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Effect of Different Kefir Grain Starter Concentration on Yield, pH, CO_2 Content, and Organoleptic Properties of Buffalo Milk Kefir

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Abstract

This research was investigated the effect of different kefir grain starter concentration on yield, pH, carbon dioxide (CO_2) content, and organoleptic properties of buffalo milk kefir, and to investigated the ideal kefir grain concentration for good quality of kefir. Complete randomized design used in this research with 4 treatments and 5 replications in concentration 2.5, 5, 7.5, and 10% g/v kefir grain. Buffalo milk, kefir grain, Na_2CO_3 solution, 1% PP solution, pH meter, burette, and analytical balance were used in this research. The yield and pH of kefir was measured. Carbon dioxide (CO_2) content was measured by Na_2CO_3 titration, while the organoleptic properties that included level of sourness, sour aroma, texture, and overall acceptance were done by 25 panelists. The results indicated that different kefir grain starter concentration significantly affected to the yield, pH, CO_2 content, and organoleptic properties ($p<0.05$). The most optimal kefir grain concentration was 5% proved by yield 81.81%, pH value 4.20, CO_2 content 0.43%, and proper organoleptic properties which were low level of sourness, acceptable aroma of kefir and texture. As conclusion, the concentration of grain relied on the kefir properties that may achieve in the optimum quality of kefir.

Introduction

Kefir is a functional food product formed through a fermentation process involving yeast and lactic acid bacteria to produce an acidic and yeasty taste, creamy texture, and containing alcohol (Sawitri, 2011). Kefir has been proven for patients with lactose intolerant, improves digestion, provides vitamin B6, B2, folic acid, reduced the risk of cancer or tumors, decreased cholesterol, and improves immune system (Sawitri, 2011).

Kefir grain had a 2-3 mm diameter, yellowish white with a springy texture (Utami *et al.*, 2017). Kefir grains consist of LAB and yeast including *Lactobacillus kefirgranum* and *Lactobacillus lactis* to break down lactose into lactic acid, *Lactobacillus kefiranofaciens* that cause coagulation, *Leuconostoc* which form diethyl and *Candida kefir* which form ethanol and CO_2 (Sawitri, 2011). Different concentration of kefir grain as a starter might affected the value of kefir yield, pH, CO_2 content produced and panelist evaluation of kefir through organoleptic tests. Therefore, most research was done in testing the quality of cow's milk kefir based on the concentration of kefir grain that represents from the total microbial, viscosity and organoleptic properties (Safitri and Swarastuti, 2013). Based on our knowledge, research that was related to buffalo milk kefir and the grain have not been done yet so this research might conducted as the initial publication of the quality of yield, pH value, CO_2 content, and organoleptic properties against kefir grains variation. Therefore, this research was investigated to find the description of yield, pH, CO_2 content, and organoleptic properties of kefir with the variation in grain concentration in order to produce the good quality of kefir based on related parameters. The benefit of this research was providing description of the kefir from buffalo milk using variation in the applied grain.

Materials and Methods

Materials

As much as 20 litres of fresh buffalo milk that was obtained from Medan, South Sumatera. Kefir grain, aquadest, Na_2CO_3 0.45 N, phenolphthalein 1%, jars, plastic wrap, measuring cup, analytical balance, pH meter (Hanna, USA), pipette, burette, autoclave

(Hirayama, Japan), ranking test form were used in this experiments.

Kefir Preparation

Sterilization was carried out on buffalo milk using autoclave. Kefir was produced using 1 liter of milk with the grain weighed 25, 50, 75, and 100 g for each treatments using anaerobic jar. The samples were kept at room temperature for 24 hours. Kefir was filtered twice to prevent remained grain in kefir. After that the jar was stored in the refrigerator (Bayu *et al.*, 2017).

Yield and pH Measurement

The yield of kefir was measured as previous researcher (Nugroho *et al.*, 2018). pH value was measured using 40 ml of sample when the temperature reached 25˚C as conducted by previous researcher (Rasbawati *et al.*, 2014).

CO_2 Content Measurement

Carbon dioxide content was measured using the titration method with Na_2CO_3 0.045 N solution as the titrant. The titration process was performed by taking 10 ml of buffalo milk kefir samples and then the sample was dripped by 1 ml phenolphthalein. The sample was dripped by titrant until the color of sample turned into a pink and calculated by titration formula (APHA, 1978).

Organoleptic Properties Test

The organoleptic properties of buffalo milk kefir were tested by ranking test. As much as 25 panelists were given a questionnaire contained panelist information, instruction, and panelist responses. Sensory attributes for this test were sour taste, sour aroma, texture, and overall preference. This ranking test was conducted by 25 semi-trained panelists (Tarwendah, 2017). Panelists were asked to rank the intensity of the sensory attributes of the sample with a score from 1 to 4 according to the number of samples given. A score of 1 for the sample with the highest intensity then a larger number indicated lower intensity.

Data Analysis Data

Data obtained from the yield, pH value, CO_2 content that analyzed by ANOVA with a significance level of 5% and continued with Multiple Region test *Duncan's* if there were differences. Organoleptic test data results (ranking method) were analyzed by *Kruskal-Wallis* with a significance level of 5% and continued with *Mann-Whitney* if there were differences.

Result and Discussion

Yield of buffalo milk kefir with different concentration of kefir grain was statistically provided significant effect ($p<0.05$) in kefir grain concentration 2.5, 5, and 7.5%, while the 10% treatment was not significantly different with a 7.5% kefir grain concentration. The yield indicated in a range value from 67.53 to 84.82 as presented in Table 1. It is shown that the yield of buffalo milk kefir decreased due to the increasing kefir grain concentration. In kefir fermentation, lactose converted into lactic acid, acetic acid, alcohol, and CO_2 so resulted in the decrease in total solids (Laureys and Vuyst, 2014). Alcohol and CO_2 had volatile properties so that the more concentration of kefir grain the more alcohol and CO_2 would be produced this may causing the kefir mass to decreased (Adiluhung and Sutrisno, 2018). In buffalo milk fat degradation occurs by lipase enzymes where the higher the concentration of kefir grain the more fat was broken down into simpler fatty acids thereby reduced the kefir mass (Kustyawati *et al.*. 2012). The breakdown of fat into fatty acids produced short chain fatty acids which were volatile and easily lost such as caproic acid, caprylic acid, capric acid (Damayanthi *et al.*, 2014)

Concentration of kefir grain was statistically provided significant effect ($p<0.05$) to the pH but 7.5% kefir grain concentration was not significantly different from the 5 and 10% kefir grain concentration. pH kefir showed the range from 4.02 to 4.30 as shown in Table 1. It is shown that the pH value decreased due to the increasing concentration of kefir grain. The degree of kefir acidity was a representation of the accumulation of organic acids in kefir (Bayu *et al.*, 2017). In the processed of lactose fermentation the bacteria will be converted by lactic acid bacteria into organic acids such as lactic acid, acetic acid, propionate which caused kefir to had an acidic taste (Sawitri, 2011). The higher concentration of starter kefir grain the more microbes that converted lactose into lactic acid, the more organic acids were produced resulted in the decrease in pH (Safitri and Swarastuti, 2013).

Table 1. The yield, pH value, and CO_2 content of buffalo milk kefir

Kefir grain (%)	Yield (%)	pH	CO_2 (%)
2.5	84.82 ± 1.44^{a}	4.30 ± 0.10^{a}	0.38 ± 0.01^{a}
5	81.81 ± 0.80^{b}	4.20 ± 0.08^{a}	0.43 ± 0.02^{b}
7.5	77.43 ± 3.62^{c}	4.09 ± 0.15^{ab}	0.48 ± 0.01^{c}
10	67.53 ± 4.75^{c}	4.02 ± 0.19^{b}	0.52 ± 0.03^{d}

Results are mean ± standard deviation (n=20). Different superscript letters in the same column indicates the significant differences ($p<0.05$).

Table 2. The organoleptic properties of buffalo milk kefir

Organoleptic attributes	Kefir grain concentration			
	2.5%	5%	7.5%	10%
Sourness	3.28 ± 1.02^{a}	3.24 ± 1.96^{a}	2.28 ± 1.06^{b}	1.64 ± 0.91^{c}
Aroma	3.24 ± 0.97^{a}	2.72 ± 0.89^{b}	2.40 ± 1.00^{b}	1.64 ± 1.04^{c}
Texture^{ns}	2.29±1.17	2.56±0.91	2.36±1.29	2.24±1.01
Overall^{ns}	2.28±1.21	2.24±1.01	2.56±1.04	2.92±1.15

Data represented mean±standard deviation (n=25). Different superscript letters in the same row indicates the significant differences ($p<0.05$). Sourness and aroma were the value for its level.

Concentrations of kefir grains was significantly affected ($p<0.05$) to CO_2 content in kefir as can be seen in Table 1. It is shown that the CO_2 content increased due to the increase concentration of kefir grain. The presence of CO_2 in kefir caused the sensation of soda that appeared when kefir was consumed. Yeast in kefir grains converted glucose into alcohol and CO_2 and a combination of both gave sensation of carbonated drink (Prastiwi *et al.*, 2018). Release of CO_2 might occurred when decarboxylation of pyruvic acid to acetaldehyde (Hasanah *et al.,* 2012).

Level of Sourness

Based on Table 2 it is known that 2.5 and 5% grain had the lowest level of sourness intensity compared to other treatments. Concentration of 7.5% kefir grain had a higher level of sourness than 2.5 and 7.5% while 10% had the highest level of sourness intensity. The sourness in buffalo milk kefir is caused by the production of lactic acid and acetic acid that occur during fermentation. Sweetness decreased by increasing the concentration of kefir grains due to decreased lactose content during fermentation (Lestari *et al.,* 2018). *Lactobacillus bulgaricus* may utilize glucose as much as 2.0-3.5 g/liter during the exponential phase and 8 g/liter in the stationary phase during fermentation (Prastiwi *et al.,* 2018).

Level of Kefir Aroma

Based on Table 2, it is known that 2.5% kefir grain concentration had the lowest level of kefir aroma intensity as compared to 5 and 7.5% but had no significant value, while 10% had the highest kefir aroma intensity compared to other treatments due to volatile compounds, acetaldehyde, and acetaldehyde which caused acidic aroma in kefir. The presence of yeast in kefir grain also produced ethanol which caused a distinctive aroma that affected to panelist acceptance (Mondang *et al.*, 2016).

Kefir Texture

Based on Table 2, it is known that texture with different concentrations of kefir grains was statistically provided not significant effect (p>0.05). The thick texture of kefir was formed because of the coagulation in kefir (Zakaria, 2009). Coagulated casein increased kefir viscosity so the texture became thicker. An increased in the number of microorganisms increased the total amount of lactic acid which triggers a decreased in pH beyond the isoelectric point, then coagulation will be formed (Prastiwi *et al.*, 2018).

Overall Acceptance

Based on Table 2, it is known that overall acceptance with different concentrations of kefir grains was statistically provided not significant effect (p>0.05). As much as 5% kefir grain concentration was the most preferred among other treatments where the level of sourness, the aroma, and texture were acceptable by panelists. The 10% kefir grain concentration was the least preferred because it had the most acidic intensity of taste, the most pungent aroma of kefir, and the thickest texture among others. The higher concentration of kefir grain produced a strong sourness and kefir aroma (Lestari *et al.*, 2018). This overall represents the overall level of panelist acceptance from sensory attributes ranged from taste, aroma, to texture.

Conclusion

The higher concentration of kefir grain, lower the yield would be produced, the pH value decreased, and CO_2 content increased. The most optimal kefir grain concentration was 5% resulted desirable organoleptic properties which were low level of sourness, acceptable aroma of kefir and texture.

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