of the total amino acids), which may account for the meat's good taste. Thirdly, the fish muscle proteins contain very high levels of both lysine (6.13 mg·g$^{-1}$ and 7.5% of the total amino acids) and arginine (7.1 mg·g$^{-1}$ and 9.7% of the total amino acids). Lysine, which is extremely rare in cereals, is an essential amino acid required for proper development and is also a precursor in the production of carnitine, a nutrient with roles in converting fatty acids into energy and in regulating cholesterol levels. Arginine is classified as a semi-essential or conditionally essential amino acid and it participates in protein synthesis and other physiological functions such as detoxification and energy conversion[19]. Fourthly, the Mandrian also contains relatively high levels of branched chain amino acids (BCAA), such as leucine (6.2 mg·g$^{-1}$ of muscle protein), isoleucine (3.8 mg·g$^{-1}$), and valine (3.96 mg·g$^{-1}$). BCAA are needed for the maintenance of muscle tissue and appear to preserve muscle stores of glycogen, and they also help prevent muscle protein breakdown during exercise[20], and the enriched branched chain amino acids may contribute to the meat soft textures.

Two-dimensional gel electrophoresis is a powerful technique to analyze proteomic patterns of specific protein distribution according to their pH isoelectric focusing and molecular sizes and to identify differentially expressed proteins[20]. In the study, we applied the technique to investigate if some proteins

are differentially expressed in the muscle tissues of the two fish species. Proteins were extracted from the muscle tissues of both of the two fish species and the urea soluble proteins were separated according to charge in Immobiline Dry strips (Amersham Biosciences) with a linear pH gradient from 3 to 10, and in SDS-PAGE gels[3,16]. We run four repeats of the 2-D gels as shown in Tab.1 and our observations revealed that the protein distribution on the gels was quite consistent. As summarized in the Tab.2 and Tab.3, the mandarin fish muscle proteins resolved into 259 spots in 2-D gels with a pI range of 3 – 10. For silver carp muscle proteins resolved into 257 spots. The best resolution and reproducibility in the mandarin fish are at pI 4 – 8.5 and MW 20 – 70 ku, and for silver carp they are at pI 4.5 – 9.0 and MW 20 – 80 ku (Fig.3). Although the numbers of spots in the two species are nearly identical, the pI distributions of the proteins are dramatically different; The mandarin fish proteins overall are more acidic and the silver carp proteins tend to be more basic; this would be consistent with the higher levels of aspartic acid and glutamic acid in the mandarin fish muscles. Especially as marked in the Fig.3-A, several protein spots were identified in the mandarin fish. Regarding protein size distribution, they are similar except that the mandarin fish has many more small proteins (< 14 MW) and the silver carp has an excess of proteins in the two classes comprising 29 – 66 MW.

**Tab.1　Amino acid compositions and relative amounts in muscle proteins**

| amino acids | sliver carps ($mg \cdot g^{-1}$) | mandarin fish ($mg \cdot g^{-1}$) | differences (Q-S) | essential amino acids | flavour amino acids |
|---|---|---|---|---|---|
| alanine[2] | 3.13 | 2.72 | -0.41 | | * |
| arginine | 7.46 | 7.80 | 0.34 | | |
| aspartic acid | 6.98 | 9.60 | 2.62 | | * |
| cysteine | 1.80 | 1.60 | -0.2 | | |
| glutamic acid[2] | 8.30 | 13.86 | 5.56 | | * |
| glycine[2] | 2.65 | 4.34 | 1.69 | | * |
| histidine[1] | 8.62 | 0.52 | -8.1 | * | |
| isoleucine[1] | 2.29 | 3.80 | 1.51 | * | |
| leucine[1] | 7.56 | 8.83 | 1.27 | * | |
| lysine[1] | 1.77 | 6.20 | 4.43 | * | |
| methionine[1] | 3.01 | 3.01 | 0 | * | |
| phenylalanine[1] | 2.20 | 4.17 | 1.97 | * | |
| proline | 4.12 | 0.94 | -3.18 | | |
| serine | 3.78 | 4.01 | 0.23 | | |
| threonine | 1.5 | 4.26 | 2.76 | * | |
| tyrosine | 3.74 | 2.81 | -0.93 | | |
| valine | 3.50 | 3.96 | 0.46 | * | |
| ∑AA | 72.41 | 82.43 | 10.02 | | |
| ∑EAA | 30.45 | 34.75 | 4.3 | | |
| ∑FAA | 21.06 | 30.52 | 9.46 | | |

Notes: * 1.Essential amino acid, in terms of EAA; 2.Flavor amino acids, in terms of FAA. (the same in following)

To test for reproducibility, muscle proteins were extracted from five different fish of both species and run on separate gels. Although some variation was noted between individuals of the same species (data not shown), we concluded that such variation was probably a result of differences in protein expression rather than an experimental artifact. Similar interspecies variation was also found for puffer fish[9], Oncorhynchus[21], different gynogenetic embryos[22], and different tissues of a single species[23].

Also the isotypes of one group of proteins of trout and sea bass were found to vary with developmental stages[24]. The results of this study give preliminary proteomic patterns for the muscles of the mandarin fish and silver carp. Further work is required to determine the identity of each protein, for example by mass

spectrometric techniques, and to identify species-specific protein markers. Such a proteomics database would both contribute to our knowledge of fish biology and be of value to aquaculture program.

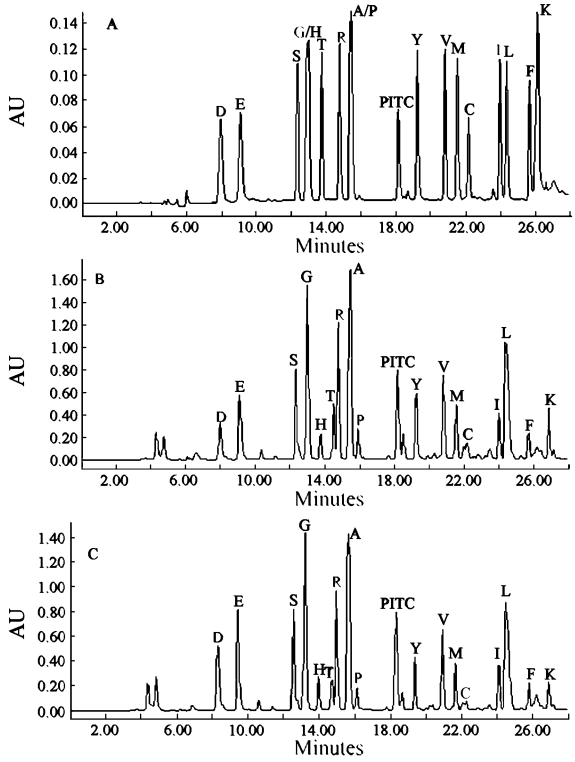


Fig.2 The spectrum profiles of the amino acid analysis with Alliance HPLC system

The amino acid standards (Sigma) were analyzed at 245 nm as controls (A). 10 μL of the muscle proteins from the mandarin fish (B) and the silver carp (C) were analyzed and the amino acids and their relative amounts were calculated according to the standards. Each sample was repeated for three times

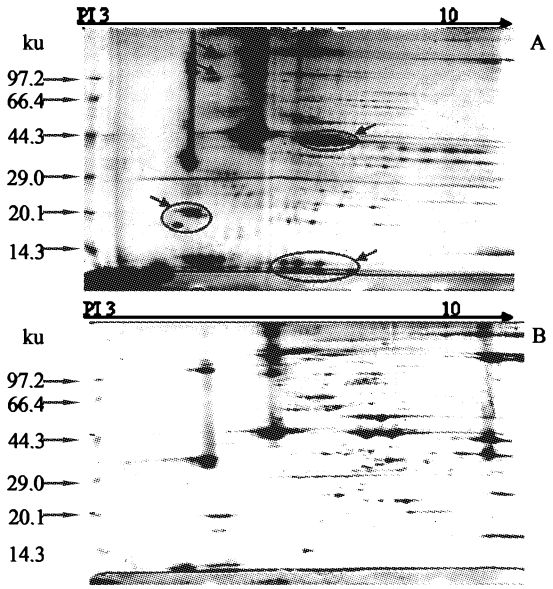


Fig.3 Two-dimensional gel electrophoresis of the muscle proteins of the two fish species

Representatives of 2-D electrophoresis gel of the mandarin fish (A) and the silver carp (B) muscle proteins. The extracted proteins (80 μg) from each fish were first separated by linear pH 3 – 10 IPG immobile Drystrips, followed by size separation on 10% SDS-PAGE gels. Distribution of protein spots were scanned and recorded with PtoXPRESS2D system. As marked in A, they are differentially expressed proteins in the mandarin fish

**Tab.2 Protein spot distribution at different pI range**

| pI range | total protein spots | pH 3 – 5 | pH 5 – 7 | pH 7 – 10 |
|---|---|---|---|---|
| mandarin fish | 259 | 74 | 128 | 57 |
| silver carp | 257 | 25 | 90 | 142 |

**Tab.3 Protein spot distribution with different molecular weight**

| protein MW (ku) | > 97.2 | 66.4 – 97.2 | 44.3 – 66.4 | 29 – 44.3 | 20.1 – 29 | 14.3 – 20.1 | < 14.3 |
|---|---|---|---|---|---|---|---|
| mandarin fish | 45 | 23 | 42 | 58 | 25 | 12 | 35 |
| silver carp | 53 | 18 | 53 | 79 | 30 | 9 | 15 |

## 4 Conclusion

Fish meat proteins have provided human beings with highly valued nutrition resources as they contain unsaturated fatty acids and are rich in essential amino acids. The mandarin fish, *S. chuatsi*, usually uncultured wild species, now has been introduced as a major species in aquaculture in China. Our study confirmed that the fish meat proteins enrich essential and taste-enhancing amino acids, as well as some rare amino acids, such as Lysine and Arginine, which are not found in cereals. The preliminary results of the proteomic profiles of the mandarin fish proteins reveal that the fish possesses quite differentially expressed

proteins. Therefore, further work will be carried out to investigate the identity of each specific proteins as well as their encoding genomic information.

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# 鳜和鲢肌肉蛋白质氨基酸组成及其蛋白质组成的比较分析

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**摘要**: 采用高压液相色谱和二维电泳方法, 对鳜和鲢肌肉组织蛋白质组成成分以及肌肉蛋白质双向电泳分布图谱进行了比较研究。研究结果证明, 鳜肌肉组织蛋白质干重为 34.7%, 而鲢肌肉干重为 33.6%; 鳜肌肉组织中必需氨基酸含量和鲜味氨基酸(天冬氨酸和甘氨酸)均高于鲢。同时, 将两种鱼肌肉蛋白进行 2D-gel 双向电泳分离, 发现鳜和鲢肌肉蛋白质组成和分布也存在明显的差异。本研究将肌肉蛋白质组成成分和蛋白的双向电泳技术结合起来, 进行蛋白质组分归类分析, 可为进一步研究两种鱼肌肉肉质研究打下基础, 为水产业提供有价值的参考, 为鱼类的养殖和肉质的改良、利用提供一个指标。

**关键词**: 氨基酸; 蛋白质组成; 二维电泳; 鳜; 鲢

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