The Effect of Fermentation Duration and Concentration Variation of *Gyrinops versteegii* Leaf Decoction on The Alcohol Content of *Gyrinops versteegii* Leaf Decoction Water Kefir and Its Integration with The Qur'anic Interpretation

Achmad Munajib

Biology department, faculty of Science and Technology, UIN Maulana Malik Ibrahim Malang, Jl. Gajayana no 50 Malang, 65144, Indonesia.. Telp. + 6285746942586. Email: achmadmunajib@gmail.com

Abstract. Water kefir is one of the drinks made from the fermentation of water kefir seeds. Alternative media is important to be found for product development. One of the potential media is agarwood leaf decoction (*Gyrinops versteegii*). The purpose of this research was to determine the effect of fermentation duration and concentration variations on the alcohol content of *Gyrinops versteegii* leaf decoction water kefir and its integration with QS. Al-Maidah [5]: 90 and QS. Al-A'raf [7]: 31. This research is an experiment with a completely randomized design (CRD) factorial pattern. The first factor was the concentration of agarwood leaf decoction (5%, 10%, 15%, and 20%). The second factor is the fermentation time (18 hours, 21 hours, and 24 hours). The second factor was combined with the addition of 6% sucrose, 5% water kefir seeds, and incubated at room temperature. The results showed that the length of fermentation and the variation in the concentration of agarwood leaf decoction had a significant effect on alcohol content with a significance value of <0.05. the alcohol content produced after fermentation is 0.4% -0.8%. The integration of these results used the interpretation method of the maudhui interpretation so that the conclusion was that beverage products below 1% alcohol content were halal and good products that were following the values contained in the Quran.

Keywords: Concentration; Fermentation; Gyrinops versteegii Leaf Decoction; interpretation; Water Kefir.

Running Title : The Effect of Fermentation Variation of *Gyrinops versteegii* Leaf Decoction on the alcohol content of *Gyrinops versteegii* Water Kefir

INTRODUCTION

Water Kefir is a fermented drink that comes from the symbiosis between yeast and bacteria that produce alcohol (ethanol), carbon dioxide, organic acids (lactic and acetic), and several other compounds from the breakdown of sugar. Initially, water kefir was only made from a mixture of water and sugar. Kefir water has many advantages over milk kefir (Ferdiaz, 1997) including the acid that is formed to extend shelf life, prevent the growth of spoilage microorganisms thus increasing the safety of kefir products, contains minerals and essential amino acids which function as elements of building, maintaining, and repairing damaged cells.

A renewable medium in the manufacture of water kefir needs to be found and tested to add new variations that are suitable and have the potential for product development, namely kefir products using agarwood leaf decoction medium (Gyrinops versteegii). Agarwood leaves (Gyrinops versteegii) were chosen because they are safe and have many benefits including as a source of natural antioxidant compounds and based on research with DPPH (Diphenil pikril Hidrazil) that Phytochemical Screening of agarwood leaf extract contains secondary metabolites such as phenol compounds, terpenoids, and flavonoids (Mega, 2010). Phenolic compounds or polyphenols can absorb and neutralize free radicals (Zheng, 2009), while in Yanti's research (2015) that Gyrinops versteegii leaves contain phenols, flavonoids, and tannins added by Silalahi (2006), these leaves contain glycoside compounds.

The fermentation process of water kefir is 18 hours, 21 hours, and 24 hours (Lathif, 2016). Khotib (2018) added

variations in the substrate concentration in the new kefir water medium, namely 5%, 10%, 15%, and 20%. This difference in concentration results in different tastes and odors in the product and the resulting compounds.

Fermentation by lactic acid bacteria in water kefir seeds can stop due to a decrease in the pH value of the medium, but the yeast in water kefir seeds is still active in a lower pH environment, so it still ferments sugar into alcohol (Sugiharti, 2014). The alcohol content in water kefir depends on the length of fermentation (Penalver, 2004). This alcohol content affects the halalness of a beverage product related to the provisions in the Qur'anic verse so that this study aims to determine the effect of fermentation time and variations in the concentration of agarwood leaf decoction (*Gyrinops versteegii*) on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*) and its integration with the verses of the Qur'an.

MATERIALS AND METHODS

Study Area

This research was conducted at the Laboratory of Microbiology, Department of Biology, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University Malang, while the materials used in this observation were water kefir seeds (obtained from Rumah Kefir Shop Batu, Malang, East Java), cotton, warp plastic, aluminum foil, sucrose (Gulaku brand), agarwood leaves (*Gyrinops versteegii*) (obtained from the Pasuruan agarwood plantation).

Procedures

Research design in making water kefir

This study used an experiment in the form of a

completely randomized design (CRD) with 2 factorial patterns. Factor 1 concerning fermentation time (18 hours, 21 hours, and 24 hours) and Factor 2 concerning the concentration of agarwood leaf decoction (*Gyrinops versteegii*) (20%, 15%, 10%, and 5%). These two factors were combined with the addition of 6% sucrose and 5% water kefir seeds and incubated at room temperature (25°C). Controls were also made without fermentation at each concentration of agarwood leaf decoction. The research design is presented in the table below:

M3 (24)
SOM3
S1M3
S2M3
S3M3
S4M3

Table 1. research design







Calculation of alcohol content

The alcohol content of the fermentation was analyzed by using a pycnometer. The pycnometer is dried in an oven for 10 minutes at 100°C. After that, let stand at room temperature until cool. Then the pycnometer weight was measured on the analytical balance. After that, the distillate is entered into the pycnometer whose weight has been known to fill the pycnometer. The pycnometer and distillate inside are weighed and the weight data is written which appears on the analytical balance screen. Other measurements in distilled water for comparison are in the same way and stages (Jhonprimen, 2012).

The results of the SG (Specific gravity) measurement of the sample are followed by conversion to the pycnometer table from the International Organization of Legal Metrology (OIML) so that the alcohol content can be determined (Marg, 2005).

Calculation of specific gravity or SG (Specific gravity) of ethanol uses the following formula (Azizah, 2012) :

SG sample = (a+b)-c

(a+d)-c

- (a + d) = weight of pycnometer containing aquades.
- (a + b) = weight of pycnometer containing distillate.
 - = empty pycnometer weight.

The results of the SG (Specific gravity) measurement of the sample are followed by conversion to the pycnometer table from the International Organization of Legal Metrology (OIML) so that the alcohol content can be determined (Marg, 2005).

Data analysis

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The data obtained were analyzed using the two-way Analysis of Variance (ANOVA) test. Then proceed with the Duncan Distance Test to determine different treatments. The results of the data analysis were integrated with the verses of the Al-Qur'an using the Maudhuii method of interpretation, namely the QS. Al-Maidah [5]: 90 and QS. Al-A'raf [7]: 31



Figure 2. Flowchart of Research Methods

RESULTS AND DISCUSSION

The data on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*) can be seen in the image below:



Figure 3. Stem Diagram of Kefir Alcohol Content of Agarwood Leaves (*Gyrinops versteegii*).

The picture above shows that there is no kefir alcohol content of agarwood leaves (Gyrinops versteegii) before fermentation. These results indicate that there is no microbial metabolic process of water kefir seeds to break down sucrose, which is different after fermentation, yeast can produce its metabolic results, it is proven that at 18 hours of fermentation the lowest alcohol content was 0.4% at a concentration of 0%, 5%, 15%, and 10%. The highest alcohol content was found at the fermentation time of 21 hours. This is evident by Khotib (2018) that the longer the fermentation time lasts, the more alcohol content is produced so that the opportunity for microbial activity to produce alcohol is greater. It is known that the alcohol content increases after fermentation for 21 hours at each concentration, which is a value 0, 8%. This value is the value of the highest alcohol content in the study. The length of fermentation afterward (24 hours) showed that the alcohol content decreased to 0.6% at a concentration of 0%, 5%, 10%, and 20%. Based on these results, the fermentation time of 21 hours is the optimal time for alcohol-producing microbes to carry out their metabolism on the substrate of agarwood leaf decoction water kefir (Gyrinops versteegii).

The resulting alcohol content in the substrate starts from the lowest value of 0.4 at 18 hours of fermentation to the highest value of 0.8 with 21 hours of fermentation caused by fermentation of sugar (sucrose) with the role of Saccharomyces cerevisiae yeast which produces ethanol (ethyl alcohol) and carbon dioxide as described by Winarno (1980) through reaction:

$$C_6H_{12}O_6 \xrightarrow{\text{Khamir}} 2 C_2H_5OH + 2 CO_2$$

(Alkohol)

In fermentation for 24 hours, the size of the alcohol value again decreased to 0.4 and 0.6 due to further fermentation by Acetobacter aceti as acetic acid bacteria which breaks down the resulting alcohol to form acetic acid through the following equation reaction (Winarno, 1980):

$$C_2H_5OH \longrightarrow CH_3COOH + H_2O$$

The decrease in alcohol during the 24-hour fementation period is reinforced by Wigyanto (2016) in his research that the size of the alcohol value is getting lower along with the longer fermentation time takes place because some of the alcohol evaporates and sucrose is reduced due to some of the sucrose being further oxidized in producing acetic acid.

Further observations were carried out an analysis of variance (ANOVA) using the SPSS 16.0 application. This was carried out to understand the significant effect of the concentration variation factor and fermentation time along with their combination on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*). A summary of the data from the analysis of variance (Anava) is presented in the following table:

 Table 2. Summary of diversity analysis of alcohol content of agarwood leaf

 decoction water kefir

Treatment	alcohol content (%)	Notation
S0M0	0,0	d
S0M1	0,4	с
S0M2	0,8	а
S0M3	0,6	b
S1M0	0,0	d
S1M1	0,4	с
S1M2	0,8	а
S1M3	0,6	b
S2M0	0,0	d
S2M1	0,4	с
S2M2	0,8	а
S2M3	0,6	b
S3M0	0,0	d
S3M1	0,4	с
S3M2	0,8	а
S3M3	0,4	с
S4M0	0,0	d
S4M1	0,6	b
S4M2	0,8	а
S4M3	0,6	b

Based on the results of the table above, it shows that

the concentration variable, fermentation time, and the combination of the two have a significant effect on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*) which shows a value less than 0.05. As for understanding the significant distance of the effect of the fermentation time treatment was carried out using the Duncan Distance Test with a significance level of 5%. The test results are shown in the following table:

Source	The Sum Of The Squares	df	Average Of Squares	F	signific ance
Duration of	6,019E9	4	1,505E9	5,856E14	,000
Fermentation					
Concentration	4,559E9	3	1,520E9	5,915E14	,000
Variation					
Combination	1,806E10	1	1,505E9	5,856E14	,000
		2			

Table 3. Results of duncan analysis on the value of alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*)

Based on the table above, it is known that the treatment of SOM2, S1M2, S3M2, S2M2, and S4M2 has the same notation (a), this shows an effect that is not significantly different in the treatment. Each treatment with the same notation still has an effect but is not significant, but the different notations from the other treatments show a significant effect. These results indicate the effect of variations in concentration and fermentation time and a combination of both on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*). The lowest alcohol content after fermentation was found in the 18-hour fermentation time treatment and the highest alcohol content was in the 21-hour fermentation time treatment, then the alcohol content decreased at the 24hour fermentation time.

Khotib (2018) explained that one of the microorganisms that affect alcohol content is Candida kefir which is classified as yeast. Kunaepah (2008) explains that high acid content can slow down the growth of microbes in the fermentation process, including yeast Candida kefir. Candida kefir is unable to break down the substrate at the beginning of fermentation, so the ability of Candida kefir to produce alcohol decreases. This is in accordance with the research that in the longest fermentation treatment (24 hours) the value of alcohol content decreased.

M3 treatment in the 24-hour fermentation time, the alcohol content decreased because it had been converted into other compounds as explained by Wigyanto (2016) that the decrease in alcohol content was due to the conversion of alcohol to acetic acid by acetic acid bacteries. Sari (2008) adds that the decrease in alcohol content is caused by the conversion of alcohol into other compounds, including esters. Reinforced by Pramita (2013), there is a decrease in the value of alcohol content produced because the ethanol obtained has been converted into organic acids, for example, acetic acid by acetic acid

bacteria in water kefir seeds, so that from the explanation above, the results of measuring the alcohol content in the study are in accordance with the literature.

The alcohol content produced in each treatment after fermentation is 0.4%, 0.6%, and 0.8% which indicates that it has met the standards set by MUI (Indonesian Ulema Council), which is below 1% which does not cause intoxicating effects on the product resulting from. Based on the ijtihad fatwa of the MUI (Majelis Ulama 'Indonesia) in 2009 and the MUI fatwa no 24 of 2003 on food products. Food and beverages with an alcohol content of less than 1% are punished halal.

The Tafsir method used in this research is the Maudhui Tafsir (thematic) method. Maudhui Tafsir is a method of interpretation that seeks to find answers to the Koran on a certain theme (Farmawi, 1977). then look for hadiths that support the theme. The first verse is QS. Al-A'raf [7]: 31

"Eat and drink but do not exaggerate, verily Allah does not like people who are extravagant".

The word ℓ with ℓ with ℓ with ℓ with ℓ and ℓ with ℓ

كُلُّ مُسْكِرِ خَمْرٌ وَكُلُّ خَمْرٍ حَرَامٌ "Everything that is intoxicating is khamr, and all khamr is haram."

The highest alcohol content in this study in each treatment is 0.8% so that this product is not intoxicating, and this is a practice of QS. Al-Maidah [5]: 90

"O people who believe, actually khamar, gambling, (sacrificing for) idols, drawing fate with arrows is a kind of satanic behavior. So stay away from that case so that you can get profit"

This verse instructs us to stay away from something intoxicating (خصر) because it is included in the رخبر) category which means impurity or in the language sense means dirty and unclean. Asy-Syinqithi explained in his interpretation that خصر is an intoxicating drink that can cover common sense and it is a heinous, disgusting, and dirty act including the act of Satan. So stay away from that action, don't let you do it so that you get good luck.

The *Gyrinops versteegii* water kefir product is far from being intoxicating for the drinker both in terms of its alcohol content and fermentation time. The length of fermentation that can cause drunkenness is more than three days as in the hadith of the prophet in the Syarh Sahih Muslim book 7/190:

وان كان قد ظهر فيه شيئ من مبادئ الاسكار والتغير اراقه لانّ اذا اسكر صار حراما ونجسا. فيراق ولا يسقيه الخادم لانّ المسكر لا يجوز سقيه الخادم كما لا يجوز شربه. واما شربه صلى الله عليه وسلم قبل اثلاث فكان حيث لا تغير ولا مبادئ تغير ولا شك اصلا

"If there had been a change which resulted in the drink becoming intoxicating and changing (into khamr), he threw it away. For if that drink causes intoxication, he shall be punished as unclean and unclean. He(peace be upon him) threw it away and did not give it to his servant (to drink). An intoxicating drink that should not be given to a servant as he is not allowed to drink it himself. Regarding the Prophet (peace be upon him) drinking it before three days, it is because it has not changed its character and character, there is no indication of change, and there is no doubt (that it is lawful) in origin"

The above hadith explains that the fermentation time before three days is still punished as halal provided that it does not change the character of the product. This is in accordance with the research that the manufacture of agarwood leaf decoction water kefir (Gyrinops versteegii), is made in a period of one day which does not cause intoxicating effects on each product made, and the alcohol content is far below 1% based on the MUI ijtihad fatwa (Majelis Ulama) 'Indonesia) in 2009 regarding the alcohol law which reads The use of alcohol or ethanol from nonkhamr industrial products (whether it is the result of chemical synthesis or non-khamr fermented industrial products) for the production process of food, beverage, cosmetics, and medicinal products, the law is: permissible , if it is not medically harmful. This law was strengthened by the MUI fatwa no 24 of 2003 on food products. Food and beverages with an alcohol content of less than 1% are punished halal. This study shows that in each treatment (18 hours, 21 hours and 24 hours) the resulting alcohol content is less than 1% so that the water kefir product is halal and good product.

CONCLUSIONS

Conclusion of this research is that the length of the fermentation process and the variation in the concentration of agarwood leaf decoction (*Gyrinops versteegii*) have a significant effect on the alcohol content of agarwood leaf decoction water kefir (*Gyrinops versteegii*) with a significance value <0.05, the alcohol content produced after fermentation is around 0.4%. - 0.8%. Based on the data obtained after testing the integration with Al-Qur'an and Hadith and the law of fiqh which is guided by both, the product of agarwood leaf decoction water kefir (*Gyrinops versteegii*) with an alcohol content of below 1% is a product that is halal and good product ($(\Delta V t du)$) according to QS. Al-Maidah [5]: 90 and QS. Al-A'raf [7]: 31.

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REFERENCES

Journal:

Mega, IM and Swastini, DA. 2010. Phytochemical Screening and Free Antiradical Activity of Agarwood Leaf Methanol Extract (*Gyrinops versteegii*). Journal of Chemistry 4 (2): 187-192.

Book:

al-Farmâwî, Abd al-Hayy. 1997. Al-Bidâyah fî at-Tafsîr al-Maudhû'i. Al-Maktabah al- Jumhûriyah, Kairo. Page: 23 Winarno, F. G. 2004. Food Chemistry And Nutrition. Jakarta: Gramedia Pustaka Utama

Thesis, Dissertation:

- Khotib, Iqbalullah Miftahul. 2018. The Effect of Long Fermentation and Variation of Concentration of Ciplukan Fruit (Physalis angulata Linn.) On Antioxidant Activity, Total Lactic Acid Bacteria and Chemical Quality of Ciplukan Fruit Juice Water Kefir (Physalis angulata Linn.) [Thesis]. Maulanan Malik Ibrahim State Islamic University, Malang. [Indonesian]
- Kunaepah, Uun. 2008. Effect of Long Fermentation and Glucose Concentration on Antibacterial Activity, Total Polyphenols and Phytochemicals of Red Bean Milk Kefir. [Thesis]. Diponegoro University. [Indonesian]
- Lathif, Yudrik. 2016. The Effect of Long Fermentation and Variation in Concentration of Kersen Leaves (Muntingia Calabura L.) on Total Acid, Medium pH and Antioxidant Activity of Kefir Kersen Leaf Tea Water (Muntingia Calabura L.) [Thesis]. Maulanan Malik Ibrahim State Islamic University, Malang. [Indonesian]