

Physicochemical Characteristics and Antioxidant Capacity in Yogurt Fortified with Red Ginseng Extract

Jieun Jung¹, Hyun-Dong Paik¹, Hyun Joo Yoon¹, Hye Ji Jang¹, Renda Kankanamge Chaturika Jeewanthi¹,
Hee-Sook Jee², Xiang Li, Na-Kyoung Lee¹, and Si-Kyung Lee*

Department of Bioresources and Food Science, Konkuk University, Seoul 05029, Korea

¹Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea

²Food R&D Center, Ilhwa Co., Ltd., Guri 11933, Korea

Abstract

The objective of this study was to investigate characteristics and functionality of yogurt applied red ginseng extract. Yogurts added with red ginseng extract (0.5, 1, 1.5, and 2%) were produced using *Lactobacillus acidophilus* and *Streptococcus thermophilus* and stored at refrigerated temperature. During fermentation, pH was decreased whereas titratable acidity and viable cell counts of *L. acidophilus* and *S. thermophilus* were increased. The composition of yogurt samples was measured on day 1, an increase of red ginseng extract content in yogurt resulted in an increase in lactose, protein, total solids, and ash content, whereas fat and moisture content decreased. The pH value and cell counts of *L. acidophilus* and *S. thermophilus* were declined, however titratable acidity was increased during storage period. The antioxidant capacity was measured as diverse methods. During refrigerated storage time, the value of antioxidant effect was decreased, however, yogurt fortified with red ginseng extract had higher capacity than plain yogurt. The antioxidant effect was improved in proportion to concentration of red ginseng extract. These data suggests that red ginseng extract could affect to reduce fermentation time of yogurt and enhance antioxidant capacity.

Keywords: red ginseng extract, yogurt, physicochemical property, microbial property, antioxidant activity

Received April 7, 2016; Revised May 25, 2016; Accepted June 6, 2016

Introduction

Ginseng (*Panax ginseng* Meyer, Araliaceae) is frequently taken orally as a traditional herbal medicine in Asian countries, and its use has been increased worldwide. Ginseng contains various pharmacological components such as ginsenoside (saponin), polyacetylene, polyphenolic compound, and acidic polysaccharides and has numerous biological activities including antitumor and antidiabetic effect, enhancing immune and brain functions, and maintaining homeostasis (Choi, 2008; Kim *et al.*, 2010). There are two types of ginseng that are commonly used in the herbal medicine market, namely, white ginseng and red ginseng. Traditionally, white ginseng is produced by sun drying of fresh ginseng and red ginseng is manufactured by steaming at 95-100°C for 2-3 h and drying (Zhang *et al.*, 2012). The red ginseng has more effective pharmaco-

logical activities than white ginseng. The differences in the biological activities of two type of ginseng may result from changes of chemical structure in ginsenosides during the steaming processes (An *et al.*, 2011; Lee *et al.*, 2012).

Many consumers have increased an interest in functional foods for healthy and various attempts may be made in dairy products (Gonzalez *et al.*, 2011). Yogurt is defined as a food produced by culturing one or more of the optional dairy ingredients such as cream, milk, partially skimmed milk, and skim milk with a characteristic bacterial culture that contains the lactic acid-producing bacteria, that lactobacilli, bifidobacteria and *Streptococcus thermophilus* (Trachoo, 2002). *Lactobacillus acidophilus* is also used as culture for fermentation of milk because of its probiotic effects such as enhancing lactose digestion, lowering serum cholesterol levels, and preventing cancer (Olson and Aryana, 2008). *Streptococcus* sp. lead to the improvement of the nutritional content and digestibility of the fermented product (Xanthopoulos *et al.*, 2012). The development of dairy product with new flavors and health benefits has the potential to increase sales (Allgeyer *et al.*, 2010; Boeneke and Aryana, 2008). Some researches have

*Corresponding author: Si-Kyung Lee, Department of Bioresources and Food Science, Konkuk University, Seoul 05029, Korea. Tel: +82-2-450-3759; Fax: +82-2-455-3082, E-mail: lesikyung@konkuk.ac.kr

been conducted to supply and process various functional yogurt products supplemented with fruits flavor, fruits, vegetal-oil and fortification of nutrients (Gonzalez *et al.*, 2011; Hanson and Metzger, 2010; Perina *et al.*, 2015). Yogurt products have a positive health effects including assimilation of cholesterol, anti-tumorigenic effect, prevention of gastrointestinal infection and release of blood pressure (Ramchandran and Shah, 2009). In the present, many dairy companies have manufactured functional yogurt that protect stomach and liver and nutritional yogurt that fortify components such as calcium, vitamin, and fruits, and reduce fat content in Korea.

Large amounts of red ginseng products are produced and consumed in Korea. However, yogurt supplemented with red ginseng has not been developed yet. In addition, no scientific studies have been conducted on red ginseng-supplemented yogurt in Korea. Therefore, the aim of this study was to develop functional yogurt supplemented red ginseng extract through investigation of characteristics and produce dairy product having the high value.

Materials and Methods

Materials

Market milk and skim milk powder were purchased from Seoul Milk Co. (Korea), and red ginseng extract was purchased from Fine Korea Co. (Korea). *Lactobacillus acidophilus* and *Streptococcus thermophilus* was used as commercial yogurt starter culture (Culture Systems, Inc., USA). Pectin from citrus was purchased from Daejung Chemicals & Metals Co., Ltd. (Korea). Ascorbic acid and 3,5-di-tert-4-butylhydroxytoluene (BHT) were purchased from Sigma-Aldrich Corp. (USA).

Preparation of yogurt

Yogurts were produced with different concentrations of red ginseng extract (0.5, 1, 1.5, and 2%). Yogurt base was made of market milk and skim milk powder and 2% skim milk powder was fortified in milk. Yogurt base was mixed with red ginseng extract, and 0.1% pectin and then pasteurized at 90°C for 10 min. Pasteurized milk was cooled and inoculated with 0.02% commercial yogurt starter culture containing *L. acidophilus* and *S. thermophilus*. Inoculated milk was incubated at 40°C until a pH of 4.4 to 4.5 and then stored in a refrigerator (4°C) overnight.

Physicochemical and microbial properties during incubation and storage

Yogurt samples were incubated at 40°C for 12 h and

analyzed at 0, 2, 4, 6, 8, 10, and 12 h. Samples were analyzed pH, titratable acidity, and viable cell count. The pH value and titratable acidity were determined by using pH meter and the method of Collins *et al.* (1991), respectively. The viable cells of yogurt were determined by using selected medium. *L. acidophilus* was colonized on MRS medium containing 1% maltose and *S. thermophilus* was colonized on M17 medium containing 10% lactose (Ashraf and Shah, 2011). Also, yogurt samples were stored for 31 d at 4°C and collected at 1, 6, 15, 22, and 31 d. Samples were determined pH, titratable acidity, and viable cell counts of *L. acidophilus* and *S. thermophilus*.

Composition of yogurt base and yogurt

The composition of market milk was determined by MilkoScan™ minor (Type 78110; FOSS Analytical A/S, Denmark). The yogurt components, namely, lactose, protein, fat, moisture, total solids, and ash were measured by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2000). Color was measured using a colorimeter (Chroma Meter CR-400 series; Konica Minolta Sensing Inc., Japan).

Preparation of samples for antioxidant activity

The yogurt samples were centrifuged at 20,760 g at 4°C for 30 min and the supernatants were then filtered through a 0.45 µm filter (cellulose acetate, Toyo Roshi Kaisha Ltd., Japan) for analysis of antioxidant activity. The supernatants were stored in freezer at -20°C before determination of antioxidant activity. Ascorbic acid and BHT were used as a positive control at a concentration of 0.1 mg/mL.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

A modified method of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was used to assay (Ye *et al.*, 2013). The sample (200 µL) was mixed with 1 mL of 100 µM DPPH solution. The mixture was shaken and incubated for 15 min in the dark condition. The absorbance of the mixture was measured at 517 nm.

DPPH radical scavenging activity (%)

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

β-Carotene bleaching assay

The antioxidant activity of the yogurt was evaluated by the modified β-carotene bleaching assay (Shon *et al.*, 2003). A solution of β-carotene was prepared by dissolving 2 mg

of β -carotene, 44 μ L of refined linoleic acid, and 200 μ L of Tween 80 in 10 mL of chloroform. Then, 5 mL of this solution was transferred to a round bottom flask and chloroform was removed using a rotary evaporator at 40°C. The obtained mixture was diluted with 100 mL of distilled water. Next, 4.5 mL aliquots were transferred to test tubes containing 0.5 mL of sample. Each tube was placed in a water bath at 50°C. The absorbance of the reaction mixture was measured at 470 nm until it was discolored.

Antioxidant activity (%)

$$= \frac{\beta\text{-Carotene content after 6 h}}{\text{Initial } \beta\text{-carotene content}} \times 100$$

Ferric thiocyanate (FTC) assay

The antioxidant activity of the yogurt samples was also determined by the ferric thiocyanate (FTC) assay with a modification of the procedure reported previously (Lee *et al.*, 2004). Yogurt samples (100 μ L), 200 μ L of linoleic acid solution in ethanol (25 mg/mL), 400 μ L of phosphate buffer (40 mM, pH 7.0), and 200 μ L of distilled water were prepared as the reaction mixture. All mixtures were incubated at 37°C. At 0, 24, 48, and 72 h, 0.1 mL aliquots were taken from the mixtures and diluted with 4 mL of 70% ethanol, followed by addition of 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM FeCl₂ in 3.5% HCl. The absorbance was measured at 500 nm.

Sensory evaluation

Sensory evaluation of samples was carried out at 1 d and samples were yogurt fortified with red ginseng extract (0.5, 1, 1.5, and 2%). A trained panel of 27 members are composed of adult male (13, age ranged from 20 to 30) and female (14, age ranged from 20 to 30). The sensory

evaluations were carried out using quantitative descriptive analysis (Lawless and Heymann, 1999). The quantitative descriptive analysis was investigated sweetness, bitterness, sour taste, texture, flavor and after taste of yogurt and red ginseng extract having graphic line scale.

Statistical analysis

The obtained data were analyzed statistically using the SPSS statistical software program version 18 (SPSS Inc., USA). Analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine differences among results.

Results and Discussion

Physicochemical and microbial properties of yogurt samples during fermentation

The physicochemical properties such as pH and titratable acidity for the studied yogurts are included in Fig. 1. The pH and titratable acidity were affected significantly ($p < 0.05$). The pH value was decreased whereas titratable acidity was increased during fermentation. The pH of all samples was similar value in initial and final fermentation, but it was difference between yogurt and yogurt added with red ginseng extract (0.5, 1, 1.5, and 2%) during fermentation (Fig. 1A). The titratable acidity was difference in initial value (Fig. 1B), however this value became similar after 8 h because of the reduction of pH value. Previous study reported that the pH value declined whereas titratable acidity rose because of the production of acid and this results were similar to our results (Bae and Nam, 2006).

The viable cells of lactic acid bacteria were determined as microbial property. Fig. 2 presents *L. acidophilus* and

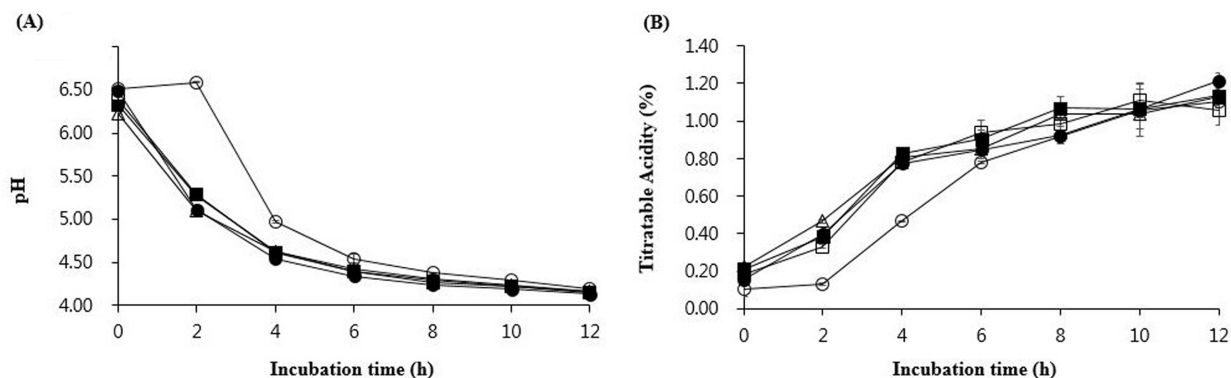


Fig. 1. The changes of (A) pH and (B) titratable acidity in yogurt fortified with red ginseng extract during fermentation. ○, control without red ginseng extract; ●, yogurt fortified with 0.5% red ginseng extract; □, yogurt fortified with 1% red ginseng extract; ■, yogurt fortified with 1.5% red ginseng extract; △, yogurt fortified with 2% red ginseng extract.

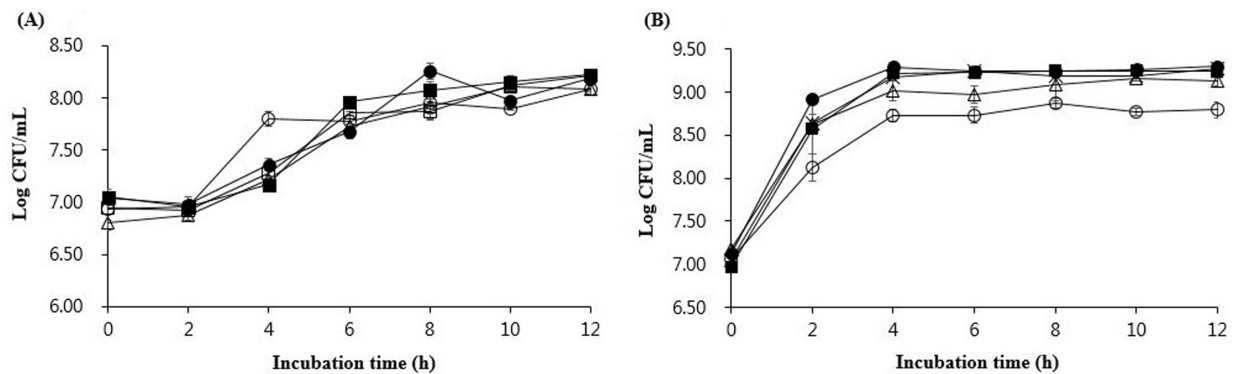


Fig. 2. The viable cells number of (A) *Lactobacillus acidophilus* and (B) *Streptococcus thermophilus* in yogurt fortified with red ginseng extract during fermentation. ○, control without red ginseng extract; ●, yogurt fortified with 0.5% red ginseng extract; □, yogurt fortified with 1% red ginseng extract; ■, yogurt fortified with 1.5% red ginseng extract; △, yogurt fortified with 2% red ginseng extract.

S. thermophilus in yogurt samples during fermentation. The viable cell counts of *L. acidophilus* and *S. thermophilus* ranged from 6.93 to 8.29 Log CFU/mL and 6.97 to 9.23 Log CFU/mL, respectively. The viable cells of *L. acidophilus* were similar level, however, the viable cell counts of *S. thermophilus* had a little increase in proportion to concentration of red ginseng extract (0.5, 1, 1.5, and 2%). Many researchers investigated about fermentation of ginseng and yogurt added with ginseng or red ginseng and their results had the similar pattern (Bae and Nam, 2006; Lee and Paek, 2003; Park *et al.*, 2006). The growth of lactic acid bacteria including *L. acidophilus* KCTC3150, *L. salivarius* subsp. *salivarius* CNU27, *Lactobacillus* sp., *Bifidobacterium longum* MG723, and mixed strains (*L. bulgaricus* KCTC3188 and *S. thermophilus* KCTC3658) was influenced by concentration of ginseng and red ginseng. A small amount of red ginseng extract promoted the growth of lactic acid bacteria, however, a large amount inhibited the growth of them (Bae *et al.*, 2005).

Composition of yogurt samples

The composition of yogurt base was 6.39% lactose,

3.78% protein, 3.72% fat, 14.43% total solids, pH 6.6, and 0.13% titratable acidity. The red ginseng extract consisted of 46.3% carbohydrate, 11.3% protein, and 0.8% fat. The composition of the yogurt supplemented with red ginseng extract was analyzed on 1 d. The lactose, protein, fat, moisture, total solids, and ash content of yogurt samples ranged from 1.51 to 2.24%, 3.93 to 4.73%, 2.60 to 3.17%, 84.43 to 85.55%, 14.45 to 15.57%, and 0.87 to 0.95%, respectively (Table 1). The component content of yogurt supplemented with red ginseng extract (0.5, 1, 1.5, and 2%) was significantly higher than that of control yogurt ($p < 0.05$). When the concentration of red ginseng extract increased, the content of lactose, protein, total solids, and ash in yogurt increased, however, the content of fat and moisture decreased. Composition of yogurt added sour cherry changed that the increase of sour cherry concentration had increase of total solids content, but fat content decreased as our data (Şengül *et al.*, 2012). Previous studies reported that fat content was decreased during fermentation because fat was changed to flavor compounds (Tuorila *et al.*, 1993; Ye *et al.*, 2013).

The color of yogurt supplemented with red ginseng extract (0.5, 1, 1.5, and 2%) was analyzed on 1 d (Table 2).

Table 1. Composition of yogurt fortified with red ginseng extract

Yogurt samples	Lactose (%)	Protein (%)	Fat (%)	Moisture (%)	Total solids (%)	Ash (%)
Control ¹⁾	1.51±0.14 ^a	3.93±0.34 ^a	3.17±0.14 ^b	85.55±0.71 ^c	14.45±0.71 ^a	0.87±0.01 ^a
RGY _{0.5}	1.70±0.15 ^a	4.21±0.51 ^{ab}	2.87±0.35 ^{ab}	84.95±0.07 ^b	15.05±0.07 ^b	0.88±0.01 ^b
RGY ₁	1.82±0.21 ^a	4.35±0.21 ^{ab}	2.69±0.09 ^{ab}	84.71±0.30 ^{ab}	15.29±0.30 ^{bc}	0.90±0.01 ^c
RGY _{1.5}	2.08±0.36 ^a	4.45±0.49 ^{ab}	2.59±0.05 ^a	84.42±0.10 ^a	15.58±0.10 ^c	0.94±0.00 ^d
RGY ₂	2.24±0.09 ^a	4.73±0.30 ^b	2.60±0.16 ^a	84.43±0.15 ^a	15.57±0.15 ^c	0.95±0.01 ^d

¹⁾Control, without red ginseng extract; RGY_{0.5}, with 0.5% red ginseng extract; RGY₁, with 1% red ginseng extract; RGY_{1.5}, with 1.5% red ginseng extract; RGY₂, with 2% red ginseng extract.

^{a-c}Means in the same column followed by different lower-case letters represent significant differences by red ginseng extract levels ($p < 0.05$).

Table 2. The color of yogurt fortified with red ginseng extract

Yogurt samples	L ²⁾	a	b
Control ¹⁾	93.99±0.60 ^e	-3.04±0.41 ^a	6.13±1.21 ^a
RGY _{0.5}	87.64±0.92 ^d	-0.31±0.10 ^b	15.49±0.09 ^b
RGY ₁	82.51±0.72 ^c	1.06±0.06 ^c	18.83±0.36 ^c
RGY _{1.5}	79.51±0.76 ^b	1.90±0.06 ^d	21.24±0.71 ^d
RGY ₂	77.86±0.64 ^a	2.46±0.06 ^e	22.42±0.60 ^e

¹⁾Control, without red ginseng extract; RGY_{0.5}, with 0.5% red ginseng extract; RGY₁, with 1% red ginseng extract; RGY_{1.5}, with 1.5% red ginseng extract; RGY₂, with 2% red ginseng extract. ²⁾L, darkness-lightness (0~100); a, greenness-redness (-60~60); b, blueness-yellowness (-60~60).

^{a-e}Means in the same column followed by different lower-case letters represent significant differences by red ginseng extract levels ($p<0.05$).

The L, a, and b values indicate changes from darkness to lightness, greenness to redness, and blueness to yellowness, respectively. Compared to the yogurts, the L values decreased from 93.99 to 77.86, whereas a and b values increased from -3.04 to 2.46 and 6.13 to 22.42, respectively, when the concentration of the red ginseng extract increased from 0 to 2%. In correlation with the amount of red ginseng extract, lightness decreased, whereas redness and yellowness increased. Thus, the color values of yogurt were affected by the addition of red ginseng extract. Kim

et al. (2008) found that color was influenced by the concentration of red ginseng extract.

Changes of yogurt during storage

The pH of the yogurt samples on 1 d ranged from 4.43 to 4.46 (Table 3). Significant differences were noted between the samples over the storage period, the pH of the yogurt decreased during storage ($p<0.05$) ranging from 4.15 to 4.29 on 31 d. The titratable acidity of control and yogurt supplemented with red ginseng extract increased significantly during the storage period (Table 3). There were no significant differences in titratable acidity between control and samples supplemented with red ginseng extract. The viable cell counts of *L. acidophilus* and *S. thermophilus* in yogurts supplemented with red ginseng extract were determined for 31 d. The changes of lactic acid bacteria of yogurt samples are represented in Table 3. At 1 d of storage, the microbial counts of *L. acidophilus* and *S. thermophilus* were not significantly different between control and yogurt supplemented with red ginseng extract; they ranged from 9.04 to 9.49 Log CFU/mL and 9.00 to 9.30 Log CFU/mL, respectively. The viable cell counts of *L. acidophilus* and *S. thermophilus* were decreased slightly during storage time, and they were ranged from 8.84 to 9.04 Log CFU/mL and 8.76 to 9.04 Log CFU/mL on 31 d.

Table 3. Changes of pH, titratable acidity, and count of lactic acid bacteria in yogurt fortified with red ginseng extract during storage at 4°C

	Samples	1 d	6 d	15 d	22 d	31 d
pH	Control ¹⁾	4.43±0.01 ^{aE}	4.35±0.01 ^{aD}	4.25±0.01 ^{aC}	4.18±0.01 ^{aB}	4.15±0.01 ^{aA}
	RGY _{0.5}	4.44±0.01 ^{bE}	4.32±0.01 ^{bD}	4.24±0.01 ^{bC}	4.24±0.00 ^{bB}	4.20±0.01 ^{bA}
	RGY ₁	4.44±0.01 ^{cE}	4.35±0.01 ^{cD}	4.29±0.01 ^{cC}	4.28±0.01 ^{cB}	4.25±0.01 ^{cA}
	RGY _{1.5}	4.43±0.01 ^{dE}	4.36±0.01 ^{dD}	4.31±0.00 ^{dC}	4.32±0.01 ^{dB}	4.28±0.01 ^{dA}
	RGY ₂	4.46±0.01 ^{dE}	4.36±0.01 ^{dD}	4.31±0.01 ^{dC}	4.29±0.01 ^{dB}	4.29±0.01 ^{dA}
Titratable acidity (%)	Control	0.88±0.01 ^{aA}	0.98±0.01 ^{aB}	1.11±0.03 ^{aC}	1.18±0.01 ^{aD}	1.12±0.01 ^{aC}
	RGY _{0.5}	0.96±0.01 ^{aA}	0.92±0.01 ^{aB}	1.09±0.01 ^{aC}	1.16±0.01 ^{aD}	1.12±0.01 ^{aC}
	RGY ₁	0.92±0.01 ^{aA}	1.00±0.01 ^{aB}	1.13±0.01 ^{aC}	1.13±0.01 ^{aD}	1.12±0.01 ^{aC}
	RGY _{1.5}	0.93±0.01 ^{aA}	1.02±0.01 ^{aB}	1.11±0.01 ^{aC}	1.13±0.01 ^{aD}	1.10±0.03 ^{aC}
	RGY ₂	0.98±0.00 ^{bA}	1.03±0.01 ^{bB}	1.14±0.02 ^{bC}	1.12±0.01 ^{bD}	1.10±0.01 ^{bC}
<i>L. acidophilus</i> (Log CFU/mL)	Control	9.04±0.01 ^{abD}	8.49±0.37 ^{abA}	8.93±0.02 ^{abB}	9.15±0.02 ^{abC}	9.04±0.04 ^{abB}
	RGY _{0.5}	9.49±0.04 ^{cd}	8.84±0.04 ^{ca}	8.86±0.04 ^{cb}	9.14±0.06 ^{cc}	8.89±0.04 ^{cb}
	RGY ₁	9.11±0.02 ^{bcD}	8.83±0.08 ^{bcA}	9.08±0.03 ^{bcB}	8.98±0.13 ^{bcC}	8.91±0.03 ^{bcB}
	RGY _{1.5}	9.16±0.05 ^{abD}	8.74±0.07 ^{abA}	8.94±0.05 ^{abB}	9.01±0.04 ^{abC}	8.93±0.05 ^{abB}
	RGY ₂	9.05±0.09 ^{ad}	8.63±0.07 ^{aA}	8.99±0.05 ^{aB}	8.90±0.07 ^{aC}	8.84±0.03 ^{aB}
<i>S. thermophilus</i> (Log CFU/mL)	Control	9.00±0.02 ^{aD}	8.94±0.01 ^{aC}	8.83±0.05 ^{aA}	8.81±0.12 ^{aB}	8.76±0.05 ^{aA}
	RGY _{0.5}	9.30±0.05 ^{cd}	9.12±0.03 ^{cc}	8.81±0.03 ^{ca}	9.21±0.03 ^{cb}	9.04±0.03 ^{ca}
	RGY ₁	9.20±0.03 ^{cd}	9.14±0.02 ^{cc}	9.04±0.03 ^{ca}	9.05±0.07 ^{cb}	9.01±0.03 ^{ca}
	RGY _{1.5}	9.13±0.02 ^{bd}	9.12±0.02 ^{bc}	8.90±0.17 ^{ba}	9.06±0.11 ^{bb}	8.94±0.05 ^{ba}
	RGY ₂	9.10±0.01 ^{bd}	9.07±0.02 ^{bc}	8.94±0.07 ^{ba}	8.89±0.05 ^{bb}	8.94±0.03 ^{ba}

¹⁾Control, without red ginseng extract; RGY_{0.5}, with 0.5% red ginseng extract; RGY₁, with 1% red ginseng extract; RGY_{1.5}, with 1.5% red ginseng extract; RGY₂, with 2% red ginseng extract.

^{a-d}Means in the same column followed by different lower-case letters represent significant differences by red ginseng extract levels ($p<0.05$).

^{A-E}Means in the same line followed by different upper-case letters represent significant differences by period ($p<0.05$).

Antioxidant activity

The antioxidant activity of yogurts supplemented with red ginseng extract was determined by the DPPH radical scavenging activity, β -carotene bleaching activity, and FTC test.

The DPPH radical scavenging activity is easy and potentially accurate with regard to measuring the free radical scavenging activity of beverages. This methodology is based on the reduction of the stable free radical DPPH that strongly absorbs at 517 nm, to the corresponding hydrazine by the free radical scavenging activity of the anti-radicals (Sendra *et al.*, 2006). The results for the DPPH radical scavenging activity of the yogurt are presented in Table 4. The antioxidant activity of yogurt supplemented with red ginseng extract (89.44 to 94.80%) was significantly higher than that of control sample (63.63%). The DPPH radical scavenging activity of the yogurt increased in proportion to increasing red ginseng extract concentration. Significant differences in the DPPH radical scavenging activity were found among the yogurt ($p < 0.05$). The antioxidant activity of red ginseng extract (0.5, 1, 1.5, and 2%) was 67.14, 91.41, 92.67, and 91.21%, respectively. The DPPH radical scavenging activity of 0.1 mg/mL of ascorbic acid was $96.29 \pm 0.18\%$. The skim milk fermented with *Lactobacillus plantarum* Gr1 and Gr2 showed DPPH radical scavenging activity of 50% (Abubakr *et al.*, 2012). However, our data showed that milk with skim milk powder fermented with *L. acidophilus* and *S. thermophilus* showed strong DPPH radical scavenging activity over 94%. Red ginseng extract had DPPH radical scavenging activity and the value was different according to extraction method. Also, DPPH radical scavenging activity of red ginseng extract depended on concentration like our results (Kim *et al.*, 2008). The antioxidant activity remained over

85% in yogurt supplemented with red ginseng extract during storage. Amirdivani and Baba (2011) and Şengül *et al.* (2012) found that herbal yogurt and yogurts supplemented with sour cherry pulp had higher antioxidant activity than plain yogurt and that the antioxidant activity increased during 7 d of storage.

The β -carotene bleaching assay is widely used to measure antioxidant activity. It is based on the principle that lipid radicals as auto-oxidation products of linoleic acid attack double bonds of β -carotene, leading to its oxidation and discoloration; however, in the presence of antioxidative substances and depending on their antioxidant ability, β -carotene retains its yellowish-orange color (Kato *et al.*, 2009). The total antioxidant activity of 5 yogurt samples is presented in Table 4. The high absorbance values indicated that the yogurts possessed antioxidant activity. Yogurt samples supplemented with red ginseng extract had higher antioxidant activity than that of control yogurt; and the results were significantly different ($p < 0.05$). The antioxidant activity of red ginseng extract (0.5, 1, 1.5, and 2%) was 16.29, 19.97, 25.09, and 26.38%, respectively. Antioxidant activity of BHT (0.1 mg/mL) was $80.91 \pm 2.42\%$ by β -carotene bleaching assay.

The FTC test was based on determination of the amount of peroxide produced at the initial stage of lipid peroxidation, a lower absorbance indicates a higher level of antioxidant activity (Lee *et al.*, 2004). Fig. 3 shows the changes in the absorbance for each fraction during incubation at 37°C. The absorbance of the control and test samples increased in proportion to the incubation time. The antioxidant activity of red ginseng extract-supplemented yogurt was always higher than that of control yogurt. Comparison of the different yogurt revealed that the antioxidant activity increased depending on the concentration of

Table 4. Antioxidant capacity of yogurt fortified with red ginseng extract during storage at 4°C

Samples		1 d	6 d	15 d	22 d	31 d
DPPH scavenging activity (Inhibition value, %)	Control	62.50 \pm 4.82 ^{aC}	62.49 \pm 5.35 ^{aC}	62.68 \pm 4.02 ^{aB}	59.02 \pm 4.84 ^{aB}	55.93 \pm 2.22 ^{aA}
	RGY _{0.5}	94.46 \pm 2.34 ^{bC}	92.42 \pm 1.58 ^{bC}	88.00 \pm 2.70 ^{bB}	86.82 \pm 2.50 ^{bB}	85.91 \pm 3.05 ^{bA}
	RGY ₁	94.85 \pm 0.11 ^{cC}	94.56 \pm 0.35 ^{cC}	94.02 \pm 0.85 ^{cB}	93.54 \pm 1.25 ^{cB}	90.19 \pm 2.04 ^{cA}
	RGY _{1.5}	94.85 \pm 0.07 ^{cC}	94.68 \pm 0.22 ^{cC}	94.55 \pm 0.10 ^{cB}	94.26 \pm 0.24 ^{cB}	92.25 \pm 2.74 ^{cA}
	RGY ₂	94.26 \pm 0.31 ^{cC}	94.23 \pm 0.29 ^{cC}	94.17 \pm 0.26 ^{cB}	94.17 \pm 0.28 ^{cB}	94.09 \pm 0.28 ^{cA}
β -Carotene bleaching activity (Inhibition value, %)	Control	45.73 \pm 4.97 ^{aC}	40.86 \pm 4.14 ^{aC}	34.50 \pm 1.42 ^{aB}	32.59 \pm 2.61 ^{aB}	32.35 \pm 2.51 ^{aA}
	RGY _{0.5}	51.30 \pm 2.09 ^{bC}	50.69 \pm 1.89 ^{bC}	46.87 \pm 3.54 ^{bB}	44.26 \pm 3.65 ^{bB}	38.05 \pm 3.00 ^{bA}
	RGY ₁	50.69 \pm 6.02 ^{bC}	48.09 \pm 5.98 ^{bC}	46.89 \pm 5.09 ^{bB}	46.64 \pm 5.81 ^{bB}	44.61 \pm 6.89 ^{bA}
	RGY _{1.5}	53.48 \pm 0.97 ^{cC}	52.49 \pm 0.42 ^{cC}	51.55 \pm 0.67 ^{cB}	50.27 \pm 0.84 ^{cB}	46.71 \pm 3.88 ^{cA}
	RGY ₂	50.25 \pm 1.92 ^{bC}	49.59 \pm 1.49 ^{bC}	47.49 \pm 3.13 ^{bB}	46.82 \pm 2.46 ^{bB}	45.07 \pm 2.73 ^{bA}

¹⁾Control, without red ginseng extract; RGY_{0.5}, with 0.5% red ginseng extract; RGY₁, with 1% red ginseng extract; RGY_{1.5}, with 1.5% red ginseng extract; RGY₂, with 2% red ginseng extract.

^{a-c}Means in the same column followed by different lower-case letters represent significant differences by red ginseng extract levels ($p < 0.05$).

^{A-C}Means in the same line followed by different upper-case letters represent significant differences by period ($p < 0.05$).

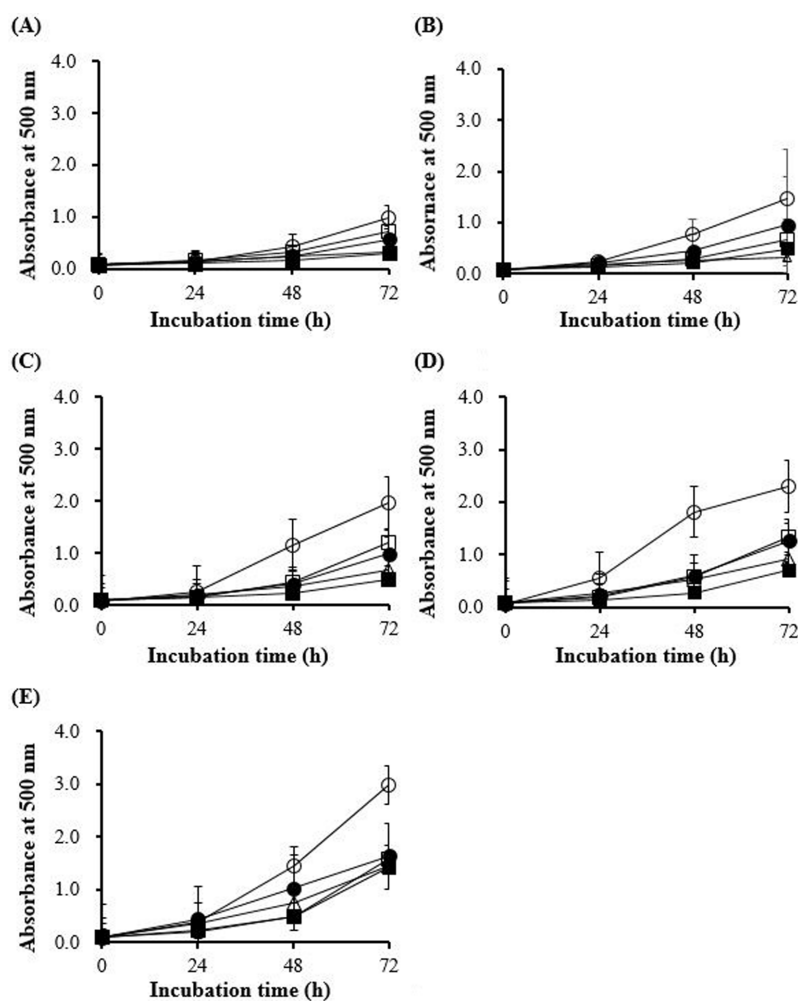


Fig. 3. Antioxidant activity of yogurt fortified with red ginseng extract during storage at 4°C determined by ferric thiocyanate assay. (A), 1 d; (B), 6 d; (C), 15 d; (D), 22 d; (E), 31 d. ○, control without red ginseng extract; ●, yogurt fortified with 0.5% red ginseng extract; □, yogurt fortified with 1% red ginseng extract; ■, yogurt fortified with 1.5% red ginseng extract; △, yogurt fortified with 2% red ginseng extract.

red ginseng extract, ranging from 38.41 to 92.80% on 1 d. However, a decrease in the antioxidant activity during storage was observed. On 6 d, yogurt did not show antioxidant activity, but yogurt supplemented with red ginseng extract showed antioxidant activity, ranging from 23.64 to 75.06%. The yogurts added with red ginseng extract had antioxidant effect until 22 d. BHT (0.1 mg/mL) had 76.89 ± 0.69% antioxidant activity in FTC test. The previous study reported that whey of skim milk fermented with *L. plantarum* Gr1 and Gr4 was measured by ferrous chelating activity (FCA) and the FCA value was 94% (Abubakr *et al.*, 2012).

Sensory evaluation

The yogurt samples were mixed with syrup that decreases bitter taste of red ginseng extract. Results from sen-

sory evaluation did not show significant differences ($p > 0.05$) in yogurt fortified with red ginseng extract (data not shown). An increase in red ginseng extract concentration resulted in increased bitterness, flavor of red ginseng extract, and after taste of red ginseng extract. However, the sweetness, flavor of yogurt, and after taste of yogurt were felt weakly.

Conclusion

The addition of red ginseng extract (0.5, 1, 1.5, and 2%) in yogurt affected to pH, titratable acidity, viable cell counts, and composition. The antioxidant activity was determined by method of different mechanism. Yogurt samples had the weak activity that inhibit auto-oxidation of linoleic acid in β -carotene bleaching assay, however, sam-

ples had the strong activity that free radical scavenging activity and inhibition of lipid peroxidation. The antioxidant activity was increased as an increase of red ginseng extract concentration. However, the antioxidant activity of yogurt fortified with red ginseng extract (0.5, 1, 1.5, and 2%) was higher than control yogurt. We found that it was to improve antioxidant activity because red ginseng extract supported the growth of lactic acid bacteria.

Acknowledgements

This paper was supported by Konkuk University in 2015.

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