

## The Effect Of Fermentation Time On Alcohol Content, Chemical Characteristic, And Halal Label Of Vanilla (*Vanilla planifolia*) Leaf Kombucha Tea

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**Keywords :** Vanilla Leaf Kombucha Tea, Alcohol Content, Chemical Characteristics, Halal Beverage Products

**Abstract :** Kombucha tea has become an innovative beverage product and popular with the public because it has many health benefits. One of the plant leaf that has the potential as an ingredient for making kombucha tea is vanilla leaf. Fermentation time will affect the alcohol content and active compounds content in vanilla leaf kombucha tea. Fermented beverage products that have an alcohol content of less than 0.5% are legally halal and medically harmless. The purpose of this study was to determine the halal label of vanilla leaf kombucha tea with variations in fermentation time based on alcohol content and chemical characteristics. The alcohol content test for vanilla leaf kombucha tea was carried out by quantitative analysis of the gravimetric method with variations in the 4 treatments including fermentation time of 0, 4, 8, and 12 days. The results of the alcohol content test showed increased results. Vanilla leaf kombucha tea with 0 day fermentation had an alcohol content of 0.12%, 4 days 0.15%, 8 days 0.21%, and 12 days 0.28%. From the results, the alcohol content in vanilla leaf kombucha tea was less than 0.5% and based on the MUI agreement on alcoholic beverages having an alcohol content of less than 0.5%, so the product is declared as a halal fermented drink. The results of the chemical characteristic test showed that there were differences in pH values and titrated total acid levels in kombucha tea. Vanilla leaf kombucha tea which had the best chemical characteristics is vanilla leaf kombucha tea with P2 treatment (4 days of fermentation) with a pH value of 3 and a Tat level of 0.012 and P3 treatment (8 days of fermentation) with a pH value of 3 and a Tat level of 0.024.

### INTRODUCTION

Currently, many diseases are caused by exposure to pollution and consumption of unhealthy foods. One of them is free radical attack (Wahdaningsih, 2011). The thing that can be controlled to reduce exposure to free radicals is to avoid consuming foods or drinks that trigger the formation of free radicals and choose healthy foods or healthy drinks. One of the healthy foods or healthy drinks is food or drinks that contain antioxidants (Nabillah, 2020). One of the innovations in beverage products that are rich in antioxidants and popular with the public is tea.

Processed tea products continue to be developed to increase the active compounds they contain, one of which is making kombucha tea.

Kombucha tea is a traditional beverage product made from fermented tea and sugar solution using kombucha culture starter (*Acetobacter xylinum* and *Saccharomyces cerevisiae* and several other types of yeast) and fermented for 7-14 days. Kombucha drinks have higher antioxidant activity when compared to unfermented tea drinks. Kombucha tea has health effects such as being an antioxidant, antibacterial, improving intestinal microflora, increasing body resistance and lowering blood

pressure. The main benefit of kombucha tea is that it can detoxify and repair the liver (liver) in the human body because it contains glucuronic acid, which is able to neutralize toxic compounds in the human body (Rosida et al., 2021).

In the manufacture of kombucha tea, one of the factors that can affect the resulting product is the fermentation time. Fermentation time will affect the alcohol content and content of active compounds in kombucha tea. As a Muslim, the alcohol content allowed in drinks is less than 0.5%. Therefore it is very important to control the fermentation time of making kombucha tea. Based on research conducted by Rosyada et al (2023), the results of the alcohol test show that the higher the concentration of sugar given to the kombucha leaf of the belimbing wuluh makes the alcohol level increase. This is because the sugar concentration affects the production of yeast consumption of sugar where the low concentration of sugar in the manufacture of alcohol causes the yeast to grow in a short time, resulting in a less than optimal fermentation process that occurs and the fermentation process will be completed in a shorter time (Rosyada et al., 2023).

In the research conducted by Priyono & Riswanto (2021) regarding testing the alcohol content in fermented kombucha drinks, it produces kombucha tea fermented drinks with halal alcohol levels below 0.5%. Thus the results of testing for kombucha tea have met the standards of the Indonesian Ulema Council (MUI), so that the fermented kombucha drink tested has been declared a halal drink and can be consumed by Muslim consumers (Priyono & Riswanto, 2021). Riswanto & Rezaldi (2021) also conducted research on the alcohol content of kombucha tea using 2 samples, that is black tea and kecombrang flowers. The results of kombucha studies conducted in the laboratory showed an ethanol/alcohol content of 0.055%.. The test results have met the regulatory standards of the Indonesian Ulema Council (MUI) where halal fermented drinks are below 0.5%. Riswanto & Rezaldi, 2021).

In the research of Sulistiawaty & Solihat (2022) which uses two types of tea, that is green tea and black tea. The results of the analysis showed that the fermentation time had an effect on the alcohol content. The longer the fermentation time will increase the alcohol content. The average alcohol content produced during 12 days of fermentation is less than 0.5% according to the 2018 LPPOM MUI standard (Sulistiawaty & Solihat 2022). In another study conducted by Pratiwi & Aryawati (2012) regarding kombucha fermentation with seaweed *Sargassum* sp. mentioned the effect of kombucha fermentation time with *Sargassum* sp. on alcohol content on day 4 to day 12 there was an increase in alcohol content that is, 0.2973%, 0.4683%, and 0.6209%. However after experiencing an increase on the 12 days, then on the 16 days it decreased again that is 0.4240% (Pratiwi & Aryawati, 2012).

One of the plant leaves that has the potential as an ingredient for making kombucha tea is vanilla leaf. Vanilla is a type of plantation crop that has high economic value with relatively stable price fluctuations compared to other plantation crops. In the food and beverage industry, the vanilla plant is well known because it has a distinctive aroma. Vanilla plant (*Vanilla planifolia*) is a plant that is widely used by the community. Generally, vanilla is used as a food and beverage flavoring ingredient, as well as a raw material for perfume (Nurcahyani, 2013). Based on research by Menon & Nayeem (2013) on the vanilla plant, the results show that the vanilla plant is able to treat and prevent toothache, menstrual pain, stomach ulcers, fever, cough and sore throat. Compounds that play a very large role in the organoleptic properties are 4-hydroxy3-methoxy benzaldehyde (vanillin). Based on the isolation results, it can be seen that the components of fresh and dry vanilla glycosides include acids, fatty aldehydes, alcohols, fatty esters, alkanes, alkanones, amines, benzene aldehydes, benzene esters, benzene alcohol and phenols, as well as other substances (Melawati, 2006).

In processed tea products, vanilla leaf have not been widely used, especially in making

kombucha tea. Therefore in this research will be made kombucha tea from vanilla leaf with variations in fermentation time, this aims to determine the halal product seen from the alcohol content and chemical characteristics.

## METHOD

### Research methods

This study used an experimental research method with a completely randomized design (CRD). Variations in the fermentation time used were 0, 4, 8, and 12 days.

### Materials and Tools

The materials used in this study were vanilla leaf, tea, kombucha culture starter, distilled water, granulated sugar, PP indicator, and NaOH.

The tools used in this study were measuring cups, beaker cups, measuring flasks, glass containers, stirrers, pipettes, volume pipettes, spoons, analytical scales, stoves, micropipette, oven, vortex, autoclave, aluminum foil, and pycnometer.

### Procedure

#### Making Vanilla Leaf Tea

Vanilla leaf are washed under running water until clean and cut into small pieces. Then dried in the sun until the leaf wilt for 30-60 minutes. The wilted vanilla leaf are dried using an oven at 55°C until the leaf are completely dry. The dried leaf are used as vanilla leaf tea.

#### Kombucha Starter Preparation

2000 ml of water is boiled until boiling and 200 grams of sugar (10% w/v) is added to the amount of water used and 10 grams of 0.5% (w/v) tea is added. Then filtered and the filtrate is covered with aluminum foil and let stand until the tea has room temperature. After that, 200 ml (10% b/v) of kombucha culture starter was added to the tea, then the container was tightly closed. The propagation of the kombucha starter culture was left for 14 days.

#### Making Vanilla Leaf Kombucha Tea

36 grams (0.5% w/v) of vanilla leaf tea is brewed using 7200 ml of boiling water. Then added sugar with a concentration of 20 grams (10% w/v) stirred. The infusion of vanilla leaf tea is tightly closed with aluminum foil and let stand until room temperature. 20 ml of kombucha liquid starter was added and fermented for 0, 4, 8 and 12 days in a closed container and filtered after the fermentation time was complete.

#### Test of Chemical Characteristics of Vanilla Leaf Kombucha Tea

##### Test Levels of Total Titrated Acid

The titrated total acid level test was carried out using the principle of acid-base titration. The test was carried out by placing 10 ml of the sample in a 100 ml volumetric flask and then adding distilled water up to the mark. After that, 10 ml of the filtrate was taken and put into the Erlenmeyer and 3 drops of PP indicator were added. The solution was titrated with 0.1 N NaOH solution until the color of the sample solution changed from clear to pink. Total acid is calculated using the formula:

$$\text{Total Acid (\%)} = \frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times \text{BM} \times 100\%}{V_{\text{sample}} \times 1000} \quad (1)$$

Information :

V NaOH : Volume NaOH for titration

N NaOH : Standard concentration of NaOH

V samples : Volume sample for titrate

BM : Molecular weight of acetic Acid

##### Test pH levels

pH measurement was measured using a pH meter. Vanilla leaf kombucha tea is put in a beaker glass and immersed in a pH meter.

##### Alcohol Test

Test the alcohol content of kombucha tea using gravimetric analysis. Gravimetric analysis, also known as quantitative testing based on weight, is the process of isolating and weighing certain elements or compounds from elements in

their purest form (Sediarso et al., 2020). Compound formulas and the atomic weights of the constituent elements can be used to calculate the weight of an element. Several methods that can be used to separate the elements or compounds contained are precipitation methods, evaporation methods, and many other methods (Rahmelia et al., 2015).

The test of alcohol content was carried out using the Skoog method (1985) by taking 25 ml of vanilla leaf kombucha tea solution and neutralizing it using 3 N NaOH. Furthermore, the solution was distilled by distillation and the result was collected as much as 25 ml. The distillation results are then put into a 25 ml pycnometer, complete with a thermometer. Before being tested, the pycnometer and thermometer were weighed first and the pycnometer was put into cold water until the temperature reached 28°C. Then, the outer surface of the pycnometer is dried with a tissue and weighed. Calculation of specific gravity can be done in the following way:

$$\frac{(Bp + destilat) - Bp \text{ empty}}{Bp + aquades) - Bp \text{ empty}} \quad (2)$$

Information :

Bp : Picnometer weight

By knowing the specific gravity of alcohol, the alcohol content can be found on a list of specific gravity.

## RESULTS

### Effect of Fermentation Time on Chemical Characteristics

This research was conducted to determine the effect of fermentation time on the chemical characteristics of vanilla leaf kombucha tea (*Vanilla planifolia*). Kombucha tea was made with 4 variations of fermentation time that is 0 day, 4 days, 8 days and 12 days, and observed with several parameters, that is total acid titration (Tat) and pH. The results of the

chemical characteristic test can be seen in the following table:

Table 1: Chemical Characteristic Test Results.

Treatment	pH ± SD	Tat Levels (%) ± SD
P1	5	0.003
P2	3	0.012
P3	3	0.024
P4	2	0.048

P1 (fermentation time 0 days), P2 (fermentation time 4 days), P3 (fermentation time 8 days), P4 (fermentation time 12 days)

The results of the chemical characteristic test in table 1 show that there are differences in pH values and titrated total acid levels in kombucha tea. Table 1 shows that the treatment with the best chemical properties was P2 treatment (4 days of fermentation) with a pH value of 3 and a Tat level of 0.012 and P3 treatment (8 days of fermentation) with a pH value of 3 and a Tat level of 0.024.

### Effect of Fermentation Time on Alcohol Levels

This study measured the alcohol content in vanilla leaf kombucha tea compared to the MUI standard number 10 of 2018 to see the halal label for the beverage product. The results of the research on the alcohol content of kombucha tea are presented in Table 2 as follows:

Table 2: Alcohol Content Test Result

Treatment	Alcohol (%)	MUI Standard No. 10 of 2018	Annotations
P1	0.12		M
P2	0.15	Alcohol content	M
P3	0.21	<0.5%	M
P4	0.28		M

P1 (fermentation time 0 days), P2 (fermentation time 4 days), P3 (fermentation time 8 days), P4 (fermentation time 12 days), M (fulfill), TM (not fulfill)

Based on Table 2, it is known that there was an increase in alcohol content during the fermentation of vanilla leaf kombucha tea.

## DISCUSSION

pH is the degree of acidity used to express the degree of acidity or degree of alkalinity of a solution (Sugeng & Sulardi, 2019). The pH level of kombucha that is acceptable and safe to consume is pH 3.00. If kombucha has a pH below this value, it needs to be diluted (Naland, 2008). The results of the pH test in Table 1 can be seen that during the fermentation time, the pH of the vanilla leaf kombucha tea decreased. The decrease in pH occurs because during fermentation there is growth and metabolic processes of acetic acid, lactic acid and yeast bacteria which produce organic acid compounds, causing a decrease in acidity (Jayabalan et al., 2014).

Total titric acid (Tat) is a better predictor of the effect of acidity on taste and aroma than pH. Total titrated acid has a relationship with the measurement of total acid contained in food. The Tat value is a measurement of the total dissociated and undissociated acids, while the pH measurement only measures the total dissociated acids (Angelia, 2017). The results of the tat test in Table 1 show that there was an increase in total titrated acid during the fermentation process. The highest tat content was vanilla leaf kombucha with a 12-day fermentation time of 0.48%. The increase in total acid in kombucha is the result of bacterial metabolism which produces acids such as acetic acid, gluconic acid, and glucuronic acid (Priyambada et al., 2012). The longer the fermentation, the organic acids that are formed increase and are detected as total titrated acid (Chakravorty et al., 2016). The longer the fermentation can produce a total acid between 0.5-2.0%. (Nurhayati et al., 2020).

According to MUI Fatwa number 10 of 2018 concerning food and beverage products that contain alcohol/ethanol, it is known that the alcohol content in fermented beverages that may be consumed is drinks that have an alcohol content of less than 0.5%. In Table 2 it is known that in all treatments during fermentation it was found that the vanilla leaf kombucha complied with the halal beverage standards set by the

MUI. Based on the results of the alcohol test, it was found that there was an increase in alcohol content during the fermentation of vanilla leaf kombucha tea. The alcohol content of vanilla leaf kombucha tea with a fermentation time of 0 day was 0.12%, 4 days 0.15%, 8 days 0.21%, and 12 days 0.28%.

The alcohol found in kombucha tea after fermentation is the result of yeast activity on the glucose found in kombucha tea. This is supported by the opinion of Sadiyah et al. (2017) which states that yeast activity will produce a certain amount of alcohol. The increase in alcohol content during the fermentation period is caused by yeast developing and metabolizing which then produces alcohol, the longer the fermentation is carried out, the more alcohol will be produced. This is supported by the opinion of Altay et al. (2013) which states that the longer the fermentation time is carried out, the alcohol content will increase. Alcohol levels can increase further supported by factors such as acidity, the type of culture used, and also the fermentation temperature which supports yeast activity during fermentation (Lestari et al., 2018).

Based on research that has been carried out by Rizqiqa et al., (2021) by making teh kombucha rhizome from green tea solution with the addition of ginger and ginger rhizome, the highest alcohol content value was obtained for the type of ginger rhizome with 12 days of fermentation (1.18%) and the lowest in the control with 12 days of fermentation (0.49%). In the treatment of the ginger rhizome type and the control, the alcohol content decreased on day 12, that is 0.70%, 0.49%, while in the type of ginger rhizome on day 12 it increased to 1.18% (Rizqiqa et al., 2021). In a study by Simanjuntak et al., (2016) the alcohol content in kombucha apu-apu still increased until day 12. This was due to the yeast still having the ability to break down carbohydrate food into alcohol and carbon dioxide (Simanjuntak et al., 2016).

Yeast is one of the microbes that plays an important role in the process of making kombucha because yeast plays an important role in converting sugar into alcohol, where the

alcohol produced by yeast will be oxidized by the bacteria *Acetobacter xylinum* into acetic acid. The longer the fermentation, the components of the media for yeast growth diminish, in growth the yeast utilizes nutrients, that is sugar as a carbon source for its growth (Lestari et al., 2018).

Fermentation in kombucha is carried out by kombucha culture by converting glucose to alcohol and CO<sub>2</sub> then reacts with water to form carbonic acid. Alcohol will be oxidized to acetic acid. Gluconic acid is formed from the oxidation of glucose by bacteria from the genus *Acetobacter*. The culture at the same time will produce organic acids such as acetic acid, lactic acid, glucuronic acid, folic acid, and vitamin C. *Acetobacter xylinum* bacteria convert sugar into cellulose which is called nata and floats on the surface of the medium (Rinihapsari, 2008)

Bacteria *Acetobacter xylinum* is one of the bacteria that plays an important role in the process of making kombucha tea. *Acetobacter* bacteria can produce acetic acid which is the main product of fermentation. Besides being able to break down alcohol into acetic acid, *Acetobacter* is also able to form cellulose from glucose available in the media. The secreted cellulose fibers will form a loose network covering the cells which gradually cover the surface of the liquid fermentation medium until it reaches a thickness of one centimeter. The cellulose or nata that is formed in the kombucha fermentation process indicates the success of making kombucha tea because kombucha has a characteristic, that is the formation of nata in kombucha products (Aini et al., 2022).

## CONCLUSION

Fermentation time can affect the halal label of vanilla leaf kombucha tea based on alcohol content and chemical properties. Vanilla leaf kombucha tea meets halal beverage standards because it has an alcohol content of less than 0.5% which is in accordance with the 2018 LPPOM MUI standards. Vanilla leaf kombucha tea has the best chemical properties,

that is the P3 treatment (8 days fermentation time).

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