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Chemical and microbiological characteristics of kombucha made from robusta cascara and green tea

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ABSTRACT

Dried coffee peel or cascara as coffee waste is a potential ingredient that still contains phytochemical substances such as polyphenol and clorogenic acid. Cascara can be used in making kombucha. Kombucha is a functional drink made of fermenting tea and sugar with using symbiotic culture of bacteria and yeast (SCOBY) as starter. The addition of cascara and combination with green tea could potentially improve nutritional values on kombucha. This study aimed to evaluate the chemical and microbiological characteristics of kombucha made from robusta cascara and green tea. This study used Completely Randomized Design with one treatment factor namely combination of robusta cascara and green tea (100%:0%; 75%:25%; 50%:50%; 25%:75%; 0%:100%). The treatment repeated three times. The parameters observed were total polyphenol content, total titrated acid, total dissolved solid, pH, and total lactic acid bacteria. The result showed that combination of robusta cascara and green tea had significant effects on all parameters. The increased of green tea proportion in treatments could increase content of total titrated acid from 0.0032% (kombucha with 100% robusta cascara) to 0.0048% (kombucha with 100% green tea) whereas total dissolved solid from 10.0667% Brix to 9.1333% Brix and pH values from 3.6000 to 3.4667 slightly decreased in kombucha. The higher polyphenol content in the raw material the higher total polyphenol obtained in kombucha reflected on 100% green tea kombucha with 0.2245 mg GAE/mL. Total lactic acid bacteria of kombucha obtained at 3.3760 log CFU/mL to 4.3917 log CFU/mL.

Key words: Coffee peel; Fermentation; Total Poliphenol; Total Lactic Acid Bacteria.

1 INTRODUCTION

Aside from the increased of production because of high demand for the past few decades, coffee industry in fact still generates waste such as pulp or coffee peel, husk, and silver skin. Mesocarp or coffee peel has become the biggest numbers of coffee waste with around 29.9% from 40-45% of total waste produced (Muzaifa et al., 2022). This coffee peel actually contained numerous nutrients namely protein (5.2%), carbohydrate (35%), fiber (30.8%), mineral (10.7%), water (84.2%), and sugar (4.1%) (Muzaifa; Rohaya; Sofyan, 2021). In addition, it also has clorogenic acid, flavonol, anthocyanidin, catechin, rutin, tanin, and ferulic acid (Heeger et al., 2017) which are beneficial phytochemical subtances making coffee peel is a potential material to be processed.

According to Nurhayati, Yuwanti and Urbahillah (2020), coffee peel has mainly processed as plant fertilizer and animal feed. Furthermore, coffee peel can also be dried then consumed by brewing like tea which is known as cascara. It has slightly coffee aroma and taste fruity with notes from citrus and cherry, consider like watermelon and blackcurrant or strawberries and raisin. Cascara contained polyphenol compounds such as antioxidants (8.9 mmol/L) which can act as functional drink (Arpi et al., 2021). It also has caffeine content (226 mg/L) and phenolic compounds dominantly protocatechuic (85.0 mg/L) and clorogenic acid (69.6 mg/L) (Heeger et al., 2017).

Recently, the consumption of traditional fermented drink named kombucha has increased due to its multiple functional properties and easy way to make. Kombucha is a beverage with slightly sweet and acidic taste produced from fermentation of generally black tea or green tea with addition of sugar and a cellulose biofilm as starter contained symbiotic culture of bacteria and yeast (SCOBY) for 7-14 days (De Miranda et al., 2022). The bacteria contained in SCOBY is mainly acetic acid bacteria (AAB) such as *Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species; and lactic acid bacteria (LAB) from *Lactobacillus* and *Lactococcus* species while for the yeasts mostly are *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis.* This symbiotic culture is capable of inhibiting the contaminating microorganism's growth in kombucha medium (Villareal-Soto et al., 2018). Thus, kombucha fermentation was a combination of alcoholic, acetic, and lactic fermentation. Kombucha has wide variety of chemical composition including polyphenols; sugars such as sucrose, glucose, and fructose; organic acids mainly acetic, gluconic, glucoronic, and L-lactic acid; amino acids and proteins; lipids; essential minerals; vitamins B1, B2, B6, B12, and C; ethanol; antibiotic substances; and enzymes (De Miranda et al., 2022).

The use of tea in kombucha making has been done widely with black tea or green tea. Both come from the same species of plant, *C. sinensis*, native to Southeast Asia, but with different type of manufacturing process. Green tea preserves more polyphenol content with a higher concentration of catechins produced from the fresh leaves as compared to black tea (De Miranda et al., 2022). In the search for diversified

substrates and novel experiences for the consumer, there are various studies with other materials as substrates to produce kombucha. According to Trihaditia, Yuliani and Priambodo (2021), kombucha with robusta cascara as substrate fermented for 6 days is reported to have pleasant sensory profiles (color, taste, aroma, and viscocity) compared to arabica cascara kombucha. Furthermore, the amount of total polyphenol in robusta cascara kombucha is higher than kombucha with arabica cascara due to high content of total polyphenol in robusta, especially tanin (Anjliany; Syafutri; Widowati, 2022).

Using robusta cascara and green tea as ingredients to make kombucha is expected to give higher nutritional values from the original kombucha made of tea. It was also expected as diversified product of kombucha and to reduce the coffee peel waste. Thus, the aim of this study was to evaluate the chemical and microbiological characteristics of kombucha resulted from combination of robusta cascara and green tea.

2 MATERIAL AND METHOD

2.1 Material

Robusta coffee peel and green tea used in this research were from Pagaralam, South Sumatera. Kombucha starter (SCOBY) were purchased from e-commerce Shopee and sugar were purchased from Indomart. Chemical ingredients for analysis such as follin chiocalteu reagent (FCR) (Merck, Germany); sodium carbonate 7% (Merck Millipore, USA); sodium hydroxide 0,1N (Merck Millipore, USA); phenolphtalein (Fisher, Singapore); gallic acid (Merck Millipore, USA); and MRSA media (Merck, Germany) were purchased from Agricultural Product Technology Laboratory, Indralaya, South Sumatera.

2.2 Methods

This research used Completely Randomized Non Factorial Design with treatment factor is different combination of robusta cascara and green tea (%). The treatment repeated three times. The treatment used in this research were shown in Table 1.

Table 1: Percentage and Weight of Robusta Cascara and Green Tea Mix.

Treatment	Percentage (%) (Robusta Cascara : Green Tea)	Weight (g) (Robusta Cascara : Green Tea)
A1	(100 : 0)	(4 : 0)
A2	(75 : 25)	(3 : 1)
A3	(50 : 50)	(2 : 2)
A4	(25 : 75)	(1 : 3)
A5	(0 : 100)	(0 : 4)

Parameters observed were chemical characteristics (total polyphenol content, total titrated acid, pH, and total dissolved solid) and microbiological characteristic (total lactic acid bacteria). In this study, the treatments before fermentation were also analyzed for total titrated acid, pH, and total dissolved solid to make a comparison with data after fermentation. In addition, the pellicle and liquid of kombucha starter (SCOBY) were also analyzed for total lactic acid bacteria.

2.2.1 Kombucha Preparation

Robusta cascara and green tea mix according to the treatments (Table 1) was infused into 500 mL of hot water for 10 minutes then filtered with 80 mesh of filter. Sugar as much as 50 g was added to robusta cascara and green tea solution and stirred thoroughly then cooled down to <38°C. The cascara and tea solution was transfered into a sterile glass jar and added 25 g (5% w/v) of pellicle kombucha starter (SCOBY) and 25 mL (5% v/v) of liquid SCOBY. The jar was covered with tissue and fermented indoor (temperature range 20-25°C) and not exposed to direct sunlight for 8 days. After fermentation completed, the kombucha was separated from the solid SCOBY. The liquid kombucha was kept in refrigerator at 4°C then analyzed (Sari; Irdawati, 2019).

2.2.2 Analysis of Total Polyphenol Content

The analysis of total polyphenol content were using the Folin-Ciocalteu method as described in Kuru et al. (2017). A total of 0.1 mL of kombucha sample was put into a test tube and 4.9 mL of distilled water was added. A total of 0.5 mL of Follin-Ciocalteu reagent was added into the test tube and vortexed then allowed to stand for 5 minutes. A total of 1 mL of 7% Na_2CO_3 solution was added and vortexed so that the solution was homogeneous. The test tube was left in a dark place for 60 minutes, then inserted into an UV-Vis sphectrophotometer using 765 nm wavelength. Measurement of the blank solution was carried out in the same way but the sample was replaced using distilled water in the same amount. Total polyphenols were calculated using standard curves made from gallic acid at various concentrations.

2.2.3 Analysis of Total Titrated Acid

Total titrated acid was tested using the principle of acid titration by base referring to Rosyada, Agustina, and Faizah (2023) with slight modification. A total of 10 mL of kombucha was taken and put into a 100 mL volumetric flask. Added distilled water up to the mark and then homogenized. Took 50 mL and put it in an Erlenmeyer then added 3 drops of phenolphthalein indicator (pp). Titrated with 0.1 N NaOH solution until a pink color was formed. Scale reading at the time the first red color was formed and lasts for 15 seconds. Total acetic acid content (%) was measured with following equation 1.

$$\text{Total Acid (\%)} = \frac{\text{V NaOH} \times \text{N NaOH} \times \text{MW} \times 100\%}{\text{V sample} \times 1000} \quad (1)$$

V NaOH is volume NaOH for titration; N NaOH is NaOH standart concentration; V sample is volume of the sample for titration; and MW is molecular weight of acetic acid.

2.2.4 Analysis of Total Dissolved Solid

The refractometer was prepared. The prism glass cover was opened and then 1-2 drops of sample were placed on it. The prism glass cover closed slowly. The refractometer was directed at a bright light and then the scale reading was seen through the binoculars. If the scale was blurred, the binoculars were rotated until the scale reading was clear. Total dissolved solids were expressed in °Brix (Official Methods of Analysis of AOAC International - AOAC, 2005).

2.2.5 Analysis of pH

A sample of 5 mL was prepared. The pH meter was calibrated using pH 4 and 7 buffers. The cathode was inserted into the sample and left until the number indicated on the digital measurement no longer changed. Each time the measurement, the cathode of the pH meter was rinsed with distilled water and then dried before being used again (AOAC, 2005).

2.2.6 Analysis of Total Lactic Acid Bacteria

Analysis of total lactic acid bacteria in this study used method from Zubaidah, Fibrianto and Kartikaputri (2021) with slightly modification. A sample of 1 mL fermented kombucha was taken and diluted with 9 mL of distilled water until 10^{-3} dilution. A total of 1 mL of 10^{-2} and 10^{-3} dilution was poured into Petri dish continued with MRSA in duplex. The Petri dish was incubated for 72 hours with temperature 37°C. After 72 hours, the formed colonies were counted. The amount of total colonies that is taken as data must be under 300 colonies.

2.3 Data Analysis

The data obtained were then processed using ANOVA. The treatment that had a significant difference was further tested using Honestly Significant Difference test at 5% level.

3 RESULTS

Analysis of kombucha using combination of robusta cascara and green tea revealed that the highest values of total polyphenol content were found in kombucha with 0% robusta cascara and 100% green tea (A5), while the rest of treatments were not significantly different. It should be noted that for total polyphenol content in raw materials before fermentation was not studied. The content of total titrated acid before fermentation ranged from 0.0001% to 0.0016% and increased after fermentation ranged from 0.0032% to 0.0048%. The highest values of total titrated acid were reported in kombucha with 0% robusta cascara and 100% green tea (A5) and the lowest were found in kombucha with 100% robusta cascara and 0% green tea (A1).

Meanwhile, the content of total dissolved solid before fermentation ranging from 10.8000% Brix to 12.0667% Brix decreased to 9.133-10.067% Brix after fermentation. The total dissolved solid values in kombucha with 75% robusta cascara and 25% green tea (A2) after fementation were significantly lower and different than other treatments. The pH values were also decreased from average of 6 before fermentation to average of 3 afterward. The lowest content of pH were observed in kombucha with 0% robusta cascara and 100% green tea (A5), while the highest were found in kombucha with 75% robusta cascara and 25% green tea (A2) and kombucha with 50% robusta cascara and 50% green tea (A3). The content of total lactic acid bacteria in kombucha with 25% robusta cascara and 75% green tea (A4) were reported the highest among all treatments.

The characteristics of treatments both before and after fementation were shown in Table 2 and Table 3, respectively. The figure of kombucha produced were shown in Figure 1.

4 DISCUSSION

4.1 Total Polyphenol of Kombucha

The average total polyphenol of kombucha ranged from 0.0800 mg GAE/mL to 0.2245 mg GAE/mL. These results were low compared to kombucha using green tea only as raw material which reported contained 1.08 GAE/mL in 15 days of fermentation (De Miranda et al., 2022) and kombucha from cascara contained between 6.3 and 36.9 mg GAE/mL in 16 days of fermentation (Nurhayati; Yuwanti; Urbahillah, 2020). The difference of fermentation time in this research that

Table 2: Characteristics of Treatment's Raw Material Before Fermentation.

Characteristic	A1*	A2	A3	A4	A5
Total titrated acid (%)	0.0001[a]	0.0003[a]	0.0007[ab]	0.0005[ab]	0.0016[b**]
Total dissolved solid (% Brix)	11.5333[b]	10.8000[a]	12.0667[c]	11.5000[b]	10.7667[a]
pH	6.0700[a]	6.2500[b]	6.3800[cd]	6.3200[c]	6.4000[d]

is only 8 days was expected to generate lower total phenol in kombucha. According to Nurhayati, Yuwanti and Urbahillah (2020), phenolic compounds that are bound to structural components of substrate's cell wall such as cellulose, lignin, and protein through ester bonds will be detached into free phenolic acids by enzyme activity of microorganism during fermentation thus increased the total phenol of kombucha.

Thereafter, polyphenol content in raw materials also affected the total phenol in kombucha. Green tea is known to have high polyphenol content, mainly flavonoid in form of catechin around 30-40% of soluble solid (Habiburrohman; Sukohar, 2018). It is reflected on kombucha with 100% green tea (A5) that has the highest total polyphenol content around 0.224 mg GAE/mL among all treatments. As for robusta cascara, the main component of polyphenol is chlorogenic acid which is only 1.88-2.45 mg/g (Trihaditia; Yuliani; Priambodo, 2021). The difference of total polyphenol content in green tea and robusta cascara is mainly caused by the processing method where green tea processed minimally with low contact from heating or high temperature procedure to prevent loss of its polyphenol. Meanwhile, for robusta cascara, specifically used in this research obtained from local farmer, is processed by drying at uncontrolled temperature that is susceptible to loss of polyphenol substances which are easily damaged by high temperature. This indicated that using substrate with high polyphenol content would determine the final total polyphenol in kombucha making. The higher substrate's polyphenol value, the higher total polyphenol in kombucha drink.

4.2 Total Titrated Acid of Kombucha

Total titrated acid indicates amount of acid substances in kombucha produced through fermentation process. The average total titrated acid of kombucha ranged from 0.0032% to 0.0048% after fermentation. It was found that total acid in substrates increased during fermentation because of the bacteria's activity in SCOBY as starter that can produce organic acids. At initial stage, yeasts contained in SCOBY, mainly from genera *Brettanomyces* and *Zygosaccahromyces,* hydrolyzed sucrose into glucose and fructose through enzyme invertase producing ethanol via glycolytic pathway (De Miranda et al., 2022). Then, the ethanol was oxidized by acetic acid bacteria from genera *Acetobacter* with enzyme alcohol dehydrogenase and aldehyde dehydrogenase through Krebs cycle producing acetic acid. On the other hand, the genus *Gluconobacter* and *Acetobacter* bacteria used glucose to produce gluconic acid while fructose that is used less contributes to sweet taste of kombucha (Leal et al., 2018).

Table 3: Characteristics of Treatments After 8 Days of Fermentation.

Characteristic	A1*	A2	A3	A4	A5
Total polyphenol (mg GAE/mL)	0.1314^{a}	0.0800^{a}	0.1149^{a}	0.1237^{a}	0.2245^{b**}
Total titrated acid (%)	0.0032^{a}	0.0034^{a}	0.0046^{bc}	0.0041^{b}	0.0048^{c}
Total dissolved solid (% Brix)	10.0667^{b}	9.1333^{a}	9.8333^{b}	9.8667^{b}	9.8000^{b}
pH	3.5333^{ab}	3.6000^{b}	3.6000^{b}	3.5333^{ab}	3.4667^{a}
Total lactic acid bacteria (log CFU/mL)	3.6928^{ab}	3.9650^{ab}	3.5507^{a}	4.3917^{b}	3.3760^{a}

*Treatments: A1 (100% robusta cascara : 0% green tea); A2 (75% robusta cascara : 25% green tea); A3 (50% robusta cascara : 50% green tea); A4 (25% robusta cascara : 75% green tea); A5 (0% robusta cascara : 100% green tea).

**Values which are followed by different superscript alphabet in the same column is significantly different at level of 5% according to Honestly Significant Difference (P<0.05).

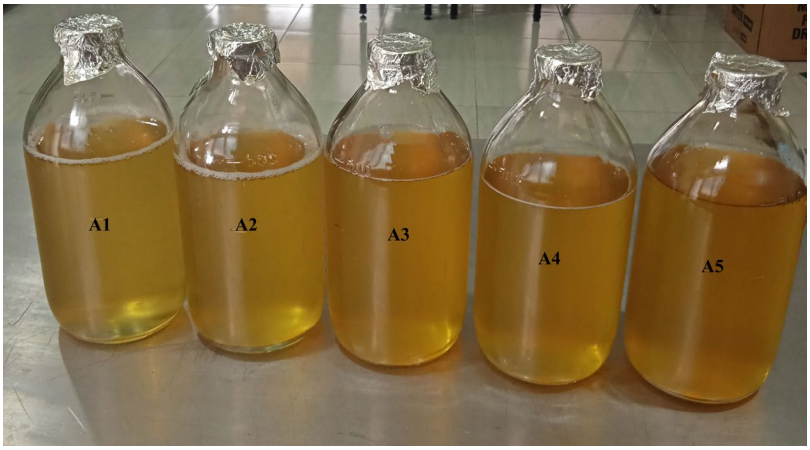

Figure 1: Kombucha of Robusta Cascara and Green Tea After SCOBY Removed.

Total titrated acid of kombucha obtained in this research was lower than quality standart for total acid of kombucha referred to Kombucha Brewers International (KBI) which is around 0.27-2.03%. Moreover, total titrated acid of kombucha with 100% robusta cascara (A1) was also lower compared to total titrated acid value of kombucha robusta cascara in Anjliany, Syafutri and Widowati (2022) which is around 0.018%. Total titrated acid of kombucha with 100% green tea (A5) also was lower than green tea kombucha's total acid value in Purnami, Jambe and Wisaniyasa (2018) that is around 0.75%.

It is assumed that concentration of initial sugar contained in all treatments were not in a proportionate amount to produce organic acids that can meet the standart of KBI. It can be seen from sugar added in kombucha making was only 50 g or 10% out of 500 mL water whereas in Leal et al. (2018), the concentration of sugar is recommended at 5-20% as the main carbon source providing the media and nutrients necessary for microorganism development. Moreover, the concentration of kombucha starter (SCOBY) also affected the content of total titrated acid in kombucha. A study from Anjliany, Syafutri and Widowati 2022). stated that 10% (w/v) of starter was the optimum concentration in making kombucha and produced the highest acetic acid. In this study, the concentration of SCOBY used was already 10% which consisted 5% (v/v) of the liquid and 5% (w/v) of the pellicle, but it is not an optimum concentration to produce suitable total titrated acid value with KBI standart.

In addition, tea leaves contained sugar about 6.5% (Piyasena et al., 2022) that is higher than sugar content in coffee peel at 4.1% (Muzaifa, Rohaya; Sofyan, 2021). This could provide more sugar supply for producing ethanol and then used to produce organic acids. It affected in total titrated acid per treatments that as green tea proportion increased on A2, A3, and A4 treatments the content of total titrated acid would be higher than A1 (kombucha with 100% robusta cascara) and thus kombucha with 100% green tea (A5) produced the highest value of total titrated acid.

4.3 Total Dissolved Solid of Kombucha

Total dissolved solid of kombucha indicates amount of water soluble materials or solids such as carbohydrate compounds, organic ions or even reduction sugar, organic acids, pectin, and protein contained in kombucha (Purnami; Jambe; Wisaniyasa, 2018). The average total dissolved solid of kombucha in this research ranged from 9.1333% Brix to 10.0667% Brix. Total dissolved solid values obtained in this research were also likely similar with total dissolved solid of green tea kombucha obtained in Purnami, Jambe and Wisaniyasa (2018) which is around 10.24% Brix and cascara kombucha obtained in Nurhayati, Yuwanti and Urbahillah (2020) around 8.82-13.75°Brix. The sugar adding into tea solution could affect total dissolved sugar in kombucha. In this study, 50 g (10% b/v) sugar was added into 500 mL water while on Purnami, Jambe and Wisaniyasa (2018) used 150 g (15% b/v) sugar in 1 L water and on Nurhayati, Yuwanti and Urbahillah (2020) 300 g (10% b/v) sugar was added into 3 L water.

It was found that total dissolved solid in substrates decreased during fermentation into kombucha. It is assumed that the microorganisms in starter (SCOBY) needed food source in order to change the sweet tea and cascara as substrate into acidic kombucha by degrading sugar and other soluble substances in substrate (Nurhayati; Yuwanti; Urbahillah, 2020). Degradation carbohydrate into smaller monomers done by yeast thereafter was utilized by bacteria to produce ethanol and organic acid (Leal et al., 2018) causing total value of dissolved solid decreasing and the increased of total titrated acid after fermentation.

This study also revealed that as proportion of green tea increased, the content of total dissolved solid was likely to decrease. Such tendency might be affected due to carbohydrate content in tea leaves that is lower than in cascara. According to Srijanto and Purwantiningsih (2008), tea leaves contained carbohydrate at 10-20%, while in Muzaifa, Rohaya and Sofyan, (2021) cascara contained 35% carbohydrate substances. Although the sugar adding for all treatments were same, the carbohydrate content in every treatments could be different due to the percentage of robusta cascara and green tea used in kombucha fermentation. This led to kombucha with 100% robusta cascara that contained higher carbohydrate value had the highest total dissolved solid at 10.0667% Brix and decreased in A2 to A5 at around 9.1333% Brix to 9.8667% Brix. Therefore, the higher percentage of sugar adding and carbohydrate content in raw material could provide more food source for microbia to convert tea solution into kombucha resulting in higher total dissolved solid value.

4.4 pH of Kombucha

pH is important parameter to pay attention in making kombucha. A good kombucha should have pH value between 2.5 to 4.2 and if the pH is under 2.5 then kombucha should be added the sweet tea until its pH value reaches ≤4.2. This low pH value enables to prevent pathogens growing in kombucha (Nummer, 2013). According to *Food and Drug Administration* (FDA) *Model Food Code* for kombucha production in Nummer (2013) suggests that kombucha fermentation time for safe consumption should not be more than 10 days. The pH values in kombucha ranged from 3.4667 to 3.6000. All pH values have fulfilled the quality standart in Nummer (2013) indicated that kombucha produced in this research are safe for consumption.

This study revealed that the pH values of substrates decreased after 8 days of fermentation into kombucha from

around 6 to 3. The decreased pH were likely caused by microbia's growth and activity to change sweet substrates into sour kombucha. At first, the medium is aerobic, high in sucrose, and slightly acidic due to the SCOBY addition. Gradually, the oxygenation of the liquid decreases because of the development of a cellulose layer on top of the fermenting liquid and the consumption of oxygen by the microorganisms inside the liquid. The growth and metabolism of yeasts, acetic acid bacteria (AAB), and lactic acid bacteria (LAB) result in the accumulation of organic acids and thus decreasing pH values (Laureys; Britton; De Clippeleer, 2020). Therefore, this result is in accordance with total titrated acid values that the higher total amount of acid produced in kombucha, the lower pH value. It is showed in kombucha with 0% robusta cascara and 100% green tea (A5) that has the highest value of total titrated acid and the lowest value of pH.

4.5 Total Lactic Acid Bacteria of Kombucha

In this study, analysis of total lactic acid bacteria was intented to see the potential of kombucha using combination of robusta cascara and green tea as probiotic drink. Total lactic acid bacteria of kombucha obtained has range from 3.3760 log CFU/mL on kombucha with 100% green tea (A5) to 4.3917 log CFU/mL on kombucha with 25% robusta cascara and 75% green tea (A4). The lactic acid bacteria in kombucha were likely derived from lactic acid bacteria contained in starter (SCOBY) that used to make kombucha. It is known after analyzing the total lactic acid bacteria on the liquid of SCOBY contained around 5.34 log CFU/mL and on the pellicle of SCOBY contained around 2.48 log CFU/mL. This showed that during fermentation, total lactic acid bacteria on kombucha increased supported by the suitable substrate's pH ranging from 6.0700-6.4000 for lactic acid bacteria to grow optimally which on Laureys, Britton and De Clippeleer (2020) reported that pH value around 4-6 is the suitable pH for lactic acid bacteria to grow. Meanwhile, microbial population in kombucha fermentation usually presents at around 10^6-10^8 CFU/mL (Laureys; Britton; De Clippeleer, 2020).

Moreover, the total dissolved solid of substrates around 10.7667-12.0667% Brix also contributed to lactic acid bacteria growth for being its nutrition and energy source (Wistiana; Zubaidah, 2015). The increased value of total titrated acid during fermentation were expected to indicate that lactid acid bacteria were also growing on kombucha, besides acetic acid bacteria, as lactic acid bacteria could hydrolyze sugar to produce lactic acid. They can also produce other metabolites such as ethanol, acetic acid, carbon dioxide, diacetyl, and mannitol (Laureys Britton; De Clippeleer, 2020).

The highest value of total lactic acid bacteria obtained in A4 (kombucha with 25% robusta cascara and 75% green tea) around 4.3917 log CFU/mL. It is affected by the substrate's total dissolved solid around 11.5000% Brix provided nutrients for bacteria to grow and pH of 3.5333 which was still in a suitable condition to the bacteria living in kombucha. Meanwhile, A5 (kombucha with 0% robusta cascara and 100% green tea) has the lowest total lactic acid bacteria. It is assumed that high content of polyphenol and caffeine in green tea could inhibit microbial growth because of their anti-microbial property. This is supported by total polyphenol contained in kombucha with 100% green tea (A5) around 0.2245 mg GAE/mL as the highest value of all treatments. In addition, the caffeine contained in green tea at 2-4% (Piyasena et al., 2022) is higher than cascara's caffeine content around 1.04-1.37% (Nurhayati; Yuwanti; Urbahillah, 2020). Moreover, pH value also affected for lactic acid bacteria to grow where in kombucha with 0% robusta cascara and 100% green tea (A5) has pH of 3.4667 as reported that the bacteria is hardly growing on pH under 3.5 (Laureys Britton; De Clippeleer, 2020).

5 CONCLUSIONS

The variation of robusta cascara and green tea combination has a significant impact on total polyphenol content, total titrated acid, total dissolved solid, pH, and total lactic acid bacteria of kombucha. Total polyphenol contents of kombucha were affected by raw material's total polyphenol which higher the polyphenol value in the raw material higher total polyphenol could present in kombucha. This can be seen in kombucha with 100% green tea that has the highest total polyphenol value at 0.2245 mg GAE/mL. The increased of green tea proportion in treatments could increase content of total titrated acid from 0.0032% to 0.0048% whereas total dissolved solid and pH values decrease in kombucha from 10.0667% Brix to 9.1333% Brix and from 3.6000 to 3.4667 respectively. Total lactic acid bacteria were affected by total dissolved solid and pH value of substrate to grow well in kombucha as in kombucha with 25% robusta cascara and 75% green tea (A4) has the highest total lactic acid bacteria at 4.3917 log CFU/mL. Moreover, a proportionate amount of initial sugar and optimum concentration of starter (SCOBY) were also affected for producing suitable total titrated acid to KBI standart. Robusta cascara and green tea combination used as treatments produced kombucha with potential as probiotic and functional drink reflected from total lactic acid bacteria and total polyphenol content obtained in this study. We presume this result is useful in exploring more diverse kombucha's substrate and to create functional drink with higher nutritional values.

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7 AUTHOR'S CONTRIBUTION

Conceptual idea: Nurrocmah, S.I.; Parwiyanti; Methodology design: Parwiyanti; Nurrocmah, S.I.; Data collection: Nurrocmah, S.I.; Data analysis and interpretation: Parwiyanti; Nurrocmah, S.I.; Malahayati, N., and Writing and editing: Nurrocmah, S.I.; Parwiyanti; Malahayati, N.

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