ATLANTIS PRESS
Advances in Biological Sciences Research, volume 20
Proceedings of the International Conference on Improving Tropical Animal Production for Food Security (ITAPS 2021)

The Effect of Different Kefir Grain Starter Concentration on pH, Alcohol Percentage, and Organoleptic Properties of Ultra High Temperature (UHT) Milk Kefir

Fitrianingsih Fitrianingsih[1*], Harapin Hafid[1], Asma Bio Kimestri[1], Andini Sulfitrana[1], Rachmita Dewi S. Toba[1]

[1] *Department of Animal Science, Faculty of Animal Science Halu Oleo University, Kendari Indonesia*
[*] *Corresponding author. Email: fitrianingsih.peternakan@uho.ac.id*

ABSTRACT

Kefir is a fermented milk product by lactic acid bacteria and yeast, rich in benefits for the human body. Kefir grains are a starter in making kefir. This research determines pH, alcohol percentage, and organoleptic properties using kefir grains with different concentrations. Ultra-high temperature (UHT) milk is the raw material for kefir in this study. Kefir produced was by using 2.5% (P1), 5% (P2), 7.5% (P3), and 10% (P4) kefir grains. The parameters measured were pH, alcohol percentage, and organoleptic properties. The research was designed based on a completely randomized design, then the data collected were analyzed using analysis of variance followed by Duncan's New Multiple Range Test. The results indicated that the concentration of kefir grains significantly affects ($p<0.05$) pH, alcohol percentage, and organoleptic properties. Seven percent (7%) is the best concentration of kefir grains.

Keywords: *Kefir, pH, UHT milk, alcohol percentage, organoleptic properties.*

1. INTRODUCTION

Nowadays, fermented milk products are top-rated because they are proven to have various benefits for human health. Fermented milk is categorized into functional food because it is scientifically proven to affect the human body positively. Lactose content in fermented milk is low, so people with lactose intolerance can consume it. Fermented milk has also been shown to improve the performance of the digestive tract, has an anti-cancer effect [1], and can increase fat digestibility, so it is perfect for people who want to lose weight [2].

Kefir is one of the fermented milk products containing essential compounds for the body's health, such as vitamins, amino acids, carbon dioxide, aceton, alcohol, and essential oils. Besides, kefir has antimicrobial properties against pathogenic microorganisms due to the formation of organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, and bacteriocins during fermentation [3] which make the shelf life of kefir to be longer. Kefir provides vitamins B6, B2, folic acid, reduces the risk of cancer or tumors, decreases cholesterol, and improves the immune system [4].

Fresh milk is a raw material commonly used in kefir producers, but ultra-high temperature (UHT) milk and powdered milk can be used as alternatives if fresh milk is unavailable. Zakaria [5] reported that using UHT milk with a concentration of 10% starter showed better quality than powdered milk kefir.

UHT milk is fresh milk that has been processed by heating at high temperatures (143^{o} C) for a few seconds. UHT milk is the most recommended than other processed milk because the chemical and organoleptic changes are kept to a minimum. UHT milk is an alternative material in fermented milk produced, especially in areas where fresh milk is unavailable such as Southeast Sulawesi.

Kefir grain is a fermentation starter in making kefir, which has a characteristic springy texture, yellowish-white with a 2-3 mm diameter, [6]. Kefir grains contain a combination of lactic acid bacteria (*Streptococcus* and *Lactobacillus sp*) and yeast (some yeasts from the genera *Kluyveromyces*, *Candida*, and *Saccharomyces*) which play a role in forming kefir with a unique taste, which tastes fresh and has a slightly alcoholic or yeasty aroma. Lactic acid bacteria and yeast work symbiotically to produce a unique taste in kefir, including proteolysis products and the degradation of lipolysis enzymes [7].

The addition of different concentrations of kefir grains will affect the nutritional content, physical properties, and organoleptic kefir. The more concentration of kefir grains added, the higher the ability of microbes to break down glucose to produce primary metabolites (lactic acid and alcohol) and secondary metabolites (antibacterial activity and polyphenols) [8]. Several studies have reported the effect of different concentrations of kefir grains on the quality of kefir. The levels of alcohol, protein, fat, ash and organoleptic kefir are significantly affected by the concentration of kefir grains [9]. Furthermore, Margareth [10] reported that yield, pH, CO_2 content and organoleptic properties were affected by the concentration of kefir grains.

This study aims to determine the effect of kefir grain concentration and the best concentration of kefir grains to give to UHT milk kefir so that later it will save on production costs, but it is still at a level accepted by consumers.

2. MATERIALS AND METHODS

The material used in the study was Ultra High-Temperature milk Full Cream from the Supermarket at Kendari city, kefir grain, and aqua dest. Tools used in this study were pH meter, glass jar, wooden spoon, measuring cup, and ranking test form.

2.1 Kefir Preparation

Kefir production was by using 1 liter of milk with the kefir grain concentration of 2.5%, 5%, 7.5%, and 10% for each treatment using an anaerobic jar. The samples should be at room temperature for 24 hours. Kefir was filtered twice to prevent remaining grain in kefir. After that, the jar was stored in the refrigerator [11].

2.2 Research Design

The experiment was designed based on a completely randomized design with four treatments, and each consisted of five replications to test the pH, alcohol content, and organoleptic quality. The treatment was the kefirs grain concentration as followed: P1 = 2.5% kefir grain, P2 = 5% kefir grains, P3 = 7.5% kefir grains and P4 = 10% kefir grains by weight of milk.

The data collected was analyzed by using Analysis of Variance with a 5% error deviation. Discovering the treatments' effects, the Duncan Multiple Range Test followed the statistical analysis of a treatment effect [12].

2.3 The Observed Parameters

The parameters observed in this study were the pH, alcohol percentage, and organoleptic properties of UHT milk kefir. Organoleptic properties observed were thickness, sourness, aroma, and color.

pH

The pH value of kefir produced was determined using a pH meter (EBRO), and the pH meter calibration was using pH 4 and 7. The pH meter was dipped into 5 mL beaker glass containing 3 ml of kefir sample [13].

Alcohol Percentage

The first step was putting 100 ml of the sample into a distillation flask for alcohol percentage measurement. Then, add 100 ml of aquadest to the distillation flask. Then the sample was distilled at a temperature of 80°C. The distillate was collected into an Erlenmeyer tube until it reached a volume of 50 ml. Furthermore, the distillate was put into the pycnometer until it was complete. The next step was weighing the pycnometer and the distillate for comparison. After that, calculating

Table 1. The rating scale of the instrument Organoleptic Properties

Organoleptic Properties	Scale				
	1	2	3	4	
Viscosity	very not thick	not thick	a bit thick	Thick	Very thick
Aroma	has no kefir scent	has little kefir scent	slightly kefir scent	kefir scent	has strong kefir scent
Sourness	Not sour	Poorly sour	Slightly sour	Sour	Very sour
Color	Very milky white	Milky White	Slightly yellow	Yellow	Dark yellow

Table 2. The Average pH and alcohol percentage of UHT Milk Kefir with Different Kefir Grains

Parameter	Kefir Percentage(%)			
	P1	P2	P3	P4
pH	5.15±0.46^{a}	4.69±0.55^{b}	4.18±0.39^{c}	3.18±0.18^{d}
Alcohol (%)	0.28±0.009^{a}	0.28±0.005^{a}	0.29±0.008^{a}	0.31 ±0.007^{b}

Table 3. The Average of Organoleptic Properties of UHT Milk Kefir with Different Kefir Grains

Organoleptic properties	Kefir Percentage(%)			
	P1	P2	P3	P4
Viscosity	2.56±0.96^{a}	3.00±1.22^{b}	3.16±0.85^{b}	3.32±0.69^{b}
Aroma	3.36±0.99^{a}	3.44±0.65^{a}	3.68±0.85^{a}	4.16 ±0.85^{b}
Sourness	2.80±0.81^{a}	3.00±0.70^{ab}	3.60±0.76^{bc}	3.48±01.12^{c}
Color	1.32±0.32^{a}	2.04±0.45^{b}	2.56±0.65^{c}	2.52±0.65^{c}

the specific gravity of alcohol by the formula:

$$density = \frac{(empty\ pycnometer\ wight)-(pycnometer\ wight+sample)}{(pycnometer\ wight+aquades)}$$

Then it is converted using the specific gravity alcohol conversion table [14].

Organoleptic Properties

Twenty-five people of semi-trained panelists determined organoleptic measurements of UHT milk kefir. The panelist criteria used were 19 - 24 years old, gender was ignored. Organoleptic properties observed were thickness, sourness, aroma, color, soda, and overall. The rating scale of the instrument presented in Table 1.

3. RESULTS AND DISCUSSION

3.1 The pH of UHT Milk Kefir

The pH of milk indicates the degree of acidity of the milk. The average pH of regular milk ranges from 6 - 7. Fermentation of milk will increase the tendency to decrease pH due to the activity of lactic acid bacteria. The results (Table 2) show that different concentrations of kefir have a significant effect ($p<0.05$) on the pH of kefir milk.

The average pH of kefir obtained ranged from 5.51 – 3.18, with the highest pH obtained from kefir with 2.5% kefir grains added, while the lowest obtained from kefir added 10% kefir grains. There was a decrease in pH along with the increasing concentration of kefir grains given.

The degree of acidity of kefir was from lactose degradation by lactic acid bacteria contained in kefir grains. Therefore, the greater the concentration of kefir grains, the kefir's pH becomes more acidic. Sulmiyati [13] stated that the lactose metabolism by lactic acid bacteria resulted in a decrease in the pH of fermented milk. The amount of lactose and lactic acid bacteria determined the amount of acid produced during fermentation. During the fermentation process, lactic acid bacteria will produce organic acids such as lactic acid, acetic acid, propionic acid, which causes the sour taste of kefir [15].

3.2 Alcohol Percentage

Alcohol is the final product of fermentation. Kefir grains consist of lactic acid bacteria and yeast that form a symbiotic matrix. Lactic acid bacteria will break milk lactose into lactic acid and some simple sugars such as glucose and galactose, while glucose will be broken down into alcohol by yeast [16]. That process caused kefir to have a characteristic fresh sour taste and smell of alcohol.

The percentage of alcohol kefir obtained in this study ranged from 0.28% - 0.31%. Statistical analysis showed that the percentage of alcohol kefir added with 10 grams (p4) of kefir grains was significantly higher (0.31%) than the percentage of alcohol kefir with 2.5%, 5%, and 7.5% of kefir grains (P1-P3). Using a higher concentration of kefir grains increased the amount of alcohol in kefir [10]. Alcohol consists of volatile compounds so the percentage of alcohol will increase with the increasing concentration of kefir grains [17].

3.3 Viscosity

The results (Table 3) show that kefir grains with different concentrations resulted in different significantly UHT milk kefir viscosity scores ($p<0.05$). The viscosity of kefir produced by 5%, 7.5%, and 10%, kefir grains (P2, P3, and P4) was not significantly but significantly different with 2.5% kefir grains (P1).

The viscosity score of kefir ranges from 2.56 (not thick) – 3.32 (slightly thick). A higher concentration of kefir grains increases the panelists' assessment scores on the viscosity of kefir, from not thick to thick. The high concentration of kefir grains increases the availability of lactic acid, which can coagulate protein to make kefir more viscous. The formation of lactic acid during the fermentation process serves as a flavor and destabilization of proteins which causes coagulation of milk proteins [18].

3.4 Aroma

The results showed that different concentrations of kefir grains had a significant effect ($p<0.05$) on the aroma of UHT milk kefir. Panelists' assessment scores on the aroma of kefir ranged from 3.36 (slightly kefir-scented) – 4.16 (kefir-scented). The scores indicate that the greater the concentration of kefir grains, the stronger the kefir aroma.

The determination of the aroma of kefir was using the composition of lactic acid bacteria and yeast in kefir grains [19]. During the kefir fermentation process, metabolites such as lactic acid, diacetyl, and acetone played a role in giving kefir a distinctive aroma [20].

3.5 Sourness

Another organoleptic property measurement was acidity. The results showed a significant difference ($p < 0.05$) between the average acidity score of UHT milk kefir and the administration of Kafir grains with different concentrations. The greater the concentration of kefir grains given, the more sour the taste of kefir. Panelists' assessment scores ranged from 2.80 (tends to be slightly acidic) – 3.6 (tends to sour). Lactic acid bacteria and yeast synergize in producing the sour taste of kefir [21]. The lactose component and the sweet taste decreased as the kefir grains' concentration increased [22].

3.6 Color

The results showed that the average score of the panelists' assessment of the color of kefir had a significant difference ($p<0.05$). The average score obtained ranged from 1.32 (very milky white) - 2.56 (slightly yellow). The greater the concentration of kefir grains, the color of kefir becomes more yellow. Yilmaz-Ersan *et al*. [23] stated that kefir grains diameter about 1 – 6 mm and are yellowish.

4. CONCLUSION

The difference in the concentration of kefir grains affects the pH and percentage of alcohol. The greater the concentration of kefir grains, the lower the pH, but the alcohol percentage increased. Different concentrations of kefir grains affect the viscosity, aroma, sourness in color. Seven percent (7%) is the best concentration of kefir grains.

REFERENCES

[1] McBean, L. D. 1999. Emerging Dietary Benefits of Dairy Foods. Nutr. Today, 34(4): 47-53.

[2] Rogeli. 2000 FERMENTED MILK AS A FUNCTIONAL FOOD. Agriculture Scientifies. ISSN 1330-7142. Vol 6 (1) : 105-107.

[3] Stephen Moses John* and Sirirat Deeseenthum. 2015. Properties and benefits of kefir -A review. Songklanakarin J. Sci. Technol. 37 (3), 275-282.

[4] Sawitri, M. E. 2011. Kajian Konsentrasi Kefir Grain dan Lama Simpan dalam Refrigerator Terhadap Kualitas Kimiawi Kefir Rendah Lemak. Jurnal Ilmu-Ilmu Pertanian . Vol 21 No 23-28.

[5] Zakaria, Y. 2009. Pengaruh Jenis Susu dan Persentase Starter yang Berbeda terhadap Kualitas Kefir. Jurnal Agribisnis Peternakan. Vol (9) No. 1: 26-30.

[6] Utami, R., Nurhartadi, E., Nursiwi, E., Andriana, M.A.M., Fitriyaningsih, I. 2017. Fermenting whey cheese using kefir seeds (kefir grains) with a variety of nitrogen sources. Agritech 37(4):377-385.

[7] Choi JW and KF Ng-Kwai-Hang. 2003. Effects of genetic variants of K-casein and B-Lactoglobulin and heat treatment on coagulating properties of milk. Asian-Aust. J. Anim. Sci. 16(10):1212-1217.

[8] Astawan, M. 2008. Susu Fermentasi untuk Kebugaran dan Pengobatan. Universitas Atma Jaya, Yogyakarta.

[9] Setyawardani, T., A. H. D. Rahardjo, M. Sulistyowati, & S.Wasito. 2014. Physiochemical and organoleptic features of goat milk kefir made of different kefir grain concentrations on controlled fermentation. Anim. Prod. 16:48-54.

[10] Margareth, L.L, Nurwantoro, H. Rizqiati.2020. Effect of Different Kefir Grain Starter Concentration on Yield, pH, CO2 Content, and Organoleptic Properties of Buffalo Milk Kefir. Journal of Applied Food Technology 7(1):15–18

[11] Bayu, M.K. 2017. Analysis of total dissolved solids, acidity, fat content, and viscosity level in kefir with different fermentation times. Journal of Food Technology 1(2):33-38.

[12] Steel, R.G.D. & J. H. Torrie. 1993. Prinsip dan Prosedur Statistika. Suatu Pendekatan Biometrik. Terjemahan. Bambang Sumantri Edisi 2. PT. Gramedia. Pustaka Umum, Jakarta.

[13] Sulmiyati, N.S. Said, D.U. Fahrodi, R. Malaka, and F. Maruddin. 2019. The Physicochemical,

Microbiology, and Sensory Characteristics of Kefir Goat Milk with Different Levels of Kefir Grain. Tropical Animal Science Journal, Vol 42(2):152-158. DOI: https://doi.org/10.5398/tasj.2019.42.2.152

[14] James, C. S. 1995. Analysis Chemistry of Food. Blackie Academic andProfessional. Great Britain.

[15] Sawitri. M.E. 2011. Kajian Konsentrasi Kefir Grain dan Lama Simpan dalam Refrigerator terhadap Kualitas Kimiawi Kefir Rendah Lemak. 2011. Animal Sciences Journal 21(1):2328.

[16] Yusriyah, N. H, & R. Agustini. 2014. Pengaruh waktu fermentasi Dan konsentrasi bibit kefir terhadap mutu kefir susu sapi (The Effect of fermentation and concentration of kefir grains of quality of cow's milk kefir). UNESA Journal of Chemistry 3:53-57.

[17] Adiluhung, W.D., Sutrisno, A. 2018. Effect of glucomannan concentration and proofing time on the texture and organoleptic characteristics of rice bread (Oryza sativa). Journal of Food and AgroIndustry 6(4):26-37.

[18] Safitri, M. F and A. Swarastuti. 2013. Kualitas Kefir Berdasarkan Konsentrasi Kefir Grain.Jurnal Aplikasi Teknologi Pangan. Vol 2. No 2 : 87-92

[19] H. Temiz and K. Dağyıldız. 2017. Effects of Microbial Transglutaminase on Physicochemical, Microbial and Sensorial Properties of Kefir Produced by Using Mixture Cow’s and Soymilk. Korean J.Food Sci.Anim. Res. 37(4): 606.

[20] T Setyawardani, J Sumarmono, A H D Rahardjo, K Widayaka and R S S Santosa. 2019. Texture Profile and Sensory Characteristics of Kefir with Colostrum Addition. The 1st Animal Science and Food Technology Conference (AnSTC) 2019. Earth and Environmental Science 372. oi:10.1088/1755-1315/372/1/012036

[21] Cais-Sokolińska. D, J. Wójtowski, J. Pikul, R. Danków, M. Majcher, J. Teichert, and E. Bagnicka. 2015. Formation of volatile compounds in kefir made of goat and sheep milk with high polyunsaturated fatty acid content. J.Dairy Sci. 98(10): 6692-6705.

[22] Lestari, M.W., Bintoro, V.P., Rizqiati, H. 2018. Effects of fermentation time on acidity, viscosity, alcohol content, and hedonic quality of coconut water kefir. Journal of Food Technology 2(1):8-13.

[23] Yilmaz-Ersan, L and E. Kurdal. 2014. The Production of Set-Type-Bio-Yoghurt with Commercial Probiotic Culture. International Journal of Chemical Engineering and Applications, Vol. 5, No. 5. DOI: 10.7763/IJCEA.2014.V5.418