Research Article
J. Ginseng Res. Vol. 34, No. 3, 168-174 (2010)
DOI:10.5142/jgr.2010.34.3.168

JGR Journal of Ginseng Research

Antioxidant Activity and Ginsenoside Pattern of Fermented White Ginseng

Seong-Il Lim[1], Chang-Won Cho[1], Ung-Kyu Choi[2], and Young-Chan Kim[1*]

[1]Korea Food Research Institute, Seongnam 463-746, Korea
[2]Pohang Center for Evaluation of Biomaterials, Pohang 790-834, Korea

Ethanol and water extracts of white and fermented ginseng were prepared and their ginsenoside composition and antioxidant effects were assessed. The main ginsenosides in white ginseng were $Rb_1 > Re > Rg_1$, and those in fermented ginseng were $Rb_2 + Rb_3 > Rd > Rg_1$. Ginsenosides Rd and Rg_3 in fermented ginseng were enriched 11 and 58 times, respectively, over that in white ginseng through fermentation with five *Bacillus* spp. The greatest levels of 2-deoxyribose and superoxide anion dismutase-like activities were found in 50% ethanol extracts of fermented ginseng. Thus, these data suggest that white ginseng has the greatest free radical scavenging activity and that fermented ginseng has the highest antioxidant activity.

Keywords: White ginseng, Fermented ginseng, Antioxidant, Ginsenosides

INTRODUCTION

Ginseng (*Panax ginseng* C.A. Meyer), one of the most widely studied medicinal herbs, contains saponin, phenolic compounds, polyacetylene, alkaloids, and polysaccharides [1]. The discovery of saponins as the active substance has led to the elucidation of ginseng's many pharmacological properties, such as antidiabetic effects [2], cardiovascular system dysfunction improvement [3], liver-protective effects [4], and various types of anti-stress activities [5]. However, with polyacetylene substances in ginseng being discovered to inhibit the growth of cancer cells, studies on the physiological effects of other active substances in ginseng have flourished [6]. In addition, reports show that saponin purified from ginseng has either no or only weak antioxidant activity, leading to the suggestion that phenolic compounds rather than saponins are responsible for ginseng's antioxidant properties [7].

Ten phenolic acids, including ferulic, and cinnamic and caffeic acids have been identified in ginseng. Maltol, a unique substance of red ginseng produced during the manufacture of red ginseng from fresh ginseng, is known to have potent antioxidant activity [8]. Also, phenolic compounds such as maltol, salicylic acid, and vanillic acid, which are of lower molecular weight than ginsenosides Rg_1, Re, and Rb_1, have stronger antioxidant effects [9]. Furthermore, ginseng enhances the activities of endogenous anti-oxidative enzymes such as superoxide dismutase (SOD), catalase, peroxidase, and glutathione peroxidase. When ginseng extracts were administered into Sprague–Dawley rats for 24 hours, liver SOD, catalase, and glutathione peroxidase activities increased significantly [10]. Furthermore, red ginseng extract increased SOD and peroxidase and catalase activities after ICR male mice were gamma-irradiated [11]. In addition, red ginseng extract administration enhanced endogenous SOD activity and significantly decreased the serum malondialdehyde level [12,13].

Thus far, about 30 different types of ginsenosides

Received 20 Jan. 2010, **Revised** 27 Jul. 2010, **Accepted** 29 Jul. 2010

***Corresponding author**
E-mail: yckim@kfri.re.kr
Tel: +82-31-780-9145, Fax: +82-31-709-9876

have been identified in ginseng [14]. These compounds are classified as diols, triols, or according to their aglycon structural characteristics. Diols and triols account for the majority of ginsenosides, and only ginsenoside Ro (0.6% of all ginsenosides) is classified as an oleanane [15]. The most prevalent ginsenosides are the diol-type Rb_1, Rb_2, Rc, and Rd, and triol type ginsenosides Rg_1 and Re. Ginsenoside Rf is absent from *Panax quinquefolius*, or American ginseng [16]. Ginsenosides Rb_1 and Rg_1 are the main index ingredients of ginseng, and these are used for standardization and quality control of health-promoting food production [17] and Korean standard (KS H 2153) [18] nationally, as well as internationally, e.g., in the *Codex Alimentarius*.

Consumer studies suggest that quality improvement entices consumers to purchase ginseng, even at a higher cost [19]. Hence, this study aimed to enhance the nutritional and functional quality of ginseng via fermentation of white ginseng using beneficial bacteria, and to convert ginseng saponins (ginsenosides) using microbial glucose lyases.

Ginsenoside structure has been elucidated [20,21], and numerous studies have uncovered a variety of beneficial effects such as anticancer activities [22,23]. Fermented ginseng has the advantage of being able to provide probiotic bacteria, and can lyse and convert ginsenosides, allowing more efficient absorption. Bacterial conversion of ginsenosides has been well documented [24-27], and *Bacillus* sp. are known to produce a plethora of degradative enzymes [28-30].

Therefore, this study used solid fermentation with *Bacillus* rather than standard ginseng liquid culture media. For solid fermentation, *Bacillus* was inoculated and heat-treated (85°C) to eliminate non-spore-forming bacteria. This was followed by the addition of ginseng powder and fermentation for 15 days. Saponin was then extracted and analyzed to evaluate ginsenoside conversion, and the antioxidant activities of fermented ginseng were compared to those of non-fermented white ginseng.

MATERIALS AND METHODS

Reagents

Ginseng powder produced from Poongi Agricultural Producers Union (Chun-Je Myoung ginseng powder; Poongi, Korea) was used in this study.

Microbial stock preparation

Prior to solid fermentation, microbial stocks were prepared using *Bacillus* spp. KS-25 (KCTC 11351BP), *B. subtilis* KS-29 (KCTC 11352BP), *B. licheniformis* KS-30 (KCTC 11353BP), *B. sonorensis* KS-33 (KCTC 11354BP), and *B. circulans* KS-80 (KCTC 11355BP) obtained from the Korea Gene Bank (Daejeon, Korea). Stock was prepared by adding water to a bacterial mixture (0.01 kg), rice bran (4 kg), molasses (2 kg), and brown sugar (4 kg; final mass 100 kg). The mixture was incubated at 22.5±2.5°C with a cycle of 2 hour aeration (10 m^3/h) and 4 hour non-aeration for 21 days.

Ginseng fermentation

Fermented ginseng was produced by mixing stock solution (1/10,000 of ginseng powder [w/w]) and sterilized water (15% of ginseng powder [w/w]), followed by heat treatment at 85°C for 2 hours. Ginseng powder was then added, homogenized, and fermented for 15 days at room temperature. Finally, fermented ginseng was dried for 4 hours at 85°C (Fig. 1).

Saponin preparation

Samples (5 g) were transferred to the extractor apparatus. After the addition of water-saturated butanol (50 mL), samples were extracted three times at 80°C for 3 hours. After extraction, the sample was filtered (Whatman No. 2), and the filtrate transferred to a 250 mL separating funnel and washed with distilled water. The butanol layer was then vacuum-dried and concentrated *in vacuo*. Ether (50 mL) was then added and samples extracted at 36°C for 30 minutes to eliminate lipid components. The debris remaining was dried at 105°C for 30 minutes, dissolved in methanol (25 mL), filtered (0.45 μm pore size), and used for analysis of saponin composition.

Ginsenoside composition analysis

Ginsenoside analysis was performed for evaluating the sample saponin composition as described previously [31]. HPLC was carried out using μ-Bondapak™

Fig. 1. Photograph of ginseng fermented by *Bacillus* sp.

C_{18} columns (10 μm, 3.9×300 mM; Waters) and a Jasco UV detector (203 nm). The mobile phase was a water (A) and acetonitrile (B) gradient, and with A as the standard, 80% (0 min), 80% (5 min), 67% (38 min), 20% (63 min), 20% (75 min), 80% (77 min), and 80% (90 min) were employed. Mobile phase flow speed was 1.0 mL per minute, the sample injection volume was 20 μL, and analysis was performed at 35°C.

Sample preparation for anti-oxidation activity determination

To constant amounts of ginseng powder samples (below 200 mesh), a tenfold volume of water (w/w), 50% ethanol (v/v), and pure ethanol (v/v) were added, extracted (with reflux) for 3 hours, followed by vacuum concentration. Samples (50 mg/mL) were then used for measuring antioxidant activity.

Determination of hydroxyl radical removal

Hydroxyl radical (•OH) removal activity was measured using a 2-deoxyribose oxidation assay, as described previously [32]. Then, 0.2 mL of 0.1 mM $FeSO_4$/0.1 mM EDTA·2Na, 0.2 mL 2-deoxyribose (10 mM), 0.2 mL sample, and 1.2 mL phosphate buffer (0.1 M; pH 7.4) were mixed. After the addition of 0.2 mL H_2O_2 (10 mM), the mixture was incubated at 37°C for 4 hours, and the reaction stopped by addition of a 1 mL trichloroacetic acid (2.8%) solution. Thiobarbituric acid/50 mM NaOH (1%; 1 mL) was then added and mixtures heated at 100°C for 10 minutes, followed by rapid cooling and measurement of OD_{532}. The final optical density of the sample was compared to that of the control group.

Superoxide dismutase-like activity

SOD-like activity was determined as described by Marklund and Marklund [33]. Tris-HCl buffer (pH 8.5) and 24 mM pyrogallol were added to the sample solution, and OD_{420} monitored for 2 minutes. Sample activities are expressed as the auto-oxidation inhibition rate (%) of pyrogallol *vs.* the control group.

RESULTS AND DISCUSSION

Change in ginsenoside content

Lactic acid bacteria and *Bacillus* have been used for most studies on ginseng fermentation, especially those using liquid culture method [34]. In this work, we fermented solid pulverized ginseng and examined changes in ginsenoside content and antioxidant activity (Fig. 2) Ten ginsenosides, Rg_1, Re, Rf, Rg_2+Rh_1, Rb_1, Rc, Rb_2+Rb_3, Rd, Rg_3, and Rh_2, were analyzed (Table 1). Ginsenoside content was altered significantly after *Bacillus* fermentation. More specifically, ginsenoside Rb_1 and Rc content decreased from 4.93 and 3.03 mg/g to 2.46 and 2.62 mg/g, respectively, after fermentation. In contrast, ginsenoside Rd increased from 0.66 to 7.21 mg/g. Ginsenoside Rb_1 is known to be converted to Rd

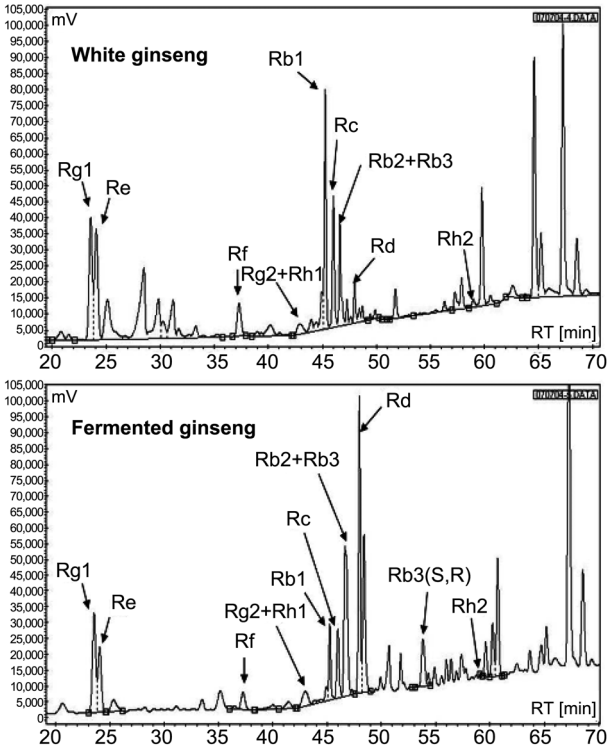

Fig. 2. HPLC chromatograms showing ginsenosides extracted from white and fermented ginseng.

Table 1. The effect of fermentation on the ginsenoside content of white ginseng

Ginsenosides	Contents (mg/g)	
	White ginseng	Fermented ginseng
Rg_1	3.21	4.04
Re	3.48	3.14
Rf	0.99	0.80
Rg_2+Rh_1	0.38	0.82
Rb_1	4.93	2.46
Rc	3.03	2.62
Rb_2+Rb_3	2.11	7.65
Rd	0.66	7.21
Rg_3	0.03	1.76
Rh_2	0.09	0.07
Sum	18.91	30.57

by β-D-glucosidase, and Rc to Rd by L-arabinofuranosidase [35]. Therefore, the decrease in Rb_1 and Rc and increase in Rd content may have been due to *Bacillus*-secreted ß-D-glucosidase and α-L-arabinofuranosidase. Our data are similar to those in a previous report [36] showing that liquid fermentation of ginseng with 21 *Bacillus* spp. resulted in a decrease in Rc content, although fermentation by 16 *Bacillus* spp. caused an increase in Rd levels. However, our solid culture showed a greater decrease in Rb_1 and increase in Rd levels compared to liquid fermentation [36]. Solid fermentation resulted in a markedly different ginsenoside content than liquid fermentation, since swelling of ginseng in liquid culture increased Rd content to 20%, although no effect occurred on Rb_1 and Rc content [37].

Ginsenoside Rd represses differentiation of preadipocyte 3T3-L1 cells. Also, AMP-activated protein kinase, an inhibitor of adipocyte differentiation and essential for metabolism [38], was activated by Rd in a concentration-dependent manner. Therefore, an increase in Rd content may contribute to inhibition of adipocyte differentiation. Solid culture fermentation increased Rg_3 content from 0.03 to 1.76 mg/g through hydrolysis of Rb_1 glucose at the carbon 20 position. Therefore, solid culture fermentation converted ginsenosides in the sequence Rb_1, Rc→Rd→Rg_3. Ginsenoside Rg_3 has anticancer and anti-metastasis activity *in vivo* [39]. Also, Rg_3 produced by microbial conversion has greater anticancer activity than Rg_3 from natural ginseng. When *Phellinus linteus*, a mushroom traditionally used in Northeastern Asia for gastroenteric disorders, lymphatic diseases, and cancer, was used for microbial conversion of ginsenosides, anticancer activity increased significantly [40,41]. Heat processing of *Panax notoginseng* converted ginsenosides into Rg_3, which inhibited proliferation of SW-480 human colorectal cancer cells [42]. Therefore, these data suggest that solid fermentation of ginseng with a mixture of five *Bacillus* spp. may enhance the health-promoting properties of ginseng.

Hydroxyl radical removal activity of ginseng

Steaming ginseng causes alterations in free sugar, polyphenol compound, acidic polysaccharide, and ginsenoside content, and increases antioxidant activity [43]. Moreover, Kang *et al*. [44] reported the removal of hydroxyl radicals by 20(S)-Rg_3, 20(R)-Rg_3, Rk_1, and Rg_5 produced from steamed ginseng.

The •OH is a highly reactive free radical that oxidizes various tissues and membranes [45]. As Fig. 3 shows, we determined the hydroxyl radical removal activity of white ginseng solvent extract and the highest antioxidant activity (>90%, similar to the positive control) was present in the post-solid fermentation solvent extract. The solid-fermented water extract showed the lowest antioxidant activity, and solvent extracts of both white and solid fermented ginseng possessed similar antioxidant activity. Moon *et al.* [46] reported that 20 mg/mL of hot water extract of citrus side-products, fermented with *B. subtilis*, inhibited more than 50% of the oxidative activity of 2-deoxyribose. These results suggest that both white and solid fermented ginseng could be used as antioxidants, since the hydroxyl radical is responsible for oxidative tissue damage.

Superoxide dismutase-like activity

SOD, an anti-oxidative enzyme with activity against reactive oxygen species (ROS), protects cells and tissues by converting the toxic superoxide radical anion (O_2^-) to hydrogen peroxide (H_2O_2) and O_2. SOD is involved in a variety of diseases, such as degenerative neurological and heart and artery diseases [47,48]. The anti-oxidative activity of solvent extracts of white and solid fermented ginseng was determined using the colorimetric pyrogallol auto-oxidation reaction (Fig. 4). The B-1 fraction of the fermented ginseng ethanol extract had the greatest SOD-like activity, followed by the B-3 fraction of the water extract. Lee [49] reported that 0.4% of the biopolymer produced by mixed *Bacillus* species was responsible for 59.4% of the total SOD-like activity. Therefore, solid fermented ginseng could be used as an antioxidant due to its marked SOD-like activity. SOD is present in tissue and blood, and removes ROS. Substances with SOD-like activity protect cells by inhibiting the activity of the superoxide radical [50].

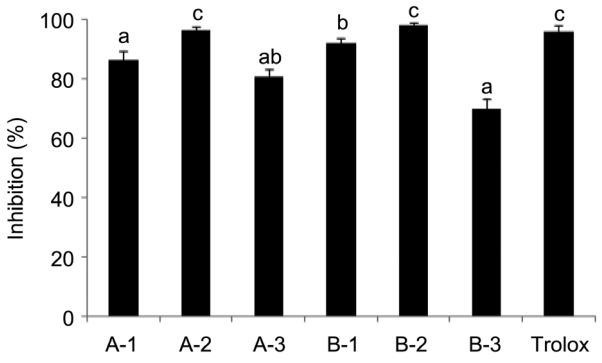

Fig. 3. Hydroxyl radical scavenging by white and fermented ginseng. A, white ginseng (1 mg/mL); B, fermented ginseng (1 mg/mL); 1, absolute ethanol; 2, 50% ethanol; 3, water. Data are expressed as the mean±SD. Column superscripts indicate a significant difference ($p < 0.05$) by Duncan's multiple range test.

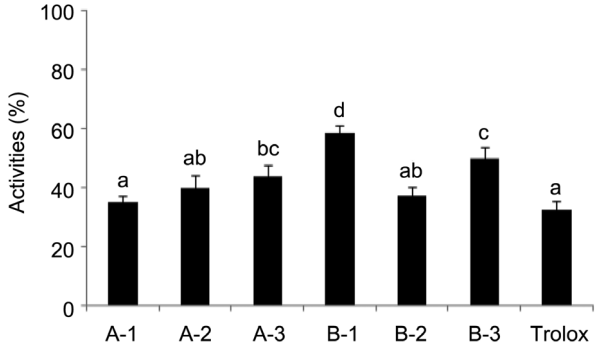

Fig. 4. Superoxide dismutase-like activity of white and fermented ginseng. A, white ginseng (1 mg/mL); B, fermented ginseng (1 mg/mL); 1, absolute ethanol; 2, 50% ethanol; 3, water. Data are expressed as the mean±SD. Column superscripts indicate a significant difference ($p<0.05$) by Duncan's multiple range test. NS, not significant.

Therefore, the SOD-like activity of solid fermented ginseng may be due to phytochemicals, e.g., phenolic compounds. Moreover, the antioxidant activity of solid fermented ginseng may also be attributable to Rg_3, which is known to possess neuroprotective and anti-oxidative activities. Furthermore, these data suggest that solid fermented ginseng could be developed as a functional raw material after further *in vitro* and *in vivo* studies.

ACKNOWLEDGEMENTS

This research was supported by the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

REFERENCES

1. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685-1693.
2. Tchilian EZ, Zhelezarov IE, Hadjiivanova CI. Effect of ginsenoside Rg_1 on insulin binding in mice liver and brain membranes. Phytother Res 1991;5:46-48.
3. Kang SY, Kim SH, Schini VB, Kim ND. Dietary ginsenosides improve endothelium-dependent relaxation in the thoracic aorta of hypercholesterolemic rabbit. Gen Pharmacol 1995;26:483-487.
4. Ju CN. The preventive effect on the saponin fraction of *Panax ginseng* C.A. Meyer against ethanol intoxication of rat liver. Proceedings of the 4th International Ginseng Symposium, 1984. p. 63-74.
5. Kim ND, Han BH, Lee EB, Kang JY. Studies on ginseng on antistress effects. Korean J Pharmacogn 1979;10:61-67.
6. Hwang WI, Oh SK. Effects of petroleum ether extract of ginseng root on some enzyme activity in human colon cancer cells. Korean J Ginseng Sci 1986;10:27-35.
7. Liu ZQ, Luo XY, Sun YX, Chen YP, Wang ZC. Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? Biochim Biophys Acta 2002;1572:58-66.
8. Shin JG, Park JW, Pyo JK, Kim MS, Chung MH. Protective effects of a ginseng component, maltol (2-methyl-3-hydroxy-4-pyrone), against tissue damages induced by oxygen radicals. Korean J Ginseng Sci 1990;14:187-190.
9. Han BH, Park MH, Han YN, Shin SC. Studies on the antioxidant components of Korean ginseng (IV) Antifatigue active components. Yakhakhoe Chi 1984;28:231-235.
10. Lee DW, Shon HO, Lim HB, Lee YG. Antioxidant action of ginseng: an hypothesis. Korean J Ginseng Sci 1995;19:31-38.
11. Chun C, Chang CC. The effect of red ginseng extracts on the superoxide dismutase, peroxidase and catalase activities in the liver of gamma ray irradiated mice. Korean J Ginseng Sci 1993;17:29-34.
12. Choi KM, Lee EJ, Kim YH, Baik SH, Kim YK, Choi DS. Effects of red ginseng on the lipid peroxidation of erythrocyte and antioxidant superoxide dismutase (SOD) activity in NIDDM patients. Korean J Ginseng Sci 1997;21:153-159.
13. Choi JH, Lee KM, Kim CB, Kim HJ. The effects of red-ginseng intake on free radicals produced during aerobic exercise in the elderly. The 7th Fall Congress of Korean Society for Exercise Nutrition, 1999. p. 21-40.
14. Kim SN, Ha YW, Shin H, Son SH, Wu SJ, Kim YS. Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control. J Pharm Biomed Anal 2007;45:164-170.
15. Hu P, Luo GA, Wang Q, Zhao ZZ, Wang W, Jiang ZH. The retention behavior of ginsenosides in HPLC and its application to quality assessment of Radix Ginseng. Arch Pharm Res 2008;31:1265-1273.
16. Ando T, Tanaka O, Shibata S. Comparative studies on the ginsenosides and sapogenins of ginseng and related crude drugs. Shoyakugaku Zasshi 1971;25:28-32.
17. Korea Food & Drug Administration. Health functional food acts and subordinate statutes, notifications. Seoul: The Korea Food & Drug Administration, 2008.
18. Korean Agency for Technology and Standards. Ginseng--Determination of ginsenosides (Rb1, Rg1) contents--Method using high performance liquid chromatography (KS H 2153). Gwacheon: The Korean Agency for Technology and Standards, 2007.

19. Jeong HB, Park SH, Ko SK, Cho SH, Im BO. Actual consumption conditions and consumer perception of ginseng in the major countries. J Ginseng Res 2005;29:152-158.
20. Sanada S, Kondo N, Shoji J, Tanaka O, Shibata S. Studies on the saponins of ginseng. I. Structure of ginsenoside—R_O, Rb_1, Rb_2, Rc and Rd. Chem Pharm Bull (Tokyo) 1974;22:421-428.
21. Shibata S, Tanaka O, Ando T, Sado M, Tsushima S, Ohsawa T. Chemical studies on oriental plant drugs. XIV. Protopanaxadiol, a genuine sapogenin of ginseng saponins. Chem Pharm Bull (Tokyo) 1966;14:595-600.
22. Kim SW, Kwon HY, Chi DW, Shim JH, Park JD, Lee YH, Pyo S, Rhee DK. Reversal of P-glycoprotein-mediated multidrug resistance by ginsenoside Rg(3). Biochem Pharmacol 2003;65:75-82.
23. Liu WK, Xu SX, Che CT. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. Life Sci 2000;67:1297-1306.
24. Bae EA, Kim NY, Han MJ, Choo MK, Kim DH. Transformation of ginsenosides to compound K (IH-901) by lactic acid bacteria of human intestine. J Microbiol Biotechnol 2003;13:9-14.
25. Chi H, Kim DH, Ji GE. Transformation of ginsenosides Rb_2 and Rc from *Panax ginseng* by food microorganisms. Biol Pharm Bull 2005;28:2102-2105.
26. Chi H, Ji GE. Transformation of ginsenosides Rb_1 and Re from *Panax ginseng* by food microorganisms. Biotechnol Lett 2005;27:765-771.
27. Luan HW, Liu X, Qi X, Hu Y, Hao D, Cui Y, Yang L. Purification and characterization of a novel stable ginsenoside Rb_1-hydrolyzing ß-D-glucosidase from the Chinese white jade snail. Proc Biochem 2006;41:1974-1980.
28. Kelly CT, O'Reilly F, Fogarty WM. Extracellular α-glucosidase of an alkalophilic microorganism, *Bacillus* sp. ATCC 21591. FEMS Microbiol Lett 1983;20:55-59.
29. Pajni S, Dhillon N, Vadehra DV, Sharma P. Carboxymethyl cellulase, β-glucosidase and xylanase production by *Bacillus* isolates from soil. Int Biodeterior 1989;25:1-5.
30. Rowe GE, Margaritis A. Enzyme kinetic properties of α-1,4-glucosidase in *Bacillus thuringiensis*. Biochem Eng J 2004;17:121-128.
31. Hong HD, Sim EM, Kim K, Rho J, Rhee YK, Cho CW. Comparison of preparation methods for the quantification of ginsenosides in raw Korean ginseng. Food Sci Biotechnol 2009;18:565-569.
32. Chung SK, Osawa T, Kawakishi S. Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Biosci Biotech Biochem 1997;61:118-123.
33. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-474.
34. Park S, Kim DH, Paek NS, Kim SS. Preparation and quality characteristics of the fermentation product of ginseng by lactic acid bacteria (FGL). J Ginseng Res 2006;30:88-94.
35. Park CS, Yoo MH, Noh KH, Oh DK. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. Appl Microbiol Biotechnol 2010;87:9-19.
36. Kim HG, Kim KY, Cha CJ. Screening for ginseng-fermenting microorganisms capable of biotransforming ginsenosides. Korean J Microbiol 2007;43:142-146.
37. Han CK, Hong HD, Kim YC, Kim SS, Sim GS. Effect of puffing on quality characteristics of red ginseng tail root. J Ginseng Res 2007;31:147-153.
38. Kim MS, Lee MS, Kim SH, Kim SH, Kim HJ, Sung MJ, Kim HY, Kwon DY, Hwang JT. Anti-obesity effects of ginsenosides Rd via AMPK and PPAR gamma. Korean J Biotechnol Bioeng 2007;22:341-344.
39. Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tono-oka S, Samukawa K, Azuma I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb_2, 20(R)- and 20(S)-ginsenoside-Rg_3, of red ginseng. Biol Pharm Bull 1995;18:1197-1202.
40. Lee JJ, Kwon HK, Lee DS, Lee SW, Lee KK, Kim KJ, Kim JL. Mycelial extract of *Phellinus linteus* induces cell death in A549 lung cancer cells and elevation of Nitric Oxide in Raw 264.7 macrophage cells. Mycobiology 2006;34:143-147.
41. Lee JJ, Kwon HK, Jung IH, Cho YB, Kim KJ, Kim JL. Anti-cancer activities of ginseng extract fermented with *Phellinus linteus*. Mycobiology 2009;37:21-27.
42. Sun S, Wang CZ, Tong R, Li XL, Fishbein A, Wang Q, He TC, Du W, Yuan CS. Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities. Food Chem 2009;118:307-314.
43. Yang SJ, Woo KS, Yoo JS, Kang TS, Noh YH, Lee J, Jeong HS. Change of Korean ginseng components with high temperature and pressure treatment. Korean J Food Sci Technol 2006;38:521-525.
44. Kang KS, Kim HY, Yamabe N, Yokozawa T. Stereospecificity in hydroxyl radical scavenging activities of four ginsenosides produced by heat processing. Bioorg Med Chem Lett 2006;16:5028-5031.
45. Heo SI, Wang MH. Antioxidant activity and cytotoxicity effect of extracts from *Taraxacum mongolicum* H. Korean J Pharmacogn 2008;39:255-259.
46. Moon YG, Lee KJ, Heo MS. Characteristics of citrus by-

product ferment using *Bacillus subtilis* as starter extracts. Korean J Microbiol Biotechnol 2007;35:142-149.
47. Balin AK. Testing the free radical theory of aging. In: Adelman RC, Roth GS, eds. Testing the theories of aging. Boca Raton, FL: CRC Press, 1982. p.137-182.
48. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;11:298-300.
49. Lee SL. Effect of biopolymer produced by mixed *Bacillus* strains on the antioxidant activity and inhibitory activities of tyrosinase and elastase. J Korean Soc Cosm 2009;15:562-570.
50. Murakami A, Takahashi D, Koshimizu K, Ohigashi H. Synergistic suppression of superoxide and nitric oxide generation from inflammatory cells by combined food factors. Mutat Res 2003;523:151-161.