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Changes in chemical characteristics of kombucha from various cultivars of snake fruit during fermentation

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Abstract. Kombucha snake fruit is a beverage of sugared snake fruit juice with a kombucha consortium called SCOBY (Symbiotic Culture of Bacteria and Yeast). This research was aimed to know the changes of chemical characteristics of kombucha from various cultivars of snake fruit during fermentation. This research used Randomized Block Design with 1 factor (*salak Suwaru*, *salak Madura*, *salak Pondoh* and *salak Bali*) and three replication. The data obtained were statistically evaluated by ANOVA test (Analysis of Variance) and continued by Fisher test to determine level of significant difference. The Effectiveness Index Test was used to select the best treatment. The results demonstrated that there were changes in the chemical characteristics of a decrease in pH, total sugar, and total soluble solids, as well as an increase in total acidity, total phenol and antioxidant activity significantly during fermentation. The best treatment was obtained on kombucha of *salak Suwaru* cultivars with total acidity 1.64%, pH 3.22, total sugar 7.76%, total soluble solids 12.88 °Brix, total phenol 535.59 mg/L GAE and antioxidant activity 88.18%.

1. Introduction

Kombucha is a beverage of sugared tea fermented for 14 days with symbiotic consortium of acetic acid bacteria and yeasts called SCOBY. *Acetobacter xylinum* and various yeast (e.g. *Saccharomyces*, *Zygosaccharomyces*, *Brettanomyces* and *Candida*) have been identified in kombucha fermentation. Commonly, kombucha is made from black tea leaves as substrate [1]. Based on several previous researches it has been proven empirically that black tea kombucha has possesses functional properties such as antimicrobial, antioxidant, antidiabetic, antidiabetic and other [1]. Besides of black tea, kombucha can be made from other substrates such as grape, orange and blackcurrant [2].

Snake fruits (*Sallacazalacca*) is one of Indonesia's indigenous commodities which contains fiber, vitamin A, B1, C, and high antioxidant activity [3]. Besides that, snake fruits have higher polyphenols compared to apples, oranges, mangosteen, and kiwi [3][4]. Based on previous research revealed that *Suwaru* snake fruit which is fermented into kombucha can increase polyphenols and organic acids as well as antioxidant activity [5].

Indonesia have many snake fruit cultivars such as *salak Suwaru* (Malang, Jawa Timur), *salak Pondoh* (Sleman, Yogyakarta), *salak Madura* (Bangkalan, Madura) and *salak Bali* (Karangasem, Bali). Each cultivar has unique characteristics. This research was aimed to know changes of chemical characteristic of kombucha from various cultivars of snake fruit during fermentation.



2. Material and Method

2.1. Material

Snake fruits of commercial maturity were of cultivars Salak Suwaru from Malang, Salak Madura from Bangkalan, Salak Pondoh from Sleman, and Salak Bali from Karangasem. Commercial Kombucha Starter was purchased from Bandung, while cane sugar was bought from a local super market in Malang.

2.2. Experimental design

The experimental design used in this research is a randomized block design with one factor that consists of 4 levels and repeated 3 times to obtain 16 units of trial. Factors used are cultivars as a raw material for making kombucha namely:

S1 = *salak Suwaru*

S2 = *salak Madura*

S3 = *salak Pondoh*

S4 = *salak Bali*

The independent variable in this research is various cultivars as a raw material. The dependent variables include pH, total acidity, total soluble solids, total sugar, total phenol and antioxidant activity. Kombucha were analysed on day 0 and 14 during fermentation.

2.3. Kombucha preparation

Kombucha were prepared according to previous research [5]. The snake fruits were peeled, washed, and cut into small sizes, from which 500 g was mixed with water (1:1, w/w), juiced (blended) and filtered (cheese cloth). Cane sugar were added 10% (w/v) in the snake fruit juice, pasteurized (Waterbath Memmert, Germany) at 65 °C for 30 min and cooled to ambient temperature before storing in a sterile jar. The sugared juices were inoculated with 10% (v/v) the liquid broth of kombucha starter aseptically. The jar covered with clean cheese cloth and fixed with rubber bands. Fermentation was conducted under aseptic conditions, carried out by incubation at room temperature ($28 \pm 3^\circ\text{C}$) for 14 days.

2.4. Kombucha analysis

2.4.1. Total acidity and pH analysis

Total acidity was measured by titration procedure [5], 10 mL of samples was taken and 2 or 3 drops of phenolphthalein indicator was added. It was then immediately titrated with 0.1 M NaOH till permanent colour appeared. pH samples were measured by pH meter (Hanna, Thermo Fischer Scientific, USA).

2.4.2. Total sugar and total soluble solid analysis

Total sugar was determined by the anthrone method according to previous research [5], 1 mL samples was transferred to a test tube and mixed with 5 mL anthrone reagent (0.05 g anthrone in 50 mL concentrated H_2SO_4). The test tube were held at 100 °C for 12 min and cooled before measuring the absorbance at 630 nm in a spectrophotometer (Spectro 20D Plus, Labomed, USA) with a glucose solution as the standard. Total soluble solids of the samples were measured by refractometry (Atago handheld refractometer N-1E, Japan).

2.4.3. Total phenolic compound analysis

Total phenolic content was determined with the Folin-Ciocalteu reagent with reference to previous research [5] using gallic acid as the standard. One milliliter methanolic extract of samples was placed into a test tube and vortexed (Nissin mixer N-20 M, USA) for 15 s with 1.5 mL Folin-Ciocalteu reagent, and allowed to stand at room temperature for 5 min, before adding 1.5 mL of 0.57 M Na_2CO_3 and incubated for 90 min at room temperature. Absorbance was measured at 750 nm using the

spectrophotometer, with the same mixture except the sample extract was replaced by methanol as the blank. Total phenolic content was expressed as mg GAE (Gallic Acid Equivalent)/L.

2.4.4. Antioxidant activity analysis

Antioxidant activity was measured in using the DPPH radical scavenging activity method reference to previous research [6] with slight modification, 100 μ L samples diluted with 3 ml of ethanol 96%, then 1 ml of 0.2 mM DPPH was added and homogenized. The reaction mixture was vortexed and left to stand at dark room for 30 min and the absorbance for the sample (Abs sample) was measured using UV-VIS spectrophotometer (Shimadzu, UV-1601, Japan) at 517 nm against buffer blank. Controls were made by replacing sample with aqua demin. Antioxidant capacity is expressed as % DPPH radical scavenging ability by the formula $[1 - (\text{Abs sample}/\text{Abs Control})] \times 100\%$.

2.5. Statistical Analysis

All the data were expressed as mean \pm standard deviation (SD) for 3 repetition. The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Fisher test (Stat View) using MiniTab Ver.16.0. Statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1. Chemical characteristic of snake fruit kombucha

Kombucha fermentation process characterized by decreasing the substrate in the form of sucrose, as well as the improvement of fermentation products such as acids and exopolysaccharide (nata phase). Table 1 shows that there were an increase in total acidity which is in line with the decrease in pH, total sugar and TSS. Based on the results of variance analysis, it is known that there were cultivar different on changes in total acidity, pH, total sugar and total soluble solids. *Salak Pondoh* kombucha had the highest change values.

Table 1. Chemical characteristics of snake fruit kombucha.

Cultivars of snake fruit	Parameters					
	Total acidity (%)			pH		
	Day -0	Day-14	Change	Day -0	Day-14	Change
Suwaru	0.57 \pm 0.14 a	1.64 \pm 0.10	1.07 b	3.91 \pm 0.19 b	3.22 \pm 0.09 ab	- 0.69 b
Madura	0.50 \pm 0.11 ab	1.60 \pm 0.06	1.11 ab	4.00 \pm 0.16 b	3.20 \pm 0.12 ab	- 0.80 a
Pondoh	0.26 \pm 0.07 c	1.72 \pm 0.17	1.46 a	4.42 \pm 0.30 a	3.12 \pm 0.02 b	- 1.3 a
Bali	0.35 \pm 0.06 bc	1.52 \pm 0.06	1.17 ab	3.76 \pm 0.17 b	3.38 \pm 0.15 a	- 0.38 b
Cultivars of snake fruit	Parameters					
	Total sugar (%)			Total soluble solids ($^{\circ}$ Brix)		
	Day -0	Day-14	Change	Day -0	Day-14	Change
Suwaru	10.1 \pm 0.62 c	7.76 \pm 0.03	- 2.30 bc	13.93 \pm 0.06 a	12.88 \pm 0.08 a	- 1.05 ab
Madura	11.6 \pm 0.35 b	8.26 \pm 0.17	- 3.36 ab	13.30 \pm 0.10 b	12.43 \pm 0.32 b	- 0.87 c
Pondoh	12.6 \pm 0.64 a	8.28 \pm 0.42	- 4.28 a	14.08 \pm 0.10 a	12.97 \pm 0.06 a	- 1.12 a
Bali	10.5 \pm 0.38 c	8.25 \pm 0.87	- 2.21 c	13.97 \pm 0.06 a	12.93 \pm 0.06 a	- 1.04 b

Values are means \pm SD (n=3 for each group).

Values in a column with the different letters are significantly different ($p < 0.05$)

The increased in total acidity that occurs during the fermentation process is caused by changes of glucose by bacteria and yeast during the fermentation process to produce organic acids, especially

acetic acid [1]. Kombucha fermentation process starts from the process of hydrolysis of sucrose to glucose and fructose by *Acetobacterxylinum*. In the pentose phosphate pathway, glucose is converted by the enzyme glucokinase to glucose-6-phosphate (G6P), then G6P is converted to glucose-1-phosphate (G1P) with the help of phosphoglucosmutase enzyme and converted back to UridinDiphosphate (UDP) with pyrophosphorylase enzyme until it is converted to cellulose with the help of cellulose synthase enzymes. The result of cellulose formation is what will be seen as a pellicle on the surface of the fermentation medium[7].

Besides producing cellulose, the metabolic process of *Acetobacterxylinum* will also produce primary metabolites in the form of acetic acid[1] and also other organic acids including gluconic acid, glucuronic acid[1], malic acid, tartaric acid, citric acid, butyrate and lactic acid[5]. While yeast (*Saccharomyces cerevisiae*) during the fermentation process will convert glucose and fructose to alcohol and CO₂ through the process of glycolysis[1]. Furthermore, CO₂ will react with water to form carbonic acid. Meanwhile, the alcohol produced will be oxidized by *Acetobacterxylinum* to acetaldehyde and then into acetic acid[1]. The accumulation of organic acids formed during the fermentation process will affect the pH value. The higher the total acid formed, the lower the pH value, this occurs because the acid in the kombucha will release protons (H⁺) so that the pH value decreases. The decreased total sugar and total soluble solids occurs during the fermentation process along with the increased in total acidity. This is because sugar in the fermentation medium is used as a carbon source for the growth of microorganism cells, in addition to the metabolic processes that produce cellulose and metabolites in the form of certain organic acids[1]. Total soluble solids value is a measure of the content of a combination of all substances (inorganic and organic) dissolved in food. Some decreases the value of total soluble solids during the fermentation process other than due to the reduced total sugar can also be caused by the deposition of protein, pectin, pigments and other minerals. This also happened in this research.

3.2. Total phenolic compound and Antioxidant activity

Fermentation process in kombucha is known to be able to change bioactive components. Several previous researches revealed that there was an increase in the concentration of phenol [1], flavonoids and tannins[1, 8] during fermentation. Based on Table 2, there was an increase in total phenols, tannins and flavonoids in all cultivars of snake fruit kombucha. The results of the analysis revealed that various types of snake fruit used as ingredients to make kombucha showed no significant differences in the parameters of changes in total phenol values and antioxidant activity.

Table 2 Total phenolic compound and antioxidant activity of snake fruit kombucha.

Cultivar of snake fruit	Parameters					
	Total phenolic compound (mg/L GAE)			Antioxidant activity (%)		
	Day -0	Day-14	Change	Day -0	Day-14	Change
Suwaru	281.01 ± 11.28 a	535.59 ± 1.96 a	254.58	85.19 ± 0.04 a	88.18 ± 1.47 a	2.99
Madura	257.48 ± 6.96 b	473.82 ± 8.55 b	216.34	73.31 ± 3.78 b	77.21 ± 1.17 b	3.90
Pondoh	164.67 ± 2.47 d	377.09 ± 16.76 c	212.42	70.37 ± 3.37 b	73.26 ± 0.91 c	2.90
Bali	185.92 ± 5.40 c	408.14 ± 16.76 c	211.11	72.77 ± 3.23 b	76.76 ± 2.47 b	3.99

Values are means ± SD (n=3 for each group).

Values in a column with the different letters are significantly different (p< 0.05)

The increased of phenol during the fermentation process is suspected because there is a biotransformation process of phenol compounds by microorganisms in kombucha. In previous research revealed that bacteria and yeast in the kombucha consortium can produce various types of enzymes that function to break down phenol complex compounds into simple forms [1]. During fermentation process, several hydrolytic enzymes such as invertase, cellulase, amylase are produced [9]

which are thought to be able to break various complex bonds between phenol compounds and the structure of the material network so as to increase the number of free phenols in fermentation media. *Candida tropicalis* which is one type of microorganism in kombucha culture is known to be able to break down various types of phenol compounds into simple forms [10]. Ferulic acid and cinnamic acid are a type of phenolic acid that is abundant in plants [11]. *Saccharomyces cerevisiae* is able to produce enzymes of cinnamic decarboxylase and vinyl reductase. Sinamic decarboxylase enzymes act to catalyze the decarboxylation process of cinnamic acid and ferulic acid to 4-vinylphenol and 4-vinylguaikol. While the enzyme vinyl reductase acts to catalyze the reduction process of 4-vinylphenol and 4-vinylguaikol to 4-ethylphenol and 4-ethylguaikol [12].

The increase of phenol compounds in line with increase of antioxidant activity. This is supported by several previous researches that have revealed that the content of phenol compounds will increase in line with the increase in antioxidant activity [8]. This is because bioactive compounds have a free ratio with hydrogen atoms from the aromatic hydroxyl group (-OH) they have [13].

3.3. Best treatment of snake fruit kombucha

The selection of the best treatment was carried out using the De Garmo / effectiveness index method [14] Based on this, it was known that the best results of kombucha of *salak Suwaru* cultivar with the following characteristics: total acid 1.64%. pH 3.22. total sugar 7.76%. Total soluble solids 12.88 °Brix. total phenol 535.59 mg / L GAE and antioxidant activity 88.18%.

4. Conclusion

The results demonstrated that there were changes in the chemical characteristics of a decrease in pH, total sugar, and total soluble solids, as well as an increase in total acidity, total phenol and antioxidant activity significantly during fermentation. The best treatment was obtained on kombucha of *salak Suwaru* cultivars with total acidity of 1.64%, pH of 3.22, total sugar of 7.76%, total soluble solids of 12.88 °Brix, total phenol of 535.59 mg/L GAE and antioxidant activity of 88.18%.

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