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Substitution of Refined Sugar in Lingzhi (*Ganoderma lucidum*) Kombucha with Honey from Riau, Indonesia: the Effects on Characteristics, Sensory Acceptance, and Antioxidant Activity

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The rising prevalence of diseases associated with elevated free radical levels highlights the need for antioxidant-rich products. Kombucha, a fermented beverage, is gaining popularity for its health benefits, including antioxidant properties. Traditionally made from tea leaves and refined white sugar (RWS) sugar, kombucha's versatility allows for the inclusion of various plant materials. Lingzhi mushroom, known for its medicinal properties, offers an intriguing alternative ingredient due to its antioxidant content. Substituting sugar with honey also provides a different flavor and may enhance the beverage's bioactive profile. This study aimed to examine the impact of honey substitution on lingzhi kombucha production. Two types of honey, sourced from different botanical origins and bee species in Riau, Indonesia, were tested alongside RWS as a control. The experimental design used a randomized block design with honey type and ratio as factors. Characteristics such as reducing sugar content, titratable acidity, and pH were analyzed before and after fermentation. Changes in the total phenolic content (TPC) and total flavonoid content (TFC) of each lingzhi kombucha were also monitored. Antioxidant activity was assessed by measuring IC_{50} using the DPPH method. Additionally, sensory evaluations provided insights into consumer acceptance. The results revealed that fermentation decreased lingzhi kombucha's reducing sugar, pH, and IC_{50} while increasing titratable acidity, TPC, and TFC. The type and ratio of honey affected the characteristics of lingzhi kombucha, except for sensory evaluation. The findings suggest that lingzhi kombucha enriched with honey has the potential to be a functional beverage with enhanced antioxidant properties, warranting further exploration in functional food development.

Keywords: antioxidant, functional food, kombucha, lingzhi, Riau honey

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INTRODUCTION

The metabolic processes within the human body generate harmful free radicals, whose excessive levels can lead to various illnesses like cardiovascular disease and cancer (Lobo *et al.* 2010). Although the body has systems in place to control these substances, outside influences like radiation, chemicals, or air pollution can raise their levels (Lobo *et al.* 2010). Hence, consuming antioxidant products is necessary to counteract and reduce the accumulation of free radicals in the body.

Kombucha, a fermented beverage made with a symbiotic culture of bacteria and yeast (SCOBY) mixed culture starter, is becoming more and more well-liked because of its sour-acidic flavor, refreshing feeling, and health advantages brought about by organic acids and bioactive substances that arise from the interaction of microbes with tea, particularly antioxidant activity (Kitwetcharoen *et al.* 2023). The SCOBY that ferments kombucha might differ from one culture to the next, although it usually consists of lactic acid bacteria, acetic acid bacteria, and yeast. Traditionally, kombucha is made from tea leaves, mainly black tea. However, it can also be crafted from a variety of plants, especially rhizomic spices like turmeric (*Curcuma longa*) (Zubaidah *et al.* 2023), blue turmeric (*C. aeruginosa*), white turmeric [*C. zedoaria* (Berg.), Roscoe] (Zubaidah *et al.* 2024), and Javanese turmeric (*C. xanthorrhiza*) (Zubaidah *et al.* 2022). Additionally, flowers such as butterfly pea flower (*Clitoria ternatea* L.) (Majid *et al.* 2023; Permatasari *et al.* 2022; Wongthai *et al.* 2021), golden-flower (*Camellia petelotii*) (Wu *et al.* 2023), honeysuckle flower (*Lonicera japonica*) (Wu *et al.* 2023), and royal lotus pollen (*Nelumbo nucifera)* (Wongthai *et al.* 2021) can be used. Snake fruit (*Salacca zalacca*) (Zubaidah *et al.* 2019), guava (*Psidium guajava*) (Khaleil *et al*. 2020), and papaya (*Carica papaya*) (Sharifudin *et al.* 2021) fruits can also be utilized in kombucha production. Then, underutilized plant materials, including soursop leaves (Candra *et al.* 2023), coffee skin (Muzaifa *et al.* 2022), and cacao pulp liquefaction (Yuliana *et al.* 2023) may be used in kombucha as well. Even though the components of kombucha naturally contain a number of bioactive substances that support its initial antioxidant activity, the fermentation process during kombucha production can enhance these bioactivities further, creating a beverage product with significantly heightened antioxidant properties.

Traditional Chinese medicine has historically utilized the medicinal fungus lingzhi, also known as *Ganoderma lucidum*. Recent research has delved into the various health advantages offered by lingzhi – including its potential anti-cancer, anti-diabetic, hepatoprotective, immunomodulatory, anti-inflammatory, anti-tumor, hypolipidemic properties, and notably, its antioxidant effects. The various bioactive substances present in lingzhi are responsible for these advantages such as phenolics, polysaccharides, and triterpenes (Oludemi *et al.* 2018). While recognized for its numerous health benefits, the predominantly bitter and earthy flavor and aroma of lingzhi may potentially reduce consumer acceptance. Nevertheless, incorporating lingzhi into kombucha could potentially modify its flavor while enhancing its bioactivity (Elfirta *et al.* 2024; Sknepnek *et al.* 2018).

In the production of kombucha, refined sugar is typically used as a substrate for the cultivation of SCOBY. Nevertheless, honey, a natural sweetener, can serve as a replacement for sugar. The chemical characteristics of the finished product were enhanced when honey was used as a carbon source, producing more significant concentrations of organic acids, essential oils, alcohols, esters, and polyphenols. Furthermore, the initial chemical compounds in honey may also change the fermentation process of kombucha (Watawana *et al.* 2017), resulting in a distinct taste and bioactivity. The characteristics of honey are influenced by the type of bee and the botanical source (Belina-Aldemita *et al.* 2023). Thus, this study aims to investigate the impact of two distinct types of honey, derived from varying botanical origins and bee species, on substituting sugar in lingzhi kombucha production.

MATERIALS AND METHODS

Sample Collection

The lingzhi mushroom powder (LMP) was acquired from a local farmer in Cianjur, West Java, Indonesia, whereas the SCOBY and kombucha solution was acquired from a local kombucha producer in Cibinong, West Java, Indonesia. Refined white sugar (RWS) (Gulaku®) was purchased from a local market in Cibinong, West Java, Indonesia. Two varieties of honey were used as sweetening agents in this research. One type was sourced from *Apis mellifera* bees (Am honey) in Siak, Riau, derived solely from the nectar of *Acacia crassicarpa* flowers (unifloral) (Handayani *et al.* 2022). The other type came from *Heterotrigona itama* bees (Hi honey) in Kampar, Riau, originating from a mixture of various flower nectars (multifloral) (Pribadi and Wiratmoko 2023). The rest of the chemicals were purchased from Merck® unless it was mentioned otherwise.

Experimental Design

A randomized block design with two components was employed in this study: the type of honey (Am and Hi) and the honey ratio of 50 and 100% (w/v) to RWS as control, with three replicates each. Each lingzhi kombucha

was characterized by its reducing sugar, titratable acidity, pH, TPC, and TFC before (Day 0) and after (Day 14) fermentation. Antioxidant activity was evaluated before and after fermentation as well. In addition, sensory evaluation of the end product of lingzhi kombucha was analyzed. The data of each formulated kombucha were analyzed by two-way analysis of variance (ANOVA) at $p < 0.05$ using SPSS 26.0 (IBM, USA) to see the influence of each factor and its interaction on lingzhi kombucha. Moreover, data from each kombucha before and after fermentation were analyzed by paired t-test using the same software.

Lingzhi Kombucha Preparation

The lingzhi kombucha preparation in this study was based on Elfirta *et al.* (2024) with slight modifications (refer to Figure 1). The starter of kombucha for this experiment was prepared by preparing sweetened lingzhi solution by boiling LMP 0.5% (w/v) and RWS 5% (W/V) in tap water for 10 min. Once the solution had reached room temperature, (± 29 °C), the solution was mixed with SCOBY 2.5% (w/v) and kombucha solution 20% (w/v) inside a sterilized glass jar. Then, the jar was covered using sterilized cheesecloth and rubber band and stored at room temperature (± 29 °C) for 14 d prior to being harvested and utilized as a starting for lingzhi kombucha.

The kombucha was prepared by boiling 0.5% (w/v) of LMP in tap water. After it was cooled to room temperature, a previously prepared kombucha starter was added to the solution, which consisted of SCOBY and lingzhi kombucha starter to the final concentration of 2.5% (w/v) and 20% (v/v), respectively. Moreover, 5% (w/v) of the source of sweetener was added to kombucha. The prepared solution was stored in a sterilized glass jar with cheesecloth to seal the glass jar mouth and allow it to incubate for 14 d at room temperature. The lingzhi kombucha beverage was harvested by filtration and centrifugation and stored at 4 °C storage before being used for sensory evaluation. In addition, Cell-free samples from before and after fermentation were taken, centrifuged (Kubota 6500, Japan), and stored at –20 °C until being thawed for characterization and antioxidant activity assay.

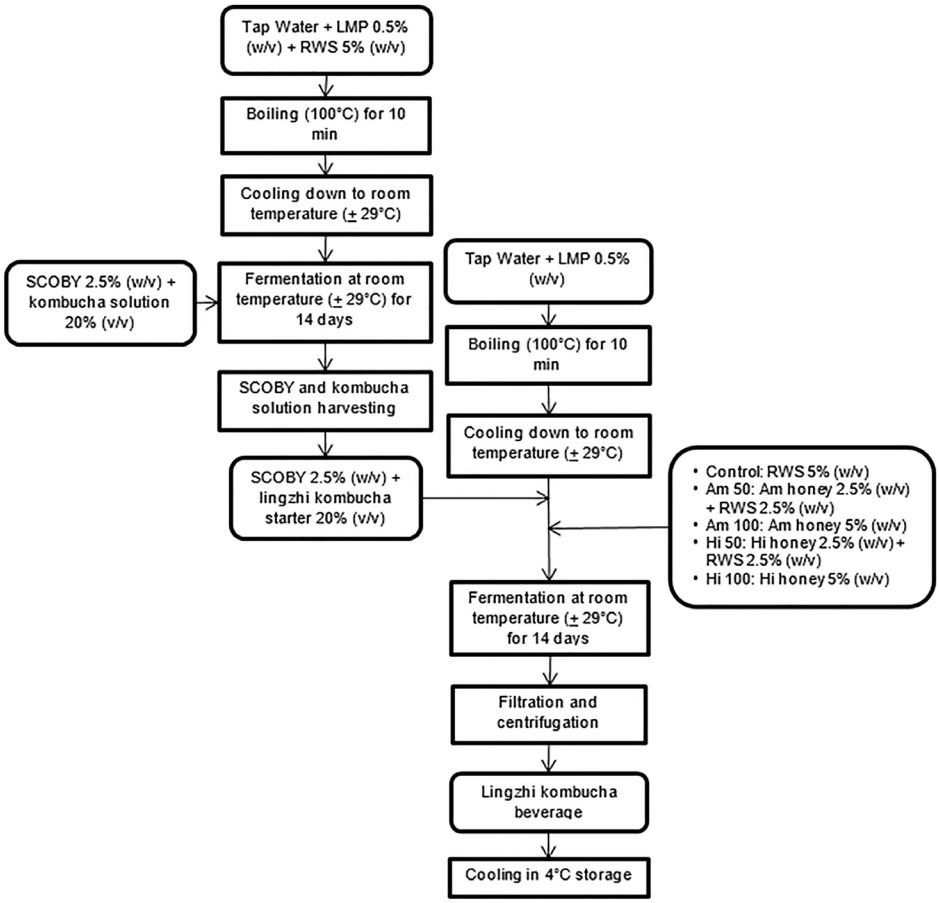

Figure 1. Lingzhi kombucha beverage flow chart. [LMP] lingzhi mushroom powder; [RWS] refined white sugar; [SCOBY] symbiotic culture of bacteria and yeast.

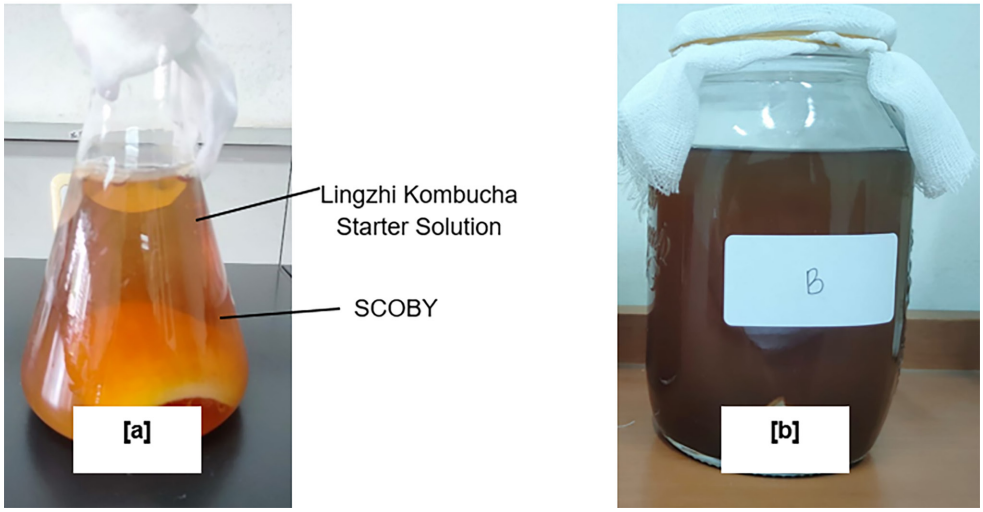

Figure 2. [a] Lingzhi kombucha starter; [b] lingzhi kombucha beverage.

Lingzhi Kombucha Characterization

Reduction sugar. The reduction sugar of kombucha was measured using a dinitro salicylic acid (DNS) assay (Elfirta *et al.* 2023) with slight modification. 0.5 mL of cell-free kombucha sample were mixed thoroughly with 1 mL of DNS reagent (DNS 7.5 g/L, sodium hydroxide 14 g/L, sodium potassium tartrate 216 g/L, phenol 5 g/L, and sodium metabisulfite 6 g/L) to be heated for 5 min at 100 °C. After being cooled to room temperature, 5 mL of distilled water was added to the mixes, and a spectrophotometer (Shimadzu UV type BioSpec 1601, Japan) was used to detect absorbance at 540 nm.

Titratable acidity and pH. Titratable acidity was calculated by titrating the cell-free kombucha using 0.1 N NaOH and phenolphthalein 1 % (w/v in ethanol). The proportion of acetic acid was used to express the results in the sample through Equation 1 (Elfirta *et al.* 2023). Cell-free kombucha's pH was determined using a pH meter (Horiba LAQUA PH1100, Japan).

$$\% \text{ acetic acid} = \frac{(\text{v titrant x N titrant x } 60.05)}{\text{v sample (mL)}} \; x \; 10 \qquad (1)$$

Total phenolic content (TPC). The quantification of TPC was determined using the Folin-Ciocalteu method (Elfirta *et al.* 2024). In brief, 200 µL of cell-free kombucha samples were prepared to 2 mL using distilled water completely combined with 200 µL of Folin-Ciocalteu reagent for 8 min, and then 2 mL of 7% sodium carbonate (w/v) was added. The mixture was allowed to stand for a further 90 min in the dark, and absorbance was measured at 750 nm using a spectrophotometer (Shimadzu UV type BioSpec 1601, Japan). The calibration curve was used to determine the TPC, and the results were reported as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

Total flavonoid content (TFC). The measurement of TFC in the kombucha was determined using aluminum chloride in a colorimetric method (Elfirta *et al.* 2024). A 10% aluminum chloride solution in methanol (w/v) was blended with 0.5 mL of cell-free kombucha in an aliquot, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. At dark room temperature, the mixture was incubated for half an hour. A spectrophotometer was then used to test the mixture's absorbance at 415 nm (Shimadzu UV type BioSpec 1601, Japan). The outcome data were expressed as mg/mL of quercetin. Equivalents in milligrams per mL (mg QE/mL) of kombucha.

Lingzhi Kombucha Antioxidant Activity

The determination of kombucha's antioxidant capacity was based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Elfirta *et al.* 2024). A serial dilution of kombucha was made (1.25–15%), and then each solution was carefully mixed with 0.2 mL with 0.6 mL of 0.1 mM DPPH solution (Himedia, India) in methanol. After letting the combination stand in the dark for half an hour, a spectrophotometer was used to measure its absorbance at 517 nm. (Shimadzu UV type BioSpec 1601, Japan). Methanol was used as blank, and the % inhibition was calculated using Equation 2. The IC_{50}, or the concentration of kombucha in lowering 50% of free radicals, was calculated using the percentage inhibition of each dilution:

$$\text{Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} x\ 100 \qquad 2$$

Lingzhi Kombucha Sensory Evaluation

The sensory evaluation of kombucha after 14 d of fermentation was tested using a seven-point hedonic scale scoring. Thirty (30) semi-trained panelists were recruited from the Department of Food Technology, Padjadjaran University, Jatinangor, West Java, Indonesia, and the sensory test was held at the Sensory Evaluation Laboratory from the same department. The recruits were food technology college students (aged 19–22) who had passed a sensory evaluation course. The panelists received random labels for each lingzhi kombucha in identical plastic containers (± 150 mL). The panelist gave a score of the acceptance of kombucha's color, aroma, bitterness, sourness, sweetness, and overall on a scale of 1 (very dislike) to 7 (very like). The panelist received mineral water and white bread to cleanse their palate between samples. In the end, each panelist received a goody bag filled with snacks as a token of appreciation from the research team. The study's ethical clearance was approved by the Chemical Ethics Committee of the National Research and Innovation Agency (number 005/KE.04/SK/12/2022), and all participants in this study provided written informed consent.

RESULTS AND DISCUSSION

Lingzhi Kombucha Characterization

Fermentation altered every parameter in lingzhi kombucha characters. Reduced levels of reducing sugar and pH were observed in all lingzhi kombucha samples, following a 14-d fermentation period. On the contrary, titratable acidity, TPC, and TFC exhibited an increase (refer to Table 1). Before and after fermentation, adding Riau honey led to elevated levels of reducing sugar, titratable acidity, TPC, and TFC in lingzhi kombucha. Meanwhile, there was a slight shift in pH observed in lingzhi kombucha with the utilization of Riau honey, both before and after fermentation.

Throughout the fermentation process, yeast enzymatically produces invertase, which hydrolyses disaccharides sucrose into monosaccharides, which bacteria will utilize. Consequently, the levels of reducing sugars in kombucha decrease. Concurrently, yeast produces ethanol and carbon dioxide as metabolic byproducts. Lactic acid bacteria secrete lactic acid during fermentation, leading to an increase in titratable acidity and a reduction in the pH of the lingzhi kombucha. Additionally, acetic acid bacteria oxidize ethanol within kombucha, yielding acetic acid and contributing to increased acidity (Bishop *et al.* 2022).

The phenolic and flavonoid levels in lingzhi kombucha increased during fermentation, leading to a corresponding rise in their antioxidant activity. This phenomenon mirrors findings from various studies that also reported an increase in polyphenol, flavonoid, and antioxidant activity in lingzhi kombucha (Elfirta *et al.* 2024; Sknepnek *et al.* 2018) or in kombucha with honey (Candra *et al.* 2023; Fernando dos Santos *et al.* 2024). The augmentation of polyphenols and flavonoids in lingzhi kombucha is attributed to polyphenol oxidation catalyzed by enzymes from the microbial activity in the SCOBY. As polyphenols undergo oxidation, their chemical structure is converted into smaller, simpler phenolic compounds such as catechins and flavonoids (Kitwetcharoen *et al.* 2023). Additionally, enzymatic activity from the SCOBY may facilitate the degradation of lingzhi cell walls, releasing more phenolic and flavonoid compounds into the kombucha (Jakubczyk *et al.* 2020).

On Day 0, lingzhi kombucha containing Am honey demonstrated a greater increase in reducing sugar and titratable acidity than the Hi honey addition, indicating higher sugar and acid content in Am honey than Hi honey. In the TPC of Day 0 lingzhi kombucha, both Am honey and Hi honey showed a similar elevating effect, whereas in Day 0 lingzhi kombucha TFC, Hi honey showed a higher elevating effect than Am honey. This trend persisted in Day 14 lingzhi kombucha, where residues of reducing sugar and titratable acidity were notably higher in Am honey samples but similar to final TPC content and higher TFC in Hi honey samples. Both honey type and honey ratio influence and interact with almost every character in the lingzhi kombucha before and after the fermentation (refer to Table 2). The type of honey did not influence the TPC of lingzhi kombucha before fermentation. The type of honey also did not show any interaction with ratio honey in the same characteristics.

In our previous investigation, the reducing sugar content of Hi honey ranged from 65.8–67.3%, with a sucrose content ranging from 2.1–3.2% (w/w) (Pribadi and Wiratmoko 2023). Conversely, the initial pH of Hi honey (3.96) and Am honey (3.89) exhibited negligible disparity, yet Hi honey demonstrated higher titratable acidity compared to Am honey (207.54 and 104.55%, respectively) (unpublished data). Notably, the significantly higher moisture content in Hi honey (30.01%) relative to Am honey (16.67%) (unpublished data) resulted in a lower quantity of honey solids added to the kombucha, thereby inducing a lesser acidifying effect on lingzhi kombucha compared to Am honey. This result is according to research conducted by Elfirta *et al*. 2023) and Fernando dos Santos *et al*. (2024), which observed the incorporation of different types of honey in kombucha, resulting in different characteristics of kombucha before and after the fermentation.

Table 1. Characteristics and antioxidant activity of lingzhi kombucha.

Measured value	Sweetener	Sugar-honey ratio	Day 0 (before fermentation)	Day 14 (after fermentation)
Reducing sugar (µg/mL)	Refined sugar (control)	100:0	0.38 ± 0.10	0.18 ± 0.005*
	Am	50:50	1.79 ± 0.03	0.47 ± 0.10*
		0:100	4.28 ± 0.07	1.48 ± 0.02*
	Hi	50:50	1.23 ± 0.07	0.24 ± 0.004*
		0:100	3.35 ± 0.02	0.29 ± 0.004*
Titratable acidity (%)	Refined sugar (control)	100:0	0.11 ± 0.01	1.54 ± 0.04*
	Am	50:50	1.11 ± 0.01	1.89 ± 0.03*
		0:100	1.24 ± 0.01	1.99 ± 0.05*
	Hi	50:50	0.67 ± 0.01	1.66 ± 0.03*
		0:100	0.79 ± 0.01	1.79 ± 0.05*
pH	Refined sugar (control)	100:0	3.64 ± 0.02	2.85 ± 0.01*
	Am	50:50	3.77 ± 0.02	2.77 ± 0.02*
		0:100	3.65 ± 0.02	2.84 ± 0.03*
	Hi	50:50	3.79 ± 0.03	2.88 ± 0.01*
		0:100	3.82 ± 0.03	3.02 ± 0.01*
TPC (mg GAE/g)	Refined sugar (control)	100:0	33.78 ± 1.39	94.52 ± 6.9*
	Am	50:50	60.07 ± 2.77	102.67 ± 1.41*
		0:100	77.85 ± 4.06	115.88 ± 2.45*
	Hi	50:50	59.21 ± 2.87	105.38 ± 2.18*
		0:100	78.59 ± 4.68	163.90 ± 2.23*
TFC (mg QE/g)	Refined sugar (control)	100:0	34.97 ± 0.94	43.96 ± 1.34*
	Am	50:50	28.3 ± 0.42	49.73 ± 0.94*
		0:100	32.41 ± 1.07	59.84 ± 1.29*
	Hi	50:50	39.86 ± 1.38	51.51 ± 1.24*
		0:100	40.97 ± 1.40	85.84 ± 0.89*
IC_{50} (µL/mL)	Refined sugar (control)	100:0	36.78 ± 0.26	12.08 ± 0.04*
	Am	50:50	33.59 ± 0.25	11.58 ± 0.08*
		0:100	31.30 ± 0.39	10.77 ± 0.32*
	Hi	50:50	32.85 ± 0.14	9.84 ± 0.06*
		0:100	27.47 ± 0.35	7.63 ± 0.06*

*Significant difference between fermentation days ($p < 0.05$, pair t-test). [TPC] total phenolic content; [TFC] total flavonoid content; [Am] *A. mellifera* bee honey; [Hi] *H. itama* bee honey

Table 2. Two-way ANOVA results for characteristics and antioxidant activity of lingzhi kombucha.

Variable	Time Point	Source of variation	SS	*df*	Mean square	F-value	*p*-value
Reducing sugar	Day 0	Honey type	2.23	1	2.23	412.91	< 0.001*
		Honey ratio	73.56	2	36.78	6809.15	< 0.001*
		Interaction	1.32	2	0.66	122.36	< 0.001*
	Day 14	Honey type	2.06	1	2.02	1157.92	< 0.001*
		Honey ratio	3.22	2	1.61	926.08	< 0.001*
		Interaction	2.38	2	1.19	684.88	< 0.001*

Table 2. Cont.

Titratable acidity	Day 0	Honey type	0.8	1	0.8	1235.01	< 0.001*
		Honey ratio	5.83	2	2.91	4485.93	< 0.001*
		Interaction	0.4	2	0.2	309.01	< 0.001*
	Day 14	Honey type	0.19	1	0.19	127.37	< 0.001*
		Honey ratio	0.75	2	0.37	245.2	< 0.001*
		Interaction	0.1	2	0.05	32.04	< 0.001*
pH	Day 0	Honey type	0.02	1	0.02	30.65	< 0.001*
		Honey ratio	0.06	2	0.03	49.99	< 0.001*
		Interaction	0.03	2	0.01	21.99	< 0.001*
	Day 14	Honey type	0.04	1	0.04	199.18	< 0.001*
		Honey ratio	0.04	2	0.02	84.66	< 0.001*
		Interaction	0.02	2	0.01	56.92	< 0.001*
TPC	Day 0	Honey type	0.02	1	0.02	0.00	0.97
		Honey ratio	11957.96	2	5978.98	616.99	< 0.001*
		Interaction	3.87	2	1.94	0.20	0.82
	Day 14	Honey type	2574.62	1	2574.62	136.73	< 0.001*
		Honey ratio	13740.31	2	6870.16	364.86	< 0.001*
		Interaction	4366.62	2	2183.31	115.95	< 0.001*
TFC	Day 0	Honey type	404.46	1	404.46	349.26	< 0.001*
		Honey ratio	42.3	2	21.15	18.26	< 0.001*
		Interaction	215.73	2	107.86	93.15	< 0.001*
	Day 14	Honey type	771.61	1	771.61	533.28	< 0.001*
		Honey ratio	5491.36	2	2745.68	1897.61	< 0.001*
		Interaction	1265.88	2	632.94	437.44	< 0.001*
IC_{50} (µL/mL)	Day 0	Honey type	10.47	1	10.47	127.86	< 0.001*
		Honey ratio	164.2	2	82.1	1002.57	< 0.001*
		Interaction	12.34	2	6.17	75.34	< 0.001*
	Day 14	Honey type	11.88	1	11.88	595.85	< 0.001*
		Honey ratio	24.98	2	12.49	626.46	< 0.001*
		Interaction	7.42	2	3.71	186.18	< 0.001*
Color		Honey type	0.01	1	0.01	0.01	0.95
		Honey ratio	0.61	2	0.30	0.14	0.87
		Interaction	0.02	2	0.01	0.01	0.99
Aroma		Honey type	< 0.001	1	< 0.001	< 0.001	0.99
		Honey ratio	0.71	2	0.35	0.18	0.84
		Interaction	0.24	2	0.12	0.06	0.94
Bitterness		Honey type	0.98	1	0.98	0.52	0.46
		Honey ratio	20.02	2	10.01	5.3	0.01*
		Interaction	0.72	2	0.36	0.19	0.83
Sourness		Honey type	3.54	1	3.54	1.62	0.21
		Honey ratio	5.47	2	2.74	1.25	0.29
		Interaction	1.78	2	0.89	0.41	0.67
Sweetness		Honey type	0.01	1	0.01	0.01	0.94

Table 2. Cont.

	Honey ratio	9.55	2	4.78	2.54	0.09
	Interaction	0.14	2	0.07	0.04	0.96
Overall	Honey type	1.66	1	1.66	0.99	0.32
	Honey ratio	17.78	2	8.89	5.36	0.01*
	Interaction	0.84	2	0.42	0.25	0.78

**p*-value significant at α = 0.05; [SS] sum of the squares; [df] degree of freedom; [MS] mean of the squares

Lingzhi Kombucha Antioxidant Activity

The fermentation process involving SCOBY in lingzhi kombucha increases the ability of lingzhi kombucha to inhibit the free radical of DPPH among the samples (Figure 3). The IC_{50} value reflects the concentration of lingzhi kombucha needed to reduce 50% of existing DPPH free radicals; a lower IC_{50} signifies higher antioxidant activity. Microbial activity in lingzhi kombucha has notably bolstered its health advantages, particularly in heightened antioxidant activity.

Prior to fermentation, the antioxidant activity of lingzhi kombucha ranged from 27.47–36.77 µL/mL and rose even higher after the fermentation (ranging from 7.62–12.08 µL/mL). Similar to Elfirta *et al.* (2024), the IC_{50} of lingzhi kombucha pre-fermentation was 77.55 µL/mL and decreased after the fermentation depending on the fermentation time. The increased antioxidant activity on lingzhi kombucha may be the effect of increasing TPC and TFC during fermentation (Table 1), as both compounds are known to contribute to antioxidant activity (Mahani *et al.* 2024). However, the presence of other phytochemicals in honey beyond those measured may contribute to its antioxidant properties when integrated into lingzhi kombucha, posing a limitation to this study. These potential contributors include vitamins C and E, glucose oxidase and catalase enzymes, organic acids, amino acids, and minerals (Kitwetcharoen *et al.* 2023).

Incorporating Riau honey significantly elevated the antioxidant activity in lingzhi kombucha both before and after fermentation, as indicated in Table 1. Both type and ratio of honey influence the antioxidant activity and show an interaction between those two factors (Table 2). Furthermore, the use of Hi honey demonstrated a greater enhancement effect on the antioxidant activity of lingzhi kombucha than Am honey.

In our prior unpublished investigation, the antioxidant activity, assessed *via* IC_{50} in DPPH analysis, was notably higher in Am honey at 60.98 mg/mL compared to Hi honey, which exhibited a lower activity at 155.65 mg/mL. Intriguingly, despite the lower overall antioxidant activity, Hi honey demonstrated elevated levels of certain compounds recognized for their antioxidant contributions, notably TFC. Specifically, the TFC of Hi honey measured at 128.77 mg QE/kg surpassed that of Am honey, which stood at 65.54 mg QE/g. Although the TPC, another contributor to antioxidant activity, showed a similar range between the two honey types, with Hi honey at 1.09 mg GAE/g and Am honey at 0.98 mg GAE/g, this did not explain the significant disparity in TFC observed in lingzhi kombucha incorporating Hi honey compared to Am honey. Consequently, Hi honey lingzhi kombucha exhibited higher antioxidant activity than its counterpart.

Consuming foods abundant in organic acids, polyphenols, and flavonoids such as kombucha has been associated with anti-hyperglycemic and anti-obesity benefits (Permatasari *et al.* 2022; Zubaidah *et al.* 2019). Furthermore, the diverse array of beneficial compounds found in kombucha serves multiple functions, including hepatoprotective (Zubaidah *et al.* 2023), antimicrobial (Candra *et al.* 2023; Khaleil *et al.* 2020; Sknepnek *et al.* 2018), immunomodulator (Zubaidah *et al.* 2021), and anticancer properties (Candra *et al.* 2023; Hartati *et al.* 2024).

Lingzhi Kombucha Sensory Evaluation

The addition of Riau honeys did not produce significant changes in the sensory acceptance of lingzhi kombucha (refer to Table 3). The honey ratio affected the acceptance of bitterness and overall attributes of lingzhi kombucha (Table 2), and there was no interaction between the type and ratio of honey on the sensory acceptance.

While the incorporation of honey did not significantly alter the sensory acceptance of lingzhi kombucha, certain characteristics typical of kombucha were observed in this product. The slight brown-yellow hue of lingzhi kombucha may be attributed to extracted polyphenols from the lingzhi mushroom and honey (Handayani *et al.* 2022; Kanyanat *et al.* 2023; Oludemi *et al.* 2018). This color change could result from the activity of polyphenol oxidase enzymes, which catalyze oxidative reactions on polyphenols, potentially enhancing color and aroma development during fermentation (Bishop *et*

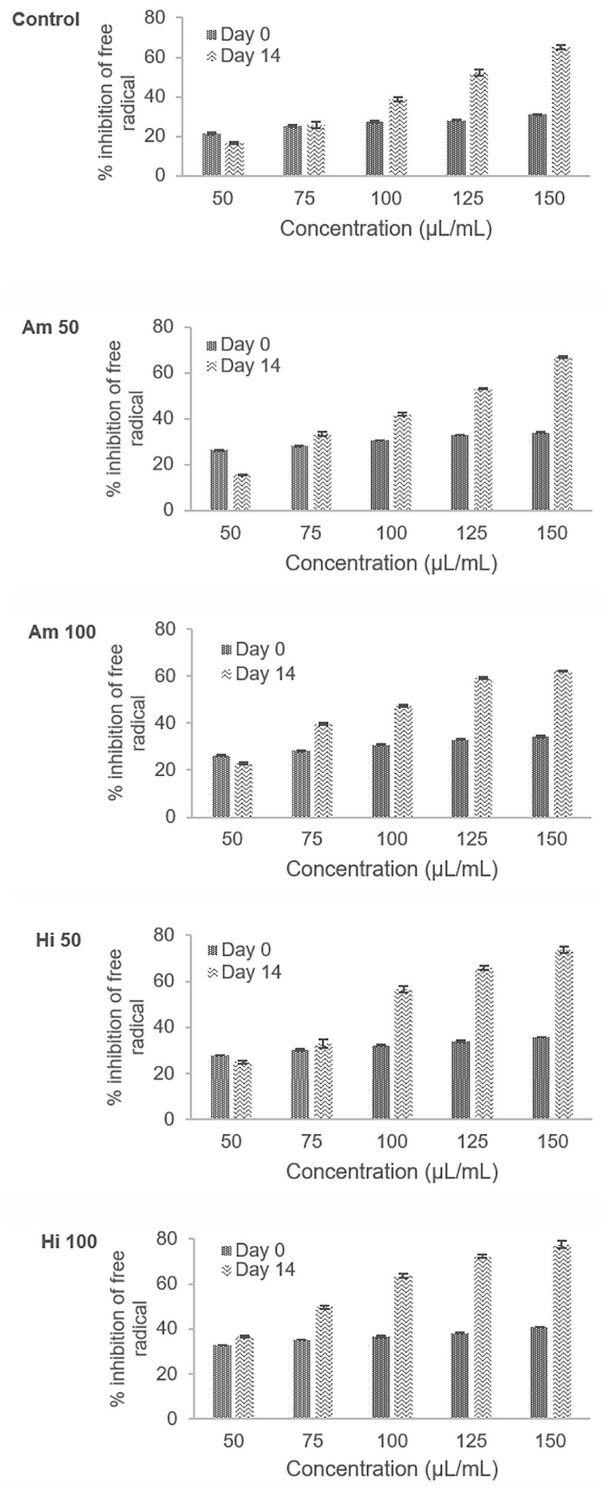

Figure 3. DPPH scavenging activity of lingzhi kombucha. [Control] refined white sugar 100%; [Am 50] *A. mellifera* bee honey 50%; [Am 100] *A. mellifera* bee honey 100%; [Hi 50] *H. itama* bee honey 50%; [Hi 100] *H. itama* bee honey 100%.

al. 2022). Moreover, lingzhi kombucha's "cidery" aroma stems from residual alcoholic compounds, organic acids, and esters formed during fermentation (Bishop *et al.* 2022). Additionally, the bitterness is likely imparted by polyphenols and triterpenoids extracted from lingzhi, contributing to an astringent sensation (Oludemi *et al.* 2018). The sourness is primarily due to organic acids produced by bacteria – predominantly acetic, glucuronic, and gluconic acids, with traces of lactic, succinic, and malic acids also present (Bishop *et al.* 2022). Lingzhi may contain ganoderic acid (Oludemi *et al.* 2018), which could be extracted during kombucha production and further enhance the acidic aroma and flavor of lingzhi kombucha. Furthermore, it should be acknowledged that organic acids may be present in the honey, with honey sourced from *H. itama* generally exhibiting lower sweetness and higher acidity levels than common honey varieties (Kanyanat *et al.* 2023; Pribadi and Wiratmoko 2023). The sweetness in the kombucha arises from residual reducing sugars in lingzhi kombucha (Bishop *et al.* 2022). Finally, the carbonated sensation results from naturally produced carbon dioxide gas by yeast in the SCOBY (Bishop *et al.* 2022).

CONCLUSION

In conclusion, the study demonstrates the potential of lingzhi mushroom kombucha enriched with honey as a functional beverage with enhanced antioxidant properties. The SCOBY aids in the fermentation process, leading to significant increases in total phenolic and flavonoid content, as well as enhancing antioxidant activity. The substitution of refined sugar with honey, particularly from different botanical origins and bee species in Riau, Indonesia, influenced the biochemical composition of lingzhi kombucha. While honey supplementation did not significantly alter the sensory characteristics, it contributed to minor color and aroma perception improvements and a slight decrease in bitterness. Importantly, lingzhi kombucha fortified with honey maintained its fermentation process, indicating compatibility with the microbial activity of SCOBY. This suggests the feasibility of incorporating honey as a natural sweetener in kombucha production without inhibiting fermentation. Future research directions could delve deeper into elucidating the mechanisms underlying the interaction between lingzhi mushroom, honey, and SCOBY during fermentation to optimize the bioactivity of the final product. Moreover, exploring the effects of different honey types and concentrations on lingzhi kombucha's sensory acceptance and health benefits could provide valuable insights for product development and consumer acceptance.

Table 3. Sensory evaluation of lingzhi kombucha.

Lingzhi kombucha	Sensory attributes					
	Color	Aroma	Sweetness	Sourness	Bitterness	Overall
Control	4.70 ± 1.49	4.47 ± 1.23	4.71 ± 1.49	4.76 ± 1.60	4.59 ± 1.23	4.82 ± 1.33
Am 50	4.82 ± 1.29	4.59 ± 1.67	4.82 ± 1.07	4.41 ± 1.42	4.59 ± 1.33	4.82 ± 1.29
Am 100	4.88 ± 1.58	4.53 ± 1.18	3.88 ± 1.57	3.94 ± 1.56	4.00 ± 1.70	3.88 ± 1.32
Hi 50	4.88 ± 1.17	4.71 ± 1.53	5.24 ± 1.09	4.94 ± 1.25	4.71 ± 1.36	5.24 ± 1.09
Hi 100	4.88 ± 1.73	4.41 ± 1.58	4.06 ± 1.43	4.53 ± 1.42	3.94 ± 1.34	4.24 ± 1.35

[Control] refined white sugar 100%; [Am 50] *A. mellifera* bee honey 50%; [Am 100] *A. mellifera* bee honey 100%; [Hi 50] *H. itama* bee honey 50%; [Hi 100] *H. itama* bee honey 100%

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