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Functional Characterizations of Extruded White Ginseng Extracts

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Abstract The antibacterial and antioxidant potentials of extruded ginseng extract (EGE) with 60% ethanol and methanol were investigated. The inhibitory activity of the EGE in Gram-positive bacteria was significantly higher than in Gram-negative bacteria. Higher antibacterial activity was observed with methanol ginseng extract when moisture content and barrel temperature were 20% and 115°C, respectively, that diameter of inhibition zone at 1,500 mg/mL was 15.40±0.13 mm for *Bacillus subtilis* and 9.31±0.05 mm for *Salmonella typhimurium*. The amount of total phenolics was highest in extruded ginseng at 20% moisture content and 115°C barrel temperature. Especially, a positive correlation was observed between the total phenolic content and antioxidant activity of the extracts. In the 2,2-diphenyl-1-picryhydrazyl radical (DPPH) system, all tested extract of extruded ginseng at 20% moisture content exhibited very strong antioxidant properties when compared to red ginseng with percent scavenging effect of 23-35% at 20 mg/mL. In conclusion, it can be said that the extracts of extruded ginseng could be used as natural antimicrobial and antioxidant agents in the food preservation.

Keywords: antimicrobial activity, antioxidant, antibiotic, extruded ginseng, β-carotene-linoleic acid

Introduction

The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. Currently there is a growing interest to use natural antibacterial compounds and sometimes show antioxidant activity as well as antimicrobial activity (1). The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents (2).

Ginseng (*Panax ginseng* C.A. Meyer) is one of the most widely taken herbal products in the Korea. The major pharmacologically active constituents of ginseng are triterpene saponins called ginsenosides. Until now, more than 30 ginsenosides have been isolated (3). The medicinal properties of ginseng have been linked to its antioxidative capability. Some glycosides found in ginseng appear to act as antioxidants. More recently, the extract of ginseng has been reported to have adjuvant properties against porcine parvovirus (PPV), *Staphylococcus aureus,* and *Erysipelothrix rhusiopathia* (4,5). Especially, red ginseng has high potent biological activities such as radical scavenging, vasodilating, and anti-tumor activities (6). Red ginseng is obtained by steaming ginseng for designated hours. Matsuda (7) reported that heated ginseng with steam into red ginseng increased the number of saponins detected from 11 to 16. Previous research described that the red ginseng and red ginseng powder prepared by extrusion process can be produced at larger amounts within a shorter time than those of the conventional method for preparing red ginseng (8). Chemical components of extruded root ginseng studies demonstrated that extruded ginseng at 25 and 30% moisture content and 110°C barrel temperature had similar components to those of red ginseng (9). Extrusion cooking is a continuous process, which utilizes high temperature and high shear force to produce a product with unique physical and chemical characteristics. It is a low cost and high temperature short time (HTST) process, used worldwide for processing of a number of food products (10). Product quality can vary depending on the extruder type, screw configuration, feed moisture, temperature profile in the barrel, screw speed, and feed rate. Kim and Ryu (11) have showed that extract yield highly of extruded white ginseng increased by extrusion treatment.

This study was performed to optimize extrusion conditions of white ginseng powder and to characterize their functions with respect to antioxidant and antibacterial effects.

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Materials and Methods

Materials Powdered ginseng (white ginseng and red ginseng) used in this work were purchased from Dong-Jin Drug and Food Co. (Gumsan, Korea). All chemicals were of analytical grade. β-Carotene, linoleic acid, 2,2-diphenyl-1-picryhydrazyl radical (DPPH), gallic acid, Folin-Ciocalteu's phenol, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extrusion process Extruded ginseng was made from white ginseng powder by using co-rotating intermeshing twin screw extruder (Incheon Machinery Co., Incheon, Korea). The process conditions were applied as follows: screw speed 250 rpm, feed rate 140 g/min, barrel temperature 100, 115, and 130°C, moisture content 20, 25, and 30%, screw diameter 31.8 mm, and die diameter 3 mm. The extrudate was directly dried in an oven at 50°C for 8 hr. The dried extrudate was ground to powder using a mechanical grinder and then sieved through 40-mesh and stored at 4°C until analysis.

Solvent extraction The powdered ginsengs (20 g) were macerated with 60% of ethanol and methanol (1:8) at 35°C. The mixture was shaken for 24 hr at 110 rpm in water bath.

The extracts were filtered through Whatman No.1 filter paper using a Buchner funnel and the solvent removed under reduced pressure with a rotary evaporator (Buchi R-200; Labortechnik, Flawil, Switzerland); this process was repeated 3 times. The dried crude extracts were stored at 4°C. The extracts were dissolved in water before use. The yield of extract was calculated by ratio weight of ginseng extract (g) to weight of ginseng powder (g).

Assay for total phenolic contents The total phenolics constituent of ginseng extracts were determined according to the Folin-Ciocalteu colorimetric method and gallic acid as standard compound (12). The reaction mixture was composed of 0.1 mL of extract (20 mg/mL), 7.9 mL of distilled water, 0.5 mL of the Folin-Ciocalteu's phenol reagent, and 1.5 mL of 20% sodium carbonate. The contents of the tubes were mixed thoroughly and stored in the dark for 2 hr. The absorbance was measured at 765 nm and converted to phenolic contents according to the calibration curve from various concentration of gallic acid (as gallic acid equivalents, GAE/mg of extract).

Antibacterial activity using disc agar diffusion method Two bacterial strains as well as mainly food-borne pathogens were used in this experiment. *Bacillus subtilis* (KCTC 1023) and *Salmonella typhimurium* (KCTC 1925) were obtained from Korean Collection for Type Cultures (KCTC) in Korea. Procedures to test antibacterial activities were as descried by National Committee for Clinical Laboratory Standards (NCCLS) (13). The microorganisms were grown for overnight at 37°C in 30 mL of nutrient broth. The cultures were adjusted with sterile saline solution (0.85% NaCl) to obtain turbidity comparable to that of McFarland No. 0.5 standard. Petri plates were prepared by pouring 20 mL of Mueller Hinton agar and allowed to solidify. Plates were dried and 0.1 mL of inoculums suspension was poured and uniformly spread. Thirty μL containing 1,000 and 1,500 mg/mL of each ginseng extracts were impregnated onto paper disc (6 mm in diameter) placed on the top of agar plate. The plates were incubated at 37°C for 24 hr. Five mg/mL of gentamycin, streptomycin, and tetracycline were included as positive controls for bacteria and water or 100% dimethyl sulfoxide (DMSO) were served as negative controls. The antimicrobial activity was assessed based on measurement of the diameter (mm) of the clear zone around the paper disc. All determinations were done in triplicate.

Radical scavenging activity using DPPH assay The extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH proposed by Brand-Williams *et al.* (14). Briefly, 100 μL of various concentrations (20 mg/mL) of the extracts in methanol was added to 5 mL of a 0.1 mM methanol solution of DPPH. After 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate and BHT was used as positive control. The DPPH radical scavenging activity was calculated according to the following:

$$\text{Scavenging activity (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Antioxidant activity using β-carotene-linoleic acid assay A stock solution of β-carotene-linoleic acid was prepared as follows (15). First, 2.0 mg of β-carotene was dissolved in 10 mL of chloroform. Two mL of this solution was pipetted into a 100-mL round-bottom flask. After chloroform was removed under vacuum, using a rotary evaporator at 40°C for 10 min, 25 μL of linoleic acid, 200 mg of Tween 40 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. Aliquots (3 mL) of this emulsion were transferred into test tubes containing 100 mL of the extracts (in ethanol). The test tubes were incubated in hot water (50°C) for 2 hr, together with 2 blanks, one containing the antioxidant BHT as a positive control and the other with the same volume of solvent instead of the extracts. The antioxidant activity was expressed as % inhibition relative to the control after 120 min incubation using the equation

$$\text{Antioxidant activity (\%)} = [1 - (A_0 - A_t)/(A'_0 - A'_t)] \times 100$$

where A_0 and A'_0 are the absorbance measured at zero time of incubation for the test sample and control, respectively, and A_t and A'_t are the absorbance measured in the test sample and control, respectively, after 120 min incubation.

Results and Discussion

Extract yield and total phenolic contents Extraction yields and total phenolics of ginseng extracts were summarized in Table 1. The extract yield was calculated with the base on the original weight of ginseng powder and the total phenolic contents was expressed as μg/mg dry weight based on a standard curve of gallic acid (as GAE) (R^2=0.99, y=0.001x−0.0042) (data not shown). The extruded ginseng extract (EGE) yields ranged from 21.86±0.28% (EtOH extracts) to 27.74±0.47% (MeOH extracts), that these values were lower than white and red ginseng extract (both ethanol and methanol extraction). For methanolic extract, the highest extraction yield of extruded ginseng was obtained at 30% moisture content and 100°C barrel temperature. Phenolics are secondary metabolites which are produced as a result of the plant's interaction with the environment (16). Among the samples obtained from different extrusion conditions, methanolic EGE from 20% moisture content and 115°C barrel temperature demonstrated the highest total phenolic contents (423.20±5.29 μg GAE/mg), following by ethanolic extruded ginseng at the same extrusion condition (385.87±5.00 μg GAE/mg). It can be observed that mostly the total phenolic contents of EGE was higher than red ginseng extract (206.87±2.52 and 220.53±5.03 μg GAE/mg by ethanol and methanol, respectively). Extruded ginseng at high moisture content of extrusion condition showed the low total phenolic contents. The extraction of ginseng by methanol was found to be the most efficient solvent to extract phenolic constituents. This result accorded with the reports of Yen *et al.* (17) and Antolovich *et al.* (18) that methanol is a widely used and effective solvent for extraction of antioxidants.

Table 1. Extrusion yield of white ginseng extract and their phenolic contents[1)]

Extrusion condition		Yield[2)] (g/100 g of plant)		Total phenolics[3)] (μg GAE/mg)	
Moisture content (%)	Barrel temp. (°C)	EtOH	MeOH	EtOH	MeOH
30	130 (Ex-1)	26.36±0.03	27.00±0.07	202.02±2.00	188.53±5.03
	115 (Ex-2)	26.55±0.18	26.41±0.10	191.87±2.08	178.20±1.73
	100 (Ex-3)	26.48±0.10	27.74±0.47	170.53±3.79	181.87±3.06
25	130 (Ex-4)	24.99±0.48	26.40±0.63	182.53±2.52	189.87±3.79
	115 (Ex-5)	26.95±1.16	26.98±0.48	255.53±3.21	220.20±4.58
	100 (Ex-6)	23.95±0.17	24.32±0.08	259.87±2.08	304.53±3.79
20	130 (Ex-7)	21.86±0.28	23.58±0.02	361.87±3.51	383.20±2.08
	115 (Ex-8)	25.42±0.18	26.89±1.05	385.87±5.00	423.20±5.29
	100 (Ex-9)	26.25±0.80	24.45±0.57	290.53±3.51	342.87±5.13
Red ginseng		38.08±0.20	41.13±0.31	206.87±2.52	220.53±5.03
White ginseng		31.45±0.20	32.46±0.51	152.53±4.51	164.20±4.36

[1)]Each value is the mean±SD.
[2)]Yield % ginseng extract were obtained from solvent extraction method.
[3)]Total phenolic contents were determined by the Folin-Ciocalteu method.

Table 2. Diameter of inhibition zone of ginseng extracts against to *B. subtilis*

Extrusion condition		Diameter of inhibition zone (mm)[1)]			
		Ginseng extract: 1,000 mg/mL		Ginseng extract: 1,500 mg/mL	
Moisture content (%)	Barrel temp. (°C)	EtOH	MeOH	EtOH	MeOH
30	130 (Ex-1)	11.10±0.11	12.06±0.05	12.38±0.13	13.32±0.06
	115 (Ex-2)	10.49±0.24	11.92±0.13	12.26±0.03	14.40±0.04
	100 (Ex-3)	10.67±0.03	12.67±0.06	11.57±0.03	14.05±0.06
25	130 (Ex-4)	10.49±0.17	12.60±0.07	12.12±0.13	14.01±0.06
	115 (Ex-5)	10.47±0.12	12.77±0.07	11.91±0.04	15.17±0.01
	100 (Ex-6)	10.13±0.13	11.70±0.16	12.11±0.06	13.43±0.12
20	130 (Ex-7)	10.18±0.23	11.43±0.12	12.86±0.06	13.95±0.14
	115 (Ex-8)	11.63±0.17	12.90±0.13	13.44±0.03	15.26±0.13
	100 (Ex-9)	10.45±0.12	12.45±0.13	12.58±0.03	13.59±0.11
Red ginseng		11.84±0.12	13.10±0.05	13.37±0.04	14.26±0.12
White ginseng		9.40±0.07	10.26±0.04	10.14±0.05	10.72±0.11

[1)]Triplicate determination±SD.

Antibacterial activity All of ginseng extracts (including red and white ginseng extracts) at 1,000 mg/mL were effective against *B. subtilis* (Table 2), but no effective to inhibitory *S. typhimurium*. The inhibition effect of methanolic ginseng extracts was lower than ethanolic ginseng extracts. For *B. subtilis*, the diameter of inhibition zone value of ethanolic EGE were between 10.13 and 11.63 mm and those of methanolic EGE were between 11.43 and 12.90 mm, respectively. The 3 ethanolic EGE and 5 ethanolic EGE inhibited the *S. typhimurium* in concentration of 1,500 mg/mL, that ethanolic and methanolic EGE obtained from 20% moisture content and 115°C barrel temperature of extrusion condition shows highest activity with diameter of inhibition zone 8.50±0.07 and 9.31±0.05 mm, respectively. Similarly, these EGE showed best antibacterial effect to *B. subtilis*. White ginseng extract at 1,500 mg/mL was not active against *S. typhimurium* whereas red ginseng extract created a zone of inhibition of about 8.36 and 8.82 mm for ethanolic and methanolic extracts, respectively. The negative control with water used in this study did not affect the growth of bacteria strains. In this study, the antibacterial properties of all ginseng extract were lower than the commercial drug used (gentamycin, streptomycin, and tetracycline). It can be noted that potential of EGE in Gram-positive bacteria was significantly higher than in Gram-negative. This result may be attributed to the fact that the cell wall in the Gram-positive bacteria consists of a single layer, whereas the Gram-negative bacterial cell wall is a multilayered structure bounded by an outer cell membrane (19,20).

Radical scavenging activity The essence of the DPPH method is that the antioxidants react with the stable radical. DPPH radical; a stable free radical with a characteristic absorption at 517 nm, was used to determine radical scavenging effect of natural compound (21). The method is based on the reduction of methanolic $DPPH^{\cdot}$ solution in the presence of a hydrogen donating antioxidant due to the formation of non-radical form DPPH-H by the reaction (22). Free radical scavenging capacities of corresponding

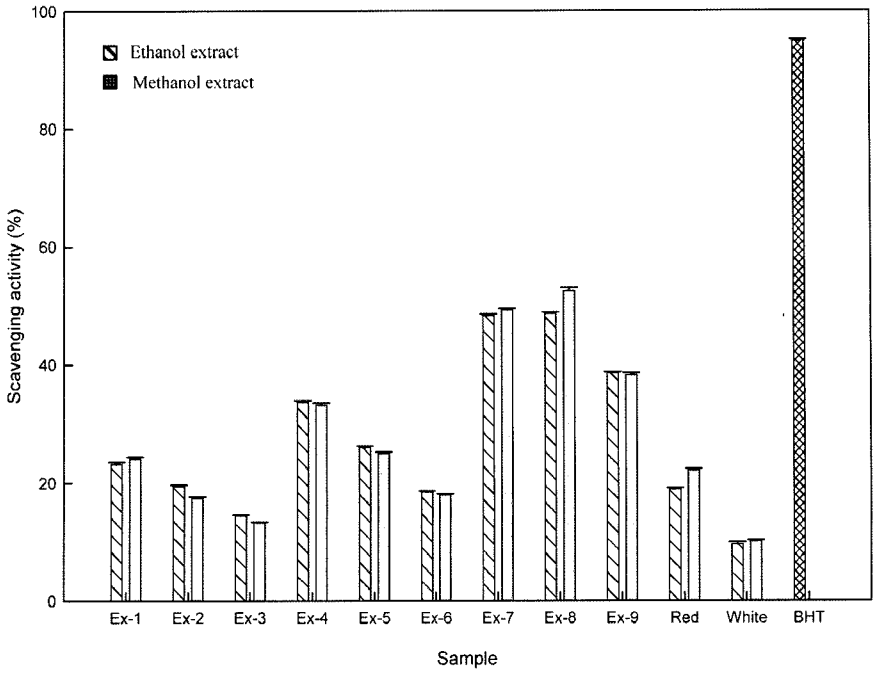

Fig. 1. Percent scavenging activity of extruded ginseng compared with red and white ginseng. Ex-1, EGE 30% MC+ 130°C barrel temp.; Ex-2, EGE 30% MC+115°C barrel temp.; Ex-3, EGE 30% MC+100°C barrel temp.; Ex-4, EGE 25% MC+130°C barrel temp.; Ex-5, EGE 25% MC+115°C barrel temp.; Ex-6, EGE 25% MC+100°C barrel temp.; Ex-7, EGE 20% MC+130°C barrel temp.; Ex-8, EGE 20% MC+115°C barrel temp.; Ex-9, EGE 20% MC+100°C barrel temp.

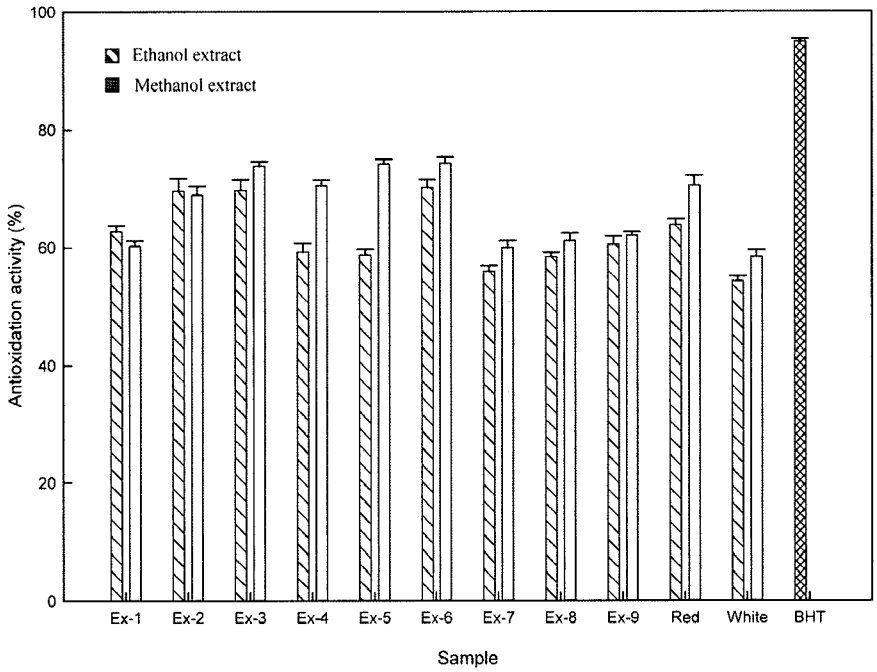

Fig. 2. Percent antioxidation activity of extruded ginseng compared with red and white ginseng. Ex-1, EGE 30% MC+ 130°C barrel temp.; Ex-2, EGE 30% MC+115°C barrel temp.; Ex-3, EGE 30% MC+100°C barrel temp.; Ex-4, EGE 25% MC+130°C barrel temp.; Ex-5, EGE 25% MC+115°C barrel temp.; Ex-6, EGE 25% MC+100°C barrel temp.; Ex-7, EGE 20% MC+130°C barrel temp.; Ex-8, EGE 20% MC+115°C barrel temp.; Ex-9, EGE 20% MC+100°C barrel temp.

ethanolic and methanolic ginseng extracts are shown in Fig. 1. All extract was able to reduce stable radical $DPPH^{\cdot}$ to the yellow-colored diphenylpicrylhydrazine. The order of methanolic EGE efficacy was 24.03, 17.42, and 13.29% (at 30% moisture content; 130, 115, and 100°C, respectively), 33.19, 25.02, and 17.98% (at 25% moisture content; 130, 115, and 100°C, respectively), 49.24, 52.50, and 38.25% (at 20% moisture content; 130, 115, and 100°C, respectively). The percentage scavenging values of ethanolic EGE were nearly with methanolic extract in the same extrusion condition. These results suggest that extruded ginseng sample at 20% moisture content and 115°C barrel temperature might contain the strongest free radical scavenger compound. None of the EGE was as effective scavengers as the

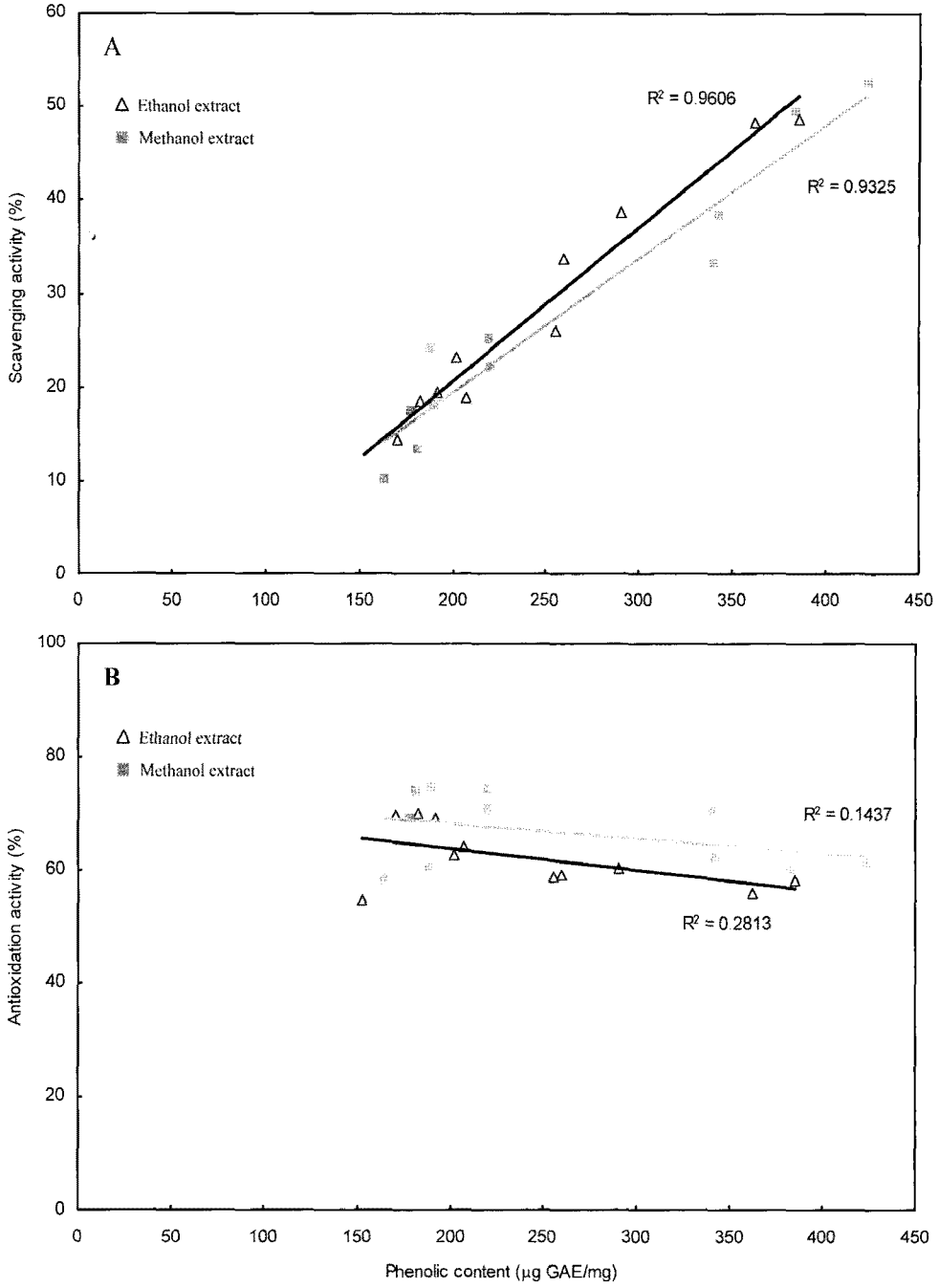

Fig. 3. Correlation of total phenolic contents with scavenging activity (A) and antioxidation activity (B). GAE, Gallic acid equivalent.

positive controls BHT (94.88%). However, all of the ethanolic and methanolic EGE at 25 and 20% moisture content were better scavenger than red ginseng extract (ethanol: 18.94% and methanol: 22.14%), a plant known to possess strong antioxidant activity (23).

Antioxidant activity The antioxidant activity of ginseng extracts and BHT at 20 mg/mL concentrations was determined using a β-carotene-linoleate model system. The mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from the hydroperoxides formed from linoleic acid (24). An extract that inhibits β-carotene bleaching can be described as a free radical scavenger and a primary antioxidant (25). The results in this study are presented in Fig. 2. It was observed that no significant differences in antioxidant activity were observed between ethanolic ginseng extracts and methanolic ginseng extracts. The amount of the antioxidant activity percentage was highest in methanolic extruded ginseng at 20% moisture content and 100°C barrel temperature of extrusion condition. The ethanolic ginseng extract from 30% moisture content and 130°C barrel temperature had the lowest inhibitory ability on the β-carotene oxidation with 55.88% when compared with all of extruded ginseng sample. All of methanolic EGE from 20% moisture content (100°C= 74.38%, 115°C=74.24%, and 130°C=70.54%) were more active than the same concentration of red ginseng extract. However, the protection of β-carotene bleaching provided

by the extruded samples was lower than that BHT standard (94.94% at 20 mg/mL). This suggests that the extracts may have potential use as antioxidative preservatives in emulsion type system.

Correlation coefficients of total phenolic (GAE), scavenging activity, and antioxidation activity The correlation coefficients between total phenolic with scavenging activity (%) and antioxidation activity (%) of the ginseng extracts were studied using a linear regression analysis. Figure 3A illustrated the scavenging activity correlated highly with total phenolic contents (R^2=0.9606 for ethanolic ginseng extracts and R^2=0.9325 for methanolic ginseng extracts), while the correlation coefficients between total phenolic with antioxidative activity value (Fig. 3B) was found to be very weak, less than 0.5. This probably is the reason that methods of measuring antioxidant activity are extremely dependent on the conditions used and the substrates or product monitored. Different methods based on different mechanism for measurement antioxidant activity may lead to different result even from the same sample (26). In this study, the antioxidative activity of various extracts of ginseng was determined with 2 assay based on different mechanisms, namely DPPH assay based on electron transfer reaction and β-carotene-linoleic acid assay based on hydrogen atom transfer reaction (27). Furthermore, Vinson *et al.* (28) held the idea that the synergism among the antioxidants in the mixture made the antioxidant activity not only dependent on the concentration of antioxidant, but also on the structure and interaction among the antioxidant. In conclusion, the *B. subtilis* was sensitive to the action of all EGE, whereas *S. typhimurium* was resistant to the action of some EGE. The DPPH scavenging and antioxidative activity data suggests that the EGE was observed to have antioxidant capacity. As known, there is a significant linear correlation between phenolic concentration and free radical scavenging activity but no correlation exists between the antioxidant activity assayed by β-carotene blenching method and total phenolics. This can be of beneficial interest in preservation of foodstuffs, their possible use as natural additives to replace synthetic antioxidants.

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