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Study on Total Protein and Amino Acid Profile of Milkfish (*Chanos chanos*) Meat and Its Product from Sidoarjo, East Java

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KEYWORDS

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Abstract Milkfish is one of the most cultivated fish species in Indonesia because these fish have a high tolerance to the various environmental conditions of tropical waters and are resistant to many pests and diseases. This preliminary research was conducted to analyze the amino acid composition contained in milkfish meat and its product. 20 fresh milkfish were obtained from ponds in Sidoarjo, East Java, in September 2020. Amino acid composition and protein content were analyzed. Based on its protein content, milkfish has been classified as a source of high protein. The amino acid profile of milkfish consists of approximately 17 types of amino acids. Glutamic acid, the amino acid present in the highest concentration, makes milkfish very popular.

Introduction

The sustainable potential of Indonesian marine fisheries is estimated at 6.5 million tons per year, which are scattered in the territorial waters of Indonesia and the EEZ (Exclusive Economic Zone) with a utilization rate of 5.71 million tons or 77.38 percent (Ministry of Marine Affairs and Fisheries, 2011). Fish plays a vital role in fulfilling nutrition sources, especially protein and amino acid content for humans in developing countries (Hafiludin, 2015). Fish also serves as a source of plural unsaturated fatty acids (PUFAs), protein, minerals, and vitamins. Although fish is rich in nutrients, it is a material that spoils quickly and has a short shelf life.

Milkfish is one of the most cultivated fish species in Indonesia because these fish have a high tolerance to the various environmental conditions of tropical waters and are resistant to many pests and diseases (Malle *et al*., 2019). One of the districts that have developed milkfish cultivation in Indonesia is the Pangkep Regency, one of the primary commodities produced. Influential external factors include cultivation location or habitat, feed formulation, and conditions of the quality of the waters of the fish farm (Hafiludin, 2015). This preliminary research was conducted to analyze the amino acid composition contained in milkfish meat and its product.

Materials and methods

The Samples

A total of 20 fresh milkfish were obtained from ponds in Sidoarjo, East Java, in September 2020. The milkfish samples that were chosen were of uniform size. The fish samples were put in an insulated box containing ice, with a fish to ice ratio of 1:2 (w/w), and transported to the Laboratory of Fisheries Product Technology, Fishery Product Engineering Division, Faculty of Fisheries and Marine Sciences, University of Brawijaya Malang.

Amino Acid Composition Analysis

The amino acid composition was determined by following Ishida *et al.* (1981).

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Muscle protein was hydrolyzed with 6N hydrochloric acid (HCl) at 110°C under anaerobic conditions for 24 h. The hydrolyzed samples were neutralized with 6N sodium hydroxide (NaOH) and were derivatized using a kit (AccQ-Fluor Reagent, WAT052880, Waters, USA). The derivatized samples were injected into high-performance liquid chromatography (HPLC) (1525, Waters), equipped with a C18-RP column and a fluorescence detector (2475, Waters). The amino acids were identified and quantified by comparing with the retention times and peak areas of standards (WAT088122, Waters). For the tryptophan analysis, the minced meat was digested with 5% (w/v) NaOH for 24 h and neutralized to pH 7.0 with 6N HCl. The tryptophan content was measured spectrophotometrically at 530 nm (Demeester *et al*., 1978).

Protein Content analysis

Protein content was determined using the modified method of Pratama *et al.* (2019). The sample was weighed as much as 2 grams in each part (head, body, tail, dorsal, ventral). Then enter the sample in a volumetric flask and add distilled water to the mark to dissolve the sample. Then enter 2 ml of sample into a centrifugal tube and add 1 ml of 10% TCA to precipitate the protein. Then centrifugation at 3000 rpm for 5 minutes, which functions to separate the residue and supernatant. Next, the supernatant was removed, and 2 ml of ethyl ether was added to break the protein in the precipitate. The sample was homogenized and centrifuged (3000 rpm for 5 minutes) to separate the residue and the supernatant. After that, leave it for 24 hours at room temperature until the precipitate is dry. Then the sample is added with 4 ml of distilled water and homogenized. Then the sample was added with 6 ml of biuret reagent as an indicator of purple color. Then the sample was taken 0.1-1ml and stored in a test tube at room temperature for 30 minutes until it was purple. The final step is to measure the absorbance value using a UV-Vis spectrophotometer with a wavelength of 520 nm. Calculation formula for % protein content:

$$\%\ Protein\ \frac{VxNx0.014x6.25xP}{Sample\ (Gram)}\ x100\%$$

While V is sample titration volume, N is solution normality of HCl, or H2SO4 0,0222 N and P is dilution factor = 100/5

Results and Discussion

The Yield

The average weight and length of the fish used in this study were 423.1 ± 12.13g and 38 ± 2.41 cm, while cooked milkfish (Using Presto pan) was 449.8 ± 10.12g Gram and 35 ± 2.13g Cm. Which is approximately the size of the milk is consumed in many households. The results of proximate analysis, that of the content of minerals and vitamins, and the amino acid and fatty acid profile of milkfish are presented in Table 1

Table 1. The average weight and length of the fish used in this study

Sample	Weight	Length
Fresh Milkfish Meat	423.1 Gram	38 Cm
cooked milkfish (Using Presto pan)	449.8 Gram	35 Cm

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Protein Level Testing Results Data

Data observations on the Basics of Fishery Product Technology for the Test Material for Protein Content of Food and Food Processed Protein test results are presented in Table 2 below, and the following data are obtained:

Table 2. Content of Food and Food Processed Protein test results

Sample	Part	Protein content (%)	Std
Fresh meat	Head	12	± 0.22
	Body	21	± 0.14
	Tail	3	± 0.12
	Dorsal	6	± 0.4
	Ventral	4.5	± 0.41
Total		**46.5**	
Cooked meat	Head	14.3	± 0.23
	Body	25.5	± 0.3
	Tail	7.5	± 0.34
	Dorsal	6	± 0.11
	Ventral	7.5	± 0.15
Total		**60.8**	

The total protein content of the edible parts of milkfish was 46.5%, and cooked milkfish was 60.8%. This indicated that the milkfish contained high protein levels and can thus be used as a source of animal protein. The high protein content of >15% of milkfish placed it in the high-protein fish category (FAO, 2016).

Amino acid content

Data observations on the Basics of Fishery Product Technology for the Test Material for Amino acid Content of Food and Food Processed Protein test results are presented in Table 2 below, and the following data are obtained:

Table 3. Content of Food and Food Processed amino acid test results

Amino Acid	Fresh meat	Cooked milkfish
Alanine	0.73	9.49
Arginine	0.29	3.77
Aspartic acid	0.80	10.4
Glutamic acid	1.27	16.51
Glycine	0.28	-
Histidine	0.49	6.37

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Amino Acid	Fresh meat	Cooked milkfish
Isoleucine	0.35	4.55
Leucine	0.67	-
Lysine.	0.57	7.41
Methionine	0.25	3.25
Phenylalanine	0.34	4.42
Proline	0.41	5.33
Serine	0.29	3.77
Cysteine	0.40	-
Tyrosine	0.45	5.85
Threonine	0.45	5.85
Valine	0.47	6.11

The protein quality of any food is judged by the ratio of essential and non-essential amino acids present. High-quality protein contains essential dietary amino acids in quantities that correspond to human requirements (WHO, 2007). The amino acid profile of milkfish consists of approximately 17 types of amino acids (Table 3). Milkfish also contains the highest essential amino acids, namely leucine. Several factors cause the difference in amino acid composition in milkfish meat.

These are internal factors, including age, size, condition of fish, and the external factor that is the habitat. The red meat and white meat, liver, heart, and other organs in milkfish have different amino acid compositions. The highest amino acid content for red meat and white meat in milkfish is histidine and taurine, while the most elevated amino acid for other organs (liver, intestine, and heart) is the amino acid taurine (Shiau *et al*., 1996). Table 3 shows that glutamic acid content in milkfish meat is relatively high, giving the brackish water fish a more savoury taste.

Seafood tastes are influenced mainly by the content of free amino acids (FAA). The FAA that is dominant in the white muscle of milkfish is histidine, taurine, and glycine (Shiau *et al*., 2001). It has been proposed that FAA regulates the main metabolic pathways to improve the health, survival, growth, development, lactation, and reproduction of organisms. FAA also plays a vital role in preventing metabolic diseases such as obesity, diabetes, cardiovascular disorders, intrauterine growth restriction, infertility, intestinal and neural dysfunction, and infectious diseases. Clandinin *et al.* (1997) reported that arginine, cystine, leucine, methionine, tryptophan, tyrosine, aspartate, glutamic acid, glycine, proline, and taurine had been classified as necessary FAA in human nutrition.

Analysis of proteins and amino acids can be a consideration for the nutritional value of milkfish. We suggest further research to use this research material to analyze several popular consumption fish such as tilapia (Insani *et al*., 2020) and Genggehek fish (Valen *et al*., 2019). Testing of Arapaima predatory fish and alligator fish is also recommended for consideration if these fish can also be consumed so that farmers do not release them into the water if they cannot maintain them

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(Fadjar *et al*., 2019). Analysis can also be carried out on non-fish aquatic animals such as mangrove snails (Islamy and Hasan, 2020), where these animals are also widely consumed by the community as a non-fish alternative food.

Conclusions and Suggestion

Based on its protein content, milkfish has been classified as a source of high protein. Glutamic acid, the amino acid present in the highest concentration, makes milkfish very popular.

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