Original Research Article

Composition of Flavour Non Volatile Compound Steamed Milk Fish (*Chanos chanos*)

ABSTRACT

This research aims to identify the composition of non-volatile flavor compounds contained in steamed milkfish *(Chanos chanos).* This research was conducted at the Fisheries Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor; IPB Integrated Laboratory. The study was conducted experimentally by treating a sample of 100 grams of steamed milkfish (at 100°C for ± 30 minutes) to identify non-volatile compounds and 1 milkfish to test the description. Product description tests were carried out on samples which included appearance, flesh, aroma, texture, and taste. Identification of non-volatile flavor compounds using High Performance Liquid Chromatography (HPLC). The results showed that steamed milkfish had 15 non-volatile flavor compounds identified from 15 amino acid compounds. The two umami-flavoring amino acids are 3.21% glutamic acid and 2.14% aspartic acid. The four sweet tasting amino acids are 1.31% alanine, 0.96% threonine, 1.13% glycine and 0.72% serine. The nine bitter amino acids are lysine 2.15%, leucine 1.74%, arginine 1.32%, valine 1.26%, isoleucine 1.16%, phenylalanine 0.95%, tyrosine 0.79%, methionine 0. .69% and 0.90% histidine. Steamed milkfish has a whole appearance with a bright yellowish color. The savory aroma of fresh steamed milkfish is in accordance with the characteristics of milkfish that live in brackish water. The texture of the meat is dense and very dense. The taste of savory steamed milkfish is according to its kind.

Keywords: non volatile flavor compounds, steaming, milk fish

1. INTRODUCTION

Indonesia has excellent freshwater, brackish water, and marine fishing potential. With ponds estimated to total 931,000 ha, nearly all of the potential for brackish water fisheries has been employed, with the majority being used for milkfish (Chanos chanos) production. Milkfish is a brackish water fisheries product that the general people frequently consumes [1]. The popularity of milkfish is growing every year, which is attributable to its excellent flavor, tender flesh that doesn't crumble when cooked, and reasonable selling price, which makes it accessible to all social classes [2].

Milkfish is frequently used by Indonesians as a source of processed food and non-food. Typically, milkfish is prepared by frying, steaming, adding vegetables, smoking, and other methods. If food has been processed, it will have gained value. Food is typically processed utilizing low, high, and fermentation techniques. Fish is a perishable food, hence processing is done to fishery products to extend the shelf life [3].

Many milkfish are processed traditionally. Traditional processing that has been widely carried out can affect the flavor characteristics of the product, for example high temperature processing such as steaming which is generally widely used in typical West Javanese dishes and is known as pepes. Processing with high temperatures (thermal processes) is one of the most important methods in processing food products because it can have the desired effect on food quality such as ripening and the formation of certain flavors, also has a preservative effect and improves nutrients in relatively simple processing [4];[5]. Steaming can affect physical changes, chemical reactions and product characteristics, especially flavor and texture. According to [5], the danger of losing vitamins and other food molecules that are sensitive to high temperatures can be reduced when using water vapor as a heat source during the steaming process. Fresh fish, a raw material, has fundamental qualities and traits that will alter and be impacted by the cooking process. According to [6], processing fisheries products at high temperatures is anticipated to influence the taste components' composition.

Flavor is a combination of taste and aroma when food is consumed. Flavor is also an important indicator in fulfilling human needs in order to increase the taste and consumer acceptance of food products. According to [7] flavor or taste is an impression or sensation produced by food ingredients that is received by the human senses, especially the impression or sensation caused by taste and aroma. In general, flavor is an important factor in the final food product, especially in processed fishery products, because flavor will affect the level of acceptance and consumption of the finished product. Flavor compounds found in fish usually come from protein and fat compounds. Flavor compounds in general can be divided into volatile and non-volatile flavors.

Non-volatile components are components that give taste sensations such as sweet, bitter, sour, salty and umami to the taste buds. The components that are the main constituents of the taste of seafood are components that are water soluble and have a low molecular weight except minerals, vitamins and pigments. Nucleotide and peptide group compounds are the main compounds of non-volatile components. Non-volatile flavor compounds can influence the taste characteristics of a food product and usually come from compounds of the amino acid, peptide, and nucleotide groups [8];[9]. Amino acids contained in fishery products can provide information regarding the types of amino acids that affect the process of flavor formation.

There has been a lot of research done on non-volatile flavors for fishery products outside of Indonesia, but less on non-volatile flavors for fishery products inside of Indonesia. [10] research, which compares the biochemical composition and non-volatile taste active components in raw and steamed oysters, is one of several studies on the non-volatile flavor composition of fisheries commodities conducted abroad. According to [11], milkfish is popular because it is excellent and reasonably priced. The high protein level of milkfish around 20–24%, comprised of 1.23% glutamic amino acid and 2.25% lysine is what gives it its savory flavor.

Research on the identification of the composition of non-volatile flavor compounds in milkfish has not been found in Indonesia. Identification of the characteristics of non-volatile flavor compounds of a commodity can be used as data for mapping the flavor composition of a product. This data can be used as a basic data for the application of a product, one of which is flavoring agent. In addition to data on analysis of non-volatile flavor compounds, organoleptic tests are also required to determine whether the product is acceptable to consumers. Organoleptic test is a method used to test the quality of a material or product using the human senses. Based on the above background, research on the composition of non-volatile flavor compounds in fresh and steamed milkfish needs to be investigated.

2. METHODOLOGY

2.1 Time and Place of Research

This research was conducted from September to November 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 milk fish (*Chanos chanos*) as much as 2.5 kg from Karangsong, Indramayu. The chemicals used for the analysis of amino acid compounds were Orthophthalaldehyde 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

The research method used is an experimental method using HPLC tools to identify non-volatile compounds and test descriptions. Amino acid profile data are discussed in a comparative descriptive manner which aims to identify a parameter by comparing more than one sample or at different times [12].

2.3.1 Sampling

The first stage of this research was sampling milkfish from fish sellers at TPI Karangsong, Indramayu. Milkfish samples obtained were 2.5 kg and then transported using a coolbox and curai ice with a ratio of 1:3 to the FPIK UNPAD Fishery Products Processing Laboratory.

2.3.2 Sample Preparation

Sample preparation was carried out at the FPIK UNPAD Fisheries Processing Laboratory. Samples of steamed milkfish were cleaned, weeded and then weighed as much as 100 grams to identify non-volatile flavor compounds and 1 milkfish for organoleptic testing, then steamed until cooked for ± 30 minutes at 100^{o}C.

2.3.3 Packaging

The weighed, steamed samples are then wrapped in aluminum foil, tagged, and then coated once more with cling wrap before being placed in zip-lock bags. Before being transported to the Integrated Laboratory at the Bogor Agricultural Institute for analysis using high performance liquid chromatography, packaged samples were placed in a coolbox with ice (HPLC).

2.4 Parameters Observation

2.4.1 Amino Acid Profile Analysis

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.

2.4.2 Snakehead Fish Product Description Test

Simple descriptive sensory analysis is a sensory analysis method in which the sensory attributes of a food ingredient are identified and described. The simple descriptive test procedure was a sample of steamed milkfish was prepared, a questionnaire was prepared, the sensory properties of the product were identified by the researcher, the results of the product sensory identification were described.

2.5 Data analysis

Steamed milkfish samples were tested using the HPLC tool. Quantitative data from the analysis of the amino acid profile are discussed in a comparative descriptive manner. The goal is to identify a parameter by comparing more than one sample or at different times [12].

3. RESULTS AND DISCUSSION

3.1 Identification of Non-volatile Flavor Compounds of Snakehead Fish

The analysis of non-volatile taste components using liquid chromatography showed that the steamed milkfish sample included 15 different types of amino acids. Essential amino acids and non-essential amino acids are the two types of amino acids found in the sample of steamed milkfish. Aspartic acid, glutamic acid, arginine, and alanine, which are non-essential amino acids, were the most prevalent amino acid compounds found in the steamed milkfish sample, accounting for 3.21%, 2.14%, 1.32%, and 1.32 %. Lysine, leucine, and valine make up the majority of the essential amino acids in steamed milkfish (2.15%, 1.74%, and 1.26%, respectively).

The factors of the processing, storage, fish type, freshness of the raw materials, feed, and environment can affect the production of these free amino acids. According to [13], fish habitat may have an impact on the chemical composition of the flesh, including the amino acids and fatty acids. In comparison to fresh milkfish, heating milkfish samples can result in a greater formation of free amino acids. The proteolysis reaction that takes place during heating to blame for this [14];[15];[16]. Table 1 shows the results of the analisys amino acid composition of steamed milkfish.

Table 1. Amino acid composition of steamed milk fish sample

Amino Acid	Value (%)
Asam aspartat	2,14
Asam glutamat	3,21
Serin	0,72
Histidin	0,90
Glisin	1,13
Treonin	0,96
Arginin	1,32
Alanin	1,31
Tirosin	0,79
Metionin	0,69
Valin	1,26
Fenilalanin	0,95
Isoleusin	1,16
Leusin	1,74
Lisin	2,15
Total Asam Amino	**20,45**

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

According to [17] the reduction in water content that occurs during the steaming process is associated to variations in protein levels in fish. The amount of water in the food will decrease as the temperature is raised while the amount of protein in the food will rise. The amount of protein damage that occurs in the food increases as processing temperature and duration increase. Milkfish has a protein level of 20–24%, which is higher than that of goldfish (16.04%) and snapper (15.61%) [11];[16].

The value of glutamic acid content identified in steamed milkfish is 3.21%. Glutamic acid and aspartic acid are the building blocks of umami (savory) taste, one of the basic tastes used in food sensory tests [18]. 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, paste, soy sauce and others [19]. This study according to [20] that the most abundant amino acid component found in steamed crab meat *(Portunus pelagicus)* is glutamic acid of 2.41%.The content of different amino acids in fish is influenced by differences in species, sex, season. , habitat, eating habits, swimming activities, gonadal maturity level, and processing [21] Glutamic acid is produced from the conversion of glutamine by glutaminase or from the results of proteolysis [22]. Milkfish has a high glutamate content so that milkfish has a savory taste [11] Glutamic acid has a very important role in food processing, because it can cause a delicious taste, in seasonings. u cook containing monosodium glutamate [23].

The high glutamic acid in meat is because glutamic acid is a natural component in almost all foods that contain high protein such as fish meat. Glutamic acid can be produced in the human body and is a very important component of human metabolism. Glutamate has the characteristic that when added to a food ingredient it will give it a strong taste characteristic and stimulate the nerves on the human tongue. The salt derived from glutamate, known as monosodium glutamate, is very well known as a food flavoring. According to [24], glutamate is a savory flavor enhancer of ~~a~~ food. Glutamic acid is an amino acid that can be obtained from glutamine and asparagine respectively, the amide group present in the glutamine and asparagine molecules can be converted into a carboxylic group through a process of hydrolysis with acids or bases.

Milkfish that had been steam cooked contained as much as 22.14% aspartic acid. According to earlier studies, seluang fish has a higher content of non essential amino acids as a result of the cooking procedure. Similar to how glutamic acid does, aspartic acid imparts a savory flavor to food [18]. Weakness and exhaustion are symptoms of aspartic acid deficiency [25]. According to [26];[27], when combined with sodium salt, glutamic acid and aspartic acid have a sour taste but impart an umami flavor.

The serine compound identified in steamed milkfish is 0.72%. [28] states that serine is associated as an amino acid that produces a sweet taste. Serine is an important amino acid needed for fatty acid metabolism and the immune system [25]. [29] stated in their research that serine and glycine were able to absorb methylglyoxal and formaldehyde in food at a pH of 7.0 and 37 C. Methylglioxal and formaldehyde reacted with glycine and serine to form imidazole salts.

The histidine compound detected in steamed milkfish was 0.90%. Histidine is an amino acid compound which is known to give a bitter taste but not as bitter as phenylalanine (Kato et al. 1989). Histidine is commonly found in migratory fish such as tuna, skipjack, mackerel and eels [21]. The histidine content in migratory fish is high to maintain muscle pH levels

during swimming. Histidine also acts as a source of energy during prolonged starvation in migratory fish. Histidine in pelagic fish muscle tissue has a buffering effect, protecting the tissue from the sudden increase in lactic acid produced during anaerobic-powered muscle activity [30];[31].

Histidine has systemic, anti-inflammatory functions in the body and plays a dual role in protein interactions. It is necessary for tissue growth and repair, maintenance of myelin sheaths, increased blood flow, removal of excess heavy metals from the body and ~~for~~ protection from radiation. Histidine also helps with memory and cognitive function, the immune system and stomach acid regulation. Important for tissue growth and repair and blood cell production [25].

The content of glycine compounds detected in steamed milkfish was 1.13%. According to [32], glycine is an amino acid component that contributes to the sweet taste of food. Glycine is commonly found in fish that live in swamps [21]. Glycine is necessary for the proper functioning of the central nervous system [25]. The glycine content in fish can function in accelerating wound healing because it contains albumin and protein. Glycine and hydroxyproline are joint indicators of the presence of connective tissue, especially collagen and play a key role in stability [25].

Threonine identified in steamed milkfish was 0.96%. Threonine is an amino acid that has a sweet taste [28]. Threonine participates in the maintenance of strong bones and teeth, accelerates wound healing and reduces fat in the liver, maintains protein balance, plays a role in the formation of collagen and elastin. Threonine is also involved in supporting various functions such as cardiovascular function, central nervous system, and immunity, and growth of the thymus [25];[31].

The arginine compound identified in steamed milkfish is 1.32%. [26] Stated that 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 below 1 can increase the salty taste and give sea urchins an umami taste [33];[34]. The high arginine content can give rise to a seafood-like flavor and increase the sweetness of the crustaceans [34]. The bitter taste elicited by arginine can be masked by glutamate, NaCl, and Adenosine Monophosphate (AMP). Arginine is able to stimulate growth hormone from the pituitary and insulin from the pancreas. Arginine has been shown to reduce urinary nitrogen loss to promote positive nitrogen balance in post-injury catabolism [35].

The amino acid alanine identified in steamed milkfish was 1.31%. Alanine is one of the active flavor components that gives a sweet taste to aquatic foods [21]. Alanine has a sweet taste and seafood, such as snow crab and clams [36]. [32] 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 [25]. 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.

Tyrosine compound detected in steamed milkfish was 0.79%. Tyrosine is an amino acid compound that produces a bitter taste [28]. The biochemical reactions of tyrosine are glycogenic and ketogenic. Tyrosine is a non-essential amino acid that is neutral and polar (hydrophilic/water loving) which is uncharged. Tyrosine is also a compound that has an aromatic chemical structure. Tyrosine can be a supplement that suppresses appetite, supports weight loss, and improves memory. Tyrosine, tryptophan and phenylalanine are useful for treating a genetic disorder called phenylketonuria [31]. Lack of tyrosine causes hypothyroidism [25]. Cysteine is useful in wound healing after surgery and in increasing white blood cell activity [25].

Methionine compound detected in steamed milkfish was 0.69%. Methionine is a taste amino acid that contributes to a bitter taste in foodstuffs [28]. Methionine is Methionine which is an amino acid that is important for fat metabolism, maintains liver health, prevents accumulation of fat in the liver and major arteries, prevents allergies and osteoporosis. Methionine prevents the formation of fat in the arteries and the development of allergies and is effective in the treatment of osteoporosis [25]. Methionine in the human body also functions as a supplier of sulfur, an intermediary in phospholipid biosynthesis, preventing liver disease, preventing inflammation, and lowering cholesterol.

Valine compound identified in steamed milkfish was 1.26%. Valine is a class of hydrophobic amino acid compounds that contribute to producing a bitter taste in food but is not as bitter as phenylalanine [15]. Valine is a type of amino acid that can accelerate the wound healing process. Valine has an important role in muscle metabolism and maintaining nitrogen balance. [26] that the removal of the active taste component of valine in food can increase the sweet and umami taste and slightly increase the sour taste. Valine is one of the three branched-chain amino acids (two of which are leucine and isoleucine) which play a role in growth, the nervous system, digestion, increasing energy, increasing endurance, and lowering blood sugar levels [37].

Phenylalanine compound identified in steamed milkfish was 0.95%. Phenylalanine is an amino acid compound that produces a bitter taste [28]. Phenylalanine plays a role in regulating mood and optimizing a balanced nervous and

cognitive work system. Phenylalanine is effective in the central nervous system and is fast learning and is used in the treatment of depression [31].

Isoleucine detected in steamed milkfish samples was 1.16%. Isoleucine is an active compound that gives a bitter sensation to food ingredients [28]. The main function of isoleucine in the body is to increase energy, help the body recover after physical activity, and regulate blood sugar levels [25];[31].

Leucine compounds in steamed milkfish by 1.74%. Leucine is an amino acid compound associated with a bitter taste but not as bitter as phenylalanine [15];[31]. 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. Leucine is an important molecule that can stimulate muscle synthesis and also has a therapeutic role in stressors such as trauma, burns, etc. The main function of leucine is as one of the 3 branches of the amino acid chain (the other 2 are valine and isoleucine). Leucine functions in increasing the synthesis of growth hormone. Leucine is a supplier of energy in the body, helps regulate blood sugar and helps muscle recovery after exercise. Clinically improves body healing, aids bone healing, aids skin healing, influences brain function, and modulates the release of enkephalins which reduce pain naturally [31].

Lysine identified in steamed milkfish was 2.15%. Lysine contributes to a bitter taste in food [15]. Lysine has been shown to have a role in growth. Lysine content in fish can function in accelerating wound healing because it contains albumin and protein. Lysine has an important role because it is part of the basic composition of antibodies, strengthens circulation and maintains normal cell growth [34]. [38] 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.

3.2 Test Description of Steamed Milk Fish

The steam milkfish description test showed that steamed milkfish had a intact appearance with a slightly yellowish bright color. The aroma of steamed milkfish is fresh, savory according to the specifics of milkfish that live in brackish water. The texture of the flesh is dense and very compact. The taste of steamed milkfish is savory according to the specific type.

Color is an important parameter in food, whether it is food that has gone through the manufacturing process or not gone through the manufacturing process. Foodstuffs that are considered nutritious will not be consumed if they have an unsightly color or give the impression of deviating from their proper color [39]. Color can provide information that a chemical change has occurred in food [40]. Meat color is one of the most important factors affecting meat quality, an indicator of the maturity of the cooking process and consumer preferences. Cooked meat is significantly lighter, less red and more yellow than raw meat. The white flesh of the fish turns slightly yellow and slightly brownish due to the Maillard reaction by free ribose. [39] states that the cooking process using high temperatures affects the color of the fish.

Aroma is one of the parameters that determine the delicacy of a food product [41]. [39] states that aroma or smell is one of the parameters of good taste 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 smell in fish is caused by nitrogen components, namely guanidine, trimethyl amine oxide (TMAO), and imidazole derivatives [42].

Taste is the tongue's response to stimuli provided by ~~a~~ food which is an important factor that can affect consumers ~~in~~ a food product. Taste is a factor that plays an important role in determining the final decision [43]. Taste is influenced by several factors, namely temperature, concentration, chemical compounds and interactions with other taste components. The savory taste of milkfish is associated with the presence of compounds such as glutamic acid or its salts in foodstuffs, for example monosodium glutamate and 5-nucleotide types such as inosine 5-monophosphate (IMP), guanidine 5-monophosphate (GMP) [44]. Glutamic acid is present in all fish species and is the most important contributor to umami taste and is enhanced by IMP and GMP [27].

The texture of food is influenced by several factors, including: the ratio of protein content, fat, type of protein, processing temperature, water content and water activity. [5] states that the texture of food is mostly determined by the water content contained in the product. The higher the water content makes the texture of the fish softer and not crunchier. The cooking process reduces excess water, thus improving the sensory, physical and chemical qualities of the product and thereby increasing the shelf life of the product. Fish meat becomes soft due to protein denaturation and release of polypeptide chains during cooking [45].

4. CONCLUSION

Based on the description of the discussion, it can be concluded that steamed milkfish has identified non-volatile flavor compounds as many as 15 amino acid compounds. Two umami-flavored amino acids are 3.21% glutamic acid and 2.14%

aspartic acid. The four sweet tasting amino acids are 1.31% alanine, 0.96% threonine, 1.13% glycine and 0.72% serine. The nine bitter amino acids are lysine 2.15%, leucine 1.74%, arginine 1.32%, valine 1.26%, isoleucine 1.16%, phenylalanine 0.95%, tyrosine 0.79%, methionine 0, 69% and 0.90% histidine. Steamed milkfish has an intact appearance with a bright yellowish color. The aroma of steamed milkfish is fresh, savory according to the specifics of milkfish that live in brackish water. The texture of the flesh is dense and very compact. The taste of steamed milkfish is savory according to the specific type.

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