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Total Phenol Content, Total Flavonoid Content, Antioxidant Activity and GC-MS Analysis of a New Variety of Rice (*Oryza sativa* L.)

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Abstract

Consuming foods rich in antioxidants plays an important role in human health, one of which is through rice. This research aimed to describe the total phenol content and total flavonoids content as well as GC-MS analysis of raw Baroma rice extract and analyze the antioxidant activity of Baroma rice in different cooking methods. Baroma rice was collected from Semarang, Central Java, Indonesia thus was analyzed to get data about total phenol content, total flavonoid content, and compouds identifies using GC-MS. We also analyzed antioxidant activity of Baroma rice among raw, rice cooked in stove and *magic com* extract using Anova test followed by Tukey test. The result proved that total phenol content was 85.64 mg/L and total flavonoid content was 78.28 mg/L, meanwhile the highest antioxidant activity was in raw extract of Baroma followed by rice cooked in magic com and stove, suggesting that cooking method effected on its property. GC-MS analysis showed the diversity of chemical compound, totaling 15 compounds and peaks.

Keywords: antioxidant activity; Baroma variety; GC-MS analysis; flavonoid; phenol.

INTRODUCTION

Food is an essential nutrient for humans, especially its nutritional content for growth. Food is also actually part of the treatment that can be done for health. Over time, the existence of EPFRs which is abbreviation of environmentally persistent free radicals has become a health threat due to their potential to damage DNA and be toxic (Qin et al., 2025). Therefore, consuming foods containing antioxidants is one way to neutralize the malignant effects of free radicals. This requires vigilance, considering that free radicals such as ROS (hydrogen peroxide (H2O2), hydroxyl radicals (•OH), (superoxide radicals (O2•−), and singlet oxygen (1O2)) are suspected of causing diseases (Pizzino et al., 2017) such as cancer (R. Huang et al., 2021), diabetes (Volpe et al., 2018), and cardiovascular disease (Elias-Llumbet et al., 2024).

Antioxidant activity has been studied in many plant species such as the Apiaceae family (Bello et al., 2025), *Bauhinia saccocalyx* (Katisart et al., 2025), *Bacopa monnieri* (Govindarasu & Murugesh, 2025), *Schefflera vinosa* (Singh et al., 2021). In *Oryza sativa*, its antioxidant activity is also worth considering that rice is a staple food consumed by more than half of the world's population, especially in Asia mainly due to its protein, carbohydrate, vitamin, and essential mineral content (Colasanto et al., 2024). In colored rice varieties such as black rice western Taiwan; Taibalang red waxy rice; Taibalang black waxy rice; black rice Thailand; red rice Thailand; Guangfu red rice; are proven to have high antioxidant activity (Y. P. Huang & Lai, 2016). In line with Huang, (Shao et al., 2014) also stated that pigmented rice, especially black rice, has higher antioxidant activity and total phenolic content in the endosperm, embryo, and bran compared to white rice and red rice. Brown and purple rice are also reported to have promising antioxidants as in the study (Gong et al., 2017) (Li et al., 2024). This antioxidant activity is closely related to the existance of phenols and flavonoids (Suryanti et al., 2020). (Yusoff et al., 2022) argued that flavonoids are bioactive compounds that contribute to antioxidant activity.

Baroma is a rice variety launched directly by the Indonesian Ministry of Agriculture in 2019 through Balai Besar Padi (Rice Center in Indonesia). Baroma is an aromatic Basmati rice that has been refined by Indonesian researchers consequently it can grow in lowlands. Baroma is claimed to have higher productivity compared to Basmati and the rice size remains long even when planted in lowlands. Research on Baroma rice is still limited, such as (Pranatami & Akmalia, 2025)'s research on the potential of Baroma rice as an anticancer, the nutritional value of Baroma rice in different cooking methods (Akmalia et al., 2024), and Baroma rice as a variety for food security (Windiyani & Rusdianto, 2020).

Information related to metabolite or compounds and the total content of Baroma rice has never been reported. Therefore, an in-depth analysis of total phenols, total flavonoids and GC-MS analysis of raw Baroma rice as well as antioxidant activity with different cooking methods is necessary. This study aims to describe the total phenol content and total flavonoids content as well as GC-MS analysis of raw Baroma rice extract and analyze the antioxidant activity of Baroma rice in different cooking methods.

MATERIALS AND METHODS

Materials

The materials used in this research were Baroma rice (*Oryza sativa* L.) collected from Semarang, Central Java, Follin ciocalteus phenol reagent, aquadest, gallic acid, sodium nitrite, aluminum nitrate, sodium hydroxide, quercetin, methanol (Merck), DPPH reagent, ethanol (Merck). The tools used were food thermometer, *magic com*, stove, spectrophotometer, and Thermo Scientific GC-MS.

Procedures

Preparation of tested rice

Two hundred g of rice (*Oryza sativa* L.) was cooked using a stove and another 200 g was cooked using a *magic com* in 20 minutes. The temperature of the cooked rice was measured using a food thermometer with the results of the stove-cooked rice having a temperature of 87.8℃, and 87.9℃ for the magic com-cooked rice. We tested the total phenols and flavonoids in the raw rice extract and for the further test, we did the antioxidant test. Baroma rice extract was obtained through the maceration method with 100 mL of ethanol solvent using 200 g of raw Baroma rice, rice cooked in magic com, and rice cooked in stove. Antioxidant activity testing was carried out on 3 extracts of raw Baroma rice, cooked magic com, and cooked stove using the DPPH method. We also tested the compound in raw extract using Gas Cromatography-Mass spectrometry (GC-MS).

Determination of Total Phenol Content Equivalent Gallic Acid using Spectrophotometric Method

A sample of 1 mL was taken then add 0.5 mL of Follin ciocalteus phenol reagent and 7.5 mL of Aquadest. It was shaken then let it stand for 10 minutes. We added 1.5 mL of 20% sodium carbonate solution and shake also let it stand for 10 minutes. Add Aquadest up to 10 mL and read the absorbance at a wavelength of 760 nm.

Determination of Total Flavonoid Content Equivalent Quercetin using Spectrophotometric Method

A sample of 1 mL was taken then add 0.3 mL of 5% sodium nitrite and wait for 5 minutes. Add 0.6 mL of 10% aluminum nitrate, wait for 5 minutes. It was then added 2 mL of 1 M sodium hydroxide and adjust it to a volume of 10 mL. The las step was the reading of absorbance at λ 510 nm.

Antioxidant Analysis Procedure by DPPH Method Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995) Method (Brand-Williams et al., 1995)

Five mL PA methanol was added to sample 0.2 g then extracted by vortexing. The extract was taken as much as 0.2 mL. Add 2.8 mL of 0.1 mM DPPH reagent, then incubated at room temperature and dark room for 1 hour. It was then calibrated with a 515 nm spectrophotometer, also did it to make a blank of 0.2 mL methanol + 2.8 ml of 0.1 nM DPPH reagent.

The DPPH free radical scavenging activity was calculated as follows and was measured equivalent in % wb:

$$\% \text{ DPPH} = \frac{1 - \text{sample absorbance}}{\text{sample absorbance}} \times 100\%$$

GC-MS Analysis Procedure

The ethanol extract of raw Baroma rice was analyzed by Thermo Scientific GC-MS. It was run using column with length 30 m, diameter 0,250 mm, and film thickness 0,25 μm. Injector temperature was 250℃, splitless injection method, and the carrier gas was helium with operating pressure of 70,69 kPa. An initial temperature we used was 40℃ for 3 min; a linear increase to 100℃ at 5℃/minutes and hold for 3 min and ramping to 250℃ at 10℃/ minutes and hold for 4 minutes (Hu et al., 2023).

Data analysis

Data related to total phenol content, total flavonoid content, and GC-MS analysis were described descriptively while the antioxidant activity was measured equivalent in % wb then the values obtained from the three extracts were analyzed using the ANOVA test using JASP software followed by Tukey test.

RESULTS AND DISCUSSION

We tested the presence of flavonoid and phenolic compounds, which play a role in scavenging free radicals, by testing total phenols and flavonoids. Groups of bioactive substances known as flavanoids are further classified as flavones, flavanols, flavanones, flavonols, and isoflavones.The results showed that the total observed phenol was 85.64 mg/L and the total flavonoid was 78.28 mg/L (Table 1).\

Table 1. Total phenol dan flavonoid content in raw Baroma rice.

No	Parameter	Calculated Amount (mg/L)
1.	Total phenol content	85,64
2.	Total flavonoid content	78,28

The results of the antioxidant activity of the raw extract and the *magic com* and stove methods are described in Table 2. Cooking significantly reduces antioxidant activity, with the lowest value in Baroma rice cooked using a stove. However, % wb values for both cooking methods are still relatively high, so these methods can be recommended for use when cooking rice.

Table 2. Antioxidant Activity of Baroma rice in raw, magic com cooking and stove cooking**.**

No	Extract	% wb
1.	Raw Baroma	2,23^{a}
2.	Magic com cooking of Baroma	1,74^{ab}
3.	Stove cooking of Baroma	1,08^{b}

Numbers followed by the same letter indicate no significant difference in the Tukey test with a 95% confidence level.

GC-MS analysis is performed to detect compounds even in small concentrations. The advantages of GC-MS include its high sensitivity and the ability to separate mixed compounds (Arsana et al., 2022), although it depends on extraction conditions, sample preparation techniques, and analytical parameters (Phuong et al., 2025). The result of GC-MS detection is summarized in Table 3.

Table 3. Compound identified in Baroma Rice using GC-MS.

Peak	Retention Time	Similarity Index	Compoound Name	Relative Area (%)	Formula
7	4,93	731	Ethanone, 2-(formyloxy)-1-phenyl-	5,87	$C_9H_8O_3$
11	6,58	819	Arsenous acid, tris(trimethylsilyl) ester	0,62	$C_9H_{27}AsO_3Si_3$
20	24,46	867	1-Dodecanol	0,24	$C_{12}H_{26}O$
21	24,52	928	Cyclododecane	3,23	$C_{12}H_{24}$
23	25,11	911	2,4-Di-tert-butylphenol	0,70	$C_{14}H_{22}O$
24	26,19	869	Dodecanoic acid, ethyl ester	0,38	$C_{14}H_{28}O_2$
27	27,40	819	2-Propenoic acid, pentadecyl ester	6,08	$C_{18}H_{34}O_2$
36	28,53	877	Tetradecanoic acid, ethyl ester	1,04	$C_{16}H_{32}O_2$
38	29,45	788	5-Octadecenal	0,17	$C_{18}H_{34}O$
40	30,58	895	Hexadecanoic acid, ethyl ester	16,93	$C_{18}H_{36}O_2$
43	30,87	809	Isopropyl palmitate	0,27	$C_{19}H_{38}O_2$
44	32,17	900	n-Propyl 9,12-octadecadienoate	8,32	$C_{21}H_{38}O_2$
45	32,21	871	Ethyl Oleate	6,06	$C_{20}H_{38}O_2$
46	32,27	808	n-Propyl 11-octadecenoate	0,94	$C_{21}H_{40}O_2$
47	32,43	836	Eicosanoic acid, ethyl ester	8,57	$C_{22}H_{44}O_2$

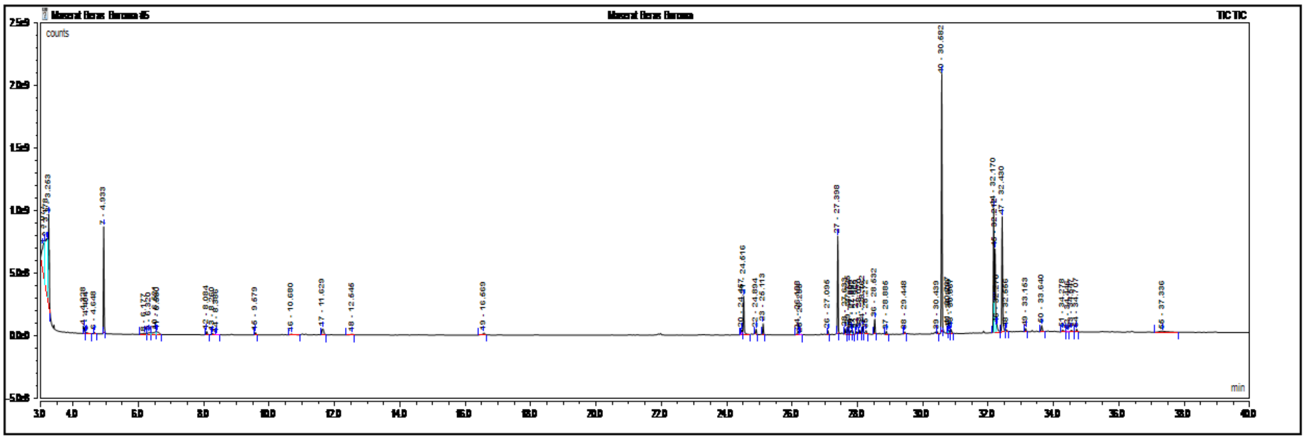

Figure 1. Chromatogram of GC-MS analysis.

Figure 1 shows that there are 55 peaks and 133 compounds of Baroma rice analyzed using GC-MS. After sorting the compounds through Similarity Index selection and comparison with the PubChem database, 15 compounds were successfully identified. These compounds were located at peaks 7, 11, 20, 21, 23, 24, 27, 36, 38, 40, 43, 44, 45, 46, and 47. As clearly seen in

Table 3 and Figure 1, peak number 40 has the largest relative area of 16.93% with the retention time is 30,582.

Discussion

Baroma rice (*Oryza sativa* L.) is white rice which, when compared to pigmented rice, apparently has fewer total phenols and flavonoids (see Table 1). This is proven in the study of (Ghasemzadeh et al., 2018) who obtained a total phenolic content of black rice of 1214.7 mg GAE/100 g DM and 811.32 mg GAE/100 g DM for red rice. Meanwhile, the total flavonoid content of black rice was mg QE/100 g DM and red rice was 457.00 mg QE/100 g DM. Phenols and flavonoids have been known as potential antioxidants in black rice (Suryanti et al., 2020) which play a role in capturing free radicals such as ROS and RNS thus they can be used for antimicrobial, anti-inflammatory, anticarcinogenic and anticancer therapy. Free radicals can damage cell components such as fat, protein, nucleic acid and ultimately cause cell death (Woo et al., 2018). (Subedi et al., 2014) claimed that free radical production induces disorders such as asthma, diabetes, cardiovascular disease, cancer, inflammatory disease, neurodegenerative diseases, and premature aging. Phenols and flavonoids work by directly scavenging ROS radicals and inhibiting ROS formation by chelating trace elements, such as quercetin, which chelates iron and stabilizes iron (Dias et al., 2021). According to (Banjarnahor & Artanti, 2014), the free hydroxyl group will provide a radical molecule its hydrogen atom, stabilizing it and producing a relatively stable flavonoid phenoxyl radical. The presence of phenol and flavonoid compounds is very linear with the antioxidant activity of plants (Kusumawardani & Luangsakul, 2024; Subedi et al., 2014).

Das et al. (2023)contended that knowledge about food nutrition is important for regular consumption. The decrease in antioxidant activity in cooked rice (see Table 2) is thought to be due to a decrease in phenol or flavonoid levels. (Fracassetti et al., 2020) studied the antioxidant activity of raw and cooked Carnaroli, Venere, and Artemide rice varieties. The results showed that the Total Phenol Index decreased to 31.7% in Carnaroli, 58.3% in Venere, and 71.7% in Artemide compared to the contents in the raw samples. Similar results were obtained by (Phanthurat & Thatsanasuwan, 2023), where the streamer and rice cooker methods significantly reduced total phenol and flavonoid content, as well as antioxidant activity. The reduction of phenolic compounds following cooking can be ascribed to various factors, including the water-rice ratio, the duration of cooking (Fracassetti et al., 2020), and temperatures (Aleixandre et al., 2022). Furthermore, the loss of total phenol content (TPC) may be due to several factors including the migration of biocompounds into the cooking water, chemical degradation or transformation into different compounds, aa well as interactions with other food components, which consequently diminishing their extractability (Bani et al., 2024).

Bani et al. (2024) also emphasized that cooking can lead to a loss of antioxidant activity, observed in rice varieties BO (brown orange); BC (brown carnaroli); BV (brown violet); MC (milled carnaroli); MV (milled violet); MO (milled orange); The varieties with the most significant reduction in AOA were Brown violet and milled carnaroli. Furthermore, (Tang et al., 2016)'s research found that anthocyanins, which also play a role in antioxidant activity, undergo degradation during high-temperature cooking using the rice cooker method. Consuming food with proper cooking methods can prevent the loss of antioxidants. Elevated level of ROS facilitate tumor progression by impairing the function of T cells and natural killer (NK) cells while also encouraging the recruitment and M2 polarization of macrophages (R. Huang et al., 2021). Therefore, it is necessary to adjust rice cooking methods to maintain high antioxidant activity.

As observed in Figure 1 and Table 3, the chromatogram shows that there are 15 peaks volatile compounds successfully extracted from raw Baroma rice. The compound in peak 40 is identified to be Hexadecanoic acid, ethyl ester which has the highest composition characterized by a relative area of 16.93% and a retention time of 30.58. Hexadecanoic acid, ethyl ester compound has a molecular weight of 284.5 g/mol and a molecular formula of $C_{18}H_{36}O_2$. This compound has been proven to have potential as an anti-inflammatory tested on model mice (Saeed et al., 2012), nematicide or pesticide (Sulochana et al., 2016) and anti-chikungunya virus (Sagna et al., 2023). In this peak, another compound actually is suspected to be Pentadecanoic acid, ethyl ester that has a molecular weight of 270.5 g/mol and a molecular formula of $C_{17}H_{34}O_2$. This compound is a derivative of palmitic acid, which is a significant element influencing the efficacy of chemotherapy in multiple myeloma (Dong et al., 2024). (Casillas-Vargas et al., 2021) also reported that this compound has antibacterial activity against both gram-positive and gram-negative bacteria. At peak 47, it is proved to be Eicosanoic acid, ethyl ester with a relative area of 8.57% and a retention time of 32.430. Eicosanoic acid, ethyl ester is a compound possessing a molecular weight of 340.6 g/mol and a molecular formula of $C_{22}H_{44}O_2$ which is formed by the esterification of long-chain straight-chain fatty acids, eicosanoic acid and ethanol. (Sokeng et al., 2020) showed that Eicosanoic acid, ethyl ester acts as an anti-inflammatory and analgesic effect.

n-Propyl 9,12-octadecadienoate is a compound in peak 44. The compound n-Propyl 9,12-octadecadienoate, also known as linoleic acid propyl ester, has a molecular weight of 322.5 g/mol and a molecular formula of $C_{21}H_{38}O_2$. This compound is an ester of a fatty acid resulting from the formal condensation of the carboxylic group of inoleic acid with the hydroxy group of propanol. Linoleic acid is the main active ingredient in Wali fruit seed oil which has various biological and pharmacological activities, and is often used to cure various cancers, such as liver, lung, and gastrointestinal cancer (Wu et al., 2017). Besides, another suspected compound in this peak is 9,12-Octadecadienoic acid that has a molecular weight of 280.4 g/mol and a molecular formula of $C_{18}H_{32}O_2$. 9,12-Octadecadienoic acid is otherwise known as omega-6 unsaturated fatty acid (Krishnaveni et al., 2014). It is also apparently useful in the treatment of hyperlipidemia and atherosclerosis (Hamilton-Amachree & Uzoekwe, 2017) and has proven antibacterial and antifungal activity against *S. aureus, S. pyogenes, S. typhi, E. coli, C. albicans*, and *A. niger* (Onoabedje et al., 2025).

Arsenous acid, tris(trimethylsilyl) ester in Baroma rice was observed at peak 11. Recent studies outlined by (Wiraswati et al., 2023) suggest that this compound was also seen in methanolic extract of *Momordica cymbalaria* containing biological activity to combat diabetes mellitus, diarrhea, skin disease, rheumatism, and ulcers. Cyclododecane, as found in peak 21, is a member of cycloalkanes and 2,4-Di-tert-butylphenol in peak 23 is the property of the class of organic compounds known as phenylpropanes. Dodecanoic acid, ethyl ester also known as ethyl laurinate or ethyl laurate belongs to fatty acid esters. This compound is a multipurpose emulsifier, moisturizer, and texture-enhancing ingredient in the cosmetics industry (Villanueva-Bermejo et al., 2025). Meanwhile, (Khromykh et al., 2022) found that 2-Propenoic acid, pentadecyl ester express antioxidant and antimicrobial activity in fruits and leaves extract of *Actinidia polygama* and *A. arguta*. Tetradecanoic acid, ethyl ester also known as Ethyl myristate, makes up a significant portion of the total fatty acids in palm kernel and coconut oils (Mohebbifar, 2021). It is a part of the fatty acid ethyl esters and is made of myristic acid. It is also used as one of biodiesels component. As established by (Guo et al., 2006), Isopropyl palmitate acts as a penetration enhancer of drugs in ethanol solution and (Chen et al., 2021) claimed that Ethyl oleate as effective acaricides for the control of spider mites. Moreover, n-Propyl 11-octadecenoate reported in *S. mombin* leaf extract acts as an organic addition in the production of biogenic soap (Ohiri et al., 2023). The research also showed the presence of 5-octadecenal in peak 38 as aldehydes. Aldehydes are odorous compounds commonly found in fragrant rice, such as Baroma rice, and are typically synthesized from the oxidation of free fatty acids or the decomposition of linoleic acid (Hu et al., 2023). Besides, Ethanone, 2-(formyloxy)-1-phenyl- in peak 7 also produces aromatic compound in the presence of ketone. Furthermore, 1-Dodecanol, a fatty alcohol, was detected, which also plays a role in producing floral odors.

The production of metabolite compounds is highly dependent on the region (Arsana et al., 2022), climate change, and environmental conditions around the plant (Pant et al., 2021). (Feng et al., 2019)'s research related to GC-MS analysis proves that secondary metabolite compounds in rice in different habitats have differences. Rice from the Jiansanjiang region has unique compounds, there are benzoic acid, glucose, fumaric acid, Myo-inositol, Xylose, xylitol, sorbitol, and Raffinose, while rice from Wuchang has Pentanoic acid, Glucopyranose, Octadecanoic acid, Eicosanoic acid, Docosanoic acid, 1-Monohexadecanoylglycerol, Trehalose, β -Tocopherol, and β-sitosterol. In this study, Baroma rice analyzed using GC-MS came from Semarang, Central Java, so it is very possible that the results will be different if using Baroma rice grown in other areas. Research related to Baroma rice can still be elaborated again with the profiling of metabolite compounds and antioxidant activity in rice with different habitat.

CONCLUSIONS

The total phenol and flavonoid content of Baroma rice were detected at 85.64 mg/L and 78.28 mg/L, respectively. The highest antioxidant activity was observed in the raw extract of Baroma rice, while the magic com cooking method had a higher % wb value than the stovetop cooking method. Meanwhile, GC-MS analysis showed that 15 compounds were successfully extracted, with peak number 40 having the largest relative area of 16.93%. The compound is identified to be hexadecanoic acid, ethyl ester. Further research can be conducted, including analyzing other compounds that play a role in antioxidant activity such as carotenoids, vitamins, or trace elements and expanding the variety of cooking methods to find the most appropriate method for maintaining the antioxidant activity of Baroma rice.

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