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Bioactive Ginseng Yogurts Fermented with *Bifidobacteria*: The Transformation of Ginsenosides to Compound K

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ABSTRACT The objective of this study was to prepare bioactive ginseng yogurts containing compound K, which is transformed from ginsenosides, and to investigate the compound's cytotoxicity against tumor cells. Milk containng ginseng was fermented by *Bifidobacteria* KK-1 and KK-2, and their activities for transforming ginsenosides to compound K were measured. Among the tested concentrations of ginseng in the milk, compound K was effectively produced in the 3% and 6% ginseng yogurts fermented for 48 hrs. These fermented ginseng yogurts were extracted with BuOH, and their cytotoxicities against tumor cells were examined. The BuOH extract of the yogurt made from the 3% ginseng milk showed cytotoxic activity against P388 and HeLa tumor cells. However, the nonfermented ginseng milk did not exhibit cytotoxicity against these cells. Therefore, we deem that the ginseng yogurt, which contained compound K, could be developed as a potential fermented drink product.

KEYWORDS: *Ginseng yogurt, compound K, ginsenosides, Bifidobacteria, cytotoxicity*

INTRODUCTION

Probiotics are defined as viable microorganisms that exhibit beneficial health effects in the host upon ingestion, by improving the properties of its indigenous microflora (5). Traditionally, yogurt is manufactured using *Lactobacillus* spp. and *Streptococcus* spp. such as *L. bulgaricus* and *S. thermophilus*. These lactic acid bacteria provide certain health benefits. However, they are neither natural inhabitants of the intestine nor survive in the gastrointestinal tract. Therefore, *Bifidobacterium* spp., which is a primary microbe in the human intestine, has been considered a probiotic for yogurt (9,10).

Ginseng is used as a traditional medicine in Korea, China, and Japan. The major components of ginseng are ginsenosides, which are divided into the protopanaxadiol group (ginsenosides Ra, Rb1, Rb2, Rc, and Rd) and the protopanaxatriol group (ginsenosides Rg1, Re, Rf, and Rg2) (8,11). These ginsenosides are regarded as the principal components responsible for the pharmacological activities of ginseng. Protopanaxadiol ginsenosides exhibit sedative, anticonvulsive, analgetic, antipyetic, antiinflammatory, and antipsychotic activities, and they can improve gastrointestinal motility (3,4). Nevertheless, if ginseng containing the ginsenosides Rb_1, Rb_2, and Rc is orally ingested by humans, these ginsenosides are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-proto-panaxadiol (compound K) by human intestinal bacteria such as *Bifidobacteria*. The transformed compound K can induce anti-metastatic and anti-carcinogenic effects by blocking tumor invasion and by preventing chromosomal aberration and tumorigenesis (6,12). However, most commercial lactic acid bacteria such as *L. acidophilus, L. bulgaricus, and S. thermophilus* do not transform these ginsenosides to compound K (1,6). Furthermore, compound K can not be transformed via chemical hydrolysis, such as by hydrochloric acid.

Therefore, the objectives of this study were to prepare bioactive ginseng yogurts using the *Bifidobacteria* KK-1 and KK-2, which were previously isolated (1), and to investigate the cytotoxic activity of compound K produced in the yogurts against tumor cells.

MATERIALS AND METHODS

Materials and starter culture for yogurt

Fresh ginseng was purchased from Kyungdong Local Market (Seoul, Korea), washed 5 times, dried in an oven at 60°C for 48 hrs, and then powdered. A general anaerobic medium (GAM) was purchased from Nissui Pharmaceutical Co., Ltd. (Japan), and Tryptic soy broth was purchased from Difco Co. (USA).

Bifidobacterium minimum KK-1 (KK-1) and *B. cholerium*

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KK-2 (KK-2), isolated from human intestinal microflora, were used as starter cultures (1). KK-1, KK-2, and a combination of KK-1 and KK-2 were inoculated into 5 mL of GAM, respectively, and incubated at 37°C for 20 hrs. These *Bifidobateria* were cultured in Tryptic soy broth for the yogurt starter.

Preparation of ginseng yogurt

The ginseng yogurt was prepared by the following procedure: ginseng powder (1, 3, or 6 g), which was sterilized by an autoclave, was added to 100 mL of milk, and 7 g of sugar was added. Then, 3% starter (approximately 5×10^{10}) was added to the mixture and fermented for 48 hrs at 37°C.

Determination of compound K in the ginseng yogurt

The compound K contents of the ginseng yogurts containing 1, 3, or 6% ginseng powder were determined. Here, yogurt sample (2 mL) and chloroform (6 mL) were homogenized and centrifuged, and then 1 mL of each sample was obtained from the bottom portion of the mixture. This sample was evaporated, dissolved with methanol, and then assayed by TLC using the following: TLC plate, silica gel $60F_{254}$ (Merck Co., USA); developing solvent, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (v/v), lower phase; spotted concentration of compound K = 0.1, 0.5, 1, 2, and 5 g. The plates were stained by spraying them with MeOH:H_2SO_4 (95 : 5, v/v), followed by heating. The stained TLC plates were then analyzed using a TLC scanner (Shimadzu Model CS-9301PC, Tokyo, Japan).

***In vitro* cytotoxicity assay**

The *In vitro* cytotoxicity of the butanol extract of the 3% ginseng yogurt was evaluated against P388 cells (mouse lymphoid bioplasma cell line) and HeLa cells (human cervix adenocarcinoma) by the 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, according to the method of Carmichael *et al.* (2). Each cultured cell line was harvested, counted, and inoculated at the appropriate concentrations (180 μL volume: 4×10^4 cells/well for the P388 cells; 3×10^4cells/well for the HeLa cells) into 96-well microtiter plates. The P338 cell line was then cultured for 2 hrs, and the HeLa cell line was cultured for 24 hrs. These cells were exposed to the test compounds for 2 days at 37°C. A 50 μL amount of MTT solution (2 mg/mL in PBS) was added to each well, and the plates were incubated for 4 hrs. After aspiration of the medium, DMSO (100 μL) was added to solubilize the MTT-formazan product. The absorbance was measured by an ELISA reader at 540 nm. The tumor cell growth was defined by comparison with the control cell culture.

RESULTS AND DISCUSSION

Ginsenosides Rb1, Rb2, and Rc are transformed to compound K by human intestinal bacteria. As shown in a previous study (1), among the human intestinal lactic acid bacteria, *B. minimum* KK-1 and *B. cholerium* KK-2 demonstrated compound K transforming activities in ginseng extract. Ginseng yogurt fermented using *Bifidobacteria* can be used as a probiotic yogurt due to its compound K content. However, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, commercial lactic acid bacteria used to manufacture yogurt, can not transform ginsenosides to compound K. Therefore, ginseng yogurt must be prepared using *B. minimum* KK-1, *B. cholerium* KK-2, or a combination of *Bifidobacteria* KK-1 and KK-2 as starters to produce probiotic yogurt. The thin layer chromatograph of the ginseng yogurts containing 1, 3, and 6% ginseng, fermented by the combination of *Bifidobacteria* KK-1 and KK-2 for 48 hrs, is shown in Fig. 1. The yogurt fermented without ginseng did not contain compound K after 48 hrs of fermentation; however, the 1, 3, and 6% ginseng yogurts contained compound K, which was transformed from the ginsenosides in the ginseng. Among the tested concentrations of ginseng, the compound K content was highest in the 6% ginseng yogurt fermented for 48 hrs.

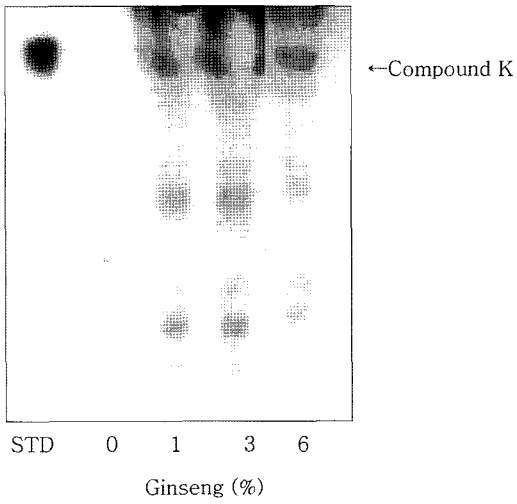

Fig. 1. Thin layer chromatograph of ginseng yogurt fermented by the combination of *Bifidobacteria* KK-1 and KK-2 for 48 hrs. TLC elution solvent, $CHCl_3$-MeOH-H_2O (65 : 35 : 10); STD, compound K standard.

Therefore, we fermented the milk containing 1, 3, and 6% ginseng using *B. minimum* KK-1, *B. cholerium* KK-2, or the combination of KK-1 and KK-2 as starters, and determined the compound K contents of the yogurts by various fermentation times (Table 1). Compound K was not detected in the nonfermented ginseng yogurt, and after 12 hrs of fermentation, compound K was not detected in the 1, 3, and 6% ginseng yogurts (Table 1). However, after 24 hrs of fermentation, compound K was detected in the yogurts. The ginseng yogurts fermented by *Bifidobacterium* KK-1, and the combination of *Bifidobacteria* KK-1 and KK-2, contained

Table 1. Transforming activities for ginsenosides to compound K in ginseng yogurts by various fermentation times

Starter	Conc.[1](%)	Compound K (M)			
		Fermentation time (hr)			
		12	24	48	72
Bifidobacterium KK-1	1	-	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
	3	-	0.07 ± 0.02	0.11 ± 0.02	0.09 ± 0.02
	6	-	0.24 ± 0.03	0.30 ± 0.01	0.26 ± 0.04
Bifidobacterium KK-2	1	-	-	0.03 ± 0.01	0.01 ± 0.02
	3	-	-	0.08 ± 0.00	0.07 ± 0.00
	6	-	-	0.19 ± 0.01	0.14 ± 0.01
Bifidobacteria KK-1 and KK-2	1	-	0.05 ± 0.01	0.06 ± 0.00	0.06 ± 0.01
	3	-	0.12 ± 0.00	0.16 ± 0.01	0.14 ± 0.00
	6	-	0.38 ± 0.00	0.42 ± 0.01	0.37 ± 0.01

[1]Concentration of ginseng in yogurt
Three percent of starter (approximately 5×10^{10}) in milk was added to the mixture and then fermented.
Values are the mean ± S.D.

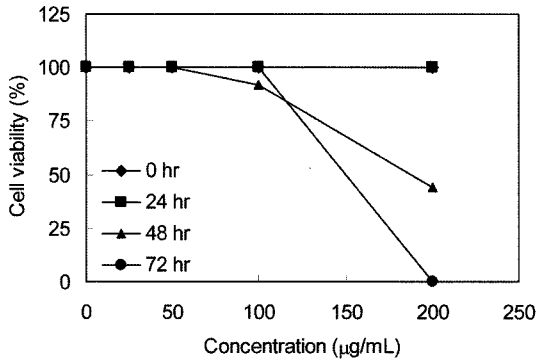

Fig 2. The cytotoxicity of the butanol extract of the 3% ginseng yogurt fermented for various times, against P388 cells.

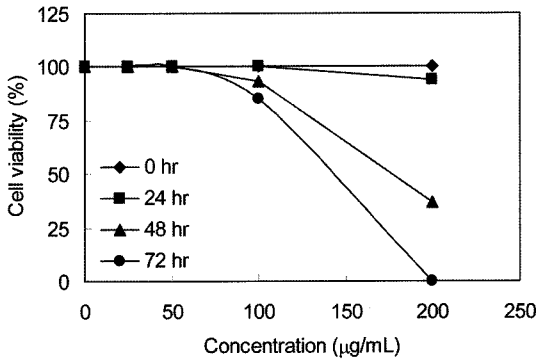

Fig 3. The cytotoxicity of the butanol extract of the 3% ginseng yogurt fermented for various times, against HeLa cells.

0.03 and 0.05 μM (1% ginseng yogurt), 0.07 and 0.12 μM (3% ginseng yogurt), and 0.24 and 0.37 μM (6% ginseng yogurt) of compound K, respectively. Yet, the ginseng yogurt fermented by *Bifidobacterium* KK-2 did not contain compound K after 24 hrs of fermentation. After 48 hrs of fermentation, all the ginseng yogurts contained compound K. For the 6% ginseng yogurt, *Bifidobacterium* KK-1 (0.30 μM) produced a higher level of compound K than *Bifidobacterium* KK-2 (0.19 μM), and the combination of *Bifidobacteria* KK-1 and KK-2 potently produced compound K (0.42 μM). Thus, these *Bifidobacteria* may synergistically transform the ginsenosides in ginseng to compound K. Nevertheless, a 6% ginseng-containing yogurt may be inappropriate, because generally, ginseng at a dose of 3 g per day is what is orally administered to humans, and the volume of commercial yogurt is approximately 90-150 ml. Therefore, to evaluate the cytotoxicity of the compound K produced in the ginseng yogurt against tumor cells, the 3% ginseng yogurt fermented by the combination of *Bifidobacteria* KK-1 and KK-2 was extracted with BuOH, and its cytotoxic activity against P388 and HeLa cells was measured (Fig. 2, 3). The ginseng yogurt extract that was fermented for 48 hrs demonstrated cytotoxic activity against the P388 cells, where the cell viabilities were 92% in 100 μg/mL and 44% in 200 μg/mL (Fig. 2). The ginseng yogurt extract fermented for 48 hrs also showed cytotoxic activity against the HeLa cells, with cell viabilities of 93% in 100 μg/mL and 37% in 200 μg/mL (Fig. 3).

Bae *et al.* (1) reported that ginseng extract did not exhibit cytotoxicity against certain tumor cell lines (L1210, P388, Me180, A549); yet, Zhou reported that compound K exhibited cytotoxic effects against tumor cells (13). On the other hand, fermented ginseng extract exhibited potent cytotoxicity against these cell lines. In a previous study (7), 3% ginseng yogurts fermented by *B. minimum* KK-1, and the combination of KK-1 and KK-2, received the highest overall sensory acceptability scores from elderly participants, among 1, 2, and 3% ginseng yogurts fermented by *B. minimum* KK-1, *B.*

cholerium KK-2, or the combined *Bifidobacteria*. The 3% ginseng yogurts fermented by *B. minimum* KK-1 and the combined *Bifidobacteria* had 8.25 and 8.85 log CFU/g of product, respectively (7). It is reported that for health benefits, probiotic bacteria must be viable and available at high concentrations, typically 6 log CFU/g of product (10).

In conclusion, the consumption of ginseng yogurt fermented by the combination of *Bifidobacteria* KK-1 and KK-2 may be used to prevent tumors, and may also have beneficial effects on human health by improving the properties of the indigenous intestinal microflora.

Therefore, we believe ginseng yogurt containing compound K can be developed as a potential fermented drink product.

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