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Improving the quality of kombucha cascara with different varieties and fermentation time in diverse arabica coffee (*Coffea arabica L*) cultivars

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ABSTRACT

One of the innovations of cascara is the kombucha cascara which is made from the fermented coffee fruit skin, which is fermented with a kombucha starter or Symbiotic Culture of Bacteria and Yeast (SCOBY). The cascara used in this study was cascara from the *Timtim, Borbor,* and *Ateng super* coffee varieties. This study aims to determine the effect of different varieties of arabica coffee and fermentation time on the quality of kombucha cascara. This study used a factorial randomized block design method consisting of two factors. The first factor is cascara from coffee varieties consisting of three levels (*Timtim, Borbor, Ateng super*). The second factor is the length of fermentation, consisting of three levels (4, 8, and 12 days). Parameters analyzed were antioxidant activity, total phenol, tannin content, pH, and total microbe. The results showed that cascara from coffee varieties significantly affected antioxidant activity, and pH. Fermentation time significantly affects antioxidant activity, total phenol, tannin content, pH, and total microbial. The best treatment was obtained in the cascara treatment of the *Ateng super* coffee variety and the fermentation time was 12 days with the following characteristics; antioxidant activity 45.74%, total phenol 132,59 mg/L, tannin content 0.46%, pH value 2.69, and total microbes 4.99x104 CFU/ml.

Key words: Fermentation; cascara; coffee; kombucha.

1 INTRODUCTION

Kombucha is a fermented sweet tea solution using a kombucha microbial starter and several types of yeast known as kombucha mushrooms. Kombucha mushroom is a microorganism that has the form of a white to yellow gelatin sheet with a thickness ranging from 0.4 to 1.3 cm (Jayabalan et al., 2014) Kombucha tea is a fermented beverage that uses microorganisms, namely by utilizing kombucha starter cultures (*Acetobacter xylinum* and *Saccharomyces cerevisiae* and several other types of yeast). The use of this fermented drink has been carried out in several places, one of which has an impact on health as an antioxidant (Rosida; Sofiyah; Putra, 2021), anti-bacterial, and improves the resistance of the human body, which contains glucuronic acid so that it can neutralize toxins in the body (Cangussu et al., 2020). In particular, kombucha is a type of fermented tea that is added with sugar and natural bio-compounds produced from the Symbiotic Culture of Bacteria and Yeast (SCOBY), which can make bio-compounds as microorganisms, including fungi that spread evenly on the surface of kombucha, can decompose sugar molecules into various acids such as *acetic, carbonate, chondroitin sulfate*, *folate, gluconate, glucocorticoid, lactate*, simple sugar compounds, vitamins, alcohol and multiple *polyphenols* with strong antioxidant effects (Muzaifa et al., 2021).

The product that has been researched and has the potentially to be developed is kombucha from cascara. Kombucha is a traditional fermented sweet tea obtained from the infusion of tea leaves by fermenting the symbiotic association of acetic acid bacteria and yeast. According to (Muzaifa et al., 2021) kombucha is made from cascara by varying the fermentation time. The resulting kombucha cascara is relatively the same as the original kombucha. The result of 8 days of fermentation of kombucha cascara is better chemically, but in taste, 12 days of fermented kombucha is preferred. Kombucha uses raw materials from Gayo cascara Arabica coffee and is commercialized as Gayo kombucha.

One of the cascara innovations is kombucha cascara fermented from coffee fruit skin with kombucha starter or SCOBY (Symbiotic Culture of Bacteria and Yeast). Kombucha cascara is a product that can prevent cell aging, prevent cancer, and contain antioxidant compounds. The kombucha cascara fermentation process is carried out at room temperature ranging from 4-to 12 days. Kombucha cascara has a unique taste, a tea-like color, rich in aromatic compounds, a sweet-sour taste, and a fragrant aroma (Prono-Widayat et al., 2021).

2 MATERIAL AND METHODS

The tools used in the analysis consisted of the incubator (Eyela LSI-170 D), colony counter (CC30), dropper, petri dish (CMSI), measuring cup (ohyo JL-180), and test tube (pyrex), vortexed (D-Lab MX-S), volumetric flasks (Pyrex), spectrophotometer (UV-I900i) In making kombucha, cloth napkins, stoves, pans, rubber bands, drying tarpaulin(drying direct in the sun) filters, trays, analytical scales (Kern ABT 220-4M), coffee pulper machines (CP-100), and glass jars.

The materials to be that was used in this study consisted of coffee husks 1 kg par each (*Timtim, Borbor, and Ateng super*), which were picked themselves from Sukaramai, Bener Meriah Regency, Indonesia, at an altitude of 1,400 m.a.s.l, kombucha seeds, Merck brand PCA (Plate Count Agar) media, distilled water and water, 0.1 M FeCl3, 3.8 ml DPPH (Aldrich D9132-IG) 0.1 Mm, 0.3 ml $K_3Fe(CN)_6$ 0.008 M, and ethanol (absolute for analysis).

2.1. Experimental design

This study aims to determine the effect of different varieties of Arabica coffee and the duration of fermentation on kombucha cascara's Physico-chemical and sensory characteristics. Cascara from the coffee variety used was V1: cascara from the Timtim variety, V2: cascara from the Borbor variety, and V3: cascara from the Ateng Super variety. The length of fermentation carried out is was F1: 4 days, F2: 8 days, and F3: 12 days. An analysis using the Multivariat Analysis of Variance (MANOVA) is carried out from the research results. If the results obtained have a significant effect, it is necessary to conduct further tests using the Duncan.

2.2. Research procedure

1. *Cascara making:* (Muzaifa et al., 2021) The coffee cherries from the *Timtim, Borbor* and *Ateng super varieties* (1 kg each variety) were sorted by separating from the damaged and young fruit; the chosen samples were taken were ripe and red fruit. The coffee cherries are washed with running water and sorted, and the coffee beans. The coffee cherries are then pulped with a coffee pulper machine to produce pulp (± 380 g for each variety). The pulp is placed in a winnowing tray and dried in the sun for ± 4 days for 7 hours (9 am to 4 pm). The dried pulp is called known as cascara

2. Making kombucha cascara: (Lestari et al., 2019) Take one liter of water and then First boiled it a litter of water to 100 degrees Celsius. Once the water was boiling, added 10 grams of cascara (for each treatment). An then Cascara was left for 15 minutes at room temperature with hot water added. The remaining pulp from the cascara is filtered, then added 100 grams of sugar and stir until dissolved. Cascara tea containing sugar is cooled to room temperature (20-25 °C). Then the cascara tea is put into a sterilized glass bottle. Put the kombucha seeds (5% of the steeping weight) into a glass bottle. The glass bottles were covered with cloth and tied with rubber. Fermentation was carried out at room temperature for 4, 8, and 12 days, Figure 1.

2.3. Analysis

Antioxidant Activity: (Guezzane et al., 2021) Determination of antioxidant activity begins with 0.1 ml of a sample taken and 5 ml of methanol pa added, then vortexed and 4 ml taken. Then added DPPH 1 ml 0.2 mM in methanol, vortexed, and incubated in a dark room for 30 minutes and obtained absorbance at a wavelength of 517 nm. Antioxidant activity is determined by the amount of DPPH radical absorption inhibition by calculating the percentage of DPPH absorption inhibition (absorbance blank) using the f100%

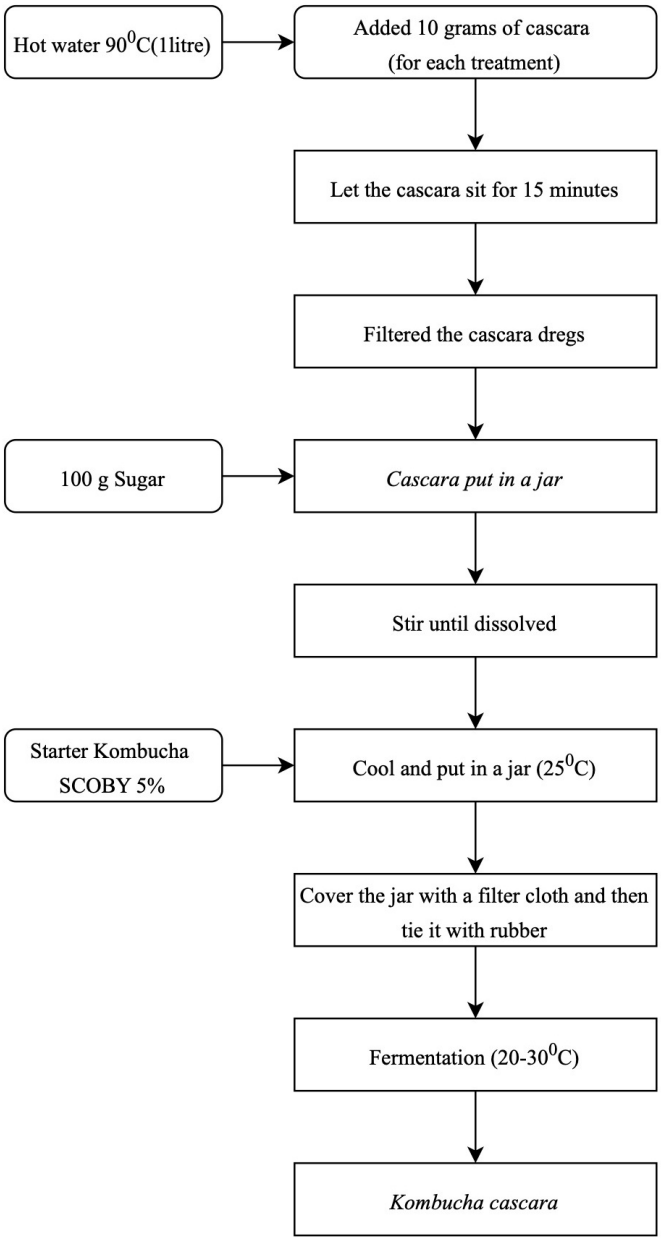

Figure 1: Diagram process kombucha.

Total Phenol: (Guezzane et al., 2021; Guiné et al., 2020) Measurement of total phenol was carried out using Folin-Ciocalteu reagent and gallic acid as standard. The measurement begins with preparing 9 ml of the folic solution to which 90 ml of distilled water is added and homogenized. And then, a gallic acid solution of 0.010 grams was designed, added to 100 ml of distilled water, and homogenized. Then, 6 grams of Na_2CO_3

solution was prepared, added to 100 ml of distilled water, and standardized. Spectrophotometry is turned on; wait 30 minutes.

The next step is to prepare 6 jars and 5 ml volumetric flasks to make a standard solution.

The 1st measuring flask was filled with 10 ml of distilled water, the 2nd measuring flask was filled with 2 ml of gallic acid and 8 ml of distilled water, the 3rd measuring flask was filled with 4 ml of gallic acid and 6 ml of distilled water, the 4th measuring flask was filled with 6 ml gallic acid and 4 ml aquadest, the 5th measuring flask was filled with 8 ml gallic acid and 2 ml aquadest, and the 6th measuring flask was filled with 10 ml gallic acid then each flask was vortexed for 30 seconds.

The standard solution was put into a UV-Vis spectrophotometer, calibrated to 760 nm. Then read the absorbance. The sample measurement was carried out by taking 0.2 ml of the sample using a micropipette and putting it into a 5 ml volumetric flask covered with aluminum foil, then adding 1.8 ml of folic and 1.8 Na_2CO_3 then vortexed for 30 seconds. Put into a cuvette and read the total phenol on a spectrophotometer with a wavelength of 760 nm. Entire phenol content was calculated using the linear regression equation of gallic acid, y = ax+b.

Tannin content: Take one ml of cascara tea and mix it with distilled water in an 18 x 150 mm test tube. Add 0.3 ml of 0.1 M $FeCl_3$, then shaken, and 0.3 ml of $K_3Fe(CN)$ 0.008 M, then shaken and let stand for 10 minutes. The measurement results (absorbance value) and samples were determined using a UV-VIS spectrophotometer at a wavelength of 720 nm. Absorbance value of blank = 0.625.

pH value: Measurement of pH (degree of acidity) with a pH meter calibrated with buffer solutions pH 4 and 7 before use. The sample was measured by taking 20 ml of the sample; then, the electrode was rinsed with distilled water, dried with a tissue, and then immersed in the sample. The electrode is left immersed for some time. The value that is read is a stable pH value.

Total Microbes: one ml of material is taken and then put into a test tube, and 9 ml of distilled water is added and stirred until evenly distributed. The results of this dilution are taken 1 ml and then added to 9 ml of distilled water. The effects of this dilution were taken with a dropper of 1 ml and added with 9 ml of distilled water. From the dilution results, one ml was taken and put into a test tube, and then 9 ml of distilled water was added. The dilution was carried out up to 10000 times (10^4). From the dilution results in the last test tube, one ml was taken and flattened on PCA agar medium, which had been prepared on a petri dish, then incubated for 24 hours at 32°C in an inverted position. The colony counter counts the number of colonies that exist.

3 RESULTS

Data analysis was carried out using SPSS version 25. This study was Analyzed using Multivariat Analysis of Variance (MANOVA) and if there was a difference, it was tested using Duncan's test. Data generated for antioxidants can see in Table 1, total Phenol in Table 2, tannin content in Table 3, pH in Table 4, and result of total microbe in Table 5.

The highest antioxidant value was found at 8 days higher than 4 and 12 days, this data can be seen in Table 1.

Table 1: Result of antioxsidant content (%).

Varieties	Fermentation time	Mean	Std. Deviation	N
Timtim	4 days	29.2967	0.1950	3
	8 days	43.4867	0.3001	3
	12 days	43.0300	0.2252	3
	Total	38.6044	6.9868	9
Borbor	4 days	31.5100	0.4928	3
	8 days	59.1167	0.3001	3
	12 days	54.9467	0.2957	3
	Total	48.5244	12.8920	9
Ateng Super	4 days	40.3000	0.5957	3
	8 days	51.8867	0.3001	3
	12 days	45.0533	0.4067	3
	Total	45.7467	5.0592	9
Total	4 days	33.7022	5.0560	9
	8 days	51.4967	6.7793	9
	12 days	47.6767	5.5293	9
	Total	44.2919	9.6003	27

The total phenol value produced is very high at 12 days and the data generated from various varieties is generally the same value produced in total at 12 days is 132 mg/L, this data can be seen in Table 2.

Table 2: Result of total phenol (mg/L).

Varieties	Fermentation time	Mean	Std. Deviation	N
Timtim	4 days	55.8167	5.0083	3
	8 days	107.1500	29.0646	3
	12 days	137.1500	10.8282	3
	Total	100.0389	38.9301	9
Borbor	4 days	60.9833	5.2042	3
	8 days	86.8167	8.6072	3
	12 days	140.3167	5.8595	3
	Total	96.0389	35.5218	9
Ateng Super	4 days	68.3167	3.7859	3
	8 days	98.8167	1.2583	3
	12 days	120.3167	3.4034	3
	Total	95.8167	22.7802	9
Total	4 days	61.7056	6.7982	9
	8 days	97.5944	17.5632	9
	12 days	132.5944	11.2901	9
	Total	97.2981	31.9085	27

The tannin content produced in the existing coffee types, it can be seen that the fermentation process greatly affects the value of tannins produced, the longer the fermentation process, the more the tannin value in kombucha will decrease. This shows the value of tannin content at 12 days decreased to 0.46%, this can be seen in Table 3.

Table 3: Result of tannin content (%).

Varieties	Fermentation time	Mean	Std. Deviation	N
Timtim	4 days	0.6567	0.0416	3
	8 days	0.5600	0.0265	3
	12 days	0.4567	0.0737	3
	Total	0.5578	0.0973	9
Borbor	4 days	0.6200	0.0755	3
	8 days	0.4800	0.0400	3
	12 days	0.4567	0.0153	3
	Total	0.5189	0.0880	9
Ateng Super	4 days	0.5800	0.0625	3
	8 days	0.5000	0.0200	3
	12 days	0.4667	0.0153	3
	Total	0.5156	0.0606	9
Total	4 days	0.6189	0.0627	9
	8 days	0.5133	0.0444	9
	12 days	0.4600	0.0387	9
	Total	0.5307	0.0825	27

The resulting pH value shows no significant change, but the total value produced is the same as the length of the fermentation process at 8 days, namely 2.8167, this can be seen in Table 4.

The total number of microbes analyzed was not significant, indicating the same number compared to the variety, length of fermentation and treatment. In general, the longer, the higher the microbial value produced. Can see in Table 5.

The results of the data above which are processed using MANOVA, which are significantly different, will be displayed after being tested with the Duncan test. In this study, the antioxidant value and pH had significantly different values and conversely data that did not differ were not shown. Tests on the length of fermentation on kombucha produced antioxidant values, total phenol, tannin levels, pH and total microbes had significant differences and the Duncan test was tested. Data in Tables 6, 7, 8, 9, 10, 11 and 12 show data on the real effect of the analysis conducted. The level of influence produced shows the number of differences in the varieties and length of fermentation produced.

The effect of antioxidant and the resulting varieties have an influence between the two, a significant value level of 1.000.

Table 4: Result of pH Value.

Varieties	Fermentation time	Mean	Std. Deviation	N
Timtim	4 days	3.0667	0.029	3
	8 days	2.8733	0.046	3
	12 days	2.7133	0.038	3
	Total	2.8844	0.157	9
Borbor	4 days	2.9367	0.012	3
	8 days	2.6600	0.087	3
	12 days	2.6233	0.065	3
	Total	2.7400	0.158	9
Ateng Super	4 days	3.1033	0.040	3
	8 days	2.9167	0.012	3
	12 days	2.7467	0.006	3
	Total	2.9222	0.156	9
Total	4 days	3.0356	0.080	9
	8 days	2.8167	0.129	9
	12 days	2.6944	0.067	9
	Total	2.8489	0.171	27

Table 5: Result of total microbe (10^4 CFU/ml).

Varieties	Fermentation time	Mean	Std. Deviation	N
Timtim	4 days	3.8800	0.106	3
	8 days	4.1733	0.046	3
	12 days	4.5200	0.212	3
	Total	4.1911	0.303	9
Borbor	4 days	4.0800	0.212	3
	8 days	4.0667	0.257	3
	12 days	5.4800	128.561	3
	Total	4.5422	0.967	9
Ateng Super	4 days	4.0400	0.200	3
	8 days	4.3067	0.197	3
	12 days	4.9867	0.311	3
	Total	4.4444	0.472	9
Total	4 days	4.0000	0.180	9
	8 days	4.1822	0.194	9
	12 days	4.9956	0.788	9
	Total	4.3926	0.638	27

The ph value with the number of samples 9 also affects the resulting value, the ph value in the timtim and ateng super varieties shows the same value. But in the borbor variety the resulting value is smaller, and different from both

The length of the fermentation process greatly affects the antioxidant value produced, this is shown in Table 8. Significant value reached 1.00. The effect of fermentation time

on total phenol (Table 9), tannin (Table 10), pH (Table 11) and total microbes (Table 12) shows that the length of fermentation is very influential on the results found, in general the longer the fermentation process, the higher the value produced.

Table 6: Effect antioxidant content and varietas.

Duncan[a,b]

Varieties	N	Subset		
		1	2	3
Timtim	9	38.6044		
Ateng Super	9		45.7467	
Borbor	9			48.5244
Sig.		1.000	1.000	1.000

Table 7: Effect ph value and varietas.

Duncan[a,b]

Varieties	N	Subset	
		1	2
Borbor	9	2.7400	
Timtim	9		2.8844
Ateng Super	9		2.9222
Sig.		1.000	.091

Table 8: Effect antioxidant content and Fermentation time.

Duncan[a,b]

Fermentation time	N	Subset		
		1	2	3
4 days	9	33.7022		
12 days	9		47.6767	
8 days	9			51.4967
Sig.		1.000	1.000	1.000

Table 9: Effect total Phenol and fermentation time.

Duncan[a,b]

Fermentation time	N	Subset		
		1	2	3
4 days	9	61.7056		
8 days	9		97.5944	
12 days	9			132.5944
Sig.		1.000	1.000	1.000

The highest phenol value was found at 12 days, the longer the fermentation process, the higher the phenol value produced.

The tannin value of the fermentation process, the longer the fermentation process, the less tannin value will be produced.

Table 10: Effect tannin content and fermentation time.

Duncan[a,b]

Fermentation time	N	Subset		
		1	2	3
12 days	9	0.4600		
8 days	9		0.5133	
4 days	9			0.6189
Sig.		1.000	1.000	1.000

Table 11: Effect pH Value and fermentation time.

Duncan[a,b]

Fermentation time	N	Subset		
		1	2	3
12 days	9	2.6944		
8 days	9		2.8167	
4 days	9			3.0356
Sig.		1.000	1.000	1.000

Table 12: Effect total microbes and fermentation time.

Duncan[a,b]

Fermentation time	N	Subset	
		1	2
4 days	9	4.0000	
8 days	9	4.1822	
12 days	9		4.9956
Sig.		.423	1.000

The pH value produced is similar to the tannin value in Table 10, the longer the fermentation process, the lower the pH value produced

The number of microbes produced in this study shows that the longer the fermentation process, the higher the total microbial value produced, this can be seen in Table 12.

4 DISCUSSION

4.1 Antioxidant Activity

Antioxidants are substances or compounds that have the ability to inhibit, delay or prevent the oxidation process of other easily oxidized materials (Prono-Widayat et al., 2021) Polyphenol compounds in coffee function as antioxidants through the primary antioxidant mechanism, namely breaking the chain of the oxidation process (Palente; Suryanto; Momuat, 2021; Santoso, 2016) The results showed that the percentage of free radical inhibition in kombucha cascara resulted in antioxidant activity ranging from 29.10 to 59.37%, with an

average of 44.29%. Analysis of variance showed that the cascara of the coffee variety, the fermentation time, and the interaction of the two had a very significant effect (P≤0.05) on the antioxidant activity of kombucha cascara.

According the results in table 6. The effect of antioxidants with coffee varieties showed that the *timtim, ateng super* and *borbor* varieties produced very significant differences and these three varieties produced significantly different values. The amount of antioxidants from these three varieties is very different, this shows the variety can affect the amount of antioxidants.

The length of fermentation affects antioxidants, on 4 days it produces 33% and when on day 8 the resulting value increases (51%), after that the amount of antioxidants will decrease, this is due to the influence of the fermentation process on the microorganisms present in kombucha, so the value obtained produced will be curved according to the growth of bacteria.

The antioxidant activity of kombucha cascara at 8 days of fermentation tends to increase compared to 4 days of fermentation. At 8 days of fermentation, metabolism occurs in kombucha culture microbes, which results in increased antioxidant activity in kombucha.

According to research by (Suhardini et al., 2016), at 8 days of fermentation, the antioxidant activity of kombucha will increase. According to (Battikh et al., 2013; Goh et al., 2012; Santos-Buelga et al., 2019; Xia et al., 2019), the metabolism of microorganisms during fermentation resulted in an increase in antioxidant activity in kombucha. The antioxidant activity of kombucha cascara tends to decrease at 12 days of fermentation. This is in line with (Puspitasari; Ririn, 2017) that the antioxidant activity of kombucha experienced an optimum point on the 7 day of fermentation and would decrease with increasing fermentation time (Nurikasari et al., 2017). The antioxidant activity of the cascara produced tends to be lower due to the drying process. As the drying process progresses, the antioxidant compounds are damaged or degraded in the cascara.

4.2 Total Phenol

The results of the total study phenol in kombucha cascara ranged from 55.81 mg/L-140.31 mg/L with an average of 97.29 mg/L. Analysis with MANOVA showed that total phenol had no effect on cascara varieties, this was because phenolic compounds did not affect the types of varieties produced. However, the total phenol value is very influential on the length of the fermentation process. The longer the fermentation process, the phenol compounds produced will increase, this is because phenol compounds can be formed during the fermentation process.

According to table 9 shows that the higher total phenol was obtained at 12 days of fermentation (132.59 mg/L) which was different from 8 days of fermentation (97.59 mg/L) and 4 days (61.71 mg/L). From Figure 2, it can be seen that the total phenol in kombucha cascara increased from 4 to 12 days of fermentation. This is presumably due to the formation of phenolic compounds that degrade matrix components due to enzyme activity obtained by SCOBY microbes in the kombucha (Bhattacharya et al., 2011). The enzymatic activity of yeast and bacteria in kombucha culture causes the formation of phenolic compounds derived from the degradation of the matrix of other components (Primurdia; Kusnadi, 2014). Some phenolic compounds will increase in the fermentation process because microorganisms such as *Saccharomyces cerevisiae* in kombucha can decarboxylate ferulic acid and cinnamic acid (Pebiningrum; Kusnadi, 2018).

The results of the total study phenol in cascara ranged from 29.90 mg/L – to 42.90 mg/L with an average of 35.82 mg/L. The higher total phenol was found in cascara from the Ateng Super coffee variety (42.90 mg/L), followed by cascara from the Borbor coffee variety (34.65 mg/L) and cascara from the Timtim coffee variety (29.90 mg/L). L). Analysis of total phenol in cascara has a low average compared to total phenol in kombucha cascara. This shows that the processing of cascara by fermentation into kombucha increases the total phenol bound to increase its functional properties (Muzaifa et al., 2021). The total phenol in the cascara produced tends to be low due to the oxidation process due to heat treatment during the drying process for the cascara under the sun.

4.3 Tannin content

The results showed that the tannin levels in kombucha cascara ranged from 0.45% -to 0.65%, with an average of 0.53%. The results of the Manova test on Duncan's test showed that the tannin content had no significant effect on the variety.

The result of fermentation time on tannin levels can be seen in Table 10. The tannin levels in kombucha cascara decreased with the longer fermentation. At 4 days of fermentation, the highest tannin content (0.62%) was obtained. Along with the fermentation process, the tannin content decreased continuously. Tannin content decreased to (0.51%) at 8 days of fermentation, then at 12 days of fermentation decreased to (0.46%). According to (Prono-Widayat et al., 2021), the content of tannins fell as the fermentation process took place because microbes degraded the tannins. Several bacteria contain tannin *acyl hydrolase or tannase*, which can break the molecular bonds in tannins. Tanase is an enzyme that acts as a catalyst in the hydrolysis reaction of tannins and can be produced by yeast, fungi, and bacteria. The tannin content during fermentation will decrease due to the physical and chemical treatment during the processing of kombucha cascara into steeping. The use of high temperatures can reduce the tannin content in kombucha cascara because it can accelerate the process of breaking the ester bond in hydrolyzed tannins.

The study results showed that the tannin levels in cascara ranged from 1.30%-1.44%, with an average of 1.38%. The higher tannin content was found in cascara from the Ateng Super coffee variety (1.44%), followed by cascara from the Borbor coffee variety (1.39%) and cascara from the Timtim coffee variety (1.30%). One factor influencing the level of tannins in cascara is the amount of extract in the brewing water. Tannins are closely related to the steeping taste, which can give a bitter taste to the brew (Azizah et al., 2019). Tannins also play a role in forming brown color and astringent taste in steeping cascara.

4.4 pH value

The study results of the pH value in kombucha cascara ranged from 2.56-to 3.15, with an average of 2.84. Analysis of variance showed that cascara from coffee varieties and duration of fermentation had a very significant effect ($P \leq 0.01$), and the interaction of the two had a significant impact ($P \leq 0.05$) on the pH value of kombucha cascara. The interaction effect of the cascara treatment of coffee varieties (V) and the length of fermentation (F) on the pH value can be seen in Figure 11.

pH has no effect on the variety of coffee produced, but does affect the length of fermentation. The length of the fermentation process greatly affects the pH level of kombucha, this is tested with Duncan showing the longer the fermentation process, the higher the resulting pH value, this data can be seen in table 11.

The highest pH value was obtained in the cascara treatment of the timtim and ateng super coffee varieties. The fermentation time was 4 days (3.035 – 3.10), significantly different from cascara from the Borbor coffee variety. The fermentation time was 12 days (2,69). This is presumably because the acid will be formed by the bacteria *Acetobacter xylinum* during the fermentation process. The pH of kombucha will decrease due to the increase in the concentration of acetic acid and the release of protons in the dissolved acetic acid.

The kombucha fermentation process will also produce other organic acids that can cause a decrease in pH. Furthermore, the reduction in the pH value is also because fermentation will support the growth of Acetobacter xylinum bacteria to fulfill its metabolic activity. To release free protons that lower the pH of the dissolved acetic acid solution, it will separate (Suhardini; Zubaidah, 2015; Wistiana; Zubaidah, 2015). According to (Pratiwi; Aryawati, 2012) an increase in the acid content of a material will decrease the pH value.

Several factors that affect the acidity of cascara are the use of the type of coffee and the processing process (Cangussu, L. B. et.al. 2020). In this study, using a uniform kind of coffee, namely Arabica coffee, but different varieties of Arabica coffee used can affect the cascara's pH value.

4.5 Total Microbes

The results of the comprehensive microbial analysis ranged from $3.88x10^4$ CFU/ml – $5.48x10^4$ CFU/ml, with a mean of 4.39x104 CFU/ml. Analysis of variance showed that the cascara of the coffee variety and the interaction of the two treatments had no significant effect ($P \geq 0.05$). In contrast, the length of fermentation (F) had a very significant effect ($P \leq 0.01$).

There was no significant difference in the total number of microbes in varieties, this is the Duncan test showed no difference at all. However, the fermentation process showed that 4 days and 8 days showed no difference at all, but changes occurred at 12 days. According to (Wistiana; Zubaidah, 2015), the total number of microbes increased from 4 days to 12 days. The increase in total kombucha cascara microbes is because kombucha contains soluble solids such as caffeine, amino acids, and sugar. These substances are used as a source of energy and nutrients to increase the development of microbes. The fermented kombucha culture showed higher antimicrobial and antioxidant activity than the unfermented infusion (Vitas et al., 2018)

5 CONCLUSION

Factors of variety and duration of fermentation have a significant effect on the analysis conducted in this study. The varietal factor is very influential on antioxidants and pH. The length of fermentation carried out significantly affected antioxidants, total phenols, tannins, pH and total microbes. the results of the study showed that the best treatment was obtained in the cascara treatment of the Ateng Super coffee variety and the fermentation time of 12 days with the following characteristics; antioxidant activity 45.67%, total phenol 132.59 mg/L, tannin content 0.46%, pH value 2.69, total microbes 4.99x104 CFU/ml.

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7 AUTHORS' CONTRIBUTION

IS wrote the manuscript and performed the experiment, SR supervised the experiment and co-worked the manuscript, and IS reviewed and approved the final version of the work, M conducted all statistical analyses.

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