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Viscosity, Total Acid, Protein and Hedonic Level of Kefir Made from Buffalo Milk with Different Concentration of Kefir Grain

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Abstract

This research aims to determine the effect of different kefir grain concentration to viscosity, total acid, protein and hedonic level of buffalo milk kefir and to determine the ideal kefir grain concentration for buffalo milk kefir. The basic ingredients used in this research were buffalo milk and kefir grain. Concentration of kefir grain at 2.5%, 5%, 7.5%, 10% (g/v) was used to measure viscosity, total acid, protein and hedonic level of buffalo milk kefir. Ostwald pipette and pycnometer were used to determine the viscosity, total acid used titration method with NaOH 0.1 N, destruction and distillation methods were used to determine the protein content and the hedonic test were done by 25 panelists. The results show that different levels of kefir grain concentration gave significant effect ($p<0.05$) on every parameter. Various levels of kefir grain concentration had highly significant effect ($p<0.05$) on kefir as a product. The conclusions from this study are the more addition of kefir grain, the more viscosity, total acid, and protein content. From the hedonic test gave specific results in sour taste, aroma, texture and overall.

Introduction

Kefir is a food product that due to its health benefits for human, kefir has become a functional food. Kefir has several health benefits such as increase endurance, reduce blood pressure, improve digestion, and as an antimicrobial for microbes that can harm human health (Lestari *et al.*, 2018). The manufacturing process is fairly easy to make, kefir is considered and recognized by many enthusiasts. Kefir is the result from mixing the solid layer and the liquid layer into one. The other health benefits are as probiotics, to control cholesterol in the body, antifungal, and antitumor (Prastiwi *et al.*, 2018).

There have been several studies on kefir made from milk other than buffalo milk. Kefir milk usually processed using cow's milk, goat's milk, sheep's milk because it contains nutrients and chemical structures (Rossi *et al.*, 2016). The fat content inside the buffalo milk is around 7-8% and the protein content in the buffalo milk is ranged between 4.2 to 4.6% which is higher than the cow milk that has about 4% fat content and 3.5% protein content inside. Buffalo milk is a good source of

minerals and vitamin A and immunoglobulins, lactoferrin, lysozyme, bifidogenic, and lactoperoxidase which are known as protein protector that can protect the body from diseases as well as repair damaged cells in body (Matondang and Talib, 2015).

The urgency of doing this research and measure the parameter is to find the right formula to make buffalo milk kefir, because kefir has a taste that is not suitable with Indonesian tongue, especially buffalo milk which is only processed as a typical cheese of North Sumatra, so that it can further examined in its processing into kefir milk. There was a study of influence from different fermentation times on kefir milk where fermentation time has a bigger effect than kefir grain concentration on the total microbial and viscosity of kefir milk (Safitri and Swarastuti, 2013).

The background of this research is to know the effect of different concentration of kefir grain in buffalo milk, to understand the characteristics of the buffalo milk kefir from each treatment. The purposes of this research are to determine the effect of different kefir grain concertation to viscosity, total acid, protein and hedonic

level of buffalo milk kefir and to determine the ideal kefir grain concentration for buffalo milk kefir. The benefits from this research is to produce kefir from buffalo milk as food diversification.

Materials and Methods

The materials used in this study were the fresh buffalo milk from buffalo with murrah breed from Medan (North Sumatra), kefir grains, distilled water, plastic wrap, paper towels, 0.1 N NaOH solution, NaOH 45%, HCl 0.1 N. The tools used in this study were pans, stove, thermometer, basin, mixer, jars, plastic wrap, filters, aluminum foil, Kjeldahl tube, destruction equipment, distillation, erlenmeyer, pycnometer, measuring cups, analytical balance and pipette.

Methods

The research was conducted in October 2019 in the Laboratory of Chemistry and Engineering Laboratory of Nutrition and Food and Agricultural Products, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang.

Making the buffalo milk kefir was done by adding buffalo milk 20 plastic jars with 1 liter of each jar. Kefir grain was weighed according to the treatment, which were 25 g, 50 g, 75 g, and 100 g for each treatments with analytical balance and mixed into each jar. Plastic jars were covered with plastic wrap, closed and labeled in accordance with the treatment. The fermentation process was carried out with all samples left at room temperature for 24 hours. Fermented kefir was filtered from the grain and stored in the refrigerator or cold temperature (Ningsih et al., 2018).

The viscosity test was done by using pycnometer and Ostwald viscometer. The test began by weighing the empty pycnometer and weighing the pycnometer that had been filled with 10 ml of distilled water. Then the weighed pycnometer was filled with 10 ml of kefir samples. The kefir sample was filled into the viscometer, pulled the sample until the upper mark by suction and allowed the sample to drain back into the lower mark. The timer was used to count the time that the kefir sample takes to pass from the upper mark into the lower mark (Sutiah et al., 2008). This step was also used to the distilled water.

The principle of the total acid test was by titration. The test was done by preparing a 10 ml sample and putting it into the erlenmeyer flask. Then 1-2 drops of phenolphthalein indicator was added. The sample was titrated with 0.1 N NaOH solution until the sample changes color to stable pink (Muawanah, 2007).

The protein test was conducted with a 0.5 g sample inserted into the Kjeldahl flask and 0.5 g selenium added with 10 ml sulfuric acid. The sample was destructed until the color turns clear green. Then distilled with the addition of NaOH and H_3BO_3 mixture and two drops of the MR MB indicator. Then, it was titrated with 0.1 N HCl until the color changes to constant purple (Kusnadi et al., 2012).

The hedonic test were tested by twenty-five trained panelists and each panelist assigns a value to each sample based on their favorite using a predetermined scale. The scale or score that used in the test is 1: strongly dislike, 2: dislike, 3: like, and 4: strongly like (Kartika et al., 1992).

Data obtained from the viscosity, total acid and protein content were statistically analyzed with SPSS 22.0 using Analysis of Variance (ANOVA) with significance level of 5%, and continued with Duncan test when there was a significant effect. The result of hedonic test were analyzed using non-parametric Kruskal Wallis test and then followed by Mann Whitney test.

Results and Discussion

Kefir Viscosity

Table 1 shows that the treatment of kefir grain concentration had a significant effect ($p<0.05$) on the viscosity of the resulting kefir. Giving too much kefir grain will increase the protein content in kefir, so giving more kefir grain will increase the viscosity of kefir by forming a thick texture on kefir. This is in accordance with the opinion of Bayu et al. (2017) which states that the protein contained in milk can bind water molecules causing an increase in viscosity. From this study it can be seen that kefir grain can increase lactic acid in kefir. Lactic acid that formed in the process of kefir fermentation cause clumping of protein in milk that affects the thickness or increases viscosity of kefir. This is in accordance with the opinion of Rumeen et al. (2017) which states that the formation of lactic acid in fermented milk products makes the distinctive taste of the product and destabilizes proteins that make the texture thicker.

Table 1. The result of viscosity test, total acid and protein on buffalo milk kefir

Treatments	Viscosity	Total Acid (%)	Protein (%)
2.5% kefir grain	0.16±0.06a	0.94±0.06a	0.54±0.42a
5% kefir grain	0.22±0.07ab	1.05±0.05ab	1.36±0.94a
7.5% kefir grain	0.29±0.10bc	1.10±0.13b	2.50±0.94b
10% kefir grain	0.38±0.11c	1.27±0.12c	4.22±0.65c

Results are mean±standard deviation; Different superscript letters in the same column indicates the significant differences ($p < 0.05$)

In the process of making kefir, kefir from all treatments were all increased in viscosity or more viscous than before. This event was proven by buffalo milk before the addition of kefir grain had a liquid or runny texture and after fermentation with kefir grain it formed into a thicker texture and was followed by changes in the taste of the product becoming more acidic. This is in accordance with the opinion of Ginting et al. (2019) which states that during the fermentation process occurs protein coagulation that makes the texture of kefir is thicker than ever, this phenomenon occurs because the fermentation process produces lactic acid makes sour taste in the kefir and affect the kefir viscosity.

Total Acid in Kefir

Table 1 shows that the different concentration of kefir grain treatments had a significant effect ($p <0.05$) to kefir total acid. Kefir with 10% kefir grain treatment had the most total acid than the other treatments. Giving too much kefir grain during fermentation increases bacterial activity, leading to even more lactose breakdown. This is

in accordance with the opinion of Prastujati *et al.* (2018) which states that the more addition of kefir grain will cause a lot of lactose is broken down by bacteria so that the total acid increased. The increase in total acid of kefir shows that the influence of a given amount of kefir grains and the ability of the starter to increase the acidity of a sample. This is in accordance with the opinion of Afriani (2010) which states that the total acid is not only influenced by the amount of starter used, but is also influenced by the type of starter used and the ability of the starter to produce lactic acid.

Protein Content

Table 1 shows that the different concentration of kefir grain treatments had a significant effect ($p < 0.05$) to its protein content. The protein content in buffalo milk kefir is formed due to the influence of a good source of protein from buffalo milk as raw material, the protein content in kefir grains, and the protein produced by bacteria during the fermentation process. This is in accordance with the opinion of Susanti and Utami (2014) which states that the proteins contained in the kefir comes from the protein in milk as the main ingredient, kefir grain that contains 40-60% protein and protein from bacteria results of fermentation process. Meanwhile, kefir with 7.5% kefir grain concentration treatment with the protein content of 2.50% almost compatible with the applicable standard and kefir with 10% kefir grain concentration treatment has passed the standard. This is in accordance with CODEX STAN 234-2003 (2003) which states that kefir as a fermented milk drink must have a minimum protein content of 2.7% contained in it.
Hedonic Level

Table 2. The result of hedonic test on buffalo milk kefir

Treatments	Sour Taste	Aroma	Texture	Overall
2.5% kefir grain	3.08[a]	3.44[a]	2.76[a]	3.16[a]
5% kefir grain	3.24[a]	3.04[c]	2.76[ab]	3.24[a]
7.5% kefir grain	2.48[b]	2.56[b]	3.12[bc]	2.44[b]
10% kefir grain	2.24[b]	2.96[c]	3.24[c]	2.36[b]

Different superscript letters in the same column indicates the significant differences ($p < 0.05$)

Table 2 shows that the different concentration of kefir grain treatments had a significant effect ($p < 0.05$) to the hedonic level of kefir. The panelist preferred the sour taste and aroma of the 5% kefir grain concentration treatment then the 7.5% kefir grain concentration treatment for the reason that kefir with these treatments had not too sour in taste and aroma. This is in accordance with the opinion of Surja *et al.* (2019) which states that panelists are more likely to like kefir with a sour taste that is not too acidic and with a kefir aroma that is not too strong or pungent aroma. Panelists preferred treatments 7.5% and 10% because the texture is thicker where the treatment 2.5% and 5% has a runny texture, so it feels good to be consumed. This is in accordance with the opinion of Aulia *et al.* (2019) which states that preference for kefir refers to kefir which has a slightly runny or slightly viscous texture and the right thickness, the process of changing the texture to thick

due to lactic acid contained in kefir coagulates protein from kefir during the fermentation process. Overall, the panelists preferred the kefir with treatment 5% then 7.5% with taste and aroma as the main factor.

Conclusion

The different concentration of kefir grain treatments gave a significant effect to all parameters that were tested on kefir. The viscosity, total acid, and protein content increased with the addition of kefir grain. From the hedonic test gave specific results in sour taste, aroma, texture and overall

References
Afriani. 2010. Effect of lactic acid bacteria starter use of lactobacillus plantarum and lactobacillus fermentum on total bacteria lactic acid, acid content and ph value of curd milk cow. Scientific Journal of Animal Husbandry. 13(6):279-285. DOI:10.22437/jiiip.v0i0.114

Aulia, S., Rizqiati, H., Nurwantoro. 2019. The effect of kefir substitution to physical characteristic, total yeast, and hedonik of ice cream. Jurnal Teknologi Pangan. 3(2):192-198. (In Bahasa Indonesia)

Bayu, M.K., Rizqiati, H., Nurwantoro. 2017. Total dissolved solid, acidity, lipid, and degree of viscosity analysis of kefir optima at different fermentation duration. Journal Food Technology. 1(2):33-38. DOI:10.14710/jtp.1.2.%p

Codex Alimentarius Commission. 2003. Codex Standard for Fermented Milks: Codex STAN 243. FAO/WHO Food Standards

Ginting, S.O., Bintoro, V.P., Rizqiati, H. 2019. Lactic acid bacteria (lab), total soluble solid, alcohol content, and hedonic quality of cow milk kefir with varied red dragon fruit juice concentration. Journal Food Technology. 3(1):104-109. DOI:10.14710/jtp.3.1.104–109

Lestari, M.W., Bintoro, V.P., Rizqiati, H. 2018. Effect of fermentation time on acidity, viscosity, alcohol concentration, and hedonic quality of coconut (*Cocos nucifera*) water kefir. Journal of Food Technology. 2(1) : 8-13. DOI:10.14710/jtp.2.1.%25p

Matondang, R.H, Talib, C. 2015. Utilzation of buffalo to support milk production. Journal of Agricultural Research and Development. 34(1):41-49. DOI:10.21082/jp3.v34n1.2015.p41-49

Muawanah, A. 2007. Effect of incubation time and variation of starter type on sugar, lactic acid, total acid and ph of soy milk yogurt. Journal of Valence Chemistry. 1(1):1-6. DOI:10.15408/jkv.v1i1.206

Ningsih, D.R., Bintoro, V.P., Nurwantoro. 2018. Analysis of total soluble solid, alcohol, ph value and total acid kefir optima with addition of high fructose syrup (hfs). Journal of Food Technology. 2(2):84-

89. DOI:10.14710/jtp.2.2.84–89

Prastiwi, V.F., Bintoro, V.P., Rizqiati, H. 2018. Characteristics of microbiology, viscosity value and organoleptic properties of kefir optima with the addition of high fructose syrup (hfs). Journal of Food Technology. 2(1):27-32. DOI:10.14710/jtp.2.1.%p

Prastujati, A.U., Hilmi, M., Khirzin, M.H. 2018. The effect of starter concentration on alcohol, ph, and total titrated acids (tta) in whey kefir. Journal of Applied Animal Husbandary. 1(2):63-69. DOI:10.25047/jipt.v1i2.893

Rumeen, S.F., Yelnetty, A., Tamasoleng, M., Lontaan, N. 2017. Utilization of several sucrose levels on sensory characteristics of cows. Journal ZOOTEC. 38(1):123-130. DOI:10.35792/zot.38.1.2018.18565

Safitri, M.F., Swarastuti, A. 2013. Kefir quality based on kefir grain concentration. Jurnal Aplikasi Teknologi Pangan. 2(2):87-92

Surja, L.L., Dwiloka, B., Rizqiati, H. 2019. Effect of high fructose syrup (hfs) addition on chemical and organoleptic properies of green coconut water kefir. Journal of Applied Food Technology. 6(1):3-8. DOI:10.17728/jaft.4189

Susanti, S., Utami, S. 2014. The influence of fermentation time on the protein content of kefir as a compiler material guideline for biochemistry courses. Journal Florea. 1(1):41-46. DOI:10.25273/florea.v1i1.371

Sutiah, K., Firdausi, S., Budi, W.S. 2008. Cooking oil quality study with viscosity and bias index as parameter. Jurnal Berkala Fisika. 11(2):53-58. (In Bahasa Indonesia)

Rossi, E., Hamzah, F., Febriyani, F. 2016. The proportion of goats' milk and soya milk in kefir production process. J. Peternakan Indonesia. 18(1):13-20. DOI: 10.25077/jpi.18.1.13-20.2016

Kartika, B., Hastuti, P., Supartono, W. 1992. Guideline For Food Test Senses Center For University in Food and Nutrition. University of Gadjah Mada. Yogyakarta. (In Bahasa Indonesia)

Kusnadi, D.C., Bintoro, V.P., Al-Baari, A.N. 2012. Water binding properties, level of elasticity and protein content of meatballs from combination of beef meat and rabbit meat. Jurnal Aplikasi Teknologi Pangan. 1(2):28-31. (In Bahasa Indonesia)