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# The effect of temperature, incubation and storage time on lactic acid content, pH and viscosity of goat milk kefir

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ABSTRACT

Kefir is a fermented beverage that has probiotic properties and often used as a cosmetic or ointment raw material. Its lactic acid content is classified as AHA (alpha hydroxy acid) which is known good for skin health. The objectives were to study the influence of temperature, fermentation and storage time on the amount of lactic acid, pH and viscosity of kefir. Fermentation of kefir was performed at two different temperatures (room temperature and 37°C) for 24 and 48 h. Storage condition of kefir products was performed at cold and room temperature for 4 to 28 d. The content of lactic acid was based on the total organic acid determined using acid-base titration. The results showed that the average content of lactic acid in 48 h-fermented kefir at room temperature and 37°C were 0.9 to 2.2% with pH and viscosity characteristics were 4.1 to 4.3 and 1400 to1600 cPs, respectively. Meanwhile, during 24 d of storage, the average content of lactic acid was 1.97 to 3.54%, where pH and viscosity characteristics were 3.5 to 4.5 and 3400 to 6400 cPs, respectively. The optimum storage time of goat milk kefir is obtained on days 4 to 12 and they can be stored for up to 24 days without deterioration of kefir products.

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## 1. Introduction

Kefir is an acidic-alcoholic fermented milk product with little acidic taste and creamy consistency that was originated in the Balkans, in Eastern Europe, and in the Caucasus (Angulo et al., 1993). Kefir beverage is commonly manufactured by fermenting milk with kefir grains. This process is supported with a complex microbial symbiotic mixture of lactic acid bacteria (e.g., *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*) and yeasts (e.g., *Kluyveromyces* and *Saccharomyces*) (Magalhães et al., 2010).

The main products of kefir fermentation are lactic acid, ethanol and carbon dioxide which confer the beverage with viscosity, acidity and low alcohol content (Firdausi et al., 2010). Kefir is a fermented product like yoghurt that has health benefits. Kefir is a dairy product produced by fermentation of various animal milk (i.e., goat and cow) with kefir grain (Yilmaz-Ersan et al., 2016). Kefir grains have a complex composition of microbial species such as the predominance of lactic acid bacteria, acetic bacteria, yeasts, and fungi (Pogačić et al., 2013).

Fatty grains in goat's milk are between 1 to 10 millimicrons the same as cow's milk. However, the number of small diameter and homogeneous fat granules is more common in goat's milk, so that goat's milk is more easily digested by human digestive organs and does not cause diarrhea to people who consume it. Physically, the difference between goat's milk and cow's milk can be seen from its color, where goat's milk is whiter than cow's milk, because goat's milk does not contain carotene (Je-Ruei Liu et al., 2005; Rattray and O'Connell, 2011).

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., 1999). Temperature and storage time affect the viability of lactic acid bacteria and yeast, and also contribute to the development of pH, titratable acidity, and taste of the product.

This study was conducted to determine the effect of temperature and incubation time during kefir production and the effect of storage time on the kefir products by analyzing the content of lactic acid and their physicochemical properties e.g. pH and viscosity as supporting indicators.

## 2. Materials and methods

### 2.1. Materials

Pure goat milk was obtained from Palalangon Farm, kefir grain was from Rumah Kefir Bandung, distilled water, buffer pH 4.7 and 9.0 for calibration, indicators of phenolphthalein, oxalic acid and NaOH were purchased from Brataco, Bandung. The tools and instruments were thermometer, stir bar, 100 g of ointment pot, erlenmeyer (Pyrex), beaker glass (Pyrex), incubator, refrigerator, pH meter (Mettler Tolledo), analytical balance (Ohaus), and viscometer (Brookfield).

### 2.2. Kefir production

Amount of 2.5 l of kefir starter was added into 47.5 l goat milk to make 50 l of mixture containing 5% of kefir. It was then divided

into four containers (10 l of each). Two respective containers were fermented in an incubator at 37°C and at room temperature for 48 h. The remaining mixture of milk was filtered again and washed with boiled water to obtain kefir starter for the next fermentation. The kefir products from each fermentation (room and incubator) were divided into smaller pots consisted of 14 pots where each pot contained 100 grams of kefir. Seven respective pots were kept at low temperature (5 to 10°C) and room temperature.

### 2.3. Determination of lactic acid content, pH and viscosity

The observations of kefir product were the measurement of pH, organic acid content, and viscosity. Unfermented goat milk was a negative control. The measurement was performed at intervals of 24 and 48 h of fermentation. In addition, the effect of storage time of kefir was observed for 4, 8, 12, 16, 20, and 24 d. The level of lactic acid in this study referred to the amount of organic acid. According to Underwood (1989), organic acid content was determined using the acid-base titration method which was carried out by filling the burette with NaOH 0.1 N. Kefir was weighed in 18 grams in Erlenmeyer, then added 10 drops of 1% phenolphthalein as an indicator. Kefir has a characteristic pH of 3.77 to 4.19. The pH of goat milk kefir was directly measured without dilution. The viscosity of kefir was measured by Brookfield viscometer with a speed of 30 rpm.

## 3. Results and discussion

### 3.1. Effect of temperature and incubation time on the lactic acid content of goat milk kefir

Optimization of the incubation time and temperature was required to obtain the optimal amount of lactic acid during kefir fermentation with the addition of 5% (w/v) starter. After 24 h of fermentation, the amount of organic acid was 0.9 and 1.3% at room temperature and 37°C, respectively (Table 1). The result was consistent with Tamime's statement and Robinson that good culture will produce 0.4 to 1.0% lactic acid after 12 to 20 h and the standard requirement for the amount of organic acid for fermented milk is at least 0.3% (Codex, 2003).

**Table 1**. Effect of temperature and incubation time on the amount of lactic acid in goat milk kefir

| Temperature | Lactic acid content, % | |
|---|---|---|
| | 24 h | 48 h |
| RT | 0.906 | 1.52 |
| 37°C | 1.35 | 2.2 |

RT: room temperature

The length of fermentation affected the product, i.e. the longer the incubation time, the more substrates that can be overhauled by the starter. This was found by the value of the amount of organic acid that increased after 48 h of fermentation, where the amount of organic acid produced by 1.5% at room temperature and 2.2% at 37°C. These were greater than the results from 24 h fermentation. Temperature at 37°C was found to be the optimal temperature for kefir fermentation due the higher organic acid level.

To our results, the level of organic acid was highly determined by the temperature and incubation time. The temperature above 30°C enabled lactic acid bacteria to optimally grow, hence produced more lactic acid. The longer time for fermentation also gave the chance of bacteria to generate more lactic acid from lactose contained in goat milk.

### 3.2. Effect of temperature and incubation time on the pH of goat milk kefir

Table 2 showed pH of goat milk kefir produced from different temperature and incubation time. Lower pH was observed in 48 h-fermented kefir compared to the kefir fermented for 24 h. It was related to the amount of organic acid content in Table 1. The longer the fermentation time, the lower the pH value due to the high level of lactic acid. Lund and Eklund (2000), reported that lactic acid bacteria convert lactose to lactic acid, thereby reducing the pH of the substrate which is beneficial for the growth of lactic acid bacteria. As said that the pH of growth of lactic acid bacteria is 4.82 to 4.39.

**Table 2**. Effect of temperature and incubation time on the pH of goat milk kefir

| Temperature | pH | |
|---|---|---|
| | 24 h | 48 h |
| RT | 4.34 | 4.10 |
| 37°C | 4.33 | 4.17 |

RT: room temperature

### 3.3. Effect of temperature and incubation time on the viscosity of goat milk kefir

The viscosity of goat milk kefir during storage is shown in Table 3. The increase of the viscosity was time-dependent. The optimum viscosity was obtained after 48 h fermentation at 37°C which was 1600 cPs, while the lowest viscosity value was 1400 cPs at room temperature for 24 h fermentaion. Harjiyanti et al. (2013) stated that the formation of lactic acid by lactic acid bacteria cause an increase in total acid so that casein undergoes coagulation with gel formation. The formation of the gel causes the texture to become semi-solid thereby increasing the viscosity of fermented milk. In addition, after a decrease in pH, the gel was formed followed by an increase in viscosity.

**Table 3**. Effect of temperature and incubation time on the viscosity of goat milk kefir.

| Temperature | Viscosity (cPs) | |
|---|---|---|
| | 24 h | 48 h |
| RT | 1400 | 1480 |
| 37°C | 1500 | 1600 |

RT: room temperature

### 3.4. Effect of temperature and storage time on the lactic acid content of goat milk kefir

The relationship between storage time, temperature and the lactic acid content in kefir products is shown in Table 4. It can be seen that the lowest lactic acid was 1.97% which was obtained from kefir fermented in an incubator and stored at room temperature for 16 d. While the highest total lactic acid was up to 3.54% which was obtained from kefir fermented at room temperature and stored at cold temperature for 24 d. However, too high lactic acid content is not good when compared to the Indonesian National Standard (SNI) of yogurt which is also applied for kefir standardization. According to SNI, the total level of lactic acid that meets the quality standards is between 0.5 to 2.0%.

**Table 4**. Effect of temperature and storage time on lactic acid content of goat milk kefir

| Storage | Temperature | Lactic acid content (%) on the storage time | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 d | 8 d | 12 d | 16 d | 20 d | 24 d |
| Incubator | Cold | 2.94 | 2.77 | 2.85 | 2.46 | 2.5 | 2.27 |
| | RT | 2.53 | 2.80 | 2.44 | 1.97 | - | - |
| Room | Cold | 2.82 | 2.82 | 2.72 | 2.78 | 3.46 | 2.18 |
| | RT | 2.72 | 2.65 | 2.93 | 2.56 | 2.49 | 3.54 |

RT: room temperature, -: kefir has gone bad, all kefir products have gone bad on 28 d

Therefore, based on this study, kefir products will have good quality until the 16 d when stored at room temperature whereby the content of lactic acid contained still meet the yogurt SNI. In addition, if kefir is stored at cold temperatures (6 to 10°C) the quality will remain good until the 24 d.

### 3.5. Effect of temperature and storage time on the pH of goat milk kefir

In Table 5, goat milk kefir had the lowest pH (3.49) after 24 d of storage at cold temperature (6 to 10°C) which could be affected by the exponential growth phase for LAB (Lactic Acid Bacteria). The bacteria metabolised lactose in milk and produced lactic acid and resulted in low pH. Temperature regime at 6 to 10°C was more favourable for the growth of lactic acid bacteria than lower temperatures regimes, hence produced more lactic acid. The pH of kefir is a reflection of organic acid accumulation (Suriasih et al., 2012). Temperature is one of the fermentation variables that has great influence of the bacterial growth (Lacroix et al., 2005).

**Table 5**. Effect of temperature and storage time on the kefir pH of goat milk kefir

| Storage | Temperature | pH on the storage time | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 d | 8 d | 12 d | 16 d | 20 d | 24 d |
| Incubator | Cold | 3.62 | 4.13 | 4.11 | 4.54 | - | - |
| | RT | 3.51 | 3.57 | 3.60 | 3.69 | 3.72 | 3.57 |
| Room | Cold | 3.55 | 3.79 | 4.30 | 3.73 | 3.56 | 3.49 |
| | RT | 3.59 | 3.62 | 3.60 | 3.72 | 3.72 | 3.81 |

RT: room temperature, -: kefir has gone bad, all kefir products have gone bad on 28 d

### 3.6. Effect of temperature and storage time on the viscosity of goat milk kefir

Sugitha and Djalil (1989) stated that factors affecting the viscosity of kefir are the concentration and state of the protein, concentration and state of fat, temperature and duration of milk stored. An increase in protein content can increase the viscosity. Kuswanto and Sudarmadji (1989) stated that the viscosity value was obtained from dairy products due to the clumping of casein because of the low acidity due to the work of the bacterial starter.

**Table 6.** Effect of temperature and storage time on the viscosity of goat milk kefir

| Storage | Temperature | Viscosity (cPs) on the storage time | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 d | 8 d | 12 d | 16 d | 20 d | 24 d |
| Incubator | Cold | n.d. | n.d. | n.d. | n.d. | n.d. | 1600 |
| | RT | 6400 | 5500 | 4200 | 3400 | - | - |
| Room | Cold | n.d. | 6000 | 5500 | 5300 | 3400 | 5000 |
| | RT | n.d. | n.d. | n.d. | n.d. | n.d. | 3800 |

RT: room temperature, -: kefir has gone bad, all kefir products have gone bad on 28 d, n.d.: not readable

Temperature and storage time have affected the viscosity of kefir. The study showed that the kefir products displayed a very high viscosity value and therefore was not readable (Table 6). The lowest viscosity was 3400 cPs which was obtained from kefir incubated at the incubator temperature and stored at room temperature for 16 d. The average viscosity values of kefir decreased during storage. The level of viscosity of kefir was caused by differences in temperature, incubation time, total solids of raw materials that affect the availability of casein and milk lactose. According to Rahayu and Christanti (1991), the longer a product is stored, the protein will form a heavy aggregate that easily settles. The decrease of viscosity according to Rahayu and Christanti (1991) is probably due to the degradation of protein during long storage. This was observed in all kefir products stored until 28 d where kefir had gone bad resulted in 2 layers of lumps at the top and liquid at the bottom.

## 4. Conclusion

Temperature, incubation and storage time did not have a major effect on the goat milk kefir. Fermentation for 48 h at different temperatures resulted in kefir products containing 0.9 to 2.2% of lactic acid, where pH and viscosity characteristics were 4.1 to 4.34 and 1400 to 1600 cPs, respectively. Meanwhile, during 24 d of storage, the average content of lactic acid was 1.97 to 3.54%, where pH and viscosity characteristics were 3.5 to 4.5 and 3400 to 6400 cPs, respectively. Therefore, the recommended storage time for kefir products is 4 to 12 d and can be stored for up to 24 d without any additional substances.

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## Conflict of Interest

The authors declare there is no conflict of interest.

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