

The Sensory of Kombucha Cacao Pulp (*Theobroma cacao*) Fermented Using Symbiotic Culture of Bacteria and Yeast (Scoby) as a Starter

Neti Yuliana^{1*}, Gusrianti Wulan Sari¹, Endang L. Widiastuti², Suharyono¹, Teguh Setiawan¹

¹ Department of Agricultural Product Technology (THP), Faculty of Agriculture, University of Lampung
Jl. Sumantri Brojonegoro No 1, Gedung Meneng, Bandar Lampung, Indonesia

*Correspondence : E-mail: neti.yuliana@fp.unila.ac.id, Phone +62-081540817199

² Department of Biology, Faculty of Math and Science, University of Lampung
Jl. . Sumantri Brojonegoro No 1, Gedung Meneng, Bandar Lampung, Indonesia

ABSTRACT

The sensory of kombucha cocoa pulp during fermentation using bacteria-yeast symbiosis as a starter was evaluated. This study used a completely random block design (CRBD), one factor with five levels of fermentation time (0-8 days). Observation included color, aroma, taste, and overall acceptance of kombucha. The data obtained were statistically analyzed using ANOVA followed by LSD at a 5% level. The results showed that the fermentation time levels significantly affected the sensory and acceptance of kombucha pulp-cacao. Among samples, the panelists preferred the kombucha drink, which was fermented for four days, with a description of a slightly sour sweet taste, slightly cloudy cream color, and a moderate level of preference. This fair amount of preference indicates that the sensory kombucha pulp of cocoa still needs to be developed to bind its sensory acceptance so that consumers are interested and get the health benefits of kombucha pulp-cocoa

Keywords: *cocoa pulp, fermentation, kombucha, sensory, scoby .*

ABSTRAK

Sensori kombucha pulpa kakao selama fermentasi menggunakan starter simbiosis bakteri-khamir telah dievaluasi. Penelitian ini menggunakan Rancangan Acak Kelompok (CRBD) satu faktor dengan lima taraf waktu fermentasi (0-8 hari). Pengamatan meliputi warna, aroma, rasa, dan daya terima kombucha secara keseluruhan. Data yang diperoleh dianalisis secara statistik menggunakan ANOVA dilanjutkan dengan LSD pada taraf 5%. Hasil penelitian menunjukkan bahwa waktu fermentasi berpengaruh nyata terhadap sensoris dan daya terima kombucha pulp-cacao. Di antara sampel, panelis lebih menyukai minuman kombucha yang difermentasi selama empat hari, dengan deskripsi rasa manis agak asam, warna krem agak keruh, dan tingkat kesukaan sedang. Preferensi yang dengan tingkat sedang ini menunjukkan bahwa kombucha pulpa kakao secara sensorik masih perlu dikembangkan untuk meningkatkan daya terima sensorisnya agar konsumen tertarik mendapatkan manfaat kesehatan dari kombucha pulp-cocoa.

Katakunci: *pulpa kakao, fermentasi, kombucha, sensori, scoby .*

INTRODUCTION

Cocoa is a quite superior agricultural commodities in Indonesia. Lampung is one of the provinces in Indonesia where cocoa production is increasing by year and in 2019 this province reached the 5th largest position in Indonesia (1). As cocoa production increases so does the amount of waste from processing cocoa beans. Various waste products are generated during the processing of cocoa pods, such as cocoa

pulp. This substance is a favorable substrate for fermentation since it contains a lot of glucose (4.58 0.12% w/v) and fructose (3.25 0.03% w/v) (2).

One of the fermented drinks that is quite well known at this time is "Kombucha". According to Coelho et al. (3), the presence of probiotic microbes, antibiotics, amino acids, polyphenols, sugars, organic acids, water-soluble vitamins, and different micronutrients created during fermentation are what give

kombucha its positive effects. Kombucha is a sweet, slightly sour drink made by fermenting tea with sugar through a symbiotic culture of bacteria and yeast (SCOBY) (4). These symbiotic cultures are Acetobacter, Lactobacillus, and Yeast (5), which are linked in a symbiotic relationship in the production of metabolites during kombucha fermentation (6).

Currently, there is very little research on kombucha made from cacao waste. Because the substrates used in the preparation of fermented beverages affect the properties of the beverage, cacao pulp kombucha is undoubtedly a different sensation of kombucha beverages. The aim of this study was to evaluate the organoleptic properties of cocoa pulp during kombucha fermentation.

MATERIAL AND METHODS

Cocoa Pulp Juice and Starter

The cocoa pulp juice was sourced from a group of cocoa farmers in Tanggamus, a notorious cocoa growing region in Lampung, Indonesia. The starter used for fermentation was commercial SCOBY.

Kombucha Production

Media preparation for kombucha manufacturing was done following Yuliana et al. (7). Granulated sucrose was added to filtered cocoa pulp juice after diluting it with water at a ratio of 1:20 (v/v) to bring the Brix to about 12. The juice was then pasteurized for 10 minutes at a temperature of 95 to 100°C. The fermentation jar was filled aseptically with the medium, which was then left to stand at room temperature. Then, 3% w/v SCOBY was added to the pasteurized cocoa pulp and allowed to ferment for eight days at room temperature. Every two days, samples were obtained to assess the

sensory quality of the kombucha beverage. The fermented kombucha beverage was pasteurized at 80°C for 10 minutes before the sensory evaluation.

Sensory Analysis

A sensory test was performed using a hedonic test and an evaluation (scoring) test. The sensory test was conducted by a total of 25 semi-skilled panelists, agricultural technology students from the Faculty of Agriculture, Lampung University. Samples are randomly presented to subjects in coded containers. Attributes assessed for scoring test included aroma, color, taste and 5 liking score for hedonic acceptance.

Experiment Design and Statistical Analysis

The CRBD was employed in this investigation, which had 4 replicates and 5 phases of fermentation duration (0, 2, 4, 6, and 8 days). Data variance was analyzed to determine if there was an effect between treatments. The BNT test at the 5% level was used to further test.

RESULT

Color and Acid Aroma

The results showed that the length of fermentation significantly affected color and the sour aroma scoring of kombucha made with scoby as a starter (Table 1). The kombucha color score ranged from 2.33 (slightly cloudy white) to 3.54 (slightly cloudy brown).

Table 1. Scoring of color, and aroma of kombucha at different fermentation time

Time (day)	Score	
	Color	Aroma
0	2.33±0.09 ^c	1.29±0.09 ^d
2	2.35±0.10 ^c	2.27±0.15 ^c
4	3.22±0.14 ^b	3.15±0.05 ^b
6	3.22±0.07 ^b	3.22±0.11 ^b

8	3.54±0.15 ^a	3.70±0.12 ^a
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The means followed by the same letter in a column do not differ significantly (LSD test; $p>0.05$).

Score of color: 1=Cloudy white, 2= Slightly cloudy white, 3 = Slightly cloudy cream, 4 =Slightly cloudy brown, 5= Slightly clear brown.

Score of aroma: 1= slightly acid; 2= acid; 3= very acid, 4 = very acid with slightly alcohol aroma, 5= very acid with alcohol aroma.

Taste Description of Cocoa Pulpa Kombucha Drink

The length of fermentation influenced the description of the taste of kombucha drinks with a range of sweet, sour, and slightly alcoholic by panelists (Table 2).

Table 2. Description of the taste of kombucha pulp according to panelist.

Taste	Time (day)				
	0	2	4	6	8
Sweet	67.00 %	31.00 %	3.00%	1.00%	0.00%
Slightl y acid	33.00 %	55.00 %	52.50 %	10.00 %	0.00%
Acid	0.00%	9.00%	22.00 %	36.50 %	17.75 %
Very acid	0.00%	0.00%	3.00%	34.00 %	37.50 %
Slightl y alcohol ic	0.00%	0.00%	0.00%	0.00%	32.75 %

Description of taste in cocoa pulp kombucha drink 0 days of fermentation was dominated by sweet taste (67%) with a little sour (33%). During fermentation, the description of sweetness decreased; for example, on the second day of fermentation, the panelist described a slightly sour taste of as much as 55%, and sweetness reduced to 31%. In 4 days of fermentation, the number of panelists who described sour taste increased, and 3% of panelists even detected very sourly. In

kombucha, after six days of fermentation, more and more panelists detected a very sour taste which reached 36%. In the 8-day fermentation, panelists who described it as very acidic were getting more and more and began to detect a slightly alcoholic taste.

Overall Acceptance of Kombucha Cacao Pulp Drink

The overall acceptance score of the kombucha ranged from 2.76 (neither like nor dislike) to 3.92 (like slightly) was significantly affected by fermentation time. The decrease in the overall acceptance score of kombucha is directly proportional to the length of fermentation time (Table 3). The percentage of panelists' preference for the color, aroma, and taste of kombucha pulp can be seen in Table 4. It can be seen from the table that the panelists preferred the four days fermented kombucha and the control (day 0). These probably due to these two kombuchas have a distinctive taste and aroma

Table 3. Skoring of overall acceptance of kombucha at different fermentation time.

Time (day)	Acceptance
0	3.92±0.09 ^a
2	3.82±0.04 ^b
4	3.84±0.00 ^{ab}
6	3.02±0.08 ^c
8	2.76±0.00 ^d

The means followed by the same letter in a column do not differ significantly (LSD test; $p>0.05$). Score of acceptance: 1= dislike very much; 2= dislike moderately 3= neither like nor dislike, 4= like slightly 5: very like.

Table 4. Panelist Responses to Kombucha's Preference of Color, Aroma, and Taste

Parameter	Time (day)				
	0	2	4	6	8
Color	49%	14%	23%	6%	8%
Aroma	44%	11%	31%	9%	5%
Taste	39%	22%	29%	6%	4%

DISCUSSION

Color and Aroma

Along with the length of fermentation time, there was an increase in the color score of kombucha. On the second day of fermentation, the kombucha color score was 2.35 (slightly cloudy white) and increased on the fourth day of fermentation, which was 3.22 (slightly cloudy white cream), and on the eighth day, the score became 3.54 (creamy white, slightly cloudy brown) (figure 1). The color of the resulting kombucha will be browner, but the turbidity level will decrease and becomes more apparent. Microbial fermentation utilized total dissolved solids as energy so that over time the dissolved solids causing turbidity in the media will decrease, and the liquid will become more apparent. In addition, during fermentation, the polyphenol oxidase enzyme produces an enzymatic browning reaction (Macedo et al., 2016) so that the color of kombucha drinks turned from cream to brown. The dark color changes of the medium during fermentation might also be attributed to phenolic and flavonoid content that increased during fermentation.



Figure 1 Color change of kombucha during fermentation

The kombucha aroma score ranged from 1.29 (acid on day 0) to 3.70 (very sour and slightly alcoholic) on day 8. The aroma score produced by kombucha increased with the length of fermentation

time. According to Zhao et al. (9), the longer the fermentation will increase the volatile components in kombucha, which affect the aroma, such as aldehydes, alcohols, aldehydes, ketones, acids, esters, and benzene compounds. The aroma of kombucha as affected by volatile compounds formed during the fermentation process was reported (10,11).

Description of Taste of Cocoa Pulpa Kombucha Drink Flavor

Description of taste in cocoa pulp kombucha drink 0 days of fermentation was dominated by sweet taste (67%) with a little sour (33%). During fermentation, the description of sweetness decreased; for example, on the second day of fermentation, the panelist described a slightly sour taste of as much as 55%, and sweetness reduced to 31%. In 4 days of fermentation, the number of panelists who described sour taste increased, and 3% of panelists even detected very sourly. In kombucha, after six days of fermentation, more and more panelists detected a very sour taste which reached 36%. In the 8-day fermentation, panelists who described it as very acidic were getting more and more and began to detect a slightly alcoholic taste.

In this study, cocoa pulp kombucha fermentation used symbiotic bacteria and yeast as a starter. The activity of acetic acid bacteria caused the increase in sour taste during fermentation as the dominant bacteria in the consortium made up SCOBY. In the course of fermentation, acetic acid bacteria create the organic acids acetic acid and gluconic acid (4,6). These two organic acids play an essential role in the sour sensory of kombucha drinks. In addition to acetic acid bacteria, lactic acid bacteria such as the *Lactobacillus* species

and *Lactococcus* genus are also present in kombucha culture (12), which might contribute to increased acidity. During fermentation, the accumulation of organic acids results in a product that tastes sour (6,3,4). In addition to the increase in sour taste, the panelists also described the reduced sweetness in fermented kombucha products. Wang et al. (13) linked organic acid production and sugar depletion by microbes during kombucha fermentation.

Overall Acceptance of Kombucha Cacao Pulp Drink

The assessment of the quality of food ingredients generally depends on several factors, including color, aroma, taste, and overall acceptance. From the aspect of color, aroma, and taste, the panelists preferred the color, aroma, and taste of kombucha with a fermentation time of 0 days, which were 49%, 44%, and 39%, respectively. This result means that the panelists prefer the color of kombucha, which is cloudy white, has a fruity aroma and tastes sweet and slightly sour. The panelists liked the cloudy white kombucha color compared to the brown kombucha; besides that, the panelists liked the more apparent appearance of the kombucha. The original color of the cocoa pulp liquid is cloudy white. However, during fermentation, an enzymatic browning reaction occurs by the polyphenol oxidase enzyme so that the color of the kombucha drink is cream to brown (8). Panelists like colors that tend to be light compared to dark colors.

Panelists preferred the taste of kombucha on fermentation on day 0, which is sour, but some liked kombucha with a sour taste and a little alcohol taste. Aroma is one of the determining factors for panelists' acceptance of kombucha study (11). In this study, panelists preferred the taste of

kombucha on fermentation on day 0, which is sour, but some liked kombucha with a sour taste and a little alcohol taste. The organic acids that are created during kombucha fermentation, including alcohol, acetic acid, and other organic acids (6,3,4). These compounds give the beverage its distinct sour flavor and scent.

Sweet taste is the most preferred taste by most people, so panelists prefer the taste of kombucha on fermentation on day 0, which is sweet and slightly sour—however, some panelists like kombucha with a sour taste and a hint of alcohol. According to Wang (13), kombucha has a distinctive taste, such as sour with a hint of alcohol and sparkling, because microbes produce CO₂ during fermentation.

CONCLUSION

The length of fermentation significantly affects how well panelists and tasters receive the cocoa pulp kombucha drink. The panelists preferred the kombucha beverage, which was fermented for four days, describing it as having a somewhat hazy cream appearance, a slightly sour sweet taste, and a moderate preference. This study suggests that more work needs to be done on the sensory kombucha pulp of cocoa to improve its sensory acceptance and pique consumer interest in and understand the health advantages of kombucha pulp cocoa.

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REFERENCES

1. Kementrian Pertanian. Produksi Kakao Menurut Provinsi di Indonesia tahun 2016-2020. Jakarta:

- Kementrian Pertanian. 2020; 1-2
2. Leite PB, Machado WM., Guimarães, AG, de Carvalho GBM, Magalhães-Guedes KTM, Druzian JI. Cocoa 's Residual Honey : Physicochemical Characterization and Potential as a Fermentative Substrate by *Saccharomyces cerevisiae* AWRI726. *The Scientific World Journal* 2019; Volume 2019 | Article ID 5698089. | <https://doi.org/10.1155/2019/5698089>
 3. Coelho RMD, de Almeida AL, do Amaral RQG, da Mota RN, da Sousa PHM. Kombucha: Review, *Int. J. Gastron. Food Sci.* 2020; Vol. 22, October: 100272. doi: 10.1016/j.ijgfs.2020.100272.
 4. Nyhan LM, Lynch KM, Sahin AW, Arendt EK. 2022.“Advances in Kombucha Tea Fermentation: A Review,” *Appl. Microbiol* Vol 2(1):73–101. doi: 10.3390/applmicrobiol2010005.
 5. Villarreal-Soto SA, Beaufort S, Bouajila J, Soucharda J-P, Renard T, Rollan S, Taillandier P. 2019. Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts. *Process Biochemistry.* 2019; Vol 83 August 2019: 44-54. doi: 10.1016/j.procbio.2019.05.004.
 6. Jayabalan R, Malbařsa RV, Lonřcar, ES, Vitas JS, Sathishkumar M. A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comprehensive Review in Food Science and Food Safety.* 2014; Vol 14: 538-550
 7. Yuliana N. Widiastuti EL, Setiawan T. Proses Pembuatan Minuman Kombucha Pulpa Kakao. Jakarta: Sertifikat Paten Sederhana. 2018: 4-11.
 8. Macedo ASL, Rocha FS, Ribeiro M. S, Soares SE, Bispo ES. Characterization of polyphenol oxidase in two cocoa (*Theobroma cacao* L.) cultivars produced in the south of Bahia, Brazil. *Food Science and Technology.* 2016; Vol 36(1): 56-63.
 9. Zhao ZJ, Sui Y, Wu H, Zhou CHX, Zhang J. Flavour Chemical Dynamics During Fermentation of Kombucha Tea. *Emirates Journal of Food and Agriculture.* 2018; 30(9): 732-41.
 10. Nurhidayah N. Pengaruh Lama Fermentasi Terhadap Mutu Kombucha Sari Buah Nanas. Doctoral dissertation, Universitas Mataram. 2018.
 11. Purnami KI, Jambe AAGNA. Wisaniyasa NW. Pengaruh jenis teh terhadap karakteristik teh kombucha. *Jurnal ITEPA.* 2018; Vol 7(2): 1–10.
 12. Bishop P, Pitts ER, Budner D, Thompson-Witrick KA. 2022. Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chem. Adv.* 2022; Vol 1, No. October 2021:100025.
 13. Wang X. Development and characteristics of green teakombucha. Thesis. Massey University. Albany, New Zealand. 2018; Pp 8-13