

## Chemical Profile and Antioxidant Activity of Rhizome-Based Kombucha

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**Abstract.** Kombucha is a fermented beverage produced through the symbiotic activity of acetic acid bacteria and yeasts within a SCOBY (Symbiotic Culture of Bacteria and Yeast). This study aimed to evaluate the chemical characteristics and antioxidant activity of kombucha produced from various rhizomes, namely turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), aromatic ginger (*Kaempferia galanga*), ginger (*Zingiber officinale*), and Javanese turmeric (*Curcuma xanthorrhiza*), with black tea kombucha used as a control. Fermentation was conducted for 12 days using black tea kombucha as the starter culture. The analyzed parameters included pH, total acidity, total sugar, total phenolic content, and antioxidant activity determined using the DPPH radical scavenging method. Data were analyzed descriptively, and the best treatment was selected using a Multi-Criteria Decision-Making (MCDM) approach with the Simple Additive Weighting (SAW) technique. The results demonstrated that rhizome-based kombucha fermentation decreased in pH and total sugar content, accompanied by increases in total acidity, total phenolic content, and antioxidant activity. Among the tested treatments, white turmeric kombucha exhibited the best overall performance, with a pH of 3.75, total sugar content of 3.11%, total phenolic content of 117.8  $\mu\text{g GAE/mL}$ , and antioxidant activity of 72.97%. Nevertheless, the antioxidant activity of white turmeric kombucha remained lower than that of black tea kombucha as the control. These findings indicate that rhizomes, particularly white turmeric, have potential as alternative substrates for functional kombucha production, although further optimization is required to enhance their antioxidant capacity.

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### INTRODUCTION

Kombucha is a tea-based fermented beverage produced through the symbiotic activity of acetic acid bacteria and yeasts which are embedded in a SCOBY (Symbiotic Culture of Bacteria and Yeast). During fermentation, this microbial consortium utilizes sucrose as a carbon source. It converts it into a wide range of bioactive metabolites, including organic acids such as acetic, gluconic, lactic, citric, and malic acids, as well as polyphenol biotransformation products, water-soluble vitamins, and other bioactive compounds that contribute to kombucha's functional characteristics (Macedo et al., 2020). The chemical composition and biological activity of kombucha are strongly influenced by the type of substrate, carbon source, and fermentation conditions, which collectively determine microbial metabolic dynamics throughout fermentation.

### Citation

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In recent years, kombucha has gained increasing popularity as a functional beverage due to its reported biological activities, including antioxidant, antibacterial, antidiabetic, antihyperlipidemic, and immunomodulatory effects, as well as its role in supporting gastrointestinal health (Coelho et al., 2020). The antioxidant activity of kombucha is primarily attributed to the presence of polyphenolic compounds and their biotransformation products generated during fermentation, which play a crucial role in scavenging free radicals and reactive oxygen species (ROS). Kombucha produced from green and black tea is well recognized for its high antioxidant capacity due to the natural abundance of catechins and polyphenols in tea leaves, which can be enzymatically released and modified during fermentation (Onsun et al., 2025). Nevertheless, reliance on tea as the primary substrate has stimulated growing interest in exploring alternative non-tea substrates that may enrich the phytochemical profile and enhance the functional value of kombucha.

Indonesia possesses a rich diversity of rhizomatous plants (*empon-empon*) that are abundant in bioactive compounds and exhibit considerable potential as alternative substrates for kombucha production. Several rhizomes, including ginger, turmeric, white turmeric, kencur (*Kaempferia galanga*), and Javanese turmeric (*Curcuma xanthorrhiza*), are known to contain phenolic compounds, flavonoids, curcuminoids, and vitamins that function as natural antioxidants. Ginger contains gingerols and shogaols, which have been reported to exhibit antioxidant and hypoglycemic activities (Shenoy & Mahurkar, 2022), while turmeric and Javanese turmeric are rich in curcuminoids that play important roles in mitigating oxidative stress and inflammation (Perez et al., 2025; Xu et al., 2025). White turmeric (*Curcuma zedoaria*) contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which have been reported to possess antioxidant activity and cytotoxic potential against certain cancer cell lines (Gharge et al., 2021). Meanwhile, kencur is known to contain flavonoids, saponins, and polyphenols with significant antioxidant potential (Ahmed et al., 2020). These phytochemical attributes highlight rhizomes as promising substrates for the development of functional kombucha.

Several previous studies have demonstrated that fermentation of rhizomes into kombucha can enhance antioxidant activity. Ginger kombucha supplemented with honey was reported to exhibit antioxidant activity ranging from 63.07% to 83.81%, while turmeric-based kombucha showed an antioxidant activity of 52.95% (Leonard et al., 2021). This enhancement in antioxidant activity is presumed to be associated with the release and biotransformation of phenolic compounds during fermentation. In the case of *Alpinia galanga* and *Curcuma xanthorrhiza*, previous studies have reported the presence of diverse bioactive compounds, including phenolics, flavonoids, and curcuminoids, which may undergo structural modifications during fermentation, thereby increasing antioxidant capacity (Shenoy & Mahurkar, 2022). However, to date, comparative studies that simultaneously evaluate the chemical characteristics and antioxidant activity of kombucha produced from white turmeric, kencur, and Javanese turmeric under a uniform fermentation system remain limited. This lack of comparative data hampers a comprehensive assessment of the functional potential of different rhizome-based kombucha formulations.

Therefore, this study aimed to evaluate the chemical characteristics and antioxidant activity of kombucha produced from five different rhizomes, namely ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), aromatic ginger (*Kaempferia galanga*), and Javanese turmeric (*Curcuma xanthorrhiza*). The findings of this study are expected to provide a more comprehensive scientific basis for the utilization of local rhizomes as raw materials for functional kombucha and to identify the rhizome with the most optimal antioxidant potential for the development of value-added fermented beverages.

## MATERIALS AND METHODS

### Experimental Design

This research is an experimental study. This study employed a Randomized Complete Block Design (RCBD) with a single factor: rhizome type. The treatments consisted of black tea kombucha as the control (S1), turmeric (*Curcuma longa*) kombucha (S2), white turmeric (*Curcuma zedoaria*) kombucha (S3), aromatic ginger (*Kaempferia galanga*) kombucha (S4), ginger (*Zingiber officinale*) kombucha (S5), and Javanese turmeric (*Curcuma xanthorrhiza*) kombucha (S6). The rhizome samples were obtained from a local market in Malang, Indonesia, and selected for freshness and uniform size. Each was prepared with a rhizome concentration of 0.8% (w/v), selected based on previous studies and preliminary trials indicating its suitability for supporting fermentation while maintaining acceptable sensory characteristics and bioactive compound stability (Zubaidah et al., 2025). All treatments were conducted in triplicate.

### Preparation of Rhizome Materials

Fresh rhizomes were peeled, thoroughly washed, and sliced to a thickness of 1–3 mm. The sliced rhizomes were dried using a cabinet dryer at 60 °C for 5 h to reduce moisture content and inhibit enzymatic activity. The dried rhizomes were subsequently ground to obtain a coarse powder and stored in airtight containers until further use. For each treatment, 8 g/L of dried rhizome powder was used to achieve a 0.8% (w/v) concentration.

### Preparation of Rhizome-Based Kombucha

Dried rhizome powder was weighed to obtain a 0.8% (w/v) concentration and placed into tea bags. A total of 500 mL of water was brought to a boil, infused with the rhizome tea bags for 4 min, then 10% (w/v) sucrose was added and stirred for 1 min. The infusion was then allowed to cool to room temperature.

The rhizome infusion was transferred into sterile glass jars and inoculated with 10% (v/v) kombucha starter containing SCOBY. Fermentation was carried out at room temperature, protected from direct light, for 12 days. Samples were collected on day 0 and day 12 of fermentation. Black tea kombucha was prepared using the same procedure and served as the control.

### Chemical Analysis

Chemical profile analysis included the determination of pH, total acidity, total phenolic content, and total sugar. The pH value was measured using a digital pH meter, calibrated with standard buffer solutions at pH 4.0 and 7.0, according to the method of Chen et al. (2025). Total acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator, following the method of Chen et al. (2025) as modified by Zubaidah et al. (2021), and the results were expressed as a percentage of acetic acid. Total phenolic content was analyzed using the Folin–Ciocalteu method with gallic acid as the standard, as described by Amelo et al. (2025).

Absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 770 nm, and the results were expressed as mg gallic acid equivalents (GAE) per gram of sample. Total sugar content was determined using the anthrone method (Chen et al., 2025), with absorbance measured at 630 nm using glucose as the standard, and the results were expressed as a percentage (%).

### Antioxidant Activity Analysis

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The samples were reacted with 0.2 mM DPPH solution in methanol and incubated for 30 min in the dark at room temperature. Absorbance was measured at the maximum wavelength of 516.4 nm using a UV–Vis spectrophotometer (Zubaidah et al., 2025). The percentage of antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity(\%)} = \frac{A_0 - A_s}{A_s} \times 100$$

where  $A_0$  represents the absorbance of the blank and  $A_s$  represents the absorbance of the sample.

### Data Analysis

The data were analyzed using analysis of variance (ANOVA) with Minitab® software version 18. When significant differences were detected ( $P < 0.05$ ), mean comparisons were performed using Fisher's Least Significant Difference (LSD) test. The selection of the best treatment based on chemical characteristics and antioxidant activity was carried out using a Multi-Criteria Decision-Making (MCDM) approach with the Simple Additive Weighting (SAW) technique.

## RESULTS AND DISCUSSION

### 1. Characteristics of Raw Materials Used in Rhizome-Based Kombucha and Black Tea Kombucha

The raw materials used in this study consisted of five types of rhizomes, namely turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), ginger (*Zingiber officinale*), kencur (*Kaempferia galanga*), and Javanese turmeric (*Curcuma xanthorrhiza*), which were obtained from Belimbing Market, Malang, Indonesia. The rhizomes were physically selected to ensure they were free of mechanical damage, decay, or fungal contamination. Subsequently, the rhizomes were peeled, washed, sliced to a thickness of 1–3 mm, and dried using a cabinet dryer at 60 °C for 5–6 h. The dried rhizomes were then ground to obtain coarse powders and weighed according to the designated treatment concentrations. Commercial black tea kombucha (Sorro®) was used as the control. Sucrose was added at 10% (w/v), and the aged black tea kombucha starter was inoculated at 10% (v/v).

The initial characteristics of rhizome-based kombucha and black tea kombucha control were analyzed on day 0 of fermentation, including pH, total acidity, total phenolic content, and total sugar (Table 1). The results showed that the initial total acidity values were relatively uniform across treatments, ranging from 0.027% to 0.039%. Variations in total acidity were closely associated with pH, with lower pH values generally corresponding to higher total acidity. This inverse relationship is consistent with the findings of Wang et al. (2022), who reported that pH is negatively correlated with total acidity in fermented beverages. The relatively low initial pH values observed in rhizome-based kombucha were attributed to the addition of a kombucha starter previously fermented for 14 days, which resulted in an acidic initial fermentation medium.

**Table 1.** Chemical characteristics of rhizome-based kombucha and black tea kombucha on day 0 of fermentation

Rhizome	Total Acidity (%)	pH	Total Phenolic ( $\mu\text{g GAE/mL}$ )	Total Sugar (%)
Turmeric	$0.027 \pm 0.024^b$	$4.53 \pm 0.11$	$56.00 \pm 13.68^c$	$9.09 \pm 0.83$
White turmeric	$0.034 \pm 0.038^{ab}$	$4.79 \pm 0.89$	$45.12 \pm 17.01^d$	$9.11 \pm 1.1$
Kencur ( <i>Kaempferia galanga</i> )	$0.033 \pm 0.029^{ab}$	$4.74 \pm 0.66$	$44.42 \pm 9.35^d$	$9.07 \pm 2.33$
Ginger	$0.033 \pm 0.034^{ab}$	$4.61 \pm 0.34$	$66.52 \pm 18.35^b$	$9.29 \pm 2.95$
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	$0.036 \pm 0.037^{ab}$	$4.81 \pm 0.71$	$50.73 \pm 16.44^{cd}$	$9.06 \pm 2.40$
Black tea control	$0.039 \pm 0.034^a$	$4.27 \pm 0.36$	$214.94 \pm 8.42^a$	$9.32 \pm 0.63$

**Note :** Different superscript letters within the same column indicate significant differences according to Fisher's Least Significant Difference (LSD) test at a 95% confidence level.

The initial total phenolic content showed significant differences among rhizome types. This variation is attributed to differences in the intrinsic phenolic compound content of each rhizome. Turmeric and *Curcuma xanthorrhiza* (Javanese turmeric) are rich in curcuminoids as the major phenolic compounds (Iweala et al., 2023; Widyastuti et al., 2020), whereas white turmeric contains phenolics at levels of 446.91–711.6  $\mu\text{g/g}$  (Anukanon et al., 2025). The high phenolic content in these samples can be attributed to the intrinsic metabolic capacity of Curcuma species to synthesize secondary metabolites, particularly phenolics, through the phenylpropanoid pathway. This pathway is highly active in rhizomatous plants as part of their defense mechanism against environmental stress, pathogens, and oxidative damage. Furthermore, factors such as plant variety, maturity stage, cultivation conditions, and post-harvest handling may also influence phenolic compound accumulation, thereby contributing to the variability observed in these samples. Lesser galangal (*Kaempferia galanga*) contains polyphenols, flavonoids, alkaloids, and tannins, with a total phenolic content of approximately 146 mg/g (Subaryanti et al., 2022), while ginger (*Zingiber officinale*) contains zingerone, eugenol, and gingerdiol, with a total phenolic content of 47.7 mg/100 g (Edo et al., 2025). In addition to raw material composition, pretreatments such as washing, drying, and processing may also affect phenolic levels due to the easily oxidizable, light- and oxygen-sensitive, and volatile nature of phenolic compounds (Perez et al., 2025).

The initial total sugar content of rhizome-based kombucha ranged from 9.06 to 9.32% and differed among treatments. Sugar concentration plays a critical role in supporting the growth of kombucha microorganisms and the formation of chemical compounds during fermentation (Chibuye et al., 2024). The observed differences in initial total sugar are likely influenced by the degree of evaporation during rhizome brewing, where water loss through evaporation can increase the concentration of dissolved sugars (Leonard et al., 2021).

## 2. Chemical Characteristics of Rhizome-Based Kombucha and Black Tea Kombucha

### 2.1 Total Acid and pH

The increase in total acid over the fermentation period exhibited a consistent pattern across all treatments. Black tea control kombucha showed the highest total acid value on day 12, whereas the lowest value was observed in Javanese turmeric kombucha. The progression of total acid during fermentation is presented in Table 2.

**Table 2.** Increase in total acid of rhizome-based kombucha during fermentation

Rhizome	Total Acid (%)		Increase
	Day 0	Day 12	
Turmeric	0.027 ± 0.024 <sup>b</sup>	0.084 ± 0.022	0.057
White turmeric	0.034 ± 0.038 <sup>ab</sup>	0.086 ± 0.018	0.052
Kencur ( <i>Kaempferia galanga</i> )	0.033 ± 0.029 <sup>ab</sup>	0.078 ± 0.043	0.045
Ginger	0.033 ± 0.034 <sup>ab</sup>	0.080 ± 0.057	0.047
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	0.036 ± 0.037 <sup>ab</sup>	0.064 ± 0.030	0.028
Black tea control	0.039 ± 0.034 <sup>a</sup>	0.091 ± 0.038	0.052

**Note :** Different superscript letters within the same column indicate significant differences according to Fisher’s Least Significant Difference (LSD) test at a 95% confidence level.

The increase in total acid during fermentation is closely associated with the synergistic activity of yeasts and bacteria within the SCOBY. Yeasts, particularly *Saccharomyces cerevisiae*, hydrolyze sucrose into glucose and fructose, which are subsequently fermented into ethanol. The ethanol is then oxidized by acetic acid bacteria, such as *Acetobacter* and *Komagataeibacter*, into acetic acid, while glucose is also converted into gluconic acid and glucuronic acid (Coelho et al., 2020; Chen et al., 2025). The accumulation of these organic acids leads to an increase in total acid alongside a decrease in pH during fermentation.

Analysis of variance indicated that the increase in total acid across treatments was not significantly different ( $P > 0.05$ ) for either rhizome-based kombucha or the black tea control. This finding suggests that fermentation duration plays a more dominant role in determining total acid accumulation than the type of rhizome used. The longer the fermentation period, the greater the formation of organic acids, particularly acetic and gluconic acids, thereby increasing the total acid content of kombucha (Aung & Eun, 2022). These results are consistent with the report by Zubaidah et al. (2023), which demonstrated an increase in total acid in kombucha prepared from various apple varieties over time.

During rhizome-based kombucha fermentation, pH values decreased in all treatments until day 12. The pH reduction exhibited a consistent pattern over time. Detailed initial, final, and the magnitude of pH decrease are presented in Table 3.

**Table 3.** pH Changes of rhizome-based kombucha during fermentation

Rhizome	Total pH		pH decrease
	Day 0	Day 12	
Turmeric	4.53 ± 0.11	3.59 ± 0.39 <sup>c</sup>	0.057
White turmeric	4.79 ± 0.89	3.75 ± 0.30 <sup>abc</sup>	0.052
Kencur ( <i>Kaempferia galanga</i> )	4.74 ± 0.66	3.69 ± 0.17 <sup>bc</sup>	0.045
Ginger	4.61 ± 0.34	4.21 ± 0.25 <sup>a</sup>	0.047
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	4.81 ± 0.71	4.12 ± 0.19 <sup>ab</sup>	0.028
Black tea control	4.27 ± 0.36	3.68 ± 0.13 <sup>bc</sup>	0.052

**Note :** ΔpH represents the difference between pH values at Day 0 and Day 12. Different superscript letters (a–c) within the same column indicate significant differences ( $p < 0.05$ ) according to Fisher’s Least Significant Difference (LSD) test.

The decrease in pH during kombucha fermentation is closely associated with the formation of organic acids resulting from microbial metabolism. Yeasts, particularly *Saccharomyces cerevisiae*, hydrolyze sucrose into glucose and fructose, which are subsequently fermented into ethanol. Acetic acid bacteria, such as *Acetobacter xylinum*, then oxidize the ethanol into organic acids, primarily acetic acid, leading to proton release and a reduction in the fermentation medium's pH (Li et al., 2025; Aung & Eun, 2022). In addition to acetic acid, kombucha contains various other organic acids, including gluconic acid, glucuronic acid, lactic acid, citric acid, malic acid, succinic acid, pyruvic acid, tartaric acid, and oxalic acid, which collectively contribute to a decrease in overall pH (Onsun et al., 2025). This is attributed to the metabolic activity of the symbiotic culture of bacteria and yeast (SCOBY), which converts available substrates into organic acids. Yeasts hydrolyze sucrose into glucose and fructose and ferment them into ethanol, which is subsequently oxidized by acetic acid bacteria into acetic acid. Meanwhile, glucose is further metabolized into gluconic and glucuronic acids, and intermediates of central metabolism contribute to the formation of other organic acids. These biochemical conversions explain the accumulation of diverse acids and the resulting decrease in pH.

Analysis of variance revealed that the pH decrease among treatments was not significantly different ( $P > 0.05$ ) for either rhizome-based kombucha or the black tea control. This indicates that fermentation duration is the primary factor influencing pH reduction, whereas rhizome type did not have a statistically significant effect. Nevertheless, variations in the magnitude of pH decrease are likely related to differences in bioactive compound content among the rhizomes, which can affect microbial activity during fermentation. Lesser galangal (*Kaempferia galanga*) is known to contain flavonoids, polyphenols, tannins, quinones, and sesquiterpenes (Vishaka et al., 2022); turmeric and *Curcuma xanthorrhiza* (Javanese turmeric) contain curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Xu et al., 2025; Perez et al., 2025); white turmeric contains curcuminoids and essential oils rich in monoterpenes and sesquiterpenes (Gharge et al., 2021); and ginger (*Zingiber officinale*) contains gingerol, shogaol, zingerone, and zingiberene ((Shenoy & Mahurkar, 2022).

The observed decrease in kombucha pH over the fermentation period in this study aligns with the findings of Zubaidah et al. (2023) on kombucha prepared from faloak bark, which reported a significant pH drop from 3.86 on day 0 to 2.78 on day 14. These results confirm that longer fermentation duration leads to greater accumulation of organic acids, resulting in a continuous decrease in kombucha pH.

## 2.2 Total Sugar

During rhizome-based kombucha fermentation, a decrease in total sugar content was observed across all treatments through day 12. The reduction in total sugar was observed across all kombucha types, including rhizome-based kombucha and the black tea control. The initial and final total sugar values, along with the magnitude of the decrease, are presented in Table 4.

Analysis of variance showed that the decrease in total sugar across treatments was not significantly different ( $P > 0.05$ ) for either rhizome-based kombucha or the black tea control. This indicates that fermentation duration is the primary factor influencing total sugar reduction, whereas rhizome type does not have a statistically significant effect.

**Table 4.** Total sugar content of kombucha from various rhizomes during fermentation

Rhizome	Total Sugar (%)		Decrease (% points)
	Day 0	Day 12	
Turmeric	9.09 ± 0.83	3.13 ± 0.60	5.96
White turmeric	9.11 ± 1.11	3.11 ± 0.92	6.00
Kencur ( <i>Kaempferia galanga</i> )	9.07 ± 2.33	2.94 ± 1.45	6.13
Ginger	9.29 ± 2.95	3.72 ± 1.69	5.56
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	9.06 ± 2.40	3.05 ± 0.08	6.02
Black tea control	9.32 ± 0.63	4.18 ± 1.72	5.14

**Note :** Different superscript letters within the same column indicate significant differences according to Fisher’s Least Significant Difference (LSD) test at a 95% confidence level

The reduction in total sugar during kombucha fermentation is related to the utilization of sucrose as a carbon and energy source by the microorganisms within the kombucha culture. Yeasts, particularly *Saccharomyces cerevisiae*, produce the enzyme invertase, which hydrolyzes sucrose into glucose and fructose. Glucose is then fermented into ethanol, which is subsequently oxidized by acetic acid bacteria, such as *Acetobacter xylinum*, into organic acids, while fructose also contributes to acid formation (Nyhan et al., 2022; Coelho et al., 2020). As microbial metabolic activity increases during fermentation, sugar consumption intensifies, leading to a continuous decrease in total sugar content in kombucha.

These findings are consistent with those of Zubaidah et al. (2024), who reported a decrease in total sugar content in kombucha prepared from various salak varieties during fermentation up to day 14, with reductions ranging from 2.21 to 4.28%. This demonstrates that total sugar reduction is a common characteristic of kombucha fermentation and is closely associated with the dynamics of microbial metabolism throughout the fermentation process.

### 2.3 Total Phenolic Content

During rhizome-based kombucha fermentation, total phenolic content increased across all treatments. This increase occurred in both rhizome-based kombucha and black tea control. The lowest increase was observed in turmeric kombucha, while white turmeric and Javanese turmeric kombucha showed relatively higher increases. Detailed initial and final total phenolic values, along with the magnitude of the increase, are presented in Table 5.

**Table 5.** Increase in total phenolic content of rhizome-based kombucha during fermentation

Rhizome	Total Phenolics (µg GAE/ml)		Increase
	Day 0	Day 12	
Turmeric	56.00 ± 13.68 <sup>c</sup>	85.47 ± 30.63 <sup>b</sup>	29.47 <sup>b</sup>
White turmeric	45.12 ± 17.01 <sup>d</sup>	117.77 ± 54.93 <sup>b</sup>	72.65 <sup>ab</sup>
Kencur ( <i>Kaempferia galanga</i> )	44.42 ± 9.35 <sup>d</sup>	85.46 ± 55.04 <sup>b</sup>	41.05 <sup>b</sup>
Ginger	66.52 ± 18.35 <sup>b</sup>	110.72 ± 36.82 <sup>b</sup>	44.20 <sup>b</sup>
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	50.73 ± 16.44 <sup>cd</sup>	123.38 ± 68.23 <sup>b</sup>	72.65 <sup>ab</sup>
Black tea control	214.94 ± 8.42 <sup>a</sup>	417.40 ± 131.75 <sup>a</sup>	202.46 <sup>a</sup>

**Note :** Values are expressed as mean ± standard deviation. Different superscript letters (a–d) within the same column indicate significant differences (p < 0.05) based on Fisher’s Least Significant Difference (LSD) test.

Analysis of variance indicated that the increase in total phenolic content among treatments was significantly different ( $P < 0.05$ ). Javanese turmeric and white turmeric kombucha exhibited higher total phenolic increases compared to other treatments, whereas turmeric kombucha showed the lowest increase. These differences are likely related to variations in the content and types of bioactive compounds in each rhizome, which influence the biotransformation processes during fermentation.

The increase in total phenolics during kombucha fermentation is presumed to result from the enzymatic activity of microorganisms in the kombucha culture. Enzymes produced by bacteria and yeasts can degrade complex polyphenolic compounds into simpler, more detectable phenolic forms, thereby increasing the measurable total phenolic content (Coelho et al., 2020). In addition, phenolic compounds bound as glycosides can be hydrolyzed by  $\beta$ -glucosidase under acidic conditions into free phenols (Liu et al., 2025). Release of phenolic acids may also occur from plant cell wall matrices, such as lignin and cellulose, through the cleavage of ester bonds by microbial enzymes during fermentation (Coelho et al., 2020).

Differences in total phenolic increases among rhizome types are likely related to the presence of specific bioactive compounds in each substrate. Javanese turmeric contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Perez et al., 2025), while white turmeric is rich in arylheptanoid compounds (curcuminoids), essential oils, and polysaccharides (Gharge et al., 2021). Turmeric is known to contain curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Xu et al., 2025). Variations in the initial phenolic composition influence the release and transformation of phenolic compounds during fermentation, resulting in differences in total phenolic increases among rhizome-based kombucha. These findings demonstrate that kombucha fermentation not only enhances total phenolic content but is also significantly influenced by the type of rhizome substrate used, through enzymatic biotransformation mechanisms during fermentation (Leonard et al., 2021).

### 2.3 Antioxidant Activity

Analysis results indicated that the antioxidant activity of rhizome-based kombucha increased during fermentation. The lowest antioxidant activity was observed in lesser galangal (kencur) kombucha, whereas relatively higher values were found in Javanese turmeric and white turmeric kombucha. Detailed initial and final antioxidant activity values, along with the magnitude of the increase, are presented in Table 6.

**Table 6.** Increase in antioxidant activity of rhizome-based kombucha during fermentation

Rhizome	Antioxidant Activity (%)		Increase
	Day 0	Day 12	
Turmeric	55.00 ± 16.66	68.32 ± 24.95	13.32
White turmeric	59.28 ± 25.23	72.97 ± 13.22	13.69
Kencur ( <i>Kaempferia galanga</i> )	53.45 ± 33.18	66.46 ± 24.60	13.01
Ginger	52.70 ± 17.60	65.45 ± 13.23	12.75
Javanese turmeric ( <i>Curcuma xanthorrhiza</i> )	58.43 ± 18.18	74.02 ± 16.56	15.59
Black tea control	70.97 ± 6.79	79.23 ± 7.50	8.26

**Note :** Different superscript letters within the same column indicate significant differences according to Fisher's Least Significant Difference (LSD) test at a 95% confidence level.

Analysis of variance indicated that the increase in antioxidant activity among treatments was not significantly different ( $P > 0.05$ ). This suggests that kombucha fermentation generally enhances antioxidant activity, regardless of the rhizome type used as the substrate. The increase in antioxidant activity during kombucha fermentation is closely associated with the elevated content of phenolic compounds. During fermentation, microorganisms produce enzymes that hydrolyze and release bound phenolic compounds, thereby enhancing the antioxidant capacity of the kombucha beverage (Zubaidah et al., 2025; Liang et al., 2023). Phenolic compounds exhibit strong antioxidant potential because they can donate electrons or hydrogen atoms from hydroxyl groups (-OH) to neutralize free radicals. The stability of the phenolic compound after electron donation is influenced by benzene-ring resonance, which allows the compound to remain in a stable form (de Oliveira et al., 2025).

The antioxidant mechanism of phenolic compounds involves the reduction of free radicals into non-reactive species. This ability is influenced by the number and position of hydroxyl groups in the phenolic structure, where a higher number of hydroxyl groups enhances the compound's hydrogen-donating ability and antioxidant activity (de Oliveira, 2025).

These findings are consistent with previous reports indicating that kombucha's antioxidant activity tends to increase with fermentation time. Li et al. (2025) reported a significant increase in kombucha antioxidant activity from day 7 to day 14, which corresponded to elevated polyphenol and flavonoid content. Similarly, Zubaidah et al. (2022) observed that the increase in antioxidant activity in salak-based kombucha was positively correlated with total phenolic content, confirming the strong relationship between phenolic compounds and antioxidant capacity.

In addition to increased phenolic content, the rise in kombucha antioxidant activity is also influenced by microbial metabolic activity during fermentation. Yeasts and bacteria in the kombucha culture produce enzymes involved in phenolic biotransformation, including vinyl phenol reductase and ferulic acid reductase, which contribute to the formation of phenolic compounds by decarboxylating cinnamic and ferulic acids (Zubaidah et al., 2025; Chen et al., 2025). Moreover, organic metabolites produced during fermentation may further enhance antioxidant activity by increasing the availability of active phenols (Onsun et al., 2025). These results indicate that rhizome-based kombucha fermentation can significantly enhance antioxidant activity, primarily by increasing and biotransforming phenolic compounds, highlighting its potential as a functional beverage rich in antioxidants.

### 3. Selection of the Optimal Rhizome-Based Kombucha Treatment

The optimal rhizome-based kombucha treatment was determined using a Multi-Criteria Decision-Making (MCDM) approach, specifically the Simple Additive Weighting (SAW) method. This technique allows systematic comparison of multiple alternatives based on multiple weighted criteria, facilitating the objective selection of the most favorable treatment among turmeric, white turmeric, lesser galangal (kencur), ginger, and Javanese turmeric kombucha. The parameters included in the analysis were total acid, pH, total phenolic content, total sugar, and antioxidant activity, collectively representing chemical and functional quality indicators of kombucha.

The SAW method operates by assigning a weight to each parameter according to its relative importance, normalizing the data, and calculating a cumulative score for each treatment. This approach ensures that the selection of the optimal kombucha treatment reflects a balance between desirable chemical properties (e.g., total acid, pH, total sugar) and biofunctional attributes (e.g., total phenolics, antioxidant activity).

Based on the SAW analysis, white turmeric kombucha emerged as the optimal treatment among the rhizome-based variants. This indicates that, overall, white turmeric kombucha exhibited the most favorable combination of chemical composition and biofunctional properties after 12 days of fermentation. The specific values for each evaluated parameter are summarized in Table 7.

**Table 7.** Optimal rhizome-based kombucha treatment after 12 days of fermentation

Parameter	White Turmeric Kombucha
Total Acid (%)	0.086
pH	3.75
Total Sugar (%)	3.11
Total Phenolics ( $\mu\text{g GAE/mL}$ )	117.77
Antioxidant Activity (%)	72.97

The superior performance of white turmeric kombucha can be attributed to several factors. Its relatively high total phenolic content and antioxidant activity suggest that white turmeric contains bioactive compounds—such as arylheptanoids, curcuminoids, essential oils, and polysaccharides—that are efficiently released and transformed during fermentation through microbial enzymatic activity. In parallel, its balanced total acid and pH values indicate optimal microbial metabolism, in which yeasts hydrolyze sucrose into glucose and fructose, which are then fermented into ethanol, which is subsequently oxidized by acetic acid bacteria into organic acids. This metabolic synergy results in a kombucha product that maintains both desirable sensory properties and functional bioactivity. Overall, the MCDM-SAW analysis provides a quantitative framework to identify the most promising rhizome substrate for functional kombucha production. White turmeric kombucha not only exhibits superior chemical and functional attributes but also serves as a promising candidate for further development and optimization in functional beverage formulations.

## CONCLUSION

Fermentation of kombucha from various rhizomes for 12 days resulted in decreased pH and total sugar content, and increased total acid, total phenolic content, and antioxidant activity. Statistical analysis indicated that rhizome type did not significantly affect pH, total acid, total sugar, or antioxidant activity, but did significantly affect total phenolic content. Among the treatments tested, white turmeric kombucha exhibited the most favorable chemical and functional characteristics, with a total acid content of 0.086%, pH 3.75, total sugar 3.11%, total phenolics 117.77  $\mu\text{g GAE/mL}$ , and antioxidant activity of 72.97%, although these values were still lower than those of black tea kombucha used as a control. Based on these results, white turmeric kombucha is recommended for further development as a functional fermented beverage, with future research focusing on optimizing fermentation time and substrate formulation, evaluating the stability of bioactive compounds, and conducting *in vivo* bioactivity assessments.

## AUTHOR CONTRIBUTION

**N.K.** conceptualized and designed the research and supervised the overall process. **E.A.R.** conducted the experiments and collected the data. **E.Z.** analyzed and interpreted the data. **N.K. E.Z.** and **I.T.** drafted the manuscript. All authors reviewed, revised, and approved the final manuscript.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the conduct of this research.

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