

CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF GOAT MILK KEFIR DURING STORAGE UNDER DIFFERENT TEMPERATURES

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ABSTRAK

Penelitian bertujuan untuk mempelajari pengaruh suhu dan lama penyimpanan yang berbeda terhadap karakteristik kimia dan mikrobiologi kefir susu kambing. Penelitian dilaksanakan dengan rancangan acak lengkap pola faktorial 3x3. Faktor pertama adalah suhu penyimpanan kefir (-1 s/d -5°C; 1 s/d 5°C dan 6 s/d 10°C) dan faktor kedua adalah lama penyimpanan (10; 20 dan 30 hari), masing-masing perlakuan diulang 3 kali. Peubah yang diamati meliputi *titratable acidity*, aktivitas air, jumlah bakteri asam laktat dan total yeast. Analisis variansi dan uji Jarak Berganda Duncan digunakan untuk menganalisis data. Hasil penelitian menunjukkan bahwa lama penyimpanan pada suhu yang berbeda mempengaruhi pH kefir. Kefir dengan pH terendah dihasilkan pada penyimpanan selama 10 hari dengan suhu 6 s/d 10°C. Bilangan asam kefir dipengaruhi oleh suhu penyimpanan, nilai tertinggi didapatkan dari kefir yang disimpan pada suhu 6 s/d 10°C. Nilai aktivitas air kefir tidak dipengaruhi oleh suhu maupun lama penyimpanan, dengan nilai rata-rata 0,875±0,028 unit. Kefir yang dihasilkan memiliki total bakteri asam laktat rata-rata 7,17±0,92 log cfu/ml. Total bakteri asam laktat dipengaruhi oleh suhu penyimpanan, namun tidak dipengaruhi oleh lama penyimpanan. Total khamir kefir dipengaruhi oleh suhu penyimpanan dengan populasi tertinggi terdapat pada kefir yang disimpan dengan suhu 6 s/d 10°C yaitu sebanyak 6,76±0,39 log cfu/ml. Penelitian ini mengkonfirmasi bahwa suhu penyimpanan secara nyata mempengaruhi karakteristik kefir dari susu kambing, sehingga harus menjadi pertimbangan pada saat menyimpan kefir dalam jangka waktu yang lama.

Kata kunci: kefir, pH, titratable acidity, bakteri asam laktat, yeast

ABSTRACT

This research was conducted to study the chemical and microbiological properties of goat milk kefir stored under different temperatures and storage time. A completely randomized design, factorial pattern 3 x 3 was used in this study. The first factor was storage temperature (-1 to -5; 1 to 5 and 6 to 10°C) and the second factor was storage time (10; 20 and 30 days). Each treatment has three replicates. Variables observed included pH, water activity (aw), total lactic acid bacteria (LAB), and total yeast. Data were subject to analysis of variance and Duncan's multiple range test. Results showed that storage time and temperature had significant effects on pH. The lowest pH of Kefir was obtained by storing it for 10 days at 6 to 10°C. Titratable acidity was significantly affected by temperature, and kefir stored at 6 to 10°C has the highest titratable acidity. Storage time and temperature had no significant effects on water activity, and the average water activity of kefir was 0.875±0.028. Total LAB and total yeast were significantly affected by temperature, but not by storage time. In average, total LAB and total yeast in kefir were 7.17± 0.92 log cfu/ml and 6.76± 0.39 log cfu/ml, respectively. In conclusion, this study

confirmed that temperature of storage has a major contribution to the characteristics of kefir made from goat milk; hence it has to be considered when handling kefir for a longer period of time.

Keywords: kefir, pH, titratable acidity, lactic acid bacteria, yeast

INTRODUCTION

Kefir is fermented milk product that contains low alcohol and carbonate produced by kefir grains. Kefir can be categorised as functional food with typical taste and flavour. Kefir grains are mixture of various microflora, mostly lactic acid bacteria (LAB) and yeasts, which is symbiotically responsible for the fermentation process to produce lactic acid and alcohol. LAB and their metabolite products can bring positive effects on health as probiotic.

Kefir grains consist of complex LAB and yeast namely *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus spp.* and the yeast includes *Kluyveromyces*, *Saccharomyces* and *Torula*, in a protein-polysaccharide matrix (Witthuhn *et al.*, 2005; Magalhaes *et al.*, 2011). Kefir grains grow during fermentation process. Peptides and exopolysaccharide, the bioactive components, which act as anticarcinogenic, antimutagenic and antiviral substances, were formed during fermentation (Fanworth, 2005).

Generally, kefir is made from cow's milk, but milk from Etawah Grade goat is one of potential milk to be made into kefir as a functional food product. Goat's milk is better than cow's milk because it has smaller diameter of fat globules and has low allergenicity. Goat's milk has different fatty acid composition because it contains higher level of short and middle chain of fatty acids.

Kefir is generally stored in a refrigerator, where cold temperature decreases the metabolism of kefir grains, thereby affecting the product characteristics. Increasing the population of microorganisms that produce lactic acid and other compounds during fermentation has contributed to the characteristic of kefir (Abraham *et al.*, 1998). Temperature and storage time affect the viability of LAB and yeast, and also contribute to the development of pH, titratable acidity, and taste of the product.

The objectives of this research were to investigate the effects of different temperature and storage time on microbiological and chemical characteristics of kefir made from goat's milk.

MATERIALS AND METHODS

Experimental Design

The research employed a completely randomized design, factorial pattern 3x3. The first factor was storage time (10, 20 and 30 days) and the second factor was storage temperatures (-1 to -5; 5 to 10 10°C and 6 to 10°C). Each treatment was replicated 3 times.

Goat Milk and Procedure of Making Kefir

A total of 54 L fresh goat milk was obtained from Etawah Grade goat farmers in Banyumas, Central Java, Indonesia. The milk was transported in a cold condition, and was pasteurized at 72°C for 15 second, then was cooled to 28°C. Kefir grains were added and incubated at 28°C for 24 hours to allow fermentation process. Milk kefir was separated from kefir grains by using a fine plastic strainer and ready for further treatment. Kefir grains were placed in a plastic container and ready for another batch of fermentation.

Enumeration of Lactic Acid Bacteria and Yeast

Total lactic acid bacteria (LAB) and yeast in kefir sample were determined according to the procedures of Burns *et al.* (2008). Kefir sample was homogenized in a stomacher (lab-blender 400, London, UK) for three minutes; then 1 mL of homogenate was taken and diluted. One ml sample of the highest three dilutions were aseptically taken then placed in sterile petri dishes. The sample was poured in De Man Rogosa and Sharpe Agar (MRS Agar, Oxoid) medium and incubated at 37°C for 48 hours. Yeast was grown in PDA (Potato Dextrose Agar, Oxoid) medium. Total LAB and yeast were enumerated using BAM method (BAM [Bacteriological analytical manual online], 2011).

Determination of pH and Water Content

pH of 10 ml kefir sample was measured using pH meter (Hanna Instrument, USA). Water content was determined by standard methods described by Harley and Prescott (2002).

Determination of Titratable Acidity

Determination of titratable acidity in kefir was aimed to measure the amount of organic acid

in the sample (Sudarmadji *et al.*, 1997). Lactic acid was measured using Mann's Acid Test. The formula used was lactic acid = (NaOH volume x N NaOH x 0.09)/(sample weight) x 100%.

Data Analysis

Analysis of variance and Duncan's multiple range test were used to analyze data using SPSS17.0 statistical package.

RESULTS AND DISCUSSIONS

Data presented in Table 1 show that the effects of temperature, storage time and their interaction on pH of kefir was highly significant ($P < 0.01$). The effect of storage temperature was highly significant ($P < 0.01$) on titratable acidity, but not significant ($P > 0.05$) on water activity and water content. The effect of storage time was significant ($P < 0.05$) on water content, but not significant ($P > 0.05$) on titratable acidity and water activity.

Goat milk kefir had the lowest pH (4.37) after 10 days of storage at 6 to 10°C. After 10 days of storage was presumed to be the exponential growth phase for LAB. The bacteria metabolised lactose in milk and produced lactic

acid and resulted in low pH. Temperature regime of 6 to 10°C was more favourable for the growth of lactic acid bacteria than lower temperatures regimes, hence produced more lactic acid metabolites. The pH of kefir is a reflection of organic acid accumulation (Suriasih *et al.*, 2012). Temperature is one of fermentation variables that has great influence of bacterial growth (Lacroix *et al.*, 2005). The initial pH of fresh goat milk in this experiment was 6.7 and decreased to 4.5 after 24 hours.

Table 1 shows that titratable acidity of goat kefir was affected by temperature of storage. Titratable acidity was the highest in kefir stored 6 to 10°C and the lowest in kefir stored -1 to -5°C. Titratable acidity is directly correlated with pH; therefore kefir having the highest titratable acidity has the lowest pH. According to Athanasiadis *et al.* (2004) and Güzel-Seydim *et al.* (2000), the acidity of kefir is the result of an accumulation of organic acids, alcohol and others volatile compounds produced by kefir microorganism. The average titratable acidity in this experiment was $0.177 \pm 0.04\%$, which was lower than those reported by Chen *et al.* (2009) who previously reported that titratable acidity was 0.7 to 1.4%. Fanworth and Mainville (2003) reported that,

Table 1. Changes in pH, Titratable Acidity, Water Activity and Water Content of Goat Milk Kefir during Storage at different temperatures

Temperature (°C)	Storage (days)	pH	Titratable acidity (%)	Water activity	Water content (%)
-1 to -5	10	4.47 ± 0.04	0.174 ± 0.03	0.874 ± 0.010	87.103 ± 1.04
	20	4.57 ± 0.04	0.154 ± 0.00	0.891 ± 0.008	89.323 ± 0.41
	30	4.73 ± 0.04	0.166 ± 0.00	0.879 ± 0.010	86.870 ± 0.85
1 to 5	10	4.68 ± 0.04	0.135 ± 0.01	0.889 ± 0.003	86.993 ± 0.05
	20	5.17 ± 0.04	0.154 ± 0.00	0.871 ± 0.020	89.841 ± 0.64
	30	4.63 ± 0.04	0.139 ± 0.00	0.863 ± 0.046	88.117 ± 2.34
6 to 10	10	4.37 ± 0.04	0.230 ± 0.03	0.878 ± 0.011	87.572 ± 0.36
	20	4.40 ± 0.04	0.217 ± 0.00	0.877 ± 0.022	87.942 ± 0.77
	30	4.65 ± 0.04	0.228 ± 0.01	0.873 ± 0.010	88.108 ± 0.94
T		**	**	ns	ns
S		**	ns	ns	*
TxS		**	ns	ns	ns

** = highly significant ($P < 0.01$); * = significant ($P < 0.05$); ns = non significant ($P > 0.05$)

normally, kefir has titratable acidity between 0.7 and 1.0 %. Low titratable acidity of kefir in this experiment could be attributed to the amount of kefir grains added, which was only 1%. Irigoyen *et al.* (2003) reported significant differences in kefir properties due to the amount of kefir grains added during manufacture.

Kefir should be stored in low temperature to minimise un-controlled metabolism of lactic acid

bacteria (Sawitri, 2011). Kefir microorganisms consists of lactic acid bacteria and yeasts that produce lactic acid, carbon dioxide, ethanol, acetaldehyde and acetone. These compounds resulted in specific flavour characteristics (Beshkova *et al.*, 2003). Lactic acid was produced mainly by *Lactobacillus lactis* and *Lactobacillus kefirgranum*, whereas ethanol and

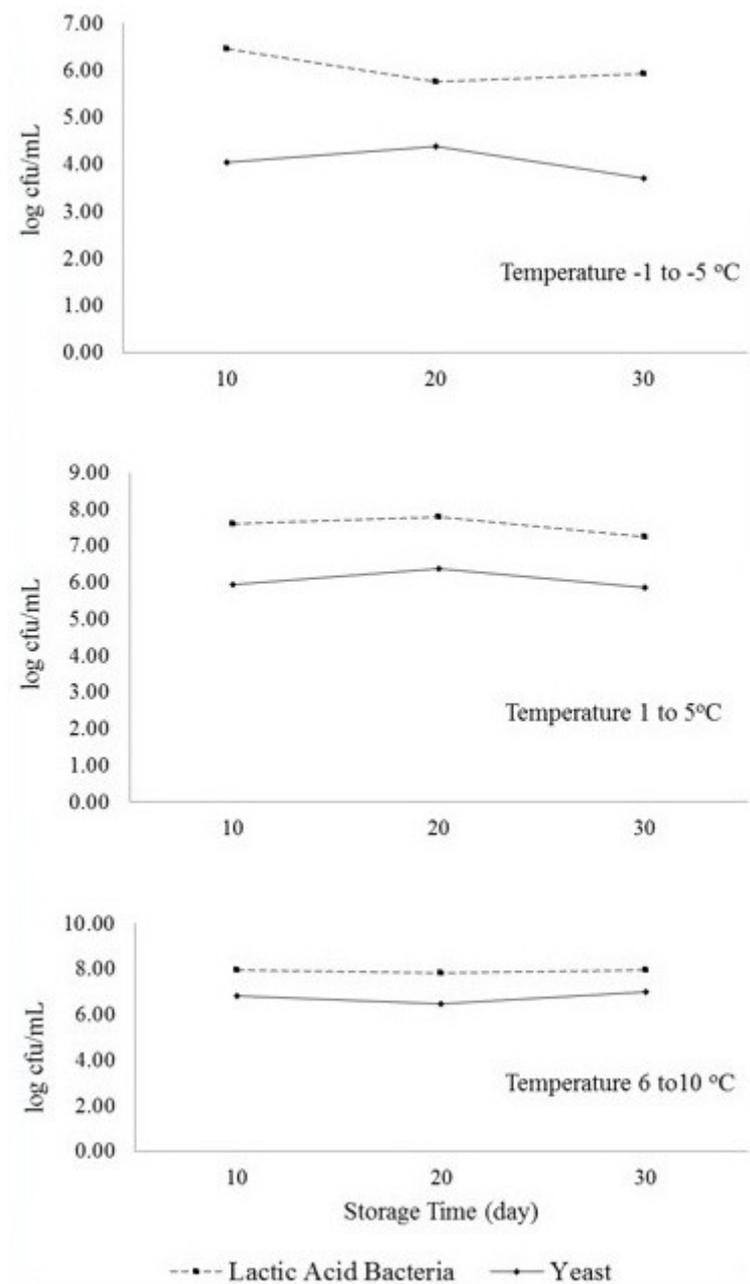


Figure 1. Number of Lactic Acid Bacteria and Yeast in Kefir during Storage at Different Temperature

CO₂ were formed by *Candida sp.* (Susilorini and Sawitri, 2005).

Water activity of kefir in this experiment was not affected by storage time, storage temperature and their interaction. The average water activity was 0.88±0.019. Only storage time showed significant effects on water content. Kefir stored for 20 days has the highest water content (89.84%), and kefir stored for 30 days has similar water content to kefir stored for 10 days. In food system, water is important component which affects appearance, texture, taste and acts as a solvent during metabolism process.

Figure 1 presents data on total lactic acid bacteria and yeast. Lactic acid bacteria were group of microorganisms frequently found in kefir, and the average was 7.17±0.92 log cfu/ml. The effects of storage temperature on total LAB was highly significant (P<0.01), but the effects of storage time was not significant (P>0.05). The highest population of LAB (7.92 log cfu/ml) was observed in kefir stored 6 to 10°C, although the differences from kefir stored 1 to 5°C were not significant. Fanworth (2005) reported that the population of LAB in kefir was 10⁷ cfu/ml. In frozen storage (-1 to -5°C) condition, the population of lactic acid bacteria was 6.05 log cfu/mL.

Type and population of microorganisms in kefir grains have a great contribution to the characteristics of kefir (Farnworth, 2005). Other factors, such as bacterial metabolite, yeast, manufacturing technology and chemical compounds also influence microbiological, physicochemical and sensory characteristics of kefir. Growth of kefir microorganisms is affected by fermentation and storage (Leite *et al.*, 2013). Under optimum temperature, LAB and yeast would increase exponentially. Growth of microorganisms is optimum under certain environmental temperature. Therefore, kefir must be stored in a low temperature to minimize the growth of bacteria and yeast, and to preserve the sensory characteristics of the product.

Figure 1 shows that total LAB remained stable at approximately 10⁷ cfu/ml during storage up to 30 days, regardless of temperature. This is not in line with Irigoyen *et al.* (2005) that lactic acid flora decreased by about 1.5 log units between day 7 and 14 of storage and then remain stable at that level. Chen *et al.* (2004) also reported that both LAB and yeast population increased with the increasing of incubation time.

The effect of storage time on total yeast was not significant. The yeast population in kefir was 10⁵ cfu/mL at 30 days of storage. The highest total yeast (6.759±0.39 log cfu/mL) was observed in kefir stored at 6 to 10°C. This finding was in line with Chen *et al.* (2009) that in traditional kefir, the viable yeast population count was 10⁶ cfu/mL for batch fermentation cycles compared to 10⁴ to 10⁵ cfu/mL for the micro capsulated kefir. The growth of yeasts was minimized when kefir was stored at temperature below 0°C. The higher temperature during storage, the higher metabolic activity and growth of yeasts did. Previously, Morgaless (2011) reported that minimum yeast population in kefir was 6.21±0.01 cfu/g, whereas Gabriela *et al.* (2011) reported that yeast population varied between 5.92 log cfu/g and 8.30 log cfu in the sample from Minas Gerais-Brazil.

Although Witthuhn *et al.* (2005) reported that storage temperature has little effects on the activity of kefir grains and kefir has a low final pH (4.3) throughout the 10-months of storage, temperature of storage is one of major factors that contributes to the metabolic activity and growth of microorganisms. Frozen temperature could damage cells and forced cells to be static or very low metabolism. Therefore, kefir grains have to be conditioned at room temperature after freezing.

CONCLUSION

This study confirmed that temperature of storage has major contribution to the characteristics of kefir made from goat milk. Kefir stored at temperature 6 to 10°C has the lowest pH but having the highest titratable acidity, total lactic acid bacteria and total yeast compared to kefir stored at lower temperature regimes.

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