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Comparative Analysis of Chemical Properties, Nutritional, Phytochemical, and Antioxidant Properties of Kombucha Teas: Black Tea, Pecah Beling Tea, and Poly-herbal Tea

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Abstract: Kombucha tea is a fermented drink made from a combination of tea, sugar, bacteria, and yeast. Many studies show that kombucha can also be developed using different types of tea. Hence, this study aimed to investigate the production of kombucha tea using black tea, Pecah Beling (*Strobilanthes crispus*) tea, and Poly-herbal tea (containing *S. crispus* and *Orthosiphon stamineus*) with three different concentrations of sugar (sucrose). The study evaluated the samples' chemical properties (pH value and sugar brix), nutritional, phytochemical, and antioxidant properties. The results showed that kombucha tea with black tea had the highest total phenolic and flavonoid content (13%-25%), while kombucha tea with Pecah Beling tea had the highest antioxidant activity (85%,88%, and 90%). Based on the analysis, the study suggests that kombucha tea can benefit due to its functional components, such as phytochemical and antioxidant.

Keywords: kombucha tea; chemical; phytochemical; antioxidant.

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1. Introduction

The rising cost of pharmaceutical products can majorly impact health, especially as technology continues to evolve rapidly. This is particularly concerning for low- and middle-income countries where the availability and affordability of medicine can be challenging. As a result, many people seek cheaper and more accessible options to maintain their health. One such option is functional food, which is a type of food that has been improved biologically or through the addition of beneficial components to enhance its positive effects on consumers [1]. Herbal products have also become increasingly popular due to their perceived health benefits, with tea being one of the oldest and most widely consumed herbal products. Tea is a rich source of antioxidants, which have been shown to reduce the risk of chronic diseases [2].

Kombucha is one of the products that have been produced involving the usage of tea leaves and the fermentation process. Kombucha tea is a fermented tea involving a symbiotic culture of bacteria and yeast. The so-called SCOBY acts as the vessel for bacteria and yeast [2]. The main ingredients of kombucha tea are tea leaves, sucrose, SCOBY, and 10% starter culture from the previous batch. The presence of sucrose in the formulation of kombucha tea

acts as the substrate for bacteria and yeast for their metabolic system, producing beneficial acid. SCOBY, which is also called tea fungus, usually contains various microorganisms that may affect the composition of kombucha tea, which possess a variety of bioactive compounds, including polyphenols, vitamins, minerals, and diverse metabolic products of yeast and bacteria [3]. Various probiotic microbes have been found in SCOBY in previous studies, such as gram-negative aerobacilli, yeasts, and lactic acid bacteria in the Acetobacteraceae family [4]. Along the fermentation process, a new layer of SCOBY will form on top of the solution and increase in thickness, providing the necessary oxygen for the microbes [5].

Although the production of kombucha tea mainly includes tea leaves (*Camelia sinensis*), there are various studies with other raw materials that act as substrates in producing this beverage using the same method. Production of kombucha tea using another alternative as a substrate, such as fruit juice [6], soy [7], and other conventional food plants, shows satisfactory results in terms of kinetics and biological properties [8]. Therefore, this study will focus on 3 types of kombucha beverages which is kombucha tea from black tea (*Camellia sinensis*), Pecah Beling tea (*Strobilanthes crispus*), and Poly-herbal tea (*Strobilanthes crispus* and *Orthosiphon stamineus*). This paper will discuss their physical properties, phytochemical and antioxidant properties throughout the fermentation duration, and the product. It is hypothesized that the fermentation process with tea residue can eventually increase the product's beneficial properties, such as the phytochemicals and antioxidants.

2. Materials and Methods

2.1. Formulation of kombucha tea using black tea, Pecah Beling tea, and Poly-herbal tea.

Pre-treatment and preparation of kombucha tea were conducted using black tea, Pecah Beling tea (*Strobilanthes crispus*), and Poly-herbal tea (*Strobilanthes crispus* and *Orthosiphon stamineus)*. The main ingredient in the formulation of kombucha tea is the starter culture, also known as SCOBY, which generally consists of *Acetobacter xylinum, Gluconobacter,* and *S. cerevisiae* [8]. Three different weights of sugar (60, 80, 120g) were prepared, diluted in 1 liter of hot water, and stirred until the sugar was fully dissolved. The next step is the addition of dried tea leaves or dried samples into the mixture. Four grams of each dried tea leaf sample were placed into the mixture and continued to be stirred for 5 minutes. The mixture was left to cool down to room temperature before adding another ingredient. Before adding the SCOBY, the beverages were stored at a temperature close to room temperature, and only sanitized utensils were used to control the growth of microorganisms and prevent unwanted contamination [9]. 250 ml of starter culture was added to the mixtures along with the daughter SCOBY from the previous batch. The acidity level of the mixture was measured, the glass bottles were covered with a paper towel, and the fermentation process was held for 10 days. The growth of the new daughter SCOBY was observed along the process. After 10 days, the SCOBY was removed, and the daughter SCOBY was kept for the next batch. The mixture was filtered and allowed to sit at room temperature for 3 days for the carbonation process. At the end of this method, there will be 3 samples for each type of kombucha tea, bringing to a total of 9 samples. Kombucha tea with black tea acts as a control sample due to its high commercial availability.

2.2. pH analysis.

50ml of each sample was taken from all three samples, including the control sample; the pH value was measured using a pH meter [10]. This analysis was conducted three times for triplicate results. The result was statistically analyzed using GraphPad Prism InStat and Microsoft Excel 2017.

2.3. Sugar brix analysis.

The brix analysis was done using a handheld refractometer [11]. A refractometer is a tool that can determine the concentration of a particular substance in a liquid solution by using the principle of refraction of light from one medium to another. This analysis was conducted on the fermentation process's 1st, 5th, and 10th day and three times each for triplicate results. To use the refractometer, place the sample between the illuminating and the measuring prisms, use a rotating knob to place the shadow boundary on the telescope crosshairs, and read the refractive index from the scale. The analysis was conducted three times for each sample to get a triplicate result. The statistical analysis was conducted utilizing GraphPad Prism InStat and Microsoft Excel 2017, and the outcome was presented as the mean value accompanied by the standard deviation.

2.4. Nutritional analysis.

The end product for all the samples was analyzed for calories, protein, total carbohydrates, and total fat; each parameter was analyzed using different methods involving Method of Analysis for nutrition Labelling for calories and total carbohydrates (AOAC, 1993), AOAC 976.05 for protein (AOAC, 1935) and In-house Method Based on Pearson's Chemical Analysis of Foods for total fat [12]. The analysis was performed in triplicate to obtain the average result, while the outcome presentation focused on the standard deviation value.

2.5. Total phenolic content (TPC)

This method was conducted using the Folin-Ciocaltue reagent [13]. 20g of sodium carbonate was dissolved in 100 mL distilled water to obtain a 20% sodium carbonate solution. 200µL of each sample was mixed with 3mL of distilled water and 0.5mL of Folin-Ciocaltue solution and left for 3 minutes. 2 mL of 20% sodium carbonate was added to the solution and left for 1 hour. All these methods were executed in a dark place. The overall solution was vortexed and measured at 650nm using UV-Vis spectroscopy. Gallic acid acts as the standard, and the results were expressed in µg gallic acid equivalents/mL (µg GAE/mL). The statistical analysis was carried out using Microsoft Excel 2017, and the results were reported as the mean value and its corresponding standard deviation.

2.6. Total flavonoid content (TFC).

Aluminium chloride colorimetric was used in determining the TFC content [14]. 1mL from each sample was taken and mixed with 4 mL of distilled water in a test tube, followed by adding 0.3mL of 5% sodium nitrate solution and remaining for 6 minutes. 0.3mL of 10% aluminum chloride hexahydrate was added to the solution and left for 5 minutes. 2mL of 1M sodium hydroxide was added and left for another 5 minutes. Then, the solution was vortexed and measured at 520nm using UV-Vis spectrometry. Rutin was used as standard, and µg rutin

equivalent /mL (µg RE/mL) will be used as the result. The statistical analysis was performed using Microsoft Excel 2017. The result was expressed in mean value and standard deviation.

2.7. DPPH free radical scavenging activity.

This method was conducted by referring to methods from earlier studies with some modifications [15]. DPPH solution was prepared in methanol and sonicated for 5 minutes to produce a stable free radical DPPH. Each sample, including the control sample, was mixed with 50µL of DPPH solution in a 1cm path-length microcuvette, and the mixture was stored in a closed and dark container for 16 minutes. The absorbency was measured after the reaction using a UV-Vis spectrophotometer at 517nm. Ascorbic acid was taken as a control in this analysis. The free radical scavenging activity percentage will be calculated using the following formula from further study [16]. The statistical analysis was performed using GraphPad Prism InStat and Microsoft Excel 2017. The result was expressed in mean value and standard deviation (SD):

$$\text{Scavenging activity}(\%) = \left[1 - \frac{A_{517\text{nm,sample}} - A_{517\text{nm,blank}} - A_{517\text{nm,standard}}}{A_{517\text{nm,control}}}\right] \times 100 \quad (1)$$

2.8. ABTS cation radical scavenging activity.

The procedure for this analysis was conducted by referring to a method previously reported [16]. First, the ABTS radical cation needed to be produced by adding 7.4mM ABTS diammonium salt to 2.6mM potassium persulphate stock solution, and the mixture was left overnight in a dark room at room temperature. The ABTS cation solution was diluted with distilled water to obtain an absorbance of 1.0 at 734nm. 4mL diluted ABTS radical cation solution was added to 20µL of the sample and left for 60 minutes for reaction activity. The absorbance of the sample was measured at 734nm on a UV-Vis spectrophotometer, and this method was conducted three times to obtain a triplicate result. The percentage of free radical scavenging activity was calculated using the following formula from a further study [16]. The statistical analysis was performed using GraphPad Prism InStat and Microsoft Excel 2017. The result was expressed in mean value and standard deviation (SD):

$$\text{ABTS Cation Radical Scavenging Activity}(\%) = \left[1 - \frac{Abs_{\text{sample}}}{Abs_{\text{blank}}}\right] \times 100 \quad (2)$$

3. Results and Discussion

3.1. pH analysis.

The fermentation period took place completely in 10 days, and the samples' pH values were recorded on days 1, 5, and 10. A statistically significant difference was discerned between the pH values at the beginning and post-fermentation, which is illustrated in Figure 1. The highest pH value for the entire sample on the first day was 2.91 to 2.82. The addition of starter culture from the previous batch of kombucha tea caused the low pH value from the first reading. Towards the end of the fermentation process, kombucha tea with Poly-herbal tea exhibited the lowest pH values for 60, 80, and 120g sugar concentrations, giving pH values 2.44, 2.43, and 2.38, respectively. This phenomenon can be explained due to the production of beneficial acids from the production of bacteria and yeast. The process overview is glucose and fructose being

fermented by yeast into ethanol, carbon dioxide, and glycerol, which affect the alcohol content in the tea. The by-product of the yeast, which is the ethanol, will be used by AAB to produce acetic acid [17] parallel to the report from previous research, where the pH value of the kombucha tea sample declined after being fermented for 10 days by way of the organic acids development [18]. Another study by Jakubczyk *et al.* [19] discerned a reduction of pH value throughout 14 days of the fermentation stage, which was approximately 2.30 to 2.53 at room temperature.

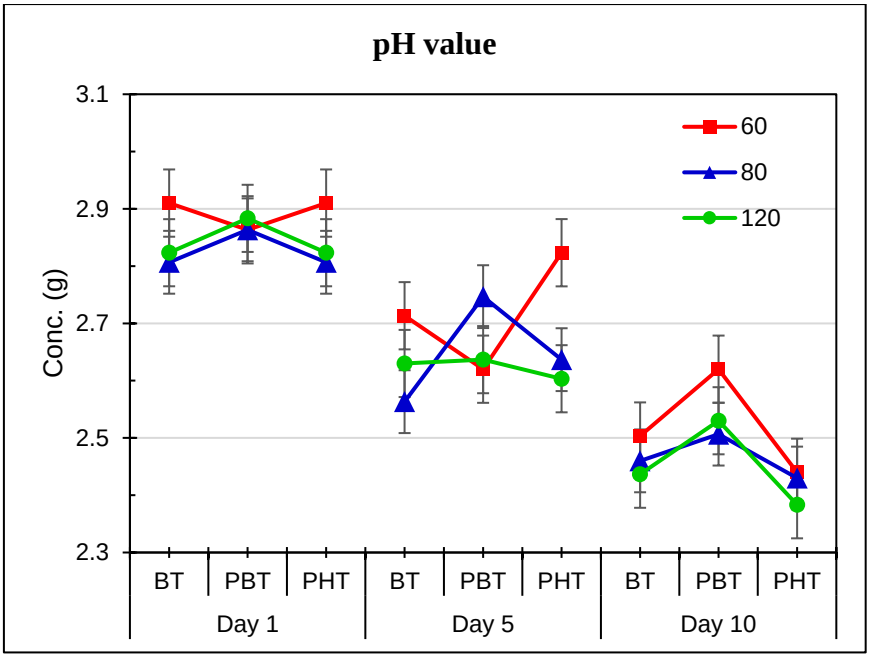

Figure 1. The result for pH during the 10-day fermentation process for kombucha tea with black te (BT), Pecah Beling tea (PBT), and Poly-herbal tea (PHT).

Fermentation is the key to changing the pH value in kombucha tea and leads to the production of organic acids [20-21]. The organic acids in kombucha tea include acetic, glucuronic, gluconic, tartaric, malic, citric, lactic, succinic, and malonic acids [10]. According to Cardoso et al. [22], acetic acid was the most common organic acid in kombucha tea on the 10th day of the fermentation process.

3.2. Sugar brix analysis.

Sugar brix analysis was conducted using a refractometer, and the samples were analyzed on the 1st, 5^{th,} and 10th day of the fermentation process. The objective of this analysis is to analyze the amount of sugar concentration throughout the fermentation process. Figure 2 shows the overall result of sugar brix analysis for all the samples. Based on the result, the entire sample shows a gradual decrease in the sugar brix percentage. Approximately 8-45% reduction of sugar brix percentage occurred along the fermentation process. Kombucha tea with Pecah Beling tea containing 60g of sucrose shows the highest drop (45.08%), which might be due to the high reaction of the fermentation process and the hydrolysis of sucrose by beneficial bacteria. Kombucha tea with Pecah Beling tea also shows the lowest reduction of sugar brix concentration (8.23%) with a sucrose concentration of 120 g. This phenomenon can be explained by the presence of SCOBY, which acts as a vessel for bacteria and yeast. Sucrose acts as the main substrate for bacteria and yeast for the fermentation process, which leads to the production of lactic acid and alcohol, explaining the reduction of sugar brix percentage throughout the fermentation process [23].

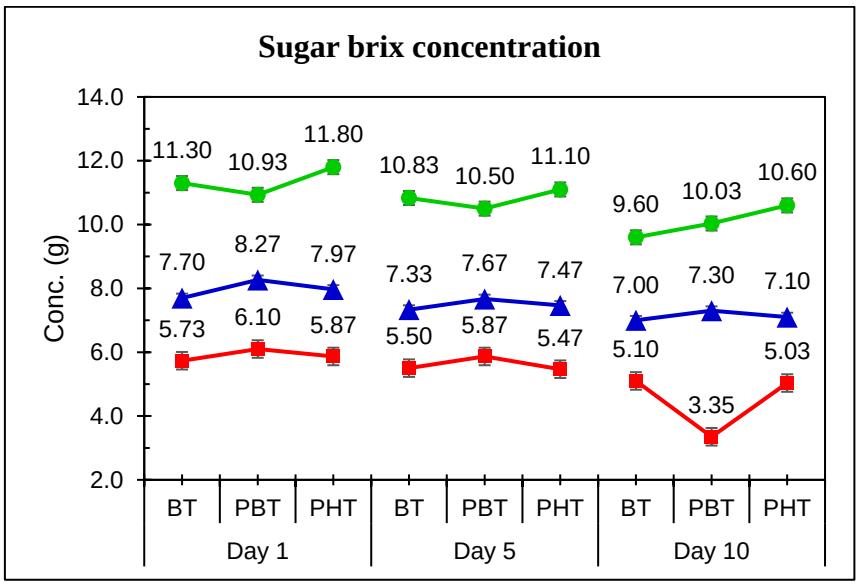

Figure 2. The result for sugar brix percentage for 10 days fermentation process for kombucha tea with black tea (BT), Pecah Beling tea (PBT), and Poly-herbal tea (PHT).

3.3. Nutritional content.

The nutritional contents of the final product of each kombucha sample were summarised in Table 1. Each sample for every sucrose concentration undergoes different methods to evaluate each parameter involving total calories, protein percentage, total carbohydrate, and total fat according to Malaysian nutritional labeling [24]. Each sample exposes an increment for each parameter linearly with the concentration of sucrose except total fat, which was not detected for all concentrations. The total calories and carbohydrates were predominantly due to the sucrose content, which also helps improve kombucha tea's flavor. Kombucha tea with black tea records the least number of calories (16 kcal/100 g, 22 kcal/100 g, 40 kcal/100 g) and the total percentage of carbohydrates (3.7%, 5.3%, 9.7%) compared to other kombucha tea. The fermentation rate elucidates this in kombucha with black tea, which is higher when sucrose acts as a substrate and is hydrolyzed by bacteria into their monomer form [25].

Table 1. The result for the nutritional content of kombucha tea with black tea, Pecah Beling tea, and Poly-herbal tea for every concentration

Test parameter	Black tea			Pecah Beling tea			Poly-herbal tea		
	60	80	120	60	80	120	60	80	120
Calories/energy (kcal/100g)	16±0.02	22±0.04	40±0.09	23±0.18	31±0.24	48±0.37	19±0.04	28±0.06	44±0.02
Protein (%)	0.2±0.73	0.2±0.52	0.2±0.03	0.2±0.06	0.5±0.03	0.6±0.06	0.6±0.04	0.4±0.45	0.3±0.09
Total carbohydrate (%)	3.7±0.05	5.3±0.03	9.7±0.12	5.6±0.04	7.2±0.05	11.5±0.11	4.4±0.74	6.7±0.13	10.7±0.06
Total fat (%)	-	-	-	-	-	-	-	-	-

Tea leaves contain multiple polysaccharides such as galactose, galacturonic acid, rhamnose, and glucose [23-26]. Although the saccharides present may affect the process of kombucha tea production, discussion on the fermentation process in fermented tea is limited to the presence of sucrose, fructose, and glucose [23]. An experimental study on amino acid content in kombucha tea was conducted by Jayabalan et al. [27]. The study revealed that

kombucha tea contained essential and non-essential amino acids, and lysine was the highest among the group [27]. The concentration of amino acid intensifies linearly with the duration of fermentation. Although this investigation fixed the fermentation duration for ten days, its miscellanies still prove the presence of protein and amino acid in the final product.

3.4. Total phenolic and flavonoid content.

Kombucha tea with black tea shows significantly higher total phenolic content (TPC) than other kombucha tea. It undergoes drastic progress during the fermentation period from the first day until the fifth day, as shown in Table 2. Kombucha tea with Pecah Beling and Poly-herbal tea presented a phenolic compound and gradual elevation within the fermentation period. Still, it was not as efficient as kombucha tea with black tea. This phenomenon may be due to the different processes involved in producing each tea leaf, especially black tea, which is fully oxidized during its production, as mentioned above [28]. The oxidizing process ensues chemical changes in the tea phenolics components, mainly from catechins to theaflavin and thearubigins [29]. This process often begins with the withering process and continues during rolling when cell structure destruction happens [30]. Fermentation is the primary process of kombucha tea production, with living microbes converting the existing polyphenol components into a simpler form. The differences in the production process of tea leaves explain the divergent total phenolic content in different types of tea leaves thus for this study. Jayabalan *et al.* [31] reported that theaflavin and thearubigin were relatively stable compared to epicatechin isomers during fermentation but uniformly degraded during the fermentation period extended to 12 days. An experimental study reported an escalation in total phenolic content during the fermentation period, which remained constant after the third day of the process [3]. The total phenolic content of kombucha tea in this study might contrast with other studies and can be interrelated to the variances between tea and kombucha starter culture employed.

Flavonoids are also one of the main chemical components in kombucha tea and their most influential quality parameters because of their color and taste [32]. In this analysis, kombucha and black tea also revealed the highest flavonoid content compared to kombucha tea with Pecah Beling tea and Poly-herbal tea. The results illustrate high flavonoid content from the first day of fermentation and lessening throughout the process; nonetheless, it is still considered high. Kombucha tea with Poly-herbal tea also indicates a promising result and minor reduction throughout fermentation. Kombucha tea with the insertion of 80 g sucrose gave the most promising result in all three types of samples. As mentioned before, black tea undergoes a fermentation process in its production, which embarks on the oxidation of flavonoids present in the tea leaf caused by the release of intracellular polyphenol oxidase, eventually affecting the product's color and flavor. The fermentation process with the presence of living microbe and microbial enzymes resulting more formation of flavonoid content [19]; therefore, flavonoid content was inferior in kombucha tea with Pecah Beling and Poly-herbal tea.

Table 2. The overall result for total phenolic content, total flavonoid content, DPPH and ABTS for each sample.

Type of tea	Fermentation period(days)	TFC (mg/ml)	TPC (mg/ml)	DPPH (%)	ABTS (%)
Black tea (60g sugar)	1	3.787 ± 0.003	0.379 ± 0.002	79.74 ± 0.42	99.294 ± 0.41
	5	3.852 ± 0.003	3.852 ± 0.023	75.64 ± 0.32	98.588 ± 0.32
	10	3.649 ± 0.010	3.648 ± 0.032	82.31 ± 0.49	99.294 ± 0.11

Type of tea	Fermentation period(days)	TFC (mg/ml)	TPC (mg/ml)	DPPH (%)	ABTS (%)
Black tea (80g sugar)	1	3.823 ± 0.006	0.382 ± 0.012	80.78 ± 0.11	99.294 ± 0.35
	5	4.075 ± 0.007	4.075 ± 0.042	75.38 ± 0.57	98.023 ± 0.26
	10	3.623 ± 0.003	3.622 ± 0.016	60.77 ± 0.11	98.588 ± 0.22
Black tea (120g sugar)	1	3.004 ± 0.009	0.3 ± 0.031	77.14 ± 0.23	99.153 ± 0.31
	5	2.988 ± 0.002	2.988 ± 0.026	66.92 ± 0.60	96.045 ± 0.57
	10	2.901 ± 0.011	2.901 ± 0.017	72.31 ± 0.24	96.893 ± 0.42
Pecah Beling tea (60g sugar)	1	0.283 ± 0.006	0.028 ± 0.014	92.47 ± 0.23	99.718 ± 0.32
	5	0.177 ± 0.015	0.178 ± 0.043	85.9 ± 0.34	99.153 ± 0.27
	10	0.564 ± 0.002	0.564 ± 0.051	84.62 ± 0.31	100 ± 0.33
Pecah Beling tea (80g sugar)	1	0.298 ± 0.011	0.03 ± 0.014	87.18 ± 0.22	99.576 ± 0.42
	5	0.426 ± 0.002	0.427 ± 0.037	85.9 ± 0.72	99.011 ± 0.37
	10	0.701 ± 0.017	0.701 ± 0.024	83.59 ± 0.27	98.729 ± 0.71
Pecah Beling tea (120g sugar)	1	0.197 ± 0.012	0.02 ± 0.051	88.31 ± 0.35	99.716 ± 0.22
	5	0.234 ± 0.003	0.234 ± 0.031	85.64 ± 0.26	99.153 ± 0.36
	10	0.437 ± 0.003	0.437 ± 0.035	78.21 ± 0.33	98.305 ± 0.87
Polyherbal tea (60g sugar)	1	1.462 ± 0.008	0.143 ± 0.023	85.97 ± 0.11	99.859 ± 0.43
	5	1.784 ± 0.009	1.784 ± 0.042	72.82 ± 0.70	97.599 ± 0.51
	10	1.712 ± 0.017	1.712 ± 0.013	73.08 ± 0.38	96.186 ± 0.12
Polyherbal tea (80g sugar)	1	1.153 ± 0.002	0.115 ± 0.013	88.83 ± 0.37	99.435 ± 0.24
	5	2.846 ± 0.002	2.845 ± 0.022	74.87 ± 0.36	96.61 ± 0.21
	10	1.54 ± 0.016	1.54 ± 0.011	79.74 ± 0.25	97.599 ± 0.26
Polyherbal tea (120g sugar)	1	0.789 ± 0.004	0.079 ± 0.014	90.65 ± 0.32	99.294 ± 0.31
	5	1.037 ± 0.002	1.037 ± 0.052	83.33 ± 0.41	95.48 ± 0.23
	10	0.917 ± 0.016	0.971 ± 0.011	81.54 ± 0.24	96.328 ± 0.72

3.5. Antioxidant analysis.

Antioxidant capacity and bioactivity were typically linked with the presence of the molecular structure of phenolic compounds, providing multiple benefits to consumers. In SCOBY, the presence of microbial components, bacteria, and yeast qualifies an enzyme creation that converts polyphenolic components into a simpler form, leading to higher antioxidant capacity [33, 34]. Compared to regular black tea and kombucha tea, kombucha tea comprises higher polyphenols and other beneficial compounds that can act as antioxidant agents. From the result in Table 2, the overall sample covers a high percentage of antioxidants based on DPPH and ABTS analysis and only reveals minor changes within the fermentation interval. For DPPH analysis, kombucha tea, in the substitution of Pecah Beling tea, unveiled a vast antioxidant percentage compared to kombucha tea with black tea and kombucha tea with poly-herbal tea.

Nevertheless, there was a slight reduction of approximately 2-10% through the fermentation process. Antioxidant levels were the highest on the first day of fermentation for entirely three 60, 80, and 120 concentrations, with 92.47%, 87.18%, and 88.31%, respectively, for DPPH analysis. The overall antioxidant content in kombucha tea with Poly-herbal tea was marginally higher than kombucha tea with black tea; however, these can be claimed as beneficial beverages attributable to the high percentage of antioxidants. This phenomenon was experimentally reported by Tan and team [35], where Pecah Beling species (*S. crispus*) contained great concentrations of antioxidants with polar extract (water and methanol) with a

sample concentration below 200µg/ml. Another study was handled converging on the antioxidant properties with a combination of multiple herbal species, including Pecah Beling (*S. crispus*), disclosed that the most optimum formulation was 23.96% of Pecah Beling (*S. crispus*), 0.62% of *P. niruri*, and 75.42% of *O. aristatus*. However, Pecah Beling does not show the highest antioxidant properties compared to other herbs; it contributed to the most optimum formulation, producing the highest antioxidant percentage, 93.95% [36].

The approach of ABTS analysis toward the achieved results displays a gradual declination of approximately 1-3% during the fermentation process. The difference in sucrose concentration in the formulation of Pecah Beling (*S. crispus*) does not differ significantly from the antioxidant concentration. The dissimilarities of antioxidant concentration in kombucha tea with Pecah Beling (*S. crispus*) and kombucha tea with Poly-herbal tea might be due to the antagonistic effect. This output happened when the combined components yielded a total effect that was less than the sum of the effect of each chemical [37]. The antioxidant in kombucha tea with Poly-herbal tea expressed a lower concentration than in other samples even though multiple herbal components were involved.

4. Conclusions

In conclusion, this study looked at the production of kombucha tea using different types of tea and sucrose concentrations. The fermentation process had a significant impact on the pH and sugar content of the tea. The sucrose concentration also affected the tea's nutritional composition, with higher sucrose concentrations promoting higher calories, protein, and carbohydrate content. Kombucha tea with black tea had the most promising results regarding phytochemical and antioxidant content, while kombucha tea with Pecah Beling tea had a superior ability to scavenge free radicals. The differences in results between Pecah Beling and Poly-herbal tea may be due to the antagonistic effects of multiple herbal components. Overall, this study highlights the potential health benefits of kombucha tea and suggests that different types of tea and sucrose concentrations can influence its nutritional and antioxidant properties.

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Conflicts of Interest

The authors declare no conflict of interest.

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