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Chemical Characteristics of White Turmeric (*Curcuma zedoria* Rosc.) and Mango Turmeric (*Curcuma mangga* Val.) Kombucha with Various Proportion of Sugar and Honey

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ABSTRACT

Background: Kombucha is a tea-based fermented beverage product made with the addition of sugar which is assisted by the activity of microorganisms in the form of Symbiotic Culture of Bacteria and Yeast (SCOBY) that has many health benefits. Honey can be an alternative substitute for sugar which offers some potential bioactive compounds that sugar does not. The purpose of the study was to determine the effects of fermentation on the chemical characteristics of white turmeric and mango turmeric kombucha with various proportions of sugar and honey.

Methods: This study used a Nested randomized with two factors and 4 replications. The variables investigated were type of turmeric (white turmeric and mango turmeric) and various proportions of sugar and honey (10 sugar:0 honey, 5 sugar:5 honey, 0 sugar:10 honey).

Result: Results showed that the best treatment for making kombucha is using mango turmeric with proportion of sugar 0:honey 10, which contains total acid (1.29%), pH (2.96), total sugar (5,82%), total phenol (196.31 mg GAE/ml), total flavonoids (84.95 mg QE/ml), antioxidant activity (IC50 46.79 ppm) and antibacterial activity (inhibition zone) against *E. coli* (4.50 mm) and *S. aureus* (5.11 mm). This study demonstrates that the type of turmeric and proportions of sugar and honey treatment significantly influence the quality of kombucha, highlighting its potential as a basis for developing high-value functional food products.

Key words: Antibacterial Activity, Antioxidant Activity (Ic50), Mango Turmeric Kombucha, White Turmeric Kombucha.

INTRODUCTION

White turmeric (*Curcuma zedoria* Rosc.) and mango turmeric (*Curcuma mangga* Val.) are rhizomes used in herbal medicine, particularly in tropical areas and Southeast Asia (Albalawi and Alanazi, 2023; Urbanova *et al.,* 2024). Both contain bioactive compounds like curcuminoids, flavonoids and saponins, exhibiting numerous therapeutic properties, including antioxidant, antimicrobial and anti-inflammatory effects (Dosoky and Setzer, 2018; Amalraj *et al.,* 2016). Honey, rich in bioactive components such as flavonoids and phenolic acids, has bactericidal properties against strains like *E. coli* and *S. aureus* (Raj *et al*., 2024; Moniruzzaman *et al*., 2012). However, the plant matrix in turmeric may limit the bioavailability of these compounds, necessitating fermentation treatments to enhance their health benefits (Sharma *et al*., 2022). A popular fermented product, kombucha, results from the symbiotic fermentation of acetic acid bacteria and yeast or SCOBY (Symbiotic Culture of Bacteria and Yeast), mainly involving *Acetobacter xylinum* and yeasts like *Zygosaccharomyces*, *Schizosaccharomyces* and *Saccharomyces* (Leal *et al.,* 2018). However, more research needs to be done regarding the optimal proportions of sugar and honey to get kombucha with best performance. Therefore, in this study a kombucha innovation was carried out based on white turmeric and mango turmeric with the proportion of sugar and honey was chosen to determine the effectiveness of the treatment to increase the bioactive compounds and is thought to increase its antioxidant and antibacterial activity.

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MATERIALS AND METHODS

The experiment was conducted in August-November 2024 at the Laboratory of Department Food Science and Biotechnology, Brawijaya University. White turmeric was obtained from Hijo Farm in Malang, Indonesia, mango turmeric from Aufastore in Surabaya, Indonesia. The kombucha starter was obtained from the Healthy Secret store, Sumbawa Forest honey (Sarang Maduku), jasmine tea (Tong Tji), sugar (Gulaku) and mineral water (Aqua) were obtained from a supermarket in Malang, Indonesia. White turmeric and mango turmeric were washed, peeled and sliced into 1-3 mm thick pieces. They were dried in a cabinet dryer at 60°C for 7 hours and then crushed. The dried rhizomes were placed in tea bags at a concentration of 0.8% b/v, using Tong Tji jasmine tea as a base. Both rhizome and tea extracts were boiled in 500 ml mineral water for 3 minutes and sweetened with sugar and honey at ratios of 10:0, 5:5 and 0:10. After cooling, kombucha starter (10% v/v) was added and fermentation occurred for 6 days, followed by analysis on day 0 and day 6 for various chemical properties. Total acid was analyzed according to Winandari *et al.* (2022), total sugar was analyzed according to Lubis *et al.* (2024), total phenol was determined according to Mihai *et al.* (2024), total flavonoid was determined according to Yeti and Yuniarti (2021), antioxidant activity IC50 was evaluated according to Molyneux (2004) and antibacterial activity was determined according to Razmavar *et al.* (2014). The data obtained were analyzed statistically using a two-factor nested analysis of variance (ANOVA) model, where the type of turmeric (Factor A) was treated as the main factor and the ratio of sugar and honey (Factor B) was nested within each type of turmeric. The nesting the experimental design, applying specific sugar and honey ratios to turmeric types, enabling evaluation of main effects and within-group variability. Statistical analysis was conducted using the statistical software Minitab version 20. Post-hoc comparisons of treatment means were performed using Fisher's Least Significant Difference (LSD) test at a 95% confidence level (p<0.05), chosen for its sensitivity and effectiveness in detecting differences among means following a significant F-test as an ANOVA result. The best treatment was selected through the Multiple Criteria Decision-Making method with the Zeleny technique based on chemical tests, which were then compared with the control sample using the t-test.

RESULTS AND DISCUSSION

Total acid

The analysis of variance indicated that the type of turmeric and the proportion of sugar and honey significantly affected the total acid value in white and mango turmeric kombucha (P<0.05). As shown in Table 1, total acid values increased during fermentation for both kombucha types. Higher honey content notably raised acidity, especially in mango turmeric kombucha, suggesting enhanced fermentation activity. This indicates that turmeric type and sugar-honey proportions influence microbial metabolism due to substrate availability and composition differences. Wang *et al.* (2022) explain that the increase in total acid value during fermentation is due to yeast converting sugars into ethanol, while acetic acid bacteria like *Acetobacter* sp. and *Gluconobacter* produce organic acids, notably acetic acid. The rise in acid concentration in both kombucha varieties can be attributed

Table 1: Chemical characteristics, antioxidant activity and antibacterial activity values of white turmeric and mango turmeric kombucha with various proportions of sugar and honey during fermentation.

Type of turmeric	Proportions of sugar: Honey	Total acid (%) Day-			pH Day-			Total sugar (%) Day-			Total phenol (mg GAE/ml) Day-			Total flavonoid (Tmg QE/ml) Day-			Antioxidant activity IC_{50} (ppm) Day-			Inhibition zone of *E. coli* (mm) Day-			Inhibition zone of *S. aureus* (mm) Day-		
		0	6	Increase	0	6	Decrease	0	6	Decrease	0	6	Increase	0	6	Increase	0	6	Decrease	0	6	Increase	0	6	Increase
White	10 : 0	0.2±	0.5±		4.6±	4.2±		9.8±	8.9±		47.7±	118.9		26.7±	54.6±		98.0±	73.1±		2.6±	1±		3.5±	3.8±	
turmeric		0.03^{d}	0.1^{d}	0.3^{d}	0.1^{a}	0.1^{a}	0.5^{c}	0.04^{a}	0.1^{a}	0.9^{b}	0.2^{d}	$\pm 0.2^{f}$	71.3^{f}	0.4^{e}	$0,3^{e}$	27.9^{f}	$1,0^{c}$	0.8^{a}	24.9^{b}	0.3	$3.0.5^{c}$	0.5^{b}	0.1^{ab}	0.1^{c}	0.3^{c}
	5 : 5	0.3±	0.7±		4.3±	3.7±		8.8±	7.5±		53.0±	144.0		30.6±	64.4±		92.7±	65.2±		3.0±		3.6 ±	3.7±	4.0±	0.4^{c}
		0.05^{c}	0.7^{c}	0.3^{d}	0.03^{b}	0.2^{b}	0.6^{bc}	0.1^{b}	0.01^{c}	1.3^{b}	0.1^{b}	$\pm 0.1^{d}$	100.0^{d}	0.4^{d}	0.4^{d}	33.9^{e}	$0,1^{e}$	0.4^{c}	27.5^{b}	0.4		0.5^{bc}	0.6^{b}	0.1^{a}	0.2^{bc}
	0 : 10	0.4±	0.8±		4.1±	3.4±		7.5±	6.0±		55.0±	175.1±		34.8±	70.6 ±		89.0±	57.9±		2.8±	4.0±		3.5±	4.7±	
		0.06^{bc}	0.1^{b}	0.4^{c}	0.1^{c}	0.03^{c}	0.8^{ab}	0.4^{c}	0.01^{e}	1.4^{c}	0.1^{a}	0.1^{b}	120.1^{c}	0.2^{b}	0.4^{c}	35.8^{d}	0.7^{f}	0.9^{d}	31.1^{ab}	0.4	0.8^{ab}	1.2^{b}	0.1^{ab}	0.9^{ab}	1.2^{ab0}
Mango	10 : 0	0.4±	0.9±		4.2±	3.6±		9.5±	8.4±		42.0±	129.7±		25.9±	64.2 ±		112.9±	66.1±		2.4±	3.2±		3.2±	4.0±	
turmeric		0.08^{abc}	0.1^{b}	0.4^{c}	0.04^{c}	0.1^{b}	0.5^{c}	0.4^{a}	0.1^{b}	1.1^{bc}	0.1^{f}	0.1^{e}	87.7^{e}	0.1^{f}	0.4^{d}	38.3^{c}	0.8^{a}	0.2^{b}	46.8^{b}	0.1	0.5^{c}	0.8^{b}	0.1^{c}	0.1^{bc}	0.8^{bc}
	5 : 5	0.4±	1.0±		4.0±	3.2±		8.4±	7.0±		46.9±	173.4±		31.6±	72.7 ±		99.1±	51.6±		2.7±		4.0±	3.5±	4.3±	
		0.05^{ab}	0.1^{b}	0.6^{b}	0.1^{d}	0.03^{c}	0.7^{ab}	0.2^{b}	0.02^{d}	1.4^{b}	0.2^{e}	0.1^{c}	126.6^{b}	0.3^{c}	0.6^{b}	41.1^{b}	0.7^{b}	0.6^{e}	47.5^{ab}	0.2	$0,4^{ab}$	1.3^{b}	$0,1^{ab}$	0.4^{abc}	0.8^{bc}
	0 : 10	0.5±	1.3±		3,6±	2.7±		7.4±	5.2±		50.0±	196.3±		35.6±	85.0 ±		95.3±	46.8±	48.5^{a}	2.6±	4.5±	1.9^{a}	3.3±	5.1±	
	0.06^{a}	0.2^{a}	0.8^{a}	0.02^{e}	0.3^{d}	0.9^{a}	0.4^{c}	0.1^{f}	2.2^{a}	0.2^{c}	0.3^{a}	146.3^{a}	0.3^{a}	0.7^{a}	49.3^{a}	0.6^{d}	$0,2^{f}$			0.2	0.3^{a}		0.2^{bc}	0.9^{a}	1.8^{a}

Remarks: *Mean values within a column followed by the same letters are not significantly different at p<0.05 according to ANOVA Test.

to *Acetobacter xylinum*, which also produces malic, tartaric, citric, butyric and lactic acids (Zubaidah *et al*., 2021; Sawab *et al*., 2017).

pH value

An increase in total acid during the fermentation process will reduce pH value of kombucha (Zubaidah *et al*., 2019). In addition, pH measurement is very important in kombucha fermentation because pH has a crucial role in controlling the fermentation process and influencing the final result of the fermentation product (Hur *et al*., 2014).

Based on (Table 1), the increase in total acid value and the largest decrease in pH are found in the mango turmeric kombucha with proportion sugar 0 : honey 10. The increasing proportion of honey will affect the increase in total acid and the lower of pH kombucha. Adding honey can speed up the fermentation process causing the increased number of microbes, resulting in the increased production of organic acids (Slacanac *et al.*, 2011). This is due to the difference in the types of sugar contain in granulated sugar and honey, where the type of sugar in honey is classified as simple sugar in the form of fructose and glucose, while granulated sugar contains more complex sugars in the form of sucrose (Evahelda *et al*., 2017). This causes microbes in kombucha to react more easily and quickly to form organic acids with the compounds contained in honey compared.

Total sugar

The variance analysis indicated that the type of turmeric and the sugar and honey proportions significantly influenced total sugar content reduction in white and mango turmeric kombucha ($P<0.05$). Based on Table 1, as fermentation progressed, total sugar levels decreased across all treatments, particularly in samples with a higher honey proportion. This suggests that honey enhances microbial sugar utilization, possibly due to its more fermentable sugar profile. Yeast and bacteria in kombucha convert sugar into organic acids, alcohol and metabolites (Zubaidah *et al.*, 2022).

The total sugar content in both kombucha types decreased linearly with higher honey proportions. Granulated sugar is around 98% sucrose, while honey comprises approximately 79% simpler sugars (Sancho *et al*., 2013; Alghamdi *et al*., 2020). Microbes degrade these simpler sugars more efficiently, lowering total sugar levels (Kaashyap *et al*., 2021). However, bioactive compounds like Zingiberene and Curcumin can inhibit microbial processes, prolonging fermentation and leaving more sugar intact (Yousfi *et al*., 2021).

Total phenol

The analysis of variance showed that the different types of turmeric and the proportion of sugar and honey significantly affect the increase in total phenol content during fermentation ($P < 0.05$). The data showed in (Table 1), total phenol increased steadily throughout the fermentation process for all treatments. The data showed that the highest increase in total phenol was in the turmeric mango kombucha with a proportion of sugar 0 : honey 10. Increasing honey proportion in both types of turmeric kombucha raised total phenol content due to phenolic compounds in honey, like syringic acid, gallic acid and flavonoid-derived phenolics (Ciulu *et al*., 2016). This indicates that ingredient composition influences microbial metabolism and is essential for enhancing kombucha's functional properties.

Total phenols in kombucha can increase because of enzymes produced by microorganisms, an acidic media environment and the breakdown of complex phenol components (Kuzu *et al*., 2023). Glucosidase, pectinase, xylanase, cellulase and glucanase are enzymes that can increase total phenol. Those enzymes can degrade complex polyphenol compounds into simpler phenolic compounds, like catechin and epicatechin (Kim *et al.*, 2023). Besides that, LAB also contributes to degrading cinnamic acid into 4-vinyl phenol and 4-vinyl guaiacol (Beek and Priest, 2000).

Total flavonoid

The analysis of variance showed that both the type of turmeric and the proportion of sugar and honey significantly influenced the increase in total flavonoid content during fermentation ($P<0.05$). This trend may be attributed to the enhanced activity of fermentative microorganisms, which can break down complex polyphenol structure into simpler ones due to microorganism enzymes such as beta-glucosidase, cellulase and pectinase which can damage the cell matrix thereby releasing more active aglycone components that are bound to plants, therefore flavonoid compounds increased (Liang *et al*., 2024). Flavonoids in plant cells are unstable; the acidic fermentation process

Table 2: Characteristics of mango turmeric kombucha as best treatment compared to black tea kombucha during fermentation.

Parameter	Mango turmeric kombucha (Sugar 0 : Honey 10)	Black tea kombucha
pH	2,72^{a}	3,60^{b}
Total acid	1,29%^{a}	0,48%^{b}
Total phenol	196,32 mg GAE/ml^{b}	625,63 mg GAE/ml^{a}
Total sugar	5,16%^{a}	6,20%^{b}
Total flavonoid	84,95 mg QE/ml^{b}	388,05 mg QE/ml^{a}
Antioxidant activity	46,79 ppm^{b}	4,62 ppm^{a}
Antibacterial activity *E. coli*	4,50 mm^{b}	4,80 mm^{a}
Antibacterial activity *S. aureus*	5,11 mm^{b}	5,50 mm^{a}

breaks down their complex structures, enhancing flavonoid bioavailability (Liang *et al.,* 2024).

As observed in (Table 2), the increase in total flavonoid content in kombucha may be attributed to the presence of flavonoid compounds in honey, such as quercetin, isorhamnetin, apigenin, alangin, myricetin, luteolin, kaempferol, chrysin and galangin (Cianciosi *et al*., 2018).

Antioxidant activity

The analysis of variance revealed that both the type of turmeric and the proportion of sugar and honey significantly influenced the increase in antioxidant activity ($P<0.05$). This trend can be attributed to microbial activity during fermentation. Yeast enzymes degrade polyphenol complexes into simpler monomers, while lactic acid bacteria release β-glucosidase to break glycosidic bonds, increasing the availability of free phenolic compounds (Mahapatra *et al*., 2016). Free phenolic compounds act as antioxidants by donating hydrogen atoms or -OH groups to stabilize free radicals (Zubaidah *et al*., 2019). A decrease in IC50 value signifies increased antioxidant activity (Beniwal *et al*., 2022). Additionally, fermentation produces organic acids like oxalic acid, which can capture free radicals (Kayashima and Katayama, 2002). The vitamin content in kombucha also rises during fermentation, particularly vitamin C, known for its potent antioxidant properties (Antolak *et al*., 2021).

The study results indicate that the increased addition of honey affects the increase in antioxidant activity of kombucha. This is due to the presence of polyphenolic compounds, derivative compounds carotenoids, organic acids and several enzymes that have properties as antioxidants in honey. Based on research by Cianciosi *et al*. (2018), honey has natural components such as phenolic compounds, flavonoids, vitamin C, α-tocopherol, beta carotene, organic acids and enzymes which play an important role in increased antioxidant activity in kombucha.

Antibacterial activity

The analysis of variance indicated significant differences in the types of turmeric and the proportions of sugar and honey regarding their inhibitory effects on *Escherichia coli* and *Staphylococcus aureus* ($P<0.05$). As shown in Table 1, inhibition increased with longer fermentation times. The mango turmeric kombucha sample, with a sugar and honey ratio of 0:10, exhibited the highest inhibition levels on the 6^{th} day of fermentation. Antibacterial activity increases with the increasing proportion of honey on both types of turmeric. This is due to the metabolic results of microbes in the form of organic acids during fermentation and because of the presence of other antibacterial compounds in honey and kombucha.

Kombucha contains antibacterial compounds such as organic acids, polyphenols, esters and aldehydes (Al-Mohammadi *et al*., 2021). Acetic acid disrupts bacterial cell functions by penetrating cell walls and damaging membranes, influencing enzymatic activity and DNA structures (Sanwal *et al*., 2023). Phenolic compounds also inhibit pathogens by altering cell membrane functions (Bensehaila *et al*., 2022). A study showed that honey enhanced antibacterial effects in turmeric kombucha; larger clear zones resulted from honey's antibacterial compounds like hydrogen peroxide, which harms bacterial DNA (Mandal and Mandal, 2011; Raj *et al*., 2024). Honey also can increase microbial growth during fermentation (Puspitasari *et al*., 2017).

Best treatment

Based on the results in the (Table 2), mango turmeric kombucha with a proportion of sugar 0 : honey 10 shows superiority in several parameters, namely pH, total acid and total sugar compared to black tea kombucha. Through paired t-test results, it shows that between mango turmeric kombucha with a sugar proportion of 0 : honey 10 and black tea kombucha, most of the parameters show significant differences.

Limitation and suggestion

This study was conducted using *in vitro* methods. We recommend that future researchers undertake in vivo assessments of kombucha to better understand its potential as a functional beverage. Additionally, the exploration of mango turmeric remains relatively underre presented in current research. Subsequent investigations could focus on comparing the bioactive compound profiles of mango turmeric with those of white turmeric, thereby enriching the body of knowledge in this area.

CONCLUSION

This study demonstrated that type of turmeric treatment, namely white turmeric and mango turmeric and the proportions of sugar and honey treatment significantly influenced the chemical, antioxidant and antibacterial properties of kombucha. The addition of honey in kombucha improved the rate of microbial activity during fermentation resulting in a faster fermentation process to release bioactive compounds compared to granulated sugar, thereby enhancing the bioavailability of bioactive compounds. These changes led to a higher total acid content (1,29%), total phenol content (196.31 mg GAE/mL), total flavonoid content (84.95 mg QE/ml), stronger antioxidant activity (IC50 46.79 ppm) and also decreased pH (2,72) and total sugar content (5,16%) in the kombucha. Additionally, the improved bioactive compound availability enhanced antibacterial properties, as shown by inhibition zone diameters of 4.50 mm against *Escherichia coli* and 5.11 mm against *Staphylococcus aureus.*

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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