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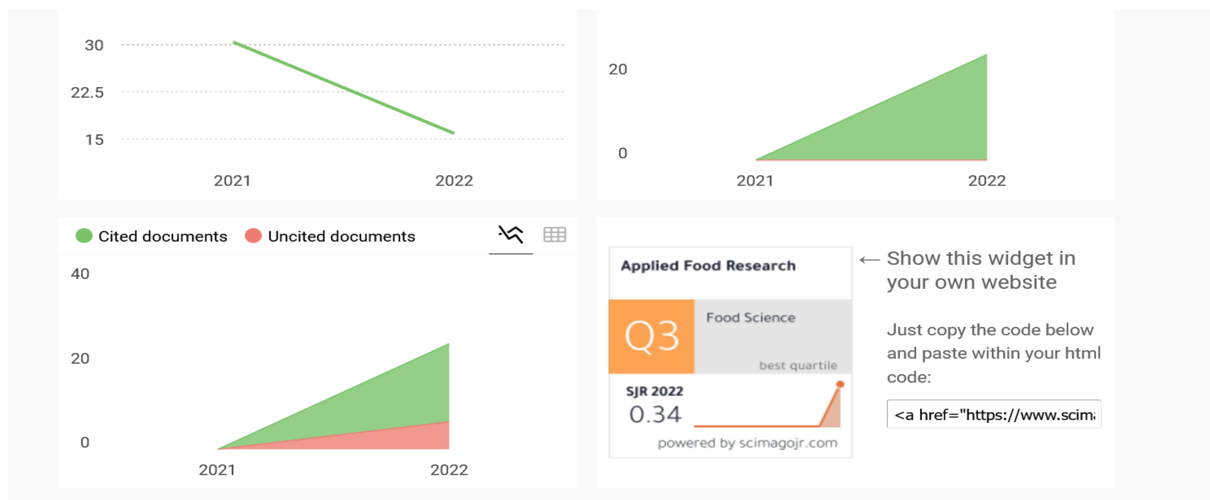

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# Total microbe, physicochemical property, and antioxidative activity during fermentation of cocoa honey into kombucha functional drink

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ARTICLE INFO

*Keywords:*
ABTS
DDPH
Flavonoid
Fermentation
Phenolic

ABSTRACT

Cocoa honey is a by-product of cocoa bean processing and is rich in sugars and minerals that is suitable as medium for fermentation, from which then it could be used for making potential kombucha functional drink. It is an approach to upgrading cocoa honey product. This study evaluated total microbes, physicochemical aspect, antioxidant activity, and phenolic and flavonoid compounds during the fermentation of cocoa honey kombucha. The fermentation time significantly affected the properties of cocoa honey functional beverages. Total acidity, cellulose weight, viable counts, and antioxidative activity increased during the 8-day fermentation. On the contrary, pH and total soluble solids decreased after 6 days of fermentation. Based on antioxidative activity, the good quality of kombucha was confirmed after 6 days of fermentation. During this time, the kombucha cacao honey drink demonstrated high antioxidant activity (70.14% ±1.15% DDPH and 43.49% ± 0.52% ABTS), which correlated with the high levels of phenols (144.75 ± 2.03 mg GAEq/mL) and flavonoids (1.776 mg QAEq/mL). It is expected to help in further research on the production of kombucha functional drink.

## 1. Introduction

Cocoa honey is a by-product of cocoa bean processing, and it is abundant in Indonesia. Cocoa is one of Indonesia's few agricultural commodities and is commonly processed into brewed chocolate or chocolate beverages or further processed into a primary raw material for other chocolate products. Various wastes are generated during the processing of cocoa pods, such as cocoa pulp, pericarp, and pulp (Indiarto et al., 2021). Cocoa honey results from pulp liquefaction due to microbial enzymatic action on pectin (De Vuyst & Leroy, 2020; Díaz-Muñoz et al., 2021; Dos Santos et al., 2014). As part of the remaining biomass, cocoa honey is discarded through on-farm processing. Disposal of this biomass residue routinely creates environmental problems such as need landfill areas, the appearance of an unpleasant odor and polluting the soil. Therefore, updating and extensively studied should be done related to this in order to solve its environmental problems, such as by utilizing it into potential functional drink.

Bio-valorization of food waste is an economic and environmental approach that can mitigate traditional waste problems (Majumder, 2020). At present, its effect on the sustainability of food production is a focus of many scientists. Valorizing food wastes can be conducted either by recovering value-added compounds from food waste or by converting wastes into processing materials to make it having more economic value. Cocoa honey is high in reducing sugar and dietary fiber, and it can be regarded a natural source of bioactive phenolic compounds with substantial antioxidant activity (Da Silva et al., 2015). To upgrade cocoa honey, some studies previously focused on non-bioprocess research, such as jelly and jam (Alves et al., 2016; Dos Santos et al., 2014). Some cocoa honey products also underwent fermentation processes (bioprocesses) because cocoa honey is rich in glucose (4.58 ± 0.12% w/v) and fructose (3.25 ± 0.03% w/v) (Leite et al., 2019), making it a suitable substrate for fermentation. Some of these products are kefir (Puerari et al., 2012), brandy, coconut and beer-fermented heaps (Nunes et al., 2020), vinegar (Ganda-putra & Wartini, 2019; Oddoye, 2013), and probiotic cocoa juice with lactic acid bacteria (Guimarães et al., 2019). Research on cocoa honey must be improved so that the scarcity of publications does not hinder the exploitation and marketing of cacao honey products (Guirlanda et al., 2021). Besides sugar, cacao honey contains phytochemicals with antioxidant capacity, phenolic and polyphenolic compounds, such as flavonoids (Da Silva et al., 2015; Endraiyani et al., 2017; Leite et al., 2019). Therefore, this by-product is potentially a production medium for functional drinks.

Nowadays, people are becoming more aware of the importance of caring for their health. When they consume drinks, they do not only look at the taste and nutritional fulfillment but also consider the functional aspects of food for health. One functional beverage that is becoming increasingly popular is kombucha. This famous fermented beverage is made initially from tea leaf extract as a substrate. Kombucha is a sweet,

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https://doi.org/10.1016/j.afres.2023.100297
Received 1 February 2023; Received in revised form 4 April 2023; Accepted 11 April 2023
Available online 21 April 2023
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slightly sour beverage made by fermenting tea with sugar added by a symbiotic culture of bacteria and yeast (SCOBY) (Nyhan et al., 2022). SCOBY is highly variable in composition, with some species frequently found from the Gluconobacter, Acetobacter, Zygosaccharomyces, Saccharomyces, and Schizosaccharomyces genera (St-Pierre, 2019). Lactic acid found in kombucha culture are Lactobacillus sp. and Lactococcus sp. (Bishop et al., 2022). These symbiotic cultures are bound in a symbiotic relationship in producing metabolites during kombucha fermentation (Jayabalan et al., 2014). The bioactive compounds of kombucha are beneficial for health. Research studies have examined organic acids, flavonoids, and phenolic compounds that describe kombucha therapeutic properties (Malbaša et al., 2013). Thus, kombucha made from cocoa honey, a by-product of the cacao fruit, will undoubtedly add to the variety of functional drinks.

This study used fermentation to valorize cocoa wastes to prepare a cocoa honey-based kombucha which has potential as a functional drink. Besides being influenced by food substances, temperature, pH, and bacterial activity, the growth of SCOBY in the fermentation process is also influenced by the fermentation time (Coelho et al., 2020). The longer the fermentation, the more secondary metabolites are formed, which will affect the resulting kombucha. This study aimed to evaluate properties of cacao honey-based kombucha functional drink and provide unique insights on total microbe, physicochemical property, in vitro antioxidant activity, and phenolic and flavonoid compounds.

## 2. Materials and methods

### 2.1. Sample preparation

Cocoa honey juice was obtained from a cocoa farmer organization in Tanggamus, a district with a notorious cacao-producing region in Lampung Province of Indonesia. Fresh cocoa beans put in a perforated fermentation box and placed at a height of one meter. The juice that comes out of the fermentation box hole is accommodated in a slightly bent plastic container so that it can be collected in a bottle. The cocoa honey juice was then frozen. Before being transported to the laboratory, the frozen juice was put into a cooler box. The cocoa honey was then stored in a freezer at −19°C for further use as growing media fermentation. A commercial SCOBY obtained from the marketplace was used as the starter for fermentation.

### 2.2. Chemicals

Plate Count Agar was sourced from Difco, ethanol, 2,2′-Azino-bis-(3-ethyl- benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2, 2-Diphenyl-1-picrylhy-drazyl (DPPH), vitamin C, quercetin, gallic acid, $K_2S_2O_8$, Folin-Ciocalteu reagent, $AlCl_3$, NaNO, $NaCO_3$, NaOH were obtained from Merck, Germany. All chemicals used were of analytical grades

### 2.3. Kombucha production

The medium preparation for kombucha production followed the methods of Yuliana et al. (2019). The cocoa honey as raw material has total soluble solid about to 16-17∘brix, pH 3.42-3.50, and antioxidative activities (85.315±0.332%). The cocoa honey was filtered and diluted by adding water in a ratio of 1:20 (v/v). Then, granulated sucrose was added to adjust the cocoa honey juice to raise a brix of approximately 12°. Thereafter, the adjusted cocoa honey was sterilized at a temperature of 95–100°C for 10 min. The medium was poured into a fermentation container aseptically and allowed to stand until room temperature (30ºC). The sterilized cocoa honey was then inoculated with SCOBY at 3% w/v base and incubated at room temperature for 8 days of fermentation. Microbial growth, physicochemical properties, antioxidative activities, and phenolic contents were monitored during the fermentation.

### 2.4. Experiment design

In this completely random block design, five levels of fermentation time (days 0, 2, 4, 6, and 8) with four replicates were considered.

### 2.5. Determination of total microbe and physicochemical property

Plate count agar (Difco) media in the plate count method were used for the enumeration of aerobic mesophilic organisms. Plates were scored after 48 h of incubation at 37°C. pH value was determined using a pH meter (Orion Star™ A215, Thermo Fisher Scientific™, Waltham, MA, USA) pre-calibrated to pH 4.0 and 7.0. Titratable acidity (TA) was determined by titration of the sample with a standard solution of sodium hydroxide to a pH endpoint of 8.20 (Li et al., 2021). The TA was expressed in % of acetic acid, calculated from this equation:

$$\% \, Acidity\,(w/v) = N \times V_1 \times Eq\ wt \times 100/V_2 \times 1000 \quad (1)$$

N = Normality of NaOH (mEq/ml), $V_1$ = volume of titrant (mL), $V_2$ = Volume of sample (mL), Eq wt = Equivalent weight of acetic acid (60.05 mg/mEq).

The total soluble solid (TSS) content was determined using a handheld analog refractometer (Atc, Jiangsu Victor Instrument Meter Co., Ltd.), and the results were expressed as brix. Changes in the clarity of the culture medium were observed every 2 days during the 8-day fermentation. Biocellulose production in the medium was monitored by gently removing it with a spatula. Excess moisture was removed with a paper towel, and the fresh weight was measured on a digital scale.

### 2.6. Determination of phytochemicals and antioxidant activity

The determination of total phenolic content, total flavonoid content, and antioxidant activity using DPPH and ABTS methods on kombucha samples begins with the preparation of the extract. Approximately 5 mL of the samples was taken, added with 20 mL of ethanol 96%, and then extracted using a shaker for ± 4 h. The kombucha extract was centrifuged for 10 min (3000 rpm) and filtered using Whatman grade 2 filter paper, with a thickness of 8 μm (Yilmaz-Ersan et al., 2018).

#### 2.6.1. *Total phenolic content*

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteau reagent (Ismail et al., 2012). For quantification, 0.2 mL of the extract sample was prepared in test tubes and diluted with 0.2 mL of distilled water. This diluted extract was mixed with 0.2 mL of 50% Folin-Ciocalteu reagent, homogenized with Vortex, and allowed to stand for 1 min. Then, 4 mL of a 2% sodium carbonate ($Na_2CO_3$) solution was added, vortexed for 1 min, and then left in the dark chamber for 30 min at room temperature. The total phenolic content was determined by reading the resulting blue absorbance using a UV-Vis spectrophotometer (Genesys 840-208100 UV, Thermo Scientific) at a wavelength of 760 nm. A standard curve was generated at various gallic acid concentrations (0–100 ppm). The relationship of gallic acid concentration is plotted in the x-axis, and the absorbance of the gallic acid reaction with the Folin-Ciocalteu reagent is plotted in the y-axis. Total phenolic concentration was expressed as mg gallic acid equivalent (GAEq) per mL extract.

#### 2.6.2. *Determination of the total flavonoid content*

The flavonoid content was determined by the method described by Sultana et al. (2009); 1 mL of the extract was added to 4 mL of distilled water in a 10-mL test tube. Then, 0.3 mL of the 5% $NaNO_2$ solution was added and allowed to stand for 5 min. Thereafter, 0.3 mL of 10% $AlCl_3$ was added and allowed to stand again for 6 min. Then, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added to the mixture and homogenized using a vortex. The absorbance of the solution was read at a wavelength of 380 nm with a UV-Vis spectrophotometer. The total flavonoid content was expressed as mg quercetin acid equivalents (QAEq) per mL.

*2.6.3. Antioxidant activity of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)*

The initial stage of antioxidant activity was the preparation of a DPPH control solution using a modified method (Shimamura et al., 2014). Approximately 0.0078 g of the DPPH solution was weighed in a dark room and dissolved in 100 mL of 96% ethanol. Then, 5 mL of the 0.02 mM DPPH solution was taken and placed into a cuvette to measure its absorbance at a wavelength of 517 nm. The results of the absorbance measurement were calculated as control absorbance (Ak). The free radical elimination activity (DPPH) of extract samples was determined by taking 1 mL of each extract, adding 2 mL of the DPPH solution, agitating in a vortex, and keeping it in the dark for 30 min. Subsequently, the absorbance was measured in a UV-Vis spectrophotometer (Genesys 840-208100 UV, Thermo Scientific) at 517 nm. The absorbance obtained was recorded as the sample absorbance (As). Then, to obtain the percentage of the antioxidant activity, the absorbance of the extract (As) was compared with the absorbance of DPPH (Ak). The percentage of the antioxidant activity against DPPH radicals at each concentration of the sample solution was calculated by the formula described by Brand-Williams et al. (1995):

$$\% \, Antioxidant \, activity = (Ak - As)/Ak \times 100\% \quad (2)$$

*2.6.4. Antioxidant capacity of 2,2′-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS)*

The antioxidant ABTS activity of the extracts was evaluated according to the method described by Brand-Williams et al. (Brand-Williams et al., 1995). First, the ABTS (7 mM) solution was mixed 1:1 with a 2.45 mM $K_2S_2O_8$ solution and left in the dark for 16 h before use. This $ABTS^+$ solution was then diluted with 96% ethanol to give an absorbance of 0.70 ± 0.02 at 734 nm and recorded as Ak. To measure the samples, 100 µL of the extract was removed, and 2.9 mL of the ethanol $ABTS^+$ solution was added. The absorbance of the samples was read on a UV-Vis spectrophotometer (Genesys 840-208100 UV, Thermo Scientific) at 734 nm exactly 30 min after initial mixing and recorded as sample absorbance (As). (2). The calibration curve was developed using ascorbic acid as a standard. The scavenging activity of ABTS was calculated using equation 3:

$$\% \, Antioxidant \, activity = (Ak - As)/Ak \times 100\% \quad (3)$$

*2.7.* Statistical analysis

Data were analyzed for variance to determine the presence of an effect between treatments and were then further tested using the BNT test at 5% level. The relationship between antioxidant parameter was determined using the Pearson correlation method.

## 3. Results and discussion

*3.1. Total microbe, biocellulose, and visual culture media*

The increase in total microbes, biocellulose and visual culture medium during fermentation are presented in Table 1. Total microbes increased from the start of the fermentation (log 4.95 CFU/mL) to the final fermentation (log 14.99 CFU/mL), indicating that kombucha culture grew well in the cocoa honey media. In addition to increasing acidity, the activities of this kombucha culture were observed in biomass yield (cellulose formation). The fermentation time had a significant effect on the kombucha cellulose ($p < 0.05$), where the highest cellulose weight value was obtained after 8 days of fermentation (3.17 g). This increase in biocellulose was in line with microbe growth during fermentation (Table 1).

**Table 2**
pH, acidity, and TTS of cocoa honey kombucha during the fermentation period.

| Time (day) | pH | Acidity (% acetic acid) | TTS (ºbrix) |
|---|---|---|---|
| 0 | 4.16±0.09[a] | 0.17±0.07[d] | 12.25±0.28[a] |
| 2 | 3.37±0.04[b] | 2.15±0.19[c] | 12.50±0.40[a] |
| 4 | 3.15±0.04[c] | 3.63± 0.56[b] | 12.25±0.28[a] |
| 6 | 3.10±0,07[c] | 5.63±0.42[a] | 11.38±0.47[b] |
| 8 | 2.97±0.08[d] | 6.14±0.41[a] | 11.00±0.41[b] |

The means followed by the same letter in a column do not differ significantly (LSD test; $p > 0.05$).

Total microbes involved in the fermentation of cocoa honey increased directly proportional to the fermentation time. Several types of bacteria can produce microbial cellulose, including *Acetobacter*. Of this genus, the dominant species is *A. xylinum*, which has been reclassified as *Gluconacetobacter xylinus* and more recently as *Komagataeibacter xylinus* (Villarreal-Soto et al., 2019). This bacterium's specific biochemical process involves oxidizing glucose to gluconic acid. Then, another biochemical process results in the synthesis of microbial cellulose, which forms the biofilm (Chakravorty et al., 2016; Jayabalan et al., 2014; Villarreal-Soto et al., 2019) that persists on the liquid surface. The biomass yield (biocellulose) directly affected the carbon source in the system; the higher the sugar content, the higher the biomass yield. A slight increase in the biocellulose biomass yield was observed during this fermentation. This yield might correlate with the slight decrease in sugar content (°brix; Table 2).

During the fermentation process, changes in the culture media of cocoa honey were monitored. Between days 0 and 2 of fermentation, cloudy white to slightly cloudy white appearances were observed (Table 1). Subsequently, the cloudiness and darkness of the medium decreased and increased, respectively, on days 4 and 6 of fermentation (slightly cloudy cream to slightly cloudy brown). Finally, on day 8, the culture media became slightly clear brown. The dark color changes of the medium during fermentation might be attributed to the phenolic and flavonoid contents. On day 6 of fermentation, kombucha cacao honey was observed to have high antioxidant activity, which correlated with the high phenolic and flavonoid contents (Table 3). Fermentation time influenced the increase in the content of these compounds.

*3.2.* pH, acidity, and TSS change

Changes in pH, total acidity, and TSS of kombucha cacao honey affect the fermentation time (Table 2). A statistically significant decrease ($p < 0.05$) was observed in the pH of kombucha samples with fermentation time. The initial pH of the cocoa honey samples was 4.16, which dropped to 2.97 during the 8-day fermentation period (Table 1). This

**Table 1**
Total microbe, biocellulose and visual culture medium of cocoa honey kombucha during the fermentation period.

| Time (day) | Total microbe (Log CFU/mL) | Biocellulose (g) | Visual culture media (color and clarity) |
|---|---|---|---|
| 0 | 4.95±0.02[e] | 0.00±0.00 [e] | Cloudy white |
| 2 | 7.00±0.02[d] | 2.01±0.35[d] | Slightly cloudy white |
| 4 | 10.06±0.02[c] | 2.46±0.46[c] | Slightly cloudy cream |
| 6 | 12.08±0.01[b] | 2.81±0.36[b] | Slightly cloudy brown |
| 8 | 14.99±0.05[a] | 3.17±0.21[a] | Slightly clear brown |

The means followed by the same letter in a column do not differ significantly (LSD test; $p > 0.05$).

**Table 3**
Total phenolic content and total flavonoid content during fermentation.

| Time (day) | Total phenolic mg GAEq/mL | Total flavonoid mg QAEq/mL |
|---|---|---|
| 0 | 8.37±0.49[e] | 1.15±0.04[c] |
| 2 | 58.18±2.03[d] | 1.55±0.03[b] |
| 4 | 88.75±0.87[c] | 1.59±0.05[b] |
| 6 | 144.75±2.03[a] | 1.78±0.06[a] |
| 8 | 117.80±1.14[b] | 1.70±0.05[a] |

The means followed by the same letter in a column do not differ significantly (LSD test; $p > 0.05$). IC50 DPPH of ascorbic acid (μg/mL) = 3.48 ± 0.078

**Table 4**
DPPH radical scavenging activity, and ABTS values during fermentation.

| Time (day) | Antioxidative activity DPPH (%) | IC50 DPPH (μg/mL) | Antioxidative activity ABTS (%) |
|---|---|---|---|
| 0 | 53.37±1.54[e] | | 29.99±0.58[e] |
| 2 | 59.73±1.02[d] | | 37.86±0.91[d] |
| 4 | 64.29±0.89[c] | | 40.77±1.00[c] |
| 6 | 70.14±1.15[a] | | 43.49±0.52[a] |
| | | 180.92±11.63 | |
| 8 | 66.23±0.69[b] | | 41.68±0.97[b] |

The means followed by the same letter in a column do not differ significantly (LSD test; $p > 0.05$). IC50 DPPH of ascorbic acid (μg/mL) = 3.48 ± 0.078

**Table 5**
Correlation (r) between parameters for cacao honey-based kombucha functional drink.

| | Phenolic | Flavonoid | DDPH | ABTS |
|---|---|---|---|---|
| Time vs | 0.910 | 0.863 | 0.886 | 0.864 |
| Phenolic vs | - | - | 0.995 | 0.961 |
| Flavonoid vs | - | - | 0.959 | 0.990 |

trend was similar to the pH of fruit-based kombuchas such as red grape kombucha and apple kombucha, with pH of 2.90 and 2.95, respectively, after 6 to 8 days of fermentation (Ayed et al., 2017; Zubaidah et al., 2018).

The pH drop that occurs during kombucha fermentation was cited in numerous studies as an effect of the increasing number of organic acids produced by the kombucha culture (SCOBY). The SCOBY is a yeast consortium with predominantly acetic acid bacteria (Chakravorty et al., 2016). On days 6–8 of fermentation, acetic acid concentration was the highest for all sample beverages (Table 2). Previous authors argued that the acetic acid bacteria found in SCOBY use sucrose as a carbon source and produce acetic acid as the primary metabolite (Jayabalan et al., 2014). In addition to acetic acid bacteria, lactic acid bacteria are present in kombucha culture, including *Lactobacillus sp.* and *Lactococcus sp.* (Bishop et al., 2022), which might contribute to increased acidity. Neffe-Skocińska et al. (2017) mentioned that in addition to acetic acid, quinic, citric, malic, and glucuronic acids are formed during fermentation by kombucha culture. In contrast to the pH, the TSS of kombucha cacao honey were observed to decrease slightly from day 6 (11.38°brix) until day 8 of fermentation (11.00°brix). The TSS might decrease with the fermentation time because many nutrients were used for the growth and metabolism of the microbes. The utilization of sugars by the kombucha culture resulted in the synthesis of various organic acids, ultimately leading to a pH reduction (Jayabalan et al., 2014).

### *3.3.* Phytochemicals

The total phenolic and total flavonoid obtained from days 0–8 of fermentation of kombucha cocoa honey are shown in Table 3. The fermentation time affected antioxidant compounds in kombucha.

As fermentation time increased until 6 days, the phenolic and flavonoid content of the cocoa honey kombucha also increased. Some studies show that fermentation could enhance the release of phenolic phytochemicals (Adetuyi & Ibrahim, 2014; Chu & Chen, 2006; Zhou et al., 2022). According to Ivanišová et al. (2020), complex phenolic compounds may be degraded to smaller molecules during fermentation, therefore total phenolics and flavonoids in the kombucha become higher.

Total phenolic levels of cocoa honey kombucha (8.37-144.75 mg GAEq/mL) were higher to those observed for tea kombucha analyzed by Cardoso et al., (2020) who found 1.09 mg GAE/mL for black tea and 7 mg.ml-1 for green tea kombucha. Therefore, cocoa honey kombucha can be considered a source of phenolic bioactive compounds. In cocoa honey kombucha samples, the flavonoids contents (1.15-1.17 QAEqmg/mL) were comparable with those in 7 days fermented tea kombucha reported by Jakubczyk et al., 2020) who found 146.8 mg/L for green tea, 90.5 ± 0.7 mg/L for black tea, 83.8 mg/L for white tea, and 198.1 mg/L for red tea.

### *3.4. Antioxidant activity*

The DPPH radical scavenging activity and ABTS values obtained from days 0–8 of fermentation of kombucha cocoa honey are shown in Table 4. The fermentation time affected the antiradical properties in kombucha.

The antioxidative activity ranged from 53.37% to 70.141% DPPH inhibition and between 29.99% and 43.49% ABTS (Table 4). These antioxidant activities correlated with the samples' phenolic and flavonoid contents (Table 5). The cocoa pulp is reported to have potential antioxidative because of its phenolic and polyphenolic compounds, such as flavonoids (Da Silva et al., 2015; Endraiyani et al., 2017; Leite et al., 2019). As fermentation time increased until 6 days, the phenolic and flavonoid content of the cocoa honey kombucha increased with strong positive correlation ($r > 0.8$) was observed between the fermentation time and the phenolic, flavonoid, DDPH, and ABTS antioxidant potential. Besides its natural substrate, the antioxidant activity of kombucha might be attributed to the metabolites of the fermentation starter cultures (Jayabalan et al., 2008; Malbaša et al., 2011; Zhao et al., 2021). This study observed a positive correlation between fermentation time and reductive potential (Table 4). In many cases, fermenting increases the beverage's antioxidant properties (Ayed et al., 2017; Zhao et al., 2021). An increase in the antioxidant potential of kombucha was also observed by Chakravorty et al. (2016) and Jakubczyk et al. (2020). The DPPH and ABTS radicals' scavenging activity increased by 39.7% and 38.36%, respectively, after 21 days (Chakravorty et al., 2016). According to Jakubczyk et al., (2020) and (Ayed et al., 2017), the increase in the diversity of microorganisms might play a significant role in the increase in the antioxidant properties of kombucha. Some studies show that fermentation could enhance the release of phenolic phytochemicals (Adetuyi & Ibrahim, 2014; Chu & Chen, 2006; Zhou et al., 2022). In this study of kombucha made from cocoa pulp, the highest value of antioxidant compounds and activity was reached on day 6 of fermentation.

Nevertheless, the antioxidant activity of kombucha cocoa honey, expressed as EC50 (DPPH), was 180.92±11.63 μg. $mL^{-1}$. The results indicated a lower efficiency of antioxidant activity compared with the mean level of EC50 ascorbic acid as a control, which showed a lower index (3.48± 0.078 μg. mL−1) (Table 4). This result could be attributed to preparing the cocoa pulp as a substrate before fermentation by adding water to the cocoa pulp at a ratio of 1:20. These facts suggest that the production of cocoa honey-based kombucha functional beverages should consider the proper preparation of the medium.

## 4. Conclusion

The fermentation time affected the total microbe, physicochemical, in vitro antioxidant activity, and phenolic and flavonoid contents of the cacao honey-based kombucha functional drink. The high quality of kom-

bucha was established after 6 days of fermentation, and kombucha cacao honey had the highest antioxidant activity and high phenol and flavonoid contents. It is expected to help for further research on the production of bioactive cacao honey-based kombucha functional drink.

## Ethical statement

This research work did not involve human and animal subjects and therefore did not have ethical approval.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Neti Yuliana:** Conceptualization, Methodology, Writing – review & editing. **Fibra Nurainy:** Data curation, Writing – original draft. **Gusrianti W. Sari:** Resources, Investigation. **Sumardi:** Supervision. **Endang L. Widiastuti:** Supervision, Visualization.

## Data availability

Data will be made available on request.

## Acknowledgments

We thank the laboratory technicians of the department for their assistance in providing the necessary resources for the research. We also thank Enago for English language editing.

## Funding

The research has received funding from the Ministry of Education, Culture, Research, and Technology, Republic of Indonesia under the program "Skema Penelitian Dasar Kompetetif Nasional" [Grant agreement no. 2147/UN26.1/PN/2022]

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