

## CHARACTERIZATION AND ANALYSIS KOMBUCHA TEA ANTIOXIDANT ACTIVITY BASED ON LONG FERMENTATION AS A BEVERAGE FUNCTIONAL

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**Background:** Kombucha is a symbiosis between bacteria (*Acetobacter*) and yeast (*Saccharomyces*), in English abbreviated SCOBY (Symbiotic Culture of Bacteria and Yeast). SCOBY in this research will be fermented using green tea media, to make kombucha tea product. Kombucha tea content is very beneficial for health, such as vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B12 (cyanocobalamin), vitamin C, acetic acid, amino acids, glucuronic acid, lactic acid and antioxidants. Product characteristic is needed to produce good product quality.

**Method:** The researchers used experimental studies and used the RAL method by varying the length of fermentation of kombucha tea.

**Result:** The result of characterization, kombucha tea pH the longer the fermentation time decreases, the longer the fermentation time the more bright, the weight of cellulose produced SCOBY more fermentation time increases weight, the reduction of sugar content the longer the fermentation time will decrease. The optimum antioxidant activity was obtained on the 7th day of fermentation of 93.79%.

**Conclusion:** Based on the results and the above discussion can be concluded, the color will be brighter with increasing length of fermentation, as well as the weight will increase with the longer fermentation. In contrast to the pH and sugar reductions the longer the fermentation time will decrease. The antioxidant activity had an optimum point on 7th day fermentation of 93.79% and will decrease with increasing fermentation time.

**Keywords:** kombucha Tea Recipe; Characteristic; Antioxidant; Long Fermentation

### INTRODUCTION

Kombucha tea is the result of fermentation of sweetened tea liquid by microorganisms from bacterial and yeast groups. The combination of *Acetobacter xylinum* and yeast bacteria is *Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Saccharomyces bisporus*, *Zygosaccharomyces* sp and several types of yeast (*Torulopsis* sp) (Aloulou et al, 2012). Kombucha culture hereinafter called SCOBY (Symbiotic Colony of Bacteria and Yeast).

The yeast cells will hydrolyze sucrose to form glucose and fructose for ethanol production, while the bacteria will convert glucose to form gluconic acid and fructose will form acetic acid. *Acetobacter* sp in kombucha culture oxidizes ethanol to acetaldehyde further into acetic acid. The accumulation of each metabolite in addition to form glucuronic acid, lactic acid, vitamins, amino acids, antibiotics, and other substances that are beneficial to health (Jayabalan et al., 2008) and specific scented. With long fermentation time it is possible to form a better composition compared to before fermentation. This study aims to determine the content of

Vitamin C and antioxidant activity of different fermentation time, to obtain kombucha tea containing Vitamin C and the most optimum antioxidant activity.

Health problems have an important role in human life and become one of the primary needs. Free radicals will make our body cells easily damaged and unable to function properly. Vitamin C in Kombucha tea is an important compound necessary to boost the immune system. Vitamin C can function as an antioxidant that can repair body cells and skin tissue damaged by free radicals. Antioxidants are useful for neutralizing free radicals (harmful particles formed as byproducts of metabolic processes, can damage genetic material and damage the immune system), inhibit the growth of cancer cells and reduce the accumulation of cholesterol in the blood and accelerate the removal of cholesterol through the feces.

## METHOD

Researchers used experimental research that is by varying the length of fermentation in Kombucha tea making. In this study to determine the optimum antioxidant activity based on the fermentation length of the authors using Completely Randomized Design (RAL), because this research is conducted indoors with uniform or controllable conditions, environmental conditions, tools, materials, and homogeneous media (Hanafiah, 2008; Siyoto & Sodik, 2015)

The study was conducted with 4 treatments and 1 control, each treatment and control was repeated 3 times. Control with 1 day fermentation, the first treatment is fermentation 3 days, the second treatment is fermentation 5 days, the third treatment is fermentation 7 days and the fourth treatment is fermentation 9 days,

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The materials used in this study are Kombucha culture (obtained from [www.wikikombucha.com](http://www.wikikombucha.com)), Sugar Sand (Gulaku Brand), Green Tea, kombucha vinegar. The tool used in this research is stainless steel pan, stove, beaker glass, stirrer, pH meter, strainer, white cloth, measuring cup, scales, organoleptic test form. Making kombucha tea 1000 ml of aquades was boiled for 10 minutes, added sugar (10% b/v) stirred until the sugar completely dissolved, then added green tea leaf (5% b/v). The tea solution is then filtered off, separated from the pulp and cooled to room temperature; the tea solution is placed in a measuring cup; added kombucha culture (10% b/v) to tea solution; tea solution containers that have been added kombucha culture are sealed with a clean cloth that has been sterilized (used cloth to prevent contamination of foreign materials, but air can still enter); fermented at room temperature (1, 3, 5, 7, 9, 11 days).

## RESULT

The fermentation process in this study is divided into 2, namely fermentation of alcohol and acetic acid. The yeast involved in kombucha fermentation is *Saccharomyces cereviceae*, whereas the acetic acid bacteria is *Acetobacter xylinum*. The yeast will break the sugar into alcohol, and the acetic acid bacteria will oxidize the alcohol to acetic acid.

### Characterization of Kombucha Tea

#### 1. Kombucha Tea Color

At the beginning of kombucha tea fermentation is dark brown, with increasing length of fermentation of dark brown color becomes lighter (figure 1). This is due to the ability of microbra to do the color degradation.



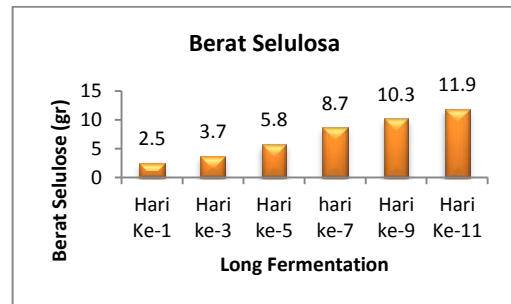
**Figure 1.** The color difference in kombucha tea based on fermentation time

Color degradation occurs because the microbes that utilize the total soluble solid as energy so that over time the solvent in the media will run out and the liquid becomes more clear or colorless (Nainggolan, 2009).

## 2. Cellulose weight generated SCOBY

The duration of fermentation influences the weight of cellulose produced by scoby on kombucha tea fermentation, this can be seen in Figure 2 which shows the increasing length of fermentation increasing the weight of cellulose. The initial weight of scoby prior to inclusion in the green tea substrate was 2.3 grams, at 1-day fermentation of cellulose weight to 2.5gr, day 2.7gr, 5th day 5.8gr, 7th day 8.7gr , day 9th 10.3gr and 11th day 11,9gr. The longer the fermentation time the weight of cellulose will increase, this is because during the process of fermentation occurs breakdown of sugar substances into simpler components of glucose and

fructose and the formation of carbon components of cellulose forming, so that in the long period of fermentation, there is accumulation of fermentation of sugar continuously.



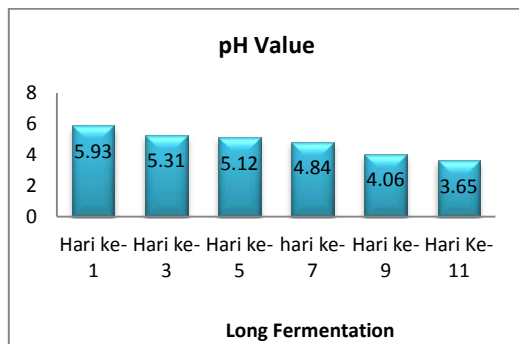
**Figure 2.** Cellulose weight generated SCOBY based on fermentation time

The process of extracellular cellulose formation by *Acetobacter xylinum* through aerobic process by using glucose as a substrate. Respiration is required for biological oxidation processes using oxygen molecules as oxidizing agents. *Acetobacter xylinum* can utilize fructose as a sugar source to synthesize cellulose. In the early formation, cellulose will first be produced in an unstructured medium, as a cell-released material, composed of randomly distributed molecules (Jayabalan, 2014).

## 3. pH value

From the data in Figure 3 it can be seen that the pH value on the 1st fermentation day (pH 5.93) gradually decreased on 3rd day fermentation (pH 5.31), 5th day (pH 5.12), day to-9 (pH 4.06) and day 11 (pH 3.65). The results of Pratiwi et al (2011) study about the effect of fermentation time on the physical and chemical properties of kombucha from Seaweed substrate yielded the data that is pH value

decreased from day 0 to day 16, from pH 4.89 decreased to pH 3, 09. The results of research conducted Sari (2014) also get the pH results decreased from the fermentation of day 4 to day 12, ie from pH 5.5 down to pH 3.52. More fermentation time will decrease the pH (acidity level ) in kombucha tea, this causes the kombucha tea more acidic taste.



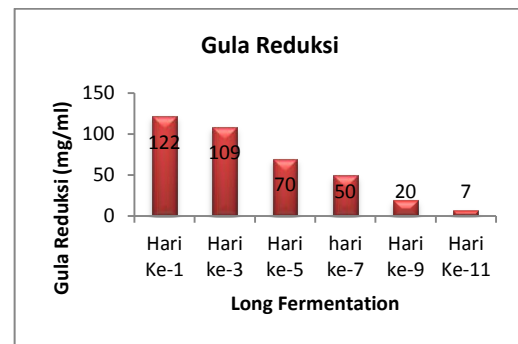
**Figure 3.** The pH value of kombucha tea based on fermentation time

The decline in kombucha tea pH occurs because during the fermentation process the yeast produces sugar into ethanol and by acetate bacteria is converted into organic acids, such as acetic acid and gluconic acid and some concentrations of organic acids leads to a decrease in the pH of the fermentation medium (Afifah, 2010). Nainggolan (2009) in his research states, the longer the fermentation takes place the concentration of acetic acid will be higher, this causes the pH value of kombucha tea tends to decrease.

#### 4. 4. Sugar reduction levels

The decrease in sugar content (figure 4) from the 1st day of fermentation by 122mg / ml continued to decline until the fermentation of the 11th day was 7mg / ml. reduction of reducing sugar explains that each microbe needs sugar as a carbon source,

because the sugar on the medium will be used by microbes as nutrients which will then be converted to alcohol and CO<sub>2</sub>. The CO<sub>2</sub> gas further reacts with water vapor and forms carbonic acid. In the process of fermentation of this sugar, yeast very active role in the decomposition of sugar into CO<sub>2</sub> and organic acids and other components (Pratiwi, 2011).



**Figure 4.** Sugar Levels Kombucha tea reduction based on fermentation time

Basically in making kombucha the most important is sugar, because sugar is the source of food for kombucha microbial culture. The type of sugar as a carbon source that is often used in making kombucha is sugar. When kombucha tea fermentation process, bacteria will convert glucose into various types of acids, vitamins, and alcohols that are beneficial to the body. This glucose is derived from the inversion of sucrose by yeast producing glucose and fructose.

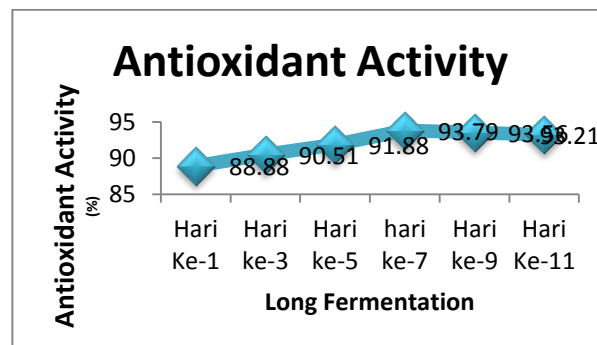
In the preparation of ethanol by yeast and cellulose by *Acetobacter xylinum*, glucose is converted to gluconic acid through the phosphate pentose pathway by acetic acid bacteria, mostly fructose is metabolized to acetic acid and a small amount of gluconic acid. Glucose here as a substrate for cell

growth and product formation (acetic acid).

The result of Marwati's research, et al (2013) about the effect of sugar concentration and kombucha starter on kombucha tea quality obtained by kombucha tea with best taste quality obtained from combination treatment of 20% sugar concentration with 20% kombucha starter concentration. The concentration of sugar and starch concentration of kombucha had significant effect on kombucha tea flavor characteristic.

### Antioxidant Activity

The antioxidant activity of kombucha tea with green tea substrate was done using DPPH method. DPPH is a free radical that is stable at room temperature that receives electrons or hydrogen and forms a stable molecule. The absorption of violet color was performed by absorbance measurement at 517 nm wavelength using visible spectrophotometer and compound as positive control. When all DPPH has binds to the antioxidant compounds in kombucha tea then the solution will lose its purple color and turn into a bright yellow color (Nur et all, 2013).



**Figure 5.** Long fermentation of kombucha tea antioxidant activity

he antioxidant activity of kombucha tea increases with increasing fermentation time (figure 5). The increase in antioxidant activity on day 1 (88.88%) and day 3 (90.51%) was 1.63%, then in the 5th day (91.88%) increased by 1.37 %, and optimum antioxidant activity in 7th day fermentation (93.79%). The increase of antioxidant activity on the 7th day is the optimum condition based on fermentation time, this can be seen with the decrease of anti oxidant activity on day 9 (93,56%) and decrease at day 11 (93,21% ).

Agus Suprijono, et al (2010) also conducted research on antioxidant activity in kombucha tea on black tea substrate, antioxidant activity on green tea kombucha showed optimum on fermentation day 7

and decreased antioxidant activity on fermentation day 10th day.

Suhardini and Zubaidah (2016) study, analyzed antioxidant activity of kombucha tea using leaf substrate containing phenol obtained optimum antioxidant activity of 88.24% up to 92.97% during fermentation day 8 and decreased antioxidant activity on day to day -14.

Wulandari (2014), conducted research on antioxidant activity of kombucha tea using coffee leaf substrate, obtained optimum antioxidant activity result on fermentation day 8 (89,51%) and decreased antioxidant activity on 12th day fermentation (53,43% ).

Increased antioxidant activity in kombucha tea is due to the metabolism of microorganisms in kombucha during the fermentation process (Goh et al, 2012). Antioxidant activity decreased after fermentation day 7, this is because acid atmosphere causes phenolic compounds become more stable and difficult to release proton that can bind to DPPH, so that antioxidant activity decreased (Ayu et al., 2013). Consuming fermented kombucha for too long can also be harmful to health because the high acetic acid content in kombucha can cause acidosis (Greenwelt et al, 2006)

### CONCLUSION

Based on the results and the above discussion can be concluded, the color will be brighter with increasing length of fermentation, as well as the weight will increase with the longer fermentation. In contrast to the pH and sugar reductions the longer the fermentation time will decrease. The antioxidant activity had an optimum point on 7th day fermentation of 93.79% and will decrease with increasing fermentation time

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