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FULL PAPER

Physicochemical and microbiological characteristics of fruit-based kombucha

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Abstract

Kombucha is a fermented drink made out of tea leaves, although recently other alternatives were considered as substitutes, such as fruits. Using different types of fruit could affect the characteristics of fruit-based kombucha. This study investigated the physicochemical and microbiological characteristics of fruit-based kombucha through a Randomized Block Design with the type of fruit (red dragon fruit, apple, snake fruit, strawberries, grapes, pear, red guava, and citrus) as the factor. The produced kombucha drinks were analyzed and compared for pH, total sugar, total phenolic compounds, total flavonoids, antioxidant activity, and total microbes. Statistical tests such as Analysis of Variance (ANOVA) and Least Significance Different (LSD) were employed ($\alpha = 5\%$). Results revealed that physicochemical and microbiological characteristics of kombucha were significantly related to the type of fruit with snake fruit kombucha showing the optimal results of each characteristics as follows: total acetic acid bacteria and yeasts of 1.53×10^9 CFU/mL, pH of 3.07, total sugar of 2.41%, total phenol of 1006.85 μg/mL GAE, total flavonoids of 1.75 mg QE/mL, and IC_{50} of DPPH scavenging activity of 5.46 μg/mL. Fermented fruit-based kombuchas are regarded as healthy substitutes to traditional kombucha as they offer rich source of nutrients that enhance human well-being.

1. Introduction

Kombucha is one of many traditional healthy drinks from China, its produced by fermenting tea with starter kombucha called SCOBY (Symbiotic Culture of Bacteria and Yeast) (Naland, 2008). The antioxidant properties of kombucha comes from organic acid, such as lactic acid, acetic acid, ascorbic acid, and gallic acid. Kombucha also contains polyphenol compounds that act as antioxidant such as flavonoids and tannins. Furthermore, the antimicrobial properties of kombucha improve the microflora in the intestine. In general, kombucha is made from tea, but recently fruit-based kombuchas have begun to be developed. Using fruits as the raw material of kombucha would produce kombucha with various functional benefits and appeal to the market.

Indonesia is a country with an abundance of fruits which can be employed to formulate beneficial functional kombucha drinks, including dragon fruit, apple, snake fruit, strawberry, grape, pear, guava, and citrus. Dragon fruit is rich in vitamins e.g., thiamine, riboflavin, niacin, carotene, and ascorbic acid which play a role as antioxidants (USDA, 2017). Rome beauty apple (*Malus sylvestris Mill*) is a fruit with high phenolic compounds, e.g., quercetin, glycosidic, procyanidin B2, chlorogenic acid, and epicatechin (Kurniawan, 2014). Suwaru snake fruit is rich in flavonoid, alkaloid, terpenoid, catechin, tannin, quinone, and ascorbic acid (Priyatno, 2006). Strawberry is cultivated worldwide and is known for its high content of vitamin C. Also, it is high in phytochemical compounds such as anthocyanin, flavanol, catechin, and epicatechin (Seeram, 2007). Grapefruit is high in vitamin C, K, A, and flavonoid compounds (Tepe and Hoover, 2015). Pear contains chlorogenic acid which is a hydroxyl cinnamic acid derivative that can prevent the formation of cancer cells (Gui *et al.,* 2016). Guava contains high phenolic and flavonoid compounds such as quercetin, guaijaverin, galic acid, leucocyanidin, and elagat acid (Javed *et al.,* 2017). Citrus contains D-limonene that prevents skin cancer, β-cryptoxanthin that improves respiratory system, and many other flavonoids and phytochemicals

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(Makni *et al.,* 2018).

As the raw material of kombucha affects the fermentation products such as organic acid, phenol, and flavonoid, using different types of fruit as raw material can affect the characteristics of fruit-based kombucha. Therefore, this study was aimed to investigate the physicochemical and microbiological characteristics of fruit-based kombucha.

2. Materials and methods

2.1 Materials

Red dragon fruit (*Hylocereus* sp.) was resourced from a local store in Malang. Rome Beauty apple (*Malus sylvestris Mill*) and strawberry (*Fragiara x ananassa*) were collected from local farmer in Bumiaji, Batu. Snake fruit (*Sallaca zallaca*) was purchased from a local farmer in Suwaru, Pagelaran, Malang. Grape (*Vitis vinifera*), pear (*Pyrus communis*), guava (*Psidium guajava*), and citrus (*Citrus reticulate*) were bought from Blimbing Market in Malang. Kombucha (control) was made from "Naga" black tea. Sugar, commercial kombucha starter, and water were resourced from the University laboratories.

2.2 Preparation of fruit juice

The preparation of fruit juice was conducted according to Esti and Sediadi (2000) with minor modifications. The fruits were washed with clean water and drained. They were then peeled, weighed in 250 g, cut into small pieces, and blended for 30 mins (water: fruit = 1:1). After that, the fruit pulp was filtered and the fruit juice was analyzed as raw material.

2.3 Preparation of fruit-based kombucha

Fruit juice (400 mL) and 10% sugar were mixed and pasteurized (Waterbath Memmert, Germany) at 85°C for 15 mins, was let cool to 25±2°C, then poured into a sterile glass jar. After that, 10% of kombucha starter (SCOBY) was inoculated, covered with cloth, and incubated at 25±2°C for 14 days. At the end of the fermentation, the kombucha was filtered (using cheese cloth) to separate it from the SCOBY.

2.4 Determination of pH and total sugar

The pH of kombucha was measured with a pH meter (Hanna, Thermo Fischer Scientific, USA). Total sugar of kombucha analysis was conducted according to Apriyantono *et al.* (1989). Samples (1 mL) were transferred to test tubes and mixed with 5 mL of anthrone reagent (0.05 g of anthrone in 50 mL of concentrated H_2SO_4). The test tubes were covered with plastic, homogenized, and heated at 100°C for 12 mins. After the samples cooled, the absorbance was measured at 630 nm in a spectrophotometer (Unico, UV-2100 Spectrophotometer) with a glucose solution as the standard.

2.5 Determination of total phenolic compounds and total flavonoid

The total phenolic compounds were determined with Follin – Ciocalteau reagent (1:10) according to Prangdimurti (2009). Samples (0.5 mL) were placed into test tubes with 2.5 mL Follin-Ciocalteau reagent, vortexed, and were put aside for 5 mins. After that, 4 mL of Na_2CO_3 7.5% (w/v) (7.5 g Na_2CO_3 in 100 mL distilled water) was added. The mixture was vortexed and put aside for 1 hr in a dark place. Absorbance was measured at 765 nm with gallic acid as standard in Unico, UV-2100 Spectrophotometer. The total phenolic compounds were expressed as mg GAE/mL (Gallic Acid Equivalent/mL).

Total flavonoid was measured according to Atanassova *et al.* (2011) with slight modification. Samples (1 mL) were placed in test tubes with 4 mL of distilled water. Then, 0.3 mL $NaNO_2$ 5% was added and put aside for 5 mins, before 0.3 mL of $AlCl_3$ 10%, 2 mL of NaOH 1M, and 2.4 mL of distilled water were added. The mixtures were vortexed and put aside for 5 mins. Absorbance was measured at 510 nm with quercetin as the standard.

2.6 Determination of antioxidant activity

The antioxidant activity was measured with DPPH method referenced from Huang *et al.* (2005). Samples (1 mL) were dissolved with distilled water in 10 mL volumetric flask. There were four series of dilutions e.g. 2000, 1500, 1000, and 500 ppm from the main solution. Then, 1 mL of 0.2 mM DPPH was added, homogenized, and put aside for 30 mins. Absorbance was measured using a spectrophotometer (Unico, UV-2100 Spectrophotometer) at 517 nm.

2.7 Determination of total microbes

The determination of total microbes was done using the standard plate count procedure according to Fardiaz (1992). Samples (1 mL) were mixed with 9 mL of sterile 0.1% peptone water and further diluted to have a serial of decimal dilutions. Each dilution (1 mL) was transferred into petri dishes and poured with sterile plate count agar (50°C), mixed, and left to solidify before incubation at 37°C for 24 hrs. Colony counts were calculated with the following equation expressed in log CFU/mL: [total colony per mL = total colony×(1/diluted factor)].

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Table 1. pH of fruit-based kombucha during the fermentation process

Types of Fruit	pH				LSD 5%
	Day-0	Day-7	Day-14	Decrease (%)	
Apple	3.43	3.13	2.83	0.17	0.36
Black Tea	4.10	3.33	3.10	0.24	
Citrus	4.27	3.63	3.23	0.24	
Dragon Fruit	4.17	3.17	2.80	0.33	
Grape	4.07	2.80	2.77	0.32	
Guava	4.07	3.30	2.90	0.29	
Pear	4.50	3.20	3.07	0.32	
Snake Fruit	4.10	3.30	3.07	0.25	
Strawberry	3.33	3.00	2.93	0.12	

2.8 Statistical analysis

The data was analyzed using Minitab 17. ANOVA (Analysis of Variance) and LSD (Least Significant Difference) with α = 5% were used to differentiate between the mean values.

3. Results and discussion

3.1 pH of fruit-based kombucha

The pH of fruit-based kombucha ranged from 3.33 – 4.50 on day-0, 2.80 – 3.63 on day-7, and 2.77 – 3.23 on day-14. According to Table 1, the pH values gradually decreased from day-0 to day-14. Based on statistical analysis, there was no significant effect (α = 0.05) of different types of fruit on the kombucha pH average. The pH of fruit-based kombucha decreased during the fermentation process. The decrease of pH during fermentation may be caused by the accumulation of organic acids as a result of yeast and bacterial metabolism. During fermentation, the yeasts involved produce ethanol and carbon dioxide. Ethanol is oxidized by acetic acid bacteria to become acetaldehyde, then oxidized to acetic acid. Acetic acid bacteria also produce gallic acid during kombucha fermentation. In addition, there are also other organic acids such as malic acid, oxalic acid, and lactic acid (Júnior *et al*., 2009). On the last day of fermentation, the lowest pH of kombucha was recorded.

Table 2. Total sugar of fruit-based kombucha during the fermentation process

Types of Fruit	Total Sugar (%)				LSD 5%
	Day-0	Day-7	Day-14	Decrease (%)	
Apple	13.72	7.03	2.38	0.83	0.55
Black Tea	10.55	5.28	1.81	0.83	
Citrus	10.59	5.35	1.83	0.83	
Dragon Fruit	14.74	7.57	2.59	0.82	
Grape	13.82	6.95	2.23	0.84	
Guava	12.47	6.18	2.09	0.83	
Pear	13.66	6.81	2.31	0.83	
Snake Fruit	14.17	7.41	2.41	0.83	
Strawberry	11.42	5.74	1.99	0.83	

3.2 Total sugar of fruit-based kombucha

Based on analysis, total sugar of kombucha with different types of fruit ranged from 8.84% to 12.96% on day-0, 4.05% to 6.47% on day-7, and 2.43% to 3.86% on day-14. The total sugar of fruit-based kombucha was shown on Table 2. Based on LSD analysis, kombucha made from different types of fruit had no significant difference on total sugar. The decrease of total sugar in fruit-based kombucha may be caused by the synergistic metabolic activity of yeast and bacteria. In such metabolism, sugar was used as a substrate in the metabolic reaction. According to Napitupulu *et al.* (2015), the sugar contained in kombucha is generally sucrose sugar. Sucrose is converted into glucose and fructose with invertase enzyme. Glucose will then be used in the Kreb's Cycle.

3.3 Total phenol of fruit-based kombucha

The average of total phenol in fruit-based kombucha ranged from 82.61 µg/mL GAE to 895.03 µg/mL GAE on day-0, 98.97 µg/mL GAE to 954.73 µg/mL GAE on day-7, and 198.67 µg/mL GAE to 1006.85 µg/mL GAE on day-14. Figure 1 demonstrates an increase of total phenol in fruit-based kombucha from day-0 to day-14. Based on LSD analysis, different types of fruit resulted in significant differences on the total phenol of fruit-based kombucha. Figure 1 shows the increase of total phenol during the fermentation period. This occurred due to the activity of microorganisms that carry out fermentation. During fermentation, microorganisms produced enzymes that can break down phenolic compounds, causing a change in complex phenolic compounds into simpler phenolic compounds, thereby increasing the average total phenolic content. According to Suhardini and Zubaidah (2016), the increase in phenolic compounds during fermentation is caused by the involvement of various types of microbes, bacteria and yeasts that can be metabolized to produce phenolic compounds through enzymatic reactions. *Saccharomyces cerevisiae*, a common yeast found in kombucha, is able to produce β-glucosidase enzymes that can break down glycoside bonds so that phenolic compounds can be released into fermentation medium. *S. cerevisiae* is also able to synthesize free phenolic compounds, namely 4-ethylphenol and 4-ethylguaiacol derived from hydroxynamic acid and ferulic acid. *S. cerevisiae* is able to produce cinnamic decarboxylase and vinylphenol reductase enzymes. Cinnamic decarboxylase enzyme catalyzes the decarboxylation process of cinnamic acid and vinylphenol reductase enzyme catalyzes the reduction of 4-vinyl phenol and 4-vynilguaiacol into 4-ethyl phenol and 4-ethylguaiacol. However, the increase in phenolic compounds is not always significant.

Nonetheless, phenols which also act as an antimicrobial compounds can also cause a decrease in the activity of microorganisms in kombucha.

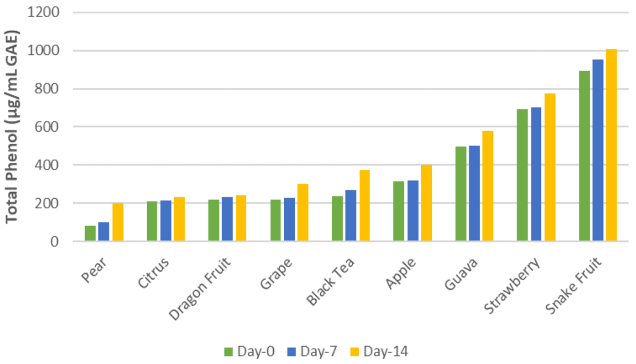

Figure 1. Total phenol of fruit-based kombucha during the fermentation process

Based on Figure 1, the kombucha with the highest total phenol on day-14 was snake fruit kombucha, while the one with the lowest total phenol was kombucha pear. The difference in total phenol between kombuchas was based on their phenolic compounds and the types of phenols they contained in each raw material (Essawet *et al.,* 2015). According to Jayabalan *et al.* (2007), the content of phenolic compounds is influenced by the flavonoid content, where flavonoid compounds are influenced by cultivation conditions and seasonality (the availability of sunlight, nutrients, pH and humidity).

3.5 Total flavonoid of fruit-based kombucha

The fermentation process from day-0 to day-14 indicated an increase of total flavonoid content in fruit-based kombucha. Based on Figure 2, the total flavonoids in fruit-based kombucha were 1.12 – 4.01 µg/mL GAE on day-0, 1.50 – 5.26 µg/mL GAE on day-7, and 1.75 – 6.05 µg/mL GAE on day-14. According to LSD statistical analysis, different types of fruits in kombucha had no significant difference on total flavonoid. Microorganisms produced enzymes that break down complex flavonoid into simple flavonoid compounds. According to Chu and Chen (2006), the total flavonoid compounds increase linearly with the length of fermentation time. Four types of catechins commonly found in kombucha such as epicatechin and epigallocatechin were converted into simpler ones by enzymes produced from kombucha fermentation. Yeast type *C. tropicalis* can degrade several types of polyphenols and flavonoids (Ettayebi *et al.,* 2003). While yeast *S. cerevisiae* has the ability to convert several types of organic acids into flavonoid compounds, such as cinnamic acid to pinocembrin, p-coumaric to naringenin, and caffeic acid to eriodictyol (Yan *et al.,* 2005).

Based on Figure 2, the highest mean of total flavonoid on day-14 was from the strawberry kombucha. Meanwhile, the one with the lowest average was snake fruit kombucha. The average total flavonoid was highest in strawberry kombucha while the lowest increase was in citrus kombucha. In Figure 2, strawberry had the highest total flavonoid while snake fruit had the lowest total flavonoid. The content of flavonoids in fruit was influenced by various factors such as growing conditions, sunlight for photosynthesis, nutrients, moisture and soil pH (Jayabalan *et al.,* 2007). According to Koopman *et al.* (2012), another factor that can affect the total flavonoids of kombucha is the sugar content in the raw material as the main carbon source in the formation of flavonoid compounds.

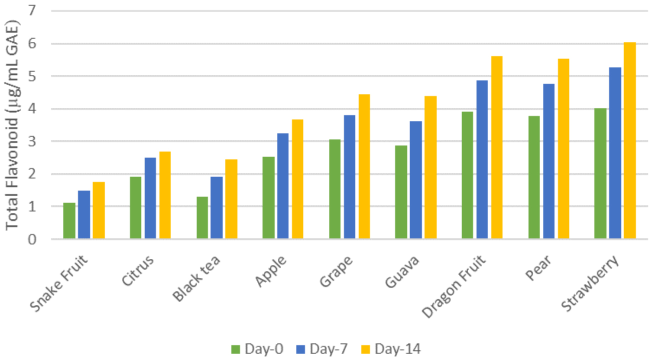

Figure 2. Total flavonoid of fruit-based kombucha during the fermentation process

3.6 Antioxidant IC_{50} of fruit-based kombucha

In this study, antioxidant activity was expressed using IC_{50} value. IC_{50} is the sample concentration which reduces the DPPH activity by 50%. The smaller the IC_{50} value, the higher the antioxidant activity. IC_{50} value of fruit-based kombucha ranged from 8.35 to 47.50 mg/mL on day-0, 6.11 to 34.76 mg/mL on day-7, and 5.46 to 31.04 mg/mL on day-14. The chart of IC_{50} value is shown in Figure 3. Figure 3 indicates the decrease of IC_{50} value of fruit-based kombucha. The antioxidant activity of kombucha was influenced by organic acids, total phenols, and total flavonoids it contained. In addition, the sugar content in kombucha as a source of nutrients for metabolism also affected antioxidant activity where the increased sugar would increase the in production of organic acids and enzymes that could degrade complex phenolic compounds into simple

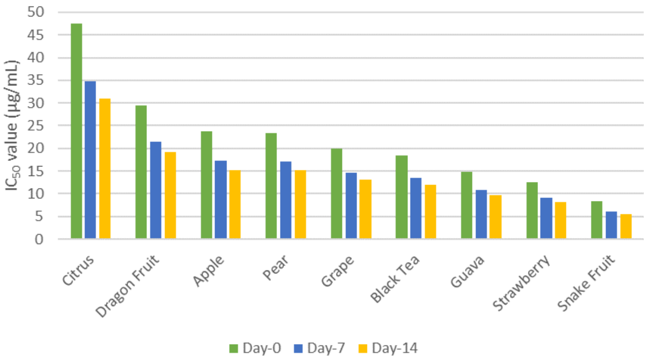

Figure 3. Antioxidant activity of fruit-based kombucha during the fermentation process

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phenolic compounds.

Apple kombucha had the highest decrease in IC_{50} value while snake fruit kombucha had the lowest decrease. The decrease of IC_{50} value was caused by an increase in phenolic compounds as antioxidants due to a biotransformation process that utilized certain enzymes in a plant cell to change the functional group of a chemical compound found in plants (Jayabalan *et al.,* 2008). Furthermore, the decrease of IC_{50} on day-7 was not significant. Based on Ayu *et al.* (2013), acidic conditions caused phenolic compounds to become more stable and difficult to release protons that can bind to DPPH.

3.7 Total microbes of fruit-based kombucha

The total microbes of fruit-based kombucha showed varied results. The microbes grown were most likely acetic acid bacteria and yeasts which are commonly found in kombucha. The average total microbes ranged from 5.6×10^{8} CFU/mL to 9.3×10^{10} CFU/mL on day-0, 2.0×10^{9} CFU/mL to 1.4×10^{11} CFU/mL on day-7, and 3.1×10^{8} CFU/mL to 2.2×10^{10} CFU/mL. There was a gradual increase of total microbes from day-0 to day-7 and a decrease of total microbes from day-7 to day-14 as shown on Figure 3. The difference in the total microbes between types of kombucha was influenced by the characteristics of the fruit. The average of total microbes is shown on Table 3. During the first week of fermentation, microbe cells would multiply first and cause an increase of total microbes. The sucrose in kombucha was then converted to glucose and fructose. The glucose broke down to organic acids and energy. The organic acid induced the optimum environment for acetic acid bacteria growth with pH around 5.0 – 6.5 up to 3 – 4. Data presented in Table 3 showed that apple kombucha has the highest level of total microbes on day-14 and citrus kombucha has the lowest value. Lončar *et al.* (2014) reported that sugar is a convenient substrate for bacteria metabolism. Organic acids and energy were the results of metabolism and were used for cell multiplication so that the higher the sugar concentration added, the higher the total microbes obtained. According to Caldwell (2000), excessive amounts of nutrients can actually have an impact on the accumulation of inhibitory compounds. The difference in total microbes between fruit-based kombucha was caused by various influencing factors such as the total sugar acts as a carbon source and energy provider, natural antioxidants in fruits, and the total content of phenolic compounds and flavonoids in kombucha.

4. Conclusion

Based on the findings, different types of fruit had no significant difference on pH, total sugar, total flavonoid in fruit-based kombucha. In contrast, different types of fruit significantly affected the total phenol and antioxidant activity. Snake fruit kombucha showed the optimal characteristics as a probiotic drink and a substitute of tea kombucha as follows: total microbes of 1.53×10^{9} CFU/mL, the pH of 3.07, total sugar of 2.41%, total phenol of 1006.85 μg/mL GAE, total flavonoids of 1.75 mg QE/mL, and IC_{50} of DPPH scavenging activity of 5.46 μg/mL.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 3. Total microbes of fruit-based kombucha during the fermentation process

Types of Fruit	Total Microbes (CFU/mL)			
	Day-0	Day-7	Day-14	Difference
Apple	9.29×10^{10}	1.20×10^{11}	2.17×10^{10}	-7.12×10^{10}
Black Tea	4.74×10^{9}	1.41×10^{11}	2.23×10^{9}	-2.51×10^{9}
Citrus	9.26×10^{8}	7.83×10^{9}	3.13×10^{8}	-6.13×10^{8}
Dragon Fruit	9.16×10^{9}	5.97×10^{10}	7.3×10^{8}	-8.43×10^{9}
Grape	1.26×10^{9}	3.59×10^{10}	4.46×10^{9}	3.20×10^{9}
Guava	5.56×10^{8}	2.01×10^{9}	4.86×10^{8}	-7.00×10^{7}
Pear	1.22×10^{10}	6.49×10^{9}	2.5×10^{9}	-9.70×10^{9}
Snake Fruit	7.3×10^{8}	6.53×10^{10}	1.53×10^{9}	8.00×10^{8}
Strawberry	3.5×10^{8}	7.15×10^{10}	2.43×10^{9}	2.08×10^{9}

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