Study of the Microbial and Chemical Properties of Goat Milk Kefir Produced by Inoculation with Taiwanese Kefir Grains

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ABSTRACT : One of the prerequisites for the successful implementation of industrial-scale goat kefir production is to understand the effects of different kefir grains and culture conditions on the microbial and chemical properties of the goat kefir. Thus, the objectives of the present study were to evaluate the characteristics of kefir grains in Taiwan on the microbial and chemical properties of goat milk kefir, as well as to understand the influence of culture conditions on production of medium chain-length triglycerides (MCT). Kefir grains were collected from households in northern Taiwan. Heat-treated goat milk was inoculated with 3-5% (V/W) kefir grains incubated at 15, 17.5, 20 or 22.5°C for 20 h, and the microflora count, ethanol content, and caproic (C6), caprylic (C8), and capric acid (C10) levels measured at 4 h intervals. Our results indicate that incubation with kefir grains results in 10^6-10^7 CFU/ml microflora count and 1.18 g/L of ethanol content at 20 h of fermentation. Incubation with 5% kefir grain at 20-22.5°C produces the highest MCT levels. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 711-715)***

Key Words : Kefir, Goat Milk, Medium Chain-length Triglycerides

INTRODUCTION

The word kefir is derived from the Turkish 'kef', which means pleasant taste (Kurman et al., 1992). Kefir is a cultured milk beverage resulting from microbial action of the wide community of microorganisms present in kefir grains on milk. These microorganisms are lodged in the grains in a polysaccharide kefiran matrix. The resulting beverage has a uniform creamy consistency, a slightly acidic taste caused mostly by the presence of lactic acid, some effervescence due to carbon dioxide and a minute (<2%) concentration of alcohol resulting from the action of the yeast cells also present in the grains. Kefir also contains a variety of aromatic substances that give it its characteristic flavor. Microorganisms present in the grains are called probiotics because they are beneficial to human health (Chen et al., 2003; Ham et al., 2003; Chen et al., 2004). These consist of lactobacilli, such as *L. brevis, L. cellobiosus, L. acidophilus, L. casei,* lactococci (including different subspecies of *Lc. Lactis*), *Streptococcus salivarius* ssp. *thermophilus*, *Leuconostoc mesenteroides* and *L. cremoris* and a variety of yeasts (fungi), such as *Kluyveromyces, Candida, Torulopsis,* and *Saccharomyces sp.*

The use of starter cultures facilitates kefir manufacture and allows product control and standardization. Kefir is a self-carbonated beverage that owes its distinctive flavor to a mixture of lactic acid, ethanol, carbon dioxide and other flavor products, such as acetaldehyde and acetone. This unique flavor is the result of the symbiotic metabolic activities of a number of bacterial and yeast species, which include both proteolytic and lipolytic degradation of milk constituents (Vedamuthu, 1977; Choi et al., 2003).

Goat milk contains around 4.5% lipids, including high levels of medium chain-length triglycerides (MCT) and short-chain fatty acids, which aid digestibility and metabolic utilization. Capric and caprylic acids and other MCT have become established treatments (Babayan, 1981) in a variety of malabsorption syndromes associated with chyluria, steatorrhea, hyperlipoproteinemia and also in cases of intestinal resection, coronary bypass, premature-infant feeding, childhood epilepsy, cystic fibrosis and gallstones, reportedly lowering serum cholesterol, inhibiting and limiting cholesterol deposition in tissues and dissolving cholesterol gallstones in growing children (Kalser, 1971; Nutting et al., 1991; White et al., 1991). Goat milk fat, with its normally higher content of short-chain fatty acids and MCT, appears to have distinct benefits in human nutrition and health, which have so far been largely unexploited (Haenlein, 1992). Likewise, some MCT and short-chain free fatty acids such as the hexanoic, octanoic and nonanoic variants, contribute to the characteristic goat flavor (Rahmat and Richter, 1996). Lipolysis can adversely affect the flavor of kefir through excess production of volatile fatty acids released by the action of lipoprotein lipase (Grappin and Beuvier, 1990).

Although bovine and caprine milk are widely used for the manufacture of many different types of fermented milk products, little information is available with respect to the effect of culture conditions on the MCT level of kefir made

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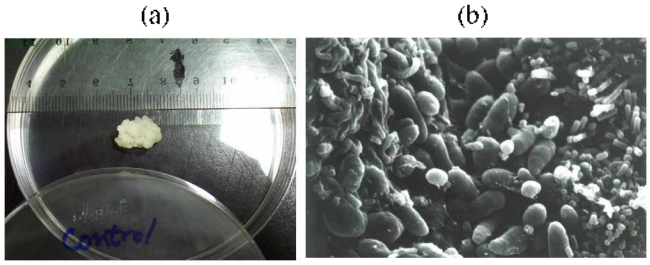

Figure 1. The appearance (a) and microphotographs (b) of kefir grains from Shinchu (×4,000).

with goat milk. Thus, the objectives of the present study were to evaluate the microbial and chemical compositions of Taiwanese sources of kefir grains in goat milk kefir, as well as the influence of culture conditions on MCT level.

MATERIALS AND METHODS

Kefir grains

Kefir grains were collected from Shinchu in northern Taiwan (Lin et al., 1999). Microflora from samples of Taiwanese kefir grain were isolated and identified in our laboratory. The lactic acid bacteria isolated from kefir grains were identified as *Lactobacillus helveticus* and *Leuconostoc mesenteroides*, and the yeasts were identified as *Kluyveromyces marxianus* and *Pichia fermentans*. In the laboratory, they were propagated at 20°C for 20 h with twice-or thrice-weekly transfers in sterilized goat milk, and kept at 4°C and -80°C for short and long-term storage, respectively.

Kefir preparation

Raw milk was obtained from the National Taiwan University Dairy Farm and heated to 90°C for 30 min in a water bath, before cooling to inoculation temperature. The heat-treated milk was inoculated with 3-5% (V/W) kefir grains and incubated at 15, 17.5, 20 or 22.5°C for 20 h, and caproic (C6), caprylic (C8), and capric acid (C10) levels were measured at intervals of 4 h. All experiments were repeated three times.

Product analysis

The lactic acid and ethanol contents of the kefir products were determined spectrophotometrically (Noll, 1974) using two enzymatic test kits from Boehlinger Mannheim (Germany).

For enumeration of the microorganisms presenting in the kefir preparations, a 1 g kefir sample was diluted with 9 ml sterilized distilled water (with 0.9% NaOH) and combined uniformly in a vortex mixer. Subsequent serial dilutions were prepared and viable numbers of the microorganisms enumerated using the pour-plate technique. Samples were plated on malt extract agar (Difco, USA) for yeasts and MRS agar (Merck, Germany) for lactic acid bacteria, with the plates incubated at 25°C and 37°C, respectively.

The caproic (C6), caprylic (C8) and capric acid (C10) contents of the kefir products were determined according to the method reported by Shantha et al. (1995). Kefir product samples were freeze-dried (Labconco Freezone 4.5 liter Benchtop, USA) and the fatty acids extracted using chloroform. After saponification and methylation, the samples were injected into a gas-liquid chromatograph (Dani, Monza, Italy; flame ionization detection) equipped with a Supelcowax-10 capillary column (60 m, 0.32 mm ID; Supelco, USA). The column temperature was programmed from 70-220°C at 10°C/min, the flame ionization detector temperature was 250°C, with nitrogen carrier gas at a flow rate of 1 ml/min. Calibration was performed up to 10 ppm for each constituent by dosed addition in sterile standard goat milk.

Scanning electron microscopy

The microstructures of the kefir grains were observed by scanning electron microscope (SEM) according to the method of Lin et al. (1999). In brief, pieces of samples were fixed in 30 g/L glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) at 25°C for 4 h. Samples were washed with 3 changes of buffer and then post-fixed with 10 g/L osmium tetroxide in the same buffer at 25°C for 1 h. After washing in distilled water, samples were dehydrated in an ethanol series: 15, 30, 50 and 70% for 10 min each; 85 and 95% for 15 min each and 100% for 1 h. The resulting specimens were critical-point dried with CO_2 using a Critical Point Dryer Samdri-PVT-3B (Tousimis, Rockville, MD, USA). The samples were fixed in stubs on a double-faced metallic tape and covered with a fine layer of gold (Ion Coater JJFC1100E; JEOL Ltd., Japan) while applying a current of 40 mA. Observations were made using an SEM (JSM-6300, JEOL Ltd., Japan).

Statistical analysis

Statistical analysis was performed using the ANOVA general linear models (GLM) procedure and Duncan's multiple range test, with significance defined at $p<0.05$ (the Statistical Analysis system Software Package version 8.1, SAS Institute, 1998). Experiments were performed three times.

RESULTS AND DISCUSSION

The microbial and chemical composition of goat milk kefir

In terms of morphology, the kefir grain cultures successively subcultured in goat milk were yellowish, irregular cauliflower-like granules with a mean diameter of 7.6 mm (Figure 1a). The microstructure revealed that kefir

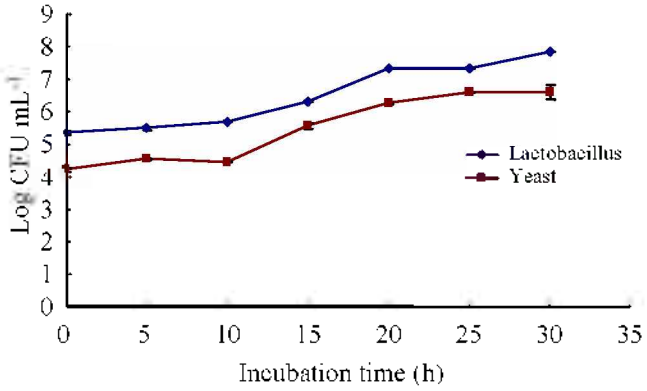

Figure 2. Microflora count during kefir incubation (n=3).

Table 1. Compositions of goat milk and goat milk kefir

Item*	Goat milk	Goat milk kefir
Crude protein (%)	3.02±0.64 [a]	2.96±0.00 [b]
Crude fat (%)	3.80±0.03 [a]	3.30±0.02 [b]
Lactose (%)	3.29±0.10 [a]	2.45±0.58 [b]
L-lactic acid (g/L)	0.61±0.09 [c]	1.52±0.76 [b]
D-lactic acid (g/L)	0.00±0.00 [c]	1.44±0.01 [a]
Ethanol (g/L)	0.00±0.00 [c]	1.18±0.01 [a]

* Each value is the mean±standard deviation of three replicate analyses.
[a-c] Values in the same row with different letters are significantly different ($p<0.05$).

grains were composed of rod-shaped bacteria and yeast (Figure 1b). The peripheral part of the granule is densely populated by a microflora composed almost exclusively of rod-shaped bacteria. Within the lactobacilli, the thermophilic homofermentative species *L. helveticus* predominated in kefir grain. Bottazzi and Bianchi (1980) indicated that both the bacteria and the yeast are arranged, with no apparent pattern, on the spongy structure of the kefir grain.

Figure 2 depicts the microflora count during kefir incubation. The study results indicate that both lactic acid bacteria and yeast counts increased with incubation time, with the *Lactobacillus* counts for goat milk higher than the yeast count. Our lab used the same kefir grains to produce cow milk kefir, and they found that the similar results. Kroger (1993) explained that when kefir grains are allowed to grow in milk, microorganisms are shed from the grains where they continue to multiply with associated production of acids, flavor components and other metabolites in the milk.

The chemical compositions of goat milk and goat milk kefir are provided in Table 1. Kroger (1993) has demonstrated that the gross composition and caloric value of kefir are identical to those of the origination milk, except that about one-quarter of the original 5% lactose is converted to lactic acid. Our chemical composition results are not in agreement, however, with lower crude protein, crude fat and lactose demonstrated for goat milk kefir

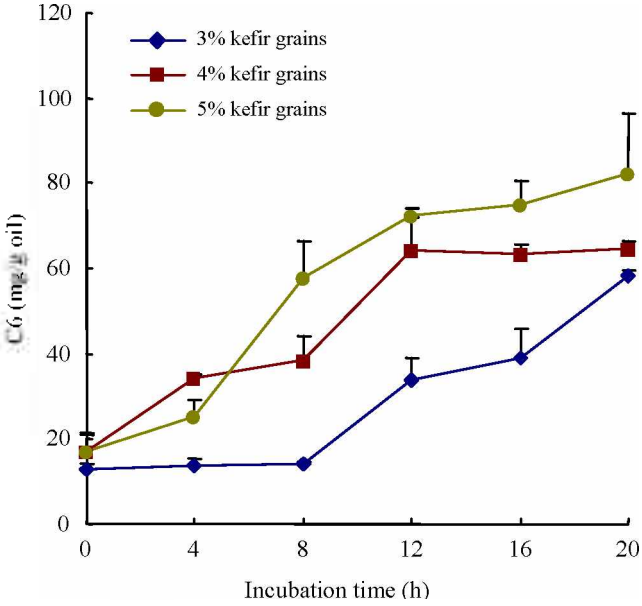

Figure 3. Effect of kefir grain concentrations on C6 content during kefir incubation (n=3).

($p<0.05$). This may be due to the release of lipase, protease and lactase from microorganisms in the kefir grains. In addition, yeast, lactose and other sugars are utilized to produce ethanol during the alcohol fermentation (Kwak et al., 1996).

Lactic acid exists as two optical isomers, D(-)-lactic acid and L(+)-lactic acid. The goat milk contained 0.61 g/L of L(+)-lactic acid, and goat milk Kefir contained 1.52 g/L of L(+)-lactic acid and 1.44 of D(-)-lactic acid (Table 1). L(+)-lactic acid, an end product of glycolysis, is usually present as a microconstituent in the blood and muscle fluids of humans and animals. Experimental findings reported by Dunlop (1967) has shown that the D-isomer is metabolized slower than L-lactate, however, both the D(-) and L(+)-isomers are absorbed at the same rate intestinally.

Goat milk kefir contained 1.18 g/L of ethanol, while no ethanol was found in goat milk samples (Table 1). Yeasts are primarily responsible for the alcohol production in kefir. The reported ethanol content of kefir varies (0.01-1.00%) depending on starter culture, fermentation time and temperature, and type of container. Our lab used the same kefir grains as used in this study to produce cow milk kefir and similar results were determined. *K. marxianus* was found in kefir grains. Participation of *K. marxianus* in the miroflora of the kefir grains ensures metabolism of lactose through alcohol fermentation and the formation of the typical yeasty flavor and aroma (Simova et al., 2002). *Pichia fermentans* is a lactose-negative yeast in the grains. This yeast assimilated DL-Lactic acid and was, therefore, completely dependent on the metabolism of lactic acid bacteria.

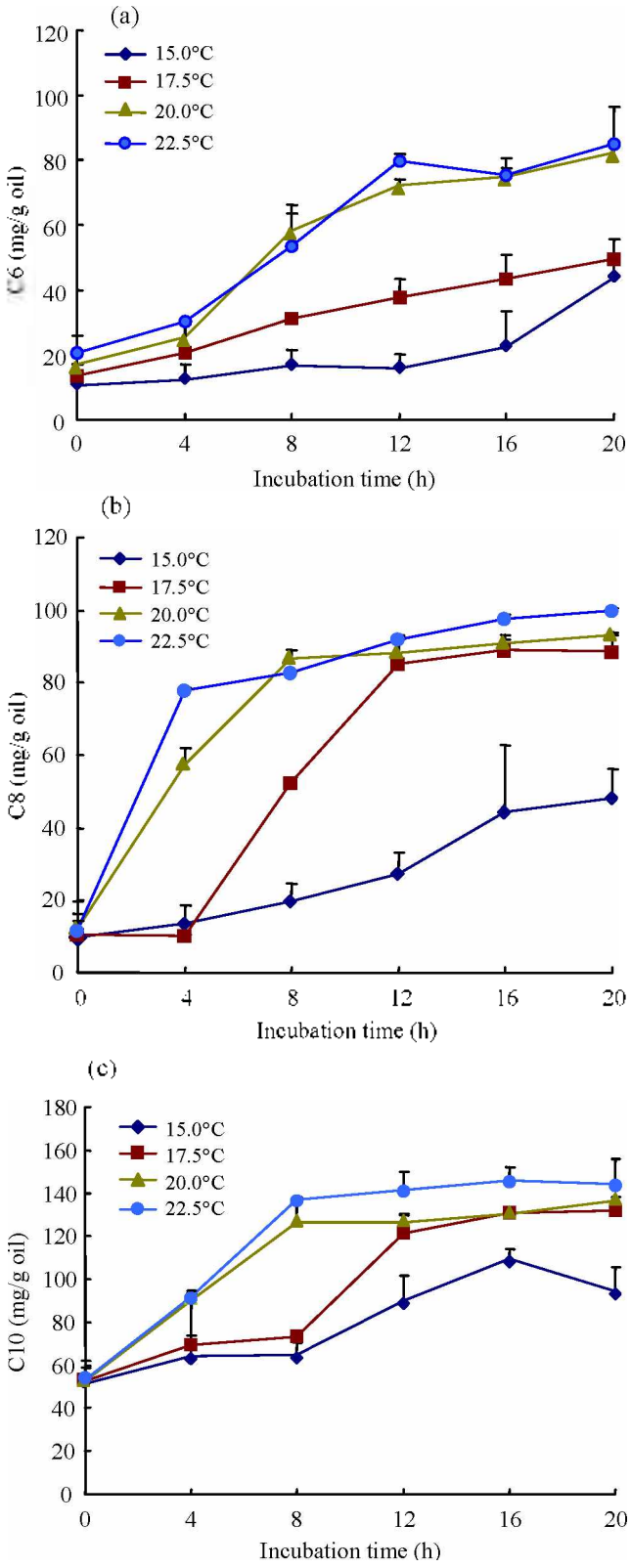

Figure 4. Effect of incubation temperatures on C6, C8 and C10 content during kefir incubation (n=3).

Influence of different culture conditions on MCT

In order to understand the effect of different culture conditions on the MCT in goat milk kefir, the heat-treated milk was inoculated with 3-5% (V/W) kefir grains incubated at 15, 17.5, 20 or 22.5°C for 20 h, and the caproic (C6), caprylic (C8) and capric acid (C10) levels were measured at 4 h intervals.

Analysis of the relationship between inoculate quantity and MCT level (Figure 3) indicates that caproic acid (C6) increases significantly with incubation time (Figure 3), with less significant increases also noted for caprylic (C8) and capric acids (C10) (data not shown). Similar increases in the levels of MCT and short-chain fatty acids have been reported for goat milk yoghurt fermentation (Hertzler and Clancy, 2003). Comparing different concentrations of kefir grains, the highest MCT concentration was obtained at 5% inoculation. This finding highlights the importance of kefir-grain concentration on lipolysis.

Figure 4 shows the effect of incubation temperature on MCT concentration in goat milk kefir. Comparing three MCT (caproic (C6), caprylic (C8) and capric acid (C10)) at the four incubation temperatures, concentrations increased sharply after 20 h of incubation, with higher temperature resulting in higher final concentrations. MCT concentration was not significantly different comparing incubation at 20 and 22.5°C ($p>0.05$). At 15°C, however, concentrations were significantly reduced ($p<0.05$). Schoevers and Britz (2003) reported that the optimal temperature for grain-mass increase in milk was 22-25°C. In this study, incubation with 5% T1 kefir grain at 20-22.5°C produced the highest MCT level. Supplementation with capric and caprylic acids and other MCT have become established treatments for patients suffering a variety of malabsorption syndromes (Babayan, 1981). Further, higher contents of short-chain fatty acids and MCT appear to have distinct and largely unexploited advantages for human nutrition and health (Haenlein, 1992).

CONCLUSION

Kefir grains were collected from a household in northern Taiwan. Heat-treated goat milk was inoculated with 3-5% (V/W) kefir grains incubated at 15, 17.5, 20 or 22.5°C for 20 h, and the microflora count, ethanol content, and caproic (C6), caprylic (C8), and capric acid (C10) levels measured at 4 h intervals. Our results indicate that incubation with kefir grains results in 10^{6}-10^{7} CFU/ml microflora count and 1.18% of ethanol content at 20 h of fermentation. Incubation with 5% kefir grain at 20-22.5°C produces the highest MCT levels.

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