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Effect of Fermentation Time on Chemical, Microbiological and Hedonic Quality of Kombucha of Arabica Coffee Cascara (*Coffea arabica* L.)

Adrina Eka Shafira*, Antonius Hintono, Siti Susanti

Department of Food Technology, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

*Corresponding author (aeshafira@gmail.com)

Abstrac

This research aims to determine the effect of different fermentation times on the chemical, microbiological and hedonic quality of Kombucha of Arabica Coffee Cascara. The material used in this study is Arabica Coffee Cascara as a raw material for making kombucha with different fermentation treatment. The research method used was a Completely Randomized Design (CRD) with 4 treatments and 5 repetitions. Fermentation time variations carried out are 2, 4, 6 and 8 days. Data from the testing of chemical and microbiological properties were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level and if there is a real influence then proceed with the Duncan Multiple Range Test (DMRT). Data from the hedonic quality test results were analyzed using Kruskal Wallis nonparametric test with a confidence level of 95% and if there was a real influence, then proceed with the Mann-Whitney test. The results showed that the different fermentation time of Kombucha of Arabica Coffee Cascara had a significant effect ($p < 0.05$) on total acid, total sugar, total alcohol, total microbes, and hedonic quality of the product. The longer the fermentation lasts, the total acid and total alcohol will increase while the total sugar, total microbes and antioxidant activity will decrease. Kombucha of Arabica Coffee Cascara with 8 days fermentation time is the best treatment product because it meets the quality standards and is preferred by panelists.

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Introduction

Kombucha is a traditional beverage made by fermenting a tea solution with sugar using a Kombucha starter for several days (Wistiana and Zubaidah, 2015). The length of time of fermentation can affect the quality of Kombucha (Puspitasari et al., 2017). Kombucha fermentation can last for 7 to 14 days (Susilowati, 2013). Chemical content contained in Kombucha, including vitamins B_1, B_2, B_3, B_6, B_{12}, B_{15}, vitamin C, folic acid, glucuronic acid, acetic acid, lactic acid, amino acids, enzymes, and antibiotic compounds (Naland, 2004). Kombucha is very beneficial for the human body because it can facilitate digestion, as an antibiotic, antioxidant and antibacterial. The various benefits of Kombucha make Kombucha known as a health drink and popular in China, Russia, Germany, and the United States (Dipti et al., 2003).

SCOBY or Symbiotic Culture of Bacteria and Yeast is a symbiotic starter between bacteria and yeast used in the fermentation of kombucha tea (Mousavi et al., 2018). SCOBY can grow in environments that contain a lot of sugar as a carbon source (Puspitasari et al., 2017). The type of sugar that is consumed by many people is High Fructose Syrup (HFS). HFS is a liquid sweetener derived from cassava with an enzymatic process that has a high sweetness level, does not form crystals, and has a high level of solubility (Prastiwi et al., 2018). HFS has a sweetness level 1.8 times higher than sucrose and has a low calorie of 3.9 calories/gram (Qonitah et al., 2016).

Kombucha can be made from various types of tea, one of them is the Arabica cascara tea derived from the Arabica coffee cherry skin. Cascara is a product derived from the outer skin of coffee cherries which is dried and processed in such a way that it can be brewed as it is brewing tea in general (Sastra and Bawono, 2018). Cascara which has been brewed into tea has a distinctive sour taste and aroma like fruits (Nafisah and Widyaningsih, 2019). The distinctive sour taste is the effect of the content of chlorogenic acid and caffeic acid

in cascara (Rohdiana, 1999). Fresh cascara generally contains crude protein, crude fiber, tannin, caffeine, lignin, fat, ash, calcium, phosphorus, and several secondary metabolite compounds, such as the polyphenol group (Sumihati et al., 2011). Polyphenol compounds contained in Cascara are flavan-3-ol, hydroxynamic acid, flavanols, anthocyanin, catechins, tannins and ferulic acids (Esquivel and Jimenez, 2012). Cascara also contains vitamins C and E which are good for human skin (Nuraini et al., 2019). This research aims to determine the effect of different fermentation times against the chemical, microbiological and hedonic quality of Kombucha of Arabica Coffee Cascara.

Materials and methods

Materials

The ingredients used are Arabica Coffee Cascara, starter SCOBY (Symbiotic Culture of Bacteria and Yeast), High Fructose Syrup, NaOH 0.1 N, PP indicators, PCA (Plate Count Agar), distilled water, physiological NaCl 0.85%, methanol and DPPH reagents. The tools used are beaker, measuring cup, analytical scales, drop pipettes, volume pipettes, test tubes, thermometers, vortices, Erlenmeyer, burettes, Kjeldahl distillation flasks, pycnometers, scales, autoclaves, petri dishes, stoves, thermometers, refractometers, and spectrophotometers.

Methods

The study was conducted in December 2019 to January 2020 in the Laboratory of Food and Agricultural Products Engineering, and the Laboratory of Chemistry and Food Nutrition, Faculty of Animal and Agricultural Sciences and Integrated Laboratory of Diponegoro University, Semarang. The analysis included total acid analysis, total sugar, total alcohol, antioxidant activity, total microbes and hedonic tests.

Arabica Coffee Cascara Tea Making

The making of The cascara tea method referred to the method of Yuwanti et al. (2018) with modification. 32.5 grams (5%) of Arabica Coffee Cascara and 650 mL of water was heated at 90°C for 5 minutes until it forms an Arabica Coffee Cascara tea. The tea was filtered to separate the pulp and then put it into a container/bottle glass. The Arabica Coffee Cascara tea was cooled to room temperature.

Kombucha of Arabica Coffee Cascara Making

The making of Kombucha of Arabica Coffee Cascara method referred to the method of Nurhayati et al. (2018) with modification. Arabica Coffee Cascara tea was mixed with 5.5% (v/v) of High Fructose Syrup and put into a sterile glass container, then added a starter SCOBY and Kombucha vinegar until the solution reached pH 4. The glass container was closed using plastic and sealed using a rubber band. The tea was incubated at room temperature with a treatment duration of fermentation for 2, 4, 6 and 8 days. The fermented tea was filtered to separate Nata (secondary product) from the Kombucha of Arabica Coffee Cascara. Kombucha of Arabica Coffee Cascara was pasteurized at 70°C for 10 minutes.

Total Acid Analysis

The total acetic acid analysis was carried out using a method that refers to Leasa and Matdoan (2015) using titration. 10 mL of Kombucha samples were put into Erlenmeyer, then diluted to 250 mL and added 2 drops of the phenolphthalein (PP) indicator. Samples were titrated using 0.1 N NaOH to form a constant pink color. The total acid was calculated using Equation

$$\text{Total Acid} = \frac{\text{ml NaOH } x \text{ N NaOH } x \text{ BM Acetic Acid}}{\text{mL sample}} \; x \; 100\% \quad \text{Equation 1}$$

Analysis of Total Alcohol

The total alcohol analysis was carried out using a method that refers to AOAC (2000) using the distillation method. 100 ml of Kombucha samples were put into the Kjeldahl distillation flask and then added to distilled water as much as 100 mL, then distilled at 80°C. The distillate was stored in an Erlenmeyer to a volume of 50 mL, then put into a pycnometer that has been weighed before. The pycnometer contained distillate was weighed and their weight is recorded. The same procedure was carried out on aquadest as a comparison. The results of the alcohol density calculation converted using the alcohol conversion table. The alcohol density was calculated using Equation 2.

$$\text{Alcohol Density} = \frac{(\text{picnometer weight} + \text{distillate}) - \text{empty picnometer weight}}{(\text{picnometer weight} + \text{aquadest}) - \text{empty picnometer weight}} \quad \text{Equation 2}$$

Analysis of Total Sugar

Total sugar analysis was carried out using a method that refers to Surtinah (2007) using a Hand Refractometer. A hand refractometer must be cleaned using distilled water and dry tissue before use. As much as 1 drop of the Kombucha sample was dropped to the lens then tightly closed (make sure there are no air bubbles was formed) and then looked at the device. A dark and light boundary scale was set so the total sugar of the product could be seen. The total sugar figure was right on the boundary line of the hand refractometer.

Analysis Total Microbe

The total microbial analysis was carried out using a method that refers to Wati (2018) using the Total Plate Count (TPC) method with PCA (Plate Count Agar) media. As much as 1 mL of Kombucha sample was put into a test tube containing 9 mL of physiological NaCl 0.85% (dilution 10^{-1}). Next, 1 mL of dilution 10^{-1} was put into a test tube containing 9 mL physiological NaCl 0.85% (dilution 10^{-2}). The same step was repeated until dilution 10^{-6}. At the last 3 dilutions, as much as 1 ml of the last 3 dilutions was put into a petri dish that has been sterilized, then added with sterile PCA media and mixed well. The plates were incubated at 37°C for 2 x 24 hours.

Analysis of Antioxidant Activity

The antioxidant activity analysis was carried out using a method that refers to Yuwanti et al. (2018) using DPPH. As much as 0.1 mL of Kombucha sample was prepared in a test tube then added 2.95 mL of methanol and 3 mL of 300 µm DPPH solution and then

homogenized using a vortex. The homogeneous samples were leavened for 30 minutes. The absorbance value was measured using a spectrophotometer at a wavelength of 517 nm. Blanks were made using methanol. The antioxidant levels were calculated using Equation 3.

$$Antioxidant\ activity\ (\%) = \frac{\text{Absorbance blanks} - \text{Absorbance samples}}{\text{Absorbance blanks}}\ x\ 100\% \qquad \text{Equation 3}$$

Hedonic Quality Test

The hedonic quality test was carried out using a method that refers to Permata and Sayuti (2016) which includes aroma, color, sour taste, and *overall* preference. The Kombucha samples were tested on 25 semi-trained panelists. The hedonic quality analysis was carried out that samples prepared in plastic cups and then given a three-digit code. Each plastic cup was filled with 15 mL samples of Kombucha of Arabica Coffee Cascara. Panelists were asked to give a favorite score using 5 likeness scales, namely a score of 1 (Dislike), a score of 2 (Somewhat Dislike), a score of 3 (Ordinary), a score of 4 (Somewhat Like) and a score of 5 (Like).

Data Analysis

The data of total acid, total alcohol, total sugar, total microbes, and antioxidant activity were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level, then followed by Duncan Multiple Range Test (DMRT) to determine the differences between the treatments given. The hedonic test data were analyzed using the Kruskal Wallis nonparametric test with a 95% confidence level, then followed by the Mann-Whitney Test. The entire analysis process was carried out using SPSS 22.0 for Windows application.

Results and Discussion

Total Acid

Based on the test results presented in Table 1, the treatment of different fermentation time on making Kombucha of Arabica Coffee Cascara had a significant effect ($p<0.05$) on the total acid value of the product. The longer fermentation of Kombucha made an increase in total acid value. This is consistent with the opinion of Simanjuntak et al. (2016) which states that the long period of Kombucha fermentation can cause a decrease in acidity and an increase in the total acid of the product.

Table 1. Total Acid, Total Sugar, Total Alcohol, Total Microbes and Antioxidant Activity of Kombucha of Arabica Coffee Cascara

Parameters	Treatments			
	2 days	4 days	6 days	8 days
Total Acid (%)	0.20 ± 0.02[a]	0.21 ± 0.01[a]	0.24 ± 0.03[a]	0.29 ± 0.04[b]
Total Sugar (°brix)	6.80 ± 0.28[b]	6.52 ± 0.48[b]	6.28 ± 0.33[ab]	5.92 ± 0.43[a]
Total Alcohol (%)	0.74 ± 0.37[a]	0.91 ± 0.39[a]	1.21 ± 0.43[a]	2.59 ± 0.36[b]
Total Microbes (10^4 CFU/ml)	12.00 ± 7.58[b]	5.80 ± 3.65[a]	3.60 ± 2.10[a]	2.22 ± 1.66[a]
Antioxidant Activity (%)	89.55 ± 0.43	89.00± 0.63	88.63 ± 0.61	87.01 ± 2.67

Notes: Numbers followed by different superscripts show significant effects ($p<0.05$).

The increase of total acid value in Kombucha of Arabica Coffee Cascara was caused by an increase in the number of organic acids due to the metabolic process of the starter SCOBY consisting of various bacteria and *yeast* with sugar. This is supported by the opinion of Ayuratri and Kusnadi (2018) which states that the sucrose contained in Kombucha will run into a recast carried out by bacteria and yeast and will then be changed to organic acids such as acetic acid and gluconic acid. The formed acetic acid would be used to stimulate the growth of *yeast Saccharomyces cerevisiae* which could metabolize and produce alcohol. This is consistent with the opinion of Nur et al. (2018) which states that *Saccharomyces cerevisiae* can be stimulated by growth due to the intake of acetic acid produced by the bacterium *Acetobacter xylinum* during aerobic fermentation.

Total Sugar

Based on the test results presented in Table 1, the different fermentation length treatment in making Kombucha of Arabica Coffee Cascara had a significant effect ($p<0.05$) on the total sugar value of the product. The longer fermentation of Kombucha caused a decrease in total sugar value. This is consistent with the opinion of Simanjuntak et al. (2016) which states that the total sugar content of Kombucha will decrease along with the increase in fermentation time. The decrease in the total sugar of Kombucha of Arabica Coffee Cascara was caused by using sugar as the main nutrient for microbes. This is supported by the opinion of Puspitasari et al. (2017) which states that the sugar content in Kombucha serves as a carbon source of microbes that can be used to grow and metabolism. Sugar used by bacteria and yeast in the SCOBY starter to metabolize and convert to organic acids and alcohol. This is supported by the opinion of Suhardini and Zubaidah (2016) which states that the level of sugar in Kombucha which is shrinking is caused by the activity of yeast which remodels sugar to be converted into alcohol as well as the activity of bacteria which remodels glucose into gluconic acid.

Total Alcohol

Based on the test results presented in Table 1, the different fermentation length treatment in making Kombucha of Arabica Coffee Cascara had a significant effect ($p<0.05$) on the total alcohol value of the product. The longer fermentation of Kombucha made an increase in total alcohol value. This is consistent with the opinion of Simanjuntak et al. (2016) which states that the total value of alcohol contained in Kombucha products will increase with increasing fermentation time. The increase in the total alcohol of Kombucha of Arabica Coffee Cascara was caused by the metabolic processes carried

out by yeast on the SCOBY starter which converts sugar into alcohol. This is in accordance with the opinion of Insani et al. (2018) which states that yeast run in a fermentation process will metabolize and remodels glucose into alcohol.

Total Microbes

Based on the test results presented in Table 1, the different fermentation length treatments in the making of Kombucha of Arabica Coffee Cascara had a significant effect ($p<0.05$) on the total product microbes. The longer fermentation of Kombucha caused a decrease in the total microbes. This is consistent with the opinion of Wistiana and Zubaidah (2015) which states that the longer the fermentation time in Kombucha, the rate of microbial growth inhibited, and the amount decreased. A decrease in the rate of microbial growth in Kombucha of Arabica Coffee Cascara was caused by a decrease in the amount of sugar which is the main nutrient for microbes. This is supported by the opinion of Ayuratri and Kusnadi (2017) which states that along with the increase in fermentation time, sugar as a source of carbon for microbes will decrease in number due to the recast process by yeast to become organic acids so that microbes lose their source of nutrients to grow and the amount decreases. Some sugar, which was the main nutrient for bacteria and yeast during the fermentation process can also be turned into an inhibitor because it has been overhauled into organic acids and alcohol which contribute to the decrease in total microbes. This is consistent with the opinion of Goh et al. (2012) which states that the sugar content in Kombucha has the potential to be an inhibitor for microorganisms because sugar that has been turned into organic acids and alcohol can reduce the ability of microorganisms to metabolize.

Antioxidant Activity

Based on the test results presented in Table 1, the different fermentation length treatments in making Kombucha of Arabica Coffee Cascara did not show any significant effect ($p>0.05$) on the antioxidant activity of the product. The longer fermentation of Kombucha caused a decrease in the antioxidant activity. This is supported by the opinion of Kusuma and Fibrianto (2019) which states that the antioxidant activity in Kombucha will tend to decrease with increasing fermentation time. A decrease in the antioxidant activity of Kombucha of Arabica Coffee Cascara was caused by a decrease in acidity (pH) so that the solution has a more acidic atmosphere. Hassmy et al. (2017) states that acidic pH values can cause phenolic compounds to become more stable so that protons that can bind to DPPH are difficult to release and the antioxidant activity of kombucha decreases.

Hedonic Test of Aroma

Based on the test results presented in Table 2, the difference in fermentation time treatment had no significant effect ($p> 0.05$) on the aroma of Kombucha of Arabica Coffee Cascara. The results of the panelist score on the aroma attributes ranged from 3.20 to 3.32, which was included in the Normal (neutral) category. The longer the fermentation lasts, the stronger the aroma becomes. This is in accordance with the opinion of Mahadi et al. (2016) which states that the fermentation process that lasts longer can affect the aroma of Kombucha becoming more acidic. The aroma of the acid was thought to cause the panelists to score "normal" because the panelists are not yet familiar with the scent of Kombucha. The aroma of acid in Kombucha of Arabica Coffee Cascara was thought to originate from the content of organic acids and volatile alcohol. This is consistent with the opinion of Purnami et al (2018) which states that Kombucha has a distinctive sour aroma derived from the content of volatile compounds, such as acetic acid, alcohol, and other organic acids. The aroma of cascara as a raw material for Kombucha also influenced the result of the Kombucha aroma. Guntoro et al. (2019) expressed the opinion that the coffee cherry or cascara skin has a strong and distinctive aroma of fruit.

Color Hedonic

Based on the test results presented in Table 2, the difference in fermentation time treatment had a significant effect ($p<0.05$) on the color preference of Kombucha of Arabica Coffee Cascara. Panelists commented on the brown color seen in Kombucha. The color of Kombucha of Arabica Coffee Cascara was thought to come up due to the content of the polyphenol compound in cascara which was the raw material for the product. This is in accordance with the opinion of Yuwanti et al. (2018) which states that cascara contains several polyphenol compounds, one of them was anthocyanin which can give a color profile to the results of steeping. The brown color in Kombucha was thought to be due to the content of catechin compounds in cascara which is easily oxidized to produce theaflavin and thearubigin compounds as a determinant of steeping color. This is supported by the opinion of Towaha (2013) which states that catechin compounds are compounds that are easily oxidized and produce theaflavin and thearubigin compounds which can affect the color yield of steeping tea products.

Table 2. Scores of Hedonic Properties of Kombucha from Arabica Coffee Cascara

Hedonic Attributes	Treatments			
	2 days	4 days	6 days	8 days
Aroma	3.28 ± 0.84	3.20 ± 0.64	3.24 ± 1.16	3.32 ± 0.98
Color	3.72 ± 0.73^{ab}	3.56 ± 0.58^{b}	4.08 ± 0.81^{a}	3.36 ± 0.70^{b}
Sour Taste	2.96 ± 1.01^{a}	3.88 ± 0.92^{b}	3.60 ± 1.11^{b}	3.36 ± 0.99^{ab}
Overall	3.24 ± 0.83^{ac}	3.68 ± 0.55^{a}	3.68 ± 0.90^{ab}	3.00 ± 0.64^{c}

Notes: Numbers followed by different superscripts show significant effects ($p<0.05$).

Sour Taste Hedonic

Based on the test results presented in Table 2, the difference in fermentation time treatment had a significant effect ($p<0.05$) on the taste of Kombucha of

Arabica Coffee Cascara. Kombucha of Arabica Coffee Cascara has a stronger sour taste along with an increase in fermentation time. This is consistent with the opinion of Sukweenadhi et al. (2019) which states that the longer the fermentation time, the sour taste that arises will be stronger while the sweet taste will increasingly disappear. The sour taste in Kombucha of Arabica Coffee Cascara was thought to arise due to the presence of organic acids. This is supported by the opinion of Lestari et al. (2019) which states that the acid taste in Kombucha products is the effect of the content of various types of organic acids, such as acetic acid, lactic acid, gluconic acids and so on, which are formed during the fermentation process.

Overall Hedonic

Based on the test results presented in Table 2, the difference in fermentation time treatment had a significant effect ($p<0.05$) on the *overall* preference of Kombucha of Arabica Coffee Cascara. Panelists stated that the *overall* preferred Kombucha of Arabica Coffee Cascara was the 4 days and 6 days fermentation length treatment allegedly due to the acidic taste and the aroma which was not too strong so that it was still acceptable. This is consistent with the opinion of Pratama et al. (2015) which states that Kombucha received and favored by panelists has a taste and aroma not too acidic. Factors that could affect the level of panelist acceptance of the product include taste, aroma, and color. This is consistent with the opinion of Nur et al. (2018) which states that taste is one of the determining factors related to the acceptance of a product by panelists.

Conclusion

The length of the fermentation lasts, the total acid and total alcohol will increase but the total sugar, total microbial and antioxidant activity will decrease. The Kombucha of Arabica Coffee Cascara with 8 days fermentation time is the best treatment product because it has a strong sour aroma, brown color, strong sour taste, total acid of 0.29%, total sugar of 5.92° brix, total alcohol of 2.59% and antioxidant activity of 87.01%.

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