

Research Article

Effect of Fermentation Time on Physicochemical Properties of Kombucha Produced from Different Teas and Fruits: Comparative Study

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This study aimed to investigate the impact of fermentation time on antioxidative activities and phenolic composition and sensory quality of kombucha fermented from different teas (green, black, and oolong) and fruits (grape, dragon, and guava). Results: the highest antioxidative activity was observed in kombucha from green tea and grapefruit fermented for 6–7 days at 25–30°C and 48 h at 37°C, respectively. Further analysis revealed that the antioxidative activity of grape kombucha was significantly improved due to an increase in polyphenols' concentration as compared to original green tea kombucha. Furthermore, the sensory evaluation of grape kombucha suggested that grape-flavoured kombucha is more acceptable by the young-aged group. In conclusion, this study provides a potential and promising method for the first time to produce fruit-flavoured kombucha with increased bioactive compounds in very short fermentation time (48 h) which could fulfil the nutritional requirement for human health.

1. Introduction

Kombucha is a fermented beverage which is prepared under aerobic conditions by fermenting tea with sugar and by applying a symbiotic culture of bacteria and yeast, generally for 10–15 days. It originated from northeast China in about 220 B.C. and spread round the globe through trade routes [1, 2]. After fermentation, kombucha becomes a cocktail of chemical components, including sugars, polyphenols, organic acids, fiber, ethanol, amino acids, essential elements, and water-soluble vitamins [3]. Due to these bioactive compounds and the chemical composition of kombucha, it is considered as a popular functional food for human health. Several *in vivo* and *in vitro* studies have been carried out to

establish the existence of antioxidant [4], antimicrobial [5], and anti-inflammatory [6] properties. Recently, kombucha has transitioned from a homemade fermented beverage to a commercialized drink in the market. In 2016, PepsiCo purchased KeVita, a popular functional probiotic and kombucha beverage maker [2].

In the USA alone, the kombucha market is expected to exhibit a strong growth rate of 17.5% between 2019 and 2024. From the year 2017 onwards, retail sales of kombucha have increased by over 30% each year globally. As per the recent report, kombucha is the fastest growing product in the functional beverage market, and in the coming time, it would become one of the most popular low-alcoholic fermented beverages in the world [2]. Despite the claim of

several health benefits of kombucha such as reduced blood pressure, healed peptic ulcer, diabetes, courage weight loss, and detoxification, the microbial contents still need to be investigated. With the growing consumption of kombucha drinks, the risk of safety is also a matter of concern. Several disease-causing microorganisms are found in kombucha drinks because of raw materials, vessels, and during packaging and fermentation methods that may sometime produce toxic metabolites and antinutritional substances such as cyanogenic glycosides, phytates, tannins, and protein inhibitors [7]. Along with these drawbacks, other hurdles in producing kombucha are the long-time fermentation period. As it has been noticed that the kombucha fermentation takes at least 3 to 60 days based on the microbial cultured used in. The fermentation of kombucha is usually performed at room temperature. Therefore, standard production methods are required to produce kombucha drinks [8].

To overcome these problems and development of kombucha with high bioactive compounds with short fermentation period, for the first time, we have tried to develop fruit-flavored kombucha drinks using a unique method and analyzed their quality characteristics. Besides the original flavor, kombucha has been developed with different flavors, which would provide a better fruit-flavored kombucha drink with improved antioxidant properties for human health. However, this approach and their comprehensive strategy has not been used before. This study aimed to develop the kombucha drink with a unique fruit flavor at different fermentation periods and compare the antioxidative activities and phenolic composition of the drink fermented from green, black, and oolong tea, along with grape, dragon, and guava fruits.

2. Materials and Methods

2.1. Materials. Green, black, and oolong tea were purchased from a local supermarket in Guangzhou. The grape, dragon, and guava fruits were also purchased from a local fresh food market in Guangzhou, China. Kombucha start culture (original kombucha mushroom solution) was purchased from Shenzhen Care Pack. Co. Ltd. (Shenzhen, China).

2.2. Preparation of Original Kombucha. Original kombucha was prepared using green, oolong, and black tea. In brief, 100 g of sucrose was dissolved in 800 mL of distilled water by maintaining the temperature at 98°C for 15 min. Then, 4 g of the tea leaves were soaked in the prepared sugar solution and heated at 98°C for 12 min. Furthermore, the tea leaves were filtered out after soaking, and the solutions were cooled down to 25°C. Furthermore, the solution was inoculated with 600 ml of kombucha start culture, and fermentation was carried out for 15 days at 25, 30, and 35°C temperatures, respectively. Samples were collected and analyzed on a daily basis during fermentation.

2.3. Preparation of Fruit Kombucha. Grape, dragon, and guava fruits were selected to produce fruit kombucha drinks due to their availability in the market and frequent

consumption by the consumers in China. In brief, the fruit was smashed, blended, and stored into a 500 mL bottle of original kombucha produced under the identified optimized processing conditions. The ratio of smashed fruit to original kombucha was 6 g to 100 mL. Bottles with original kombucha and smashed fruits were sealed with caps. The sealed bottles were placed into a water bath at a temperature of 37°C and fermented for 72 h. Samples were collected and analyzed at 0, 24, 48, and 72 h during fermentation. The formulated grape kombucha beverage was prepared with a formulation of 40% (w/w) grape kombucha, 55% (w/w) sparkling water, and 5% (w/w) sugar, without fermentation, and considered as a control sample.

2.4. Measurement of the Total Polyphenol Content. The total phenolic content (TPC) of kombucha drink was determined using the method of Ahmed and Hikal [9] with slight modifications. In brief, 250 μ L of kombucha was mixed with 125 μ L of 0.2 mol/L Folin-Ciocalteu phenol reagent and then incubated in the dark at room temperature for 5 min on a shaking table. Furthermore, after 5 min of incubation, 250 μ L of 5% (w/v) sodium carbonate was added, which was prepared in deionized water. The mixture was gently shaken in the dark at room temperature for 60 min. Furthermore, the phenolic content was recorded at the absorbance of 725 nm using a spectrophotometer (Ultraviolet visible spectrophotometer; UV-2100; Beijing Ruili Analytical Instrument Co. Ltd.). The TPC was determined in micrograms of gallic acid equivalents (GAE) per gram of barley flour (μ g GAE/g).

2.5. Measurements of the Total Flavonoid Content. The total flavonoid content of kombucha was determined according to Jakubczyk et al. [10] with slight modifications. In brief, 4 mL of distilled water was mixed with 1 mL of kombucha in test tubes. Additionally, 0.3 mL of 5% sodium nitrite solution was added to 0.3 mL of 10% aluminum chloride solution followed by incubation at room temperature for 5 minutes and further addition of 2 mL (1 M sodiumhydroxide). This stock solution was diluted with 10 mL of water. The solution turned into pink color, was mixed with vortex, and transferred into the glass cuvette, and the absorbance was recorded at 510 nm using a spectrophotometer. Aqueous solutions of catechin concentrations in the range of 50–100 mg/L were used for calibration, and the results were shown as milligrams of catechin equivalents (CEQ) per gram of the sample.

2.6. Measurement of Antioxidative Activity. The methods determining ferric-reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging ability were applied to measure the antioxidative activity of kombucha samples.

2.6.1. FRAP Assay. The FRAP assay was analyzed according to Sethi et al. [11] with slight modifications. The FRAP reagent contained 10 mM of TPTZ in 40 Mm of hydrochloric acid/20 mM ferric chloride/acetate buffer (0.3 M, pH

3.6), mixed in a ratio of 1:1:10 (v/v). Test tubes containing 1 mL of each sample in 5 ml of FRAP reagent were incubated at 37°C for 20 min, and the absorbance was recorded at a wavelength of 593 nm with a spectrophotometer. The results were calculated using standard solutions of ferrous sulphate at different concentrations (100–1400 μM) as FRAP values ($\mu\text{M Fe}^{2+}$). The standard curve equation was $y = 2.01x + 0.081$ ($R^2 = 0.999$).

2.6.2. ABTS Radical Scavenging Ability Assay. The ABTS radical scavenging ability of kombucha was examined according to Jayabalan and Subathradevi [12] with slight modifications. In brief, ABTS solution (7 mM aqueous solution of ABTS with 2.45 mM aqueous solution of $\text{K}_2\text{S}_2\text{O}_8$) was made in the dark and left for incubation at room temperature for 16 h. Furthermore, ABTS solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30°C. Furthermore, 0.3 ml of each kombucha sample was added with 1.2 ml of ABTS solution and left for 6 min incubation, and then the absorbance was recorded at 734 nm using a spectrophotometer. The capability of scavenging ABTS was calculated using the following equation:

$$\text{ABTS radical scavenging ability (\%)} = \left[\frac{A_{\text{control}} - B_{\text{sample}}}{A_{\text{control}}} \right] \times 100\%. \quad (1)$$

Here, A is the absorbance of the blank sample, and B is the absorbance of the kombucha samples.

2.7. Phenolic Analysis by High-Performance Liquid Chromatography (HPLC). Quantification of individual polyphenolic compositions was carried out by reverse-phase HPLC analysis using the method described by Garzoli et al. [13] with slight modifications. In brief, kombucha samples were injected into a Waters HPLC system. It was composed of a 1525 binary pump, a thermostat, and a 717+ auto-sampler linked to the Waters 2996 diode array and an EMD 1000 single quadrupole detector with an ESI probe (Waters, Milford, MA, USA). Polyphenols were separated using a Symmetry C-18 RP column (125 mm \times 4 mm i.d., 5 μm particle size). The mobile phase was prepared from 0.1% formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 1.2 mL/min. The gradient profile was as follows: 0–20 min, linear gradient from 10 to 25% B; 20–40 min, linear gradient up to 45% B. Then, the gradient returned to 15% B followed by an additional 5 min of equilibration time. An optimal mobile phase inflow was obtained by a post-column flow splitter (ASI, Richmond, CA, USA) with a 5/1 split ratio for the ESI probe. Chromatograms were constructed by employing a 3D mode equipped with extracted signals at specific wavelengths for different compounds (367, 326, 309, and 204 nm). To compare the literature, data, retention times, were used to qualitatively measure the compounds detected in the samples. The pure standard method and compounds were used as references for the concentration and retention time for quantitative analysis [14]. The peaks from the data acquisition and spectral evaluation were identified using the Waters Empower2 Software (Waters, Milford, USA).

2.8. Food Safety Tests. Standard United States Environmental Protection Agency (USEPA) methods 6010B and 6020 (USEPA 1996) were used to examine the trace element content by inductively coupled plasma mass spectrometry and inductively coupled atomic emission spectrometry. Australian Standard/New Zealand Standard 1766: Food Microbiology (Standards of Australia and Standards, New Zealand 1998) was applied for microbiological testing.

2.9. Sensory Tests. Sensory tests were conducted according to Qinzhu et al. [15] with slight modifications. In brief, forty volunteers in 2 different age groups (<30 years old and >30 years old) were recruited. The sensory evaluation was performed in private booths equipped with Sensory Management System hardware (2006) and computerized sensory software (Sensory Integrated Management System, Morristown, N.J., U.S.A.). The volunteers received 1 h training on discrimination testing. The sensory evaluation involved rating the color, taste, and aroma. Each characteristic was rated from 0 (extreme dislike) to 1 (extreme like).

2.10. Statistical Analysis. All parameters of each sample were measured in triplicate. The least significant difference (LSD) and one-way variance analysis (ANOVA) was performed on the data using the means and standard deviations, and the statistical significance of the F value ($P < 0.05$) was determined. These analyses were performed using v15 MINITAB statistical software.

3. Results and Discussion

3.1. Process Optimization of Fruit Kombucha Production. The grape, dragon, and guava fruits were used to develop the fruit kombucha. These three fruits are very commonly consumed by consumers throughout China. The original green tea kombucha was produced under optimal conditions and served as the control shown in Figures 1 and 2. It was found that the antioxidative activity of the control showed a slight decline after fermentation for 7 days. However, further fermentation by blending smashed fruits with original green tea kombucha (used as the start culture) for fruit flavour extraction showed significantly increased antioxidative activity in comparison with control. The highest level (4.5 mM Fe^{2+} to 7.5 mM Fe^{2+}) of antioxidant activity was observed in grape kombucha measured by the FRAB method as shown in Figure 1. The same trend of antioxidant capacity was observed by the ABTS radical scavenging method as shown in Figure 2. All three types of fruit kombucha demonstrated a substantial increase in antioxidative activity during fermentation in comparison with the control. The highest level of antioxidant capacity was observed in grape kombucha fermented for 48 h. Therefore, the optimum fermentation processing condition for grape kombucha was identified as 48 h at 37°C. Grape kombucha was produced for further development of a formulated grape kombucha beverage (see Figure 3).

The highest level of antioxidative activity of kombucha was demonstrated in green tea kombucha fermented at 25

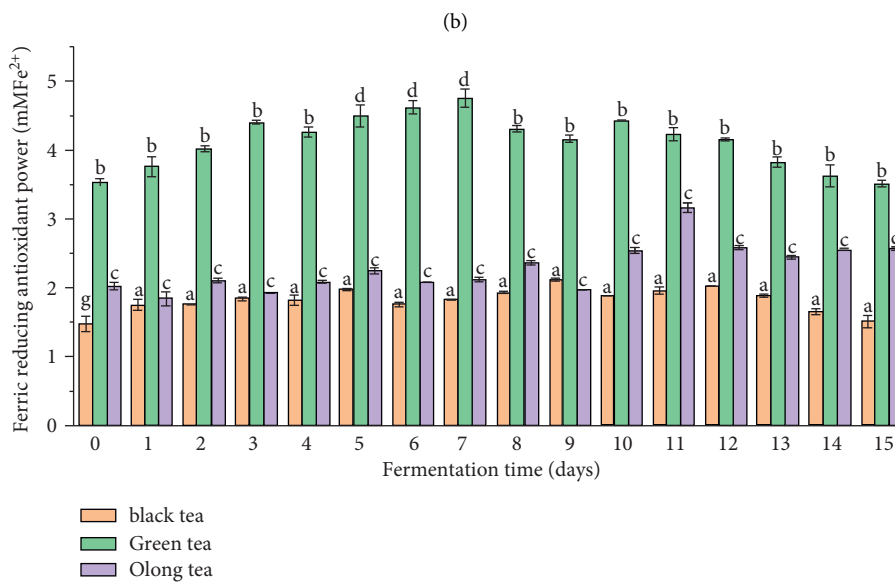
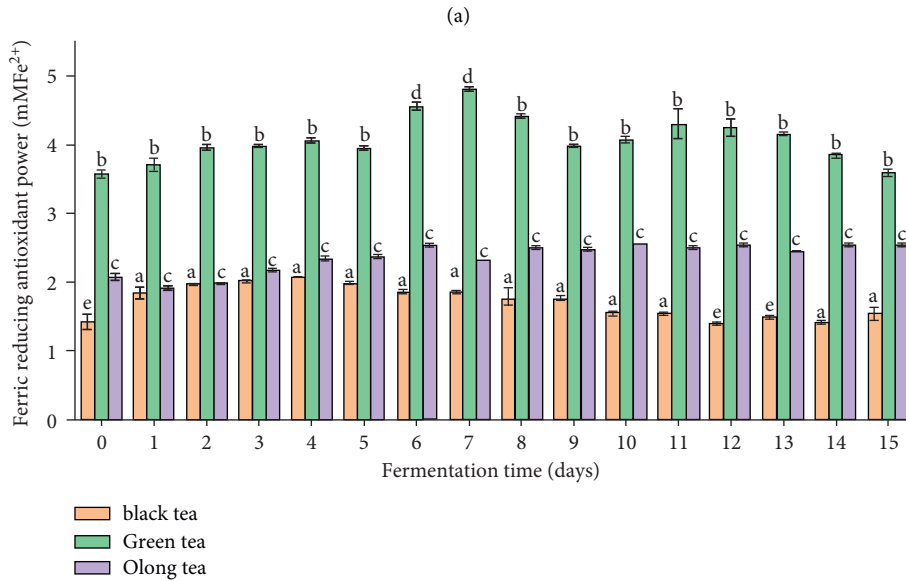
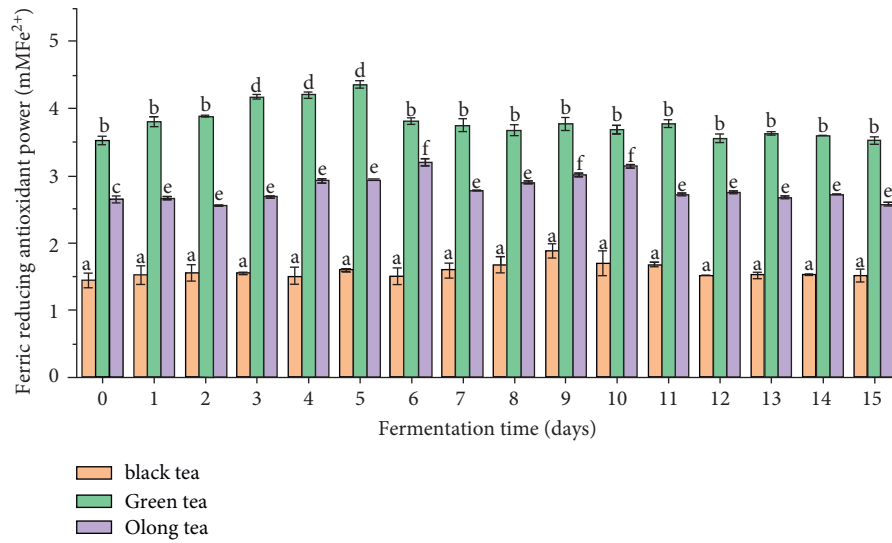
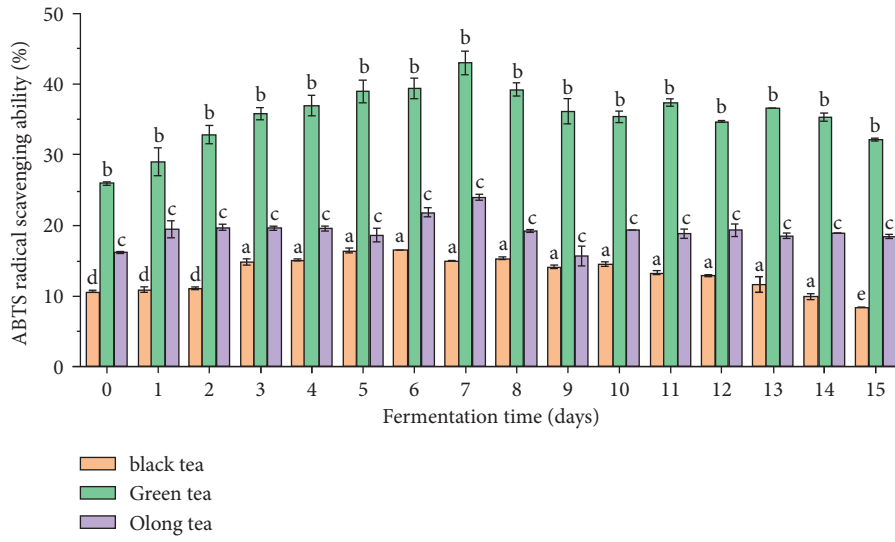
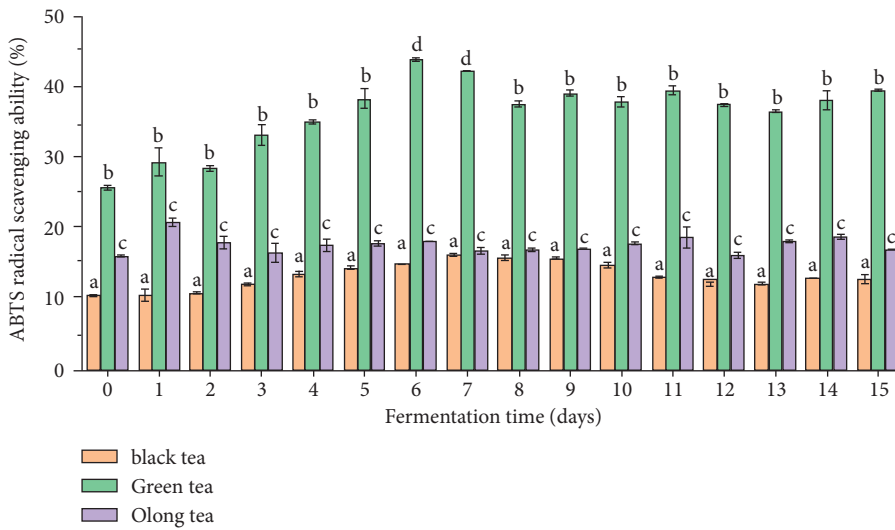


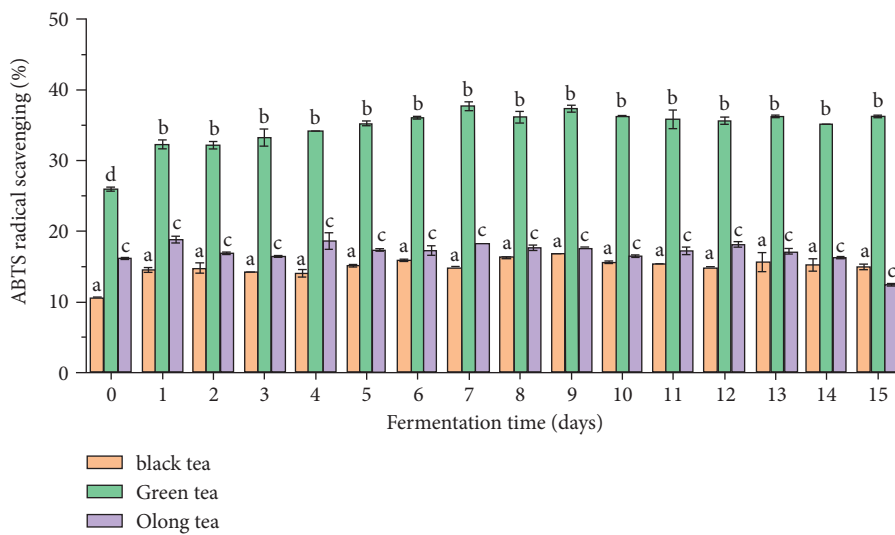
FIGURE 1: Changes in antioxidative activity of kombucha fermented from three different teas at different temperatures ((a) 35°C; (b) 30°C; (c) 25°C) measured by ferric reducing antioxidant power. Different letters in the same graph indicate significant difference ($P < 0.05$).



(a)



(b)



(c)

FIGURE 2: Changes in antioxidative activity of kombuchas fermented from three different teas at different temperatures ((a) 35°C; (b) 30°C; (c) 25°C) measured by ABTS radical scavenging ability. Different letters in the same graph indicate significant difference ($P < 0.05$).

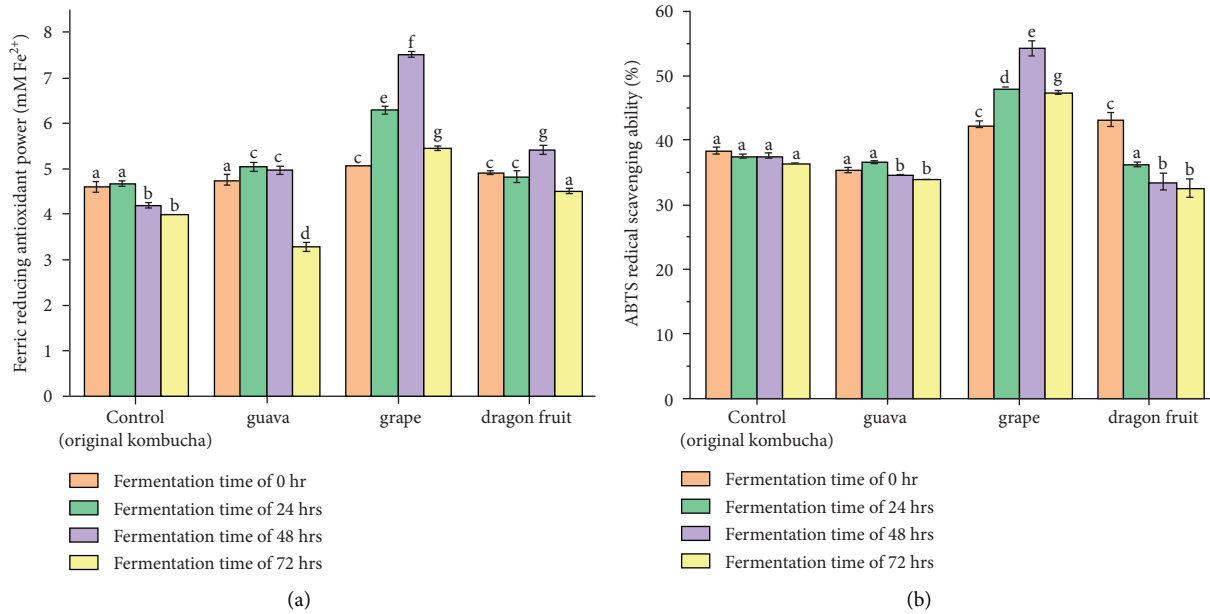


FIGURE 3: Antioxidative activity of original green tea kombucha and three fruit kombuchas fermented for different durations measured by ferric reducing antioxidant power (a) and ABTS radical scavenging ability (b). Different letters in the same graph indicate significant difference ($P < 0.05$).

and 30°C for 7 days (4.6 mM Fe²⁺) (Figure 1) and 45% after fermentation at 30°C for 6 days (Figure 2). Previous studies also reported that the optimum temperatures for original kombucha fermentation are 25 and 30°C [16]. A recent study suggested that kombucha fermentation was 28°C [17]. Furthermore, an investigation proved that the optimum temperature for fermentation of black tea kombucha was 28 ± 2°C. It can be seen in Figures 1 and 2, initially, that the antioxidative activity of green tea kombucha sharply increased up to 7 days of fermentation. However, after 7 days, the antioxidative activity of green tea kombucha either declined (Figure 1) or stagnated (Figure 2). The same trend has also been reported by Jakubczyk [10]; the author stated that the antioxidative activity of green tea and white tea kombucha during fermentation was highest at 7 days. Therefore, the optimal fermentation temperature and time were 25/30°C and 6 to 7 days, respectively. The green tea kombucha was made under this optimal condition and used as a starter culture to produce fruit-flavoured kombuchas.

3.2. Total Polyphenol Content and Flavonoid Content. The total polyphenol content in the original kombucha and grape kombucha processed under optimized conditions is shown in Figure 4. Green tea without fermentation (the green tea sample with a fermentation time of 0 days in Figures 1 and 2) served as the control sample as shown in Figure 4. The active antioxidants in plant-based products are exhibited mainly due to polyphenols. The mechanisms involved in the antioxidant activity of polyphenols include the suppression of reactive oxygen species (ROS) formation by either inhibition of enzymes involved in their production or upregulation of antioxidant defenses [18]. The content of polyphenols and the antioxidant properties were determined in fermented

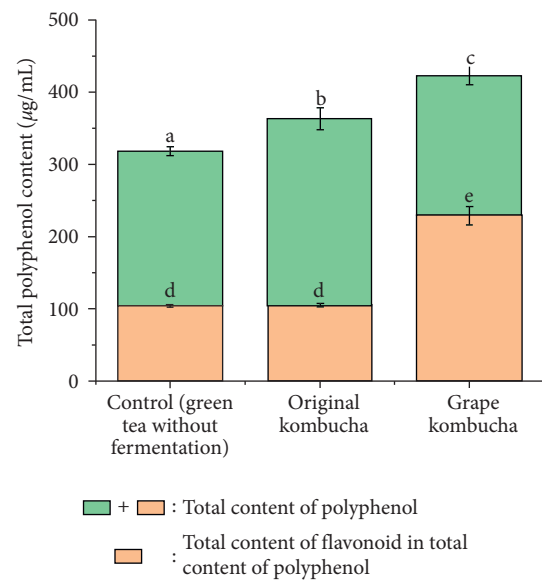


FIGURE 4: Total polyphenol and flavonoid in green tea without fermentation (control), original green tea kombucha, and grape kombucha. Different letters indicate significant difference ($P < 0.05$).

soybean and tempeh. The highest concentration (5.307 mg/g) of polyphenols was observed in soybean fermented for 4 days, and the strength of antioxidant activity was also the highest and exhibited 12 times higher than in unfermented material after fermentation for 4 days [19].

The same correlation has also been reported in fermented cornelian cherry beer, Joanna et al. [10] and *Asparagus cochinchinensis* root [20]. Wu et al. [18] compared polyphenol-rich cranberry fruit extract and non-

polyphenol-rich cranberry fruit extracts and revealed that the former offered an efficacious and safe means to prevent colonic tumorigenesis in humans due to its greater antioxidative activity attributed to polyphenols. Hence, green tea was identified as the optimal type of tea for original kombucha production.

Our findings also suggested that increasing polyphenolic contents lead to an increase in the antioxidative activity of kombucha drinks, as shown in Figures 1, 2, and 4. The polyphenol concentration increases from 318.21 to 362.89 $\mu\text{m}/\text{mL}$ in green tea before and after fermentation (kombucha). A similar result was reported by Hassoun et al. [21]; the author stated that the antioxidants found in green tea powder exhibited higher antioxidant activity followed by rosemary and mate at a given concentration due to the higher content of polyphenols in the green tea. Green tea contained approximately 30% of polyphenols on the dry weight basis. However, the oolong and black tea both contain only 5% of polyphenols on a dry weight basis [21]. This number was further elevated to 428.68 $\mu\text{m}/\text{mL}$ in grape kombucha. Among the different categories of polyphenols, flavonoids have been reported as an undisputedly superb antioxidant effect. It exhibits anti-inflammatory properties and supports the immune system [22]. It can be seen from Figure 4 that the increasing polyphenols from original kombucha to grape kombucha is due to the increase in flavonoids.

3.3. Polyphenolic Compositions of Green Tea, Original Kombucha, and Grape Kombucha. The polyphenolic compositions of green tea without fermentation (control), original kombucha, and grape kombucha are shown in Table 1. Major differences in the polyphenolic profiles of the original kombucha are the reductions in epicatechin gallate and epigallocatechin gallate and increasing epicatechin and epigallocatechin compared to control. It has been reported that the biotransformation of epicatechin gallate and epigallocatechin gallate to epicatechin and epigallocatechin by enzymes excreted by start culture during fermentation is the reason for the increasing content of epicatechin and epigallocatechin in original kombucha [10]. There are some flavonoid compounds which exist in grape kombucha, such as quercetin, narigenin, and hesperetin. This explains the increased contents of flavonoids and polyphenols in grape kombucha as shown in Figure 4. It has been reported that quercetin, narigenin, and hesperetin uniquely exist in grapefruit [23]. Many studies have proven that most of the flavonoid compounds that exist in plants are combined with protein, fat, and insoluble fibers. Fermentation induces structural changes in the plant cells which causes a reaction between enzymes secreted from microorganisms in the start culture and the complex combined with flavonoids and other materials. This reaction facilitates the release of flavonoids from the plant to the fermented grape kombucha.

3.4. Analysis of the Formulated Grape Kombucha Beverage. Among all tea and fruits, the grape kombucha was selected to produce at industrial scale for the consumers' after optimizing the processing conditions and due to the best

physiochemical properties shown after analysis and short fermentation period. The sparkling water was applied in the final grape kombucha beverage to achieve a similar mouth feel of kombucha beverages available in the market. The final formulation was fixed as 40% (w/w) grape kombucha blended with 55% (w/w) sparkling water and 5% (w/w) sugar. The antioxidative activity of the grape kombucha formulated in this study was compared with grape kombucha flavor that currently exists in the market (Table 2). Regardless of the measurement methods, the former showed superior antioxidative activity. This finding concludes that the grape kombucha formulated in this study would provide more benefits to human health.

3.5. Food Safety Tests for the Formulated Grape Kombucha Beverage. Trace element content and microbiology testing were carried out for the formulated grape's kombucha beverage prior to the sensory test. Table 3 presents the acceptable standards for the trace element of the formulated grape kombucha beverage according to Australia/New Zealand Food Standard 1.4.1. Human body needs to obtain a sufficient number of essential elements, such as sodium and calcium to maintain adequate physiological functions. However, humans might be exposed to health risks from harmful nonessential elements by drinking water and consuming fresh and processed foods [24]. For instance, skin cancer and organ cancers can be initiated by excess arsenic intake. In addition, excess cadmium intake can affect renal tubule function, and reabsorption of proteins, sugars, and amino acids leads to irreversible impairment. Having an excessive amount of trace elements in food may lead to serious health damage; however, as shown in Table 3, all trace element contents in the formulated grape kombucha beverage were lower than the standard levels.

The microbial analysis of grape kombucha is shown in Table 4. These can be compared with the reference standards obtained from Australia/New Zealand Food Standard 1.3.4 in Table 4 as well. The comparative reference standards for total viable counts are indicated in the guidelines for the microbiological examination of ready-to-eat foods for *Escherichia coli*, *Salmonella*, *Enterobacteriaceae*, yeast, and molds. It was found that the overall formulated grape kombucha beverage is fit for consumption by considering all food safety standards. Food safety is the paramount component of any food product. Therefore, it is necessary to carry out food safety tests for the formulated grape kombucha beverage prior to its sensory evaluation.

3.6. Sensory Evaluation of the Formulated Grape Kombucha Beverage. The sensory evaluation of grapes kombucha was performed by choosing two groups of panels. The sensory evaluation by these two groups revealed that the acceptance of the formulated grape kombucha beverage was susceptible for the young age group (<30) as the average score can be seen in Figure 5. There was a significant difference regarding the acceptance of the formulated grape kombucha beverage between different age groups. Although, the acceptance of the color was similar between the two age groups. However, the

TABLE 1: The concentration of monomeric phenolic compounds determined in green tea without fermentation (control), original green tea kombucha, and grape kombucha.

Phenolic compounds	Concentration ($\mu\text{g/mL}$)*		
	Green tea without fermentation (control)	Original green tea kombucha	Grape kombucha
Acid compounds			
Gallic acid	34.12 ^a \pm 0.02	48.13 ^b \pm 0.01	44.33 ^c \pm 0.01
Isoferulic acid	5.96 ^a \pm 0.01	5.24 ^b \pm 0.01	6.83 ^c \pm 0.01
<i>p</i> -coumaric acid	5.36 ^a \pm 0.03	9.63 ^b \pm 0.02	11.69 ^c \pm 0.02
Caffeine	86.63 ^a \pm 1.23	92.32 ^b \pm 0.23	82.33 ^c \pm 0.01
Flavonoid compounds			
Epicatechin gallate	28.08 ^a \pm 0.03	17.50 ^b \pm 0.03	20.80 ^c \pm 0.01
Gallocatechin gallate	5.23 ^a \pm 0.02	6.58 ^b \pm 0.02	4.50 ^c \pm 0.01
Epigallocatechin gallate	51.19 ^a \pm 0.36	40.39 ^b \pm 0.23	38.71 ^c \pm 0.52
Catechin gallate	6.96 ^a \pm 0.01	4.41 ^b \pm 0.01	6.17 ^c \pm 0.01
Gallocatechin	6.23 ^a \pm 0.02	4.86 ^b \pm 0.02	3.88 \pm 0.02
Epigallocatechin	4.96 ^a \pm 0.03	13.61 ^b \pm 0.04	15.97 ^c \pm 0.02
Catechin	3.01 ^a \pm 0.01	5.07 ^b \pm 0.01	3.66 ^c \pm 0.01
Epicatechin	2.56 ^a \pm 0.01	9.61 ^b \pm 0.01	7.56 ^c \pm 0.01
Rutin	1.98 ^a \pm 0.02	2.68 ^b \pm 0.02	4.88 ^c \pm 0.02
Quercetin	Nd	Nd	2.34 ^c \pm 0.01
Naringenin	Nd	Nd	26.92 ^c \pm 0.23
Hesperetin	Nd	Nd	3.03 ^c \pm 0.02

nd: not detected; *: mean \pm standard deviation ($n = 3$); different letters in each row indicate significant difference ($P < 0.05$) according to one-way ANOVA and LSD test.

TABLE 2: Comparison of antioxidative activity between grape kombucha in this study and kombucha with grape flavor in market.

	Antioxidative activity*	
	FRAP (mM Fe ²⁺)	ABTS radical scavenging ability (%)
Grape kombucha in this study	1.53 ^a \pm 0.24	23.68 ^d \pm 2.25
Kombucha with grapeflavor in market	0.52 ^b \pm 0.31	12.54 ^c \pm 2.51

*: mean \pm standard deviation ($n = 3$); different letters in each column indicate significant difference ($P < 0.05$), according to one-way ANOVA and LSD test.

TABLE 3: Trace elements determined in grape kombucha.

Trace elements	Sample	Content (mg/L)
		Reference (Australia New Zealand Food Standard 1.4.1)
Arsenic	0.58	2
Cadmium	<0.01	0.05
Lead	0.004	0.5
Mercury	0.033	0.5
Tin	0.24	250

TABLE 4: Microbiological test (cfu/ml) of grape kombucha.

Microbes	Sample	Cell counts
		Reference (Australia New Zealand Food Standard 1.3.4)
Total viable count (g)	230	<10 ⁴
<i>E. coli</i> (mL)	Not detected	<3
<i>Salmonella</i> (25 mL)	Not detected	Not detected
<i>Enterobacteriaceae</i> (g)	<10	<10 ²
Yeast (g)	<10	<10
Mould (g)	<10	<10

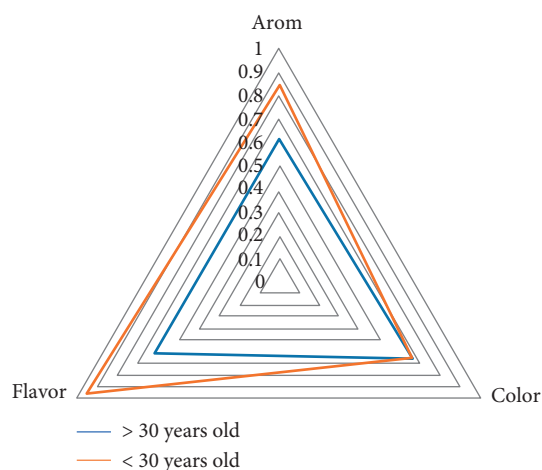


FIGURE 5: Sensory evaluation of grape kombucha by two age groups of participants.

higher acceptance of grape kombucha was noticed among young age group volunteers (<30 years) based on aroma and flavor. It was reported that kombucha is more favorable for the younger generation. This sensory evaluation further confirmed that the market promotion of the formulated grape kombucha beverage developed in this study should be focused on new generations, such as millennials.

4. Conclusion

A comprehensive analysis of antioxidative activities and phenolic composition and sensory analysis in kombucha beverages fermented from different teas and fruits have been carried out in this study. The processing conditions to produce tea and fruit kombucha are optimized. It was found that the best processing and fermentation conditions for the production of green tea kombucha would be at 25–30°C for 6–7 days. Furthermore, the investigation stated that the fermentation at 37°C for 48 h would be the more reliable processing condition for the development of grape kombucha with better physicochemical properties such high antioxidant, phenolic compounds, and texture which may help to fulfil the nutritional requirement for the consumers. The antioxidative activity of grape kombucha was significantly improved in comparison with the original green kombucha due to the increase in the polyphenol contents. After the comparative physicochemical analysis of kombucha from green, black, and oolong tea along with dragon and guava fruit, the formulated grape kombucha beverage was selected for industrial scale production. The sensory test of the formulated grape kombucha suggested that people at the age of <30 would be the most preferable consumers in the market. This study has found a novel channel to produce value-added grapes' kombucha drinks in a short fermentation period for consumers with improved nutritional quality at an industrial scale.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All the authors have read and approved the final manuscript. Siying Li and Yang Zhang contributed equally to this work.

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