



Research article

Fermentation of red ginseng extract by the probiotic *Lactobacillus plantarum* KCCM 11613P: ginsenoside conversion and antioxidant effects



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ABSTRACT

Background: Ginsenosides, which are bioactive components in ginseng, can be converted to smaller compounds for improvement of their pharmacological activities. The conversion methods include heating; acid, alkali, and enzymatic treatment; and microbial conversion. The aim of this study was to determine the bioconversion of ginsenosides in fermented red ginseng extract (FRGE).

Methods: Red ginseng extract (RGE) was fermented using *Lactobacillus plantarum* KCCM 11613P. This study investigated the ginsenosides and their antioxidant capacity in FRGE using diverse methods.

Results: Properties of RGE were changed upon fermentation. Fermentation reduced the pH value, but increased the titratable acidity and viable cell counts of lactic acid bacteria. *L. plantarum* KCCM 11613P converted ginsenosides Rb₂ and Rb₃ to ginsenoside Rd in RGE. Fermentation also enhanced the antioxidant effects of RGE. FRGE reduced 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and reducing power; however, it improved the inhibition of β-carotene and linoleic acid oxidation and the lipid peroxidation. This suggested that the fermentation of RGE is effective for producing ginsenoside Rd as precursor of ginsenoside compound K and inhibition of lipid oxidation.

Conclusion: This study showed that RGE fermented by *L. plantarum* KCCM 11613P may contribute to the development of functional food materials.

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1. Introduction

Korean ginseng (*Panax ginseng* Meyer) is one of the most popular medicinal plants and has been used as a traditional medicinal plant in East Asian countries, including Korea, Japan, and China, for more than 2,000 yr [1,2]. Ginseng is cultivated for 4–6 yr and is classified according to the processing method: fresh ginseng is unprocessed, white ginseng is dried after peeling, and red ginseng is steamed and dried [3]. Red ginseng has various biological and pharmacological activities, including antiapoptotic, antitumor, immunomodulatory, antiallergic, anti-inflammatory, and antioxidant effects, which are enhanced by heating and steaming processes [4–6]. Red ginseng contains polysaccharides, polyacetylenes, polyphenolic compounds, and ginsenosides [7,8].

Ginsenosides, which are one of the pharmaceutical components of red ginseng, are divided into protopanaxadiol type (e.g., ginsenosides Rb₁, Rb₂, Rb₃, Rc, Rd, Rg₃, Rh₂, and compound K) and protopanaxatriol type (e.g., ginsenosides Re, Rf, Rg₁, Rg₂, and Rh₁, and oleanolic acid including ginsenoside Ro) [7–9]. So far, more than 150 ginsenosides have been identified in ginseng. Major ginsenosides, which include ginsenosides Rb₁, Rb₂, Rd, Re, and Rg₁, comprise more than 80% of the ginsenosides and have low pharmacological activities and low absorption in the human body. Some minor ginsenosides, including ginsenosides Rg₃, Rh₂, F₂, and compound K, are more pharmacologically active than major ginsenosides [5,10]. Major ginsenosides can be converted to more pharmacologically active ginsenosides using diverse methods including heating, mild acid hydrolysis, alkali hydrolysis, microbial

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conversion, and enzymatic treatment [11]. Previous studies have investigated the conversion of ginsenosides using various microorganisms, including *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Aspergillus* sp., *Lactobacillus* sp., and *Bifidobacterium longum* [9,12–15]. Many studies have demonstrated the biotransformation of major ginsenosides into smaller deglycosylated forms, e.g., Rb₁, Rb₂, and Rc to Rd; Re to Rg₁ and Rg₂, Rb₁ to Rg₃ and Rh₂; Rb₁ to Rd, F₂, and Rg₃; and Rb₁ to Rd and compound K [9,12,13,15].

It has been described that fermentation is an ideal process of biochemical alteration using microbial enzymes and microorganisms. Fermentation is conducted to improve the storage period, nutrition, and sensory characteristics related to foods [16]. *Lactobacillus plantarum* is used in starter cultures as a probiotic [17]. *L. plantarum* is tolerant to acid and bile salt, produces lactic acid, and has antioxidant activity [18]. Some strains of *L. plantarum* have been isolated from fermented foods [18]. The *L. plantarum* SY12 (also called *L. plantarum* KCCM 11613P) used in this study was isolated from kimchi, which is a fermented food, and identified as a probiotic strain. *L. plantarum* SY 12 has been reported to produce various enzymes, including β-glucosidase, β-galactosidase, and β-glucosaminidase, and has antiallergic effect [19].

The purpose of this study was to examine the bioconversion of ginsenosides into smaller deglycosylated forms by *L. plantarum* KCCM 11613P and prepare fermented red ginseng extract (FRGE) with improved antioxidant effects.

2. Materials and methods

2.1. Microorganism and materials

The lactic acid bacterium *L. plantarum* KCCM 11613P, which was isolated from kimchi in our laboratory, was used as a starter culture for the production of FRGE [19]. Red ginseng extract (RGE) was purchased from Fine Korea Co. (Seoul, Korea), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid, ascorbic acid, and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Gallic acid was purchased from Tokyo Chemical Industry Co., Ltd. (Kita-ku, Tokyo, Japan).

2.2. Fermentation of RGE

L. plantarum KCCM 11613P was cultivated in lactobacilli MRS (de Man, Rogosa, Sharpe) broth (Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 12 h, and the activated culture was inoculated into MRS broth at 37°C for 12 h, twice. One gram of RGE was added to 99 mL distilled water and then sterilized at 121°C for 15 min. The RGE solution (pH 6.5) was inoculated with *L. plantarum* KCCM 11613P culture [1×10^8 colony-forming units (CFU)/mL] to a final concentration of 1% (v/v) and fermented at 37°C for 24 h. Samples were collected for analysis at 0 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h. The viable cells of *L. plantarum* KCCM 11613P were counted on MRS agar using the plate counting method [20], and the pH and titratable acidity during fermentation were determined using a pH meter (inoLab pH 720, Weilheim, Germany) and following the method described by Collins et al [21].

2.3. Preparation of samples for high performance liquid chromatography analysis

Samples were extracted twice with water-saturated *n*-butanol and freeze-dried to eliminate the solvent [22]. Crude ginsenosides were dissolved in solution of mobile phase, and the samples were filtered (0.45 μm; Econofltr RegCel, Agilent Technologies, CA, USA) prior to high performance liquid chromatography (HPLC) injection.

2.4. HPLC analysis of ginsenosides

HPLC analysis was performed according to the method described by Chang et al [22] with modifications. The analysis was performed using an Agilent HPLC system (1100 Series). Ten microliters of each sample was injected into Poroshell 120 EC-C18 column (4.6 × 250 mm, 4 μm; Agilent Technologies). The elution profile was obtained using an ultraviolet /visible detector set at 203 nm. The operating temperature was set at 30°C, and the flow rate was 1.2 mL/min. Mobile phase A and B consisted of water (J.T. Baker, Avantor Performance Materials, Inc., PA, USA) and 100% acetonitrile (J.T. Baker), respectively. Samples were eluted with the following gradient: 0 min, 28% B; 3 min, 28.3% B; 5 min, 28.3% B; 8 min, 41.5% B; 11 min, 41.6% B; 14 min, 41.6% B; 15 min, 43% B; 20 min, 71% B.

2.5. Measurement of total phenolic content in FRGE

The total phenolic content was measured using the method described by Lee et al [23]. One hundred microliters of sample was mixed with 2 mL of a 2% sodium carbonate solution. After 3 min, 100 μL of 50% Folin & Ciocalteu's phenol reagent (Sigma-Aldrich Co.) was added to the mixture. After 30 min, the absorbance of samples was measured at 750 nm using a spectrophotometer (Optizen 2120UV Plus; Mecasys Co., Ltd, Daejeon, Korea). The total phenolic content was determined using a standard curve of gallic acid.

2.6. Measurement of antioxidant activity

2.6.1. DPPH radical scavenging activity assay

DPPH radical scavenging activity was determined using the method described by Lee et al [23]. Two hundred microliters of sample was mixed with 1 mL of 100 μM DPPH solution. After 15 min, the absorbance of the mixture was measured at 517 nm. DPPH radical scavenging activity was calculated as follows.

DPPH radical scavenging activity (%)

$$= \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

2.6.2. Reducing power assay

Reducing power was investigated according to the method described by Xiao et al [24] with modifications. One hundred microliters of sample was mixed with 500 μL of 0.2M sodium phosphate buffer (pH 6.6) and 500 μL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min and then cooled for 5 min. Next, 500 μL of 10% trichloroacetic acid was added, the mixture was centrifuged at 3,560g for 5 min, and 500 μL of the supernatant was mixed with 100 μL of 0.1% ferric chloride and 500 μL of distilled water. The absorbance was read at 700 nm.

2.6.3. β-Carotene bleaching assay

The β-carotene bleaching assay was performed according to a modification of the method reported by Park et al [25]. Two milligrams of β-carotene was dissolved in 10 mL chloroform and mixed with 44 μL linoleic acid and 200 μL Tween 80. Five milliliters of the solution was taken and concentrated to remove the chloroform. The concentrate was dissolved in 100 mL distilled water, and this solution was used as the β-carotene assay reagent. Five hundred microliters of sample was mixed with 4.5 mL reagent and incubated in water bath at 50°C for 6 h. The absorbance was read at 470 nm, and antioxidant activity was calculated as follows.

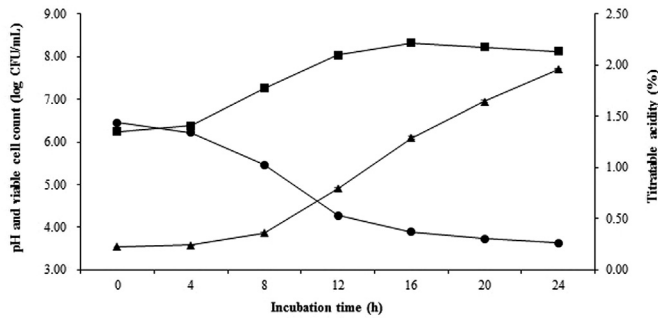


Fig. 1. Fermentation pattern of red ginseng extract by *Lactobacillus plantarum* KCCM 11613P. ●, pH; ■, Viable cell count (log CFU/mL); ▲, Titratable acidity (%). All values are presented as mean \pm standard deviation. CFU, colony forming units.

$$\text{Antioxidant activity (\%)} = \left(\frac{\beta\text{-Carotene content after 6 h}}{\text{Initial } \beta\text{-Carotene content}} \right) \times 100$$

2.6.4. Ferric thiocyanate assay

The ability to inhibit lipid peroxidation was evaluated using the method described by Jung et al [26]. One hundred microliters of sample was mixed with 200 μ L linoleic acid (25 mg/mL in ethanol), 400 μ L of 40mM potassium phosphate buffer (pH 7), and 200 μ L distilled water. The mixture was incubated at 37°C for 72 h, and the absorbance was read at 500 nm. One hundred microliters of the reaction mixture was mixed with 4 mL of 70% ethanol, 100 μ L of 30% ammonium thiocyanate, and 100 μ L of 20mM ferrous chloride (in 3.5% HCl). Inhibition of lipid peroxidation was calculated as follows.

$$\text{Inhibition of lipid oxidation (\%)} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

2.7. Statistical analysis

All experiments were performed three times independently, and three technical replicates of each of the samples were analyzed. The results are expressed as the mean \pm standard deviation. The obtained data were analyzed using the SPSS statistical software program version 18 (SPSS Inc., Chicago, IL, USA). Analysis of variance and Duncan's multiple range tests were performed to determine the significance of differences.

3. Results and discussion

3.1. Fermentation of RGE by *L. plantarum* KCCM 11613P

The pH values, titratable acidity, and viable cell number were measured during the fermentation of RGE by *L. plantarum* KCCM 11613P for 24 h. The characteristics of the fermentation are shown in Fig. 1. During fermentation, the pH value decreased from 6.45 to 3.64, and the titratable acidity increased from 0.23% to 1.96%. Previous studies have determined the physicochemical properties of the fermentation products of lactic acid bacteria. Lactic acid bacteria have been used for the starter culture of fermentation, and the pH of the products was reported to decrease owing to the production of lactic acid [24]. In another previous study, when a mixture of *Lactobacillus acidophilus* KFRI 128 and red ginseng was fermented by *Rhodiola sachalinensis*, the pH value decreased, whereas the titratable acidity increased [27].

The viable cell counts increased from 6.24 log CFU/mL to 8.31 log CFU/mL, and then decreased from 8.31 log CFU/mL to 8.12 log CFU/mL

after 16 h. Thus, the fermentation reached stationary phase after 16 h of culturing at 37°C. In a previous study, soy whey was fermented by *L. plantarum* B1-6, and a similar pattern in viable cell counts was observed [24]. When soy whey was fermented by *L. plantarum* B1-6, the viable cell number was the highest at 10 h, and then it decreased. In a previous study, when RGE was fermented using *L. acidophilus*, a similar pattern was observed, and the viable cell counts were highest at 16 h [28]. In our fermentation experiment, the viable cell counts of *L. plantarum* KCCM 11613P were highest at 16 h. Fermentation is thought to improve the biological effects of *L. plantarum* KCCM 11613P, and change ginsenosides Rb₂ and Rb₃ to ginsenoside Rd. FRGE was prepared for 16 h, and then we estimated the ginsenosides in the product and their antioxidant capacity compared to that of the control (unfermented RGE).

3.2. Changes in ginsenosides in RGE during fermentation

The ginsenosides in RGE were analyzed by HPLC. Fig. 2 shows the changes in ginsenoside compositions in the fermentation product. The unfermented RGE primarily contained ginsenoside Rb₂ and Rb₃ at a content of 52.67 ppm and 19.11 ppm, respectively. The amount of ginsenoside Rb₂ was greater than that of ginsenoside Rb₃. Ginsenoside Rb₂ was not detected in FRGE; however, ginsenoside Rd was newly detected and the amount of ginsenoside Rb₃ was increased. In FRGE, the content of ginsenoside Rb₃ and Rd was 149.86 ppm and 55.74 ppm, respectively. In a previous study, a mixture of ginsenosides (Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, and Rg₂) was fermented by lactic acid bacteria, including *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *B. longum*, *L. delbrueckii* subsp. *lactis*, and *Leuconostoc mesenteroides* subsp. *mesenteroides* for 5 d. After fermentation, the amount of ginsenosides changed, and that of ginsenosides Rb₁, Rb₂, and Rc decreased during fermentation [9]. In another study, red ginseng powder was fermented by *L. plantarum* M1 for 1 d, and the amount of ginsenoside compound K, Rh₂, Rh₁, Rg₅ + Rk₁, and Rg₃ increased, whereas that of ginsenoside Rg₂, Rg₁, Rf, Re, Rd, Rb₂ + Rc, and Rb₁ decreased [8]. The present study concluded that ginsenoside Rb₂ and Rb₃ were converted to ginsenoside Rd in the FRGE. Ginsenosides Rb₂ and Rb₃ were metabolized because the oral bioavailability of major ginsenosides such as ginsenosides Rb₁, Rb₂, and Rg₁ from the intestines is extremely low [29]. Ginsenoside Rd has pharmaceutical effects and is a precursor of other compounds such as Rg₃ and F₂ [30].

3.3. Measurement of total phenolic content and antioxidant activities of FRGE

The total phenolic content of RGE and FRGE samples is shown in Table 1. The total phenolic content in RGE and FRGE was 35.16 \pm 0.12 mg GAE/g and 37.67 \pm 0.37 mg GAE/g, respectively. Thus, the total phenolic content increased during fermentation and was higher in FRGE than in RGE. Xiao et al [24] reported that the total phenolic content increased during fermentation, when soy whey was fermented by *L. plantarum* B1-6. Jhan et al [31] reported that the total phenolic content increased after fermentation, when red beans were fermented by *B. subtilis* and *L. delbrueckii* subsp. *bulgaricus*. In addition, a previous study [32] showed that the total phenolic content in black ginseng fermented by *S. cerevisiae* was higher than that of raw ginseng and black ginseng. Therefore, our results were similar to those of the previous studies. It was previously demonstrated that the change in pH influenced the release of the bound phenolics and caused an increase in total phenolic content [33].

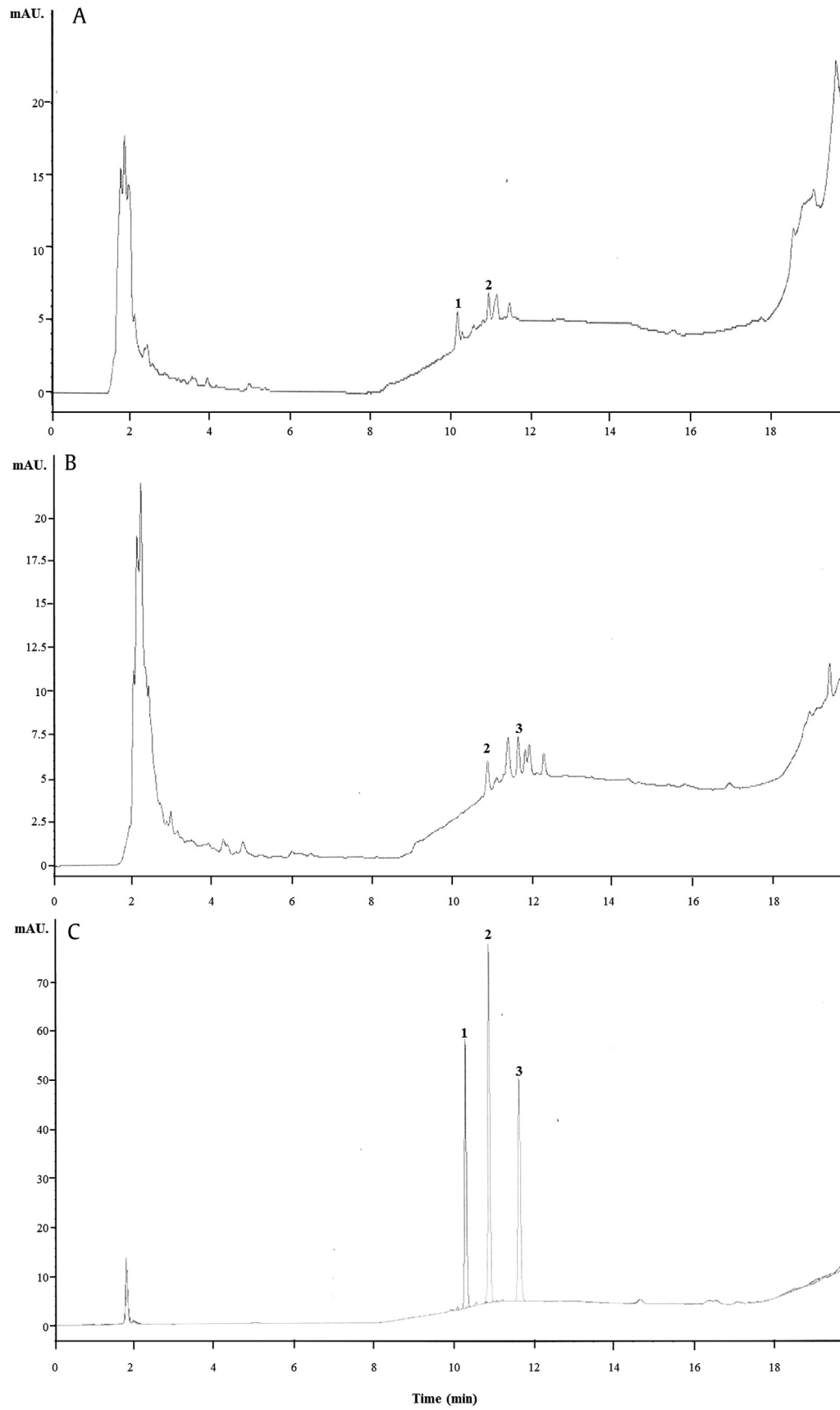


Fig. 2. Ginsenoside composition, as measured by HPLC. (A) In red ginseng extract. (B) In red ginseng extract fermented by *Lactobacillus plantarum* KCCM 11613P. (C) In standard ginsenosides. **1**, Rb₂; **2**, Rb₃; **3**, Rd.

Table 1
Total solids and total phenolic content of red ginseng extract fermented by *Lactobacillus plantarum* KCCM 11613P

Sample	Total solids (mg/g)	Total phenolic content (mg of gallic acid/g of sample)
Red ginseng extract (RGE)	7.99 ± 0.03	35.16 ± 0.12
Fermented red ginseng extract (FRGE)	7.84 ± 0.08	37.67 ± 0.37*

Values are presented as mean ± standard deviation

* $p < 0.05$

Antioxidant activities were determined by diverse methods, including the DPPH radical scavenging activity, reducing power, β -carotene bleaching, and ferric thiocyanate (FTC) assays.

The DPPH radical scavenging activity assay is the simplest experiment to determine antioxidant activity. In this assay, the purple chromogen radical DPPH is reduced by antioxidant compounds to pale yellow hydrazine [34]. The result of DPPH radical scavenging activity assay of RGE and FRGE is shown in Table 2. Both RGE and FRGE showed low DPPH radical scavenging activity. The DPPH radical scavenging activity of RGE at 0.25–4 mg/mL ranged from 4.33% to 35.54%, whereas that of FRGE ranged from 3.94% to

22.94%. For comparison, the antioxidant activity of positive control ascorbic acid (at 0.1 mg/mL) was 96.30 ± 0.18%. FRGE showed lower antioxidant activity than RGE. A previous study reported that red beans fermented by *B. subtilis* and *L. delbrueckii* subsp. *bulgaricus* had higher antioxidant activity than unfermented red beans [31]. Black ginseng fermented by *S. cerevisiae* for 25 h had stronger DPPH scavenging activity than unfermented black ginseng [32]. Another study noted that DPPH radical scavenging activity of bovine skim milk fermented by *Lactobacillus helveticus* was reduced by fermentation after 12 h [35]. These results indicated that the antioxidant activity of crude peptide extracts released during fermentation may have the ability to neutralize free DPPH radicals either by hydrogen atom or by direct reduction through electron transfer [36].

The reducing power assay is based on the conversion of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [37]. Fig. 3 shows the reducing power of RGE and FRGE samples. The absorbance of RGE and FRGE samples at 0.25–4 mg/mL ranged from 0.020 to 0.459 and from 0.012 to 0.399, respectively. The absorbance of the positive control ascorbic acid (at 0.1 mg/mL) was 0.610 ± 0.008. The absorbance of FRGE was lower than that of RGE, indicating that the reducing power of FRGE had decreased. In previous studies, the fermentation process formed reductants that reacted with free radicals for stabilization; therefore, fermented soy whey, red beans, and soy beans had higher reducing power [24,31,38]. However, our study showed

Table 2
Antioxidant effects of red ginseng extract and fermented red ginseng extract by *Lactobacillus plantarum* KCCM 11613P

Sample	Concentration (mg/mL)	DPPH radical scavenging activity (%)	Inhibition of β -carotene and linoleic acid oxidation (%)	Inhibition of lipid peroxidation (%)
Red ginseng extract	0.25	4.33 ± 0.12 ^a	30.38 ± 0.47 ^{ab}	46.30 ± 1.16 ^a
	0.5	8.01 ± 0.69 ^b	33.42 ± 0.93 ^{ab}	59.54 ± 0.70 ^b
	1	14.31 ± 0.44 ^c	35.43 ± 0.35 ^c	71.19 ± 2.49 ^c
	2	23.40 ± 0.48 ^d	43.19 ± 0.62 ^d	82.69 ± 2.06 ^d
	4	35.54 ± 1.20 ^e	47.27 ± 0.06 ^e	87.61 ± 0.65 ^e
Fermented red ginseng extract	0.25	3.94 ± 0.34 ^a	44.05 ± 0.81 ^{ab}	53.08 ± 1.54 ^a
	0.5	7.17 ± 0.67 ^b	47.92 ± 1.19 ^{ab}	71.83 ± 0.62 ^b
	1	12.81 ± 0.61 ^c	55.98 ± 0.36 ^c	79.17 ± 0.42 ^c
	2	17.78 ± 0.16 ^d	57.71 ± 0.10 ^d	87.77 ± 1.53 ^d
	4	22.94 ± 0.71 ^e	61.95 ± 0.34 ^e	93.99 ± 0.27 ^e
Ascorbic acid	0.1	96.30 ± 0.18		
BHT	0.1		80.91 ± 2.43	76.90 ± 0.70

Values are presented as mean ± standard deviation

^{a–e} Means in the same column followed by different letters represent significant differences by concentration ($p < 0.05$)

BHT, 2,6-di-*tert*-butyl-4-methylphenol; DPPH, 2,2-diphenyl-1-picrylhydrazyl; SD, standard deviation

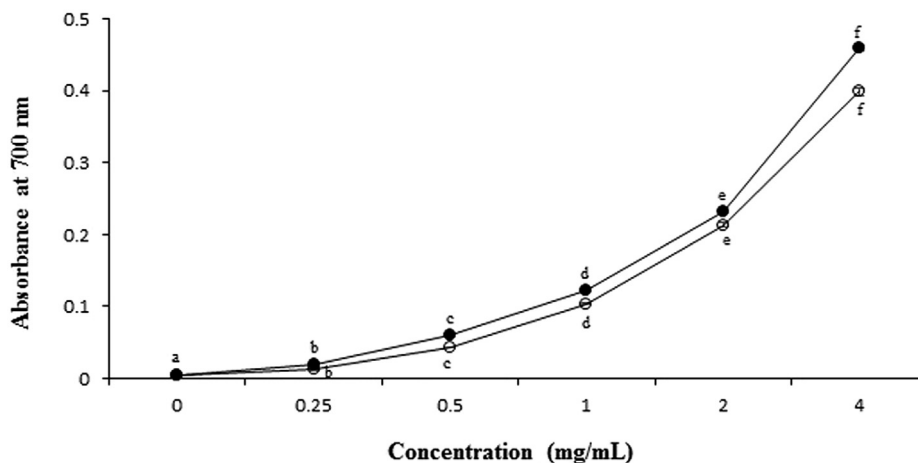


Fig. 3. Antioxidant activity of red ginseng extract fermented by *Lactobacillus plantarum* KCCM 11613P as determined by reducing power assay. All values are presented as mean ± standard deviation. ●, red ginseng extract; ○, fermented red ginseng extract. ^{a–f} Represent significant differences in concentration ($p < 0.05$).

opposite results—DPPH radical scavenging activity and reducing power were decreased after fermentation.

The β -carotene bleaching assay is conducted to assess the antioxidant capacity of extracts, and it determines the inhibition of the coupled auto-oxidation of linoleic acid and β -carotene [39]. The result of the β -carotene bleaching assay is presented in Table 2. The antioxidant activity of RGE and FRGE at 0.25–4 mg/mL ranged from 30.38% to 47.27% and from 44.05% to 61.95%, respectively. Antioxidant activity of BHT as positive control (at 0.1 mg/mL) was $80.91 \pm 2.43\%$. Previous studies reported that lactic acid fermentation increased the antioxidant activity of Magnolia flower petal extract and Malaysian herbal teas [25,40].

The FTC assay determines the content of peroxide production, and it assures the oxidation from ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) [23]. Table 2 shows the antioxidant activity of RGE and FRGE samples, as determined by the FTC assay. The antioxidant activity of RGE and FRGE at 0.25–4 mg/mL ranged from 46.30% to 87.61% and from 53.08% to 93.99%, respectively. The BHT as positive control (at 0.1 mg/mL) was $76.90 \pm 0.70\%$. These results showed that the antioxidant activity of FRGE was higher than that of RGE, and FRGE had strong antioxidant activity against lipid oxidation because of the increase in total phenolic content and ginsenoside Rd level. Earlier studies also reported that ginsenoside Rd is related to lipid peroxidation inhibitory activity, increase of antioxidant enzyme [41], and protection of neuronal cells from hydrogen peroxide and oxygen-glucose deprivation owing to oxidation suppression [42]. Phenolic compounds were shown to be a defensive barrier against lipid oxidation [43]. The phenolic compounds chelated metals in the lipid pathway and reacted with the carbonyl compounds generated by the lipid oxidation.

4. Conclusion

Ginsenosides have many pharmacological effects, such as cholesterol lowering, immune-stimulating, anticancer, and antioxidant activities. Fermentation of RGE with *L. plantarum* KCCM 11613P affected the pH, titratable acidity, viable cell number, total phenolic content, ginsenoside composition, and antioxidant effects. After fermentation, the pH value of RGE decreased, whereas its titratable acidity, lactic acid cell number, and total phenolic content increased. Ginsenoside composition was also changed by fermentation with *L. plantarum* KCCM 11613P. The levels of ginsenoside Rb₂ decreased, that of ginsenoside Rb₃ increased, and ginsenoside Rd was newly formed during fermentation. Antioxidant activity was assessed by different methods. FRGE showed strong inhibitory activity against the auto-oxidation of linoleic acid. This research demonstrated that FRGE by *L. plantarum* KCCM 11613P generated ginsenoside Rd and strongly inhibited lipid oxidation. Therefore, ginsenoside Rd in this fermented product might be used to convert to ginsenoside Rg₃, ginsenoside Rh₂, ginsenoside F₂, and compound K and to make inhibitor material in oxidation.

Conflicts of interest

The authors have no conflicts of interest with any parties or individuals.

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