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Physicochemical Characteristics and Anti-Oxidant Activities of Farm-Cultivated and Mountain-Cultivated Ginseng Seeds

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ABSTRACT

Physicochemical Characteristics and Anti-Oxidant Activities of Farm-Cultivated and Mountain-Cultivated Ginseng Seeds

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Background: Ginseng (*Panax ginseng* Meyer) has been well known for a medicinal plant. Effect of cultivations on chemical characteristics of ginseng plants has been studied. However, seed oils and defatted seed meals from farm-cultivated (FG) and mountain-cultivated ginsengs (MG) have not been studied.

Methods: Fatty acid compositions, carotenoids, tocols, squalene, and phytosterols of seed oils from two FG and one MG were analyzed. Crude saponin contents, phenolic and flavonoid contents, and anti-oxidant activities of defatted seed meals from the FG and MG were also determined.

Results: Crude lipids, crude proteins, and ash in the seeds were 17.9-22.1% (dry basis), 11.5-15.2%, and 1.4-1.7%, respectively. The major fatty acid in the seed oils was oleic acid (77.9-78.5%), followed by linoleic acid (16.6-17.4%). The seed oils from MG had higher Hunter b value, carotenoids (4.3 μg β-carotene equivalent/g oil), β/γ-tocotrienol (3.7 mg/100 g oil), and δ-tocotrienol (0.2 mg/100 g oil), and

lower α-tocotrienol (20.8 mg/100 g oil) than the others. The MG seed oils had also more squalene (405.3 mg/100 g oil) than the others. The major phytosterol in the oils was β-sitosterol (48.1-83.6 mg/100 g oil) with MG the highest. Crude saponins in the defatted ginseng seed meals were 7.9-8.5 mg/g dried meal. Defatted seed meal extracts from MG had more total phenolics (28.1 mg gallic acid equivalent/g dried extracts) and flavonoids (5.5 mg quercetin equivalent/g dried extracts), and higher DPPH or ABTS radical scavenging activities and ferric reducing anti-oxidant power than the others.

Conclusion: Physicochemical characteristics of ginseng seed oil and anti-oxidant activities of defatted ginseng seed meal might be affected by cultivation environment.

Keywords: Anti-oxidant, Defatted ginseng seed meal, Farm-cultivated ginseng, Ginseng seed oil, Mountain-cultivated ginseng

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INTRODUCTION

Ginseng (*Panax ginseng* Meyer) has been widely used as a medicinal plant because it has been known to have various biological activities such as anti-aging, anti-inflammatory, anti-oxidative, and anti-tumor activities [1]. There are two types of ginseng that are commonly consumed in Korea, farm-cultivated (FG) and mountain-cultivated ginsengs (MG). FG is cultivated on a farm using partially sun-blocking shields and harvested after 5-6 years of cultivation. MG is sown or transplanted in a deep mountain as planned and grows naturally without any man-made facilities. It is collected after 10-20 years or more [2, 3].

Influence of the different cultivations on chemical properties of ginseng seeds, seed oils, leaves, and roots has been studied [3-11]. Roots and leaves of MG have been shown to possess greater anti-cancer and anti-oxidant activities than those of FG [3-6]. However, seed oils of FG and MG have not been studied.

Ginseng seeds contain 15.0-26.6% oil on a dry basis [11, 12]. Ginseng seed oil contains more than 90% unsaturated fatty acids, including oleic (61.2-87.7%) and linoleic acids (8.9-18.8%) [10-13]. It also has phytochemicals such as fat-soluble vitamins, squalene, and phytosterols, which determine quality of an oil and have been known to be beneficial to human health [11-13]. Ginseng seed oil has been suggested to be a potential specialty resource of functional foods and cosmetics [12, 13].

Most defatted seed meals have been discarded or used as low-value by-products after oil extraction [14]. A substantial amount of anti-oxidant substances such as phenolics and flavonoids still remain in defatted seed meals [15]. Studies on

evaluating the utilization of the defatted seed meals have increasingly gained interest in the aspect of added value of ginseng by-products [14, 15]. However, studies on anti-oxidant activities and potential anti-oxidants of defatted seed meals of FG and MG are still limited. The objectives of this study were to determine physicochemical characteristics of seed oils from FG and MG and to determine anti-oxidant properties and amounts of potential anti-oxidants in the defatted seed meals from the FG and MG.

MATERIALS AND METHODS

1. Materials and reagent

FG seeds (4 years grown, FGS1; and 5-6 years grown, FGS2) collected from Hongcheon (Korea) in August, 2016 and MG seeds (7-13 years grown, MGS) collected from Pyeongchang (Korea) in August, 2016 were obtained. The seeds were dried in a freeze dryer (Clean Vac12, Hanil Scientific Inc., Gimpo, Korea) for 5 days and stored at -70°C until analyzed.

β-Carotene, α-, γ-, and δ-tocopherols, 5α-cholestane, gallic acid, quercetin, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), and standard mixture of 37 fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis, MO, USA). α-, γ-, and δ-Tocotrienol kits were purchased from Chromadex (Irvine, CA, USA). Squalene and stigmasterol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). β-Sitosterol and campesterol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetonitrile and methanol were purchased from Avantor Performance Materials (Center Valley, PA, USA). Boron trifluoride (BF_3)-methanol, tert-butyl methyl ether, Folin-Ciocalteu reagent, 2-aminoethyl diphenylborinate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, chelex 100 sodium form, and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Chemical Co. Iron (III) chloride was purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea) and sodium sulfate from Yakuri Pure Chemicals Co., Ltd. (Osaka, Japan). The other reagents used in the study were purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). All chemicals were of analytical reagent grade.

2. Preparation of samples

The dried seeds were ground by a food processor (SMX-G770, Shinil Industrial Co., Ltd., Seoul, Korea) for 3 min, followed by sieving with an 18 mesh (1 mm) testing sieve. Seed powder (100 g) was extracted by stirring with 500 mL 95% n-hexane at room temperature for 2 h and filtered through a Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, England). This extraction was repeated twice more. The collected filtrate was concentrated using a rotary evaporator (Eyela N-1000, Tokyo Rikakikai, Tokyo, Japan) at 50°C. The oils were flushed with nitrogen and stored at -70°C until used. Yield of the ginseng seed oil was calculated as percentage of weight of extracted oil to weight of used seed powder (dry basis).

Defatted seed meals were kept in a hood for 2 days to remove the residual solvent completely. Eight grams of each defatted seed meal was twice extracted using a reflux condenser with 160 mL 70% ethanol for 2 h at 85°C. The extracts were filtered by a Whatman No. 2 filter paper and concentrated by the rotary evaporator. The residues were dried in the freeze dryer for 5 days and stored at -70°C until used. Yield of the defatted ginseng seed meal extract was calculated as percentage of weight of freeze-dried extract to weight of used defatted seed meal (dry basis).

3. Analysis of proximate composition of ginseng seeds

Crude lipids, crude proteins, and ash in the dried seeds were analyzed according to AOAC [16].

4. Analysis of fatty acid composition of ginseng seed oils

The seed oils were methylated using BF_3-methanol solution according to AOCS

[17]. The fatty acid composition was analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column (DB-23, 30 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA, USA) and flame ionization detector (FID). Split ratio was 1:50. Injector and detector temperatures were kept at 240 and 280°C, respectively. Carrier gas was helium and flow rate was 1.3 mL/min. Column temperature was programmed as follows: 50°C held for 2 min, from 50°C to 175°C at 25°C/min, to 230°C at 4°C/min held for 5 min, and to 250°C at 25°C/min held for 3 min [13]. Fatty acids were identified by comparing their retention times with those of the standards.

5. Analysis of color of ginseng seed oils

The oils were evaluated for Hunter L (lightness), a (redness), and b (yellowness) values using a colorimeter (CM-5, Konica Minolta Co., Tokyo, Japan).

6. Analysis of carotenoids in ginseng seed oils

Carotenoid contents of the oils were determined by AOAC [18] method with a slight modification. Carotenoid content was calculated using a calibration curve prepared by β-carotene solutions in hexane. Absorbance was measured at 440 nm with a microplate reader (SpectraMax 190, Molecular devices, Sunnyvale, CA, USA) using hexane as blank.

7. Analysis of tocols (tocopherols and tocotrienols) in ginseng seed oils

Each oil (60 mg) was dissolved in 1 mL 2-propanol, followed by filtering the solution using a 0.2 μm hydrophobic syringe filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) before analysis of tocols [19]. Identification of tocols was carried out using an HPLC (Agilent 1260 infinity, Agilent Technologies) coupled with a single quadrupole mass spectrometer (6130, Agilent Technologies) with an APCI

interface. The separation was operated on an Eclipse XDB-C18 column (5 μm, 4.6 mm × 150 mm, Agilent Technologies) at 40°C. Mobile phase consisted of deionized water (solvent A) and methanol (solvent B). Flow rate was 0.7 mL/min with the following gradient elution program: 0-38 min, 95% B; 38-43 min, linear gradient from 95 to 100% B; 43-43.5 min, 95% B; and 1.5 min, reconditioning. Injection volume was 5 μL. The mass spectroscopy was performed in positive mode. Full mass scan was m/z 100-620 (0.6 s/scan) and target ions generated by tocols were: $(M+H)^+$: m/z 431 (α-tocopherol), 417 (β/γ-tocopherol), 403 (δ-tocopherol), 425 (α-tocotrienol), 411 (β/γ-tocotrienol), and 397 (δ-tocotrienol). Mass parameters were optimized as follows: vaporizer temperature: 325°C; capillary voltage: 4,000 V; corona current: 4 μA; nebulizing pressure (N2): 30 psi; gas temperature (N2): 350°C; gas flow (N2): 8 L/min; and fragmentor: 150 V [20]. Quantification of tocols was carried out using an HPLC (Ultimate 3000, Thermo Scientific Dionex, Waltham, MA, USA) equipped with a ZORBAX Eclipse Plus C18 column (5 μm, 4.6 mm × 150 mm, Agilent Technologies). Mobile phase was methanol and acetonitrile (1:1) at a flow rate of 1.5 mL/min. Injection volume was 20 μL. A fluorescence detector was set at an excitation wavelength of 295 nm and an emission wavelength of 325 nm [19]. Tocols were quantified by comparing peak areas with those of corresponding standards.

8. Preparation of unsaponifiable fraction of ginseng seed oils

Saponification for analyzing squalene and phytosterols in the oils was performed using a method of Beveridge et al [12] with some modification. One milliliter of 0.02% (w/v) 5α-cholestane (internal standard) in tert-butyl methyl ether was added to 1 g of the oil with 20 mL 1 M methanolic potassium hydroxide solution, stirring overnight. This solution was mixed with 40 mL distilled water and

extracted 3 times with 30 mL tert-butyl methyl ether using a separatory funnel. The combined supernatant was washed with 40 mL distilled water repeatedly until the wash water was neutral to 1% (w/v) phenolphthalein in 95% ethanol. The tert-butyl methyl ether extract was passed through sodium sulfate to eliminate water. The solvents were removed by a speed vacuum concentrator (Scanvac, LaboGene, Lynge, Denmark) at 2,000 rpm and 30°C for 4 h. The residue was dissolved in 2 mL hexane, followed by filtering the solution using a 0.2 μm hydrophobic syringe filter.

9. Analysis of squalene and phytosterols in ginseng seed oils

Identification of squalene and phytosterols (campesterol, stigmasterol, and β-sitosterol) in the oils was determined using a QP2010 Plus gas chromatograph (Shimazdu Co., Kyoto, Japan) equipped with a capillary column (DB-5, 30 m × 0.25 mm × 0.25 μm, J&W Scientific) and a mass selective detector. Split ratio was 1:10 and injection volume was 1 μL. Transfer line from GC to mass selective detector was set at 300°C. Injector and detector temperatures were 280 and 300°C, respectively. Carrier gas was helium and flow rate was 1 mL/min. Column temperature was programmed as follows: 100°C held for 3 min, from 100°C to 275°C at 15°C/min held for 25 min, and from 275°C to 280°C at 3°C/min held for 3 min. The mass spectrometer was operated at electron impact ionization mode (EI, 70 eV) and mass range was 50-600 amu [12]. Mass peaks of squalene and phytosterols were identified by matching with mass spectrum and similarity indices of the National Institute of Standards and Technology (NIST) library. Quantification of squalene and phytosterols was determined using the Agilent 6890 gas chromatograph equipped with the DB-5 column and FID. Split ratio was 1:15 and flow rate was 1.3 mL/min. Injection volume, carrier gas, temperatures of

injector, detector, and column were as described for the GC-MS analysis. Squalene and phytosterols were quantified against those of corresponding standards.

10. Determination of crude saponin in defatted ginseng seed meals

Crude saponin in the defatted ginseng seed meals were determined by methods of Shibata et al [21] and Kwon et al [22]. Defatted ginseng seed meal (1 g) was twice extracted using a reflux condenser with 10 mL 80% methanol for 1 h at 70°C. The extracts were filtered by a Whatman No. 4 filter paper (Whatman International Ltd.) and removed solvents to 5 mL of volume by a speed vacuum concentrator at 2,000 rpm and 30°C. This solution was mixed with 10 mL distilled water and extracted 3 times with 10 mL water-saturated 1-butanol using a separatory funnel. The combined supernatant was concentrated using a rotary evaporator at 50°C. The residue was moved to a round bottom flask and dried at 105°C until its weight was constant. The crude saponin content was calculated as constant weight of crude saponin to weight of used defatted seed meal.

11. Determination of total phenolics and flavonoids in defatted ginseng seed meal extracts

The freeze-dried defatted seed meal extracts were diluted with 70% ethanol (10 mg/mL) for analyzing total phenolics and flavonoids. Total phenolics were determined by method of Singleton et al [23]. Twenty microliters of the diluted extracts were mixed with 1.58 mL distilled water and 100 μL Folin-Ciocalteu reagent. After 5 min, the mixture was reacted with 300 μL 20% (w/v) sodium carbonate solution and incubated for 30 min at 40°C. Absorbance was measured at 765 nm. Total phenolic content was expressed as gallic acid equivalent (GAE) calibrated. Total flavonoids were determined by method of Jiang et al [24]. One

milliliter of the diluted extracts was diluted with distilled water to 2 mL of final volume, followed by adding 1 mL 1% (w/v) 2-aminoethyl-diphenylborate in methanol. Absorbance was measured at 404 nm. Total flavonoid content was expressed as quercetin equivalent (QAE) calibrated.

12. Anti-oxidant activities of defatted ginseng seed meal extracts

The freeze-dried defatted seed meal extracts were diluted with 70% ethanol to proper concentrations for analysis of antioxidant activities. DPPH free radical scavenging activities were measured according to the method of Brand-Williams et al [25]. Fifty microliters of the diluted samples were mixed with 100 μL 0.2 mM DPPH in 70% ethanol. After 30 min at room temperature in the dark, absorbance was measured at 517 nm. ABTS free radical scavenging activities were determined by the method of Re et al [26]. To make ABTS solution, 2.45 mM potassium persulfate solution was added into 7 mM ABTS (1:1, v/v) and kept overnight at room temperature in the dark. The ABTS solution was diluted with 70% ethanol to an absorbance of less than 0.70 at 734 nm before use. Twenty microliters of the diluted samples were mixed with 180 μL of the diluted ABTS solution. After 5 min at room temperature, absorbance was measured at 734 nm. DPPH or ABTS free radical scavenging activity (%) was calculated as follows:

DPPH or ABTS free radical scavenging activity (%) = (1 - sample absorbance/control absorbance) × 100. DPPH IC_{50} or ABTS IC_{50} value is the amount of the sample (expressed on the weight basis of the freeze-dried extracts) necessary to decrease the initial DPPH or ABTS free radical concentration by 50%. Ferric reducing anti-oxidant power (FRAP) was measured according to the method of Benzie and Strain [27]. All reagents were diluted with chelex-treated deionized

water (2 g/L). TPTZ reagent was made by diluting 10 mM TPTZ in 40 mM hydrochloric acid. FRAP reagent was made by mixing 300 mM sodium acetate buffer (pH 3.5), TPTZ reagent, and 20 mM ferric chloride anhydrous (10:1:1, v/v/v). The solution was incubated at 37°C for 10 min. Twenty microliters of the samples or standards were mixed with the FRAP reagent (180 μL) and incubated at 37°C for 10 min. The absorbance was measured at 593 nm. FRAP value was expressed by trolox equivalent antioxidant capacity (TEAC) using a trolox equivalent.

13. Statistical analysis

All experiments except for color analysis of the oils were carried out in triplicate. The results were expressed as means±standard deviations. One-way analysis of variance (ANOVA) was conducted using SPSS 23 software (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ($p<0.05$) was used to determine significance between the samples.

RESULTS AND DISCUSSION

1. Proximate composition of ginseng seeds

Proximate composition of the dried ginseng seeds is shown in Table 1. The ginseng seeds in the study had 17.9-22.1% crude lipids, 11.5-15.2% crude proteins, and 1.4-1.7% ash on a dry basis. This was similar to a previous study [10], reporting that 19.1-19.3% crude lipids, 13.7-14.3% crude proteins, and 2.2-8.7% ash on a dry basis were in seeds from 3- and 4-year-old FG collected in Geumsan (Korea). MGS and FGS2 had significantly more lipids, proteins, and ash than FGS1 ($p<0.05$). The longer the cultivation duration, the higher crude fat, crude protein, and ash in the roots from 4-, 5-, and 6-year-old FG collected in Gwacheon (Korea) [7]. Proximate composition of the ginseng seeds may be affected by the cultivation duration.

2. Yields of oils and defatted meal extracts from ginseng seeds

Yields of oils from FGS1 (FGSO1), FGS2 (FGSO2), and MGS (MGSO) were 16.3±1.9, 18.1±0.5, and 19.0±1.2% on a dry basis, respectively. Previous studies reported that yields of oils from ginseng seeds were 15.0-26.6% on a dry basis [11, 12]. Yields of defatted meal extracts from the FGS1 (FGME1), FGS2 (FGME2), and MGS (MGME) were 7.4±0.5, 7.0±0.4, and 6.0±0.3% on a dry basis, respectively.

3. Fatty acid composition of ginseng seed oils

Fatty acid composition of the ginseng seed oils is shown in Table 2. All the oils in the study contained more than 95% unsaturated fatty acids. The major fatty acid in the tested oils was oleic acid (77.9-78.5%), followed by linoleic acid (16.6-17.4%). This was similar to fatty acid composition of ginseng seed oils reported in

Table 1. Proximate composition of seeds from farm-cultivated and mountain-cultivated ginsengs (%, dry basis)

	FGS1[1)]	FGS2	MGS
Crude lipid	17.9±0.8[2)b3)]	21.4±0.2[a]	22.1±0.4[a]
Crude protein	11.5±0.6[b]	14.5±0.5[a]	15.2±0.5[a]
Crude ash	1.4±0.1[b]	1.7±0.1[a]	1.5±0.1[ab]

[1)]FGS1, farm-cultivated ginseng seeds (4 years grown); FGS2, farm-cultivated ginseng seeds (5-6 years grown); and MGS, mountain-cultivated ginseng seeds (7-13 years grown).

[2)]Means±standard deviations of three determinations.

[3)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

Table 2. Fatty acid composition of seed oils from farm-cultivated and mountain-cultivated ginsengs

Fatty acid (%, w/w)	FGSO1[1)]	FGSO2	MGSO
C16:0 (palmitic acid)	1.92±0.01[3)a4)]	1.92±0.00[a]	1.85±0.00[b]
C16:1 (palmitoleic acid)	0.26±0.00[a]	0.26±0.00[a]	0.21±0.00[b]
C18:1 (oleic acid)	77.96±0.03[b]	78.49±0.12[a]	77.88±0.01[b]
C18:2 (linoleic acid)	16.76±0.26[b]	16.61±0.03[b]	17.43±0.08[a]
C18:3 (γ-linolenic acid)	0.15±0.00[b]	0.16±0.00[a]	0.12±0.00[c]
C18:3 (α-linolenic acid)	0.07±0.00[b]	0.07±0.00[b]	0.09±0.00[a]
C20:0 (arachidic acid)	0.03±0.00[ab]	0.03±0.00[b]	0.03±0.00[a]
C20:1 (eicosenoic acid)	0.11±0.00[a]	0.10±0.00[b]	0.09±0.00[c]
SFA[2)]	1.96±0.01[a]	1.95±0.00[b]	1.89±0.00[c]
MUFA	78.33±0.03[b]	78.85±0.12[a]	78.18±0.02[c]
PUFA	16.98±0.26[b]	16.84±0.03[b]	17.65±0.08[a]

[1)]FGSO1, farm-cultivated ginseng seed (4 years grown) oil; FGSO2, farm-cultivated ginseng seed (5-6 years grown) oil; and MGSO, mountain-cultivated ginseng seed (7-13 years grown) oil.

[2)]SFA, saturated fatty acids; MUFA, monosaturated fatty acids; and PUFA, polyunsaturaturated fatty acids.

[3)]Means±standard deviations of three determinations.

[4)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

previous studies [10, 11, 13]. MGSO had significantly more linoleic acid and less oleic acid than the other oils ($p<0.05$), while FGSO2 had significantly more oleic acid and less linoleic acid ($p<0.05$). Similar results were reported for fatty acid compositions in oils from various ginseng seeds from 3- and 4-year-old FG collected in Geumsan (Korea), 4-year-old FG in Jilin (China), and 4-year-old farm-cultivated American ginseng (*Panax quinquefolium* L.) in Wisconsin (USA) [10]. A previous study also reported that the higher oleic acid, the lower linoleic acid in seed oils from 4-year-old FG collected in Geumsan (Korea) [13].

4. Color and carotenoid contents of ginseng seed oils

Color and carotenoid contents of the ginseng seed oils are shown in Table 3. MGSO was significantly higher in Hunter b value and lower in L and a values than the other oils ($p<0.05$). Carotenoids are natural pigments present in seeds and responsible for yellow color [28]. Carotenoids in the MGSO were significantly higher than those in the others ($p<0.05$). There was no significant difference in carotenoid content between FGSO1 and FGSO2. It was reported that the more carotenoid contents in sweet potatoes [29] or olive oils [30], the higher Hunter b or CIE b* values. The possible reason for significantly lower Hunter b value of used sunflower oil was reported to be reduction of carotenoids [31]. Therefore, the degree of yellowness of the ginseng seed oils may be related to the carotenoid content.

5. Tocols in ginseng seed oils

Retention times of α-, β/γ-, and δ-tocotrienols in the ginseng seed oils were matched with those of corresponding standards using HPLC (Fig. 1). However, tocopherols were not detected. The ginseng seed oils in the study had 20.8-24.9 mg

Table 3. Color values and carotenoid contents of seed oils from farm-cultivated and mountain-cultivated ginsengs

	FGSO1[1)]	FGSO2	MGSO
L	97.8±1.1[2)a3)]	98.6±0.1[a]	95.7±0.2[b]
a	-1.3±0.1[b]	-1.0±0.0[a]	-2.4±0.0[c]
b	11.1±0.8[b]	10.5±0.4[b]	27.3±0.3[a]
Carotenoids (μg β-carotene equivalent/g oil)	0.9±0.1[4)b]	0.7±0.1[b]	4.3±1.0[a]

[1)]FGSO1, farm-cultivated ginseng seed (4 years grown) oil; FGSO2, farm-cultivated ginseng seed (5-6 years grown) oil; and MGSO, mountain-cultivated ginseng seed (7-13 years grown) oil.

[2)]Means±standard deviations of two determinations.

[3)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

[4)]Means±standard deviations of three determinations.

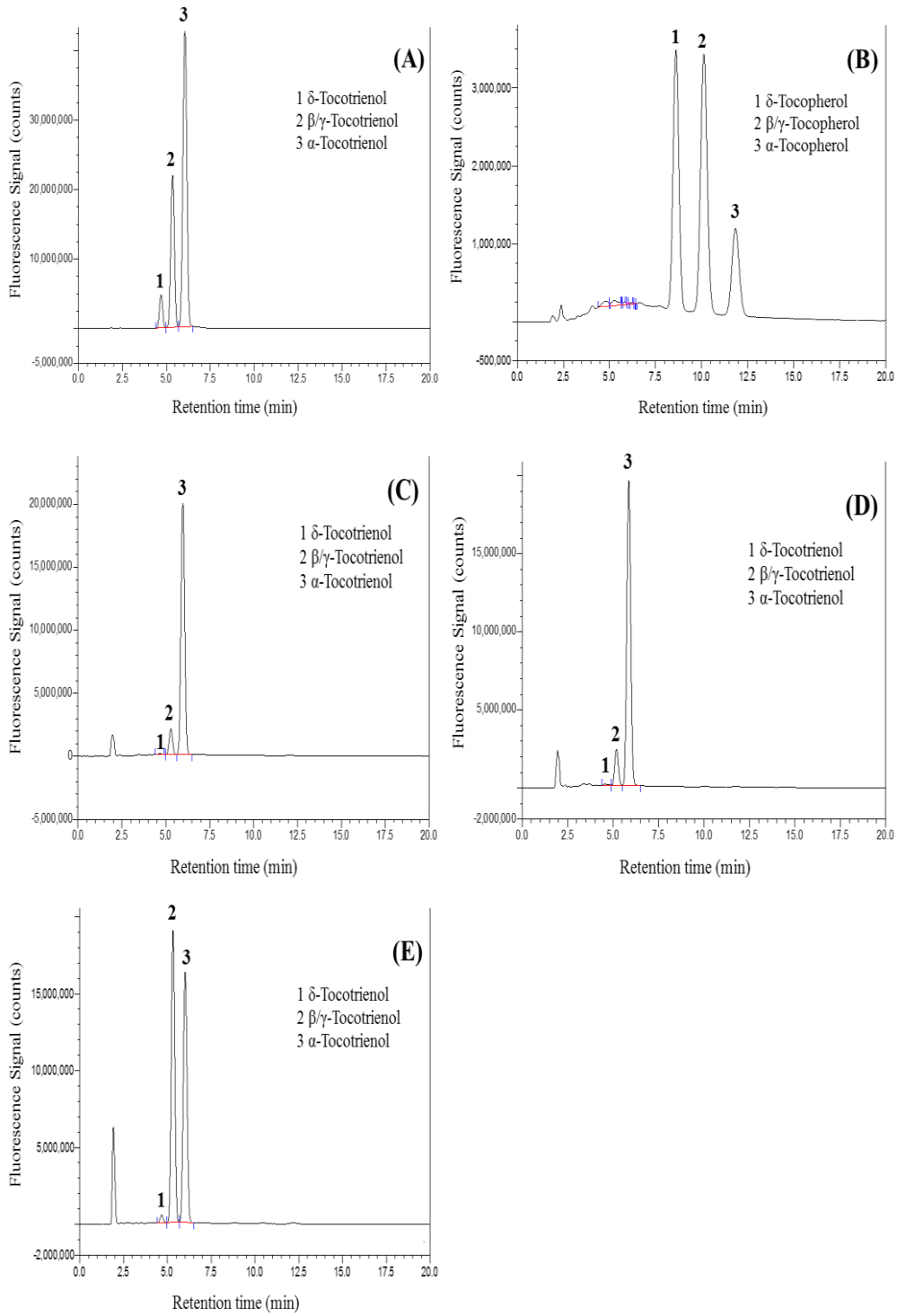


Figure 1. HPLC chromatograms of tocols in standards (A and B), farm-cultivated ginseng seed (4 years grown) oil (C), farm-cultivated ginseng seed (5-6 years grown) oil (D), and mountain-cultivated ginseng seed (7-13 years grown) oil (E).

α-tocotrienol, 0.4-3.7 mg β/γ-tocotrienol, and 0.02-0.23 mg δ-tocotrienol per 100 g oil (Table 4). A previous study reported that only γ-tocopherol was detected in seed oils from 4-year-old FG [13]. MGSO had significantly higher β/γ- and δ-tocotrienols and lower α-tocotrienol than FGSO1 and FGSO2 ($p<0.05$). There were no significant differences in tocotrienol contents between the FGSO1 and FGSO2. Previous studies reported that duration, environment, and system (application of fertilizer and pesticide) of cultivation may influence tocotrienol contents in plants [32-35].

6. Squalene and phytosterols in ginseng seed oils

Mass peaks of squalene and phytosterols (campesterol, stigmasterol, and β-sitosterol) were identified in the ginseng seed oils by matching with mass spectra and similarity indices of NIST library. Retention times of squalene and phytosterols in the ginseng seed oils were matched with those of corresponding standards using GC-FID (Fig. 2). The ginseng seed oils in the study had 298.7-405.3 mg squalene per 100 g oil (Table 5). Shark liver oils are primary commercial sources of squalene [36]. However, vegetable oils have been regarded as another desirable source of squalene due to reduction of fishery [12, 37]. Olive oil, amaranth oil, and pumpkin seed oil, which have been considered as desirable sources of squalene, contain 0.3-0.7, 0.1-0.4, and 0.09% squalene, respectively [38, 39]. Seed oil from farm-cultivated American ginseng collected in British Columbia (Canada) has been also expected to be a desirable source of squalene due to its high amount of squalene (0.5-0.6%) [12]. The oils in the study contained 0.3-0.4% squalene, while the MGSO had significantly more squalene than the others ($p<0.05$). Thus, ginseng seed oil, especially MGSO, could be regarded as a useful source of squalene. The

Table 4. Tocols (mg/100 g oil) in seed oils from farm-cultivated and mountain-cultivated ginsengs

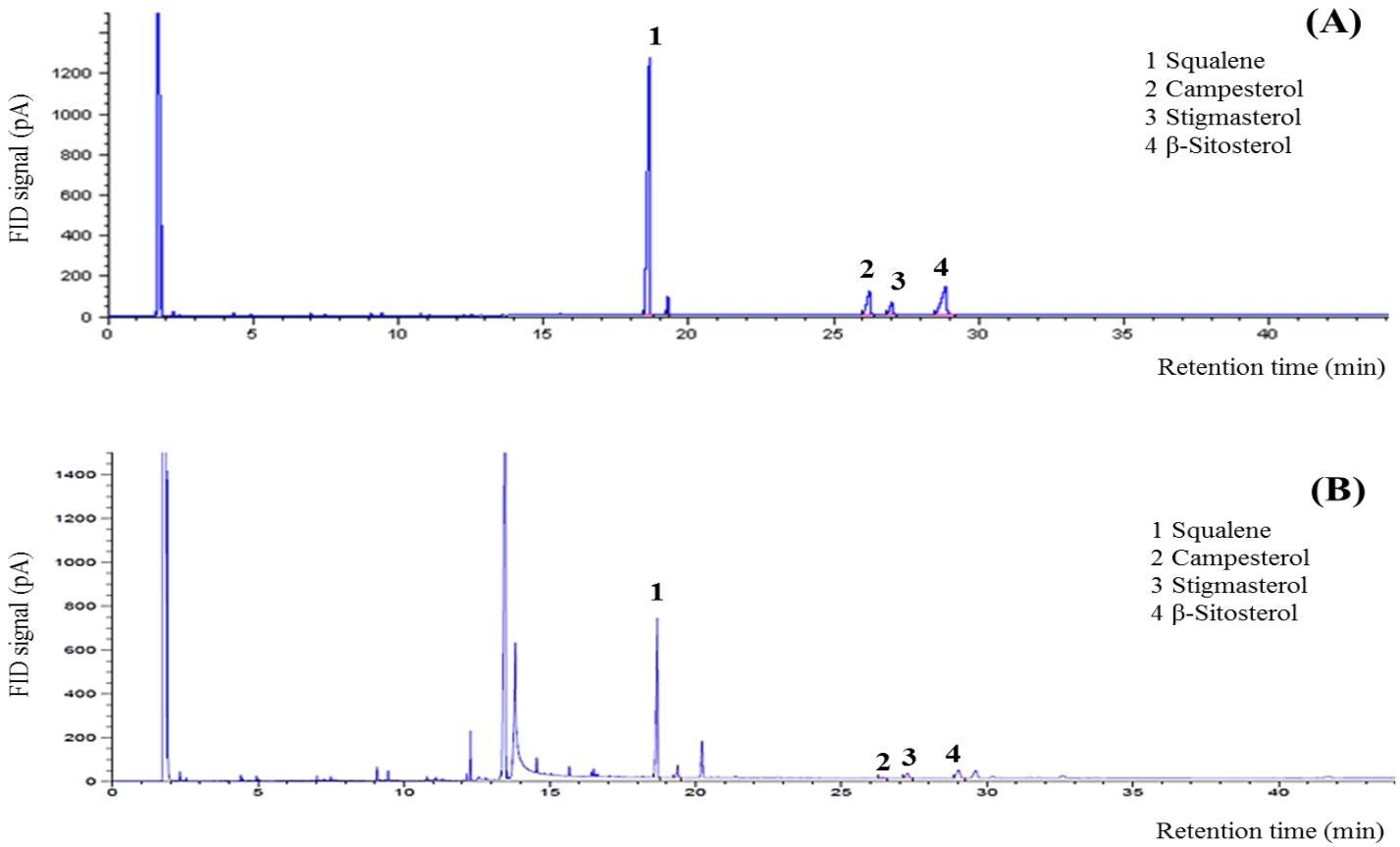
	FGSO1[1)]	FGSO2	MGSO
α-Tocotrienol	24.4±0.4[2)a3)]	24.9±0.5[a]	20.8±0.3[b]
β/γ-Tocotrienol	0.4±0.01[b]	0.4±0.01[b]	3.7±0.06[a]
δ-Tocotrienol	0.04±0.00[b]	0.02±0.01[b]	0.23±0.01[a]
Tocopherols	ND[4)]	ND	ND

[1)]FGSO1, farm-cultivated ginseng seed (4 years grown) oil; FGSO2, farm-cultivated ginseng seed (5-6 years grown) oil; and MGSO, mountain-cultivated ginseng seed (7-13 years grown) oil.

[2)]Means±standard deviations of three determinations.

[3)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

[4)]ND, not detected.


(A)
1 Squalene
2 Campesterol
3 Stigmasterol
4 β-Sitosterol
FID signal (pA)
Retention time (min)
(B)
1 Squalene
2 Campesterol
3 Stigmasterol
4 β-Sitosterol
FID signal (pA)
Retention time (min)

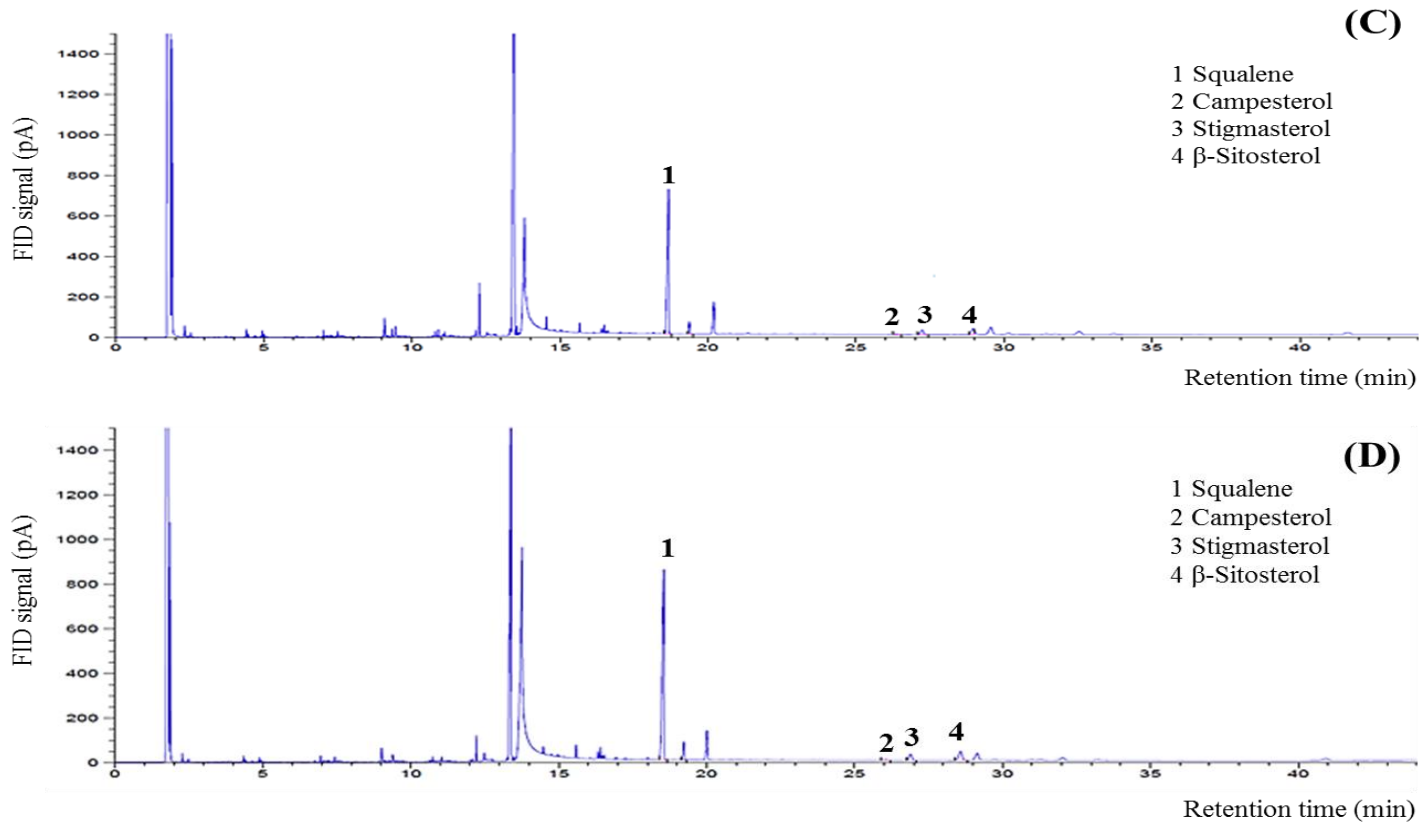


Figure 2. GC chromatograms of squalene and phytosterols in standards (A), farm-cultivated ginseng seed (4 years grown) oil (B), farm-cultivated ginseng seed (5-6 years grown) oil (C), and mountain-cultivated ginseng seed (7-13 years grown) oil (D).

Table 5. Squalene and phytosterols (mg/100 g oil) in seed oils from farm-cultivated and mountain-cultivated ginsengs

	FGSO1[1)]	FGSO2	MGSO
Squalene	327.7±17.0[2)b3)]	298.7±22.5[b]	405.3±19.0[a]
Campesterol	8.1±0.4[a]	5.9±0.3[b]	7.5±0.2[a]
Stigmasterol	39.0±2.8[b]	32.9±4.0[c]	46.6±1.6[a]
β-Sitosterol	83.5±5.3[a]	48.1±2.0[b]	83.6±2.4[a]

[1)]FGSO1, farm-cultivated ginseng seed (4 years grown) oil; FGSO2, farm-cultivated ginseng seed (5-6 years grown) oil; and MGSO, mountain-cultivated ginseng seed (7-13 years grown) oil.

[2)]Means±standard deviations of three determinations.

[3)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

ginseng seed oils in the study had 5.9-8.1 mg campesterol, 32.9-46.6 mg stigmasterol, and 48.1-83.6 mg β-sitosterol per 100 g oil (Table 5). Campesterol, stigmasterol, and β-sitosterol in seed oil from farm-cultivated American ginseng collected in British Columbia (Canada) were 8.5 -12.4, 93.2-113.2, and 125.8-186.4 mg/100 g oil, respectively [12]. Previous studies reported that species of plants may influence their phytosterol contents [40-43]. The differences in phytosterol contents between the oils in this study and farm-cultivated American ginseng seed oil might be attributed to their cultivation origins. The MGSO and FGSO1 had significantly higher phytosterols than FGSO2 ($p<0.05$). The major phytosterol in the tested oils was β-sitosterol. Previous studies reported that β-sitosterol was the most abundant in various seed oils such as linseed, rapeseed, sesame, pumpkin, and sunflower seed oils [39, 44].

7. Crude saponin in defatted ginseng seed meals

Crude saponin in the defatted meals from FGS1, FGS2, and MGS were 8.5±2.3, 7.9±0.6, and 8.3±1.2 mg/g meal on a dry basis, respectively. There was no significant difference in crude saponin content of the defatted ginseng seed meals. This was similar to a previous study [45], reporting that roots from 4-year-old fresh FG collected in Geumsan, Eumseong, Jinan, Hongcheon, Ganghwa, and Punggi (Korea) had more crude saponin than those from 5 and 6-year-old fresh FG; however, there was no significant difference in crude saponin content among them.

8. Total phenolics and flavonoids in defatted ginseng seed meal extracts

Total phenolics and flavonoids in the defatted ginseng seed meal extracts are shown in Table 6. MGME was significantly more in total phenolics and flavonoids than the other extracts ($p<0.05$). There were no significant differences in total

Table 6. Anti-oxidant contents and activities of defatted seed meal extracts from farm-cultivated and mountain-cultivated ginsengs

	FGME1[1)]	FGME2	MGME
Total phenolics (mg GAE[2)]/g)[3)]	19.8±1.0[4)b5)]	20.3±0.4[b]	28.1±1.2[a]
Total flavonoids (mg QE/g)	3.8±0.3[b]	3.9±0.3[b]	5.5±0.3[a]
DPPH IC_{50} (mg/mL)	1.2±0.2[a]	1.0±0.1[ab]	0.8±0.2[b]
ABTS IC_{50} (mg/mL)	1.7±0.03[a]	1.5±0.1[b]	1.0±0.1[c]
FRAP (mM TEAC/g)	103.4±6.1[b]	98.7±2.7[b]	136.6±6.3[a]

[1)]FGME1, defatted farm-cultivated ginseng seed (4 years grown) meal extract; FGME2, defatted farm-cultivated ginseng seed (5-6 years grown) meal extract; and MGME, defatted mountain-cultivated ginseng seed (7-13 years grown) meal extract.

[2)]GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH or ABTS IC_{50}, concentration of the extract required to scavenge 50% of DPPH or ABTS radical; and TEAC, trolox equivalent anti-oxidant capacity.

[3)]Based on the freeze-dried extract.

[4)]Means±standard deviations of three determinations.

[5)]Different small letters indicate significant differences among the samples ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

phenolics and flavonoids between FGME1 and FGME2. Previous studies [3, 4] comparing total phenolics and flavonoids in roots or leaves from FG and MG reported that ethyl acetate extracts of leaves from 12-year-old MG collected in Gangwondo (Korea) contained higher total phenolics (9.7±1.0 g GAE/100 g, dry basis) and flavonoids (3.0±0.1 g kaempferol equivalent/100 g, dry basis) than those from 6-year-old FG collected in Gyunggido (Korea) (4.9±0.6 g GAE and 2.3±0.1 g kaempferol equivalent/100 g dry basis, respectively) and that ethanol extracts of roots from 10-year-old MG collected in Seocheon (Korea) contained higher total phenolics (34.8 nmol GAE/mg, dry basis) than those from 5-year-old FG collected in Hongcheon (Korea) (12.0 nmol GAE/mg, dry basis). It implies that total phenolics and flavonoids in the defatted ginseng seed meal extracts may be affected by the cultivation place and duration.

9. Anti-oxidant activities of defatted ginseng seed meal extracts

Anti-oxidant activities of the defatted ginseng seed meal extracts are shown in Table 6. The DPPH IC_{50} and ABTS IC_{50} values of MGME were significantly lower than those of FGME1 and FGME2 ($p<0.05$), indicating the MGME had higher anti-oxidant activities than the others. MGME had significantly higher FRAP value than the others ($p<0.05$). Ethanol extracts of roots from 10-year-old MG collected in Seocheon (Korea) had lower DPPH IC_{50} value (4.9 mg/mL) than those from 5-year-old FG collected in Hongcheon (Korea) (14.6 mg/mL) [4]. Additionally, ethanol extracts of roots from 8-year-old MG collected in Goesan (Korea) had lower DPPH IC_{50} value (3.3 mg/mL) than those from 4-year-old FG collected in Goesan (Korea) (10.1 mg/mL) [5]. It was reported that the root extracts of MG had higher anti-oxidant activities than those of FG, which might be due to higher content of anti-oxidants such as phenolics and flavonoids in those of MG [4, 5].

The higher anti-oxidant activities of the MGME than those of the others may be attributed to their higher total phenolics and flavonoids.

CONCLUSION

Oleic acid was the major fatty acid in the ginseng seed oils. Hunter b value and carotenoid content of MGSO were significantly higher than those of FGSO. The MGSO had significantly more β/γ- and δ-tocotrienols, and squalene than the others. The major phytosterol was β-sitosterol in the tested oils. MGME had significantly higher anti-oxidants and anti-oxidant activities than FGME. In conclusion, cultivation environment of ginseng might affect physicochemical characteristics of the ginseng seed oils and anti-oxidant activities of the defatted ginseng seed meals.

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국문초록

인삼 및 산양삼 씨앗의 이화학적 특성 및 항산화능

김유정

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인삼(*Panax ginseng* Meyer)은 항노화, 항염증, 항산화, 항종양과 같은 생리 활성을 지닌 약용 식물로 널리 사용되어 왔다. 재배 조건이 인삼의 씨앗, 씨앗 기름, 잎, 뿌리의 화학적 특성에 미치는 영향에 대한 연구들이 보고 되었으나, 인삼 및 산양삼의 씨앗 기름과 씨앗 탈지박에 대한 연구는 부족하다. 따라서 본 연구에서는 인삼 및 산양삼 씨앗 기름의 이화학적 특성을 분석하고 탈지한 씨박의 항산화 물질과 항산화능을 측정하였다. 인삼 및 산양삼 씨앗의 조지방, 조단백질, 조회분의 함량은 각각 17.9−22.1, 11.5−15.2, 1.4−1.7%(dry basis)이었다. 인삼 및 산양삼 씨앗 기름의 95% 이상이 불포화 지방산이었고, 주요 지방산은 올레산(77.9−78.5%)과 리놀레산(16.6−17.4%)이었다. 산양삼 씨앗 기름은 인삼 씨앗 기름보다 Hunter b 값이 유의적으로 높았고, carotenoid (4.3 μg β−carotene equivalent/g oil), β/γ−tocotrienol (3.7 mg/100 g oil), δ−tocotrienol (0.2 mg/100 g oil), squalene (405.3 mg/100 g oil)

함량이 유의적으로 더 많았다($p<0.05$). 씨앗 기름의 주요 phytosterol은 β-sitosterol (48.1-83.6 mg/100 g oil)이었고 산양삼 씨앗 기름에 가장 많았다. 인삼 및 산양삼 탈지 씨박의 조사포닌 함량은 7.9-8.5 mg/g dried meal이었다. 산양삼의 탈지한 씨박 추출물은 인삼의 탈지한 씨박 추출물보다 총 폴리페놀(28.1 mg gallic acid equivalent/g dried extracts)과 플라보노이드(5.5 mg quercetin equivalent/g dried extracts)가 유의적으로 많았다($p<0.05$). 또한, 산양삼의 탈지한 씨박 추출물의 DPPH와 ABTS 라디칼 소거능, FRAP 값이 가장 높았다($p<0.05$). 결론적으로 재배 환경은 인삼 및 산양삼 씨앗 기름의 이화학적 특성 및 탈지한 씨박의 항산화물질과 항산화능에 영향을 미칠 수 있을 것으로 판단한다.

주요어: 인삼, 산양삼, 인삼 씨앗 기름, 탈지한 인삼 씨박, 항산화능

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