

## Study on Skin Care Properties of Milk Kefir Whey

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**ABSTRACT** : The purpose of this research was to study the effects of kefir whey (kefir whey, peptides, lactic acid) on skin care properties including skin lightening effect and acne treatment. The final aim was to develop a new cosmetic product and enhance the value of dairy products. The results of skin lightening tests showed that all three kefir whey components (kefir whey, peptides and lactic acid) had inhibitory ability against melanin synthesis. Furthermore, copper chelating analysis demonstrated that both kefir whey and kefir whey peptides could chelate the copper in tyrosinase, which might explain the mechanism of inhibition. The ability for acne treatment indicated that lactic acid level higher than 60 mg/ml could inhibit the growth of *Propionibacterium acne*, whereas no inhibition was found with other components. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 6 : 905-908)

**Key Words** : Milk Kefir Whey, Tyrosinase, *Propionibacterium acne*

### INTRODUCTION

Skin properties including skin lightening effect and acne treatment were getting more attention lately. Skin color is a function of the size, number and distribution of melanins (Curto et al., 1999). Tyrosinase, a bifunctional copper protein complex, is the key enzyme of melanin biosynthesis. This enzyme catalyzes two different reactions: cresolase activity, or hydroxylation of monophenols to *o*-diphenols and catecholase activity, or oxidation of *o*-diphenols to *o*-quinone (Sánchez-Ferrer et al., 1995).

Acne is a follicular rash that starts as a comedo, then prospers inflammation which leads to the formation of red papules and pustules. Inflammatory lesions probably begin when the proliferation of *Propionibacterium acnes* attracts neutrophils to the sebaceous follicles. In most cases, the inflammation gradually fades, remain about a few days to 2 weeks. As has been widely known, predominant organism is *Propionibacterium acnes*, the overproduction of sebum, and follicular hyperkeratinization are three consequential physiological factors in the pathogenesis of acne. Topical antibiotics and erythromycin in particular are extensively used in the treatment of the inflammatory component of acne (Dreno et al., 2001). However, a number of studies in the literature (Eady et al., 1993; Nishijima et al., 1994) have reported the occurrence in acne patients, of strains of *Propionibacterium acnes* resistant to antibiotics and notably to erythromycin, with an increasing percentage prevalence.

Kefir is a cultured milk beverage produced by microbial action of a wide community of microorganisms presented in

kefir grains on milk. In the kefir grains, lactic acid bacteria and yeasts are embedded in a slimy polysaccharide matrix named kefiran, thought to be produced by the lactobacilli in the grain. It is believed to contain many functional substances and it has been postulated that the longevity of Bulgarian peasants is partially due to their frequent consumption of this fermented milk (Liu et al., 2005). In a previous study, we demonstrated that orally administered kefir not only inhibited tumor growth and induced an apoptotic form of tumor cell lysis, but it also reduced glutathione ferrous-ion chelating ability and superoxide dismutase activity. These findings have indicated that kefirs possess certain functionalities (Liu et al., 2005).

Kefir can be considered to be a carrier of probiotics and various bioactive compounds, including peptide, polysaccharide and organic acid that may play a functional role for skin care. Thus, the purpose of this research was to study the effects of different kefir whey components (kefir whey, kefir whey peptide, lactic acid) on skin care properties including skin lightening effect and acne treatment. The final aim was to develop a new cosmetic product for its possible commercialized and enhance the value of dairy products.

### 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* (Lin et al., 1999). In the laboratory, they were propagated at 20°C for 20 h with twice-or thrice-weekly transfers in sterilized

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**Table 1.** Inhibitory effect of whey and peptides on tyrosinase

Concentration	Inhibition (%)			
	Whey <sup>1</sup>		Peptides <sup>2</sup>	
	10% (v/v)	20% (v/v)	2 mg/ml	5 mg/ml
Milk	19.9±1.1 <sup>b</sup>	55.6±0.7 <sup>b</sup>	0	49.42±4.63 <sup>b</sup>
Kefir	35.2±1.1 <sup>a</sup>	91.4±2.0 <sup>a</sup>	0	63.11±2.10 <sup>a</sup>

<sup>1</sup> 10% and 20% whey to 250 µl solution (included 25 U/ml tyrosinase, 1 mM dopa and 25 or 50 µl whey).

<sup>2</sup> 2 mg/ml and 5 mg/ml peptides to 250 µl solution (included 25 U/ml tyrosinase, 1 mM dopa and 100 µl peptides).

\* Each value is the mean ± standard deviation of three replicate analyses.

<sup>a,b</sup> Values in the same column with different letters are significantly different (p<0.05).

goat milk, and kept at 4°C and -80°C for short and long-term storage, respectively (Chen et al., 2005).

### Preparation of milk kefir whey

Raw milk was obtained from the National Taiwan University Dairy Farm and heated to 80°C for 30 min in a water bath, before cooling to inoculation temperature. The heat-treated milk was inoculated with 5% (V/W) kefir grains and incubated at 20°C for 20 h. After fermentation, kefir was filtrated through three layers of cheesecloth to remove the kefir grains. Kefir whey was the supernatant of milk kefir centrifuged under 8,000×g for 30 min. All experiments were repeated three times.

### Preparation of peptides

The preparation of peptides was modified the method described by Amiot et al. (2004). Equal volume of acetone and kefir whey were mixed and refrigerated at 4°C for 2 h, and then the mixture were centrifuged at 3,000×g for 10 min at 4°C. The pellet was resuspended in sterilized water, passed through 0.45 µm filter and stored at 4°C before use.

### Tyrosinase assay

Tyrosinase activity was measured as described by Maeda and Fukuda (1996). Briefly, tyrosinase assays were performed in 96-well microtiter plates by adding 100 µl of each sample with phosphate buffer (pH 6.8) containing 1 mM L-dopa and 25 U/mL tyrosinase. The plates were incubated at 37°C for 30 min, and the absorbance was measured at 475 nm in a model 3550 microplate reader (Bio-Rad Laboratories, Richmond, CA).

### Copper chelating analysis

Copper chelating analysis was performed as described by Shimada et al. (1992). One milliliters of sample solution was added to 1 ml of 20 mM hexamine buffer containing 20 mM KCl and 3 mM CuSO<sub>4</sub>, and then 0.25 ml of 1 mM tetramethyl murexide (TMM) was added. Absorbance at 485 and 530 was measured. TMM was a chelating reagent, showing an absorption maximum at 530 nm, and formed a complex with free Cu<sup>2+</sup> except Cu<sup>2+</sup> bound by samples. The TMM-Cu<sup>2+</sup> complex showed an absorption maximum at

485 nm. The copper chelating ability was determined by the ratio of 485 nm to 530 nm. The lower the values showed the better the copper chelating ability.

### Inhibition test for *Propionibacterium acnes*

Inhibition test was modified the method described by Mitsunashi and Murata (1991). *Propionibacterium acnes* (CCRC10723) was purchased from the Culture Collection and Research Center, Hsinchu, Taiwan. Each test sample (0.1 ml) was punched (8 mm in diameter) in Reinforced Clostridial Medium (RCM, OXOID), which has previously plated 0.1 ml *Propionibacterium acnes* (10<sup>7</sup>-10<sup>8</sup> CFU/ml) culture solution. After deposition, plates were incubated respectively at 37°C for 7 days under anaerobic conditions and the inhibition zones were recorded. Zones of inhibition of minimum with 10 mm were expected (the width is the distance between the edge of the disk and the outer limit of the zone of inhibition).

### Statistical analysis

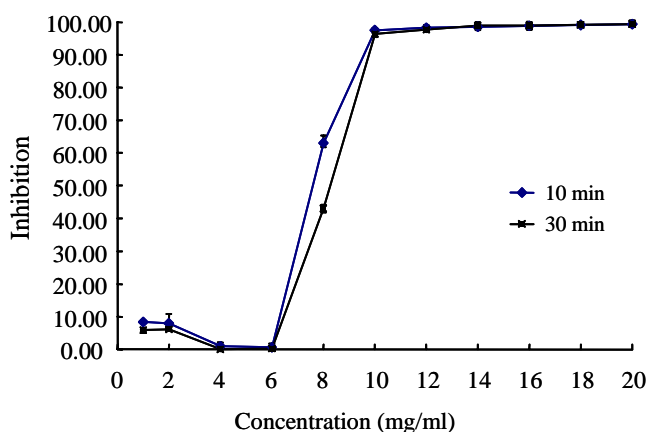
Data were analyzed using the general linear model procedure of the SAS software package (SAS/STAT, 1999), and Duncan's multiple range test (Montgomery, 1991) were used to detect differences between treatment means. Statistical significance was tested at the 5% level. All experiments were replicated three times.

## RESULTS AND DISCUSSIONS

### The effect of kefir components on the skin lightening

*Tyrosinase assay* : The skin lightening tests were performed by tyrosinase assay. The melanin synthesis is regulated by tyrosinase, which catalyzes the conversions of tyrosine to dopa and dopa to dopaquinone. Inhibition of tyrosinase activity reduced the melanin production.

Table 1 shows that inhibitory effect of kefir whey on tyrosinase. Results indicated that kefir whey had better inhibitory ability against melanin synthesis than milk whey with significantly different (p<0.05). Furthermore, the higher concentration of kefir whey showed the better inhibitory ability. The 50% inhibitory (IC<sub>50</sub>) value for kefir whey was 15 mg/ml. The chemical compositions of kefir



**Figure 1.** Inhibitory effect of lactic acid on tyrosinase, added sample to 250  $\mu$ l solution (included 25 U/ml tyrosinase, 1 mM dopa, and 100  $\mu$ l sample).

whey contain water, protein, peptides, lactic acid and minerals. Since the lactic acid and peptides were the major components of kefir whey, it was necessary to analyze the inhibitory ability for both components.

Peptides showed a concentration-dependent reduction in tyrosinase activity (Table 1). There was no inhibition against melanin synthesis at 2 mg/ml and the 50% inhibitory ( $IC_{50}$ ) value was 4.23 mg/ml. More recent investigations (Lintner and Peschard, 2000) have shown that proper modification of peptide sequences with potential cosmetic activity have commercial potential. Certain peptides (Park et al., 1998) including tyrosine peptides could inhibit the tyrosinase and provide the decomposition of existing pigmentation disturbances and to visibly lighten the skin. Those peptides behave as competitive inhibition, inhibiting the oxidation of L-DOPA by tyrosinase.

Lactic acid also shows a concentration-dependent reduction in tyrosinase activity (Figure 1). The 50% inhibitory ( $IC_{50}$ ) value was 8 mg/ml. Lactic acid especially the natural L(+) form has been widely investigated (Usuki et al., 2003). In theory, three different mechanisms are involved in the operation of lightening ingredients: 1. direct reduction of melanin, 2. inhibition of tyrosinase activity, and 3. suppression of the formation of tyrosinase. The lightening action of lactates is based on the suppression of the formation of tyrosinase (action 3). This mechanism is

completely different from the lightening action of conventional ingredients such as Vitamin C, Arbutin and Kojic acid (action 2) and Hydroquinon (action 1). Usuki et al. (2003) reported that lactic was shown to inhibit tyrosinase enzyme activity directly, but this effect was not due to the acidity of GA or LA, because adjusting the pH to 5.6 did not affect tyrosinase activity. Lactic acid might work on pigmentary lesions not only by accelerating the turnover of the epidermis but also by directly inhibiting melanin formation in melanocytes.

**Copper chelating analysis :** Since tyrosinase is a bifunctional copper protein complex, inhibition of tyrosinase activity can be determined by the ability to chelate copper in the enzyme (Kubo and Kinst-Hori, 1999).

Results in Table 2 demonstrate that both kefir whey and peptides could chelate copper in tyrosinase. Higher concentration of both components showed an increasing ability of chelation. The results, consisting with the tyrosinase assay, could explain the mechanism of inhibition against melanin synthesis. In addition, copper chelating results also provided the antioxidative activities of kefir components. A vast amount of evidence has implicated oxygen-derived free radicals as important causative agents of aging. Liu et al. (2005) studied the antioxidative activities of kefir and concluded that kefirs possessed antioxidant activity.

#### Acne treatment

The acne treatment was performed by inhibition of *Propionibacterium acne*, which commonly isolated from pustular acne lesions. The results indicated that lactic acid level higher than 60 mg/ml could inhibit the growth of *Propionibacterium acne*, while no inhibition was found for other components. Higaki (2003) reported that the plenty of free fatty acids detected in acne lesions forms as a result by the effect of *Propionibacterium acne* lipase on sebaceous triglycerides. Free fatty acids stimulate the follicular epithelium sufficiently to result in its breakage, which then enable those acid to get through the dermis and to induce inflammation. *Propionibacterium acne* lipase is stable and active at the pH between 5-8. If pH lowers than 5, the activity of *Propionibacterium acne* lipase is feebler. Wang et al. (1997) reported that low concentration of alpha

**Table 2.** The chelating effect of kefir whey and peptides on  $Cu^{2+}$

Concentration	485 nm/530 nm			
	Kefir whey <sup>1</sup>		Peptides <sup>2</sup>	
	10% (v/v)	20% (v/v)	2 mg/ml	5 mg/ml
Milk	2.44±0.11 <sup>a</sup>	2.14±0.12 <sup>a</sup>	2.14±0.12 <sup>a</sup>	1.89±0.13 <sup>a</sup>
Kefir	1.85±0.12 <sup>b</sup>	1.73±0.14 <sup>b</sup>	1.64±0.11 <sup>b</sup>	1.33±0.11 <sup>b</sup>

<sup>1</sup> 10% and 20% whey to 250  $\mu$ l solution (included 25 U/ml tyrosinase, 1 mM dopa and 25 or 50  $\mu$ l whey).

<sup>2</sup> 2 mg/ml and 5 mg/ml peptides to 250  $\mu$ l solution (included 25 U/ml tyrosinase, 1 mM dopa and 100  $\mu$ l peptides).

\* Each value is the mean  $\pm$  standard deviation of three replicate analyses.

<sup>a, b</sup> Values in the same column with different letters are significantly different ( $p < 0.05$ ).

hydroxyl acids (AHAs) reduced the thickness of stratum conreum by diminishing corneocyte cohesion. Lactic acid, one of AHAs, could lower the pH and inhibit the growth of *Propionibacterium acne*.

### CONCLUSIONS

This present study demonstrated that kefir whey, kefir whey peptides and lactic acid had skin lightening ability, while only lactic acid inhibited the growth of *Propionibacterium acne*. The inhibition of tyrosinase activity was due to the chelation of copper in tyrosinase. Although the present study proved that certain kefir components had skin care properties, further studies are necessary for developing a new commercialized cosmetic product.

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