Antioxidative Activities of Kefir

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ABSTRACT : This study aims to evaluate the antioxidative activities of cow-milk kefir and goat-milk kefir. Antioxidative mechanisms, including radical-scavenging effects, ferrous-ion chelating ability, reducing power and antioxidant activity, were investigated herein. Kefirs demonstrated significantly greater scavenging effects upon 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals, an inhibition effect upon linoleic-acid peroxidation, and more substantial reducing power, but reduced glutathione peroxidase (GSH-Px) activity than was the case for milks. There was no significant difference between milks and kefirs as regards ferrous-ion chelating ability and superoxide dismutase (SOD) activity. These findings have demonstrated that kefirs possess antioxidant activity, thereby suggesting that kefirs are potential candidates for the role of useful natural antioxidant supplements for the human diet.
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Key Words : Kefir, Antioxidative Activity, Free Radical

INTRODUCTION

As a consequence of aerobic metabolism, small amounts of reactive oxygen species, including superoxide radicals, hydroxyl radicals, hydrogen peroxide and peroxide radicals and its related radicals, are constantly generated within certain cells of certain organisms. The accumulation of peroxidants in the human body has been reported to be associated with disorders such as cancer, atherosclerosis, hypertension, and arthritis (Halliwell and Gutteridge, 1984; Frenkel, 1992, Ham et al., 2003). To avoid cellular damage by these peroxidants, most biological systems have developed inherent antioxidant systems, for example, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and uric acid, in order to protect from damage caused by the peroxidants. Such systems, however, are not totally effective and do not prevent damage universally (Simic, 1988), hence, there is an increasing interest world-wide in finding natural food-based antioxidants that are able to protect the human body from attack by free radicals and thus retard the progress of many chronic diseases, as well as retarding the lipid oxidative rancidity in foods (Pryor, 1991).

Fermented milk has often been studied for its possible beneficial physiological effects, such as its antimutagenicity, immune-potentiating activity, antitumor activity, and its contribution to the prevention of pathogenic infection (Gilliland, 1990; Sanders, 1993). Kefir is a fermented milk product that is popular in many countries. It is believed to contain many functional substances and it has been postulated that the longevity of Bulgarian peasants may be partially due to their frequent consumption of this fermented milk. Kefir differs from other fermented dairy products in that it is not produced as a result of the metabolic activities of an evenly distributed microflora, but is the product of fermentation by a mixed group of microflora confined to a matrix of discrete "kefir grains" (Marshall and Cole, 1985). In the kefir grains, lactic acid bacteria and yeasts are embedded in a slimy polysaccharide matrix named kefiran, which is thought to be produced by the *lactobacilli* in the grain (La Riviere et al., 1967; Marshall et al., 1984). The microflora of kefir grains include *Lactobacillus brevis*, *L. helveticus*, *L. kefir*, *Leuconostoc mesenteroides*, *Kluyveromyces lactis*, *K. marxianus*, and *Pichia fermentans* (Angulo et al., 1993; Lin et al., 1999). The function of the microorganisms making up the flora of the kefir grains may include the production of lactic acid, natural antibiotics and bactericides, all of which are able, to varying degrees, to inhibit the growth of undesirable and pathogenic microorganisms in kefir milk (Angulo et al., 1993).

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 increased the total IgA levels in tissue extracts from the wall of the small intestine of mice (Liu et al., 2002). To the best of our knowledge, however, a specific antioxidative effect of kefir has not yet been reported. The objectives of this study were to investigate the antioxidant properties of kefir, including its antioxidant activity, its reducing power, its scavenging effect upon radicals, and its chelating effect upon ferrous ions. The activity of certain antioxidative enzymes, such as catalase, GSH-Px and SOD present in kefir were also investigated.

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MATERIALS AND METHODS

Kefir grains

Kefir grains were collected from various households in northern Taiwan. In the laboratory, these were propagated at 20°C for 20 h with twice- or thrice-weekly transfers in sterilized cow-milk or goat-milk (50 g/l), and kept at either 4°C or -80°C for short or long-term storage, respectively.

Kefir manufacture

Sterilized cow-milk and goat-milk were both obtained from the National Taiwan University Dairy Farm and inoculated with 10% kefir grains. All inoculated cow-milk and goat-milk samples were incubated at 20°C until a pH of 4.6 had been attained. At the end of the fermentation process, the cow-milk kefir and goat-milk kefir were filtered through three layers of cheesecloth in order to remove the kefir grains. The unfermented cow-milk and goat-milk were treated with 6% lactic acid to pH 4.6, following which the acidified-milk and the filtered-kefir samples were separately centrifuged at 4°C and 17,000×g for 45 min in order to remove precipitated proteins. The resulting supernatant were then freeze-dried and stored at -80°C prior to their analysis.

Scavenging effect upon DPPH radicals

The effect of cow-milk kefir and goat-milk kefir upon DPPH radicals was measured according to the method of Shimada et al. (1992). Various concentrations of milk or kefir samples (0.8 ml, 0-4 mg/ml) were separately mixed with 0.2 ml of a methanolic solution containing DPPH radicals to give a final concentration of the DPPH radicals of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the mixture's absorbance was than measured at 517 nm. The capability to scavenge DPPH radicals was calculated as (%)=[1-(absorbance of sample at 517 nm)/(absorbance of control at 517 nm)]×100.

Chelating effects upon ferrous ions

The ferrous ion chelating ability was determined according to the method of Decker and Welch (1990). Various concentrations of milk or kefir samples (5 ml, 0-4 mg/ml) were separately mixed with 0.1 ml of a 2 mM solution of $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The mixture was then shaken and left to stand for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. The capability to chelate ferrous ions was calculated as (%)=[1-(absorbance of sample at 562 nm)/(absorbance of control at 562 nm)]×100. A greater percentage value for the equation suggested a more pronounced chelating capability.

Scavenging effect upon superoxide radicals

The effect of cow-milk kefir and goat-milk kefir upon superoxide radicals was determined by means of the PMS-NADH superoxide generating system (Robak and Gryglewski, 1988). Various concentrations of milk or kefir samples (0.5 ml, 0-4 mg/ml) were separately mixed with 0.5 ml of NBT (300 μM), 0.5 ml of NADH (936 μM), and 0.5 ml of PMS (120 μM) in 0.1 M phosphate buffer (pH 7.4). Subsequent to incubation for a period of 5 min at room temperature, the absorbance of the solution was measured at 560 nm. The capability to scavenge the superoxide radical was calculated as (%)=[1-(absorbance of sample at 560 nm)/(absorbance of control at 560 nm)]×100.

Inhibition of lipid peroxidation

The inhibition of lipid peroxidation of cow-milk kefir and goat-milk kefir was determined by the linoleic acid system (Yen et al., 2000). The linoleic acid emulsion was made up by mixing equal volumes of linoleic acid, Tween 20 and 0.02 M phosphate buffer (pH 7.0). Milk or kefir samples (0.5 ml, 2 mg/ml) were mixed with 2.5 ml of a linoleic acid emulsion (0.002 M) and 2 ml of a phosphate buffer (0.2 M; pH 7.0). The reaction mixture was incubated at 50°C for 10 min in the dark, and the degree of oxidation was measured according to the thiocyanate method, by sequentially adding 4.7 ml of ethanol (75%), 0.1 ml of ammonium thiocyanate (30%), 0.1 ml of sample solution, and 0.1 ml of ferrous chloride (20 mM) in 3.5% HCl. Follow this, the mixture was stirred for 3 min, subsequent to which the peroxide value was determined by reading the solution's absorbance at 500 nm. The inhibition of linoleic acid peroxidation was calculated as (%)=[1-(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)]×100. A greater value for this percentage indicates a greater level of antioxidant activity.

Reducing power

The reducing power of cow-milk kefir and goat-milk kefir was determined according to the method of Oyaziu (1986). Milk or kefir samples (2.5 ml, 2 mg/ml) were mixed with an equal volume of sodium phosphate buffer (200 mM; pH 6.6) and 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 min. Following this, an equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged at 1,400×g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of a 0.1% ferric chloride solution, and the absorbance of the solution was measured at 700 nm. A greater level of absorbance reflected a greater reducing potential of the solution.

SOD activity

The SOD activity was determined according to the method of Granelli et al. (1995). Milk or kefir samples (0.1

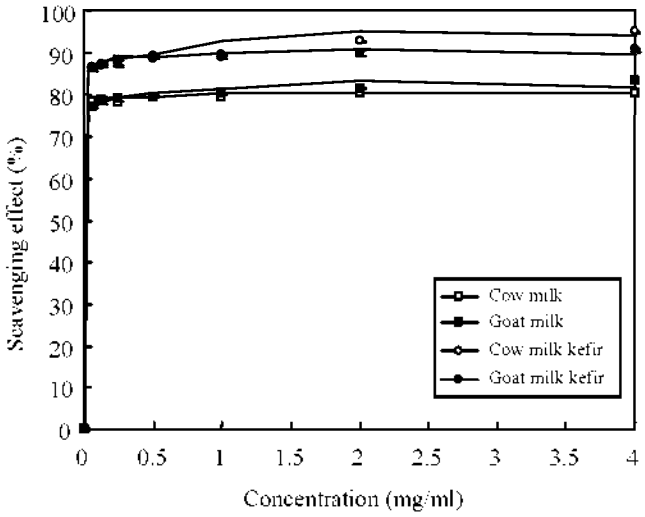

Figure 1. Scavenging effects of milks and kefirs upon DPPH radicals. Values shown are means±standard deviation from triplicate experiments.

ml, 1 mg/ml) were mixed with 2.13 ml of reaction solution containing cytochrome-c (24 μM), xanthine (100 μM), diethylentriaminpentaacetic acid (DTPA; 100 μM), and xanthine oxidase (11.2 mU/ml) in tris-HCl buffer (50 mM; pH 8.5). The absorbance of the test solution was measured at 340 nm. One unit of SOD activity was defined as the quantity of SOD required to elicit a 20% inhibition of the cytochrome-c reduction.

GSH-Px activity

The GSH-PX activity was measured using a coupled enzymatic assay as described by Lindmark-Mansson et al. (2001). Milk or kefir samples (0.2 ml, 1 mg/ml) were mixed with 0.8 ml of reaction solution containing reduced glutathione (GSH; 0.63 mM), *tert*-butylhydroperoxide (BHPx; 0.1 mM), glutathione reductase (5 μg/ml), and NADPH (0.25 mM) in phosphate buffer (50 mM; pH 7.6). Subsequent to incubation for a period of 5 min at 37°C, the absorbance of the test solution was measured at 340 nm. One unit of GSH-Px activity was defined as 1 nmole NADPH oxidized per min.

Statistical analysis

The results were analyzed using the general linear-model procedure available from the Statistical Analysis System software package version 8.1 (Statistical Analysis System Institute, 1998). Duncan's multiple range test (Montgomery, 1999) was used to detect differences between treatment means. Each experiment was conducted in triplicate.

RESULTS AND DISCUSSION

Scavenging effect upon DPPH radicals

Proton-radical scavenging is recognized as being an important mechanism for antioxidation. DPPH is a compound that possesses a proton free radical and this feature of DPPH was used to determine its proton-radical scavenging action. DPPH exhibits a characteristic absorption at 517 nm and its purple color fades when it encounters proton radical scavengers (Yamaguchi, 1998). Figure 1 reveals the dose-response curve for the radical-scavenging activity of milks and kefirs. At a dosage of 4.0 mg/ml, kefirs showed a significantly greater level of scavenging activity of DPPH radicals than did milks ($p<0.05$). It has been reported previously that skim milk possessed the radical-scavenging activity for DPPH, and heat treatment of the milk enhanced this activity. It has also been found that skim milk fermented by *L. casei* raised the DPPH radical-scavenging activity, suggesting that the casein-derived peptides may be one of the factors enhancing radical-scavenging activity (Nishino et al., 2000). In addition, Lin and Chang (2000) found that both intact cells and intracellular cell-free extracts of *Bifidobacterium longum* and *L. acidophilus* demonstrated the ability to scavenge DPPH free radicals. In this study, we found that milks fermented by kefir grains demonstrated an enhanced activity as regards scavenging the DPPH radical as compared to unfermented milks. Such a result implies that kefirs demonstrate a more substantial proton-donating ability than do milks and, thus, kefirs can afford protection against proton free radicals.

Chelating effects upon ferrous ions

Several injurious conditions may have a component that is caused by the influence of transited metal-catalyzed radical formation. It has been proposed previously that metal ions may be implicated in human cardiovascular disease by the contribution the ions make to the promotion of free radical production. Cancer and arthritis may also correlate with the catalysis of metal ions *in vivo* (Halliwell, 1984). Transition metal ions are capable of generating free radicals from peroxides by the Fenton reaction and can thus initiate lipid peroxidation and start a chain reaction by means of the decomposition of hydroperoxides to peroxyl radicals (Halliewll and Gutteridgu, 1990). Minimizing the metal ions concentration affords protection against oxidative damage by free radicals by limiting the Fenton reaction. Figure 2 indicates the chelating effect of milks and kefirs upon ferrous ions. No significant difference between milks and kefirs as regards chelating effect upon ferrous ions at a level of 4.0 mg/ml was noted in this study. Of the total milk components, lactoferrin and serum albumin have been reported to demonstrate a great degree of iron chelating activity (Gutteridge et al., 1981; Meucci et al., 1991), and the phosphoseryl residues located on the surface of casein micelle sequestering of iron and copper ions may also contribute to the antioxidant activity of milk (Allen and

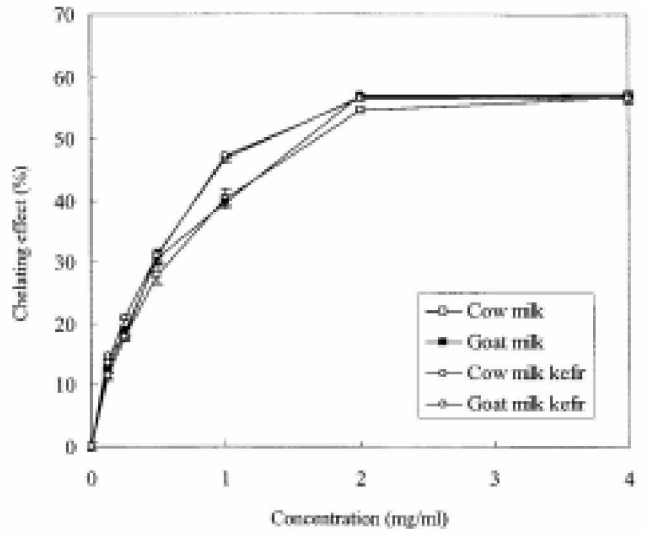

Figure 2. Chelating effect of milks and kefirs upon ferrous ions. Values shown are means±standard deviation from triplicate experiments.

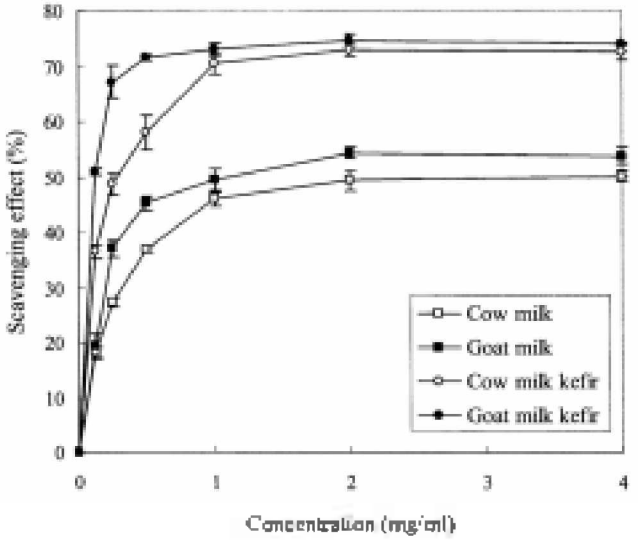

Figure 3. Scavenging effects of milks and kefirs upon superoxide radicals. Values shown are means±standard deviation from triplicate experiments.

Wrieden, 1982). In general, those milk fractions containing a greater level of phosphoseryl serine groups demonstrate a greater affinity for iron-ions, although the carboxyl group of the amino acids asparagine and glutamine are also able to bind iron-ions as well (Wong and Kitts, 2003). To the best of our knowledge, only a few previous studies have focused on the effect of milk fermentation upon the iron-ion chelating activity of milk. Lin and Yen (1999) demonstrated that the intracellular cell-free extract of several strains of lactic acid bacteria possessed iron-ion chelating ability, although we did note that milks fermented by kefir grains did not significantly affect their ferrous ion chelating ability.

Scavenging effect upon superoxide radicals

Superoxide, the single-electron reduced form of molecular oxygen, is the primary free radical in most biological systems. Although superoxides are rather unreactive compounds when compared to other free radicals, they are effective oxidants for ferrous ions, especially when involved in supporting ferrous ions catalyzed lipid peroxidation. The biological system can convert superoxide to certain reactive species including hydroxyl, peroxyl and alkoxyl radicals all of which demonstrate the potential to react with biological macromolecules and thereby induce tissue damage (Halliwell, 1994). The effect of different concentrations of milks and kefirs upon the scavenging superoxide radical is shown in Figure 3. At a dose level of 4.0 mg/ml, the scavenging effect of kefirs upon the superoxide was significantly higher than was the corresponding effect of milks ($p<0.05$). These results suggest that kefirs are good scavengers for superoxide radicals. Ye and Ng (2000) reported that lactoperoxidase, α-lactalbumin, β-lactoglobulin, and casein were highly potent as regards inhibiting superoxide formation, these authors also reporting a novel glycoprotein designed glycolactin, which inhibited superoxide radical generation *in vitro*. The scavenging effect of kefirs on the superoxide radical in this study appeared to be significantly greater than the corresponding scavenging effect of milks, although there appeared to be no significant difference between kefirs and milks ad regards SOD activity. Thus, it would appear likely that the greater ability of kefirs as regards their superoxide radical scavenging ability compared to milks must be contributed to by other antioxidative compounds other than SOD. In a previous study, we found that *L. helveticus* isolated from kefir grains possessed highly proteolytic activity (Lin et al., 1999). Whether milk proteins digested by proteolytic enzymes derived from *Lactobacillus* increased milk's free radical scavenging ability yet remains to be elucidated.

Inhibition of lipid peroxidation

Virtually all cellular components appear to be sensitive to oxidative damage. Lipid, proteins, nucleic acids, and carbohydrates are all known to undergo oxidative modification (Pacifici and Davies, 1991). Lipids peroxidation was the first type of oxidative damage to be studied in detail (Halliwell, 1994). Membrane phospholipids are continually subjected to oxidant challenges. The process of lipid peroxidation is typically initiated by an attack on a fatty acid or fatty acyl side chain by any chemical species that has sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain. The greater the number of double bonds in a fatty acid side chain, the easier is the removal of a hydrogen atom, this being the reason that polyunsaturated fatty acid

Table 1. Inhibition of linoleic acid peroxidation by cow-milk, goat-milk, cow-milk kefir and goat-milk kefir*

Sample	Absorbance at 500 nm	Inhibition ability (%)
Cow-milk	1.075±0.003	51.37±0.11[d]
Goat-milk	0.955±0.024	56.80±1.06[c]
Cow-milk kefir	0.253±0.010	88.57±0.45[a]
Goat-milk kefir	0.531±0.017	75.96±0.78[b]

* The concentration was 2 mg/ml.
[a-d] Means in a column with different superscripts are significantly different ($p<0.05$).

Table 2. Reducing power of cow-milk, goat-milk, cow-milk kefir and goat-milk kefir

Sample	Absorbance at 700 nm
Cow-milk	0.690±0.002[c]
Goat-milk	0.709±0.002[b]
Cow-milk kefir	0.760±0.001[a]
Goat-milk kefir	0.763±0.015[a]

* The concentration was 2 mg/ml.
[a-c] Means in a column with different superscripts are significantly different ($p<0.05$).

are particularly susceptible to peroxidation. Under such circumstances, the resulting lipid radicals undergo molecular rearrangement, followed by reaction with oxygen to give peroxyl radicals, which are capable of abstracting hydrogen from adjacent fatty acid side chains and so propagating a chain reaction of lipid peroxidation (Halliwell, 1984). Therefore, the inhibition of lipid peroxidation is of great important to the prevention of disease processes involving free radicals. As shown in Table 1, kefirs demonstrated a significantly greater inhibitory effect upon linoleic acid peroxidation than did milks ($p<0.05$). The inhibition rate of cow-milk kefir and goat-milk kefir upon linoleic acid peroxidation was 88.6% and 76.0% respectively for this study, suggesting that kefirs demonstrated a high antioxidative activity by inhibiting lipid peroxidation. The relative dimension of the antioxidant potential of proteins derived from dairy products is reasonably well known (Wong and Kitts, 2003), and recent studies have shown that peptides derived from milk proteins possess antioxidant activity directed toward the peroxidation of lipids or fatty acid (Suetsuna et al., 2000; Tong et al., 2000; Pena-Ramos and Xiong, 2001). The mechanism of the inhibition of lipid oxidation by such milk-derived peptides, however, is not entirely clear. Pena-Ramos and Xiong (2001) suggested that such activity was attributable to the chelation of pro-oxidative metal ions and termination of certain radical chain reactions by the presence of antioxidative peptides either through specific amino acid residue side-chain groups or through the specific peptide structure. Lin and Yen (1999) found that intact cells or an intracellular cell-free extract of some intestinal lactic acid bacteria that were known to inhibit linoleic acid oxidation, revealed a high level of antioxidative ability. Ahotupa et al. (1996) suggested that *Lactobacillus rhamnosus GG* inhibited lipid peroxidation *in vitro* due to iron-ions chelation and the superoxide anion scavenging ability. The specific mechanism underpinning the enhanced ability of kefirs to inhibit lipid peroxidation as compared to milks remains unclear at present, but may be attributable to the contribution to the cell components of microorganisms in kefir grains or by the milk-derived peptides produced during kefir fermentation.

Reducing power

Some reports in the literature have reported that reducing power may be associated with antioxidant activity (Yen et al., 2000), thus, it would appear necessary to determine the reducing power of kefirs in order to elucidate the relationship between their antioxidant effect and their reducing power. In the assay to determine kefir's reducing power, the presence of reductants in the sample would likelyresult in the reduction of the ferric/ferricyanide complex to the ferrous form. The ferrous ions concentration can therefore be monitored by measuring the level of formation of Perl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power (Oyaizu, 1986). The relative reducing power of milks and kefirs are shown in Table 2, the reducing power of kefirs at 0.5 mg/ml appearing to be significantly higher than that for milks ($p<0.05$). It has been reported previously that milk contains several antioxidants, such as lactoferrin, ascorbic acid, α-tocopherol, and carotenoids (Lindmark-Mansson and Akesson, 2000). Some milk proteins and peptides may also demonstrate antioxidative activity (Ye and Ng, 2000; Pena-Ramos and Xiong, 2001). Wong and Kitts (2003) have suggested that the reducing activity observed with buttermilk solid was attributable, in part, to the total sulfhydryl content of the compound, and to the proteins or peptides containing free hydroxyl groups which could have also contributed to buttermilk's reducing activity. Lin and Yen (1999) have also reported that several strains of lactic acid bacteria demonstrate a relatively pronounced reducing activity, these authors suggesting that such reducing activity probably derived from the actions of intracellular antioxidants and proteins.

Antioxidative enzymes activity

Antioxidative enzymes can scavenge or prevent the formation of radicals, or catalyse the synthesis or regeneration of non-enzymatic antioxidants. Among antioxidative enzymes, the presence of catalase, GSH-Px, and SOD have been demonstrated in milk (Lindmark-Mansson and Akesson, 2000). Catalase is one of the most heat-labile enzymes occurring in milk, with most of its activity being destroyed by heat treatment. In this study, the catalase activity of milks or kefirs was undetectable (results

Table 3. The specific activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) form cow-milk, goat-milk, cow-milk kefir, and goat-milk kefir*

Sample	Specific activities (U/ml)	
	SOD	GSH-Px
Cow-milk	0.511±0.016[a]	1.016±0.017[a]
Goat-milk	0.515±0.011[a]	1.045±0.019[a]
Cow-milk kefir	0.533±0.016[a]	0.603±0.016[c]
Goat-milk kefir	0.532±0.023[a]	0.804±0.014[b]

* The concentration was 2 mg/ml.
[a-c] Means in a column with different superscripts are significantly different ($p<0.05$).

not shown). The GSH-Px and SOD activities of milks and kefirs are shown in Table 3, it appearing here that no significant difference between the SOD activity of milks and kefirs existed. Nevertheless, the GSH-Px activity of kefirs was noted to be significantly lower than that of milks ($p<0.05$).

SOD is the enzyme responsible for the degradation of toxic superoxides present in organisms. The concentration of SOD in milk varies between different cows and different breeds. The average SOD activity in milk serum from Holsteins cows has been reported to be 0.92 U/ml and that from Jerseys 1.27 U/ml (Hoolbrook and Hicks, 1978). SOD is very resistant to various types of denaturing stress. Commercial pasteurized milk retains SOD enzymatic acitivity at a similar level to that for unpasteurized milk (Asada, 1976). The decomposition of excess superoxides by SOD is an important physiological antioxidant defense mechanism for aerobic organisms, it having been previously reported that *Latococcus* also expressed SOD activity (Sanders et al., 1995). Milks fermented by kefir grains, however, did not appear to influence SOD activity in this study. Therefore, the increase in the superoxide radical scavenging ability of kefirs as compared to milks must be attributable to antioxidative compounds other than SOD.

GSH-Px removes hydrogen peroxide and a variety of other peroxides at a high rate, thus providing protection against oxidative damage (Burk, 1997). GSH-Px activity has been detected in raw cow's milk at levels of between 12 and 32 U/ml (Lindmark-Mansson et al., 2001). Only a few studies focusing upon the stability of GSH-Px in milk processing have been conducted. Hojo (1982) reported that heating milk to 80°C for a period of 10 min inactivated all of the milk's GSH-Px activity, although Lindmark-Mansson et al. (2001) have reported that the GSH-Px in milk and whey remains fairly stable following several kinds of storage and heat treatment currently used in the modern dairy industry. To the best of our knowledge, no study has yet been conducted in the area of the determination of what change in GSH-Px activity occurs in milk during fermentation. In this study, we have found that the GSH-Px activity of kefirs was significantly lower than that for milks.

Milk contains various components with physiological functionality. Besides being a source of bioactive proteins, milk is also a source of bioactive peptides. Milk-protein derived bioactive peptides are inactive within the sequence of the parent protein and can be released and activated by enzymatic proteolysis, gastrointestinal digestion, or food processing. Such peptides can exert a wide range of effects, such as antimicrobial, antihypertensive, antithrombotic and immunomodulatory effects, in addition to aiding in the mineral absorption of calcium (Fiat et al., 1993; Lahov and Regelson, 1996; LeBlanc et al., 2002). Recent studies have also shown that peptides derived from milk proteins possess antioxidant activity (Suetsuna et al., 2000; Tong et al., 2000; Pena-Ramos and Xiong, 2001). In a previous study, we have found that *L. helveticus* isolated from kefir grains possessed highly proteolytic activity (Lin et al., 1999). Studies focusing upon whether milk proteins digested by proteolytic enzymes derived from kefir grains increase the bioactivities are now in progress.

CONCLUSIONS

This study demonstrates that kefirs are potential antioxidants that interact with a wide range of species directly responsible for oxidative damage. The antioxidative activity of kefirs may be attributed to their proton-donating ability, their reducing power and SOD-like activity as evidenced through DPPH and superoxide radical-scavenging and lipid peroxidative inhibition results. Therefore, kefirs are potential candidates for the role of useful and natural antioxidant supplements in the human diet.

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