Original Research Article

Composition of Flavour Non Volatile Compound Steamed Gourami (*Osphronemus gouramy*)

ABSTRACT

This research aims to identify the composition of non-volatile flavor compounds contained in the steamed gourami (*Osphronemus gouramy*). The research was carried out experimentally with the treatment of steamed gourami samples (at 100℃ for ± 30 minutes). This research was conducted at the Fisheries Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor; IPB Integrated Laboratory. A simple product description test was carried out on samples which included the appearance of the meat, aroma, texture, and taste. Identification of non-volatile flavor compounds using tools High Performance Liquid Chromatography (HPLC). The results showed that steamed gourami has 15 identified non-volatile flavor compounds of 15 amino acid compounds. Two umami-flavored amino acids are 4.09% glutamic acid and 2.67% aspartic acid. The four sweet tasting amino acids are 1.53% alanine, 1.19% threonine, 1.13% glycine and 1.01% serine. The nine bitter amino acids are lysine 2.54%, leucine 2.11%, arginine 1.64%, valine 1.30%, isoleucine 1.29%, phenylalanine 1.14%, tyrosine 0.91%, methionine 0, 79% and histidine 0.66%. Steamed gourami has an intact appearance and is pale white in color. The aroma of steamed gourami is fresh and tasty according to the specific gourami. The texture of the meat is dense, soft and very compact. The taste of steamed gourami is savory according to the specific type.

Keywords: non volatile flavor compounds, steaming, gourami

1. INTRODUCTION

Indonesia is a maritime country that has vast territorial waters, very large land, and sea fisheries resources. Inland aquaculture is one of Indonesia's fishery production potentials which has increased every year. Types of fish farming developed in Indonesia include gourami, carp, catfish, tilapia, and milkfish [30]. One of the very potential commodities is gourami (*Osphronemus gouramy*). Gourami is one of the main commodities of freshwater aquaculture with a production volume of 149,170 tonnes in 2021, as well as cultivated catfish, carp, tilapia [59].

Gourami has a high economic value, when compared to other freshwater fish commodities [4];[40]. According to [79], many gourami are traded fresh, both alive and dead. The market demand for gourami is quite high, because gourami has a good taste of meat, relatively easy maintenance, and a relatively stable price. In addition, gourami is a commodity with high nutritional content which is beneficial for humans for growth and formation of energy. Traditionally, gourami is usually processed by frying, steaming, or baking, with added spices.

Traditional processing that is commonly carried out can affect product flavor characteristics, such as high temperature thermal processing in the manufacture of pepes which is a typical food menu of West Java. This thermal process is one of the most important methods in food processing because it results in the ripening and formation of specific flavors required for food quality, product shelf life, improved nutrient availability, and relatively easy to control processing conditions [17];[20]. The thermal process that is often used is the steaming method (wet heating). According to [65], steaming is a high-temperature processing method using water vapor as a heat-conducting medium. The use of water vapor as a heat source can reduce the risk of possible nutritional loss or loss of vitamins and food compounds that are sensitive to high temperatures [20]. The heating process can affect and change the basic characteristics of fresh fish used as raw materials. Raw materials that are heated will experience changes in physical, chemical, and product characteristics, especially flavor and texture.

Flavor is all impressions or sensations that can be felt by the human senses, especially through taste and smell when consuming a food product [53]. According to [8], flavor is formed from a combination of experiences and sensations received on the characteristics of the material. Flavor is an important factor in fresh and processed food, especially in products made from fish. This happens because flavor can affect the level of consumer acceptance, preference, and consumption of a product. According to [49], the chemical composition and flavor of various types of fishery commodities have differences both in fresh and processed forms. The flavor characteristics of a commodity are influenced by compounds which are divided into two groups, including volatile and non-volatile flavor compounds.

Volatile components contained in flavors are components that give the sensation of aroma or smell, give an initial impression, and can evaporate quickly. Non-volatile components produce sensations of taste, such as sweet, sour, salty and bitter. This component does not provide an aroma sensation but becomes a medium for volatile components, and holds back the evaporation of volatile components. The taste characteristics of a product are influenced by non-volatile flavor compounds which usually come from peptide group compounds, amino acids, and also nucleotides [9];[50];[51]. Non-volatile extractive components containing nitrogen such as free amino acids play an important role in flavoring fishery products [77]. Based on opinion [13], amino acids and peptides play a direct role in the flavor of processed fishery products. The types of amino acids that influence the flavor formation process can be identified from the amino acids contained in fishery products [50].

Research on the non-volatile flavor of fishery commodities has been widely carried out abroad, but research on the non-volatile flavor of fishery commodities has not been widely carried out in Indonesia. An example of research on the non-volatile flavor composition of fishery commodities abroad was conducted by [6], namely regarding the effect of cultivating seawater with different salinities on non-volatile active flavor compounds in pacific oysters (*Crassostrea gigas*), as well as research [36] regarding the influence of high hydrostatic pressure and storage temperature on fatty acids and non-volatile flavor active compounds crayfish red claw (*Cherax quadricarinatus*).

Research on the identification of the composition of non-volatile flavor compounds in gourami (*Osphronemus gouramy*) has not been found in Indonesia. Characteristics of non-volatile flavor compounds of a commodity are identified to be used as mapping data for the flavor composition of a food product. Information regarding non-volatile flavor compounds is important to analyze the effect of the steaming method on the composition of non-volatile flavor compounds that affect the taste of a product. This data can be used as basic data as well as a reference in various applications, including for the manufacture of a product, one of which is flavor extract. In addition to data analysis of non-volatile flavor compounds, it is also necessary to carry out organoleptic tests. Organoleptic test is also called subjective measurement and has a high relevance to product quality and quality control. The organoleptic test used is a descriptive test which functions to identify and get a complete picture of the important sensory characteristics of a product, as well as provide information about the degree or intensity of these characteristics. Based on the background that has been described, it is necessary to conduct research on the composition of non-volatile flavor compounds in fresh gourami and its steamed products.

2. METHODOLOGY

2.1 Time and Place of Research

This research was conducted from December 2022 to February 2023, taking place at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University for sample preparation and organoleptic tests. While the analysis of the amino acid profile using the method High Performance Liquid Chromatography (HPLC) was conducted at the Integrated Laboratory, Bogor Agricultural University.

2.2 Materials

The main ingredient used is gourami (*Osphronemus gouramy*) as much as 2.5 kg from the fish cultivation site in the Cirata Reservoir, Purwakarta. The chemicals used for the analysis of amino acid compounds were Orthophthaldehyde or (OPA) 50 mg, sodium hydroxide, 10 g boric acid, 1 ml Brij-30 30% solution, 1 ml 2-mercaptoethanol, 0.5 standard amino acid solution. μmol/ml 1 ml, Na-EDTA 5 g, Methanol 200 mL, tetrahydrofuran (THF) 10 mL, Na-acetate 5 g and HP 2 L water. The tools used in this study were steamer, HPLC Ultra Techspere, erlenmeyer, knife, plastic wrap, aluminum foil, scales (Tanita) with an accuracy of 0.1 gram, plastic zip-lock, stove, label paper and cool box.

2.3 Research Methods

The method used in this research is an experimental method including analysis of non-volatile flavor compounds and simple descriptive tests. Data analysis of non-volatile flavor compounds with the method of analysis of amino acid compounds through High Performance Liquid Chromatography (HPLC) which will then be analyzed descriptively comparatively.

2.3.1 Sampling

The first stage of this research was taking gourami samples from fish farms in the Cirata Reservoir, Purwakarta. The 2.5 kg gourami samples obtained were transported alive and fresh in plastic filled with water and oxygen to the FPIK UNPAD Fishery Products Processing Laboratory, Jatinangor.

2.3.2 Sample Preparation

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

2.3.3 Packaging

Samples of steamed gourami that have been weighed are packed using aluminum foil, labeled and re-coated using cling wrap then put in plastic zip-lock. The packed sample is put inside a coolbox containing ice, then taken to the Integrated Laboratory, Bogor Agricultural University. Samples were analyzed using High Performance Liquid Chromatography (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 sample that has been measured in a 50 mL flask, then filter it with millipore paper. After that, potassium borate buffer pH 10.4 was added in a 1:1 ratio. a 10 µL sample was put into a clean empty vial and added 20 µL OPA reagent and left for 1 minute so that the derivatization was complete. The sample was then injected into the HPLC column as much as 5 µL and then waited until the separation of all amino acids was complete. The time required is about 25 minutes.

2.4.2 Gourami Fish Product Description Test

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

2.5 Data Analysis

Quantitative data from the analysis of amino acid profiles will be discussed in a comparative descriptive manner. Comparative descriptive research is research that compares one or more variables in two different samples or at different times [61].

3. RESULTS AND DISCUSSION

3.1 Identification of Non-volatile Flavor Compounds of Gourami Fish

The results of liquid chromatography analysis of non-volatile flavor compounds showed that there were 15 types of amino acids detected in the steamed gourami samples. The amino acids contained in the steamed gourami samples were divided into two, namely essential amino acids and non-essential amino acids. The dominant amino acid compounds identified in the steamed gourami sample were glutamic acid of 4.09%, aspartic acid in steamed gourami of 2.67%, arginine 1.64% and alanine 1.53% which belong to the non amino acids. essential. The dominant essential amino acids in steamed gourami were lysine (2.54%), leucine (2.11%), and valine (1.30%).

There are several factors that affect the amino acid content in fish meat such as season, type of fish, body size, age, stage of maturity, environmental temperature, level of feeding, digestible content in feed, stages of the organism's life cycle, and availability of food ingredients [35];[46];[60]. According to [43], the formation of free amino acids is influenced by parameters of fish species, feed, habitat, freshness of raw materials, storage and processing.The heating process on gourami samples can increase the amount of free amino acids formed compared to the amount in fresh gourami. The formation of free amino acids can be increased due to a proteolytic reaction during the heating process [37];[68].

Table 1. Amino Acid Composition Of Steamed Gourami Sample

Amino Acid	**Value (%)**
Asam Aspartat	2,67
Asam Glutamat	4,09
Serin	1,01
Histidin	0,66
Glisin	1,13
Treonin	1,19
Arginin	1,64
Alanin	1,53
Tirosin	0,91
Metionin	0,79
Valin	1,30
Fenilalanin	1,14
Isoleusin	1,29
Leusin	2,11
Lisin	2,54
Total Amino Acid	**23,98**

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

Changes in protein levels in fish are associated with reduced water content in fish during the steaming process. The higher the temperature and the longer the processing time can result in the protein content in the food being damaged and decreasing [14]. According to [54], the protein content of gourami is 18.93% greater than goldfish (16.04%) [50], and tilapia (17.33%) [19].

The value of glutamic acid content detected in steamed gourami was 4.09%. Glutamic acid can be found in foods that have a high protein content such as meat, seafood, boiled meat (broth) and soy sauce without the addition of any components, glutamate can be used to add to the taste of food such as increasing the savory taste [67]. In fishery products, glutamic acid is an amino acid that is commonly found and can create aroma and taste characteristics in food ingredients [47]. According to [10], aspartic acid and glutamic acid greatly determine the taste of umami. Glutamic acid can provide flavor umami if the concentration in the food product is above the taste threshold [31];[32];[74];[80]. Meanwhile, serine, glycine, alanine, and tyrosine contribute to the sweet taste. In addition, the interaction of several sweet amino acids such as serine, glycine and alanine with IMP can improve umami taste [33].

Glutamate ions stimulate several types of nerves that are on the human tongue. This property is used as a flavor enhancer in food [73]. According to [64], if the glutamic acid content found in fish meat is low, then the taste of fish meat will be less savory. Several fish processing methods, such as steaming can increase glutamic acid levels in fish meat. The steaming method can increase the glutamic acid content in gourami meat because the steaming process uses high heat which can change the protein structure in gourami and change the amino acids contained therein. There is an increase in the content of glutamic acid and aspartic acid during the processing at certain temperatures and cooking times [2]. The increase in glutamic acid content in meat is caused by deamination between the amino acids glutamine and asparagine which form glutamic acid so that glutamic acid levels in meat increase [55].

The content of aspartic acid identified in steamed gourami was 2.67%. In addition to glutamic acid compounds, aspartic acid also contributes to the taste of umami. Aspartic acid is a non-essential amino acid which is found in fish protein. According to [66], aspartic acid and glutamic acid work together to create a savory taste in food, especially the flavor of the seafood product. Taste of umami which is perfectly produced through a combination of glutamic acid (savory-salty) and aspartic acid (savory-sweet) [15].

The leucine compound detected in steamed gourami was 2.11%. Leucine is an amino acid that contributes to a bitter taste but not as bitter as phenylalanine compounds [31]. Leucine is an essential amino acid that belongs to the class of ketogenic amino acids, namely amino acids that can produce ketone compounds in the liver [38]. Leucine is able to increase insulin secretion and improve glycemic control in patients with uncontrolled blood glucose levels [44];[58].

Lysine compound identified in steamed gourami was 2.54%. Lysine has a contribution in giving a bitter taste [42];[78]. The most destructive processing method is heating and the most affected amino acid is lysine [5]. There are several factors that affect the lysine content of fish in processing processes such as bacterial activity, changes in pH, oxygen, heat and light or a combination thereof [27]. Lysine is an amino acid that has a function as a basic material for blood antibodies, becomes a framework in the formation of niacin (Vitamin B3), strengthens the circulatory system, maintains normal cell growth, works together with proline and vitamin C to form collagen tissue, lowers blood triglyceride levels. excess.

The content of arginine compounds in steamed gourami is 1.64%. Arginine is part of the essential amino acids which are alkaline and give food a bitter taste [48];[57]. Arginine is an amino acid that belongs to the group of glucogenic amino acids, namely amino acids that can be converted into glucose and glycogen [38]. According to [80], concentrations of arginine compounds that are below the threshold increase saltiness and contribute to the taste of umami on pig's hair [32]. The high content of arginine compounds can provide a sweet taste and distinctive flavor of seafood on crabs and scallops [74]. Arginine is an essential amino acid that the body needs to make seminal fluid (semen), as well as increase growth hormone production [69].

The value of the alanine compound identified in steamed gourami was 1.53%. Alanine is an amino acid that contributes to the sweet taste of food [57]. Alanine is a compound with a nonpolar R group that is used as a glucogenic precursor and nitrogen carrier from surface tissues for nitrogen excretion [34]. The high alanine content is found in seafood like snow crab, clam, and scallop [22];[56];[75]. Alanine is an important source of energy that can boost immunity, supply energy to muscle tissue, the brain, and the human central nervous system.

Valine compound identified in steamed gourami was 1.30%. Valine is an amino acid compound that gives a bitter taste but not as bitter as phenylalanine [31];[57]. Valine is a branched-chain amino acid that functions as a glucogenic precursor, so it is needed for the growth and maintenance of muscle tissue, stimulates mental abilities, muscle coordination, helps repair damaged tissue, and maintains nitrogen balance [26]. According to [70], deficiency of valine compounds can cause loss of muscle coordination and the body becomes very sensitive to pain, cold and heat.

The isoleucine compound detected in steamed gourami was 1.29%. Isoleucine is an amino acid that can give food a bitter taste [57]. Isoleucine compounds are needed for optimal growth, repairing damaged tissue, developing intelligence, maintaining the body's nitrogen balance, forming other non-essential amino acids, forming hemoglobin, and stabilizing blood sugar levels [18]. Deficiency of isoleucine compounds can trigger symptoms of hypoglycemia.

The content of glycine compounds in steamed gourami is 1.13%. According to [52], glycine is classified as a non-essential amino acid for humans. Glycine works together with glutamic acid and aspartic acid to give fish products a distinctive taste. Glycine is an active flavor component that gives characteristic sweet taste to various aquatic products [48]. Glycine plays an important role in stimulating the release of growth hormone, helping muscle development and growth, and wound healing. Glycine is needed in Hb synthesis and can be used to reduce stomach acidity.

Threonine compound identified in steamed gourami was 1.19%. Threonine is an amino acid compound that gives a sweet taste to foodstuffs [57]. According to [28], threonine is able to improve the ability of the intestines and digestive processes, maintain protein balance, is important in the process of forming collagen and elastin, helps the function of the heart and central nervous system and prevents epileptic seizures. Threonine compounds also function to maintain the proper protein balance in the body and are important in the maintenance of the intestinal mucosa [39].

The detected value of the phenylalanine compound in steamed gourami was 1.14%. Phenylalanine is a compound that contributes to giving food a bitter taste [57]. Phenylalanine is an amino acid that has a dominant function in the body's work system. The amino acid phenylalanine also plays a role in relieving pain and alleviating depression. Phenylalanine is needed by the thyroid gland to produce thyroxine which has the benefit of preventing goiter, playing a role in increasing basal metabolism, and regulating body temperature [3]. A deficiency in phenylalanine amino asthma can cause the body to become weak, lethargic, liver damage, and stunted growth.

The content of serine compounds in steamed gourami was 1.01%. Serine compounds play a role in giving a sweet taste [57];[76]. Serine is one of the amino acids found in fish meat which functions as a component of protein, plays a role in various biochemical processes in the body, is needed for fatty acid metabolism, and boosts the immune system [18]. According to [34], serine is a component of phospholipids containing hydroxyl groups. Serine is used as a precursor to ethanolamine and choline.

Tyrosine compounds identified in the steamed gourami sample was 0.91%. The amino acid tyrosine has a similar function to phenylalanine which produces a bitter taste [76]. Tyrosine is an amino acid that has a phenol group and is weakly acidic. Tyrosine belongs to a group of non-essential amino acids which function to reduce stress, antidepressants, drug detoxification, and cocaine [1];[34]. According to [41], tyrosine also acts as a high antioxidant against peroxy free radicals. Lack of tyrosine compounds can cause hypothyroidism [18].

The methionine compound of steamed gourami was 0.79%. The methionine compound is an amino acid that gives a bitter taste [57]. Methionine can be found in large quantities in animal food sources. Methionine plays an important role in fat metabolism, maintains liver health, prevents accumulation of fat in the liver and main arteries, prevents allergies and osteoporosis [64].

Histidine detected in steamed gourami was 0.66%. Histidine is known to give a bitter taste but not as bitter as phenylalanine [31]. Histidine gives a very weak taste and it is thought that this amino acid does not play a direct role in taste characteristics [51]. Histidine content can be reduced due to fat oxidation [7]. Histidine is one of the essential amino acids needed by the human body for protein synthesis and various physiological functions. Histidine plays a role in the nervous system and carnosine is an amino acid [63].

3.2 Test Description of Steamed Milk Fish

The test data for the description of steamed gourami showed that steamed gourami had a complete appearance with a pale white color. The aroma of steamed gourami is fresh and tasty according to the specific type of gourami. The texture of the steamed gourami meat is dense, soft and very compact. The taste of steamed gourami is savory according to the specific type.

Color or shape is one of the important parameters in food ingredients, both those that go through a manufacturing process and those that do not go through a manufacturing process. According to [45], color can provide information about the conditions of chemical changes in a food. A food ingredient will not be consumed if it has an unsightly color, so that color can be used to determine quality [72]. Discoloration of fish meat is caused by physical and chemical changes during storage [24]. [25] stated that the binding of the solution by the meat tissue resulted in a change in the color of the meat. The color of the fish meat fades because the protein in the meat experiences aggregation which can inhibit the formation of milking in the spine [16]. According to [11], meat color can affect meat quality, consumer preferences, and indicators of maturity of the cooking process. The cooking process using high temperatures can affect the color of fish meat [62].

Aroma is one of the parameters that determine the good taste of a food product [23]. Aroma or smell is one of the delicious parameters of a food and has its own charm to determine the good taste of a food product. The fishy smell is a characteristic fish odor caused by nitrogen components, trimethylamine oxide (TMAO), guanidine, and imidazole derivatives.

The gourami meat that has been steamed has a dense, soft and very compact texture. Changes in the texture of steamed fish became soft and decreased elasticity due to protein denaturation which resulted in the release of large amounts of water. According to [29], the texture of food ingredients is influenced by several things such as the ratio of protein content, type of protein, fat, water content, water activity, and processing temperature. The texture of a food is mostly determined by the water content contained in the product [21]. The higher the water content in the fish makes the texture softer and not crunchy.

Taste is the tongue's response to stimuli provided by a food and can influence consumers on a food product so that it has an important role in determining the final decision [12]. The savory taste of gourami is related to the presence of amino acid compounds such as glutamic acid or its salts in a food ingredient, for example monosodium glutamate and 5-nucleotide types such as guanidine 5-monophosphate (GMP), inosine 5-monophosphate (IMP) [71]. Taste is influenced by several factors such as chemical compounds, temperature, concentration, and interactions with other taste components. According to [77], free glutamic acid is contained in all fish species and is the most important flavor contributor.

4. CONCLUSION

Based on the description of the discussion, it can be concluded that steamed gourami has identified non-volatile flavor compounds as many as 15 amino acid compounds. Two umami-flavored amino acids are 4.09% glutamic acid and 2.67% aspartic acid. The four sweet tasting amino acids are 1.53% alanine, 1.19% threonine, 1.13% glycine and 1.01% serine. The nine bitter amino acids are lysine 2.54%, leucine 2.11%, arginine 1.64%, valine 1.30%, isoleucine 1.29%, phenylalanine 1.14%, tyrosine 0.91%, methionine 0, 79% and histidine 0.66%. Steamed gourami has an intact appearance and is pale white in color. The aroma of steamed gourami is fresh and tasty according to the specific gourami. The texture of the meat is dense, soft and very compact. The taste of steamed gourami is savory according to the specific type.

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