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Effect of Long Fermentation to Titrated Acid Total (TAT) in Lemongrass Kombucha Tea (*Cymbopogon citratus* (DC.) Stapf.)

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ABSTRACT

The lemongrass plant is one of the plants that is usually used as an additional kitchen spice to strengthen the taste. However, as time passes, lemongrass is widely used as an ingredient in drinks and medicines that benefit the body. To further increase the benefits of kitchen lemongrass, lemongrass kombucha tea is made. Currently, people need to learn more about lemongrass tea. Therefore, innovations have been made to make fermented drinks that utilize kombucha culture using a SCOBY (Symbiotic Culture of Bacteria and Yeast) starter. The lemongrass kombucha product is an innovation as a fermented beverage product which is expected to be able to compete in the processed beverage product industry in Indonesia. This research aims to determine the effect of fermentation time on titrated acid total (TAT) in lemongrass kombucha tea.

This research used different fermentation time treatments, namely 4 days, 8 days, 12 days and 16 days of fermentation with 4 repetitions each. Titrated acid total (TAT) testing is carried out when harvesting kombucha per fermentation treatment.

TAT and pH testing were carried out using a linear regression test, which has a solid relationship; the longer the fermentation time for lemongrass kombucha tea causes a decrease in the pH value and an increase in the total amount of titrated acid (TAT), the most significant total amount of titrated acid is the product with a fermentation time of 16 days, namely 2.16%.

Keywords: Kombucha, Lemongrass (*Cymbopogon citratus*), Titrated acid total (TAT), pH

INTRODUCTION

(*Cymbopogon citratus* (Dc.) Stapf.) is known by the public as Lemongrass, which is often called Lemongrass because it has a lemon-like aroma. Indonesians often use this plant as a kitchen spice, salad and beverage ingredient. Apart from being a processed food and beverage product, this plant is very rich in nutrients and is often used by people as an alternative to cure diseases and to prevent the body from getting sick quickly[1][2][3]. Lemongrass has good nutrition for the body; Lemongrass stems have a water content of 76.78%, ash content of 0.79%, and essential oils of 0.25%. Moreover, the vitamin A content is around 0.1 IU/100g, vitamin B is around 0.8 mg, and vitamin C is 4 mg. Lemongrass also provides essential minerals such as iron, magnesium, potassium, copper, calcium and zinc[4][5].

Apart from fresh drinks, one alternative to lemongrass is a healthy probiotic drink called kombucha. Kombucha is a traditional drink fermented with tea and sugar with a distinctive aroma and taste sour and sweet[6]. It contains various vitamins, minerals, and

organic acids. Kombucha tea is fermented aerobically by adding a cellulose layer/pellicle to the sweet tea called a SCOBY (Symbiotic Culture of Bacteria and Yeast)[7][8]. Acetobacter xylinum and the yeast Saccharomyces cerevisiae were the most abundant bacteria in this culture. Apart from that, Schizosaccharomyces, Candida, Torulospora, Pichia, Mycotorula, Brettanomyces, Zygosaccharomyces, Kloeckera, and Mycoderma are also found in kombucha[9][10].

During fermentation, Kombucha will experience a change in taste from refreshingly sour to sour like vinegar[11][12]. The reason is that the bacteria and yeast in Kombucha will convert sugar into several main compounds, namely ethanol, acetic acid, and glucuronic acid, as well as minor compounds, namely vitamin B, phenolic acid, lactic acid and enzymes. Organic acid compounds will increase during the fermentation process. This will result in a decrease in pH and an increase in total acidity in Kombucha[13].

The biological activity of Kombucha and its health-promoting properties have been described. The beneficial effects of this drink partly come from antioxidant activity, which restores radical production and balances the body's defence mechanisms[14][15]. As a result, Kombucha can contribute to the reduction of health disorders such as cancer, cardiovascular disease, and neurodegenerative diseases[16]. In general, Kombucha shows positive effects on digestion and gut microbiota, relieves arthritis, has antimicrobial activity, reduces insomnia, relieves headaches, detoxifies the body, shows hepatoprotective effects, relieves haemorrhoids, and positively impacts mood[14][15][17][18].

Fermentation time is one of the factors that contribute to making Kombucha. Kombucha fermentation time generally takes 6-12 days. The highest phenolic content in Kombucha was obtained during the 8 day kombucha fermentation period[19]. Moreover, soursop leaf kombucha is known to produce the highest vitamin C value, namely in the 12-day fermentation time treatment. Changes in microbial composition associated with the physicochemical characteristics of kombucha tea, such as pH and titratable acidity, influence the level of liking or organoleptic[20][21].

People need to learn more about lemongrass tea (*Cymbopogon citratus* (Dc.) Stapf.); therefore, innovations have been made in making fermented drinks that utilize kombucha culture to create quality and nutritious products. Therefore, it is necessary to research the effect of long fermentation treatment to determine the titrated acid total (TAT).

MATERIALS AND METHODS

This research used the Completely Randomized Design (CRD) method using a fermentation-length experiment of 4 treatment groups, each carried out 4 times in 4, 8, 12 and 16 days.

Preparation of starter culture media for the kombucha and SCOBY

2000 ml of water is boiled, 200g of granulated sugar is added at 10% w/v of the water used, and 10g of 0.5% (w/v) black tea is added. The SCOBY that has been evenly divided is placed in a glass cup containing tea water with 10 ml of liquid kombucha culture and one tablespoon of apple cider vinegar. Using a rubber band, the glass is covered with a napkin tie and stored in a room protected from direct sunlight for 7 days. The SCOBY formed and the kombucha solution in which the tea is fermented for 7 days is used as a starter to make kombucha.

Lemongrass tea

The base of the stem of Lemongrass (*Cymbopogon citratus* (DC.) Stapf.) is peeled to obtain the part used to make tea. The base of the lemongrass stem is cut into pieces and then dried in the sun for one day. Then, in the oven at 55°C for 6 minutes and cool. Processing lemongrass into dried lemongrass tea is intended to extend its shelf life.

Lemongrass kombucha tea

Make lemongrass kombucha tea using 12 grams of 0.5% (w/v) lemongrass tea, then brew using 2400 ml of boiling water. 200 ml of lemongrass tea is poured into each container, adding 10% (w/v) granulated sugar. A total of 20 ml of kombucha starter liquid was added and, covered and fermented for 4, 8, 12 and 16 days at room temperature.

pH analysis test

50 ml of lemongrass tea was taken from each treatment; the pH was measured using universal pH. Colour changes on pH paper are adjusted to colour standards.

Titrated Acid Total Amount (TAT) Test

10 ml of lemongrass kombucha tea into a 100 ml measuring flask and add distilled water to the mark. 50ml and put it in an Erlenmeyer, then add three drops of phenolphthalein (pp) indicator. Titrate with 0.1 N NaOH solution until the colour changes to pink. The scale reading is when the pink colour first forms and lasts 15 seconds. Total acetic acid content (%) was measured using the formula:

$$Total\ Acid\ (\%) = \frac{V_{NAOH} \times N_{NAOH} \times MW \times}{V_{Sample} \times 1000}\ 100\%$$

V NaOH is the volume of NaOH for titration, MW is the Molecular weight of acetic acid, N NaOH is NaOH standard concentration, and Vsample is the volume of the sample for titration.

STATISTICAL ANALYSIS

The pH and Total Titrated Acid (TAT) tests were the Regression and Correlation Test and the F Test (ANOVA).

RESULT AND DISCUSSION

During fermentation, chemical changes will occur, forming the pH value and total acid titration (TAT). Long fermentation treatment affects the pH and TAT of lemongrass kombucha tea. The longer the time used for fermentation, the TAT value increases, and the pH value of lemongrass kombucha tea decreases. One factor that needs to be considered when making lemongrass kombucha tea is the degree of acidity (pH). The initial and final pH values of kombucha tea are in Figure 1.

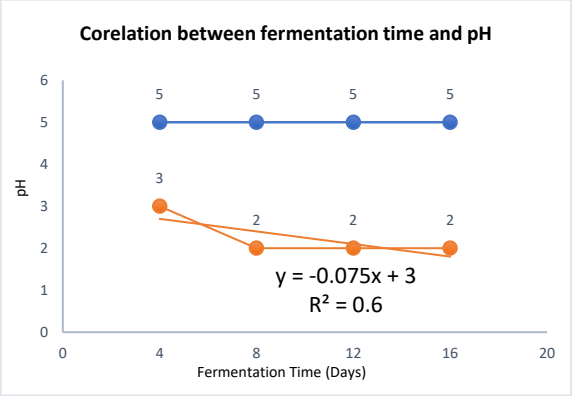

Figure 1. Initial pH value and final pH value and scatter plot of the corelation between fermentation time and pH of lemongrass kombucha tea.

Figure 1. above shows that the initial pH (before fermentation) and final pH (after fermentation) decrease during the fermentation process. This can happen because lemongrass kombucha tea produces accumulated acidic substances during fermentation. The acids formed result from the metabolism of acetic acid bacteria, so the accumulation of the acids formed will later reduce the pH of the media[22].

The decrease in pH during fermentation occurs due to the growth of lactic acid bacteria and yeast and the acetic acid metabolism process, which can increase organic acids[9]. The pH value in the kombucha fermentation process is significant; a pH value of 2.5-4.6 is the safe acidity limit for consumption[23][24]. From Figure 1, it can be seen that the pH decreases further, where on day 0 (control), the pH shows a value of 5, then in product A with a fermentation time of 4 days, the pH value shows a value of 5. The value is 3; at a fermentation time of 8 days, the pH value is assessed as 2. However, During the fermentation period from day 8 to day 16, which includes product C and product D, the pH value showed no change; it still showed a value of 2.

This is possible because the sucrose content in lemongrass kombucha tea has run out, and

breaking down glucose and fructose into organic acids and alcohol will continue until the sugar in the kombucha solution runs out[25]. So, the increase in organic acids will be higher if the time used for the fermentation process is longer, provided there is still sucrose in the lemongrass kombucha tea solution. The decrease in pH will continue to decrease if the amount of organic acids increases[26]. During the beginning of the fermentation process, the decrease in pH is caused by bacteria and yeast, which metabolize sucrose into organic acids. The decrease in pH during fermentation is possible because the sugar substrate is converted into alcohol and acetic acid products[22].

The correlation coefficient value that appears, namely r = - 0.775, is in the interval 0.600 – 0.799, which means that the two variables have a strong level of relationship. The coefficient of determination (R2) in Figure 1., the value of R2 = 0.600, shows that the pH value is influenced by the length of fermentation time. The pH value is influenced by the length of fermentation time by 60%, and other variables determine the remaining 40%. If seen from the 5% significance level (α = 0.05), it can be concluded that Ho is accepted because Sig. > α with a result of 0.775 > 0.05, which means there is an insignificant influence between the length of fermentation time and the pH value. The ANOVA test (F test) carried out using a linear regression test shows that if Fcount ≤ Ftable with a value of 3 ≤ 3.49, then it can be concluded that there is no real difference between the length of fermentation time and the pH value, so there is not enough evidence to reject Ho.

Kombucha's pH ranges from 2.62–3.27. So, in this study, the pH value found in lemongrass kombucha tea was included in the low category at a pH value of 2. The appropriate category at a pH value of 3, low pH values were found in products B (fermentation time 8 days), C (fermentation time 12 days) and D (fermentation time 16 days) is 2[27]. Another study says that kombucha has a pH value of 2.34-2.43[28]. Low acidity levels will hurt the walls of the digestive tract of consumers, especially ulcer sufferers. The pH of kombucha is acceptable and safe for consumption at a pH value of 3. The amount of TAT indicates that the formation of organic acids occurs during fermentation. The longer the fermentation time, the more TAT in kombucha tea will increase because more acetic acid will be formed. The results of the TAT calculation are in Figure 2.

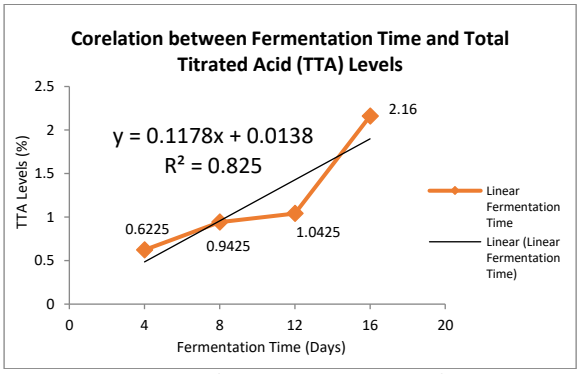

Figure 2. Scatter plot of the corelation between fermentation time and TAT of lemongrass kombucha tea.

Figure 2. shows that the longer the fermentation time, the more TAT produced increases. This can be proven by looking at the increase in TAT occurrences in each treatment. In the 4 days of fermentation time, the amount of TAT contained in the lemongrass kombucha tea solution was 0.6225%, 8 days of fermentation time was 0.9425%, 12 days of fermentation time was 1.0425%, 16 days of fermentation time was 2.1600%. The increase in TAT in lemongrass kombucha tea is closely related to the increasing activity of acetic acid bacteria in degrading sucrose and other organic materials into organic acids.

Figure 2., the results of linear regression statistical calculations, show that if the length of fermentation time gives an increase in TAT levels, from the line equation Y = 0.473x + 0.0138, the result is that the longer the time used for fermentation, the more TAT levels will increase by 0.473 times or increased by 47.3% with an initial total acid content of 0.0138%. The estimated correlation coefficient (r) obtained is r = 0.914 and has a positive value (+). This

shows that the length of fermentation time has a relationship that increases the value of TAT levels, where if the fermentation time increases, the TAT levels will increase. The correlation coefficient value that appears, namely r = 0.914, is in the interval 0.800 – 1.000, which means that the two variables have an extreme level of relationship. So, the results of the correlation analysis of these two variables show a positive relationship between the length of fermentation time and TAT levels.

The coefficient of determination (R2) in Figure 2., the value of R2 = 0.835, shows that the TAT level is influenced by the length of fermentation time. TAT levels are influenced by the length of fermentation time by 83.5%, and other variables determine the remaining 16.5%. If seen from the 5% significance level ($\alpha = 0.05$), it can be concluded that Ho is accepted because Sig. > α with a result of 0.086 > 0.05, there is an insignificant influence between the length of fermentation time and TAT levels.

The ANOVA test (F test) carried out using a linear regression test shows that if Fcount ≥ Ftable with a value of 10.133 ≥ 3.49, then it can be concluded that there is a fundamental difference between the length of fermentation time and the TAT level, so there is enough evidence to reject Ho.

This increase in total acid is proportional to the length of fermentation time; the increase in total acid is also related to the microorganisms in kombucha whose growth is in the logarithmic phase, which is also often called the log phase where this phase occurs after 2-10 hours of fermentation, the growth of these microorganisms increases rapidly with a short and constant generation time, the metabolism is most active, the synthesis of cell material is very fast in constant quantities until the nutrients contained in the kombucha tea solution run out or there is an accumulation of metabolic products which causes stunted growth[29]. The growth rate of microorganisms increased after two days, and the growth rate of yeast cells increased. The growth rate of yeast cells increased due to the availability of substrate and the pH of kombucha tea, which was suitable for the growth of yeast cells in converting glucose into alcohol and organic acids, likewise, with the activity of acetic acid bacteria, where after day 2, the substrate conditions are suitable for the growth of acetic acid bacterial cells[30].

Acetic acid bacteria convert glucose to gluconic acid and ethanol to produce acetic acid. The activity of acetic acid bacteria has begun to occur because kombucha tea contains the results of yeast cell metabolism, which converts sucrose into glucose and fructose with the help of intervase enzymes. The yeast breaks down sugar (sucrose) during the fermentation process. Glucose will then be metabolized to produce ethanol and carbon dioxide. Acetic acid bacteria will oxidize ethanol to acetaldehyde, which is then converted into acetic acid[31].

The TAT calculated in the lemongrass kombucha tea solution is considered acetic acid, which results from the metabolism of microorganisms in kombucha. This is because acetic acid is the most significant type of acid produced from the metabolic process of microorganisms in kombucha. Acetic acid is the most significant part of the acid produced during fermentation[23]. If the substrate has reduced nutrients, the bacteria will stop growing but not die. The acid concentration in kombucha only increases to a specific limit and then decreases[32]. This can happen because the sugar used by the acetic acid bacteria in the kombucha medium has been used up. However, research on lemongrass kombucha tea has not shown a decrease in the amount of TAT, but this may happen because the sugar (nutrient source) in the medium has yet to be thoroughly used up.

CONCLUSION

Lemongrass kombucha products have fundamental differences between each product; the longer the fermentation time, the more TAT increases. The total amount of titrated acid (TAT) in the treatment with a fermentation time was the most significant amount obtained in products with a

fermentation time of 16 days, which was 2.16%.

Declaration by Authors

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