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IDENTIFICATION OF NONVOLATILE FLAVOR COMPOUNDS IN FRESH INDIAN MACKEREL (Rastrelliger sp.)

Identifikasi Senyawa Flavor Nonvolatil Pada Ikan Kembung Segar (Rastrelliger sp.)

Anisa Dwi Septhyani*, Rusky Intan Pratama, Kiki Haetami, Evi Liviawaty

Fisheries Study Program, Padjadjaran University, Indonesia

Main Bandung Sumedang street KM 21, Jatinangor District, West Java 45363

*Coresponding author: anisa19017@mail.unpad.ac.id

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ABSTRACT

Nonvolatile flavoring ingredients greatly affect food flavor and can affect food acceptability among consumers. The purpose of this study aimed to determine the mackerel's nonvolatile taste components. High Performance Liquid Chromatography (HPLC) was used to analyzed fish samples. 15 different kinds of amino acids were identified in the study: seven non-essential amino acids (glutamic acid, aspartic acid, arginine, alanine, glycine, serine, and tyrosine) and eight essential amino acids (lysine, leucine, valine, isoleucine, threonine, phenylalanine, methionine, and histidine). Glutamic acid (3.65%), aspartic acid (2.35%), lysine (2.37%), leucine (1.90%), arginine (1.46%), and alanine (1.41%) were discovered to be the main nonvolatile components in mackerel. Moreover, it was found that aspartic acid and glutamic acid provide the umami flavor, arginine, leucine, and lysine give the bitter taste, and alanine provides a sweet taste.

Keywords: Amino Acid, Mackerel, Nonvolatile Flavor Compounds

ABSTRAK

Komposisi flavor nonvolatil memiliki pengaruh besar terhadap cita rasa makanan dan dapat mempengaruhi daya terima konsumen. Tujuan dari penelitian ini adalah untuk menentukan komponen flavor nonvolatil pada ikan kembung sebagai salah satu ikan yang banyak digemari masyarakat. Sampel ikan kembung segar dianalisis menggunakan alat HPLC (*High Performance Liquid Chromatography*). Hasil penelitian mengidentifikasi adanya 15 jenis asam amino pada ikan kembung segar: tujuh asam amino nonesensial (asam glutamat, asam aspartat, arginin, alanin, glisin, serin, dan tirosin) dan delapan asam amino esensial (lisin, leusin, valin, isoleusin, treonin, fenilalanin, metionin, dan histidin). Asam glutamat (3,65%), asam aspartat (2,35%), lisin (2,37%), leusin (1,90%), arginin (1,46%), dan alanin (1,41%) merupakan komponen asam amino dengan nilai tertinggi pada ikan kembung segar. Asam aspartat dan asam glutamat memberikan rasa umami, arginin, leusin, dan lisin memberikan rasa pahit, dan alanin memberikan rasa manis.

Kata Kunci: Asam Amino, Ikan Kembung, Komposisi Flavor Nonvolatil

INTRODUCTION

Mackerel is one of the small pelagic fish species that has important economic value and is widely found in Indonesian waters. According to the Center for Data, Statistics, and Information of the Ministry of Marine Affairs and Fisheries (2022), the marine capture fisheries sector in Indonesia obtained 6,494,140 tons of fish, this figure includes mackerel production which reached 362,779 tons. Mackerel is in great demand by the public as seen from the amount of mackerel consumption in Indonesia which reached 2.83 kg/capita/year (Center for Data, Statistics and Information of the Ministry of Marine Affairs and Fisheries, 2017). The use of mackerel as a fish for consumption is favored by the public because of its high nutritional content, easy to obtain, and affordable price (Indaryanto *et al*., 2018).

According to Zhang *et al*. (2019) flavor is the key to the quality of fish products in consumer acceptance. Each fish contains a different composition of flavor compounds. Flavor is a complex component involving volatile and nonvolatile compounds. Nonvolatile flavor compounds can provide an impression of taste, namely sweet, bitter, sour, salty, and savory. Common nonvolatile flavor compounds found in fish include amino acids, peptides, and nucleotides (Pratama *et al*., 2018; Zhang *et al*., 2019). The composition of amino acid compounds found in a food product can affect the taste characteristics of the product. The savory taste of fish can be obtained from the protein hydrolysis process which produces glutamic acid (Ramadhani *et al*., 2022). Fishery commodities that have been tested for the composition of nonvolatile flavor compounds in previous studies include gourami, yellowfin tuna, squid, processed crab sauce, oysters, and gazami crab (Chen *et al*., 2022; Liu *et al*., 2021; Liu *et al*., 2019; Pratama *et al*., 2018; Yue *et al*., 2016; Zhang *et al*., 2019).

Research on the composition of nonvolatile flavor compounds can produce information related to the taste characteristics of mackerel as one of the fish that is in great demand by the public. Identification of nonvolatile flavor compounds in mackerel can be the beginning for the development of flavor extract production. This study was conducted with the aim of identifying the composition of nonvolatile compounds that are the flavor components in mackerel.

METHODS

Tools and Materials

The main material in this study was mackerel taken from PPI Karangsong, Indramayu. The materials used for the analysis of amino acid compounds included orthophthalaldehyde (OPA) 50 mg, sodium hydroxide 10 g, boric acid 10 g, 30% Brij-30 solution 1 ml, 2-mercaptoethanol 1 ml, 0.5 µmol/ml amino acid standard solution 1 ml, Na-EDTA 5 g, methanol 200 ml, tetrahydrofuran (THF) 10 ml, Na-acetate 5 g, and 2L HP water. The tools used in this study included High Performance Liquid Chromatography (HPLC) Ultra Techspere, Erlenmeyer flask, knife, scales (Tanita) with an accuracy of 0.1 g, cling wrap, aluminum foil, zip-lock plastic, and cool box.

Research Stage

Experimental methods were carried out by testing non-volatile flavor compounds and descriptive tests. Fresh mackerel samples were analyzed for their non-volatile flavor compounds using the amino acid compound analysis method using High Performance Liquid Chromatography (HPLC). Primary data were obtained from the treatment of fresh mackerel meat. Secondary data on amino acids were collected through literature reviews, literature, previous research results, and books. The resulting data will be analyzed descriptively comparatively.

Research Procedure

Sampling of mackerel fish from PPI Karangsong, Indramayu. Mackerel fish samples were transported using a cool box and crushed ice with a ratio of 1:3 to the Fisheries Product Processing Laboratory, FPIK Unpad. Sample preparation was carried out at the Fisheries Product Processing Laboratory, FPIK Unpad. Mackerel fish were cleaned and cleaned. Mackerel meat samples were weighed as much as 100 grams for identification of nonvolatile flavor compounds. The weighed mackerel samples were then packed using aluminum foil, labeled, and coated with cling wrap and then put into zip-lock plastic. Layered packaging is carried out to inhibit changes and damage to the sample that can be caused by external influences, such as air, light, and temperature (Pratama, 2011). The packaged samples were then put into a coolbox filled with ice. The samples were then taken to the Integrated Laboratory, Bogor Agricultural University for analysis of nonvolatile flavor compounds.

Amino Acid Profile Analysis

High Performance Liquid Chromatography (HPLC) was used for amino acid profile analysis. The HPLC instrument conditions used: Ultra Techspere column, mobile phase flow rate of 1 ml/min, and the detector used was fluorescence. The amino acid profile results were identified using 15 standard amino acid solutions expressed in µmol units based on the sample peak area and the amino acid standard peak area. The calculation is determined as follows:

$$\text{AA Concentration} = \frac{\text{sample peak area}}{\text{standard peak area}} \times \text{Standard concentration}$$

$$\text{AA Concentration} = \frac{\text{sample peak area}}{\text{standard peak area}} \times 0.5\ \mu\text{mol/mL} \times 5\ \text{mL}$$

Meanwhile, the percentage of amino acids in the sample is determined by the calculation:

$$\text{AA (\%)} = \frac{\mu\text{mol Amino Acids} \times \text{Amino Acids Relative Mass}}{\mu\text{g sample}} \times 100$$

RESULT

Amino acid profile analysis identified 15 types of amino acids in mackerel samples. The results of amino acid analysis in Table 1 show that the amino acid content in mackerel meat consists of nine essential amino acids and six non-essential amino acids. Essential amino acids are lysine, leucine, valine, isoleucine, threonine, phenylalanine, methionine, and histidine. Non-essential amino acids are glutamic acid, aspartic acid, arginine, alanine, tyrosine, glycine, and serine.

Table 1. Results of Amino Acid Analysis of Fresh Mackerel

Types of Amino Acids	Amount (%)
Aspartic acid	2.35
Glutamic acid	3.65
Serine	0.78
Histidine	1.07
Glysine	1.14
Threonine	1.04
Arginine	1.46
Alanine	1.41
Tyrosine	0.87
Methionine	0.87
Valine	1.34
Phenylalanine	1.05
Isoleucine	1.30

Types of Amino Acids	Amount (%)
Leucine	1.90
Lysine	2.37
Total amino acids	22.60

Amino acids play an important role in the taste of food because they have different tastes such as sweet, bitter and umami (Zhuang *et al*., 2016). Based on the results of the analysis using HPLC, it was found that glutamic acid with a concentration of 3.65% was the highest percentage of amino acids in mackerel. The lowest amino acid content obtained was serine with a concentration of 0.78%. The amino acid with the least amount in a material is called the limiting amino acid. Fish can have different flavors depending on the components, concentrations, and thresholds of amino acids (Chen *et al*., 2022).

DISCUSSION

According to Cheung & Mehta (2015), amino acids have nutritional value, taste, medical benefits, and broad chemical properties so that they are widely used as food additives. There are two large industries that utilize amino acids, namely the flavoring industry that uses monosodium glutamate, alanine, aspartate, and arginine to enhance flavor, and the feed industry that uses lysine, methionine, threonine, tryptophan, and other amino acids to improve the nutritional quality of feed.

The amino acid profile of each fish can also vary due to several factors, namely the species of fish, the age of the fish, the fish's diet, and the environment in which the fish live (Cheung & Mehta, 2015). Mohanty *et al*. (2014) also stated that fish species that live in low-temperature water are rich in lysine and aspartic acid and marine fish are rich in leucine. Anchovies and mackerel have higher aspartic acid, serine and leucine content compared to freshwater fish. Mackerel also has a high histidine content.

Amino acid content with high percentage in mackerel includes glutamic acid, aspartic acid, and lysine. These three amino acid compounds are also found in high concentrations in grouper, catfish, and several other marine fish (Astuti *et al*., 2023; Erkan *et al.*, 2010). High essential amino acid content in a product indicates good protein quality. Essential amino acids cannot be produced by the body so the body requires a source of essential amino acids from food. Amino acids play an important role in the formation and function of proteins. Protein quality is influenced by the profile of its constituent amino acids (Pratama *et al*., 2018). According to Cheung & Mehta (2015) fish is a source of protein that contains all types of essential amino acids. The three highest essential amino acid contents found in mackerel are lysine, leucine, and valine.

Lysine, leucine, and valine give a bitter taste to food ingredients (Chen *et al*., 2022). The content of the amino acid lysine identified in mackerel is 2.37%. According to Dale *et al.* (2019) the presence of lysine and leucine in fish is very high. The content of the amino acid leucine in mackerel is 1.90%. Leucine can stimulate the formation of muscle protein and also affect brain function (Cobas *et al*., 2022; Šimat *et al*., 2020). The valine content in mackerel is 1.34%. Valine is one of the branched-chain amino acids that acts as a glucogenic precursor (Liu *et al*., 2023). The presence of valine in the body's metabolism has a significant positive impact on various aspects of health, especially related to muscle and cognitive function. Valine has an important role in supporting tissue repair and muscle coordination (Šimat *et al*., 2020).

The content of the isoleucine compound identified is 1.30%. Isoleucine is an amino acid that gives a bitter taste to food (Cai *et al*., 2021). Isoleucine functions to increase energy and help the body recover after high-intensity physical activity. In addition, isoleucine also plays an important role in the production of hemoglobin (Šimat *et al*., 2020). This is also supported by the statement of Abdullah *et al*. (2013) that isoleucine plays a role in the healing and repair

process of muscle tissue, as well as accelerating blood clotting in injured areas.

The threonine content in mackerel is 1.04%. Threonine is known to provide a sweet taste to food (Zhang *et al*., 2019). According to Sarower *et al*. (2012) reduced threonine is also associated with a slight increase in umami taste, but the overall taste profile remains decreased. In addition to affecting taste, threonine is an essential amino acid that plays an important role in several body functions. Threonine plays a role in maintaining bone and tooth strength, accelerating wound healing and reducing fat in the liver. Threonine is also involved in supporting various body functions such as cardiovascular function, central nervous system and immunity (Šimat *et al*., 2020).

The phenylalanine compound identified in mackerel is 1.05%. Phenylalanine is an amino acid that contributes to the bitter taste of food (Sarower *et al*., 2012). Phenylalanine is an essential amino acid that plays a role in the body's metabolism. The thyroid gland requires phenylalanine to produce thyroxine in the body (Abdullah *et al*., 2013). Phenylalanine also functions as a precursor in the synthesis of the neurotransmitter epinephrine and norepinephrine in the brain which can improve the balance of nerve and cognitive function (Abdullah *et al*., 2013; Šimat *et al*., 2020).

The content of methionine compounds identified in mackerel is 0.87%. According to Xiao *et al*. (2021) methionine produces a bitter taste in food. Methionine is an amino acid that contains sulfur (Liu *et al*., 2023). The sulfur content in methionine is important to support the body's natural antioxidant function. In addition, methionine also acts as a precursor in the production of the amino acid cysteine. Methionine is used as a treatment for liver disorders, depression, allergies, asthma, copper poisoning, and drug addiction (Šimat *et al*., 2020).

Histidine contained in mackerel is 1.07%. Histidine gives a bitter taste to food (Zhang *et al*., 2019). Histidine is commonly found in migratory pelagic fish such as mackerel. Histidine has a buffering effect that can protect tissues from sudden increases in lactic acid during anaerobic muscle activity (Šimat *et al*., 2020). Histidine plays an important role as an anti-inflammatory, antioxidant, and anti-secretory in the body. Histidine is useful in the growth and repair of damaged body tissues, increasing blood flow, and removing excess heavy metals from the body (Erkan *et al*., 2010; Šimat *et al*., 2020).

The results of the amino acid analysis in Table 1 show seven non-essential amino acids in mackerel consisting of glutamic acid, aspartic acid, arginine, alanine, tyrosine, glycine, and serine. Glutamic acid is the amino acid with the highest percentage in mackerel (3.65%). Glutamic acid is a component of natural proteins found in high-protein foods such as fish. Glutamic acid is also quantitatively the main amino acid in other fish species (Nopiyanti *et al*., 2023; Pratama *et al*., 2018; Yu *et al*., 2017). Glutamic acid is a component that has a major influence on the formation of taste in fish. Glutamic acid contributes to the umami taste in food. Umami is a taste produced by a number of substances, namely the amino acid glutamic acid and 5'-ribonucleotides such as inosinate and guanylate (Ninomiya, 2015). According to Cobas *et al*. (2022) glutamic acid is a source of nitrogen and plays an important role in amino acid metabolism.

The aspartic acid content in mackerel is 2.35%. Aspartic acid is an umami amino acid whose content has an important influence on the taste of fish meat (Liu *et al*., 2015). Aspartic acid plays an important role in the functional properties of proteins, aspartic acid plays a role in the metabolism and function of leukocytes, lymphocyte proliferation, and the synthesis of purine and pyrimidine nucleotides (Li *et al*., 2007).

The arginine content in mackerel is 1.46%. Arginine gives a bitter taste to food (Zhang *et al*., 2019). In addition to the bitter taste, arginine also has a slightly sweet taste, and the bitter taste of arginine is easily masked by NaCl and glutamate (Zhuang, 2016). As an essential amino acid, arginine plays an important role in the human body. Arginine is involved in cell division, wound healing, ammonia removal, immune function, and the process of hormone release

(Mohanty *et al*., 2014; Yu *et al*., 2017). Arginine also stimulates biological synthesis in nitric oxide, circulatory function, blood clotting, and blood pressure maintenance (Šimat *et al*., 2020).

The alanine content identified in mackerel is 1.41%. Alanine gives a sweet taste to food. According to Zhuang *et al*. (2016) the amino acid alanine can stimulate the taste buds to produce a stronger sweet taste and neutralize bitter and salty tastes. Alanine in the body plays a role in supporting gluconeogenesis, leukocyte metabolism, and glucose metabolism in the body (Cobas *et al*., 2022).

Tyrosine identified in mackerel is 0.87%. Tyrosine is a hydrophobic amino acid that can cause a bitter taste (Hu *et al*., 2022). In addition to affecting taste, the amino acid profile of fish plays an important role in maintaining health and various biological and physiological activities of the human body. Hydrophobic amino acids can work on the lipid layer of the membrane to reach targets and help cleanse toxins (Ryu *et al*., 2021). Aromatic amino acids such as tyrosine and phenylalanine have quite high antioxidant abilities.

Glycine contained in mackerel is 1.14%. Glycine produces a sweet taste in food (Zhang *et al*., 2019). This statement is supported by Zhuang *et al*. (2016) that the amino acid glycine can stimulate the taste buds to produce a stronger sweet sensation and neutralize bitter and salty tastes. Glycine has an important role in the wound healing process (Cobas *et al*., 2022).

The serine content identified in mackerel is 0.78%. Serine is a non-essential amino acid with the lowest amount in mackerel. The hydrolysis process that occurs during amino acid analysis causes serine to not be completely recovered (Lamp *et al*., 2018). Serine is one of the amino acids that contributes to the sweet taste in food (Zhang *et al*., 2019).

CONCLUSION

The results of the analysis of nonvolatile compounds identified 15 amino acid compounds identified in mackerel. Essential amino acids consist of lysine, leucine, valine, isoleucine, threonine, phenylalanine, methionine, and histidine. Non-essential amino acids include glutamic acid, aspartic acid, arginine, alanine, tyrosine, glycine, and serine. The highest percentage of amino acids in mackerel samples was obtained from glutamic acid which contributed to the umami taste of fish meat.

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