Kombucha fermentation test used for various types of herbal teas

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Abstract. Tea is a common drink in the community, the benefits of tea can be improved by processing fermented tea called kombucha. Kombucha is a refreshing drink made from tea water plus sugar, obtained through the fermentation process by acetic acid bacteria and fungi, is consumed for its health benefits. The common Kombucha starter is called *SCOOBY (Simbyotic of Bacteri and Yeast)*. Kombucha research using herbal tea is very rarely done, it is necessary kombucha research using a variety of herbal teas to determine the quality of its inhibitory power against bacteria and its quality compared with kombucha berkomposisi tea Camelia sinensis in general. The purpose of this research was to know kombucha quality with ph parameter, thickness of nata, total acid and its inhibitory power to Escherchia coli and *Staphylococcus aureus* bacteria. This research used Randomized Block Design (RAK) method using 1 tea type treatment, with 3 replications. The first type of green tea (P1), roselle tea (P2), mangosteen peel tea (P3), soursop leaf tea (P4), moringa leaf tea (P5) and yellow leaf tea (P6), so there are 18 treatment combinations. Based on statistical analysis, there was influence of tea type to kombucha quality. Treatments of P1 and P2 were found to have an optimum and significant effect for kombucha. the results in level of acidity [pH] on green tea (P1) was 3.05, nata thickness of 4.63 and total acid of 0.69. Acidity in roselle tea (P2) was 2.86, nata thickness of 3.83, and total acid of 0.71. While the lowest quality was found in the treatment of mangosteen peel (P3) tea with pH 2.57, nata thickness of 0.35, and total acid of 0.79.

1. Introduction

The lifestyle of modern Indonesian people tends to prefer products that are safe for health. Herbal health products are more prevalent in the community to prevent or major treatment of diseases, because it is considered safe from chemicals that are toxic to the body. In addition, the price of drugs that are sometimes expensive to trigger the community to try other alternatives in maintaining health through herbal products.

Tea is one of health herbal drinks that have high appeal in Indonesia. Tea is generally derived from Camelia sinensis plant species that have a variety of phenol and non-phenol compounds that benefit the body [1]. The benefits of tea can be improved by processing tea into a probiotic drink that is kombucha. Kombucha flavor is fresher and many active compounds in kombucha are not found in tea, while the active compound in its own tea does not change [2]. Kombucha affects uric acid levels in serum rattus norvegicus, decreased uric acid levels by 5 ml / day, 8 ml / day and 11 ml / day respectively 50,33%, 54,00%, and 26, respectively, 17% [3].

According to Aditiwati and Kusnadi [4], Kombucha is a fermented beverage product containing a number of vitamins, minerals, enzymes, and organic acids. Stater kombucha is often referred to as Scooby Symbiotic Culture of Bacteri and yeast. Jayabalan et al [5] states that kombucha tea is a functional beverage that occupies a position between conventional drinks and drugs, so it can be used in the prevention of a disease. Making kombucha tea itself often uses local natural ingredients ie tea from the leaves of Camelia Sinensis which is processed into black tea, green tea or oolong tea.

Over time there is one term of tea in the community called herbal tea. According Ravikumar [6] herbal tea is generally a mixture of some substances commonly called infusion/tisane. It is made from a combination of dried leaves, seeds, wood, fruit, flowers and other plants that have benefits. Herbal tea in Indonesia is widely interpreted by the community as a tea made of leaves and flowers from certain plants that are dried and used in the healing of a disease, such as peppermint tea, rosela tea, bay leaf tea and others.

Kombucha research using a variety of herbal teas is very rare, it is necessary kombucha research using a variety of herbal teas to know its quality compared with kombucha berkomposisi of the general (C. sinensis). Making kombucha from steeping herbal tea is expected to improve the effectiveness of fermented beverages as traditional medicine is cheap and easily made by the community. In addition, it can improve the utilization of materials from the environment [7], because it is easy to find and practical in the manufacturing process.

One of kombucha research using herbal tea is kombucha fermentation that is Widyasari [8] in its sketch describes kombucha moringa leaf with treatment of 30 ml leaf and fermentation time 8 days has high antioxidant activity. Another study was conducted by Hidayanti et al [9] which stated that there was a tendency to increase the number of erythrocytes and hemoglobin, and the decrease of leukocyte mice due to the treatment of kombucha rosella tea. But not all effective herbal teas are used as kombucha-making medium, some herbal teas have an unfavorable effect on Scooby's growth. Cultures for Health [10] says herbal tea itself is technically much more difficult to control kombucha pH levels and some nutritional herbal teas are not needed for Scooby, such as peppermint, chamomile and ginger. Exposure to these data at the same time provide an initiative to test / research the anti-bacterial reaction and quality of kombucha tea using various herbal teas.

The fermentation process begins with a yeast activity that breaks down sucrose into glucose and fructose. Chemical content of Kombucha with the help of extracellular enzyme invertase and subsequently glucose is reduced to ethanol and carbon dioxide formed reacts with water to form carbonic acid [4].

The purpose of this research is to know the influence of herbal tea type on kombucha quality including pH analysis, Nata thickness, total acetic acid and antibacterial activity against Echercia coli and Staphylococcus aureus bacteria.

Usefulness of research for researchers can be used as a source of learning biotechnology materials, for the Institute as a science of applied biotegi field, and for Food Industry and society can be used as a literature in the manufacture of conventional food products and optimization of the utilization of herbal medicine products / conventional medicine that is environmentally friendly and economical.

2. Materials and Methods

2.1. Place and Time of Research

Research and Activity of kombucha fermentation was conducted in Biology Laboratory 2 University of PGRI Madiun, conducted on 12 June to 5 July 2016.

2.2. Research Design

The study used Randomized Block Design (RAK) with 1 treatment 3 repetition. The research used experimental approach with 6 types of tea. The total treatment was 1 treatment with 4 replications, so the total amount of treatment was 18.

2.3. Data collection techniques

The data collecting technique is observation of kombucha quality from various types of local leaf, green tea leaf (P1), rosella (P2), mangosteen leather (P3), steeping of soursop leaf (P4) P5) and seduhan leaf yellow (P6). Measured by observing the pH parameters, the total acid and thickness of nata formed during 8 days of fermentation. Then continued antibacterial test of each treatment.

2.4. Research Instruments

Tools used for kombucha fermentation are freezers, measuring cylinders, scales, pans, cloth cover, rubber bands, gas stove, panic aluminum, 300 ml glasses, and filters. test tube, cup bekker, erlenmeyer, stirrer, petri dish, autoclave, pH meter, tweezers, dropper pipette, volume pipette, stative, measuring cup, ruler, digital scales and complete stationery. The ingredients used for kombucha fermentation are 6 types of local natural ingredients: green tea leaves, rosella roses, mangosteen leather, soursop leaf, moringa leaf brew, and seduhan leaf yellow, sugar, water, stater, alcohol 70% distilled water, NaOH, indicator of phenoltalein. The stages in the research procedure are as follows:

1) Media Sterilization Stage. Simple sterilization by flowing hot water on a glass container as a fermentation medium. The stage procedure can be seen in the appendix.
2) Kombucha Fermentation Stage. Fermentation is carried out for 8 days in a humid place without exposure to sunlight. The stage procedure can be seen in the appendix.
3) Kombucha Quality Test Stage includes:
   a) PH test. Observation of kombucha pH was performed after day 8 using pH meters that have been calibrated.
   b) Nata Thickness Test. The observed nata thickness was measured using a ruler on 3 sides of nata then calculated on average.
   c) Total Acid Tests using titration method Apriyantono et al., 1989. The procedure of the step can be seen in the appendix.
   d) Kombucha Antibacterial Test against Escherichia coli and Staphylococcus aureus bacteria using agar diffusion method.

2.5. Data Analysis Technique

The result of data obtained from quality test on kombucha with various herbal tea was processed using SPSS version 23. All data were analyzed statistically descriptive and inferential / probability anava one path and further test analyzed coefficient of diversity using LSD test.

3. Results and Discussion

Exposed data is quantitative data using statistical data (number), which is obtained from research from kombucha quality test, with pH parameter, thickness of nata and total kombucha acid. The following table 1 is the average result of kombucha research using local natural ingredients:

Table 1. Research Data Kombucha uses various types of herbal tea

No	Medium Type	Kombucha Quality		
		pH	Thickness Nata	Total Acid
1.	(P_1) Green Tea Leaves	3,05	4,63	0,69
2.	(P_2) Rosela	2,86	3,83	0,71
3.	(P_3) Mangosteen Skin	2,57	0,35	0,79
4.	(P_4) Soursop leaves	2,61	1,33	0,76
5.	(P_5) Leaves of moringa	2,61	1,43	0,74
6.	(P_6) Leaves Kemuning	2,58	1,16	0,77

3.1. Effect of Tea Type on kombucha pH

Based on the results of the pH test in Table 4.1 shows the pH present in the treatment of P1 (green tea) as a control ie with an average pH of 3.05. The highest pH of the other five herbal teas was shown in

the treatment of P2 (rosela tea) that has a pH with an average of 2.86 while the lowest pH is found in kombucha mangosteen peel tea with an average pH of 2.57.

During the fermentation the pH of each treatment decreases, according to Sutarmi [11] exposure that the pH value decrease indicates the ongoing fermentation process. The decrease in pH is due to the process of fermentation of A. xylinum bacteria in kombucha to form organic acids and other secondary metabolite compounds as a result of kombucha fermentation products according to the exposure of Sopandi and Wardah [12] that each microbial in synergistic growth is able to grow in independent and producing metabolites at low rates. With low pH, Greenwal et. al [13] states that the acid in kombucha has the ability to limit pathogenic bacteria from various other microorganisms, including contaminants that may be present in the growing medium.

Based on anova analysis with significant level 0,05 has probability value (sig) 0.000 hence there is influence of tea type to kombucha pH, kombucha pH data can be seen in Figure 1.

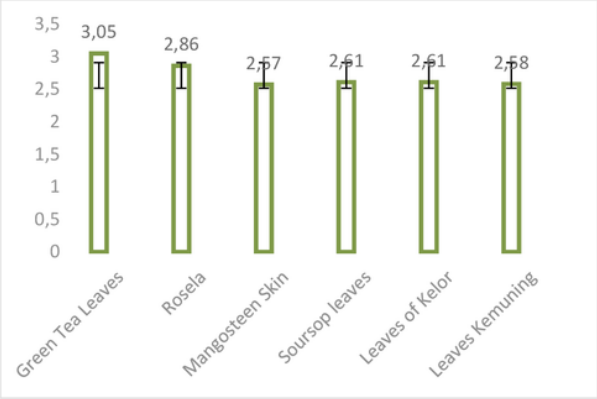


Figure 1. Chart for pH values of kombucha with various types of herbal tea.

Based on the above graph the decrease in kombucha pH for 8 days of fermentation has significant difference of pH and significantly different in each treatment. Each organism has a certain pH that allows optimal growth, according to Bazarewske 1995 in Nainggolan [14] kombucha ideal pH of 2.7-3.2, with the statement can be known kombucha tea that has the most ideal pH is kombucha green tea with pH 3.05 and kombucha rosela tea with a pH of 2.86. When the pH is ideal it can be concluded that the type of medium green tea and roselle flowers can be the optimal medium for bacterial growth in kombucha fermentation.

pH can also be influenced by tea type composition, according to Adriyani [15] Tea type effect on kombucha tea pH caused due to differences in the amount of polyphenol and catechin compounds in tea that cause bitter taste and sepat, thus affecting the activity of microbes and yeasts in decomposing sucrose into monosaccharides will be converted into ethanol and carbon dioxide. Ethanol is oxidized to form acids. Thus the pH difference in kombucha fermentation above may indicate the effectiveness of a tea type being used as a medium in kombucha fermentation.

3.2. Effect of Tea Type on Nata Kombucha Thickness

Nata thickness is one indicator of microbial growth, because the consortium of kombucha microbes is found in Scooby which will produce new nata. During the fermentation in the tea medium, the bacteria A. xylinum on the kombucha starter (Scooby) will produce an increasingly thick nata. The resulting white nata is somewhat transparent and floats on the surface of the fermentation container. Diagram of thickness nata kombucha with various types of the herbs can be seen in the Figure 2.

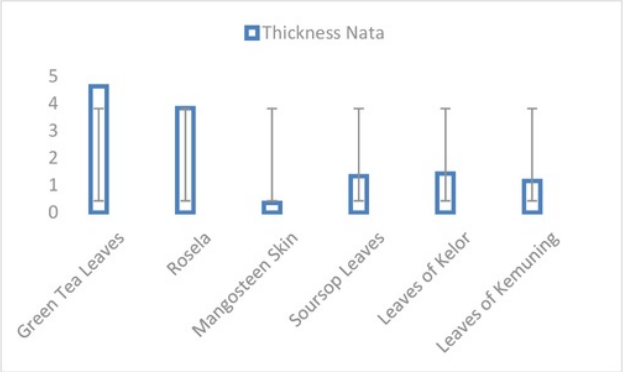


Figure 2. Diagram of Kombucha nata thickness

Based on the above images of nata on kombucha produced from various herbal teas, the thickest nata is on the controlled green tea kombucha (P1), from the five herbal teas, roselle tea (P2) has the highest thickness of 3.8 mm, while the lowest thickness was on kombucha mangosteen peel (P3) tea of 0.2 mm. The thickness of nata above is caused by the main polyphenol compound content of green tea that is catechin. According Sutarmi [11] This catechin is a compound capable of inhibiting the growth of bacteria. In addition to these catechins in green tea has abundant amounts because it does not experience the oxidation process like other teas. This results in green tea kombucha nata thicker than other tea nata, because the ability of catechin compounds against pathogenic bacteria, so that the activity of cellulose nata formation by Acetobacter xylinum bacteria is not disturbed by pathogenic bacteria. Figure 3 is a thick picture of green tea kombucha (P1), roselle (P2) and mangosteen (P3) from the side.

(a) (b) (c)

Figure 3. Nata Kombucha Observation of Side Side of Glass Container
(a) Thick Nata Kombucha Green Tea (P1)
(b) Thick Nata Kombucha Rosela Flower (P2)
(c) Thick Nata Kombucha Mangosteen Leather (P3)

The black brace marks from the picture above show the thickness of nata on the kombucha from the side. Kombucha green tea (P1) with a thickness of nata of 4.63 mm is the treatment that has the thickest nata, the second is on kombucha Seduhan rosela (P2) with thick nata of 3.83 mm, while the nata is the thinnest in kombucha Seduhan the skin of mangosteen (P3) with a thickness of nata of 0.35 mm. Here in Figure 4 is a thick kombucha appearance from the top side.

Figure 4. Nata Kombucha Observations from the Top of the Glass Container
(a) Thick Nata Kombucha Green Tea (P1)
(b) Thick Nata Kombucha Rosela Flower (P2)
(c) Thick Nata Kombucha Mangosteen Leather (P3)

Based on the arrow in Figure 4. above showed the appearance of nata kombucha from the top side. In green tea kombucha (P1) the structure of nata kombucha looked thick and flat, and the density is full, so the treatment is assumed to be the thickest nata. In kombucha the roselle flower (P2) of nata formed from the upper side appears full density, but uneven, from side-side measurements the treatment has a second nata thick after green tea kombucha. While on kombucha mangosteen peel (P3) nata seen from top side had nata that thick uneven, and texture similar nata not perfect fermented. Then it can be concluded that the type of medium can affect the activity of bacteria Acetobacter produce nata. In accordance with the analysis of anava, different types of tea have a significant effect on the thickness of nata. Other reasons that influence the thickness of nata one of them is the cause of high pH low, According to Fitria et al [16] low pH in kombucha possibly disrupt the formation of nata kombucha by A.xylinum. It can also be concluded that the low thickness of the nata is directly proportional to the low pH, according to the results of the study showed that the lowest thickness of nata is on the mangosteen peel tea with the lowest pH of 2.57.

3.3. Effect of Tea Type on Total Kombucha Acid

The growth of microbial cells is used in the confection of the tea product into kombucha and eventually has several follow-up products such as acetic acid, lactic acid and other secondary metabolite compounds. Here is a total picture of kombucha acid with various types of herbal tea:

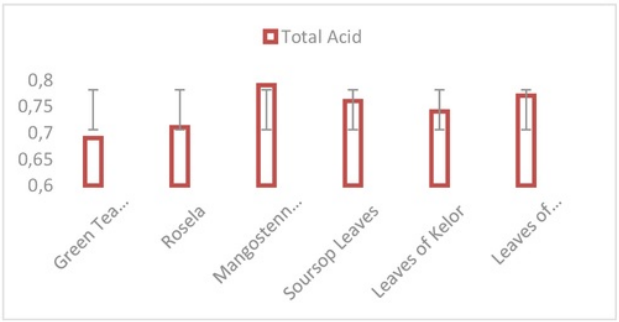


Figure 5. Total Chart of Kombucha Acid

Afifah [17] states that the higher the organic acid contained in kombucha the higher the total acid. Based on the data obtained the highest total acid is on kombucha mangosteen peel tea of 0.79 while the lowest in green tea kombucha of 0.69. Then it can be concluded from the pH data and the previous thickness of the nata that the higher the total acid in kombucha the lower the pH in kombucha

fermentation, and if the more acid then it can affect the thickness of nata formed. Although in anava analysis the results are not significant, but the effect of total kombucha acid can be analyzed using pH data and previous thickness of nata.

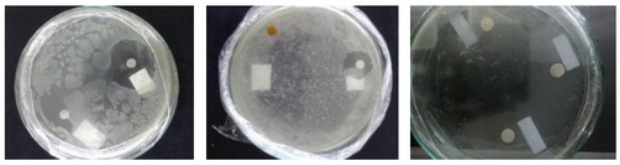
The high acid in kombucha of mangosteen leather causing the difficulty of yeast and bacteria in forming nata, it is because during the process pembenutkan cellulose nata yeast and bacteria in kombucha not able to survive with high acid conditions. So it can be concluded that the type of tea has an effect on total kombucha tea acid. This is due to differences in the amount of polyphenol and catechin compounds in tea, thus affecting the activity of microbes and yeasts in decomposing sucrose into monosaccharides which will be converted to ethanol and carbon dioxide. Ethanol is oxidized to form acids. Here is the picture 4 is a kombucha that changes color.

Figure 6. Color Change Changes Process Total Acid

Based on Figure 6 above the visible change of color to pink on the sample titrated with 0.1 M NaOH solution. The volume amount of NaOH solution for titration is used to know the total acetic acid in kombucha.

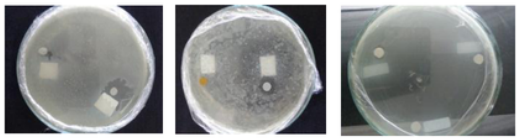
3.4. Effect of Tea Types on Kombucha Antibacterial Activity Against Escherchia coli and Staphylococcus aureus Bacteria

Greenwalt et. all (1998) stated that the antibacterial activity in kombucha against pathogenic microorganisms is mostly contributed by the acid in the kombucha, this can be known by the tests performed on some similarly inhibited microorganisms, but when the kombucha tea sample is neutralized, this antibacterial disappears. Antibacterial activity test was performed with 2 types of bacteria including gram negative bacteria (Escherchia coli) and gram positive (Staphylococcus aureus). Below in Figure 7 and 8 is the documentation of the kombucha clear zone against the bacteria *E. coli and S. aureus*:



(a) (b) (c)

Figure 7 Zone antibacteria Kombucha on *Escherichia coli* Bacteria
(a) Kombucha Green Tea (P1)
(b) Kombucha Tea Rosela (P2)
(c) Kombucha Manggis Leather Tea (P3)



(a) (b) (c)

Figure 8. Kombucha antibacteria Zone on *Staphylococcus aureus* Bacteria
(a) Kombucha Green Tea (P1)
(b) Kombucha Tea Rosela (P2)
(c) Kombucha Manggis Leather Tea (P3)

Differences in the drag zone can be caused due to the content of secondary metabolite compounds owned by different medium / tea. Some compounds are sometimes ineffective for scooby growth [9]. Watawana (2015) also explains it relates to antimicrobial activity and pH value, mainly due to the presence of acetic acid in particular and the range of organic acids, catechins and proteins produced during fermentation. The difference in the content of the secondary metabolite compounds indicates that catechin compounds in green tea have a major influence in the inhibition of pathogenic bacteria. In another study Bhattacharya et al (2016) mentioned that the catechin compound in tea is a polyphenolic compound and isorhamnetin is detected as the main antibacterial compound of kombucha belonging to flavan-3-ol and flavonoid flavonols, respectively, also detect catechins as one of the compounds in the active polyphenolic fraction of kombucha.

Based on these data it can be seen that kombucha antibacterial activity is more effective in Escherchia coli bacteria than Staphylococcus aureus, according to Branen and Davidson's statement, 1983 in Grace (2005) ie Gram negative bacteria is generally more susceptible to hydrogen peroxide compared to Gram positive bacteria.

4. Conclusion

There was significant effect on types of herbal tea in kombucha quality measued by parameters of pH changed, nata thickness, total acid and antibacterial tests. The most effective medium and best quality were green tea and roselle flower, while the lowest were kombucha treatment of mangosteen peel.

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