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Full Length Article

The Effect of Various Concentrations of Kefir Grains on Buffalo Colostrum Kefir's Microbiological, Physicochemical and Sensory Properties

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Abstract

This study was aimed at analyzing buffalo colostrum kefir's physicochemical and microorganism properties with different concentrations of kefir grains. The concentration of the kefir grains used was 10, 20 and 30% (w/v) which affected microbiological characteristics, including total lactic acid bacteria (LAB), total yeast and total microorganisms. Physicochemical characteristics included viscosity, pH, total acid, total dissolved solids, protein, fat, and sensory characteristics *i.e*., hedonic. The results showed that the total LAB, yeast, microorganisms and acids increased. In contrast, the viscosity, pH, total dissolved solids, protein and fat decreased with increasing concentration of kefir grains. The best treatment was buffalo colostrum kefir, with a grain concentration of 20%. This research is useful to determine the optimal concentration of kefir grains for functional beverage products with the best characteristics and obtain functional drinks that can help improve quality health. © 2023 Friends Science Publishers

Keywords: Buffalo colostrum; Grain kefir; Microbiological; Physicochemical; Sensory

Introduction

Kefir is one of the probiotic drinks because it contains various microorganisms that can improve body health, such as *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Saccharomyces kefir*. Probiotics are perfect for the body because they can find symbiosis with microorganisms in the intestinal tract to facilitate and balance the microflora in the digestive tract (Rizky and Zubaidah 2015). The probiotics in kefir also act as immunomodulators, namely substances that can boost the immune system by stimulating or activating lymphocytes, natural killers, and other cells that play an essential role in immunity (Adnan 2020). One of the ingredients that can also be used as an ingredient in making kefir is buffalo colostrum.

Colostrum is the first fluid from the mammary glands of dairy animals such as buffalo, cows, or goats, which is secreted about 1–3 days after giving birth. Buffalo colostrum also supports the functionality of kefir as a healthy drink because the content of immunoglobulin (Ig) and lactoferrin in buffalo colostrum is very high, even higher than buffalo milk and cow's milk. In addition, the protein activity of lysozyme in buffalo colostrum is five times greater than in cow's milk (Ahmad *et al.* 2013). Therefore, it is necessary to develop kefir products using buffalo colostrum which can benefit health.

The concentration of kefir grains influences the process of making kefir. Kefir is made by inoculating a starter called kefir grains into milk and then incubating it anaerobically. Kefir grains contain microorganisms from lactic acid bacteria (LAB) and yeast which convert the substrate into metabolite compounds. Different concentrations of kefir grains can produce different characteristics of the kefir. Moreover, the content of antibacterial compounds in colostrum is relatively high. So research was needed to determine optimal kefir grain concentrations to obtain kefir with microbes that achieve the minimum standard of probiotic kefir products.

Materials and Methods

Production of buffalo colostrum kefir

Buffalo colostrum kefir production begins with the frozen colostrum being thawed first by soaking it in warm water (Morrill *et al.* 2015). The colostrum was put into a jar and then added kefir grains at different concentrations. According to the treatment, the concentration of kefir grains was 10, 20 and 30% (w/v) (Kurniati *et al.* 2020). Colostrum was then stored anaerobically at 28℃ for 48 h. Fermented kefir was then filtered to separate the kefir grains.

Microbial analysis

Analysis of total LAB: Analysis of total BAL was done according to Hafsan (2014). The total LAB used MRSA (Merck, Germany) medium. The test was performed by diluting 1 mL of buffalo colostrum kefir sample in 9 mL of distilled water, called a 10^{-1} dilution. Dilutions were made up to 10^{-6} concentration. The last three series of dilutions were taken as much as 1 mL with a micropipette, then put in a petri dish and added the MRSA. The plates incubate upside down for 24 h at 37°C. Total LAB was calculated using the following formula:

$$\text{Total colonies (CFU/g)} = \text{total colony} \times \frac{1}{\textit{dilution factor}}$$

Analysis of total yeast: In a total yeast analysis by plate count method (Maturin and Peeler 2001), 1 mL sample of kefir was put into a test tube containing 9 mL of 0.85% physiological NaCl with a sterile pipette (10^{-1} dilution). The same is done until a dilution of 10^{-4} is obtained. The last three dilutions were taken as much as 1 mL and added with 15 mL of Sabouraud Dextrose Agar (SDA) media at 50℃, then incubated at 37℃ in an inverted position for 72 h. Total yeast was calculated using the following formula:

$$\text{Total colonies (CFU/g)} = \text{total colony} \times \frac{1}{\textbf{dilution factor}}$$

Analysis of total microorganisms: Total microbial analysis was made using the total plate count (TPC) method (Fardiaz 1993). A sample of buffalo colostrum kefir was taken as much as 1 mL into a test tube using a 1 mL micropipette. Then the dilution was carried out until the specified dilution level was 10^{-6}. The last three dilution levels were cultured in 15 mL of sterile PCA media. The plates were incubated upside down for 72 h at 37℃. Total microorganisms were calculated using the following formula:

$$\text{Total colonies (CFU/g)} = \text{total colony} \times \frac{1}{\textbf{dilution factor}}$$

Physicochemical analysis

Measurement of viscosity: Analysis of viscosity refers to Bayu *et al.* (2017) using the Ostwald viscometer (Pyrex, England). The buffalo colostrum kefir sample was diluted up to 5 times the dilution, then sucked using an Ostwald pipe to the upper calibration mark, and the time of dropping the sample to the lower calibration mark was calculated. The flow time of the tested sample was compared with the time required for another liquid of known viscosity. To measure the density of the sample, a 10 mL pycnometer (Pyrex, England) was used. Viscosity was calculated using the following formula:

$$\text{Viscosity (cp)} = \frac{\rho \text{ sample} \times \text{t sample} \times \eta \text{ water} \times \text{dillution factor}}{\rho \text{ water} \times \text{t water}}$$

$$\rho \text{ sample} = \frac{\text{m`} - \text{m}}{\text{v}}$$

Measurement of pH: The sample pH was measured using a calibrated pH meter (Herma, Germany). The pH of the sample was measured by inserting the pH meter electrode into the bottle containing the sample until the scale or number on the pH meter shows a constant reading, then the results are recorded.

Analysis of total acids: The total acid test referred to the Indonesian Standards 2981 (Indonesian Standards 2009). The analysis used the titration method, and the results were calculated as lactic acid equivalent. A 10 mL of kefir sample was put in 100 mL of distilled water and then titrated with 0.1 N NaOH until it became a persistent pink color. The total acid concentration was then calculated using the following formula:

$$\text{Total acid (\%)} = \frac{\text{Volume of NaOH} \times \text{N NaOH} \times \frac{100}{10} \times 90}{\text{Volume of sample} \times 1000} \times 100$$

Estimation of total dissolved solids (TDS): Testing for TDS referred to Retnowati and Kusnadi (2014) using a hand refractometer (Atago, Japan). The sample is dripped on top of the prism until the prism is covered and the degree of Brix will appear on the screen.

Analysis of crude proteins: Protein analysis used the Kjeldahl method by weighing approximately 0.3 g of the macerated sample and putting it into the Kjeldahl flask. The selenium mixture catalyst was added in the amount of approximately 0.3 grams. Technical sulfuric acid (concentrated) was added to as much as 10 mL. The sample was crushed to a clear green color in a fume cupboard, then cooled. The sample was distilled using a 5 mL 4% H_3BO_3 trap and two drops of MR and MB indicators were added. The digested sample was put into a distillation flask then added 100 mL of distilled water and 40 mL of 45% NaOH. Distillation was carried out until the catcher changed color from purple to green, and 40 mL of distillate was obtained. The procedure was repeated for blanks. The crude protein content was calculated by the formula (Legowo *et al.* 2005):

$$\text{Protein Content} = \frac{(\text{sample titrant} - \text{blank}) \times \text{N HCl x } 14{,}008}{\text{sample weight} \times 1000} \times 100\%$$

Analysis of fat content: The fat test referred to Legowo *et al.* (2005) method, which used the soxhlet extraction. A 0.2 g macerated sample weighed (recorded as weight A). The sample was wrapped in fine filter paper. Samples were heated in an oven at 100℃ for 4 h, then put in a desiccator for ± 15 min and weighed. The sample was reheated in the oven at 100℃ for 1 h, then put in a desiccator for ± 15 min, weighed until it reached a constant weight (recorded as weight B). All samples were included in the Soxhlet extraction apparatus. A 500 mL of *n*-hexane solution was put into the extraction apparatus. The condenser was installed, and heating flask was connected to the heating source. The extraction process was carried out for at least 6 h. The samples were aerated for ± 30 min in the open air. The sample was dried in an oven at 100℃ for 1 h, then put in a

desiccator for ± 15 min, then weighed (recorded as weight C). Fat content was calculated by the following formula:

$$\text{Fat content (\%)} = \frac{\text{Weight B} - \text{Weight C}}{\text{Weight A}} \times 100$$

Sensory attributes testing: Sensory testing uses the hedonic method, refers to Merawati *et al.* (2013). The hedonic test measured the level of preference for sensory attributes in buffalo colostrum kefir. The number of panelists was 25. Panelists were given four samples of kefir and then asked to give their preference rating on a scale of dislike (1), rather like (2), like (3) and really like (4) on the attributes of taste, aroma, texture and overall.

Statistical Analysis

The data obtained were analyzed using the SPSS 26.0 application at a significance level of 5%. Data analysis used the parametric analysis of variance (ANOVA) test. Experimental design was completely randomized factorial with seven replicates. If there was a significant effect, Duncan's Multiple Range test was carried out to determine whether there was a significant difference among treatments. The hedonic organoleptic test data analysis used a non-parametric test, namely the Kruskal Wallis test. In case there was a significant effect, Mann-Whitney test was conducted.

Results

Microbial analysis

Results showed that differences in the use of kefir grain concentrations had a significant effect ($P < 0.05$) on total LAB, total yeast and total microorganisms (Table 1). Total LAB populations obtained at a concentration of 10, 20 and 30% were 8.23×10^6, 2.88×10^7 and 2.24×10^7 CFU/mL, respectively. Total yeast obtained at a concentration of 10, 20 and 30%, was 6.52×10^5, 1.36×10^6 and 1.79×10^6 CFU/mL, respectively. Total microorganism's populations obtained at a concentration of 10, 20 and 30% were 8.52×10^6, 1.69×10^7 and 7.52×10^7 CFU/mL, respectively. These results indicated that increasing concentration of kefir grains significantly affected the microbial characteristics of buffalo colostrum kefir.

Physicochemical analysis

Differences in the use of kefir grain concentrations had a significant effect ($P < 0.05$) on viscosity, TDS, total acids, pH, and protein but had no significant effect (P > 0.05) on fat (Table 1). Viscosity values obtained at a concentration of 10, 20 and 30% were 0.92, 1.93 and 0.78 cPs, respectively. TDS obtained at a concentration of 10, 20 and 30% were 27.20, 22.40 and 21.26ºBrix, respectively. Total amount of acid obtained at a concentration of 10, 20 and 30% was 2.03, 2.07 and 2.19%, respectively. The pH value obtained at a concentration of 10, 20 and 30% was 4.11, 3.93 and 3.81, respectively. Crude protein content obtained at a concentration of 10, 20 and 30% was 17.39, 15.96 and 13.94%, respectively. Fat content obtained at a concentration of 10, 20 and 30% was 12.08, 11.44 and 9.56%, respectively.

Table 1: Effect of kefir grains on total LAB, yeast, and microbes of buffalo colostrum kefir

Microbiological properties (CFU/mL)	Kefir grain concentration		
	10%	20%	30%
Lactic acid bacteria	8.23×10^6a	2.88×10^7b	2.24×10^7b
Yeast	6.52×10^5a	1.36×10^6b	1.79×10^6b
Total microbes	8.52×10^6a	1.69×10^7a	7.52×10^7b

Data shown as the mean value of 7 replicates
Different lowercase superscripts show a significant effect (P<0,05)

Table 2: Effect of kefir grains on viscosity, total dissolved solid, total acid, pH, protein, and fat of buffalo colostrum kefir

Physioco-chemical properties	Kefir grain concentration		
	10%	20%	30%
Viscosity (cPs)	0.92 ± 0.08b	1.93 ± 0.11b	0.78 ± 0.10a
Total dissolved solid (ºBrix)	27.20 ± 0.34c	22.40 ± 0.38b	21.26 ± 0.40a
Total Acid (%)	2.03 ± 0.04a	2.07 ± 0.15a	2.19 ± 0.04b
pH	4.11 ± 0.10c	3.93 ± 0.03b	3.81 ± 0.04a
Protein (%)	17.39 ± 1.41c	15.96 ± 0.76b	13.94 ± 1.71a
Fat (%)	12.08 ± 5.83	11.44 ± 2.65	9.75 ± 2.34

Data shown as the mean value of 7 replicates
Mean ± standard deviation
Different lowercase superscripts show a significant effect (P<0,05)

Table 3: Analysis of Variance (ANOVA) of the effect of kefir grains on viscosity, total dissolved solid, total acid, pH, protein and fat of buffalo colostrum kefir

Sensory properties	Kefir grain concentration		
	10%	20%	30%
Flavor^{ns}	2.16 ± 0.99	1.68 ± 0.69	1.88 ± 0.83
Aroma^{ns}	2.92 ± 0.81	3.04 ± 0.73	2.76 ± 0.78
Texture^{ns}	2.68 ± 0.80	2.64 ± 0.81	2.60 ± 0.91
Overall^{ns}	2.52 ± 0.71	2.20 ± 0.64	2.36 ± 0.76

Data shown as the mean value of 7 replicates
Mean ± standard deviation
^{ns}, non-significant ($P > 0.05$)

Sensory analysis

Results revealed that the differences in the use of kefir grain concentrations had a non-significant effect ($P > 0.05$) on the sensory parameters analyzed (Table 3). The average value of the taste parameter ranged from 2.16 to 1.88, which indicated that taste of kefir was favorable. The average value of the aroma ranged from 2.76 to 3.04, indicating that the aroma was relatively like to like. The average value of the texture parameter ranged from 2.6 to 2.68, which indicated that the texture of kefir was relatively favorable. The average value of the overall parameter ranged from 2.2 to 2.52, which indicated general preference for kefir.

Discussion

Treatment of different concentrations of kefir grains had a significant effect ($P < 0.05$) on the total LAB, total yeast and total microorganism in buffalo colostrum kefir. These microbial populations showed an increase with the addition of kefir grains (Table 1). LAB contained in grains metabolized colostrum by converting lactose into pyruvic acid and then breaking it into lactic acid (Ginting *et al.* 2019). An increase in LAB was also caused by protein degradation into peptides by proteolytic enzymes (Bintsis 2018). The yeast also affects LAB because it can produce essential nutrients for LAB growth (Suriasih *et al.* 2012). Increase in yeast population is also caused by a decrease in pH so that it becomes more acidic (Tania and Parhusip 2022). The protein content in colostrum also supports yeast growth, yeast can degrade proteins such as casein contained in colostrum into simpler ones, namely free amino acids and peptides so that yeast can survive (Rahayu *et al.* 2020). An increase in total microbes was in line with the increase in total LAB and yeast. Microbes in kefir consisted of bacteria and yeast, so the total microbes showed a combination (Table 1). According to Nurliyani *et al.* (2015), total microbes in kefir were higher because they comprised a combination of bacteria and yeast.

Data regarding physicochemical properties indicated that the resulting viscosity of colostrum kefir decreased as the addition of kefir grains increased. A decrease in crude protein content can caused a decrease in viscosity (Table 2). According to Putri and Mardesci (2018), the viscosity of kefir can be affected by several conditions, such as protein content, fat content and storage time. The viscosity of kefir decreases due to protein degradation due to proteolytic enzymes. Alves *et al.* (2021) suggested that differences in kefir grain concentrations affect viscosity. The higher the grain concentration, the acider and the lesser the viscosity. Decrease in viscosity was also in line with the decrease in TDS because the more the concentration of kefir grains used, the more lactose was converted into lactic acid because decrease in TDS decreases the viscosity (Sholichah *et al.* 2019).

Different concentrations of kefir grains increased the TDS value of buffalo colostrum kefir (Table 2). Also, increasing in concentration of kefir grains causes an increase in the population of living microorganisms, which utilize more lactose during metabolism. Purba *et al.* (2012) stated that an increase in the starter would increase the population of microorganisms so that more total solids such as lactose and protein are broken down into lactic acid and amino acids (Sholichah *et al.* 2019).

Addition of more kefir grains means more the population of microorganisms, which metabolize to produce acid. According to Yusriyah and Agustini (2014), *Streptococcus* and *Lactobacillus* contained in grains break down lactose through the glycolysis pathway, where lactose is converted into pyruvic acid and then broken down into lactic acid. During fermentation, LAB hydrolyzes carbohydrates into glucose and galactose with the help of the β-galactosidase enzyme (Hikmetoglu *et al.* 2020). LAB uses glucose to produce lactic acid. Besides lactic acid, kefir also contains smaller amounts of acetic acid. According to Magalhaes *et al.* (2011), acetic acid is also produced during the fermentation process by hetero-lactic bacteria, which increase the acidity. Acetic acid bacteria produce acetic acid by oxidizing ethanol produced by the yeast group. This led to a decrease in pH value of colostrum kefir with an increase in the concentration of kefir grains (Table 2). Irigoyen *et al.* (2005) stated that a decrease in pH was due to the activity of lactic acid bacteria, which produce lactic acid, and acetic acid bacteria, which produce acetic acid. Iraporda *et al.* (2017) also showed that the use of lactose by microorganisms converted into organic acids during fermentation causes to decrease pH.

The crude protein content decreased as the concentration of kefir grains increased (Table 2). This is due to the metabolism of lactic acid bacteria, which utilize protein as a substrate. According to Purba *et al.* (2012), the growth of Lactobacillus bacteria can cause a decrease in protein content because these bacteria have proteolytic enzymes that degrade protein into amino acids and peptides. Bintsis (2018) stated that protein degradation could occur due to the activity of protease enzymes which convert proteins into simple peptides and amino acids, for which other microorganisms can convert into alcohols, aldehydes, and ester acids.

Likewise, higher kefir grain concentration lowered the fat content. The decrease in fat content and the increase in the concentration of kefir grains are due to the activity of microorganisms that break down triglycerides. According to Dewi *et al.* (2020), an increase in the concentration of kefir grains causes an increase in the microorganisms to produce more lipase enzyme. The resulting lipase enzyme converts fat into fatty acids to reduce fat content. Gonzales-Sanchez *et al.* (2010) stated that a decrease in fat content after fermentation could be caused by the formation of lipase by kefir microorganisms and can also be suspected due to the growth of yeast. Fauziyyah *et al.* (2018) showed that the yeast *Saccharomyces cerevisiae* also produces lipolytic enzymes, namely esterases, which cause hydrolysis of fatty acids.

Taste analysis showed no significant differences between treatments (Table 3). Based on the total acid value, the difference between treatments only resulted in a slight difference. The dominant taste in kefir products is a sour taste caused by the formation of lactic acid by bacteria. The sour taste was not liked by the panelists. The panelists did not like the too-sour taste possessed by kefir on average (Haliem *et al.* 2017).

The results of aroma analysis showed no significant differences between treatments (Table 3). The average values obtained ranged from 2.76 to 3.04, indicating that the aroma parameter was slightly preferred to the preferred category. The aroma of kefir is generally sour, resulting from the formation of lactic acid by kefir microorganisms. The fermentation process produces volatile compounds that

trigger the aroma of kefir. According to Ginting *et al.* (2019), kefir has a distinctive aroma of fermentation caused with the formation of volatile compounds such as lactic acid, acetic acid and also alcohol which can sharpen the aroma of kefir. Likewise, results of the texture (thickness) showed no significant differences among treatments. The average texture ranged from 2.6 to 2.68, indicating that it is rather a favorable category, which is assessed in hedonic test because viscosity or thickness can affect consumer acceptance of a product (Sawitri 2011). This thick texture is caused by protein clumping due to the formation of acids during fermentation due to a decrease in pH, which has reached the isoelectric point of the protein (Prastiwi *et al.* 2018). Overall, the results showed no significant differences between treatments. Acids only have differences that slightly affect other parameters, namely texture, and aroma, which lead to the overall parameters (Table 3).

Conclusion

Total LAB, total yeast, total microorganisms, and total acids increased. However, the viscosity, pH, total dissolved solids, protein and fat decreased with increasing concentration of kefir grains. The best treatment was buffalo colostrum kefir, with a grain concentration of 20%.

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Author Contributions

HR, N, EP, and VA planned the experiment, interpreted the results, made the write-up, and statistically analyzed the data.

Conflict of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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