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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 μg/mL) was lower than in M (2074.0 ± 28.4 μg/mL). The H_2O_2-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; Aloğlu 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 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 compared with

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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, antidiabetes, 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 ± 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, Gyonggi-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 × *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 Content 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's 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% Na_2 CO_3 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.