



Composition of Flavour Non Volatile Compound Steamed Snakehead Fish (*Channa striata*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to identify the composition of non-volatile flavor compounds contained in steamed snakehead fish (*Channa striata*). This research was conducted at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor; Integrated Laboratory of IPB. The study was conducted experimentally by treating samples of fresh snakehead fish and steamed snakehead fish (at a temperature of 100°C for ± 30 minutes). A simple product description test was carried out on samples which included appearance, meat, aroma, texture, and taste. Identification of non-volatile flavor compounds using High Performance Liquid Chromatography (HPLC). The results showed that the product description test of steamed snakehead fish has an external appearance that is almost the same as fresh snakehead fish, the skin peels easily and the eyes of the fish are cooked. The texture of the meat is easy to tear. The distinctive aroma of a specific type of snakehead fish. Steamed snakehead fish has a distinctive taste mixed with the sensation of mud. The non-volatile flavor compounds identified as many as 15 amino acid compounds in the steamed snakehead fish sample. The two umami amino acids are 3,40% glutamic acid and 2,23% aspartic acid. The four sweet amino acids are alanine 1,28%, threonine 1,00%, glycine 0,94% and serine 0,84%. Nine bitter amino acids are lysine 2,12%, leucine 1,76%, arginine 1,37%, valine 1,08%, isoleucine 1,08%, phenylalanine 0,95%, tyrosine 0,76%, methionine 0,66% and histidine 0,55%.

Keywords: Non volatile flavor compounds; steaming; snakehead fish.

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1. INTRODUCTION

Snakehead fish (*Channa striata*) is a type of freshwater fish that is often consumed by the public. National snakehead fish production in 2020 reached 89.292,82 tons, which came from catches in inland public waters [1]. Indonesian people usually use snakehead fish as a source of processed food and non-food products. Snakehead fish is usually processed by frying, steaming, vegetable or smoking. Foodstuffs will have added value if they have undergone a processing process. Food processing is usually carried out using low, high temperature, and fermentation methods. Processing of fishery products is carried out to increase the shelf life because fish is a food that is easily degraded (perishable food) [2].

Food processing by heating divided by temperature and time has four principles: blanching, pasteurizing, sterilizing, and cooking [3]. Cooking aims to improve the taste and preserve food ingredients. The cooking process in terms of shape and cooking method is divided into three: cooking with 100°C hot oil media or frying, cooking with 100°C hot water or steaming, and cooking with keying method 100°C or more [4].

Steaming is a cooking process with hot water steam to ripen food ingredients. Steaming can affect the physical, chemical and product characteristics, especially flavor and texture. The steaming process has the advantage that the nutrients contained are only slightly lost from the food. Steaming has a less significant effect on flavor reduction compared to other heating processes. The steaming process with the right combination of time and temperature can inactivate enzymes properly without preventing excessive changes in texture and taste in food ingredients [3]. Fresh fish raw materials have basic properties and characteristics that will change depending on the heating process carried out. Processing using high temperatures can affect the composition of flavor compounds in fishery products [5].

Flavor is one of the important indicators in human needs in order to improve the taste and consumer acceptance of food products. There are three main components of flavor there are taste, aroma, and oral stimulation [6]. Flavor is an important component in the acceptance of food products by the public. Flavor compound

components are divided into two, namely volatile flavor compounds or components of aroma compounds and non-volatile flavor compounds or taste components [7].

Non-volatile flavors give the impression of sweet, sour, bitter, salty and umami flavors to the sense of taste. Each food product, both fresh and processed, has a specific non-volatile flavor compound with each type. The composition of non-volatile flavor compounds commonly found in fishery food products are amino acids, peptides, organic acids and others. The composition of amino acid compounds that affect the savory or umami taste in fishery products is glutamic acid [7].

Many studies on the composition of amino acid compounds have been carried out such as proximate analysis, amino acid profiles and albumin concentrations of various weights of snakehead fish [8], protein profile of snakehead fish (*Channa striata*), toman fish (*Channa micropeltes*), and betutu fish (*Oxyeleotris marmorata*) [9]; amino acid composition of snakehead fish (*Channa striatus*) [10]. These studies only analyzed the amino acids from fresh snakehead fish without a cooking process and did not discuss their effect on non-volatile flavor compounds or flavor active components.

Research on non-volatile flavor compounds in Indonesia is still dominated by agricultural or plantation products. Fishery products in Indonesia that have been studied for volatile flavor compounds are carp [5], catfish and mackerel, carp [7]. Research on non-volatile flavor compounds has not been widely studied in Indonesia. This study aims to identify the composition of non-volatile flavor compounds contained in steamed snakehead fish.

2. METHODOLOGY

2.1 Time and Place of Research

This research was conducted from February to April 2022, at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University for the sample preparation process. Amino acid profile analysis using the High Performance Liquid Chromatography (HPLC) method was carried out at the Integrated Laboratory, Bogor Agricultural University.

2.2 Materials

The main ingredient used is snakehead fish (*Channa striata*) as much as 2.5 kg from fish sellers around Gedebage, Bandung City. The fish obtained were caught around rice fields and rivers in the Rancaekek area, Bandung. The chemicals used for the analysis of amino acid compounds were Ortho phthalaldehyde or (OPA) 50 mg, sodium hydroxide, 10 g boric acid, 1 ml Brij-30 30% solution, 1 ml 2-mercaptoethanol, 0.5 amino acid standard solution. mol/ml 1 ml, Na-EDTA 5 g, Methanol 200 mL, tetrahydrofuran (THF) 10 mL, Na-acetate 5 g and HP water 2 L. The tools used in this study were Steamer pan, HPLC Ultra Techspere Erlenmeyer flask, knife, plastic wrap, aluminum foil, scale (Tanita) with 0.1 gram accuracy, zip-lock plastic, stove (Rinai), label paper and cool box.

2.3 Research Methods

This research method used is the experimental method. The parameters tested include a simple description test [11] and amino acid profile analysis [12]. Amino acid profile data were analyzed using t test. If $t_{\text{value}} < t_{\text{table}}$, then H_0 is accepted or the steaming process has no significant effect on the amino acid content of snakehead fish. If $t_{\text{value}} > t_{\text{table}}$ then H_0 is rejected or the steaming process has a significant effect on the amino acid content of snakehead fish.

2.3.1 Sampling

The first stage of this research is taking samples of snakehead fish from fish sellers around the Gedebage area, Bandung. The fish obtained were caught around the dam in the Rancaekek area. The snakehead fish sample obtained was 2.5 kg and transported alive using a plastic bag filled with oxygen to the Fishery Products Processing Laboratory, Faculty of Fisheries and Marine Science, Padjadjaran University

2.3.2 Sample preparation

Sample preparation was carried out at the Fishery Products Processing Laboratory, Faculty of Fisheries and Marine Science, Padjadjaran University. Steamed cork fish. Fresh snakehead fish samples were cleaned, weeded, steamed until cooked for ± 30 minutes at 100°C then the meat was weighed 50 grams.

2.3.3 Packaging

The samples tested were weighed by 50 grams. Steamed samples that have been weighed are

packaged using aluminum foil, labeled and covered with cling wrap and then put in zip-lock plastic. The packaged samples were put in a coolbox filled with ice, then brought to the Integrated Laboratory, Bogor Agricultural University, where they were analyzed using High Performance Liquid Chromatography (HPLC).

2.4 Parameters Observation

2.4.1 Snakehead fish product description test [11]

Simple descriptive sensory analysis is a sensory analysis method in which the sensory attributes of a food ingredient are identified and described simply by two semi-trained panelists. The simple descriptive test procedure is that a sample of steamed snakehead fish is prepared, two questionnaires are prepared, the sensory properties of the product are identified by the panelists, the results of the sensory identification of the product are described simply.

2.4.2 Amino acid profile analysis [12]

The first step of amino acid analysis is to dissolve 2 g of the calibrated sample into a 50 mL flask, then filter it with millipore paper. After that, potassium borate buffer pH 10.4 was added in a ratio of 1:1. A sample of 10 L was put into a clean empty vial and added 20 L of OPA reagent and left for 1 minute for complete derivatization. The sample was then injected into the HPLC column as much as 5 L and then waited until the separation of all amino acids was complete. The time required is about 25 minutes.

2.5 Data Analysis

Quantitative data from the analysis of amino acid profiles will be tested using a significance test (t test). The test using the t test is classified as a comparative test which aims to analyze the significance of the effect of the steaming method on the amino acid content of snakehead fish.

3. RESULTS AND DISCUSSION

3.1 Test Description of Steamed Snakehead Fish

The description test data showed that steamed snakehead fish had a body color appearance similar to that of fresh snakehead fish. The aroma of steamed snakehead fish has a specific

aroma of snakehead fish in swamp habitat. The texture of the flesh is compact, dense, and white. Steamed snakehead fish without the addition of seasonings and preservatives has a specific type of taste with a slightly muddy sensation when tested. The characteristic taste of snakehead fish has a balanced intensity of savory and sweet flavors.

Appearance or color is an important parameter in good food. Appearance or color can provide information that there has been a chemical change in the food [13]. Meat color is one of the most important factors affecting meat quality, an indicator of the maturity of the cooking process and consumer preferences [14]. Cooked meat is significantly lighter, less red and more yellow than raw meat [15]. The white flesh of the fish turns slightly yellow and slightly brownish due to the maillard reaction by free ribose [16,17].

Food texture is influenced by several factors including: ratio of protein content, fat, type of protein, processing temperature, water content and water activity [18]. Furthermore [3] added that the texture of food is mostly determined by the water content contained in the product. The higher the water content, the softer the texture of the fish and not crunchy. The cooking process reduces excess water, thereby improving the sensory, physical and chemical qualities of the product and thus increasing the shelf life of the product [19]. Fish meat becomes soft due to protein denaturation and release of polypeptide chains during cooking [17,20] stated in his research that the texture of steamed shrimp is affected by cooking loss due to heat-induced denaturation and protein coagulation.

Aroma is one of the parameters that determine the delicacy of a food product [21] stating that aroma or smell is one of the parameters of delicacy in a food. Aroma or smell has its own charm to determine the delicacy of a food product. Aroma can stimulate the sense of smell to increase appetite. The fishy aroma in fish is caused by nitrogen components, namely guanidine, trimethyl amine oxide (TMAO), and imidazole derivatives [22,23].

The savory taste is associated with the presence of compounds such as glutamic acid or its salts in foodstuffs, such as monosodium glutamate and 5-nucleotide types such as Inosine 5-monophosphate (IMP), guanidine 5-monophosphate (GMP) [24]. Free glutamic acid is present in all fish species and is the most

important contributor to the umami taste and is enhanced by IMP and GMP [39,25]. The sweet taste in food comes from sugar or sucrose, monosaccharides and other disaccharides [24]. Fish usually contain free sugars, namely glucose and ribose [26]. Glycine and alanine are some examples of amino acids that affect sweetness [26]. Free amino acids are generally produced from proteolysis or certain metabolic pathways. Proteolysis can cause an increase in the concentration of amino acids, while amino acid metabolism can decrease the concentration of certain amino acids [27]. Food processing can cause denaturation of muscle proteins such as myosin, actin, and sarcoplasmic proteins [28]. Taste is the response of the tongue to stimuli given by a food which is an important factor that can affect consumers in a food product. Taste is one of the factors that play an important role in determining the final decision [29]. Taste is influenced by several factors, namely temperature, concentration, chemical compounds and interactions with other flavor components [25].

3.2 Identification of Non-volatile Flavor Compounds of Snakehead Fish

The results of the calculation of free amino acids of fresh and steamed snakehead fish can be seen in Table 1. The t-test is used to analyze whether or not the steaming method significantly affects the amino acid content of snakehead fish. The results of the t-test comparison of amino acids of fresh and steamed snakehead fish were obtained $t_{\text{value}} -4.23 < t_{\text{table}} -1.70$. Because $t_{\text{value}} < t_{\text{table}}$, then H_0 was accepted or the steaming method had no significant effect on the free amino acid content of snakehead fish. The free amino acid content of snakehead fish after steaming has increased compared to the amino acid content of fresh snakehead fish. The formation of these free amino acids can be influenced by parameters of processing, storage, fish species and freshness of raw materials. The heating process in snakehead fish samples can increase the amount of free amino acids formed compared to the amount in fresh snake head fish. This can be caused by proteolysis reactions that occur during heating [30,31,5]. Treatment of the cooking process with long hot temperatures can cause protein degradation and modification of meat products [32].

The value of the identified glutamic acid content in steamed snakehead fish is 3.40%. Glutamic acid and aspartic acid are constituents of the

umami (savory) taste, one of the basic tastes used in food sensory testing [33]. Glutamic acid is commonly found in meat, smoked meat, fish, shellfish, seaweed, vegetables (such as mushrooms, tomatoes, chicory, spinach, celery and others or fermented products such as cheese, petis, soy sauce and others [34]. low glutamate can result in a decrease in the savoriness of fishery products [35,7]. The loss of glutamic acid in food is very influential because glutamic acid is an important source of nitrogen and taste perception [36]. Glutamic acid, aspartic acid, alanine, and glycine affect the active components of the taste of raw fish and salted fish [37]. The glutamic acid content in snakehead fish can be used as a flavoring for food because it produces a savory taste (Fitri 2018) [23]. Glutamic acid with glucose is important for brain function [38].

Table 1. Amino acid composition of steamed snakehead fish sample

Amino acid	Value
Aspartic acid	2,23%
Glutamic acid	3,40%
Serine	0,84%
Histidine	0,55%
Glycine	0,94%
Threonine	1,00%
Arginine	1,37%
Alanine	1,28%
Tyrosine	0,76%
Methionine	0,66%
Valine	1,08%
Phenylalanine	0,95%
Isoleucine	1,08%
Leucine	1,76%
Lysine	2,12%
Total amino acid	20,00%

Amino acid composition (mg/100g) of steamed snakehead fish sample

Aspartic acid of steamed snakehead fish was identified as much as 2.23%. Aspartic acid gives a savory taste to food just like glutamic acid [33]. Aspartic acid deficiency causes fatigue and weakness [38,40] in [41] state glutamic acid and aspartic acid have a sour taste but give an umami taste when mixed with sodium salt.

Alanine identified in steamed snakehead fish is 1.28%. Alanine is one of the active components of taste that gives sweetness to aquatic foods [36]. Alanine has a sweet and seafood taste, such as snow crab and shellfish [42,43,67,44]. [45] in his research stated that alanine, glycine, threonine, serine and proline characterize the sweet taste of food. Alanine is used in glucose

metabolism in the body [38]. The amino acid alanine is able to stimulate insulin secretion through two pathways, namely through the sodium co-transport system and the TCA cycle pathway.

Threonine identified in steamed snakehead fish 1.00%. Threonine is one of the amino acids that has a sweet taste [46]. Threonine participates in the maintenance of strong bones and teeth. it accelerates wound healing and reduces fat in the liver. Threonine is also involved in supporting various functions such as cardiovascular, central nervous system, and immune functions, and thymus growth [38,47].

The content of glycine compounds detected in steamed snakehead fish was 0.94%. Glycine is associated with sweetness [48]. Glycine is commonly found in fish that live in swamps [10] [36]. Glycine is required for the proper functioning of the central nervous system [38]. The content of glycine in snakehead fish was identified as being able to heal wounds [10]. Glycine and hydroxyproline are co-indicators of the presence of connective tissue, especially collagen and play a key role in stability [49].

The serine identified in steamed snakehead fish is 0.84%. Serine is associated with a sweet taste-producing amino acid [46]. Serine is an essential amino acid required for fatty acid metabolism and the immune system [38,50] serine and glycine are able to absorb methylglyoxal and formaldehyde in food at pH 7.0 and 37 C. Methylglyoxal and formaldehyde react with glycine and serine to form imidazole salts.

Arginine identified in steamed snakehead fish is 1.37%. [41] stated arginine is one of the amino acids that produces a bitter taste and has a weak sweet taste sensation. The concentration of the active value of arginine taste below 1 can increase the salty taste and give the sea urchin an umami taste [51,52,7]. High free arginine content can cause a seafood-like flavor and increase the sweetness of crustaceans [7]. The bitter taste caused by arginine can be masked by glutamate, NaCl, and Adenosine Monophosphate (AMP) [53]. Arginine is very important in the wound healing process [10]. Arginine is able to stimulate growth hormone from the pituitary and insulin from the pancreas [54]. Arginine has been shown to reduce urinary nitrogen loss to promote positive nitrogen balance in post-injury catabolism [55]. Arginine has another function as a fat destroyer [8].

Lysine identified in steamed snakehead fish was 2.12%. Lysine contributes to the bitter taste in foods [31]. Lysine has been shown to have a role in growth [56,57,8]. Lysine is known to be one of the amino acids that can induce albumin synthesis [58]. The amino acid lysine is the largest constituent of albumin protein in snakehead fish which is easily damaged due to heat which causes a decrease in protein levels [9]. Lysine has an important role because it is part of the basic composition of antibodies, strengthens circulation and maintains normal cell growth [7,59] showed that treatment with a mixture of the amino acids lysine and arginine resulted in higher neuroendocrine activation in response to psychosocial stress in subjects with high trait anxiety. Lysine is a useful drug preparation for the prevention of herpes and herpes [47]. A mixture of lysine and yeast extracts can reduce the sensory unpleasant taste in salted meat [60]. Lysine present in chicken feed can increase the glutamic acid content in broiler breasts [61].

The content of steamed snakehead fish leucine compound is 1.76%. Leucine is an amino acid compound associated with bitter taste [31,45]. Leucine is very effective in producing other important biochemical compounds in the body. These chemicals are important for producing energy for the body and mental alertness. The main function of leucine is as one of the 3 branched chain amino acids (2 others are valine and isoleucine), leucine has all the properties discussed with isoleucine, because it relates specifically to the function of branched chain amino acids, insulin protein simulator, helps bone healing, helps increase healing skin, modulating the release of natural pain-reducing enkephalins [62]. Leucine functions in increasing the synthesis of growth hormone [63]. Leucine is a source of energy in the body, helps regulate blood sugar and aids muscle recovery after exercise. Clinically improves body healing and affects brain function (Šimat et al. 2020). Leucine and isoleucine regulate blood sugar levels [38].

Valine identified was 1.08% in steamed snakehead fish. Valine is one of the free amino acid compounds that produces a bitter taste in food [31]. Valine is one type of amino acid that can heal wounds [10]. Valine has an important role in muscle metabolism and maintaining nitrogen balance [64]. The removal of the active flavor component valine in food can sweeten and umami tastes and increase a little sour taste [41]. Valine and Leucine belong to the

hydrophobic amino acid group [65]. Valine deficiency can cause muscle coordination and the body becomes very sensitive to pain, heat and cold [62].

The isoleucine detected in the steamed snakehead fish sample was 1.08%. Isoleucine is associated with bitter taste [46]. The main function of isoleucine in the body is to promote energy levels and to help the body recover after physical activity [47]. Isoleucine and leucine regulate blood sugar levels [38].

Phenylalanine identified in steamed snakehead fish was 0.95%. Phenylalanine is one of the amino acid compounds that produces a bitter taste [46]. Phenylalanine plays a role in regulating mood and promoting balanced neural and cognitive function. Phenylalanine is effective in the central nervous system and rapid learning and is used in the treatment of depression [47].

The content of tyrosine compounds detected in steamed snakehead fish was 0.76%. Tyrosine is an appetite suppressant supplement, supports weight loss, and improves memory. Tyrosine, tryptophan and phenylalanine are useful for the treatment of a genetic disorder called phenylketonuria [47]. Lack of tyrosine causes hypothyroidism [38]. Cysteine is useful in wound healing after surgical operations and in increasing white blood cell activity [38].

The content of methionine compounds detected in steamed snakehead fish was 0.66%. Methionine is one of the bitter-tasting amino acids detected in meat foods [46]. Methionine prevents the formation of fat in the arteries and the development of allergies and is effective in the treatment of osteoporosis [38]. Methionine is an amino acid that is important for fat metabolism, maintaining liver health, preventing fat accumulation in the liver and main arteries, preventing allergies and osteoporosis [35].

The content of histidine compounds detected in steamed snakehead fish was 0.55%. Histidine is associated with a bitter taste [31]. Histidine is found in many migratory fish such as tuna, skipjack, mackerel, and eel [36]. Histidine is very important because it has physiological and nutritional functions for fish. High histidine content in migratory fish to maintain muscle pH levels during swimming. Histidine also acts

as an energy source during prolonged starvation in migratory fish. Histidine in the muscle tissue of pelagic fish has a buffering effect, protecting the tissue from the sudden increase in lactic acid produced during the process of anaerobic powered muscle activity [66,47].

Tryptophan is very important as an amino acid compound that provides feelings of comfort, pleasure and reduces stress [68,69]. Tryptophan helps in weight loss and sleep disorders [47]. Tryptophan is required for the production of growth hormone and vitamin B6 [38].

4. CONCLUSION

Based on the description of the discussion, it can be concluded that steamed snakehead fish has a body color appearance similar to fresh cork fish. The aroma of steamed snakehead fish is specific to the type of snakehead fish in the swamp habitat. The texture of the flesh is compact, dense, and white. Steamed snakehead fish has a savory and sweet taste that is equally dominant. The non-volatile flavor compounds identified as many as 15 amino acid compounds in the steamed snakehead fish sample. The two umami flavored amino acids are 3.40% glutamic acid and 2.23% aspartic acid. The four sweet-tasting amino acids are alanine 1.28%, threonine 1.00%, glycine 0.94% and serine 0.84%. Nine bitter amino acids are lysine 2.12%, leucine 1.76%, arginine 1.37%, valine 1.08%, isoleucine 1.08%, phenylalanine 0.95%, tyrosine 0.76%, methionine 0, 66% and histidine 0.55%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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