

Bioavailability of Fermented Korean Red Ginseng

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Abstract

In an effort to improve ginsenoside bioavailability, the ginsenosides of fermented red ginseng were examined with respect to bioavailability and physiological activity. The results showed that the fermented red ginseng (FRG) had a high level of ginsenoside metabolites. The total ginsenoside contents in non-fermented red ginseng (NFRG) and FRG were 35715.2 µg/mL and 34822.9 µg/mL, respectively. However, RFG had a higher content (14914.3 µg/mL) of ginsenoside metabolites (Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2) compared to NFRG (5697.9 µg/mL). The skin permeability of RFG was higher than that of NFRG using Franz diffusion cells. Particularly, after 5 hr, the skin permeability of RFG was significantly ($p < 0.05$) higher than that of NFRG. Using everted intestinal sacs of rats, RFG showed a high transport level (10.3 mg of polyphenols/g sac) compared to NFRG (6.67 mg of polyphenols/g sac) after 1 hr. After oral administration of NFRG and FRG to rats, serum concentrations were determined by HPLC. Peak concentrations of Rk1, Rh1, Rc, and Rg5 were approximately 1.64, 2.35, 1.13, and 1.25-fold higher, respectively, for FRG than for NFRG. Furthermore, Rk1, Rh1, and Rg5 increased more rapidly in the blood by the oral administration of FRG versus NFRG. FRG had dramatically improved bioavailability compared to NFRG as indicated by skin permeation, intestinal permeability, and ginsenoside levels in the blood. The significantly greater bioavailability of FRG may have been due to the transformation of its ginsenosides by fermentation to more easily absorbable forms (ginsenoside metabolites).

Key words: fermented red ginseng, bioavailability, ginsenoside metabolites, Franz cell, everted intestinal sac

INTRODUCTION

Ginseng (the root of *Panax ginseng* C.A. MEYER, Araliaceae) is one of the most commonly used traditional medicines in China, Korea, Japan, and other Asian countries for the treatment of various diseases. Ginseng ginsenosides (saponins) are regarded as the principal components responsible for the pharmacological activities of ginseng, and a large number of ginsenoside derivatives have been identified in *Panax ginseng* and other *Panax* spp. (1). When steamed, the root is called red ginseng. Red ginseng contains polysaccharides and ginsenosides such as Rg3, Rb1, Rb2, and Rc as main constituents (2).

These ginsenosides have shown various biological activities including anti-inflammatory activity (3) and anti-tumor effects (4,5). The pharmacological actions of these ginsenosides have been explained through their bio-transformation by human intestinal bacteria (6-8).

Orally administered ginsenosides are hardly decomposed by either gastric juices or liver enzymes, and their

absorption rates from the intestine are very low. Like other plant glycosides, ginsenosides are hydrolyzed by intestinal bacteria followed by absorption. Ginsenosides act as precursors that are metabolized to bioactive forms by intestinal bacterial deglycosylation (9,10) and fatty acid esterification (11,12) in the body. Intestinal microflora are very changeable depending on host conditions (diet, health, and even stress). Therefore, it is hypothesized that individual differences in ginseng efficacy may be partly associated with the intestinal microflora of patients. The efficiency of conversion and transformation pathways may differ greatly due to the diversity of resident microflora between individuals (13).

These processes are performed by lactic acid bacteria such as *Bifidobacterium* sp. and *Lactobacillus* sp., as well as some molds such as *Saccharomyces* sp. (14). The microbes transform certain components of foods and convert sugars to alcohol and lactic acid. For example, ginseng fermentation by lactic acid bacteria produces lactic acid as well as compound K (CK), which is trans-

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formed from ginsenosides Rb1, Rb2, and Rc, and exhibits potent cytotoxicity against tumor cells (6,15). Therefore, microbial conversion (fermentation) can be conducted to convert major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) to minor ginsenosides (Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2) that may have more profound physiological properties.

In the present study, the physiological activities of fermented red ginseng (FRG) and non-fermented red ginseng (NFRG) were investigated, and their bioavailabilities were compared via *in vitro* tests and *in vivo* tests.

MATERIALS AND METHODS

Materials

The FRG and NFRG were gifted from Sejong Korea Ginseng Co. (Incheon, Korea). Standard ginsenosides, including the compounds Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rd, Rg3, F2, CK, Rk1, Rg5, and Rh2, were purchased from Embo Laboratory (Daejeon, Korea). All other chemicals were of reagent grade and obtained from local suppliers.

HPLC analysis of ginsenosides

Each FRG and NFRG formulation (approx. 10 mg) was accurately weighed and dissolved in 3 mL of methanol. After extraction in an ultrasonic bath for 15 min, the samples were transferred to centrifuge tubes and centrifuged at 4000 rpm for 10 min. The supernatants were collected and the residues were extracted two more times by the same procedure. The supernatants obtained from these three extractions were combined in a vial and evaporated slowly to dryness under a flow of pure nitrogen gas. The residue was reconstituted with 2 mL of water and applied to an SPE C18 cartridge for sample clean-up (16).

The levels of 16 major ginsenosides were analyzed using an HPLC-based technique developed by Kim et al. (17). A Varian Prostar 200 HPLC system (Varian

Inc., Palo Alto, CA) equipped with a quaternary solvent delivery system, an autosampler, and UV detector was used. The column configuration consisted of an IMtakt Cadenza CD-C18 (4.6 × 75 mm, Imtakt Corporation, Kyoto, Japan). UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40°C; the gradient program was as follows: 0→11 min, 11% B (isocratic); 11→15 min, 11→16% B; 15→16 min, 16→20% B; 16→18 min, 20→21%; 18→24 min, 21% B (isocratic); 24→25 min, 21→22% B; 25→35 min, 22% B (isocratic); 35→36 min, 22→23% B; 36→40 min, 23% B (isocratic); 40→41 min, 23→24%; 41→45 min, 24% B (isocratic); 45→53 min, 24→37% B; 53→61 min, 37→45% B; 61→66 min, 45→46%; 66→73 min, 46→48% B; 73→75 min, 48% B (isocratic); 75→77 min, 48→11%; 77→85 min, 11% B (isocratic). The flow rate was kept at 1.3 mL/min and the sample injection volume was 5 μL. The level of total ginsenosides was determined by the sum of the 15 ginsenosides. Fig. 1 shows the HPLC chromatograms of the 15 standard ginsenosides.

Skin permeation across Franz-type diffusion cells

Skin permeation was determined by the method of Sonavane et al. (18) with some modification. Male Wistar rats weighing 250~300 g (Nara Biotech., Daejeon, Korea) were used for the study. The abdominal hair of the rats was removed with an electric clipper and an electric razor 1 day before the study. The rats were anaesthetized with ether and then decapitated. The abdominal skin was excised immediately. The excised skin was mounted in a Franz-type diffusion cell. Then, 4.9 mL of 0.1 M sodium phosphate buffer (pH 7.4) was used as a receptor medium and 100 μL of ginseng sample was placed on the donor side. The receptor medium was kept at 37°C and stirred with a magnetic stirrer at 400 rpm. Aliquots (0.5 mL) of the receptor medium were withdrawn at 0.5 hr, 1~6 hr, and 24 hr. Immediately after collection of the medium, 0.5 mL of fresh buffer

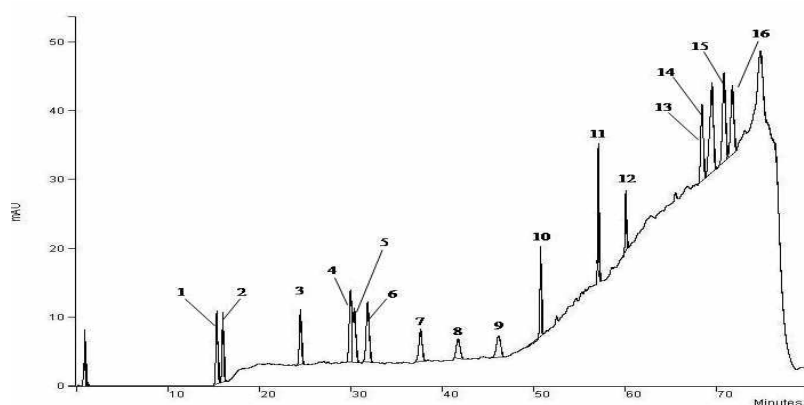


Fig. 1. Chromatogram of standard ginsenosides by HPLC assay. The column configuration consisted of a IMtakt Cadenza CD-C18 (4.6 × 75 mm). UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40°C. 1: Rg1, 2: Re, 3: Rf, 4: Rh1, 5: Rg2(r), 6: Rg2(s), 7: Rb1, 8: Rc, 9: Rb2, 10: Rd, 11: F2, 12: Rg3, 13: CK, 14: Rg5, 15: Rk1, 16: Rh2.

was added to the receptor cell. The absorbance of the permeates was measured at 203 nm.

Intestinal transport across everted intestinal sacs

Everted intestinal sac experiments were performed according to the method of Tandon et al. (19) with some modification. Male Sprague-Dawley rats, weighing 220 ~250 g (Nara Biotech.), were fasted overnight with free access to water until they were anaesthetized with urethane. Then, a middle abdominal incision was made and the jejunum was quickly taken. After the underlying mesenterium was removed, the jejunum was flushed with ice-cold Krebs-Henseleit bicarbonate (KHB) buffer to remove the intestinal contents. The jejunum was gently stretched and cut into segments (10 cm long each). Each of the sacs was carefully everted with a glass rod. One end was ligated, and the other end was ligated with a conical rubber stopper that housed one port for the removal and addition of serosal fluid, and another port for continuous supply of 5% CO₂ and 95% O₂ throughout the experiment. After being filled with 1 mL of KHB buffer (inner compartment), the sacs were incubated in 29.5 mL of the same buffer (outer compartment) that contained 0.5 mL of ginseng sample at 37°C in a water bath. Two hundred microliters of the serosal fluid inside the sacs was taken at 30 min and 1 hr, followed by immediate replacement with fresh KHB buffer of the same volume. The transfer of serosal fluid was reflected by the increase in volume inside the sac, and the gut fluid uptake was determined by measuring the increase of fluid volume in the gut. Intestinal transport of the ginseng sample was expressed as mg of polyphenols/g tissue dry weight. The polyphenol content of the transports was determined according to the Folin-Ciocalteu method (20).

Oral administration of FRG and NFRG to SD rats

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee (KUIACUC-2009-62). The experimental animals were Sprague/Dawley rats, weighing 200 g, from Nara Biotech. The FRG or NFRG solution was administered orally at a dose of 8 g/kg to the rats deprived of food but given free access to water for 18 hr before the experiments. Blood samples were collected at 30 min, 1 hr, 2 hr, 4 hr, and 24 hr after P.O. dosing. The blood samples were pretreated with solid phase extraction (SPE) cartridges (OASIS, Waters, MA, USA). The ginsenoside were assayed using the above HPLC method.

Statistical analysis

All expressed values are the means of triplicate determinations. All statistical analyses were performed

using the Statistical Package for Social Sciences version 12.0 (SPSS Inc., IL, USA). The t-test was used to assess the differences between two samples. All data were two-sided at the 5% significance level and are reported as means \pm standard deviations (SD).

RESULTS AND DISCUSSION

Ginsenosides in NFRG and FRG

The ginsenoside compositions of the NFRG and FRG were compared using HPLC (Table 1). The total ginsenoside contents of the NFRG and FRG were 35715.2 μ g/mL and 34822.9 μ g/mL, respectively, but these levels were not significantly different. Rb1 and Rg1 are the two main ginsenosides contained in red ginseng. The sum of Rb1 and Rg1 in NFRG was higher (9096.5 μ g/mL) than that in FRG (5562.0 μ g/mL). However, the level of ginsenoside metabolites (Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2) was higher level in FRG (14914.3 μ g/mL) compared to NFRG (5697.9 μ g/mL).

Ginsenosides are classified into the following categories according to their chemical constitutions: protopanaxadiols (PPD), protopanaxatriols (PPT), and oleanolic acids, and more than 40 ginsenoside variants have been reported (21,22). Among them, six major ginsenosides, including Rb1, Rb2, Rc, Rd, Re, and Rg1, account for 90% (w/w) of the total saponins in white and red ginseng (1).

With the development of new methods for ginsenoside

Table 1. Ginsenosides of non-fermented and fermented red ginseng

| Ginsenosides | Concentration (μ g/mL) | |
|--------------|-----------------------------|----------------------|
| | NFRG | FRG |
| Rg1 | 1272.1 \pm 126.1 | 751.8 \pm 93.0 |
| Re | 3367.2 \pm 351.1 | 1882.4 \pm 222.6 |
| Rf | 1223.6 \pm 113.2 | 1121.7 \pm 130.7 |
| Rh1+Rg2(s) | 1621.8 \pm 104.2 | 2095.3 \pm 204.4 |
| Rg2(r) | 568.9 \pm 247.7 | 994.1 \pm 75.4 |
| Rb1 | 7824.4 \pm 854.6 | 4810.2 \pm 537.0 |
| Rc | 6199.6 \pm 662.3 | 3807.5 \pm 413.5 |
| Rb2 | 5394.8 \pm 521.9 | 3424.9 \pm 408.3 |
| Rd | 4166.7 \pm 425.4 | 3116.0 \pm 234.3 |
| Rg3 | 1671.7 \pm 150.2 | 4903.4 \pm 584.0 |
| F2 | 18.3 \pm 0.7 | 47.1 \pm 2.1 |
| CK | 34.7 \pm 2.4 | 49.8 \pm 11.5 |
| Rk1 | 1244.3 \pm 121.7 | 4043.6 \pm 478.1 |
| Rg5 | 1107.1 \pm 114.6 | 3775.0 \pm 454.1 |
| Rh2 | 0.0 \pm 0.0 | 0.0 \pm 0.0 |
| Total | 35715.2 \pm 3235.5 | 34822.9 \pm 3650.2 |
| Rg1+Rb1 | 9096.5 \pm 980.4 | 5562.0 \pm 629.8 |
| Metabolites | 5697.9 \pm 394.9 | 14914.3 \pm 1566.0 |

NFRG: non-fermented red ginseng, FRG: fermented red ginseng, CK: compound K, Metabolites: sum of Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2.

isolation along with better ginseng processing technologies, various minor active ginsenosides have been discovered. However, it is quite difficult to separate such minor saponins from ginseng, as they are rare or non-existent in most ginseng samples. Therefore, further research into the modification of ginsenosides has been conducted in order to convert major ginsenosides into minor saponins, which may have more profound physiological properties. Recently, several investigators have reported that Rb1, Rb2, and Rc are metabolized by intestinal bacteria in rats and humans after oral administration, and that the main metabolite of PPD-type ginsenoside is CK (8,9). The metabolic pathways of conversion for these three ginsenosides by intestinal bacteria are as follows: Rb1→Rd→F2→CK; Rb2→CO→CY→CK; and Rc→Mc1→Mc→CK. The resulting CK has been shown to inhibit lung metastasis of melanoma cells and *in vitro* tumor cell invasion and migration at non-toxic or marginally toxic concentrations (9).

Skin permeability of NFRG and FRG

A permeability study of NFRG and FRG through rat skin was carried out using Franz diffusion cells. The absorbances of the permeates through the rat skin are presented in Fig. 2, which shows that absorbance increased with respect to time. The skin permeability of FRG was higher than that of NFRG. Particularly, after 5 hr, the skin permeability of FRG was significantly ($p < 0.05$) higher compared to NFRG.

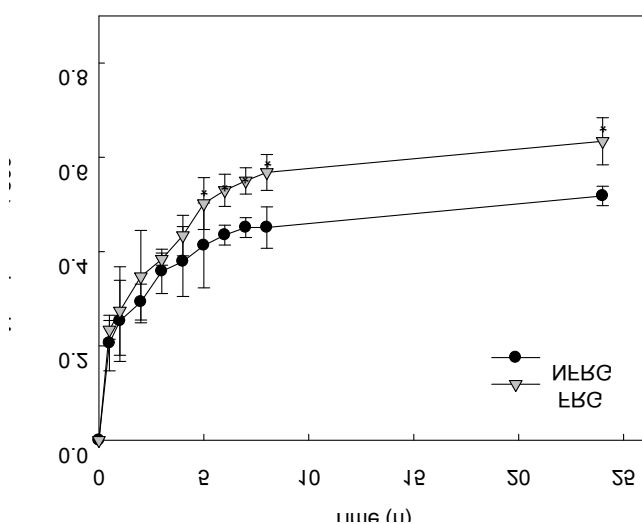


Fig. 2. Skin permeability of NFRG and FRG. 100 μ L of ginseng sample was placed on the donor side of a Franz cell. The receptor medium was kept at 37°C and stirred with a magnetic stirrer at 400 rpm. Aliquots (0.5 mL) of the receptor medium were withdrawn at 0.5 hr, 1~6 hr, and 24 hr. The absorbance of the permeates was measured at 203 nm. NFRG: non-fermented red ginseng, FRG: fermented red ginseng. *FRG showing a significant difference compared with NFRG ($p < 0.05$).

In general, the low bio-activity of extracts has limited the practicability of their industrial application in cosmetics. Therefore, several attempts have been made to enhance the bio-activity of extracts in various ways. The bio-active ingredients of plants, existing in the form of glycosides, are hydrophilic and soluble in water due to glycosyl groups. However, these properties of glycosides make them disadvantageous ingredients for skin cosmetics due to their low skin permeability. Meanwhile, aglycone ingredients are hydrophobic and can permeate human skin (23). Thus, the hydrolysis of glycoside ingredients into aglycone forms has attracted attention as an effective means for enhancing the bio-activity of extracts (24).

Pandjaitan et al. (25) studied the enzyme reaction of β -glucosidase to hydrolyze genistin (genestein 7-O- β -d-glucopyranoside) into its aglycone genestein based on varying concentration, pH, and temperature. As a result, genestein content was enhanced almost 50%. Investigations of bacterial fermentation have shown that intestinal degradation of protopanaxadiol ginsenosides proceeds stepwise via cleavage of sugar moieties, liberating mainly the monoglucosylated ginsenoside CK (8,26). This was verified in our study. The observed intestinal degradation product of protopanaxadiol ginsenosides suggests the presence of bacterial β -glucosidase enzymes that hydrolyze glycosidic linkages. By fermentation, ginsenoside metabolite contents can be enhanced about 260%.

Permeability of NFRG and FRG using everted intestinal sacs

The everted (gut) intestines of rats are a suitable *in vitro* model to study the intestinal transference of nutrients and drugs, and have been widely used (19). After 30 min, the transport level of FRG was slightly higher than that of NFRG, but there was no significant difference between them (Fig. 3). After 1 hr, FRG showed a higher ($p < 0.05$) transport level (10.31 mg of polyphenols/g sac) compared to NFRG (6.67 mg of polyphenols/g sac).

The mechanism of action of saponins on intestinal membranes *in vivo* is not yet clearly understood. Ingested saponins are exposed to many potential ligands in the intestine, such as bile salts, dietary cholesterol, and membrane sterols of mucosal cells as well as nutrients or antinutrients from food, all of which may reduce or enhance their effectiveness. It also remains to be confirmed whether traces of the compound itself enter the body through permeabilized membranes, even though all evidence up until now points to their non-absorption (27). However, the bodily absorption of saponin metabolites, produced in the intestine by micro-organisms, has

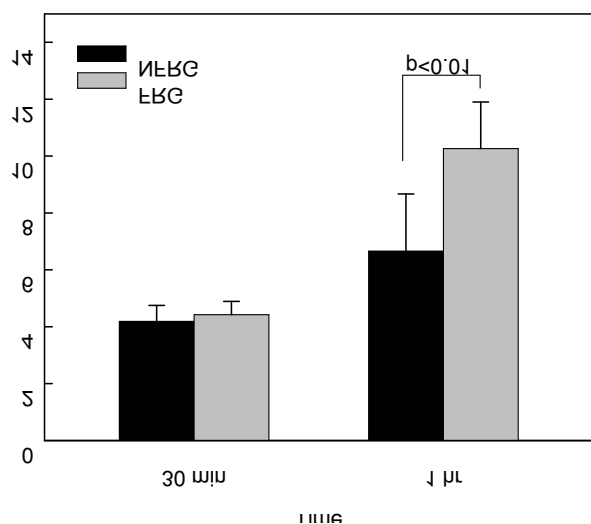


Fig. 3. Intestinal permeability of NFRG and FRG using the everted intestinal sac model. Intestinal transport of the ginseng sample is expressed as mg of polyphenols/g tissue dry weight. NFRG: non-fermented red ginseng, FRG: fermented red ginseng.

been demonstrated in ruminants (28) and human subjects (29). Therefore, FRG, which had a high level of ginsenoside metabolites, showed a high level of intestinal permeability when compared with NFRG.

Ginsenosides in the blood of rats orally administered NFRG and FRG

After oral administration of NFRG and FRG, serum concentrations were determined by HPLC (Fig. 4). For FRG, peak concentrations of Rk1, Rh1, Rc, and Rg5 were approximately 1.64, 2.35, 1.13, and 1.25-fold higher, respectively, compared to NFRG. Rk1 and Rc de-

creased more rapidly in the blood than Rg5, which increased with increasing time. Rk1, Rh1, and Rg5 increased more rapidly in the blood by oral administration of FRG than NFRG. Other ginsenosides were not detected in the blood after the administration of NFRG and FRG.

These products have great importance for the biological activity of ginseng since Rg3 shows strong vaso-relaxation properties and anti-platelet aggregation activity while Rg5 exhibits anti-cancer activity through the induction of apoptosis. Ginsenosides F4, Rg3, and Rg5, which are absent in raw ginseng, can be detected after steaming. In particular Rg3 and Rg5 are the most abundant in material that is steamed at 120°C, accounting for 39 and 19% of total ginsenoside content, respectively (30). From investigations of pathways, it was confirmed that the sugar on C-20 is more easily eliminated under acidic conditions than the sugars found at other positions (31). Ginsenoside Re is mainly hydrolyzed to yield Rg2 under acidic conditions, and then ginsenoside Rg2 is transformed to 20(R)-Rg2 by epimerization, or changed to ginsenosides F4 and Rg6 via a dehydration reaction. The intensities of other metabolites, such as ginsenosides Rg1 and Rh1, are quite low. Similar results were found in the acidic hydrolysis of ginsenoside Rb3, which is mainly converted to ginsenosides Rd, Rg3, 20(R)-Rg3, Rk1, and Rg5, and less to ginsenosides Rh2 and 20(R)-Rh2.

Inefficient absorption is one of several serious problems in the delivery of highly hydrophilic drugs. Low membrane permeability is an important factor dominating their poor absorption. Permeability is proportionally

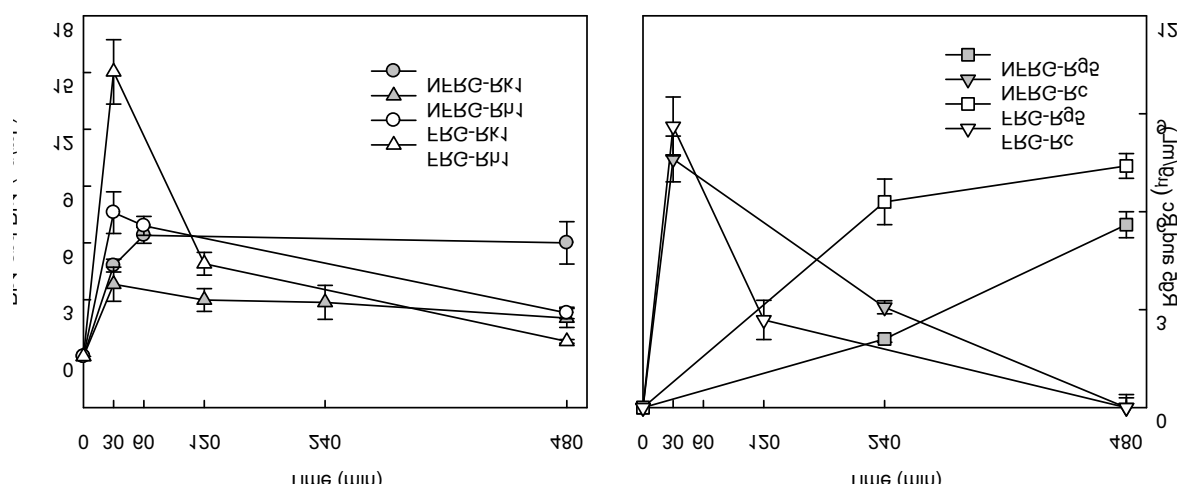


Fig. 4. Rk1, Rh1, Rc, and Rg5 contents in serum of SD rats orally administered non-fermented and fermented red ginseng. FRG or NFRG solution was administered orally at a dose of 8 g/kg to rats deprived of food but given free access to water for 18 hr before the experiments. Blood samples were collected at 30 min, 1 hr, 2 hr, 4 hr, and 24 hr after P.O. dosing. The blood samples were pretreated with solid phase extraction (SPE) cartridges. The ginsenosides were assayed using the above HPLC method. NFRG: non-fermented red ginseng, FRG: fermented red ginseng.

related to molecular size (molecular weight) or partitioning into the lipid cell membrane. Most ginsenosides are poorly absorbed from the gastrointestinal tract. After the oral administration of red ginseng extracts in healthy volunteers, plasma concentrations of ginsenosides were examined by EIA-HPLC and the results showed that ginsenoside Rb1 was not detected in the blood (32). Akao et al. (33) reported that when ginsenoside Rb1 (200 mg/kg) was administered orally to germ-free rats, neither CK nor any other metabolites were detected in the plasma, and the majority of the administered ginsenoside Rb1 was recovered from the intestinal tract, indicating its poor absorption. These results suggested that the absorption of naturally occurring ginsenosides is scant, and plasma concentrations necessary for exhibiting their reported pharmacological activities may be difficult to reach.

Orally ingested ginsenosides are activated by intestinal bacterial deglycosylation followed by fatty acid esterification. Therefore, the deglycosylation process of ginsenosides is crucial for their pharmacological expression. Indeed, bacterial ginsenoside-hydrolyzing potentials are shown to differ among humans and experimental mice, and it is easily hypothesized that individual differences in bacterial ginsenoside-hydrolyzing potentials may affect ginseng efficacy.

FRG showed dramatically improved bioavailability compared to NFRG as indicated by skin permeation, intestinal permeability, and ginsenoside levels in the blood. The significant increase in the bioavailability of FRG appears to have been due to the fermentation process and the transformation of its ginsenosides (ginsenoside metabolites) into more easily absorbed forms. FRG products containing ginsenoside metabolites may have more effective physiological activities than NFRG. Further studies comparing the physiological activities of FRG and NFRG are currently underway.

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