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Qualities of arabica and robusta cascara kombucha with different concentrations of starter

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ABSTRACT

Coffee waste consisted of coffee peel (45%), mucilage (10%) and seed peel (5%). Generally, coffee peel is used in the manufacture of fertilizers. Coffee peel waste is a potential material that can be the producer of caffeine, polyphenol, bioethanol, antioxidantand antimicrobial. Coffee peel has many benefits including ward off free radicals, protect the stomach and give a firming effect on skin. This research aimed to create functional drink with high antioxidant activity by optimizing arabica and robusta coffee peel waste. This research used Completely Randomized Factorial Design with two treatment factors namely type of cascara (arabica and robusta) and starter concentration variation (5%, 10% and 15%). The treatment repeated three times. The parameters observed were total polyphenol, total titrated acid, total dissolved solid, pH and total mesophilic aerobic bacteria. The results showed that coffee peel type, starter concentration variation and the interaction between two factors had significant effects on total titrated acid and total dissolved solid cascara kombucha. Cascara kombucha with robusta coffee peel and starter concentration at 10% was chosen as the best treatment with total phenol 93.18 µg/mL, TTA 0.82%, TDS 3.80°Brix, pH 4.21, and TMAB 7,7 log CFU/mL.

Key words: Coffee peel; Fermentation; Functional drink; Starter.

1 INTRODUCTION

The increasing of coffee-based drink popularity also has an impact on the increasing of coffee waste. Muzaifa et al. (2019) stated that coffee waste is the non-bean part obtained from the results of coffee processing. Saisa and Maliya (2018) added that the coffee waste consisted of coffee peel (45%), mucilage (10%) and seed peel (5%). Generally, coffee peelis used in the manufacture of fertilizers. In addition, coffee peel is also used as animal feed ingredients after undergoing quality improvement by the *Phanerochaete chrysosporium* fermentation method.

Coffee peel waste is a potential material that can be optimized as a producer of caffeine, polyphenols, bioethanol (Bonilla-Hermosa et al., 2014), antioxidants and antimicrobials (Jimenez-Zamora; Pastoriza; Rufián-Henares,2015). Coffee peel also contained considerable amounts of phenolic compounds that can be valorised to create functional drink (Oktaviani et al., 2020). The high intensity of coffee peel waste generated from coffee processing creates the potential for the development of coffee peel derivative products, including cascara. The infused cascara has been aromatically described as floral, sweet, tea-like and reminiscent of dried fruit. The caffeine content of cascara is 226 mg caffeine/L, while the dominant phenolic compounds, namely protocatechuic and chlorogenic acid, are 85.0 and 69.6 mg/L (Heeger et al., 2017).

One of the functional drink that are currently favored by the public, especially healthy lifestyle activists, is kombucha. Kombucha is a drink fermented from tea liquid containing sugar by kombucha starter which is a consortium of microorganisms from the acetic acid bacteria group, namely *Acetobacter xylinum* and yeast *Saccharomyces* sp. (Yanti et al., 2020) with a fermentation process carried out for 1-2 weeks (Sun; Li; Chen,2015). This symbiotic culture between bacteria and yeast is known as SCOBY or symbiotic culture of bacteria and yeast. The amount of addition of starter will affect the production of organic acids in kombucha. The amount of 10% (w/v) starter and 10% (w/v) added sugar are the optimum amounts for making kombucha. This treatment will produce the highest acetic acid, which is about 0.78%. Optimum results are obtained because the availability of nutrients is proportional to the number of microorganisms.

According to Viviandari et al. (2015), he bacteria involved in the kombucha fermentation process are acetic acid bacteria and lactic acid bacteria. This shows that kombucha has potential as a probiotic drink. The fermentation process will cause changes in physical and chemical properties which include sugar, alcohol content, pH and antioxidant levels (Nguyen et al., 2015).This change was caused by *Saccharomyces* sp. which breaks down glucose into ethanol while *Komagataeibacter xylinus* will oxidize ethanol to acetic acid (Ayuratri; Kusnadi, 2017). The fermentation process will be able to increase the total polyphenols due to microbial enzymatic activity which can free bound polyphenolic compounds so that more will be detected. Fermentation will also increase the total titrated acid caused by the breakdown of sugar compounds into organic acids. An increase in the total titrated acid will have an effect on a decrease in pH.

Research on kombucha with tea-based ingredients has been widely carried out, even now the manufacture of kombucha with raw materials other than tea. The manufacture of kombucha cascara is expected to produce a diversified product of processed coffee peel that has functional properties such as high antioxidant activity. It was also expected as one way to implement zero-waste. The use of different types of coffee and various concentrations of starter was thought to affect the physicochemical and microbiological characteristics of kombucha cascara.

2 MATERIAL AND METHODS

2.1 Materials

Coffee peel used in this research were Pagaralam Robusta coffee and Pagaralam Arabica coffee cultivated on Pagaralam, South Sumatera, harvested in 2021. Samples of kombucha starter (SCOBY) and sucrose were purchased from retail market. Chemical ingridients such as folin-ciocalteu reagent (FCR), sodium carbonate, sodium hydroxide, phenolphtalein, acetic acid, gallic acid and nutrient agar media were purchased from Agricultural Product Technology Laboratory, Palembang, South Sumatera.

2.2 Methods

This research used Completely Randomized Factorial Design with two treatment factors namely (A) type of cascara (arabica and robusta) and (B) starter concentration variation (5%, 10% and 15%). The treatment repeated three times. Parameters observed were total polyphenol, total titrated acid, total dissolved solid, pH and total mesophilic aerob bacteria (TMAB).

A1B1 = arabica coffee peel, 5% starter concentration
A1B2 = arabica coffee peel, 10% starter concentration
A1B3 = arabica coffee peel, 15% starter concentration
A2B1 = robusta coffee peel, 5% starter concentration
A2B2 = robusta coffee peel, 10% starter concentration
A2B3 = robusta coffee peel, 15% starter concentration

The parameters observed are physical characteristic (dissolved solids), chemical characteristics (titrated acid, total phenolic compounds, pH) and microbiological characteristic (total mesophilic aerobic bacteria). 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.

2.2.1 Cascara kombucha preparation

A total of 10% of sucrose (w/v) was boiled with 3 L of water, then 1% cascara (w/v) was added while stirring for 5 minutes. The cascara solution was filtered using an 80 mesh filter and then cooled down to 40°C. The cascara solution was put into a glass jar with a tap and inoculated with 5%, 10% and 15% (w/v) kombucha starter. Aerobic fermentation was done indoor (temperature range 20-25°C) and not exposed to direct sunlight for 8 days. The fermented cascara solution was separated from the kombucha starter and packaged in glass bottles and then analyzed (Nurhayati; Yuwanti; Urbahillah,2020).

2.2.2 The Analysis of Total Phenol

The analysis of total phenol content were using the Folin-Ciocalteu method as described in Villarreal-Soto et al. (2019).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. 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 The Analysis of Total Titrated Acid

A total of 10 mL of kombucha cascara 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 (Andreson et al., 2022).

2.2.4 The 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, 1995).

2.2.5 pH Analysis

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, 1999).

2.2.6 The Analysis of Total Mesophilic Aerob Bacteria

A sample of 1 mL fermented kombucha was taken and diluted until 10^{-3} dilution. A total of 10 gram nutrient agar was poured to Petri disc and continued with the sample. The Petri discs was incubated for 24 hours. After 24 hours, the formed colonies were counted (Sadiyah; Lestari, 2020).

3 RESULT

Robusta cascara kombucha which was fermented with 15% of starter had the highest total phenol value and arabica cascara kombucha which was fermented with 5% ofstarter had the lowest value of total phenol. Meanwhile robusta cascara kombucha which was fermented with 10% of starter had a value that was closest to the standard kombucha. The characteristic of cascara kombucha were shown in Table 1.

Different from the other parameters, the type of coffee peel and starter concentration variation had no significant effect to total mesophilic aerobic bacteria. The TMAB value of arabica cascara kombucha ranged from 7,32-7,61 log CFU/ml while TMAB of robusta cascara kombucha has higher value ranged from 7,46-7,72 log CFU/ml. The figure of arabica cascara kombucha and robusta cascara kombucha were shown in Figure 1 and Figure 2, respectively.

4 DISCUSSION

4.1 Total Polyphenol of Cascara Kombucha

The average of total phenol value of kombucha cascara ranged from 11.12 µg/mL to 97.07 µg/mL. The highest total phenol content was obtained by treatment A2B3 (kombucha cascara robusta fermented with 15% starter concentration) of 97.07 µg/mL. Meanwhile, the lowest total phenol content was obtained by A1B1 treatment (fermented kombucha cascara arabica with 5% starter concentration) of 11.12 µg/mL.

Phenol levels in raw materials, namely arabica and robusta coffee peel affected the total phenol content in kombucha. Meanwhile, based on the analysis of raw materials that has been carried out, Arabica coffee-peel tea contained phenol levels of 32.70 µg/mL. This amount was smaller than the phenol content in robusta coffee-peeltea, which was 33.14 µg/mL. This was directly proportionalto the measurement

Table 1: Characteristic of Cascara Kombucha.

Characteristic	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
Phenol (µg/mL)	11.12±٩,83^{a}	25.63±٦.92^{a}	18.56±٢٢.55^{a}	48.36±٧.69^{a}	93.18±٣٧,48^{a}	97.07±٤٣,31^{a}
TTA (%)	0.16±0.000^{a}	0.13±0.000^{a}	0.15±0.009^{a}	0.28±0.054^{b}	0.82±0.039^{d}	0.74±0.021^{c}
TDS (ºBrix)	4.03±0.06^{c}	3.90±0.00^{b}	3.90±0.00^{b}	3.77±0.06^{a}	3.80±0.00^{a}	3.77±0.06^{a}
pH	4.72±0.03^{b}	4.81±0.06^{b}	4.87±0.18^{b}	4.22±0.10^{a}	4.21±0.03^{a}	4.22±0.02^{a}
TMAB (log CFU/mL)	7.34±0.23	7.61±0.27	7.32±0.51	7.46±0.52	7.70±0.36	7.72±0.30

*Data shown is average value ± deviation standard

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

Figure 1: Arabica Cascara Kombucha.

Figure 2: Robusta Cascara Kombucha.

results of the total phenol of kombucha cascara where the total phenol of kombucha cascara robusta was greater than the total phenol of kombucha cascara arabica. Based on the research of Pua et al. (2021), cascara contained 34 phenolic compounds such as pyrogallol, pyrocatechol, 4-hydrobenzoic acid and chlorogenic acid. Other polyphenols such are benzenediols, benezetriols, protocatechuic, flavonoid and xanthonoids were also detected.

Based on Farah (2012), indirectly, total phenol interpreted chlorogenic acid levels as the main phenolic compounds contained in cascara. The higher value of total phenol, the higher the chlorogenic acid content. Based on Suhardini and Zubaidah (2016), the increase in phenolic compounds in tea of various types of leaves that were processed into kombucha was caused by the activity of various groups of bacteria and yeasts that could metabolize to produce flavonoid compounds through enzymatic reactions. Hunandra (2016) also stated the same thing that on the 10^{th} day of kombucha fermentation there was an increase in phenol levels caused by biotransformation of several phenolic compounds due to enzymes released and the release of catechins from acid-sensitive microorganisms. Nurhayati, Yuwantiand Urbahillah(2020) stated that the enzymatic activity produced by kombucha starter degraded matrix components to form phenolic compounds.

Nurhayati Yuwantiand Urbahillah(2020) also explained that the increase in phenol levels occurred due to the degradation of chlorogenic acid by microorganisms into caffeic acid. Caffeic acid is a flavonoid phenolic compound. Caffeic acid is then broken down into cinnamic acid. Meanwhile, according to Ayuratri and Kusnadi (2017),*Saccharomyces cerevisiae*can produce phenol reductase enzymes and decarboxylate cinnamic acid. Cinnamic acids such as pyrulic acid and p-kaumaric acid are decarboxylated to form phenolic compounds such as 4-vinyl guaiacol (4-VG) and 4-vinyl phenol (4-VP).

4.2 Total Titrated Acid of Cascara Kombucha

Cascara arabica tea contained 0.042% titrated acid and cascara robusta tea contained 0.018% titrated acid. Afterbeing processed into kombucha, the total titrated acid increased significantly. This increase occurred because yeast and bacteria in kombucha starter are involved in metabolic activities that use substrates in different way. Yeast hydrolyze sucrose into glucose and fructose, using the enzyme invertase to produce ethanol. The acetic acid bacteria use glucose to produce gluconic acid and ethanol, the latter used by them in production of acetic acid (Barbosa et al., 2022).The total titrated acid of kombucha cascara ranged from 0.132% to 0.824%. Based on the total titrated acid standard set by Kombucha Brewers International (KBI), total titrated acid value of arabica cascara kombucha has not met the standard. Meanwhile total titrated acid value of robusta cascara kombucha has met the standard ranged 0,27-2.03%.

The higher total titrated acid in kombucha cascara robusta indicated that robusta coffee peel contained higher gluconic and acetic acid as the main organic acid found in kombucha (Sknepnek et al., 2021).Beside gluconic and acetic acid, six organic acids were also detected in coffee peel. The organic acids are citric acid, tartaric acid, malic acid, succinic acid, fumaric acid, and gallic acid (Pua et al., 2021). Those acid were the key compounds that contribute to the formation of the flavour quality of kombucha (Vitas et al., 2018).

The increasing of total titrated acid directly proportional to the total phenol value obtained in this study, where the total phenolic content of kombucha cascara robusta washigher than the total phenol of kombucha cascara arabica which indicated that the content of chlorogenic acid as the main phenolic compounds in robusta coffee-peel washigher than arabica coffeepeel. The total value of titrated acid in fermented kombucha with a concentration of 10% of starter resulted in the highest total titrated acid. This was because the concentration of starter would affect the production of acetic acid in kombucha where the concentration of starter at 10% and the used of sucrose at 10% was the optimum concentration in making kombucha and produced the highest acetic acid. The concentration of starter at 10% was proportional to the amount of substrate so that the substrate could be used for growth, cell multiplication and optimal production of organic acids. Ardheniati, Andrianiand Amanto(2009) also explained that the decrease in acetic acid levels was influenced by the decreasing supply of sugarbecause acetic acid bacteria oxidized acetic acid in obtaining energy for growth. Saputra et al. (2017) in his research also explained that the concentration of acetic acid in kombucha only increased to a certain extent. This was because the sugar content had been exhausted and the acetic acid was further utilized by *Acetobacter xylinum*.

4.3 Total Dissolved Solid of Cascara Kombucha

The total dissolved solids showed the content of dissolved substances in the solution. Based on Muzaifa et al. (2019), total dissolved solids interpreted the sugar content in cascara diversification product including kombucha cascara. The total dissolved solids of kombucha cascara ranged from 3.77 - 4.03°Brix. The total soluble solids of cascara arabica tea which was initially 4.3°Brix decreased to 3.90-4.03°Brix after being processed into kombucha. The total dissolved solids of cascara robusta tea also decreased from 3.90°Brix to 3.77-3.80°Brix after being processed into kombucha.

According to Heeger et al. (2017) *in* Nurhayati, Yuwantiand Urbahillah(2020), the components contained in cascara consisted of water-soluble components, such as glucose, fructose, protein and caffeine. The higher total dissolved solids of kombucha cascara arabica indicated that cascara arabica contained more water-soluble components.

Murlida et al. (2021) stated that the sugar content of cascara arabica was higher than cascara robusta. Sugars, pigments, vitamins, organic acids and proteins were water-soluble components (Ismawati et al., 2016).

In addition to internal factors, namely the content of cascara itself, one of the external factors that affected the total dissolved solids was the heating temperature. The used of a manual stove caused the specific temperature could not be regulated. Muzaifa et al. (2019)stated that the higher the temperature, the faster the breaking of long chains of carbohydrate compounds into sugar. This results in higher total dissolved solids.

Based on Napitulu and Setyohadi (2015), an increased of microbes was accompanied by a decrease in the amount of substrate because it was consumed for metabolism which caused the value of total dissolved solids decreased. Nurhayati et al. (2017) eplained the activity and growth of microorganisms that degrade substrates such as sugar and the content of solutes in cascara and were used for microorganism metabolism so that the higher number of microorganisms would cause lower dissolved solids.

4.4 pH of Cascara Kombucha

Cascara arabica tea had a pH value of 5.79, while cascara robusta tea has a higher pH of 6.33. After being processed into kombucha, the pH decreased. Organic acid such as acetic and gluconic acid produced during fermentation could decrease the pH of kombucha. It was also known to have an influence on the growth of fermenting microbes and caused structural changes to phytochemical compounds which can impact the antioxidant activity (Bishop et al., 2022).Nurhayati et al. (2017) also stated this decrease in pH was caused by the increasing total acid. Ayuratri and Kusnadi (2017) explained that during fermentation, the yeast would remodel sucrose into glucose and fructose. Glucose was converted to gluconic acid by acetic acid bacteria. Meanwhile, most of the fructose was converted to acetic acid and a small amount of gluconic acid. It was the breakdown of sucrose into organic acids that increased acid levels and at the same time lowers pH. This statement was also supported by Nurikasari et al. (2017) who explained that yeast and bacteria broke down sucrose into organic acids such as gluconic acid and acetic acid during the fermentation process, so that the total acid increased and the pH decreased. The pH of a fermented product was closely related to the level of acid produced and had an inverse relationship with the total titrated acid where the higher the TAT value, the lower the pH value.

4.5 Total Mesophilic Aerobic Bacteria

Based on the measurement of total mesophilic aerobic bacteria of kombucha cascara ranged from 7.32 log CFU/mL to 7.72 log CFU/mL. Treatment factor A2B3 (fermented kombucha cascara robusta with 15% starter concentration) had the highest TMAB value of 7.72 log CFU/mL or 5.23 x 10^7 CFU/mL while the lowest TMAB value was obtained by treatment A1B3 (fermented kombucha cascara arabica). with 15% starter concentration) of 7.32 log CFU/mL or 2.09 x 10^7 CFU/mL.

Zubaidah et al. (2021) in their research reported that black tea kombucha recorded 1.3 x 10^8 CFU/mL total microbes on day-14. Meanwhile, Sadiyah and Lestari (2020) reported that the TPC value of kombucha tea fermented for 7 days was 79 x 10^3 CFU/mL.

Each microorganism and microflora had a different shape and size of the colony after incubation. In some samples it was possible to contain more microflora which made the colonies large so that the number of colonies that could be counted was low. There were at least 14 species of bacteria and 10 species of yeast in kombucha starter. In general most kombucha had 2-3 dominant bacteria and yeast in their composition. The most abundant genera among kombucha consortia refer to *Komagataeibacter, Lactobacillus* and *Bacillus* (Andreson et al., 2022). While the dominant yeast strains found within kombucha are *Saccharomyces cereviseae*, *Kloeckera* sp., *Schizosaccharomyces pombo* and *Saccharomyces ludwigii*(Bishop et al., 2022).

In kombucha cascara robusta, the higher starter concentration resulted in a higher total mesophilic aerobic bacteria which could indicated the amount of acetic acid bacteria and lactic acid bacteria as the main bacteria in kombucha. Based on Wistiana and Zubaidah (2015), this increase of TMAB value was caused by the presence of soluble solids in kombucha cascara such as sugar, amino acids and caffeine. These substances were used by microorganisms as a source of energy and nutrients so that the number of microorganisms increases. Meanwhile, the decrease in the number of microorganisms from A1B2 (fermented kombucha cascara arabica with 10% of starter concentration) to A1B3 (fermented kombucha cascara arabica with 15% of starter concentration) was due to the fermentation process producing organic acids, alcohol and other substances that could inhibit the growth of microorganisms. In addition, the decrease in TMAB in the A1B3 treatment was also influenced by the content of phenolic compounds formed in the fermentation process where the phenol content had antimicrobial properties that could inhibit the growth of microorganisms (Simanjuntak; Mutiara, 2016).

5 CONCLUSIONS

The variation of starter concentration and cascara in making cascara kombucha were significantly different from unfermentation tea control treatments of parameters total polyphenol, total titratic acid, total dissolved solid and pH, but did not significantly affect total plate count. The parameter values of cascara kombucha in this study were: total polyphenol of 11,12 – 97,07µg/mL, total titrated acid of 0,13 – 0,82%, total dissolved solid of 3,77 – 4,03ºBrix, pH 4,21 – 4,87 and total plate count from 7,32 – 7,72 log CFU/mL. Cascara kombucha with robusta coffee peel and starter concentration 10% was chosen as the best treatment with total phenol 93.18 µg/mL, TTA 0.82%, TDS 3.80°Brix, pH 4.21, and TPC 7.7 log CFU/mL. We presume this result is useful to create functional coffee drink product made from coffee peel waste.

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7 AUTHOR'S CONTRIBUTION

MA performed laboratory experiments, analyzed the data, conducted statistical analyses and drafted the manuscript. **MIS** participated in design of the study, sequence alignment and supervised the research. **TWW** conceived the study and participated in the sequence alignment. All authors read and approved the final manuscript.

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