Effect Fermentation Time of Halal Label Wuluh Starfruit Leaves Kombucha Tea (Avverhoa bilimbi Linn.) Based on Alcohol Content and Chemical Characteristic

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Abstract: Kombucha is a fermented drink of tea and sugar with the help of tea mushrooms. Wuluh starfruit leaf is one of the ingredients can be used to make kombucha. Kombucha contains chemical compounds have health effects. However, the fermentation process makes kombucha also produce alcohol, which can affect the halal label. The purpose of this study is to determine the effect of fermentation time of halal label wuluh starfruit leaf kombucha tea based on alcohol content and chemical characteristics. This study is an experimental study with a complete randomized design consisting (CRD) of 4 treatments including fermentation time of 0, 4, 8 and 12 days with sugar concentration 15%. The chemical characteristics tested include pH, titrated acid levels (tat), phenolics were analyzed using kruskal wallis and mann whitney test. Data on alcohol content is analyzed descriptive. The results of the kruskal wallis test have a significant difference in the chemical characteristics test. The best result from chemical charactheristic and alcohol content that fullfil MUI standard are a 4 days fermentation time treatment with a pH value of 3, a tat content of 0.016%, phenolic content of 61,6 mg/ml GAE and an alcohol content of 0.30%.

1 INTRODUCTION

Kombucha is a traditional drink fermented from tea liquid containing sugar with the help of tea mushrooms commonly known as Scoby (Symbiotic culture of bacteria and yeast). Microbes that have a role in making kombucha include *Acetobacter xylinum*, *A. aceti*, *A. pausterianus*, and *Bacterium gluconium* from the group of acetic acid bacteria and *Candida* sp., *Kloeckera* sp., *Schizosaccharomyces pombe*, *S. ludwigii*, *S. cerevisiae*, *Torulospora sp.*, *Zygosaccharomyces bailii* and *Phichia* species which belongs to the type of yeast. Kombucha has a sweet-sour taste like sparkling apple cider. During the fermentation period, kombucha will undergo a change in taste from refreshing acid to vinegar-like acid (Goh *et al.*, 2012). This is because bacteria and yeast will convert sugars into several organic acid compounds such as acetic acid, glucoronic acid, lactic acid and gluconic acid. Organic acid compounds will increase during the fermentation time, this will also affect the pH and total titrated acid (Wistiana and Zubaidah, 2015).

In the fermentation process, in addition to producing some organic acid compounds, kombucha will also produce alcohol. The alcohol content produced in kombucha will cause problems for the Muslim community about the halal label of the product. Halal is any object or activity that is allowed to be used or carried out in the Islamic religion. Food and beverage products that are allowed to be consumed according to Islam can be seen from the type of food and how to obtain it (Majidah *et al*, 2022). According to the MUI Fatwa number 10 of 2018 fermented beverage products containing more than 0.5% alcohol are not allowed to be consumed. In research conducted by Priyono and Dody (2021), it is known that there are several kombucha products that have halal certification.

Kombucha produces a good taste is generally made with the basic ingredients of black tea because black tea has a fragrance compared to other types of tea. However, for health purposes, making kombucha can use other herbal plants that have a higher content of chemical compounds in the form of natural antioxidants (Naland, 2008). The quality of dry tea based on scent and taste can be influenced

by the content of chemical compounds in it such as caffeine, tannins and essential oils. One of the plants that has the potential to be the basic ingredient of kombucha is wuluh starfruit leaves. According to Misfadhila *et al* (2020) wuluh starfruit leaves have a total flavonoid compound of not less than 0.7%. Star fruit leaves also have a higher tannin content of 10.92% compared to green tea leaves by 1.44% and orange leaves by 1.8% (Adriani, 2019).

Research on the effect of fermentation time on the halal label of wuluh starfruit leaf kombucha products has never been carried out. Thus, it is necessary to conduct research on the influence of fermentation time on the halal label of wuluh starfruit leaf kombucha wuluh based on alcohol content and its chemical characteristics with the aim of knowing kombucha products that are beneficial for body health and can be consumed by the Muslim community.

2 METHODS

Research Methods

This research used an experimental method with a complete randomized design (CRD) consisting of 12 treatments with 3 repetitions, namely a fermentation time of 0, 4, 8, 12 days with a sugar concentration of 15%.

Materials and Tools

The ingredients used in this research were wuluh starfruit leaves (*Avverhoa bilimbi* Linn.), black tea, kombucha culture starters, aquades, granulated sugar, methanol, Na_2CO_3, ethanol 96%, gallic acid, Folin-ciocalteu, DPPH, pp indicators and NaOH.

The tools used in this study included glass containers, rubber bands, clean cloths, uv-Vis spectrophotometers, autoclaves, cameras, label paper, vortexes and ovens.

Procedure

Tool Sterilization Process

Glass containers that have been covered with aluminum foil are sterilized with an autoclave at a temperature of 121℃ at a pressure of 1 atm for 15 minutes. Sterilized glass cups will be used as containers in fermentation process of wuluh starfruit leaf kombucha (*Avverhoa bilimbi* Linn.).

The identification Process of Wuluh starfruit plants

Identification of wuluh starfruit plants is carried out by taking parts of leaves, stems, flowers and fruits on plants that are guided by wuluh then matched with the characteristics of star fruit plants in the book Tropical Flowering Plants (A Guide to identification and cultivation) (Llamas, 2003).

Manufacturing stages of the kombucha starter

2000 ml of water is boiled to a boil, then 200 grams of sugar is added to 10% (w/v). after that, 10 grams of 0.5% tea (w/v) is added. then filtering is carried out and poured on a glass container that has been sterile. After the tea has the same temperature as the room temperature, the addition of a kombucha culture starter of 200 ml of 10% (w /v) to the tea brew is carried out. Then cover the container with a clean cloth then tie it with an elastic band. Propagation of the kombucha culture starter is fermented for 14 days.

Stages of making leaves wuluh starfruit tea

Separating wuluh starfruit leaves with branches. The leaves of wuluh starfruit are taken for making tea, namely young leaves. Then wash the leaves with running water thoroughly. After that, the leaves are cut into small pieces and then dried using an oven at a temperature of 55℃

Stages of making wuluh starfruit leaf kombucha

Wuluh starfruit leaf tea as much as 36 grams 0.5 % (w/v) brewed using boiling water as much as 7200 ml. then poured into a container of 200 ml each and added sugar with a concentration of 15% (w/v) 30 grams. Then a kombucha liquid starter of 20 ml each is added and then the glass container is covered with a clean cloth and fermented for 0, 4, 8 and 12 days.

Analysis test Stage wuluh starfruit kombucha leaves tea

Test of Total Titrated Acid Levels

The measurement of total titrated acid is carried out by the principle of acid titration by base referring to Cahyaningtyas (2018). The test was carried out by taking 10 ml of the sample into a 100 ml measuring flask then adding aquades to the limit mark and then filtered. After that, 10 ml of filtrate is taken and put in erlenmyer and added pp indicator 3 drops. After that, titration is carried out with a solution of NaOH 0.1 N. Titration is carried out until the solution turns

pink. The calculation is carried out with the following formula:

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

Where :
V_{NaOH} : volume NaOH for titration
N_{NaOH} : standard concentration of NaOH
V_{sample} : volume sample for titrasi
BM : Molecular weight of acetic acid

pH test

PH measurements in kombucha tea samples are carried out by taking about 100 ml of kombucha solution and then putting it into a glass beaker. After that, the pH is measured using universal pH, waited until the pH paper changes color and then matches the standard color shown on the packaging

Phenolic Test

The measurement of total titrated acid is carried out by the principle of acid titration by base referring to Cahyaningtyas (2018). The test was carried out by taking 10 ml of the sample into a 100 ml measuring flask then adding aquades to the limit mark and then filtered. After that, 10 ml of filtrate is taken and put in erlenmyer and added pp indicator 3 drops. After that, titration is carried out with a solution of NaOH 0.1 N. Titration is carried out until the solution turns pink. The calculation is carried out with the following formula:

$$\text{TPC} = \frac{c.v.fp}{g}$$

Where :
TPC : total phenolic content (mg/g GAE)
C : concentration (value x) (ppm)
V : extract volume (ml)
Fp : dilution factor
G : sample weight (grams)

Alcohol Test

Testing alcohol content using the Skoog method (1985) by taking a solution of star fruit leaf kombucha as much as 25 ml and then neutralized with NaOH 3 N. then continued with the distillation process by distillation and the results were accommodated as much as 25 ml.the distillation results were then put into a 25 ml picnometer equipped with a thermometer. Before the treatment of the picnometer and thermometer are weighed first. The picnometer is put in cold water until the temperature reaches 28. Then weighed in weight, the calculation of specific gravity can be carried out by the following formula:℃

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

Where :
Bp : picnometer weight

Data Analysis

The research data included pH, Tat levels (Total titrated acid), and phenolic levels of non-parametric test *kruskal Wallis* with a value of $\alpha < 0.05$. If there are differences, continue with *the Mann whitney* test kto find out the differences between treatments. Qualitative data, namely alcohol content, were analyzed descriptively and compared with halal requirements based on MUI Fatwa Number 10 of 2018.

3 RESULTS

Effect of fermentation time on Chemical characteristics

This study was conducted to determine the effect of fermentation time, a chemical characteristic test of wuluh starfruit leaf kombucha tea (Avverhoa bilimbi Linn.) was carried out. Kombucha tea that has been made with 4 variations in sugar concentration, namely 0, 4, 8 and 12, observations were made with several parameters, including: Total titrated acid (Tat), pH and phenolic levels. The results of the chemical characteristics test can be seen in the following table:

Table 1 Chemical characteristic test results

Treatment	pH ± SD	Tat Levels (%) ±SD	Phenolic Levels (mg/ml GAE) ±SD
P1	6	0,014±0,0014	68,27±3,601
P2	3	0,016±0,14	61,6±2,227
P3	2	0,074±0,005	91,87±8,105
P4	2	0,12±0,0038	85,2±6,593

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

The results of the Kruskal wallis test on chemical characteristics showed differences in the pH value, total levels of titrated acid and phenolic levels in kombucha tea. In table 1, it can be seen that the treatment that has the best chemical characteristics is in the P2 treatment (fermentation time of 4 days) with a pH value of 3, a Tat level of 0.016 and a phenolic level of 61.6 mg/ml GAE.

Effect of fermentation time on alcohol content

This study also measured the alcohol content in wuluh starfruit leaf kombucha and will be compared with MUI standard number 10 of 2018 to see the halal label of the beverage product. The results of the study on the alcohol content of kombucha tea are presented in Table 2 as follows:

Table 2 Alcohol content test results

Treatment	Alcohol	Standard MUI No 10 Tahun 2018	Annotation
P1	0,13		M
P2	0,30	Kadar	M
P3	0,98	alkohol	TM
P4	0,43	<0,5%	M

Where:

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 is an increase in alcohol content during the time when the fermentation of kombucha takes place. However, too long a fermentation time will make the alcohol content in kombucha decrease.

4 DISCUSSIONS

pH is the degree of acidity used to express the level of acidity or the degree of alkalinity in a solution (Karangan *et al*, 2019). According to Naland (2004) the pH level of kombucha that is acceptable and safe to consume is between 3-5.5. If the kombucha has a pH below this value, it is necessary to dilute it. The results of the pH test in Table 1 can be seen that during the fermentation time, the pH in the wuluh starfruit leaf kombucha has decreased. This is comparable to research that has been carried out by Pratiwi et al (2012) it is known that the pH number has decreased successively during the fermentation time on kombucha from seaweed saggarsum sp. The decrease in pH occurs because during fermentation there is growth and metabolic processes of acetic acid bacteria, lactic acid and yeast that produce organic acid compounds, causing a decrease in acidity (Jayabalan *et al.*, 2014).

Total titrated acid (Tat) is an estimator of the effect of acidity on taste and aroma which is better than pH. This is because tat is a measurement of total dissociated and non-dissociated acids, while pH only measures the total acid in the dissociated condition (Angelia, 2017). The results of the tat test in Table 1 know that there is an increase in the total titrated acid during the fermentation process. The highest tat content is wuluh starfruit leaf kombucha with a fermentation time treatment for 12 days of 0.12%. This is in accordance with research conducted by Pratiwi *et al* (2012) that there is an increase in total levels during fermentation time in seaweed kombucha *Saggarsum* sp. The increase in total acid levels is thought to occur because the time of sugar fermentation in kombucha will be overhauled into organic acids.

Based on the results of phenolic tests in table 1, it is known that the longer the fermentation time in wuluh starfruit leaf kombucha tea causes phenolic levels to increase until the fermentation time of the 8th day. According to Suhardini and Zubaidah (2016) the increase in phenolic levels is thought to be due to microbes of the bacterial and yeast groups that can metabolize to produce flavonoid compounds through enzymatic reactions, thus affecting the total amount of phenols in kombucha tea. In the phenolic level results shown in Table 1, it is also known that at the time of the 12th fermentation there was a decrease in phenolic levels. This is thought to be caused because during the fermentation process an oxidation reaction occurs. Decreased phenolic levels are also related to the number of microbial cells that have decreased because reduced sugar as an energy source in microbes has also decreased (Ardheniati *et al*, 2009).

The kombucha fermentation process will also undergo oxidative chemical changes from microorganisms in the substrate with the result of their breakdown in the form of more complex compounds and a change in sugar into alcohol

involving the work of enzymes (Herwin *et al*, 2013). According to Fatwa MUI number 10 of 2018 concerning food and beverage products containing alcohol/ethanol, it is known that the alcohol content in fermented beverages that are allowed to be consumed is drinks that have an alcohol content of less than 0.5%. In Table 2, it is known that in all fermentation time treatments except the 8th days fermentation, it is known that the wuluh starfruit leaf kombucha fulfill the halal standards of drinks that have been set by the MUI. According to Kim and Adhikari (2020) in order for kombucha to fulfill MUI standards with an alcohol content of less than 0.5% there are several ways that can be done, including dilution, distillation of alcohol, pasteurization and filtering bacteria or yeast that produce alcohol. Based on Table 2, it is also known that there is an increase in alcohol content during the time the fermentation of kombucha takes place. However, too long a fermentation time will make the alcohol content in kombucha decrease. This is because during the fermentation process the yeast *Saccharomyces cerevisiae* produces alcohol anaerobically, then alcohol stimulates the growth of *Acetobacter xylinum* to produce acetic acid which stimulates the growth of *Saccaromyces cerevisiae.* Then the alcohol will be used by Acetobacter bacteria for the formation of acetic acid. So that the alcohol content decreases (Pratiwi *et al*, 2012).

5 CONCLUSIONS

Fermentation time affects the halal label of wuluh starfruit leaf kombucha wuluh based on alcohol content and chemical characteristics. The kombucha which has the best chemical characteristics and has an alcohol content allowed by the MUI, namely in the P2 treatment (fermentation time is 4 days). More research is needed regarding the effect of sugar concentration on the halal label of wuluh starfruit leaf kombucha.

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