Characteristic of microbiological, chemical, and antibacterial activity of turmeric (Curcuma longa) kombucha

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Abstract Kombucha is a fermented tea beverage with the addition of a kombucha starter called SCOBY (Symbiotic Culture of Bacteria and Yeast). This research aimed to know the microbiological, chemical and antibacterial activity from various concentrations of turmeric kombucha. The data result was analyzed by descriptive analysis with one factor of turmeric concentration (0.4%; 0.8%; 1.2%; 1.6%; 2%). The best treatment was selected using Multi-Criteria Decision-Making (MCMD) method with the Simple Additive Weighting (SAW) technique. The best treatment was obtained on turmeric kombucha 0.8%. Based on analysis, the best treatment is turmeric kombucha 0.8% with characteristics as follows: pH 2.92; total acid 0.28%; total phenol 147.45 µg GAE/ml; total sugar 8%, total microbial 2.5 x 10^7 CFU/ml, inhibition zone diameters for E.coli 3.13 mm; whereas black tea kombucha has pH 2.81; total acid 0.58%; total phenol 716.02 µg GAE/ml; total sugar 7.83%, total microbial 1.3 x 10^8 CFU/ml and inhibition zone diameters for E.coli 2.50 mm.

1. Introduction
Kombucha is a beverage made from sugared tea infusion fermented by a symbiotic culture of bacteria and yeast (SCOBY). Various yeasts (Pichia, Candida, Zygossaccharomyces, Saccharomyces and Brettanomyces) and acetic acid bacteria (A. xylinum) have been identified in kombucha [1]. During the fermentation process, acetic acid bacteria and yeast form biofilm on the surface of kombucha [2] and produce organic acids such as gluconic acid and glucuronic acids [3] that reduce the pH of kombucha [4] and increase the phenolic compound and the antioxidant activity because of degradation of phenolic compounds through hydroxy acids and ferulic acids [5].

Kombucha provides many benefits as it serves as antibacterial, antioxidants, improves intestinal microflora and increases body resistance [6]. Kombucha substrate is generally made from black tea. However, it varies as time passes and develops as a form of food diversification, such as green tea kombucha, ginger kombucha, coffee leaf tea kombucha, snake fruit kombucha and turmeric kombucha.

Turmeric is one of the beneficial herbs used as traditional medicine and colouring agent. It has high productivity in Indonesia (reaching 108 million tons per year) [7] and also contains bioactive compounds such as curcuminoids and essential oils, which have functional properties as a source of antioxidants, antimicrobial, anti-inflammatory, and anti-cancer [8]. Curcuminoid compounds, along with essential oils in turmeric, acts as antimicrobials by inhibiting microbial metabolism. In addition, terpenes derivatives in the essential oils (sesquiterpenes and monoterpenes) undermine the structure of bacterial cell membranes [9].
Turmeric-based kombucha fermentation is expected to increase the functional value and increase the nutritional value of kombucha. Turmeric is used to substitute tea as the main ingredient to produce kombucha. Fermentation will increase the bioavailability of several nutrients from raw materials such as antioxidants and antibacterial, making it easier for the substance to be absorbed into the digestive system. Therefore, a study is necessary to determine the characteristics of kombucha made from various turmeric concentrations to obtain high-quality kombucha.

2. Material and methods

2.1. Preparation of kombucha
Turmeric rhizome was purchased from a traditional market in Malang, East Java, Indonesia. Washed, sliced 1-3 mm, dried in a cabinet dryer (60°C, 5 hours), ground into a powder and put in a teabag. Black Tong Tji tea (2 g/bag) is used as control (tea kombucha). Turmeric and black tea kombucha were prepared according to Ardheniati et al. [10] by extracting turmeric powder (0.4%; 0.8%; 1.2%; 1.6%; 2%) and 0.4% of black tea in 500 ml of boiling water for 5 minutes and sweetening (10% sugar). The sugared turmeric and tea extract were put into a sterile jar, cooled at room temperature before adding a kombucha starter (10%). Each kombucha was incubated for 12 days at room temperature. The sample analysis was carried out on day 0 and day 12 [11].

The turmeric concentrations (0.4%; 0.8%; 1.2%; 1.6%; 2%) were the only treatment factor used in this study, where black tea kombucha performed as the control. The data were analyzed descriptively where the best concentration of turmeric was selected through the Multiple Criteria Decision-Making method with the Simple Additive Weighting (SAW) technique.

2.2. Evaluation of kombucha
The analysis of kombucha consists of total microbial (modified from Fardiaz) [12] by counting the colonies grown on PCA agar. Titratable acidity (modified from Apriyantono) [13] using direct titration with NaOH solution 0.1 N. pH was measured using a pH meter [13]. Total phenol was determined by the complex compound formed after reaction with Folin-Ciocalteau reagent and Na2CO3 solution, spectrophotometrically at 500-600 nm [14]. Total sugar was determined with Anthrone reagent and H2SO4 spectrophotometrically at 600-700 nm [13]. Antibacterial analysis [15], and the best treatment [16]. The data were analyzed descriptively where the best concentration of turmeric was selected through the Multiple Criteria Decision-Making method with the Simple Additive Weighting (SAW) technique. K1 = Turmeric Concentration of 0.4%; K2 = Turmeric Concentration of 0.8%; K3 = Turmeric Concentration of 1.2%; K4 = Turmeric Concentration of 1.6%; K5 = Turmeric Concentration of 2%; BTK = 0.4% Black Tea Kombucha.

3. Results and discussion

3.1. Total microbial
The microbes found in kombucha are generally bacteria and yeast. According to Table 1, there was an increase in the total microbial from about 10^2 CFU/ml to around 10^7 CFU/ml after 12 days of fermentation, whereas the highest value of total microbial in turmeric kombucha is 2.5 x 10^7 CFU/ml given at a concentration of 0.8%. At a concentration of 1.2%, it started declining along with the increase of turmeric concentration. The decrease of total microbes is due to the escalating microbes inhibiting compounds contained in turmeric [17]. The total microbial of tea kombucha (1.3 x 10^8) is higher than in turmeric kombucha (1.1 x 10^7 CFU/ml – 2.5 x 10^7 CFU/ml) due to differences in raw materials, which provide different nutrients such as sugar content, vitamin and minerals that affecting microbial growth.

The increase of total microbe (1.1 x 10^7 CFU/ml – 1.3 x 10^8 CFU/ml) occurred as the microbes utilized sugar in the solution as a source of energy for cell synthesis. According to Ardheniati et al. [10], as the sugar is hydrolyzed into fructose and glucose by yeast, glucose will be fermented into ethanol.
which then is oxidized by bacteria to become organic acid (acetic acid) [18]. This process allows an acidic condition suitable for A. xylinum in carrying out metabolic activities [6].

**Table 1.** Total microbial of turmeric and black tea kombucha.

| Kombucha concentration | Total microbes (CFU/ml) | Increasing of total microbial (CFU/ml) |
|------------------------|-------------------------|----------------------------------------|
|                        | Day-0       | Day-12             |                                |
| Turmeric 0.4%           | 1.8 x 10^3  | 1.1 x 10^7         | 1.1 x 10^7                      |
| Turmeric 0.8%           | 1.3 x 10^3  | 2.5 x 10^7         | 2.5 x 10^7                      |
| Turmeric 1.2%           | 1.6 x 10^3  | 2.0 x 10^7         | 2.0 x 10^7                      |
| Turmeric 1.6%           | 1.3 x 10^3  | 1.7 x 10^7         | 1.7 x 10^7                      |
| Turmeric 2%             | 8.0 x 10^4  | 1.1 x 10^7         | 1.1 x 10^7                      |
| Black Tea 0.4%          | 1.2 x 10^2  | 1.3 x 10^6         | 1.3 x 10^6                      |

3.2. **Titratable acidity**

After 12 days of fermentation, the total acid appeared to rise (0.13%-0.39%) due to the presence of organic acids produced during the fermentation process. Based on Table 2, the total acid level reached its peak at the concentration of 0.4% and 0.8% and the averages of total acid on the 12th day of fermentation are above 0.2%. However, the total acetic acid in black tea kombucha is twice as high as turmeric kombucha. According to Aditiwati and Kusnadi [19], sucrose will be enzymatically hydrolyzed into glucose and fructose during kombucha fermentation. After that, yeast (S. cerevisiae) will metabolize glucose into ethanol and CO₂. This ethanol is then oxidized to acetaldehyde by acetic acid bacteria (Acetobacter) and into acetic acid. Other types of organic acids such as gluconic acid, glucuronic acid, and lactic acid are also produced and increase with the length of fermentation time.

**Table 2.** Titratable acidity of turmeric and black tea kombucha.

| Kombucha concentration | Titratable Acidity (%) | Increasing of titratable acidity (%) |
|------------------------|------------------------|-------------------------------------|
|                        | Day-0      | Day-12             |                                |
| Turmeric 0.4%           | 0.15       | 0.28               | 0.13                            |
| Turmeric 0.8%           | 0.16       | 0.28               | 0.12                            |
| Turmeric 1.2%           | 0.15       | 0.22               | 0.08                            |
| Turmeric 1.6%           | 0.17       | 0.26               | 0.09                            |
| Turmeric 2%             | 0.14       | 0.24               | 0.10                            |
| Black Tea 0.4%          | 0.19       | 0.58               | 0.39                            |

**Table 3.** pH of turmeric and black tea kombucha.

| Kombucha concentration | pH       | Decreasing of pH |
|------------------------|----------|------------------|
|                        | Day-0    | Day-12           |                                |
| Turmeric 0.4%           | 3.98     | 2.95             | 1.04                            |
| Turmeric 0.8%           | 4.00     | 2.92             | 1.08                            |
| Turmeric 1.2%           | 4.03     | 3.02             | 1.01                            |
| Turmeric 1.6%           | 3.99     | 3.00             | 0.99                            |
| Turmeric 2%             | 3.98     | 3.08             | 0.90                            |
| Black Tea 0.4%          | 3.96     | 2.81             | 1.16                            |

After 12 days of fermentation, there was a reduction in pH level. Based on Table 3, the highest pH reduction rate in turmeric kombucha is at a concentration of 0.8%, but this value was relatively the same among the samples. While in tea kombucha, the decrease was slightly more significant. The decrease in pH in kombucha tea and kombucha turmeric in this study is thought to be due to organic acids and secondary metabolites produced during fermentation. According to Jayabalan et al. [1], bacteria and yeast will metabolize sucrose to produce organic acids such as acetic acid, gluconic acid, and glucuronic
acid during the fermentation process. The conversion of glucose into gluconic acid and other organic acids by acetobacter causes a decrease in the pH value because dissolved organic acids can release protons (H⁺) so that the pH value decreases [20].

3.3. Total phenol
Total phenolic components were measured by using the Folin-Ciocalteu method, and the results were given as µg Gallic Acid Equivalent (GAE)/ml. Based on Table 4, Total phenol reached the highest peak at a concentration of 0.8%. After that, it is beginning to decline at a concentration of 1.2% as the curcuminoid content is relatively high to inhibit the microorganisms in turmeric kombucha. The increase in phenol content is due to enzymes produced by bacteria and yeast during the fermentation process, which degrade complex compounds. According to Essawet et al. [21], during the fermentation process, there was biotransformation of catechin compounds and other types of polyphenolic compounds by enzymes in the Kombucha culture [22], such as invertase, cellulases, amylases, which can break the complex bonds between phenolic compounds and tissue structures so that the total phenolic increases [21]. This biotransformation process is also influenced by pH because catechin and theaflavin compounds are less stable in tea solutions at pH ≤ 5 [23].

| Table 4. Total phenol of turmeric and black tea kombucha. |
|-----------------------------------------------------------|
| Kombucha concentration | Total phenol (µg GAE/ml) | Increasing of total phenol (µg GAE/ml) |
|-------------------------|---------------------------|------------------------------------------|
|                         | Day-0                     | Day-12                                   |
| Turmeric 0.4%           | 62.31                     | 108.01                                   | 45.70 |
| Turmeric 0.8%           | 76.26                     | 147.45                                   | 71.19 |
| Turmeric 1.2%           | 81.55                     | 132.89                                   | 51.34 |
| Turmeric 1.6%           | 95.28                     | 141.99                                   | 46.71 |
| Turmeric 2%             | 99.21                     | 155.95                                   | 56.74 |
| Black Tea 0.4%          | 386.07                    | 716.02                                   | 329.95|

3.4. Total sugar
After 12 days, the total sugar in turmeric kombucha and tea kombucha changed. Based on Table 5, the decrease of total sugar in kombucha appears to have very slightly different as the metabolic activity of microorganisms changes during fermentation. This decrease in total sugar is thought to be because microbes use sugar as a source of nutrition and metabolic processes. The decrease in total sugar during the fermentation process cannot be caused by microbial activity for the metabolic process. Besides that, microbes also use sugar to grow cells and form cellulose on the surface of kombucha by acetic acid bacteria [24].

| Table 5. Total sugar of turmeric and black tea kombucha. |
|-----------------------------------------------------------|
| Kombucha concentration | Total sugar (%) | Decreasing of total sugar (%) |
|-------------------------|----------------|-----------------------------|
|                         | Day-0 | Day-12 |                              |
| Turmeric 0.4%           | 8.98  | 8.07   | 0.91                         |
| Turmeric 0.8%           | 9.17  | 8.00   | 1.17                         |
| Turmeric 1.2%           | 8.95  | 7.91   | 1.04                         |
| Turmeric 1.6%           | 8.78  | 7.82   | 0.96                         |
| Turmeric 2%             | 9.00  | 7.94   | 1.06                         |
| Black Tea 0.4%          | 9.22  | 7.83   | 1.39                         |

3.5. Antibacterial
The present study measured the effectiveness of antibacterial activity by calculating the inhibition zone formed around the disc paper. The wider the inhibition zone, the more effective the antibacterial is [25]. The inhibition zone is a clear zone without microorganisms' presence [26]. Thus, a wide inhibition zone indicates a high antibacterial activity. As shown in Figure 6, The diameter of the inhibition zone on day
0 of turmeric kombucha for *E. coli* was around 1-1.75 mm and on day 12 was around 1.63-3.13 mm. In black tea kombucha, there was antibacterial activity on *E. coli* bacteria on days 0 and 12, indicated by the inhibition zone diameter of 1.75 mm and 2.50 mm, respectively.

The antibacterial activity of turmeric kombucha against *E. coli* after 12 days of fermentation was different for each concentration of turmeric. The antibacterial activity of turmeric kombucha improved along with increased turmeric concentration up to a certain extent (0.8%). However, the antibacterial activity declined afterwards. The antibacterial compounds in turmeric kombucha are different in each concentration. The antibacterial activity of turmeric kombucha against *E. coli* was 0.8%, more significant than the antibacterial activity of tea kombucha, which was only 0.4% in *E. coli* bacteria.

### Table 6. The antibacterial activity of turmeric and black tea kombucha.

| Kombucha Concentration | The Inhibition Zone (mm) | Increasing of the Inhibition Zone (mm) |
|------------------------|--------------------------|----------------------------------------|
|                        | Day-0                    | Day-12                                 |
| Turmeric 0.4%          | 1.75                     | 2.25                                   |
| Turmeric 0.8%          | 1.00                     | 1.75                                   |
| Turmeric 1.2%          | 1.25                     | 2.00                                   |
| Turmeric 1.6%          | 1.50                     | 1.75                                   |
| Turmeric 2%            | 1.25                     | 1.25                                   |
| Black Tea 0.4%         | 1.75                     | 2.00                                   |

3.6. The best treatment

The best treatment was selected based on Multi-Criteria Decision-Making (MCMD) method with the Simple Additive Weighting (SAW) technique [16]. All parameters (total microbial, total acid, pH, total phenol, total sugar, and antibacterial activity) were considered to have an equal value to determine the best concentration of turmeric kombucha. The compared concentrations of turmeric kombucha were 0.4%; 0.8%; 1.2%; 1.6%, and 2%. Based on the calculation results, turmeric kombucha with a concentration of 0.8% was chosen as the best treatment, denoted in Table 7. Experimental studies also demonstrated that, although low concentrations of curcumin induce antioxidant effects, higher concentrations of this compound increase the cellular levels of reactive oxygen species (ROS) [27].

### Table 7. Characteristics of the best treatment of kombucha.

|                       | Turmeric kombucha 0.8% | Black tea kombucha |
|-----------------------|------------------------|--------------------|
| Total Microbial       | 2.5 x 10^7 CFU/ml      | 1.7 x 10^11 CFU/ml |
| Titratable Acidity    | 0.28%                  | 1.4%               |
| pH                    | 2.92                   | 2.8                |
| Total Phenol          | 147.45 µg GAE/ml       | 418.1 µg GAE/ml    |
| Total Sugar           | 8%                     | 8.4%               |
| Antibacterial Activity Against *E. coli* | 3.13 mm                | 12.33 mm            |

4. Conclusions

Based on Multi-Criteria Decision-Making, turmeric kombucha 0.8% is the best concentration to process turmeric kombucha. This kombucha has characteristics as follows; pH 2.92; total acid 0.28%; total phenol 147.45 µg GAE/ml; total sugar 8%; total microbial of 2.5 x 10^7 CFU/ml; 3.13 mm inhibition zone diameter against *E. coli*.

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