Antioxidant and antigenotoxic effect of dairy products supplemented with red ginseng extract

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ABSTRACT

The purpose of this study was to evaluate the antioxidant and antigenotoxic effect of dairy products milk (M) and yogurt (Y) after the addition of 2% red ginseng extract to milk (RM) and to yogurt (RY). Total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, oxygen radical absorbance capacity, and total radical trapping antioxidant potential were determined in the samples. Furthermore, antigenotoxic effect of samples was measured, using comet assay in human leukocytes. Total phenolic content and total flavonoid content of RM [38.3 ± 0.8 mg of gallic acid equivalents (GAE)/100 g, 23.6 ± 0.1 mg of quercetin equivalents (QE)/100 g] and RY (41.1 ± 0.9 mg of GAE/100 g, 18.7 ± 0.1 mg of QE/100 g), respectively, were higher than those of M (6.31 ± 0.2 mg of GAE/100 g, 10.4 ± 0.1 mg of QE/100 g) and Y (8.1 ± 0.9 mg of GAE/100 g, 8.4 ± 0.2 mg of QE/100 g), respectively. The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and oxygen radical absorbance capacity values increased significantly after the addition of 2% red ginseng in both. Additionally, the total radical trapping antioxidant potential in RM (787.7 ± 7.0 μ g/mL) was lower than in M (2074.0 ± 28.4 μ g/mL). The H₂O₂-induced DNA damage in RY (0.1 ± 0.0 mg/mL) was less than the damage in Y (0.4 ± 0.0 mg/mL), but we found no significant difference between M and RM. This study indicates that supplementation with red ginseng can fortify the antioxidant and antigenotoxic effects of dairy products effectively.

Key words: dairy product, red ginseng, antigenotoxic effect, flavonoid

INTRODUCTION

Functional foods have been defined as foods that provide an additional physiological benefit that may prevent disease or promote health and wellbeing (Joseph, et al., 2018; Li et al., 2018). Many products, such as milk supplemented with vitamins, CLA, or omega-3, in addition to yogurt and dairy beverages enriched with probiotics, are now available in the market. These products are considered functional dairy products (Mattila-Sandholm and Saarela, 2005). The health benefits of milk have been known for a long time. In particular, beyond the presence of valuable nutrients, milk also contains antioxidant factors. Many researchers (Songisepp et al., 2004; Liu et al., 2005; Aloglu and Öner, 2011) reported the antioxidant effects of dairy products such as milk, yogurt, cheese, and kefir. It has been known that dairy products contain CLA, α -linolenic acids, α -tocopherol, and carotenoids antioxidants (Butler et al., 2008). Some studies showed that antioxidative effects for health benefit occur as a result of the complexation reaction between phenolic compounds and milk proteins after ingestion (Abd El-Maksoud et al., 2018; Lorenz et al., 2007). Phenols and polyphenolics are bioactive compounds as antioxidants that can be dissolved easily in milk. Generally, the use of plant extracts as natural and safe sources of phenols and polyphenols is preferred (Gad and El-Salam, 2010). New types of milk-based beverages are being introduced into the market with natural flavorings, such as chocolate, various herbs, and fruits. Such products contain plant polyphenols such as rutin from fruits and (–)-epigallocatechin gallate from tea, which are known mainly as antioxidants in lipid oxidation (Becker et al., 2004; Habtemariam and Belai, 2018). Dairy products containing n-3 (omega-3), phytosterols, isoflavins, CLA, minerals, and vitamins also play an important role in the development of functional foods. Dairy-based beverages with biofunctional activities are being offered increasingly all over the world, although the dairy-based beverage market is still a niche market

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compared with the sales of regular yogurt and plain milk (Eom et al., 2017a,b).

Red ginseng is made via steaming and drying fresh ginseng, prompting a chemical transformation via heat (Liu et al., 2016). During the steaming process, ginseng starch is gelatinized, causing an increase in saponin content. Traditionally, red ginseng has been used to refresh and enhance human wellbeing, and has been often referred to as an “adaptogenic” (Coon and Ernst, 2002). Korean red ginseng (*Ginseng Radix Rubra*) has several pharmacological and physiological effects that are being gradually discovered. In particular, the saponin fraction of Korean red ginseng shows a variety of effects, such as anticancer, antihypertension, anti-diabetes, antinociception, and improving weak body condition effects (Jung and Jin, 1996). In addition, red ginseng protected smokers from oxidative damage and reduced cancer risk associated with smoking (Jiang et al., 2017).

Therefore, the aim of our investigation was to evaluate the antioxidant and antigenotoxic effect of dairy products supplemented with red ginseng in vitro as compared with regular dairy products. Our hypothesis was that the products supplemented with red ginseng extract may have higher antioxidant and antigenotoxic effect than nonsupplemented ones, and the results from our study can be applied for the functional dairy food industry.

MATERIALS AND METHODS

Milk and Yogurt Ingredients

Whole milk (protein = 3%, fat = 4%, TS = 12%) and skim milk powder (protein = 34%, fat = 0.5%, TS = 95%) were purchased from Seoul Milk Co. (Seoul, Korea). Red ginseng extract (solid contents = $62 \pm 1.5\%$, wt/vol) was purchased from Fine Korea Co. (Seoul, Korea). Commercial starter culture (ABT-L yogurt starter culture; Culture Systems, Inc., Mishawaka, IN) was obtained from Samik Dairy and Food Co., Ltd. (Gimje-si, Jeollabuk-do, Korea). Low methoxy (LM) pectin powder from citrus was purchased from Daejung Chemicals and Metals Co., Ltd. (Siheung-si, Gyeonggi-do, Korea).

Preparation of Supplemented Milk and Yogurt Samples

Supplemented milk was produced by adding (2%, wt/vol) red ginseng extract. City milk and red ginseng extract was mixed together at 25°C with a blender (GMFC-670, Hanil Co., Seoul, Korea) and refrigerated at 4°C.

Supplemented yogurt was produced by adding 2% red ginseng extract. The market milk was mixed with skim milk powder and red ginseng extract and was pasteurized at 90°C in a water bath (SBC-24, EYELA, Tokyo, Japan) for 10 min. Pasteurized milk was cooled to 37°C in a water bath before being inoculated with yogurt starter culture containing *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*. Inoculated milk samples (initial cell concentration = 10^4 cfu/mL) were incubated until its pH reached 4.4 to 4.5 and stored in refrigerator (4°C) for overnight. We abbreviated samples of milk, red ginseng extract-supplemented milk, yogurt, and red ginseng-supplemented yogurt as M, RM, Y, and RY, respectively.

Preparations for Determination of Antioxidant Activity

Four types of liquid samples were diluted 2 fold with 99.5% methanol and heated at 30°C for 1 h in a water bath. Samples were centrifuged at $2,100 \times g$ for 30 min at 4°C and were filtered through filter papers (Whatman No. 1; GE Healthcare Co. Ltd., Chicago, IL). The solvent was removed using a rotary evaporator (N-1000V, EYELA), and the samples were freeze-dried and stored at -20°C until use.

Analysis of Phenolic and Flavonoid Contents of the Extracts

Total phenolic content was determined according to the method of Folin and Denis (1912). The M, RM, Y, and RY extracts were dissolved in distilled water at a concentration of 100 mg/mL. A sample diluted 2 fold was mixed with 0.4 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO), after which the mixture was allowed to stand at 25°C for 3 min. Subsequently, 0.4 mL of 2% Na₂CO₃ was added to the mixture. The absorbance was measured with an ELISA reader (Sunrise, Tecan Co. Ltd., Grödig, Austria) at 690 nm after letting the mixture stand for 1 h. The measurement was compared with a standard curve of prepared gallic acid solution and was expressed in the form of milligrams of gallic acid equivalents (GAE) per gram for the triplicate extracts.

Additionally, the total flavonoid content was determined in all the samples using the colorimetric Davis method (Davis, 1947). In this method, extracts reacting with diethylene glycol in alkaline solution produce a yellow chalcone, which is measured at a wavelength of 420 nm. The measurement was compared with a standard curve of prepared quercetin solution and was expressed in the form of milligrams of quercetin equivalents (QE) per gram for the triplicate extracts.

DPPH Assay

A solution of 0.8 mL of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (**DPPH**) in ethanol was added to 0.2 mL of each sample at different concentrations (100–5,000 µg/mL). The test tubes were vortexed and incubated for 10 min at 25°C in the dark, and then the absorbance (Abs) values were determined at 492 nm in an ELISA reader (Sunrise, Tecan Co. Ltd.). The radical scavenging activity (**RSA**) was assessed by the formula

$$\text{RSA (\%)} = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}}) \times 100.$$

Ethanol (0.8 mL) added to each sample solution (0.2 mL) was used as a blank. DPPH solution (0.8 mL) with the added ethanol (0.2 mL) was used as a control.

Oxygen Radical Absorbance Capacity Assay

Antioxidant activities of M, RM, Y, and RY extracts in different concentrations between 250 and 1000 µg/mL were examined for their peroxy and hydroxyl radical scavenging capacities, using the oxygen radical absorbance capacity (**ORAC**) assay system. The FLUOstar OPTIMA microplate fluorescence reader (BMG Labtech, Offenburg, Germany) was used with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. In the final assay mixture, fluorescein (40 nM) was used as a target of free radical attack with either 2,2'-azobis (2-amidinopropane) dihydrochloride (**AAPH**; 20 mM) as a peroxy radical generator in peroxy radical scavenging capacity (**ORAC_{ROO}**) assay. Trolox (1 µM) was prepared fresh daily and used as a control standard. The analyzer was programmed to record the fluorescence of fluorescein every 2 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. All data were expressed as micromoles of Trolox equivalents (**TE**). One ORAC unit is equivalent to the net protection area provided by 1 µM Trolox.

Total Radical Trapping Antioxidant Potential

Total radical trapping antioxidant potential (**TRAP**) was measured, using a modified version of the photometric method described by Rice-Evans and Miller (1994). The method for measuring antioxidant activity is based on the antioxidant inhibition of the absorbance of the radical cation of 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) (**ABTS^{•+}**). The **ABTS^{•+}** radical cation was formed through the interaction of **ABTS^{•+}**

(150 µM) with the ferryl myoglobin radical species generated by the activation of metmyoglobin (2.5 mM) with H₂O₂ (75 µM). Twenty microliters of each sample (250–1,000 µg/mL) was added to a tube containing 748 µL of PBS, 32 µL of metmyoglobin, and 450 µL of ABTS. The tubes were then mixed by vortexing. To prepare a standard curve, 10 µL of Trolox standards (0.5–2.5 mM), 758 µL of PBS, 32 µL of metmyoglobin, and 450 µL of ABTS were used. The reaction was started by adding 250 µL of H₂O₂, and absorbance was measured at 734 nm using a spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan).

Cell Culture and Treatments

We purchased HepG2 cells from the American Type Culture Collection (ATCC, Rockville, MD). The cells were maintained in Dulbecco's modified Eagle's medium (Welgene Inc., Seoul, Korea) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), streptomycin (100 µg/mL), and 2 mg/mL of NaHCO₃ in a humidified 37°C incubator gassed with 5% CO₂. The M, RM, Y, and RY extracts were dissolved in dimethyl sulfoxide (**DMSO**) and added to the culture medium so that the final concentration of DMSO was less than 1%.

Intracellular Reactive Oxygen Species Measurement with Dichlorofluorescein-Diacetate Assay

Cellular oxidative stress resulting from reactive oxygen species (**ROS**) generated by 2,2'-AAPH was measured fluorometrically, using the dichlorofluorescein-diacetate (**DCFH-DA**) method. The DCFH-DA diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterase to the non-fluorescent DCFH, which can be rapidly oxidized to the highly fluorescent dichlorofluorescein in the presence of ROS. First, HepG2 cells were cultured in 96-well plates (5 × 10⁵ cells/mL) for 24 h. After the cells were incubated with M, RM, Y, and RY extracts at different concentrations for 30 min, the medium was discarded and the wells were gently washed twice with PBS. Hanks' balanced salt solution (Sigma-Aldrich Chemicals, Poole, UK), which is stable to fluorescence, was added to each well. The AAPH was used to induce peroxy-radical oxidative stress. After wells were treated with 80 µM AAPH for 30 min, DCFH-DA was added to the culture plates at a final concentration of 40 µM, and the plates were incubated in the dark for 30 min at 37°C. Dichlorofluorescein fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission

wavelength of 535 nm using the FLUOstar OPTIMA microplate fluorescence reader.

Preparation of Human Leukocytes for Comet Assay

Peripheral venous blood was collected in heparinized vials from 3 healthy male donors with no history of smoking, drinking, or chronic use of medication. Blood samples were centrifuged at $250 \times g$ for 5 min at 4°C, and then leukocytes were isolated from the fraction of mononuclear cells containing leukocytes and monocytes via gradient centrifugation (Histopaque 1077, Sigma Chemical Co., St. Louis, MO). Isolated leukocytes were used for the comet assay after being washed with 1 mL of PBS.

Treatment of Human Leukocytes and Stimulation with Oxidative Stress

Leukocytes were incubated with M, RM, Y, and RY extracts, which were dissolved in distilled water, and the final concentration was adjusted to 1, 5, 10, and 50 µg/mL, respectively, in a dark incubator for 30 min at 37°C. For oxidative stimulus, they were resuspended in PBS with 200 µM H₂O₂ for 5 min on ice. After each treatment, leukocytes were centrifuged at $1,000 \times g$ at 4°C for 5 min and washed with PBS. We used 1% DMSO without oxidative stimulus as the negative control (NC).

DNA Damage Determination via Alkaline Comet Assay

The antigenotoxic effects of M, RM, Y, and RY extracts on human leukocytes were measured using the alkaline comet assay. Following the treatment, the leukocytes were mixed with 75 µL of 0.7% low-melting agarose. We then added this to slides precoated with 0.7% agarose. The slides were then immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosine mixture; subsequently, 1% Triton X-100 (Sigma Chemical Co.) and 10% DMSO mixture) for 1 h at 4°C. Thereafter, slides were placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na₂EDTA (pH 13.0) for 20 min. An electric current of 25 V/300 ± 3 mA was applied for 20 min at 4°C for DNA electrophoresis. The slides were washed 3 times with a neutralizing buffer (0.4 M Tris buffer, pH 7.5) for 5 min at 4°C and treated with ethanol for another 5 min. We evaluated the results using image analysis (Komet version 5.0, Kinetic Imaging, Liverpool, UK) and fluorescence microscopy (Leica DMLB, Bensheim, Germany) after staining the slides with 50 µL of ethidium bromide (20 µg/mL), and determined

the percentage of fluorescence in the tail (tail intensity; 50 cells from each of 2 replicate slides).

Statistical Analysis

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL). The data were subjected to 1-way ANOVA to determine the differences of samples. Significant differences were compared using the Tukey test at the level of $P < 0.05$.

RESULTS AND DISCUSSION

The aim of our study was to evaluate the ability of dairy products supplemented with and without red ginseng to act as antioxidants and antigenotoxic material. We conducted DPPH RSA, ORAC, TRAP, and cellular antioxidant capacity assays. The antigenotoxic ability of dairy products supplemented with and without red ginseng was estimated using a comet assay, which is a simple and sensitive technique for finding DNA damage.

Total Phenolics and Flavonoid Content

Phenolic compounds are good sources of natural antioxidants and are the products of secondary metabolism in plants. Their role is important in preventing chronic diseases such as chronic vascular disease, cancer, inflammatory bowel syndrome, and Alzheimer's disease. Flavonoids have powerful antioxidant activities in vitro and are able to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide, hydroxyl radical, peroxy radicals (Pannala et al., 1997; Boersma et al., 1999). The total phenolic and total flavonoid contents of M, RM, Y, and RY extracts are presented in Table 1. These results showed that RY had the highest total polyphenol content (TPC; 41.1 ± 0.9 mg of GAE/100 g), followed by RM, Y, and M (38.3 ± 0.8 , 8.1 ± 0.9 , and 6.3 ± 0.2 mg of GAE/100 g, respectively). Based on our results, RM had the highest total flavonoid content (TFC; 23.6 ± 0.1 mg of GAE/100 g), followed by RY, M, and Y (18.7 ± 0.1 , 10.4 ± 0.1 , and 8.4 ± 0.2 mg of GAE/100 g, respectively). The TPC and TFC of RM and RY were statistically higher ($P < 0.01$) than those in M and Y. Supplementing the samples with red ginseng had a significant effect ($P < 0.01$) on the TPC and TFC in M and Y. The interaction between polyphenol and protein was affected by (1) the form of polyphenol, (2) projection of phenolic hydroxyl groups, addition of galloyl groups, and (3) position of peripheral groups imposed by the stereochemistry of the pyranic ring of the polyphenol molecule (Kawamoto et al., 1995, 1996;

Table 1. Total phenol content (TPC) and total flavonoid content (TFC) of red ginseng extract and M, RM, Y, and RY samples at 100 mg/mL

Item	Sample ¹				
	R	M	RM	Y	RY
TPC (mg of GAE ² /100 g)	1,610.3 ± 2.5 ⁴	6.3 ± 0.2	38.3 ± 0.8**	8.1 ± 0.9	41.1 ± 0.9**
TFC (mg of QE ³ /100 g)	520.4 ± 1.8	10.4 ± 0.1	23.6 ± 0.1**	8.4 ± 0.2	18.7 ± 0.1**

¹R = red ginseng extract; M = milk; RM = red ginseng + milk; Y = yogurt; RY = red ginseng + yogurt.

²GAE = gallic acid equivalents.

³QE = quercetin equivalents.

⁴All values are means ± SD.

***P* < 0.01: significant difference between M and RM or between Y and RY based on Student's *t*-test.

Bacon and Rhodes, 1998; Kroll et al., 2000; de Freitas and Mateus, 2001; Rawel et al., 2001).

Antioxidant Effect

The DPPH[•] is a relatively stable organic radical and has been widely used to test the free radical scavenging ability of various samples. Results of DPPH radical scavenging ability of all the samples are presented in Table 2. All samples used in our study have shown direct DPPH RSA at a concentration range of 100 to 5,000 µg/mL. It appeared that M, RM, Y, and RY DPPH RSA ranged from 11.8 ± 0.0 to 14.6 ± 1.7 µg/mL, 15.1 ± 0.5 to 40.9 ± 1.1 µg/mL, 5.8 ± 0.5 to 13.1 ± 0.9 µg/mL, and 18.7 ± 1.1 to 41.1 ± 1.0 µg/mL, respectively. The results revealed that the RSA of dairy products supplemented with red ginseng were significantly higher (*P* < 0.01) than that of regular dairy products at all concentrations. Supplementation with red ginseng has been reported to yield high antioxidant activity (Kim et al., 2002). Similarly, it has been reported that herbal yogurts had higher antioxidant activity than plain yogurt (Amirdivani and Baba, 2011). It is likely that the presence of red ginseng influenced the dairy products with antioxidant activities.

The TRAP assay results for all the samples are presented in Table 3. The concentrations in samples ranged between 250 and 1,000 µg/mL. The TRAP for M, RM, and RY ranged from 33.3 ± 8.1 to 308.1 ± 6.4 µg/mL, 178.7 ± 4.3 to 903.5 ± 6.7 µg/mL, and 34.4 ± 8.7 µg to 549.4 ± 3.0 µg/mL, respectively; however, it was not detected in Y. This result indicates that the antioxidant effect of dairy products supplemented with red ginseng was greater than that of other dairy products at all concentrations.

The results for ORAC are summarized in the Figure 1. The DPPH RSA of M, RM, Y, and RY ranged from 1.9 ± 0.1 to 9.0 ± 0.5 µg/mL, 7.7 ± 0.4 to 24.1 ± 1.1 µg/mL, 2.0 ± 0.2 to 6.1 ± 0.5 µg/mL, and 8.1 ± 0.4 to 25.1 ± 1.4 µg/mL, respectively. As evidenced, ORAC of dairy products supplemented with red ginseng were significantly higher (*P* < 0.05) than other dairy products at all concentrations.

The cellular antioxidant capacity against 80 µM AAPH-induced oxidative stress in HepG2 cells (Figure 2) was evaluated at different concentrations (1, 5, 10, and 50 µg/mL) of the extracts. All the samples tested significantly decreased AAPH-induced oxidative stress in HepG2 cells, and the addition of red ginseng to dairy products augmented the cellular antioxidant capacity at the highest concentration (50 µg/mL).

Table 2. The 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (means ± SD, n = 3; %) of M, RM, Y, and RY samples at different concentrations

Concentration (µg/mL)	Sample ¹			
	M	RM	Y	RY
100	11.8 ± 0.0 ^{NS}	15.1 ± 0.5 ^{***}	5.8 ± 0.5 ^a	18.7 ± 1.1 ^{***}
250	12.3 ± 0.5	15.5 ± 0.8 ^{***}	10.1 ± 0.3 ^b	19.3 ± 0.7 ^{ab***}
500	12.4 ± 1.0	17.3 ± 1.0 ^{***}	11.1 ± 0.9 ^b	19.5 ± 0.5 ^{ab***}
1,000	13.1 ± 1.2	21.2 ± 1.5 ^{***}	10.1 ± 0.9 ^{bc}	21.1 ± 0.2 ^{b***}
2,500	14.1 ± 1.0	29.6 ± 0.5 ^{c***}	11.5 ± 1.0 ^{bc}	31.2 ± 1.2 ^{c***}
5,000	14.6 ± 1.7	40.9 ± 1.1 ^{d***}	13.1 ± 0.9 ^c	41.1 ± 1.0 ^{d***}

^{a-d}Different superscripts within a column indicate significant difference with Tukey's post hoc test (*P* < 0.05).

¹M = milk; RM = red ginseng + milk; Y = yogurt; RY = red ginseng + yogurt.

***P* < 0.01: significant difference between M and RM or between Y and RY at each concentration by Student's *t*-test.

Table 3. Total antioxidant activity (means $\pm$ SD, $n = 3$; mM TEAC¹) of M, RM, Y, and RY samples at different concentrations

Concentration ($\mu\text{g/mL}$)	Sample ²			
	M	RM	Y	RY
250	33.3 $\pm$ 8.1 ^a	178.7 $\pm$ 4.3 ^{***}	ND ³	34.4 $\pm$ 8.7 ^a
500	124.9 $\pm$ 10.7 ^b	503.0 $\pm$ 9.2 ^{***}		287.5 $\pm$ 6.8 ^b
750	152.9 $\pm$ 4.6 ^c	744.2 $\pm$ 7.5 ^{***}		491.2 $\pm$ 3.9 ^c
1,000	308.1 $\pm$ 6.4 ^d	903.5 $\pm$ 6.7 ^{***}		549.4 $\pm$ 3.0 ^d

^{a-d}Different superscripts within a column indicate significant difference based on Tukey's post hoc test ($P < 0.05$).

¹Trolox equivalent antioxidant capacity.

²M = milk; RM = red ginseng + milk; Y = yogurt; RY = red ginseng + yogurt.

³Not detected.

^{***} $P < 0.01$: significant difference between M and RM based on Student's t -test.

It is well known that milk contains antioxidant compounds, such as naturally occurring β -carotene, vitamins (i.e., E and C), and enzymatic systems (Calligaris et al., 2004). Additionally, yogurt has been considered by nutritionists to have high nutritional value and positive bioactive effects (Lourens-Hattingh and Viljoen, 2001).

The higher free radical scavenging activity for Korean ginseng extract seen in our study may not only be due to ginsenosides, but also phenolic compounds such as maltol, salicylic acid, and vanillic acid, which have the activity to scavenge free radicals (Zhang et al., 1996; Kim et al., 2002). When a natural source of phenols was mixed with skim milk, a complex compound could be formed because the phenolic compounds would act as a polydentate ligand on the protein surface involving hydrogen bonding, as well as the presence of hydrophobic interactions enough to bind to milk protein. The type of this interaction depends on the type and the molar ratio of both phenolic compound and protein (Ricardo-

da-Silva et al., 1991; Prigent et al., 2003). Ginsenosides Rb1 and Rg1 in red ginseng have antioxidant activities on lipids (Kim et al., 2004). In addition, ginsenoside Rh2 can significantly increase antioxidant enzymatic activity, thus showing antioxidant effects (Kim et al., 1996; Lai et al., 2006). The potential of RSA of dairy products supplemented with red ginseng at all tested concentrations demonstrated a possible synergistic action.

Antigenotoxic Effect

The comet assay is a rapid and sensitive method for analyzing DNA damage at the level of individual cells; the introduction of lesion-specific enzymes, which induce strand breakage at the sites of oxidized bases, allows the measurement of endogenous oxidative DNA base damage (Collins et al., 1993, 1997).

The antigenotoxic effect against H₂O₂-induced DNA damage in leukocytes (Figure 3) was evaluated with

Figure 1. Oxygen radical absorbance capacity (ORAC) of milk (M), red ginseng and milk (RM), yogurt (Y), and red ginseng and yogurt (RY). The ORAC values expressed as Trolox equivalents (mM) according to different concentration of M, RM, Y, and RY; means $\pm$ SD ($n = 3$). Values not sharing the same letter (a–c) are significantly different ($P < 0.05$) based on the Tukey's post hoc test. ^{***} $P < 0.001$: significant difference between M and RM and between Y and RY at each concentration based on Student's t -test.

Figure 2. Cellular antioxidant capacity of milk (M), red ginseng and milk (RM), yogurt (Y), and red ginseng and yogurt (RY) extracts against 80 µM 2,2'-azobis (2-amidinopropane) dihydrochloride-induced oxidative stress in HepG2 cell mode. NC = negative control (without oxidative stimulus); PC = positive control; means $\pm$ SD (n = 3). DCF = dichlorofluorescein. Values not sharing the same letter (a–d) are significantly different ($P < 0.05$) based on the Tukey's post hoc test. ** $P < 0.01$: significant difference between M and RM and between Y and RY at each concentration based on Student's *t*-test.

the extracts at different concentrations (1, 5, 10, and 50 µg/mL). Our results showed that a protective effect against H₂O₂-induced DNA damage in human leukocytes was found in M, RM, and RY. Tail intensity of RY was significantly lower than that of Y at a concentration of 50 µg/mL, whereas no additional protective effect was found.

Recent studies on the effectiveness of saponin components that are newly produced and converted in red ginseng fermentation (Trinh et al., 2007) have shown that 30 types of metabolic factors, including Rb1, Rb2, Rc, and Rd, are converted to chemical components, such as compound K, to enable endogenous absorption by intestinal microbial flora. In addition, the bioconverted saponin components have recently attracted increasing interest because of its intriguing biological

actions. Previous research has demonstrated that compound K has antigenotoxic activity, which is mediated by these metabolites (Hasegawa and Uchiyama, 1998; Lee et al., 1998). Antigenotoxic effects may also be due to other components of fermented milks. For example, kefir contains water-soluble polysaccharides that have been shown to exert antitumor activity in vivo (Lopitz-Otsoa et al., 2006).

Some studies have shown the potential of lactobacilli and bifidobacteria to inhibit the genotoxic activity of chemical compounds (Pool-Zobel et al., 1996; Burns and Rowland, 2000; Tavan et al., 2002). Various lactobacilli occur in commercial dairy products such as yogurt, and it has been indicated that lactic acid bacteria may have shown antigenotoxic activity (Pool-Zobel et al., 1996; Lankaputhra and Shah, 1998).

Figure 3. Antigenotoxic effect of milk (M), red ginseng and milk (RM), yogurt (Y), and red ginseng and yogurt (RY) extracts. NC = negative control; PC = 200 µM H₂O₂-treated positive control; means $\pm$ SE (n = 4). Values not sharing the same letter (a–d) are significantly different ($P < 0.05$) based on Tukey's post hoc test. * $P < 0.05$: significant difference between Y and RY at each concentration based on Student's *t*-test.

CONCLUSIONS

A positive correlation was observed between total phenolic and flavonoid content, antioxidant potential, and free RSA in dairy products supplemented with and without red ginseng. The present study specifically revealed the antioxidative (via DPPH, ORAC, TRAP, and CAC assays) and the antigenotoxic effect (via comet assay) of dairy products supplemented with red ginseng and showed that these capacities were similar in DPPH, ORAC, and TRAP assays. Based on these results, a recommendation can be made about dairy products to be enriched with red ginseng because of its high antioxidant activity. Thus, dairy products supplemented with red ginseng might be a promising natural source of antioxidants and could be used for the development of value-added products, such as functional dairy products.

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