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EFFECTS OF KEFIR GRAINS ON FERMENTATION AND BIOACTIVITY OF GOAT MILK

– Research paper –

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Abstract: The effects of kefir grains from different regions in China on fermentation and bioactivity were studied by using pH value, acidity degree, protein hydrolysis degree, antioxidant activity, angiotensin converting enzyme (ACE, EC 3.4.15.1) inhibition rate and sensory evaluation as indexes to select the most suitable kefir grains as starter for goat milk. The parameters of kefir fermented goat milk were optimized by single factor experiment constantly. The results showed that kefir grain K1, which performed better in antioxidant activity, ACE inhibitory activity than the other four kefir grains and sensory evaluation was inferior to kefir grain K5 only, was suitable for the fermentation of goat milk. And the optimum fermentation conditions were found to be as 3% inoculation size at 25 °C for 22h.

Keywords: ACE inhibitory activity; antioxidant activity; goat milk; kefir grain

INTRODUCTION

Kefir is an ancient fermented milk product with refreshing sour taste and aroma, originating from the North Caucasus Mountains of Russia. It has been popular in Russia and central Asian countries such as Kazakhstan and Kyrgyzstan for centuries (Otles et al., 2003). And kefir has gradually spread to Japan, the United States and some European countries (Ahmed et al., 2013), which is becoming one of the most popular fermented milk products currently. Milk are employed as a main material fermented by a kind of irregular granular starter culture, denominated kefir grain, to make kefir. Kefir grains are milky white to creamy and formed naturally. The complex folded surface makes it look like cauliflower at the size of 0.5-3.5cm in diameter (Farnworth, 2006). As a living organism in which natural immobilized microorganisms coexist, some lactic acid bacteria, acetic acid bacteria and yeasts are grown in kefir grains. These bacteria and yeasts are surrounded by a water-soluble polysaccharide matrix, which been named as kefiran (Enikeev, 2012; Hsieh et al., 2012; Irigoyen et al., 2005).

A mixture of flavor substances such as lactic acid, ethanol, carbon dioxide, acetaldehyde is formed since the lactate fermentation and alcohol fermentation of lactose take place simultaneously in the process of kefir production (Güzelseydim et al., 2000). Kefir fermented milk not only possesses a unique taste, but also has many a variety of probiotic functions. Probiotic and prebiotic properties, antimicrobial properties, anticarcinogenic properties, antidiabetic properties, antiallergic properties are verified in many researches (Güzelseydim et al., 2011), which mainly due to nutrients in liquid milk itself and metabolites secreted by microorganisms. There is evidence that one of the reasons for the general longevity of the population in the Caucasus is the long-term consumption of kefir fermented milk products.

For a long time, owing to distinguishing growth and metabolic environment in different regions, so do proliferation methods, diversity of kefir grain is observed inevitably. Compared to other

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dairy, goat milk has an unparalleled advantage, in which nutrients such as protein, fat, minerals, vitamins are in higher levels than other milk (Belewu et al., 2002, Park et al., 2007, Lópezaliaga et al., 2010). The overall protein particles of goat milk is small and fat are composed of short-chain fatty acids. Thus, screening of suitable starter culture for goat milk fermentation is very necessary. In the present study, kefir grains were employed as starters to ferment goat milk. The effects of kefir grains from different regions on the biological activities of fermented product were studied. Fermentation conditions (temperature, fermentation time, inoculation size) were optimized by single factor experiment constantly after selecting the most suitable kefir grains as fermenter for goat milk. It provided a reference for the subsequent development of the kefir goat's milk product.

MATERIALS AND METHODS

Strains: Kefir grains (K1, K2, K3, K4, K5) were collected from different regions in China and preserved by the school of Food and Biological Engineering, Shaanxi University of Science & Technology (Xi'an, China).

Preparation of kefir: Kefir grain was used as starter to ferment reconstituted skim goat milk pasteurized with 2% inoculum. It was incubated at 25 °C for 22 h.

Determination of pH: The pH was measured using pH meter (Shanghai INESA Instruments Co., Ltd, Shanghai, China).

Determination of acidity: Acidity was using standard sodium hydroxide (0.1 mol/L) titration.

Determination of protein hydrolysis degree: The degree of protein hydrolysis (DH) was measured using pH-state. It was conducted in triplicate.

Determination of DPPH free radical scavenging capacity: Kefir were collected, adjusted pH, vigorously stirred, and centrifuged at 8000 rpm/min for 15 min to obtain the corresponding whey fractions. 1 ml whey fractions were mixed with 1 ml ethanol solution containing 0.1 mmol/L DPPH radical. Absorbance was measured at 517 nm after dark reaction. The DPPH radical scavenging rate was calculated by using the following equation:

DPPH free radical scavenging rate=$(1-A_1/A_2)\times100\%$ (1)

All the DPPH free radical scavenging capacity were measured in triplicate.

Where A_1 is the absorbance of the control, A_2 is the absorbance of the compared.

Determination of the capacity to chelating effect: 1ml whey fractions were taken and add 3.7mL distilled water, 0.1mL 3mmol / L ferrous chloride solution, 0.2mL 5mmol / L phenanthroline solution, respectively. The absorbance of samples was measured at 562nm after fully reaction. The chelating effect was calculated as the following equation:

Chelating effect= $(A_b-A_a)/A_b\times100\%$ (2)

All the chelating effect were measured in triplicate.

Where A_b is the absorbance of the control, A_a is the absorbance of the compared.

Determination of ACE inhibitory activity: 100 μL whey fractions were added with 20 μL 0.1 UN/mL ACE and 200 μL 5mmol / L HHL solution. 250 mL 1mol/L HCl was provided to terminate the reaction. The sample groups were added 1.7 mL ethyl acetate. 1mL ethyl acetate layer was taken and dissolved in 3mL deionized water after evaporated at 120 °C. The absorbance was measured at 228nm. The ACE inhibition rate was calculated as follow:

ACE inhibition rate=$(X_1-X_2)/(X_1-X_3)\times100\%$ (3)

All the ACE inhibition rate were measured in triplicate.

Where X_1, X_2, X_3 represent absorbance without the whey fraction, absorbance without ACE and the absorbance in the presence of both ACE and the whey fraction, respectively.

Sensory evaluation: Sensorial evaluation of kefir was carried out by a panel of 12 experienced assessors. 50 mL kefir was collected in glass and numbered randomly before evaluation. Assessors were asked to taste. Taste and smell (total score 40), state of the tissue (total score 50), color (total score 10) of samples were evaluated in turn. Overall score was calculated and done averaged.

Single factor experiment design: Temperature (21 °C, 23 °C, 25 °C, 27 °C, 29 °C), fermentation time (18h, 20h, 22h, 24h, 26h), inoculation size (1%, 2%, 3%, 4%, 5%) were selected as factors

of single factor experiment. Acidity degree, pH value, protein hydrolysis degree, ACE inhibitory activity and sensory evaluation of kefir were measured under these conditions to optimize the parameters of kefir fermented goat milk.

RESULTS AND DISCUSSIONS

Screening of suitable kefir grains for goat milk fermentation

Five kinds of kefir grain were inoculated into pasteurized skim goat milk at inoculation size of 2% and fermented at 25 °C for 22 h, respectively. pH value, acidity degree, protein hydrolysis degree, antioxidant activity, ACE inhibitory activity and sensory evaluation of kefir were measured. The results were showed in Figure 1.

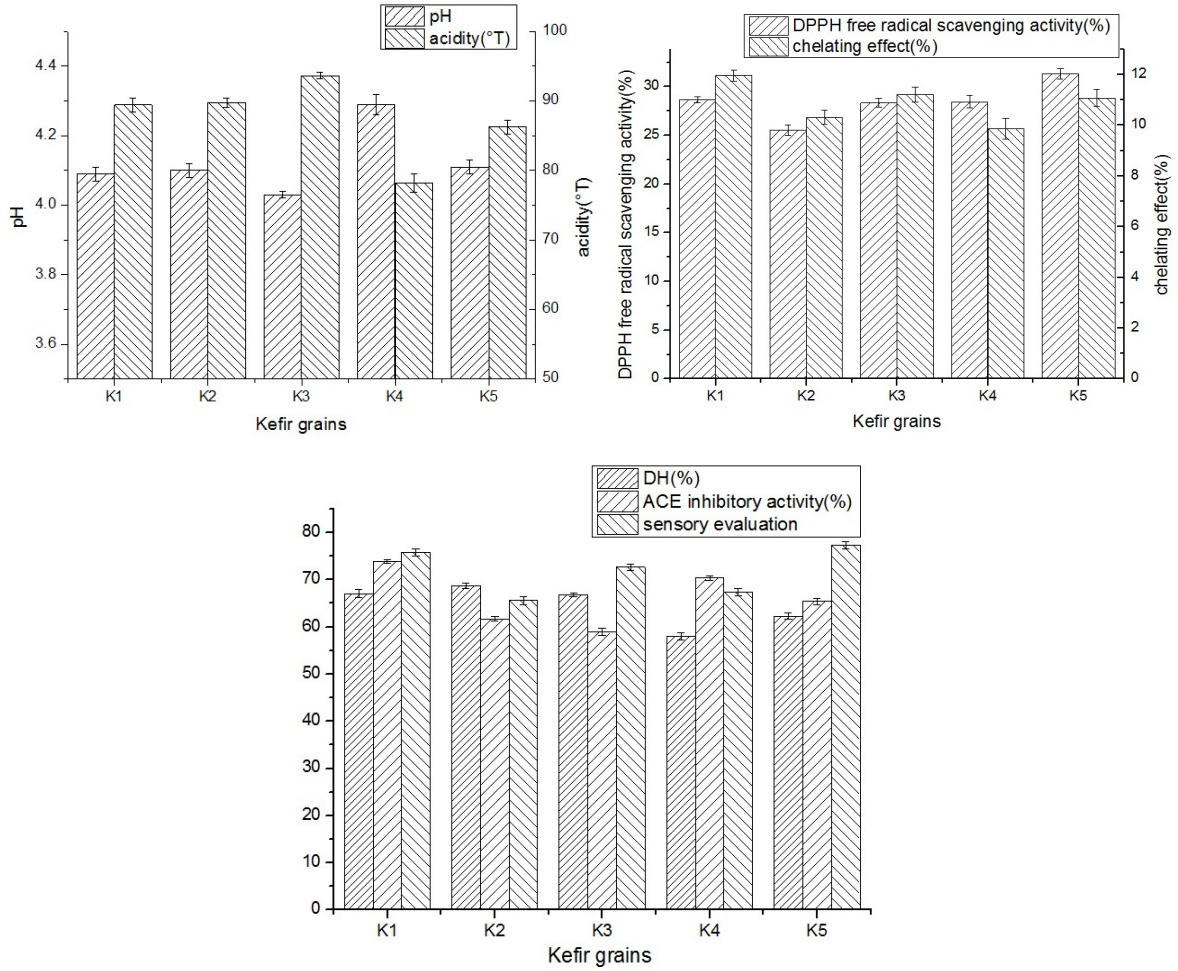

Figure 1 Effects of different kefir grains on the pH, acidity (a), DPPH free radical scavenging activity, chelating effect (b), DH, ACE inhibitory activity and sensory evaluation (c) for fermented goat milk.
The error bars represent standard deviation of means (n = 3).

As shown in Figure 1, several indexes (pH, acidity, DH, DPPH free radical scavenging activity, chelating effect, ACE inhibition rate, sensory evaluation) of the goat milk fermented by kefir grain K1 were obtained as 4.09±0.02, 89.42±1.02 °T, 67.02±0.84%, 28.67±0.31%, 11.97±0.22%, 73.83%, 75.83±0.77, respectively, after incubation with 2% inoculum at 25 °C for 22h. Both antioxidant activity and ACE inhibitory activity were superior to the other four kefir grains. Therefore, kefir grain K1 was more suitable for goat milk fermentation than other kefir grains and it was selected for further optimization.

Effect of temperature on fermented goat milk by kefir grain K1

After the recovered goat milk was sterilized and cooled, the kefir grain K1 was employed to inoculate with 2% inoculum size and fermented at 21 °C, 23 °C, 25 °C, 27 °C and 29 °C for 22 hours, respectively. pH, acidity, DH, ACE inhibition rate and sensory evaluation of samples were measured. And the results were showed in Figure 2. With the increased of fermentation temperature, the pH decreased continuously changed at a range of 4.25 to 3.89. The acidity kept rising from 73.24°T to 94.13°T. DH increased rapidly from 58.01% to 68.47% at the temperature range of 21°C to 25°C and it increased slowly after 25 °C. The ACE inhibition rate increased rapidly from 54.95% to 72.37%. Afterwards, the ACE inhibitory activity increased slowly with the increase of temperature. The maximum of ACE inhibition rate was obtained at 29 °C as 73.56%. Temperature had a significant effect on the sensory evaluation of kefir fermented goat milk. With the score increasing first and then decreasing, it reached the highest value at 25 °C. By comparing the effects of temperature on the pH value, acidity, proteolytic degree, ACE inhibitory activity and sensory evaluation of Kefir fermented goat milk, 25 °C was selected for subsequent response surface optimization.

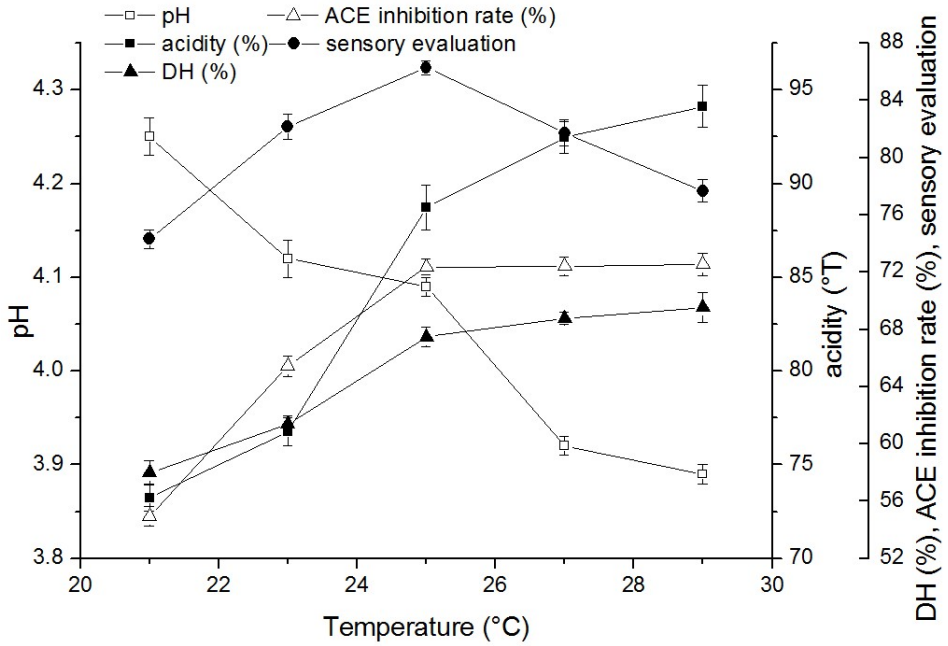

Figure 2. Effects of temperature on the pH, acidity, degree of hydrolysis, ACE inhibition rate and sensory evaluation of kefir. The error bars represent standard deviation of means (n = 3).

Effect of fermentation time on fermented goat milk by kefir grain K1

Kefir grain K1 was inoculated into pasteurized skim goat milk reconstituted at inoculation size of 2% and fermented at 25 °C for 18h, 20h, 22h, 24h and 26h, respectively. pH, acidity, DH, ACE inhibition rate and sensory evaluation of sample were measured and the results as shown in Figure 3. pH of kefir fermented goat milk decreased continuously from 4.22 to 3.99 during the fermentation period of 18 to 26 hours. The acidity increased rapidly within 18 to 20 hours. And the growth rate tended to be gentle after 20 hours, which reached to 87.02 °T at the end of the fermentation. DH had the same tendency as acidity. It increased rapidly from 55.75% to 66.89% in 18 to 22 hours. After 22 hours, the growth rate tended to be flat. ACE inhibitory activity increased rapidly from 72.03% to 72.88% within 18 to 20 hours. After 22 hours, growth rate tended to be stable and it reached finally 73.21%. Fermentation time had a significant effect on the kefir sensory evaluation as well, with the score increasing first and then decreasing, reaching a maximum of 81 at 22 h. Therefore, 22 hours was selected for further optimization.

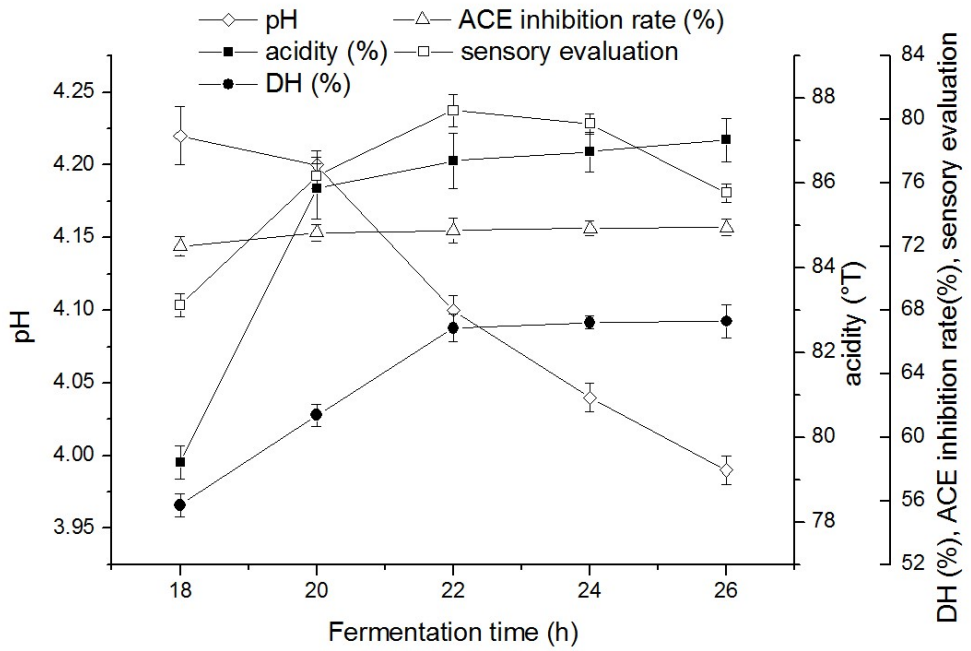

Figure 3 Effects of fermentation time on the pH, acidity, degree of hydrolysis, ACE inhibition rate and sensory evaluation of kefir. The error bars represent standard deviation of means (n = 3).

Effect of inoculation size on fermented goat milk by kefir grain K1

Kefir grain K1 was inoculated into sterilized goat milk with 1%, 2%, 3%, 4% and 5% inoculum, separately, and fermented at 25 °C for 22 hours. The pH, acidity, DH, ACE inhibition rate and sensory evaluation of samples were measured, and the results showed in Figure 4.

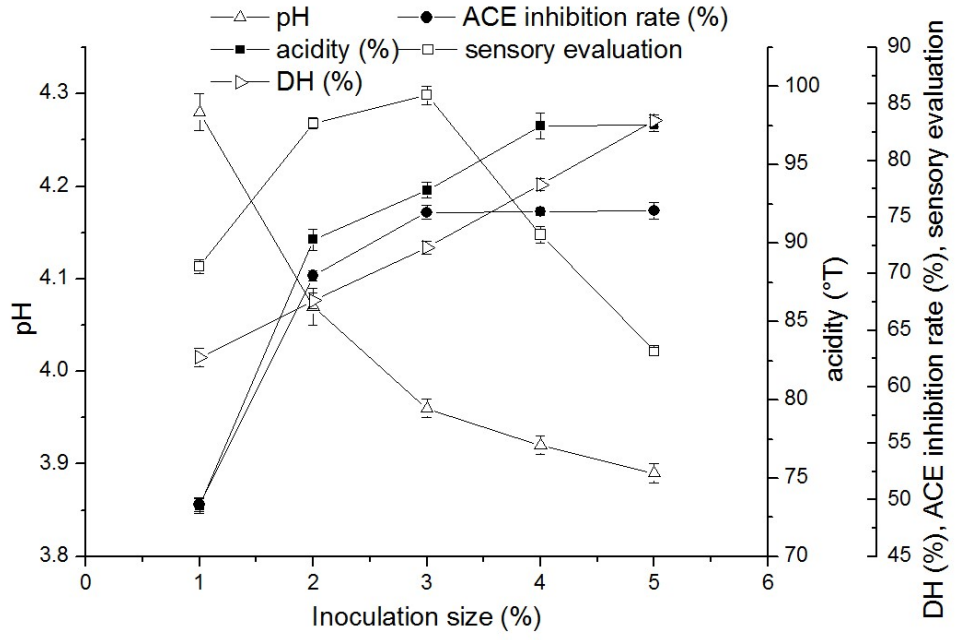

Figure 4 Effects of inoculation size on the pH, acidity, degree of hydrolysis, ACE inhibition rate and sensory evaluation of kefir. The error bars represent standard deviation of means (n = 3).

As shown in Figure 4, with the increase of inoculation size, the pH decreased continuously, while the acidity continued to increase. The lowest pH was 3.89 and the highest acidity was 97.58 °T when inoculated with 5%. As the inoculum size increased, DH changed from 62.21% to 83.54% with a linear increase. The ACE inhibition rate increased rapidly from 49.61% to

75.42% in the range of 1% to 3%. After that, the ACE inhibitory activity increased steadily and slowly. The largest ACE inhibition rate, 75.56%, was obtained when it was inoculated with 5%. The inoculation size significantly affected the sensory evaluation of kefir fermented goat milk, with the score increasing first and then decreasing. The highest value was got at 3% inoculation size.

Discussion

Kefir grain is a complex mixture of abundant lactic acid bacteria, yeasts and acetic acid bacteria (Arslan, 2015). The presence of various bacteria and yeasts in kefir leads to lactic acid and alcohol fermentation of lactose during incubation. Microbial diversity of kefir grain can be owing to distinct environments and culture incubation methods in different regions, which results to the distinctness of fermentation process and bioactivity for kefir. Besides, the protein source also has effect on quality of kefir. The value of pH reached to 4.28 and proteolytic activity was 64.4±0.8 % when made kefir using cow milk (DiNkçİ et al., 2015). Proteolytic activity exhibited obvious differences ranged from 58.01% to 68.7% when using kefir grains from different region in this study. Appreciable proteolytic activity was measured in all kefir samples which suggested proliferation of kefir grain being dependent on a proteolytic system.

It can release lots of functional peptides with bioactivity from milk source protein during fermentation. It has been reported that the biological activity of hydrolysate increased significantly as the DH increased (Lee et al., 2008). Antioxidant properties can be assessed by both DPPH free radical scavenging activity and chelating effect, which were lower than other research (Liu et al., 2005). The fact could be attributed to the origins of kefir grain and differences between measure methods. Moreover, it was found that duration of fermentation had a significant effect on the increase in antioxidant activity (Monajjemi et al., 2012). ACE inhibition rate were ranged from 58.88% to 73.83%. Thence, kefir fermented goat milk could be applied for anti-hypertension. It was reported that the value of whey kefir was run up to 73.07±0.91 % (Febrisiantosa et al., 2013). The high ACE inhibitory activity determined in kefir could be owing to the combined action of several strains of lactic acid bacteria and yeast during goat milk incubation (Quirós et al., 2005).

Studies have shown that fermentation conditions have a significant impact on the quality of kefir. A research by Schoevers et al. (2003) showed that the best proliferation for kefir grains was obtained at 25 °C, which was consistent with the results of this paper. In fact, the effect of temperature on the quality of kefir can be attributed to the influence of temperature on the growth of bacteria and yeasts in kefir grains. In other words, microbial proliferation required a suitable temperature. Inoculation size and fermentation time are also viewed as the effect factors. The results of the distinguishing studies may show some differences due to the diversity of kefir grains. Köktaş et al. (2003) found that optimal inoculation rates for kefir grains were 2%.

Kefir has become an important functional dairy food since health benefits were possessed. Kefir grains have extensive uses in food products worldwide, such as whey-based kefir beverages (Magalhães et al., 2011), white pickled cheese (Goncu et al., 2005), sourdough bread (Plessas et al., 2011), beer (Rodrigues et al., 2016). Screening of kefir grains from different regions can provide a reference for the development of kefir goat milk functional products.

CONCLUSIONS

pH value, acidity, degree of protein hydrolysis, DPPH free radical scavenging activity, chelating effect, ACE inhibitory activity, sensory evaluation of fermented milk were measured using five different kinds of kefir grains to ferment goat milk under the same conditions. The results indicated kefir grain K1 was more suitable for goat milk fermentation. Fermentation conditions were optimized by single factor experiments. The optimum fermentation conditions for kefir grain K1 were found to be as 3% inoculation size at 25 °C for 22h, which provided a reference for the subsequent production of kefir fermented goat milk.

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