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Analysis of Volatile Component Profile of Bekasam Seluang Fish (*Rasbora argyrotaenia*)

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ABSTRACT

Seluang fish is a freshwater fish commonly found in Southeast Asian waters, including Indonesia. Fermentation, autolytic and enzymatic degradation of protein and fat produce volatile compounds such as organic acids, carbonyls, nitrogen compounds, and sulfur. The research aims to analyze the identification of volatile compounds in bekasam seluang fish. The research was conducted at the Integrated Laboratory, Lambung Mangkurat University, Banjarbaru, using a four-treatment design and three replications, namely: O (without roasted rice), A (50% roasted rice), B (60% roasted rice), and C (70% roasted rice). Analysis of volatile components was carried out using Gas Chromatography-Mass Spectrometry (GC-MS), an instrumental technique for separating and identifying compounds in complex samples. Volatile components using GC-MS Headspace revealed that the addition of roasted rice affected the complexity of the volatile compounds formed. The higher the concentration of roasted rice, the more volatile compounds typical of fermentation were formed, such as pyrazine, methanol, methyl uronate, and isoleucinol. The 60% roasted rice treatment produced the most complex but balanced volatile compound profile, while 70% produced compounds with a strong aroma but potentially masked the characteristic aroma of fish.

INTRODUCTION

Seluang fish is a freshwater fish commonly found in Southeast Asian waters, including Indonesia. This fish is known for its small size and is often used as a food ingredient by local communities. Seluang fish also contains high nutritional value, making it a potential raw material for processed food products. The nutritional content per 100 grams of fresh seluang fish includes 113 kcal of energy, 13.9 grams of protein, 4.9 grams of fat, 3.4 grams of carbohydrates, 642 mg of calcium, and 646 mg of phosphorus (Ministry of Health of the Republic of Indonesia, 2017). Seluang fish production in South Kalimantan is quite high, amounting to 1,800.8 tons from capture fisheries and 3,813.4 tons from swamp waters.

Currently, seluang fish utilization is still limited, mostly processed as fried fish for household consumption. However, given its high nutritional content and abundant production, diversification of seluang-based processed products is necessary. One processing alternative is fermentation to produce bekasam, a traditional Indonesian food product produced by fermenting fish using salt and a carbohydrate source such as rice or roasted rice. Functioning as a preservation method, fermentation also produces a unique and distinctive flavor favored by most people (Rahayu, 1992).

The conventional bekasam production process involves spontaneous fermentation using microorganisms naturally present in fish, salt, and carbohydrate ingredients. The addition of carbohydrates in the form of rice, crust, or roasted rice aims to support the growth of lactic acid bacteria (LAB), which play a crucial role in producing metabolites during fermentation. LAB converts carbohydrates into various compounds such as lactic acid, ethanol, acetic acid, and carbon dioxide, which impart characteristic flavor and aroma to the final product (Lestari et al., 2018; Nuraini et al., 2014).

Previous research has shown that variations in the type and amount of carbohydrates influence the chemical and sensory quality of bekasam. The use of roasted rice as a carbohydrate source in bekasam of sepat toakang fish produces the best chemical and organoleptic quality, with a protein content of 16.12%, fat 6.86%, and pH 4.51. Sudaryanti et al., (2023), variations in carbohydrate concentration and fermentation time affect the protein content and pH value of bekasam, where the highest protein content (20.08%) and the lowest pH (5.12) were obtained in the treatment of 60% carbohydrate and fermentation for 10 days.

The addition of carbohydrates in the form of roasted rice is important because it provides energy for the growth of lactic acid bacteria, which is key to a successful fermentation process. Lasekhan et al. (2018) explain that lactic acid bacteria are capable of fermenting carbohydrates into lactic acid, which not only acts as a natural preservative but also contributes to bekasam's distinctive sour flavor. Common genera of lactic acid bacteria found in food fermentation include Lactobacillus, Streptococcus, Pediococcus, and Leuconostoc (Mulyani et al., 2019).

Lactic acid bacteria fermentation is divided into two types: homofermentative, which produces only lactic acid, and heterofermentative, which produces a mixture of metabolites such as acetic acid, ethanol, and CO_2. This fermentation process not only grows acid-forming microorganisms but also suppresses spoilage microorganisms such as proteolytic and lipolytic bacteria. This results in changes in the texture, flavor, and aroma of bekasam, making it softer, more sour, and possessing a unique, distinctive flavor.

During fermentation, autolytic and enzymatic degradation of proteins and fats produces volatile compounds such as organic acids, carbonyls, nitrogen compounds, and sulfur. These compounds contribute significantly to the aroma and flavor of bekasam. However, the intensity of the aroma produced is greatly influenced by the amount of salt used. Salt has a dual function, namely as an inhibitor of spoilage microorganisms and a regulator of the activity of fermentative microorganisms. Excess salt can suppress the growth of lactic acid bacteria and inhibit lactic acid production (Desniar et al., 2012). The research aims to analyze the identification of volatile compounds in bekasam seluang fish with a salt concentration of 15% (w/w) and the addition of roasted rice of 50%, 60%, and 70% (w/w).

LITERATURE REVIEW

Seluang fish are easily recognized by their body shape which is long and slightly flat on the stomach, while the back is bulging. His mouth was upturned with a gap that wasn't too long. Male seluang fish are lighter than female seluang fish before spawning in the rainy season because the gonads of female fish are full of eggs which are immediately released (Pratiwi, 2014). The nutritional content of 100 grams of fresh seluang fish consists of water content 74.8 g, protein 13.9 g, fat 4.9 g, carbohydrates 3.4 g, iron 1.9 mg, energy 113 calories, phosphorus (P) 646 mg, potassium (K) 182 mg, calcium 642 mg, zinc (Zn) 3.6 mg, copper 0.80 mg and Thiamine (Vitamin B1) 0.21mg (TKPI, 2019).

The benefits of seluang fish, based on its high nutritional content, according to nutritional data from the Indonesian Ministry of Health (TKPI, 2019), include energy, protein, calcium, phosphorus, iron, copper, zinc, and thiamine. These nutritional benefits include maintaining a healthy circulatory system, improving the health of pregnant women due to its high protein and iron content, and minimizing the risk of osteoporosis due to its high protein, calcium, phosphorus, copper, and zinc content. It maintains acid-base (pH) balance in the body due to its high phosphorus content. It supports the immune system due to its high protein, iron, copper, zinc, and thiamine content. Its high zinc content acts as an antioxidant.

Bekasam fish is a food product produced by spontaneous fermentation using a high salt content. Bekasam has a distinctive taste and aroma. The raw material used is freshwater fish, which is relatively inexpensive and abundant (Hidayati et al., 2012).

The nutritional content of 100 grams of Bekasam consists of 65.5 g of water, 11.9 g of protein, 4.9 g of fat, 6.1 g of carbohydrates, 7.5 mg of iron, 116 calories of energy, 757 mg of phosphorus (P), 176 mg of potassium (K), 704 mg of calcium, 1.9 mg of zinc (Zn), 2.1 mg of niacin, 0.03 mg of riboflavin (Vitamin B2), 3.5 g of fiber, and 0.80 mg of copper (TKPI, 2019). The benefits of Bekasam based on its high nutritional content according to nutritional data from the Indonesian Ministry of

Health (TKPI, 2019) are the content of protein, fiber, calcium, phosphorus, iron, copper, zinc, niacin. The benefits of these nutritional contents are: Maintaining stable blood sugar Benefits from the high content of fiber and zinc. Plays a role in hormone production Benefits from the high content of protein and zinc. Strengthens the immune system. Benefits from its high protein, iron, copper, and zinc content. Helps and maintains the growth and development of organ/body cells. Benefits from its high protein and niacin content. Helps utilize/utilize protein for cell growth and repair. Benefits from its high phosphorus content.

The addition of carbohydrates to the bekasam fermentation process aims to stimulate the growth of lactic acid bacteria, which then break down the carbohydrates into several acidic compounds such as lactic acid, acetic acid, propionic acid, and alcohol compounds such as ethyl alcohol. The number of bacteria that grow also depends on the salt used in bekasam production. Salt can slow the decay process and select for microorganisms that cause decay that cannot tolerate salt. Priyanto and Djajati (2018) state that salt is a component that can bind water in ingredients, thereby increasing osmotic pressure. The acidic compounds resulting from the breakdown of carbohydrates can impart a sour taste to the final product and act as a preservative for bekasam. Carbohydrates used in the bekasam fermentation process include white rice, roasted rice, or sticky rice tape, which are then fermented anaerobically (Candra et al., 2007).

Fermentation time can affect the texture, appearance, taste, and aroma of bekasam. The fermentation process can make the meat very soft and pale in appearance. The longer the process, the stronger the characteristic sour taste and aroma of bekasam. Lestari et al. (2018) found that prolonged fermentation can lead to increased acid production in the product. This occurs because lactic acid bacteria have more time to break down the nutrients contained in the substrate and can potentially lead to the accumulation of organic acids.

The presence of lactic acid bacteria is crucial during fermentation. Lactic acid bacteria not only contribute to acidity but also produce various volatile compounds that enhance flavor. The growth conditions of lactic acid bacteria can be optimized by adjusting factors such as salt concentration and fermentation time. The length of fermentation significantly influences the formation of volatile compounds. For example, squid bekasam fermented for three days showed significant changes in pH, total bacterial count, and sensory properties (Ramadhanti, 2024).

Fermented food products typically have higher nutritional value than their original ingredients. This is because the microorganisms in fermented foods can break down complex components into simpler components. Lactic acid bacteria play a role in improving the flavor of fermented foods, providing preservative properties, and increasing nutrient digestibility. This is due to the hydrolysis of proteins into free amino acids during fermentation.

METHOD

Identification of volatile components of fish bekasam was conducted at the Integrated Laboratory, Lambung Mangkurat University, Banjarbaru. The research design consisted of four treatments with three replications, as follows:

- Treatment 0 = Bekasam without added roasted rice
- Treatment A = Bekasam with added roasted rice (50%)
- Treatment B = Bekasam with added roasted rice (60%)
- Treatment C = Bekasam with added roasted rice (70%)

Volatile component analysis using Gas Chromatography-Mass Spectrometry (GC-MS) is an instrumental analytical technique used to separate and identify compounds in complex samples. The process begins with sample preparation and injection into the system, where the sample components are separated in a gas chromatography column. The separated compounds are measured using mass spectrometry to produce a mass spectrum that reflects the composition of the compounds in the sample. The volatile component test used was qualitative by describing the GC-MS results using descriptive data, namely by using a table by looking at the pattern (trend) of the data displayed.

Table 1. Metode GCMS-HS

INSTRUMENT CONTROL PARAMETERS: GCMSD	
Injection Source	Headspace Sampler
Oven Temperature	100 °C
Loop Temperature	110 °C
Transfer Line	115 °C
Vial Equilibration	15.00 min
Injection Duration	0.50 min
GC Cycle Time	15.00 min
Vial Size	20 ml
Column Information	HP-5MS UI (30 m x 250 µm x 0.25 µm) Temperature Range -60 °C—325 °C (350 °C)
Setpoint Temperature	(Initial) 60°C Hold Time 1 min, Rate 10°C/min to 150°C Hold Time 2 min
GC Summary Run Time	12
Injection Volume	1 µL
Split Ratio	50:1
Flow	1.0 mL/min
Pressure	8.2317 psi
Control Mode	Constant Flow
Carrier Gas	He
Average Velocity	36.623 cm/sec
Heater	250 °C
Thermal Aux 2 (G3520 Transfer Line) Temperature	200 °C

INSTRUMENT CONTROL PARAMETERS: GCMSD	
MSD Transfer Line Temperature	280 °C
MS Source	230 C maximum 250 C
MS Quad	150 C maximum 200 C
[Scan Parameters]	Start Time : 0
	Low Mass : 50
	High Mass : 550
	Threshold : 150

RESULT AND DISCUSSION

Gas Chromatography-Mass Spectrometry (GC-MS) is a commonly used analytical method to identify and quantify volatile compounds in fermented food products. The Headspace (HS) GC-MS method allows for the analysis of volatile compounds without disturbing the solid/liquid matrix, making it ideal for fermented products. In the context of Bekasam fermentation, volatile compounds such as organic acids, esters, alcohols, aldehydes, and ketones play a key role in shaping aroma and flavor characteristics (Lapsongphon et al., 2015).

The fish fermentation process produces volatile compounds from the degradation of proteins and lipids by microorganisms, particularly lactic acid bacteria (LAB) and proteolytic bacteria. Volatile component testing of Bekasam Seluang fish showed the following results:

Table 2. Data Library Results of GCMS-HS Bekasam Seluang Fish

NO	COMPONENT	REAL AREA (%)			
		O	A (50%)	B (60%)	C (70%)
1	Dicyclopentadine diepoxide	29,75			
2	Benzedrex	15,37			
3	propanoic acid	7,6	5,70	5,6	4,87
4	hepten				1,13
5	cyclohexanol	9,02	4,14	4,48	6,07
6	cyclotrisiloxane	2,81			
7	cyclotetrasiloxane	0,30	1,02		
8	pentanone			2,64	2,37
9	propanal	21,07	16,18	13,32	14,47
10	tetradecane	21,7			
11	carmabic acid			1,97	1,3
12	metylamine		2,04	3,17	3,52
13	methane		2,66	4,28	
14	cyclobutane		45,92	24,59	35,14
15	hexen		7,61		15,2
16	hydroxy methoxy methyl phenyl chromenone		1,61	1,03	1,67

NO	COMPONENT	REAL AREA (%)			
		O	A (50%)	B (60%)	C (70%)
17	ethyl methylheptane		2,33		2,06
18	butanoic acid		1,7	1,32	1,79
19	methanol		4,81		
20	amino methyl butanol		1,06		
21	methyl uronate		1,29	4,88	
22	pyrazine		1,37		2,8
23	octanoic acid				1,8
24	valeric acid		0,94	1,57	
25	cyclobutanecarboxylic acid			4,9	4,58
26	Benzene			1,07	
27	valeric anhydride			1,17	
28	thiourea			0,84	2,99
29	isoluecinol			5,13	9,26
30	thiadiazole			3,13	
31	oxy pyrrolidinedione			1,7	
32	isovaleric acid			0,85	
33	pivalic acid			0,53	
34	heptadine				0,87
35	nonane				0,64
36	methyl hexanoic acid				1,44
37	dimethyl propyl				0,78

Concentration O, i.e., without roasted rice, shows the main compounds such as dicyclopentadiene diepoxide (29.75%), benzedrex (15.37%), propanal (21.07%), tetradecene (21.7%), and cyclohexanol (9.02%). Aldehydes and alcohols from fat and protein decomposition dominate. Propanal and cyclohexanol impart a pungent and sharp aroma. The absence of carbohydrate fermentative compounds (such as pyrazine or methanol) indicates fermentation is limited to endogenous microflora with protein and lipid substrates (Ravyts et al., 2012).

Concentration A (50% roasted rice) shows the emergence of volatile compounds such as cyclobutane (45.92%), propanal (16.18%), methanol (4.81%), hexene (7.61%), pyrazine (1.37%), and methylamine (2.04%). Cyclobutane and methanol are indicators of active fermentation of roasted rice carbohydrates. Pyrazine is a product of the Maillard reaction or the activity of microorganisms that contributes to the roasted or savory aroma. The decrease in propanal indicates a shift in the metabolic pathway from lipid dominance to carbohydrate fermentation.

Concentration B (60% Roasted Rice) contains the most complex volatile compounds, such as cyclobutane (24.59%), methyl uronate (4.88%), thiadiazole

(3.13%), isoleucinol (5.13%), cyclobutane carboxylic acid (4.9%), pentanone (2.64%), and methylamine (3.17%). This indicates the simultaneous fermentation of proteins, carbohydrates, and amino acid degradation reactions. Thiadiazole and isoleucinol are formed through sulfur pathways and branched amino acids (Song et al., 2020). Methyl uronate is a polysaccharide derivative, indicating compound modification in roasted rice.

Concentration C (70% Roasted Rice) dominant compounds such as Cyclobutane (35.14%), Hexen (15.2%), Isoluecinol increased (9.26%), Pyrazine (2.8%), Octanoic acid (1.8%) and Thiourea (2.99%). The increase in aromatic compounds such as hexen and pyrazine indicates a more dominant contribution of roasted rice. Octanoic acid and methyl hexanoic acid contribute to a strong fruity and fermentative aroma. The dominance of cyclobutane and thiourea has the potential to mask the distinctive aroma of fish.

Analysis of volatile components in fermented products such as Bekasam Seluang fish (Rasbora argyrotaenia) is an important approach in evaluating the sensory quality of the product. One method commonly used for this purpose is Gas Chromatography-Mass Spectrometry Headspace (GC-MS HS). GC-MS HS is a combination of two analytical techniques, namely gas chromatography (GC) and mass spectrometry (MS), with the addition of a headspace sampling technique that allows the detection of volatile compounds from the gas space above the sample without the need for direct contact with the solid or liquid phase (Kolb & Ettre, 2006). Headspace sampling reduces contamination and increases the accuracy of analytical results, especially in complex matrices such as fermented fish. In the GC process, volatile compounds released from the headspace are separated based on their polarity and boiling point through a chromatography column, then these compounds are identified and quantified by a mass spectrometer based on their mass-to-charge ratio (m/z). The application of GC-MS HS in Bekasam analysis provides an overview of the volatile profile, such as aldehydes, alcohols, ketones, organic acids, and esters, formed from the fermentation reactions of proteins, fats, and carbohydrates by microorganisms during the fermentation process.

In fermented Bekasam fish (Rasbora argyrotaenia), volatile compounds are produced primarily by the activity of fermentative microorganisms such as Lactic Acid Bacteria (LAB) and proteolytic bacteria, which break down proteins and lipids into distinctive aroma components. The addition of carbohydrate-rich ingredients such as roasted rice can also stimulate the formation of compounds from the carbohydrate metabolism pathway, such as methanol, pyrazines, and compounds resulting from the Maillard reaction (Lasekan et al., 2018). GC-MS HS is an important tool for evaluating changes in product aroma quality due to fermentation treatments and additives.

The results of GC-MS-HS analysis on Seluang fish bekasam without the addition of roasted rice (Concentration O) showed the dominance of compounds from lipid and protein decomposition such as Dicyclopentadiene diepoxide (29.75%), Benzedrex (15.37%), Propanal (21.07%), and Cyclohexanol (9.02%). These compounds are general indicators of fermentation processes involving lipid oxidation and protein degradation by the activity of microorganisms. Propanal is known to be the main product of the oxidation of unsaturated fatty acids, especially linoleic and linolenic fatty acids, during fermentation or storage of fish products. Cyclohexanol, as a volatile alcohol compound, is also formed from the ketone reduction process or fatty acid oxidation, which is often found in fish undergoing fermentation or long-term storage (Edirisinghe et al., 2007). In addition, compounds such as Dicyclopentadiene diepoxide and Benzedrex are included in the group of heterocyclic and aromatic compounds that can be formed from the degradation reaction of complex organic compounds during the fermentation process by certain microorganisms (Karki et al., 2017).

The activity of proteolytic microorganisms plays a crucial role in the formation of volatile compounds in fermented fish products. Microorganisms such as Bacillus spp., Pseudomonas spp., and Lactobacillus spp. are known to produce protease and lipase enzymes that break down proteins and lipids into peptides, amino acids, and free fatty acids, which then undergo further reactions to produce volatile compounds (Ray et al., 2012). Amatullah et al. (2023) found that a Bacillus cereus isolate from rusip (traditional Indonesian fermented fish) exhibited high proteolytic activity and contributed to the formation of a distinctive flavor through the production of volatile compounds.

The addition of 50% roasted rice (concentration A) introduced a carbohydrate substrate that triggered different fermentation pathways, as evidenced by the presence of compounds such as cyclobutane (45.92%), methanol (4.81%), hexen (7.61%), and pyrazine (1.37%). Methanol is typically produced through the fermentation of simple sugars by microorganisms such as yeast or bacteria, particularly when glucose or maltose is converted to light alcohols under anaerobic conditions (Pinu & Villas Boas, 2017). Pyrazines are volatile heterocyclic compounds formed either through the Maillard reaction between amino acids and sugars or by microbial biosynthesis during the fermentation of grain-based foods (Van Lancker et al., 2010). In Bekasam with the addition of roasted rice, the presence of pyrazines indicates a non-enzymatic reaction, imparting a characteristic savory and roasted aroma. The decrease in propanal (from ~21% to 16.18%) confirms that fermentation has shifted from a lipid decomposition pathway to carbohydrate fermentation, as the microbes begin to utilize the sugar substrate pressure from the rice.

Concentration B (60% roasted rice) exhibits a more complex spectrum of volatile compounds. Compounds such as Cyclobutane (24.59%), Pentanone (2.64%), Methyl uronate (4.88%), Thiadiazole (3.13%), and Isoluecinol (5.13%) indicate the involvement of various metabolic pathways, including carbohydrate fermentation, amino acid degradation, and sulfur reactions. Thiadiazole and isoleucinol are derivatives of amino acid metabolism containing sulfur groups and branched chains, indicating fermentative activity towards proteins and amino acids, which enhance the characteristic aroma of fermented products. Methyl uronate, a polysaccharide derivative, indicates carbohydrate modification in roasted rice, confirming that fermentation occurs complexly and simultaneously on all substrate components.

In treatment C (70% roasted rice), the dominant volatile compounds formed included Cyclobutane (35.14%), Hexen (15.2%), Isoluecinol (9.26%), Pyrazine (2.8%), and Octanoic acid (1.8%). The significant increase in aromatic compounds such as hexen and pyrazine indicates that the intensity of savory, fruity, and fermentative aromas increases with increasing carbohydrate proportions. Pyrazine again appears higher at this concentration, providing a stronger roasted impression, while octanoic acid and methyl hexanoic acid contribute to the characteristic fruity and fermentative aromas. The dominance of compounds such as thiourea (2.99%) and cyclobutane has the potential to mask the characteristic fish aroma and create an overly intense or complex aroma profile.

Changes in the volatile compound profile in each treatment indicate fermentation dynamics influenced by substrate availability, particularly carbohydrates from roasted rice. The addition of roasted rice increases the availability of glucose and starch as energy sources for microorganisms, triggering the formation of volatile compounds characteristic of fermentation. In traditional fermentation systems, such as bekasam, the interaction between lactic acid bacteria and endogenous microflora with the substrate significantly determines the final aroma of the product. Fermentation compounds such as pyrazines, thiadiazoles, and volatile fatty acids contribute significantly to desirable or undesirable flavors.

The higher the addition of roasted rice, the more complex and varied the volatile compounds produced. This is due to the increased availability of fermentative substrates, particularly carbohydrates, which support the growth of microorganisms such as Lactobacillus spp., Bacillus spp., and other aroma-forming microorganisms. Carbohydrate availability also increases the formation of volatile compounds from secondary metabolic pathways, such as amino acids, sulfur compounds, and alcohols (Lapsongphon et al., 2015).

Fermentation in the treatment with the addition of roasted rice not only increased the intensity of the aroma compounds but also enriched the chemical profile with typical fermentative compounds such as pyrazine, methanol, and methyl uronate, which were not found in the treatment without rice. The results showed that the composition of the starting material directly influences the final volatile compound yield. In addition, compounds such as octanoic acid, valeric acid, and methyl hexanoic acid also began to appear at high treatment levels, contributing to the fruity and fermentative aromas commonly found in fermented fish products.

CONCLUSION

Volatile components using GC-MS Headspace revealed that the addition of roasted rice affected the complexity of the volatile compounds formed. The higher the concentration of roasted rice, the more volatile compounds typical of fermentation were formed such as pyrazine, methanol, methyl uronate, and isoleucinol. The 60% roasted rice treatment produced the most complex but balanced volatile compound profile, while 70% produced compounds with a strong aroma but potentially masked the characteristic fish aroma. The combination of sensory, physical, and chemical data indicated that the treatment with 50–60% roasted rice was the best choice for producing Seluang fish bekasam with optimal characteristics.

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