

Comparative Study of Volatile Compounds in Mayas Rice Under Organic Fertilizer Treatment Using GC-MS Analysis

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Abstract.

Rice aroma is an important quality attribute determined by volatile compounds, which are influenced by cultivation practices, including fertilization. However, information regarding the effects of organic fertilization on the volatile profile of local aromatic Mayas rice remains limited. This study aims to identify volatile compounds produced from Mayas rice treated with organic fertilizer. This study was conducted in Kutai Lama Village, East Kalimantan, Indonesia, from March 2022 to March 2023. Mayas rice was cultivated under two fertilization systems: inorganic fertilizers (200 kg ha⁻¹ urea, 100 kg ha⁻¹ SP-36, and 100 kg ha⁻¹ KCl) and shrimp shell-based organic fertilizer (20 kg ha⁻¹). Rice grains were harvested at maturity, dried, milled, and analyzed for volatile compounds. Aroma profiling was performed at the Indonesian Center for Biodiversity and Biotechnology (ICBB) using GC-MS. Volatile compounds were extracted with n-hexane, identified using the NIST library, and compared based on their relative peak areas. The results of GC-MS analysis of aromatic compounds in Mayas rice in organic fertilizer treatment obtained 81 compounds, while in inorganic fertilizer treatment, only 59 aromatic compounds were obtained. GC-MS analysis revealed that the aromatic compound profiles in the organically fertilized Mayas Rice were more complex than those in the inorganic fertilized Mayas Rice. The differences were seen based on the diversity of aldehyde compounds, terpenoids, esters, aromatic alcohols, and heterocyclic compounds identified.

Keywords: Aromatic compounds, mayas rice, agricultural product, organic fertilizer and volatile compounds.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food that has an important contribution as a main energy source for the Indonesian people. Apart from being a strategic commodity for national food security, rice also has high economic value, especially local rice with distinctive characteristics. Along with the increased consumer awareness of rice quality, the aroma aspect is now one of the main indicators in the determination of rice sensory quality [1, 2]. Especially in local rice varieties like Mayas rice, which have a distinctive aromatic compound. Aroma rice is the release of volatile compounds (chemical compounds) that are easily evaporated, formed in the metabolism of the plant during growth, and stored in the grains. Various chemical classes, including aldehydes, esters, and aromatic derivatives, form the complex sensory profile of rice [3-5].

The presence of volatile compounds is influenced by several factors, such as genotype, growth environment, and agronomic practices, such as the type of fertilizer used [6,7]. In the context of sustainable agriculture, the use of organic fertilizers has gained more attention over time for the impact on soil fertility, improving soil microbiota, and their potential influence on the formation of secondary metabolites, including volatiles [8]. Some studies have shown that the application of organic fertilizers, such as compost, manure, and biofertilizer, can modulate the expression of genes related to aroma biosynthesis, whether directly or indirectly through their effect on plant physiology and nutrient availability [9].

Specifically, the application of organic fertilizer alters the aroma profile of food and horticultural crops [10, 11]. Organic fertilizers application on tomato produced significant changes in the volatile compound composition that contributed to the improvement of flavor quality [11]. The application of biofertilizers increases the levels of aromatic compounds in strawberries through the enhancement of the

biosynthetic gene expression [12]. Although these studies provide important information, similar investigations have not been carried out on rice plants, especially local aromatic varieties like Mayas rice. Actually, understanding the influence of organic fertilizers on the volatile composition of rice is crucial for the development of an environmentally friendly production system that can produce a sensor superior product [13].

Previous studies have shown that much research has focused on horticultural crops or superior rice using conventional agronomic approaches. There is limited research that specifically examines the effect of organic fertilizer treatment on the changing profile of volatile compounds of local aromatic rice and the identification of key compounds with an instrumental approach based on GC-MS (Gas Chromatography–Mass Spectrometry) [14]. Although GC-MS is the most accurate and sensitive technique for detecting and quantifying volatile compounds in low concentrations, it is very precise for exploring the aroma of rice [15].

In view of the background, the necessary research was conducted to analyze and compare the profiles of volatiles of Mayas rice on various organic fertilizer treatments using GC-MS analysis. This study aims to identify volatile compounds produced from Mayas rice treated with organic fertilizer.

II. METHODS

This research was conducted in Kutai Lama Village, Anggana District, Kutai Kertanegara, and analysis of the aromatic compounds of Mayas rice was conducted at the Indonesian Center for Biodiversity and Biotechnology Laboratory in Bogor. The research was conducted from March 2022 to March 2023.

This experiment was carried out using two cropping periods: the first planting season used inorganic fertilizers while the second used organic fertilizers. Land preparation involved plowing, harrowing, and leveling of the rice field. Seedlings of rice were cultivated in a nursery for about 25 days before transplanting into the rice field in rows that measured 25 cm x 25 cm.

The inorganic fertilizer treatment provided a total of 200 kg.ha⁻¹ of urea, 100 kg.ha⁻¹ of SP-36, and 100 kg.ha⁻¹ of KCl, with all of the SP-36 and KCl being applied at the end of land preparation. Urea was applied in three applications of; 40% - seven days after transplanting (DAT), 30% - maximum tillering stage (30 DAT), and 30% - panicle formation stage (45 DAT). For organic fertilizers, shrimp shell-based organic fertilizers were applied before rice planting at an application rate of 20 kg.ha⁻¹.

Cropping maintenance includes irrigation management, weed management, and monitoring for pests and diseases. It also involves water management based on traditional methods used in rice farming, except using organic fertilizers and plant-based insecticides. Rice is harvested when approximately 85-90% of the grains turn golden. After harvesting, rice is processed through milling and drying until the moisture content drops to around 12-14%. The next thing to do is to test the sample with a GC-MS instrument.

GC-MS is one of the most reliable analytical techniques for identifying and characterizing volatile organic compounds (VOCs) responsible for rice aroma due to its high sensitivity, selectivity, and capability to separate complex volatile mixtures [16, 17]. Rice samples were cleaned to remove impurities and foreign materials before analysis. The grains were then ground into a fine and homogeneous powder using a sterile grinder. Approximately 1 g of rice powder was weighed and transferred into an extraction vial, followed by the addition of 10 mL n-hexane (pro analysis grade) as the extraction solvent. The mixture was homogenized using a vortex mixer for 2 min and subsequently macerated for 24 h at room temperature to facilitate the extraction of volatile compounds. Similar solvent extraction approaches have been widely applied for the isolation of volatile and semi-volatile compounds in aromatic rice before chromatographic analysis [17, 18].

After extraction, the samples were centrifuged at 4,000 rpm for 10 min. The resulting supernatant was collected and filtered through a 0.22 µm membrane filter to remove particulate matter before instrumental analysis. Subsequently, 1 µL of the filtered extract was injected into the GC-MS system using splitless injection mode to enhance the detection sensitivity of volatile compounds [18].

The analysis was performed using an Agilent Technologies Gas Chromatography–Mass Spectrometry system equipped with an autosampler and Mass Selective Detector (MSD). Separation of volatile compounds was achieved using an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min⁻¹. The injector

temperature was maintained at 250°C. The oven temperature program was initiated at 50°C and held for 2 min, followed by an increase of 5°C min⁻¹ until reaching 150°C. The temperature was then raised at 10°C min⁻¹ to 280°C and maintained for 10 min to ensure complete elution of volatile constituents [16, 19].

Mass spectrometric detection was carried out using Electron Impact (EI) ionization at 70 eV. The mass spectra were acquired in full-scan mode within a mass range of *m/z* 35–550. Identification of volatile compounds was performed by comparing the obtained mass spectra with reference spectra available in the National Institute of Standards and Technology (NIST) Mass Spectral Library. Compound identification was further confirmed based on retention time, molecular formula, molecular weight, and similarity index values generated by the software database [16, 20].

The relative abundance of each volatile compound was determined based on the percentage peak area obtained from the chromatogram. The percentage peak area was calculated using the following equation:

$$\text{Relative Peak Area (\%)} = \frac{\text{Peak Area of Individual Compound}}{\text{Total Peak Area of All Detected Compounds}} \times 100$$

The resulting chromatographic data included retention time (RT), peak area, percentage peak area (% area), compound name, molecular formula, and molecular weight. These data were subsequently used to characterize the volatile profile of rice samples and to evaluate differences in aroma-related compounds among treatments. Rice aroma is largely influenced by volatile compounds such as aldehydes, alcohols, ketones, esters, furans, hydrocarbons, and heterocyclic compounds, which contribute significantly to flavor quality and consumer acceptance [16, 20, 21].

III. RESULT AND DISCUSSION

The results of the research on the results of the analysis of aromatic compounds of Mayas variety rice in inorganic fertilizer treatment and organic fertilizer treatment are presented in Tables 1 and 2.

The volatile profile of Mayas rice with inorganic treatment (Table 1) is dominated by aromatic compounds, including phenol, benzene, and the aromatic heterocycle pyridine, as well as 3-butyl-1-oxide, with the largest area corresponding to a roasted, sharp, and slightly smoky aroma. Pyridine compounds are consequently formed by protein degradation and the Maillard reaction, which can enhance the aroma but sometimes reduce the softness of the rice aroma.

The existence of methoxy-phenyl oximes and metacetamol indicates the predominance of nitrogen aromatic compounds that are often associated with medicinal and phenolic aromas. This indicates that the inorganic fertilization system possibly causes stress and oxidative metabolite formation. The α -curcumene and aR-turmerone compounds are sesquiterpenes that give off a woody spicy fragrance. Although in relatively small amounts, this compound added complexity to the odour. Naphthalene derivatives produce a strong aromatic-hydrocarbon flavor. However, the sharp aroma can be caused by the presence of other ingredients or be inconsistent with the aroma character of premium rice.

Table 1. Test results of aromatic compounds of Mayas rice before organic manure treatment (inorganic manure)

Peak	RT (min)	Area	% Area	Compound Name	Molecular Formula	MW (g/mol)
1	7.09	537311177	3.12	Benzene, methyl (Toluene)	C ₇ H ₈	92.14
5	9.56	36727097	0.21	o-Xylene	C ₈ H ₁₀	106.17
13	12.96	59938593	0.35	Phenol, 2,4-dimethyl-	C ₈ H ₁₀ O	122.16
15	13.82	11863453	0.07	p-Cymene	C ₁₀ H ₁₄	134.22
16	14.19	43932628	0.26	Mesitylene	C ₉ H ₁₂	120.19
28	18.77	67176219	0.39	Cresol	C ₇ H ₈ O	108.14
41	28.02	177166830	1.03	Naphthalene	C ₁₀ H ₈	128.17
42	28.9	245652673	1.43	α -Curcumene	C ₁₅ H ₂₂	202.34
43	30.55	499877355	2.91	Oxime-, methoxy-phenyl-	C ₈ H ₉ NO ₂	151.16
44	31.1	582711783	3.39	Pyridine, 3-butyl-, 1-oxide	C ₉ H ₁₃ NO	151.21
47	32.49	86384301	0.5	Butylated hydroxytoluene	C ₁₅ H ₂₄ O	220.35

Peak	RT (min)	Area	% Area	Compound Name	Molecular Formula	MW (g/mol)
52	37.06	245449408	1.43	p-Isopropoxyaniline	C ₉ H ₁₃ NO	151.21
53	37.19	267380157	1.56	Metacetamol	C ₈ H ₉ NO ₂	151.16
55	40.83	168227981	0.98	α R-Turmerone	C ₁₅ H ₂₀ O	216.32
56	41.91	153123033	0.89	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32
58	42.37	70975829	0.41	Galaxolide	C ₁₈ H ₂₆ O	258.4
59	43.13	44011626	0.26	Cinnamaldehyde, α -hexyl-	C ₁₅ H ₂₀ O	216.32

The research results in Table 2 show that the hexanal compound is the dominant compound that contributes to green and fresh aromas. This compound is produced from the oxidation of linoleic fatty acid, believed to be the source of high-quality, aromatic rice. 8-Nonen-2-one has a very high impact and possesses a fatty, creamy, and slightly floral odor that enhances the soft rice fragrance of Mayas with organic fertilizers treatment. Limonene, α -terpinolene, and (+)-4-carene indicate a monoterpene dominance with citrus, sweet, and herbal aromas. The levels of these compounds generally increase in organically grown plants because they have a higher secondary metabolic activity. The aroma of cooked rice is characterized by a roasted, nutty aroma of furan and 2-pentyl. Benzaldehyde imparts an almond-like aroma and sweet aromatic notes, thus enhancing sensory quality.

Table 2. Test results of aromatic compounds of Mayas rice after organic manure treatment

Peak	RT (min)	Area	% Area	Compound name	Molecular formula	MW (g/mol)
1	1.16	4842155	0.02	Acetaldehyde	C ₂ H ₄ O	44.05
3	4.67	1006443773	3.77	Toluene	C ₇ H ₈	92.14
5	5.3	6894218041	25.82	Hexanal	C ₆ H ₁₂ O	100.16
14	7.82	5.47E+14	20.5	8-Nonen-2-one	C ₉ H ₁₆ O	140.22
15	8.07	939953045	3.52	Limonene	C ₁₀ H ₁₆	136.24
18	9.15	2083643175	7.8	Furan, 2-pentyl-	C ₉ H ₁₄ O	138.21
20	10.21	244354665	0.91	p-Cymene	C ₁₀ H ₁₄	134.22
23	10.61	1502093943	5.63	α -Terpinolene	C ₁₀ H ₁₆	136.24
24	10.73	614284457	2.3	(+)-4-Carene	C ₁₀ H ₁₆	136.24
37	17.89	3116234165	11.67	Benzaldehyde	C ₇ H ₆ O	106.12
43	18.96	1507290109	5.64	Linalool	C ₁₀ H ₁₈ O	154.25
57	25.17	1293000319	4.84	Methyl salicylate	C ₈ H ₈ O ₃	152.15
61	26.61	1809191429	6.77	Anethole	C ₁₀ H ₁₂ O	148.2
74	39.1	57600073	0.21	Isoeugenol	C ₁₀ H ₁₂ O ₂	164.2
75	39.37	388332351	1.45	Cinnamaldehyde, α -hexyl-	C ₁₅ H ₂₀ O	216.32
79	40.16	44559277	0.17	Tonalid	C ₁₈ H ₂₆ O	258.4
81	41.06	257154375	0.96	Indole	C ₈ H ₇ N	117.15

These three compounds can produce floral, minty, and sweet-spicy aromas, or a dark, sweet flavor. These three compounds are usually associated with premium-quality aromatic rice. Low indole concentrations impart a floral character that enhances aroma complexity. Mayas rice has a sweet and grainy aroma reminiscent of walnuts. Smooth and not pungent. This profile demonstrates the use of organic fertilizers. Mayas rice contains high levels of aldehydes, especially hexanal, benzaldehyde, and menthol. Hexanal is the principal oxidation product of linoleic acid and originates from the lipoxygenase (LOX pathway) and is known to contribute to a green, fresh, grassy aroma in aromatic rice. The high hexanal concentration in Mayas rice shows that lipid metabolism occurs actively in an organic farming system.

The GC-MS analysis showed clear differences in the volatile profiles of Mayas rice grown using inorganic and organic fertilizers. Seventeen volatile compounds were found in rice from inorganic fertilization, while rice from organic fertilization had 16 key volatile compounds. These differences highlight how fertilization management affects the production and buildup of aroma-related molecules in rice grains. Previous research has shown that cultivation methods can change the makeup of volatile organic compounds

(VOCs) due to their impact on nutrient availability, soil microbial activity, and plant metabolic processes [22-24].

In the inorganic fertilization method, the volatile profile was mainly made up of aromatic hydrocarbons, phenolic compounds, and nitrogen-containing heterocyclic compounds like toluene, cresol, pyridine derivatives, methoxy-phenyl oxime, and 2,4-di-tert-butylphenol. Among these, pyridine, 3-butyl-, 1-oxide was one of the most abundant. Pyridine derivatives typically bring roasted, strong, and smoky aromas and often come from amino acid breakdown and Maillard-type reactions [25]. While these compounds may add to aroma strength, they are not usually considered key indicators of high-quality aromatic rice. High-quality aromatic rice usually features a balanced mix of aldehydes, alcohols, ketones, and heterocyclic compounds rather than an overabundance of nitrogen-containing aromatic compounds [26].

The presence of cresol, dimethylphenol, and other phenolic compounds indicates higher oxidative degradation in rice from inorganic fertilization. Phenolic volatiles typically add medicinal, smoky, and phenolic notes, which can dull the sweet and floral aromas that consumers prefer [27]. Similarly, naphthalene and aromatic hydrocarbon derivatives might contribute undesirable hydrocarbon-like odors, which are generally less favored in premium aromatic rice. Studies comparing aromatic and non-aromatic rice cultivars have found that hydrocarbon-rich volatile profiles tend to score lower in sensory preference [28].

Interestingly, α -curcumene and ar-turmerone were found in rice fertilized with inorganic materials. These sesquiterpenes are known for their woody, spicy, and herbal aromas. Although they were present in smaller amounts, they could enhance the complexity of the aroma profile [29]. However, their effects are likely overshadowed by the stronger phenolic and heterocyclic aroma compounds from this treatment.

In contrast, rice cultivated with organic fertilizer showed a very different volatile composition dominated by aldehydes, ketones, furans, terpenoids, and phenylpropanoid derivatives. Hexanal was the most abundant compound, making up 25.82% of the total volatile profile. It is a significant oxidation product of linoleic acid from the lipoxygenase (LOX) pathway and is known for providing fresh, green, and grassy notes in rice [30, 31]. Recent studies have identified hexanal as one of the main aroma-active compounds that enhance rice aroma quality and consumer acceptance [30, 32].

The high level of 8-Nonen-2-one (20.50%) further set apart the rice grown organically. Ketones contribute creamy, fatty, and slightly floral aromas, softening the overall aroma profile and increasing sensory appeal [33]. The combination of hexanal and 8-nonen-2-one indicates a well-balanced volatile composition, creating a pleasant and smooth aroma profile.

Organic fertilization also fostered the buildup of monoterpene compounds like limonene, α -terpinolene, and (+)-4-carene. These terpenoids add citrus, floral, sweet, and herbal notes. Terpenoids greatly influence aroma complexity and are often found in high-quality aromatic crops [34]. The increased accumulation of terpenoids under organic farming may result from improved soil biological properties and nutrient cycling [22, 23]. Organic fertilization boosts soil organic carbon, encourages microbial diversity, and enhances micronutrient availability, which supports the pathways needed for producing secondary metabolites [35].

Benzaldehyde was another key volatile identified in organically grown Mayas rice, accounting for 11.67% of the total volatiles. It adds sweet, almond-like, and pleasant aromatic notes and is essential for high-quality aromatic rice [36]. The notable presence of benzaldehyde in this study suggests better aroma quality in rice grown with organic fertilizers.

Additionally, linalool, methyl salicylate, anethole, and isoeugenol were found either exclusively or at higher levels in rice from organic cultivation. Linalool is famous for its floral aroma, while methyl salicylate contributes sweet and mint-like notes [34]. Anethole and isoeugenol belong to the phenylpropanoid group and provide sweet, spicy, and floral characteristics, enriching aroma complexity. These compounds are typically linked to appealing sensory qualities in aromatic crops and are often associated with heightened secondary metabolism in environmentally balanced farming systems [35].

The presence of 2-pentylfuran in organically cultivated rice is also significant. Furan compounds are critical for cooked-rice aroma, known to create roasted, nutty, and toasted notes [37]. Previous research has

highlighted 2-pentylfuran as a distinctive compound that sets aromatic rice apart from non-aromatic varieties [38]. The mix of aldehydes, furans, ketones, and terpenoids in organically grown Mayas rice points to a more complex and appealing aroma profile.

The observed differences between the two fertilization systems likely stem from their effects on plant metabolism. Organic fertilizers release nutrients gradually and enhance soil physical, chemical, and biological properties. Increased microbial activity in organically tended soils can boost the biosynthetic pathways that produce volatile aldehydes, terpenoids, and phenylpropanoids [23, 24]. On the other hand, heavy use of inorganic fertilizers tends to promote primary metabolism linked to rapid plant growth, reducing the carbon allocated to making secondary metabolites [39]. Thus, rice grown with inorganic fertilizers had a volatile profile dominated by aromatic hydrocarbons and phenolic compounds, in contrast to rice grown organically, which had more aroma-active compounds associated with appealing sensory traits.

Recent metabolomic research also found that nitrogen management greatly affects the volatile composition of rice. Too much nitrogen can change amino acid metabolism and lower the formation of several aroma-related compounds, while balanced nutrient levels support the buildup of aldehydes, terpenoids, and other aroma-active metabolites [40]. Similar findings have emerged for fragrant rice cultivated organically, where organic methods changed the VOC composition and improved grain aroma quality compared to traditional farming.

IV. CONCLUSION

The results of GC-MS analysis of aromatic compounds in Mayas rice in organic fertilizer treatment obtained 81 compounds, while in inorganic fertilizer treatment, only 59 aromatic compounds were obtained. GC-MS analysis revealed that the aromatic compound profiles in the organically fertilized Mayas Rice were more complex than those in the inorganic fertilized Mayas Rice. The differences were seen based on the diversity of aldehyde compounds, terpenoids, esters, aromatic alcohols, and heterocyclic compounds identified.

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