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CHEMICAL COMPOSITION AND AMINO ACID PROFILE OF FRESH AND STEAMED COBIA (*Rachycentron canadum* L.)

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ABSTRACT

Cobia (*Rachycentron canadum*) is economically important fish with good prospects because of fast growing and easily cultured. Cobia is usually processed by many methods including steaming. The purpose of this research was to determine the effect of steaming process on proximate and amino acids composition. The chemical composition of fresh and steamed cobia's meat were determined by proximate analysis. Amino acid compositions of fresh and steamed cobia's meat were measured using HPLC (high performance liquid chromatography). Steaming process reduced 11.31% of water, 1.06% of ash, 26.04% of fat, and 9.11% of protein. Fresh and steamed cobia's meat contained 17 amino acids consisting of 9 essential amino acids and 8 nonessential amino acids. The highest essential amino acid in fresh cobia's meat was arginine (2,262 mg/100 g) and the highest nonessential amino acid was glutamic acid (3,894 mg/100 g). Steaming process reduced amino acids generally. The highest essential amino acid in steamed cobia's meat was leucine (1,379 mg/100 g) and the highest nonessential amino acid was glutamic acid (2,370 mg/100 g). Taurine content of fresh cobia's meat was 120.84 mg/100 g and changed into 94.33 mg/100 g after steaming process.

Keywords: Amino acid, cobia, essential, nonessential, steaming

INTRODUCTION

Cobia fish (*Rachycentron canadum* L) is a very important fish for the people of Indonesia. Cobia fish have an economic value of US $ 0.5 per seed size of 10 cm, US $ 6 per kg for consumption and US $ 4-6 per kg in frozen form (Liao and Leano 2008). This fish attracts the attention of the public both in research and commercial fields to be cultivated. The advantages such as rapid growth, high conversion efficiency, easily changed in maintenance in cages and very resistant to disease.

Fish is a raw material that has nutrients that are beneficial to humans. Fish contain protein in sufficient quantities, which is 18-20%. Protein as one of the macro nutrients has a function in the body that is to create new tissue and maintain existing tissue. Protein can be used as a required fuel energy that not met by fat and carbohydrate. Proteins are composed of twenty different amino acids. The human body cannot synthesize nine amino acids containing isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine. Amino acids known as essential amino acids are only obtained through food intake (Abdullah *et al*. 2013).

The excess protein of the fish is easily digested by the body and has complete amino acids. The excess released by this protein does not need to be supported by its nature which is easy to repair changes and damage. Physical or chemical treatment of fish food ingredients from the beginning, processing, storage and finally reaching consumers is often the cause of damage to nutritional value, especially protein.

Steaming is one of the processing method that uses high temperature which has an impact on low nutrient loss, but this depends on the type of food and steaming method. The steaming process will affect the levels of amino acids contained in food so it needs to know about the content of amino acids lost during processing. This research is based on the research of Nurjanah *et al*. (2014) which defines the steaming factor for the chemical composition and mineral red wine, and Jacoeb *et al*. (2012) about steaming factors for crab amino acids. This study aimed to determine the content of yield, proximate, and amino acids of fresh and steamed cobia fish.

MATERIALS AND METHODS

Tools and Materials

The material used in this study was adult cobia fish taken from the Kalianda area, Lampung with a length of 80-100 cm totaling 5 individuals with a harvest age of 80 days. The ingredients used in proximate analysis, amino acids and taurine are selenium, concentrated H_2SO_4 (Merck), NaOH (Merck), H_3BO_3 (Merck), HCl 0.1 N, bromcresol green methyl red (Merck), hexane solvent, HCl 6 N (Merck, Germany), 0.01 N HCl (Merck, Germany), potassium borate buffer pH 10.4 (Merck, Germany), orthoflaaldehyde (OPA) reagents (Merck), methanol (Merck), mercaptoethanol (Merck), 30% Brij-30 solution (Merck), acetonitrile 60% (Merck), sodium carbonate buffer (Merck), Carrez reagents (Merck) , danzil chloride solutions (Merck) and methylamine hydrochloride (Merck).The instrument used is a tool for proximate testing as well as amino acid and taurine testing using HPLC (Shimadzu, Japan).

Research Method

This research consists of several stages, namely: 1) Measurement of dimensions and weights of cobia fish; 2) Calculation of yield of cobia fish by separating meat, viscera, bones and fins, and heads; 3) Analysis of chemical composition consisting of analysis of water content, ash content, fat content, and protein content (AOAC 2007); 4) Analysis of the composition of amino acids and taurine.

Procedural Analysis

Amino Acids (Abdullah *et al*. 2013)

Amino acid analysis using HPLC (Shimadzu, Japan) consists of four stages, namely: the stage of making protein hydrolyzate, drying, derivatization and injection and analysis of amino acids.

a. Hydrolizate Protein

Samples were weighed as much as 0.1 g and crushed. The destroyed sample was added with HCl 6 N (Merck, Germany) of 10 mL which was then heated in an oven at 100ºC for 24 hours. Heating is done to accelerate the hydrolysis reaction.

b. Sample drying

Filtering aims to make the resulting solution really clean, separate from the solid. The filter results are taken as much as 30 µL and added with 30 µL drying solution. Drying solutions are made from a mixture of sodium carbonate, carrez reagents (merck), danzil chloride solutions and methylamine hydrochloride (merck, Germany) in a ratio of 4: 4.

c. Derivatization

A 30 µL derivatization solution was added to the drying product, the derivatization solution was made from a mixture of methanol (Merck, Germany), sodium acetate (Merck, Germany) and triethylamine (Merck, Germany) in a ratio of 3: 3: 4. The derivatization process is carried out so that the detector is easy to detect compounds in the sample, then dilution is done by adding 20 mL of 60% acetonitrile (Sigma, UK) or 1M sodium acetate buffer (Merck, Gemrany), then left for 20 minutes.

d. Injection to HPLC

The filter results were taken as much as 40 µL to be injected into the HPLC (Shimadzu, Japan). Calculation of the amino acid concentration present in the material is done by making a standard chromatogram using ready-made amino acids that undergo the same treatment as the sample. Amino acid levels in ingredients can be calculated by the formula:

$$Amino\ acids\ (\%) = \frac{sample\ area\ x\ Cx\ FP\ x\ BM}{standard\ area\ x\ sample\ weight}\ x\ 100\%$$

Information:
C = standard amino acid concentration (µg / mL)
FP = dilution factor
BM = Molecular weight of each amino acid (g / mol)

Taurine Analysis (AOAC, 2007)

The content of taurine can be analyzed using a HPLC device (Shimadzu, Japan). Testing the level of taurine, the sample was weighed as much as 0.5 g and put into a 100 mL measuring tube, then added 80 mL of distilled water and 1 mL of carrez reagents (Merck, Germany) and then shaken until homogeneous. Then dilution is done by adding distilled water to the mark and shaking it until it is homogeneous. Then the solution is filtered using whatman filter paper. The filtrate is stored in an erlenmeyer and stored in a dark place. The derivatization step is then carried out by taking 1 mL of sample extract into 10 mL measuring flask, then adding 1 mL of sodium carbonate buffer and 1 mL of dansil chloride solution. After that the sample was allowed to stand for 2 hours then shaken and added 0.5 mL of methylamine hydrochloride latency (Merck, Germany) then shaken again until homogeneous. The result of derivatization is taken as much as 40 µL and then injected into the HPLC to determine the content of taurine in the sample.

Data Analysis

Data from the research of chemical composition and amino acids used descriptive statistics with 3 replications and standard deviations. Data results were compared with relevant literary studies.

RESULT AND DISCUSSION

Size and Weight of Cobia

The cobia studied were 5 fish and were taken from the Center for Sea Water Cultivation Development, the Ministry of Maritime Affairs and Fisheries, Lampung with a harvest age of 80 days. Cobia fish samples are presented in Figure 1. The average size and weight of cobia fish are presented in Table 1. Chuang *et al*. (2010) stated that cobia fish can reach 8 kg in weight per year from the initial seed size weighing 50-100 g. This is different from the study sample fish which have an average weight of 1.83 kg with an age of 8 months. According to Nurjanah *et al*. (2014), differences in feed, habitat, and age of harvest lead to differences in weights.

Table 1. Morfometrix cobia fish, n=5

Parameter	Value
Long (cm)	64.3±3.77
Wide (cm)	7.42±1.20
Height (cm)	14.4±1.86
Weight (kg)	1.83±12.1

Rendement of Cobia

Cobia fish used in the study were prepared and the yield was calculated. The yield of cobia fish is presented in Table 2. The meat yield is the largest yield, so the cobia fish meat is suitable for processing. These results prove that the cobia fish can be utilized for processing. Amiza and Aishah (2011) stated that the cobia fish processing waste consisting of skin and bones, has high collagen levels. This skin can be used as a gelatin ingredient that provides benefits in the food industry.

Table 2. Average size and weight of cobia fish, n=5

Part	Value (%)
Meat	36.83±0.45
Head	28.67±0.03
Bone and fin	16.42±0.5
Offal	11.21±0.4
Skin	6.87±0.9

Chemical Composition of Cobia

Comparison of the chemical composition of cobia fish meat results of research with the results of research by Chuang *et al*. (2010) are presented in Table 3. The difference in results is thought to be due to several internal and external factors. Internal factors include fish species, age, and genetics, while external factors are environmental conditions, both the availability of food, and its competitors, as well as the water quality of their habitat.

Figure 1. Cobia Fish and its component
A= cobia ; B.= Gill cobia; C=Offal Cobia; D= meat cobia

The 11.31% decrease in water content is influenced by several factors, namely surface area, concentration of solutes in hot water and stirring of water. The decrease in water content is thought to be caused by the steaming process which makes the water contained in the meat of the fish release and evaporate because of the heat transfer process in fish meat (Pepino *et al*. 2015). Heat transfer and movement of water flow causes the process of evaporation and drying of food ingredients (Selcuk *et al*. 2010). Warming up the protein can cause both expected and unexpected reactions. Provision of heat causes the protein to be denatured, which in turn loses the binding power of the water and decreases its solubility, so that the protein will be released from meat (Silva *et al* 2017).

The decrease in ash content by 0.2% is caused by the breakdown of mineral particles that are bound to water due to heating so that the minerals in the meat of the cobia fish dissolve into the water when steaming and the water molecules are released from the fish meat tissue (protein). Steaming also causes a decrease in nutrition in an ingredient.

Decrease in fat content by 4.12% due to the giving of heat to cobia fish causes volatile compounds, for example aldehydes, ketones, alcohols, acids and hydrocarbons to evaporate when heating. Fat melts when given heat so that the fat contained in the meat of the cobia fish comes out of the tissue. High temperature heating in the presence of air, fatty acids, aldehydes and ketones which are volatile volatile with water, therefore a decrease in fat content after steaming (Nurjanah *et al*. 2014).

An increase in protein content of 2.16% is thought to be caused by applying heat during steaming of cobia fish meat. Meat protein is divided into 3 based on its solubility namely water soluble protein (sarcoplasma), salt soluble protein (myofibril) and connective tissue protein (stroma).

Table 3. Proximate result of cobia fresh meat and steaming without skin, n=5

Component	Wet basis Fresh (%)	Wet basis Steam (%)	Dry basis Fresh (%)	Dry basis Steam (%)	Fresh* (%)
Moisture content	77.64±0.04	66.33±0.02	0	0	77.14±1.9
Ash	1.1±0.13	0.9±0.768	4.89±0.43	31.39±0.11	1.39 ± 0.11
Protein	10.34±0.45	12.50±0.13	46.22±0.9	19.21±1.1	9.21 ± 1.10
Fat	9.19±0.15	5.07±0.98	41.1±2.7	52.64±1.46	2.64 ± 1.46
Carbohydrate	1.73±0.7	14.81±0.7	7.73±3.2	43.99±3.2	9.62 ± 0.2

*Source: Chuang *et al*. (2010), n=9

Table 4. Essential amino acids content of cobia fish

No.	Amino acids	Fresh (%)	Steam (%)	Fresh * (%)	*Scomber japonicus* steamed (g.a.a./16g N)**
1	Leucine	2.26±0.02	1.38±0.6	3.6±0.5	8.87
2	Arginine	2.26±0.05	1.29±1.0	7.1±7.2	5.58
3	Lysine	1.98±0.4	1.22±0.3	16.0±15.5	7.44
4	Threonine	1.49±0.5	0.96±0.2	2.1±0.3	4.47
5	Isoleucine	1.49±0.1	0.90±0.57	2.3±0.5	4.59
6	Valine	1.41±0.3	0.92±0.42	2.7±0.4	5.85
7	Phenylalanine	1.24±0.45	0.73±0.3	1.7±0.5	4.56
8	Methionine	0.94±0.34	0.58±0.8	2.3±0.7	2.33
9	Histidine	0.85±0.46	0.51±0.9	2.0±1.0	6.49

*Source: Chuang *et al*. (2010) **Sumber: Oduro *et al*. (2011)

Table 5. Non-essential Amino Acid Content of cobia fish

No.	Amino acids	Fresh (%)	Steam (%)	Fresh *	*Scomber japonicus* Steam (g.a.a./16g N)**
1	Glutamic acid	3.90±0.23	2.38±0.3	3.3±0.1	15.99
2	Aspartic acid	2.34±0.34	1.56±0.5	0.3±0.2	10.53
3	Alanine	1.39±0.5	1.14±0.4	11.4±2.0	5.85
4	Glycine	1.38±0.7	1.07±0.7	12.7±2.5	6.19
5	Serine	1.16±0.54	1.29±0.8	1.7±0.7	4.74
6	Proline	1.03±0.34	0.64±0.2	5.2±4.6	5.28
7	Tyrosine	0.99±0.47	0.63±0.4	2.5±0.4	3.11
8	Cysteine	0.36±0.29	1.00±0.5	0.4±0.4	-
9	Taurine	0.12±0.12	0.94±0.9	37.3±12.0	-

*Source: Chuang *et al*. (2010) **Sumber: Oduro *et al*. (2011)

Water-soluble proteins have relatively low molecular weights, high isoelectric pH and spherical structures. These physical characteristics may be responsible for the high solubility of sarcoplasms in water, so that the water-soluble protein contained in the flesh of cobia fish dissolves into steamed water. Water-soluble proteins can inhibit the formation of gels, because these proteins have a low water-binding capacity, so to remove the sarcoplasmic protein, washing can be done with water, for example washing with water, as in the processing of surimi and kamaboko products.

Salt-soluble protein is the largest part in the aquatic meat network of commodities that function for muscle contraction. This protein plays an important role in clumping and gel formation during processing. The effect of heating from the steaming process causes denatured proteins, so that the salt-soluble protein loses its functional properties. Some of the protein myofibrils dissolved during steaming takes place due to high temperatures, but the amount dissolved is not as much as the sarcoplasmic protein. This is because the general nature of this protein is salt soluble. Protein solubility depends on the temperature, the higher the temperature the more denatured proteins.

Amino acids composition

The content of essential amino acids in cobia meat is presented in Table 4. The highest essential amino acid content in fresh cobia meat is leucine and arginine with a value of 2.26%, whereas in steamed cobia meat that is leucine with a value of 1.38%. Cobia fish are fish whose habitat is at sea so they have high levels of ureum in their blood to adjust the osmotic pressure in the seawater environment (Nurhayati *et al* 2017). Arginine in fish is involved in the synthesis of ureum in the liver (Nurjanah *et al* 2014). This is what causes high arginine content. Leucine is the most common amino acid found in food sources of protein, especially fish and meat. The function of leucine in fish is that it plays a role in the body's energy production process and controls protein synthesis, as it is known that the amount of protein is the most in the meat of cobia fish, including amino acids leucine (Schweigert *et al*. 2010).

The non-essential amino acid content of cobia fish meat is presented in Table 5. The highest non-essential amino acid of fresh and steamed cobia meat is glutamic acid of 3.90% and 2.38%. Nonessential amino acids are mostly found in animal muscle tissue, namely alanine, glycine and glutamic acid (Abdullah *et al*. 2013). High levels of glutamic acid are caused by an analysis process that uses acid hydrolysis with a higher degree of analysis. Glutamine amino acids undergo deamination to form glutamic acid so that glutamic acid levels increase. Hidayat (2010) states that glutamic acid is the most important component in the formation of flavor in seafood so that the food tastes delicious. Glutamic acid contains glutamic ion which can stimulate several types of nerves on the human tongue. Cobia fish meat can be said to have a delicious taste.

The results of the analysis of the composition of essential and nonessential amino acids in cobia meat differed from the results of a study conducted by Chuang *et al*. (2010). The difference in amino acid content is thought to be due to various factors namely; type of organ observed, age of harvest and physiological processes of the organism itself (Nurjanah *et al*. 2015). Three amino acids that are not detected are asparagine, glutamine and tryptophan, this is due to the hydrolysis process. The hydrolysis process destroys all tryptophan and cysteine, and if there are metal ions, there will be a loss of methionine and tyrosine (Abdullah *et al* 2017).

Each type of food has a limiting amino acid. This amino acid is an amino acid with the least amount, so it is called a limiting amino acid (Harris and Karmas 1989). The limiting essential amino acid in fresh and steamed cobia fish is histidine with values of 0.85% and 0.51%. Limiting nonessential amino acids in fresh and steamed cobia are cystine with values of 0.36% and 1.00%.

Amino acids of cobia fish meat both essential and nonessential have decreased due to processing. Decreased amino acid content in fish meat after processing is caused by the use of high temperatures. Meat processing using high temperatures causes protein denaturation, so the protein loses its functional properties. Some of the protein dissolved during steaming takes place due to high temperatures (Conrat *et al*. 2010).

Heat treatment aims to make it easier for the body to digest food because complex proteins and carbohydrates change their structure, but the levels of vitamins, minerals and amino acids are reduced. The decrease in amino acid levels is also caused by the formation of the Maillard reaction which is different from the product during heating (Oduro *et al*. 2011).

Each type of amino acid has different characteristics from each other. Effect of processing in general using heat can cause a decrease in the amount of amino acids depending on the type of processing, temperature, and length of processing (Nurjanah *et al*. 2014). A decrease in amino acids that exceeds 10% has a significant effect on the quality of the foodstuff.

Taurine composition

The content of taurine of fresh cobia fish is 120.84 mg / 100 g while steamed cobia meat is 94.33 mg / 100 g. The decreasing taurine content is caused by food processing, namely steaming (Jiancheng *et al*. 2010). Water vapor produced by heat dissolves the taurine that is in food. Abdullah *et al*. (2017) states that taurine is a type of amino acid that is soluble in water. Cooking with high temperatures causes taurine to be released from food and then dissolved in water and come out carried away by water vapor so that its content is reduced. Washing cobia fish meat can also dissolve amino acids.

The levels of taurine in the cobia fish meat results of the study differed from those of Chuang *et al*. (2010). This is allegedly due to differences in age, catch season and stages in the organism's life cycle (Nurjanah *et al* 2015). Taurine in marine animals has the function of regulating osmoregulation to remain balanced (Abdullah *et al*. 2013).

CONCLUSION

Cobia fish meat contains 9 types of essential amino acids and 8 types of nonessential amino acids and taurine. The highest essential amino acid content in fresh cobia meat is leucine and arginine. The highest nonessential amino acid content in fresh cobia meat is glutamic acid. The highest proximate composition is protein. Steaming affects the proximate content, amino acids, and taurine.

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