Physicochemical characteristics and anti-oxidant activities of farm-cultivated and mountain-cultivated ginseng seeds

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Received: 17 August 2017 / Revised: 7 February 2018 / Accepted: 25 March 2018 / Published online: 2 April 2018
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Abstract Physicochemical characteristics and anti-oxidant capacities of seeds from two farm-cultivated and one mountain-cultivated ginsengs (*Panax ginseng* Meyer) (MG) were determined. The seeds had 17.9–22.1% (dry basis) crude lipids, 11.5–15.2% crude proteins, and 1.4–1.7% ash. Oleic acid (77.9–78.5%) was the predominant fatty acid in the seed oils, followed by linoleic acid (16.6–17.4%). The highest Hunter b value, carotenoids, ($\beta + \gamma$)-tocotrienol, and δ -tocotrienol, and the lowest α -tocotrienol were observed in the seed oils from MG. Squalene was also the most abundant in the MG seed oils. β -Sitosterol was the major phytosterol in the seed oils with MG the highest. Defatted seed meal extracts from MG possessed the most total phenolics and flavonoids, and the highest DPPH and ABTS radical scavenging activities. These results suggest that MG seeds may be a novel source of functional foods.

Keywords Anti-oxidant · Farm-cultivated ginseng · Ginseng seed · Mountain-cultivated ginseng · Physicochemical characteristics

Introduction

Ginseng (*Panax ginseng* Meyer) has been regarded as a medicinal plant due to its physiological activities such as anti-oxidative, anti-inflammatory, anti-aging, and anti-

cancer activities [1]. Koreans mainly consume two types of ginseng, farm-cultivated (FG) and mountain-cultivated ginsengs (MG). FG is cultivated for 5–6 years before harvest in a farm, where the sunlight is partially blocked. MG grows by itself in a mountain area after being sown or transplanted as planned. It is harvested after 10–20 years or more [1, 2].

There have been several studies on effect of the different cultivations on chemical characteristics of ginseng seeds, seed oils, leaves, and roots [2–6]. It has been reported that roots and leaves of MG exhibit higher anti-cancer and anti-oxidant properties than those of FG [1–3]. However, studies on seed oils of FG and MG are still limited.

Ginseng seeds contain 15.0–26.6% (w/w, dry basis) oil [6, 7]. Ginseng seed oil contains more than 90% (w/w) unsaturated fatty acids, with oleic (61.2–87.7%) and linoleic acids (8.9–18.8%) as the major fatty acids [4, 6–8]. It also possesses fat-soluble vitamins, squalene, and phytosterols. These substances affect quality of an oil and have been known to have various health benefits [7, 8]. Ginseng seed oil has been considered to be a potential specialty source of functional foods and cosmetics [6–8].

Defatted seed meals have been mostly discarded or used as low-value by-products after oil extraction [9]. A considerable amount of phenolic compounds, well-known as anti-oxidants, are still present in defatted seed meals [10]. Studies have increasingly paid attention to added value of ginseng by-products with growing interest in evaluation of defatted seed meals [9–11]. However, anti-oxidant properties and potential anti-oxidant substances of defatted seed meals of FG and MG have been little studied. The aims of this study were to evaluate physicochemical properties of seed oils from FG and MG and to evaluate anti-oxidant capacities and amounts of potential anti-oxidant substances

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in the defatted seed meal (DSM) extracts from the FG and MG.

Materials and methods

Materials and reagents

Seeds from FG (4 years grown, FGS1; and 5–6 years grown, FGS2) and MG (7–13 years grown, MGS) harvested in August, 2016 in Hongcheon (Korea) and Pyeongchang (Korea), respectively, were purchased. The seeds were dried in a freeze dryer (Clean Vac12, Hanil Scientific Inc., Gimpo, Korea) for 5 days, which was then stored at -70°C until used.

β -Carotene, α -, γ -, and δ -tocopherols, 5α -cholestane, gallic acid, quercetin, standard mixture of 37 fatty acid methyl esters, boron trifluoride (BF_3)-methanol, Folin-Ciocalteu reagent, 2-aminoethyl diphenylborinate, DPPH, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, and *tert*-butyl methyl ether were obtained from Sigma Chemical Co. (St. Louis, MO, USA). α -, γ -, and δ -Tocotrienol kits and sodium sulfate were obtained from Chromadex (Irvine, CA, USA) and Yakuri Pure Chemicals Co., Ltd. (Osaka, Japan), respectively. Squalene and stigmasterol were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). β -Sitosterol and campesterol were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetonitrile and methanol were obtained from Avantor Performance Materials (Center Valley, PA, USA). The other chemicals used in the study were of analytical grade and obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea).

Preparation of samples

The dried seeds were powdered using a food processor (SMX-G770, Shinil Industrial Co., Ltd., Seoul, Korea) for 3 min, which was then passed through an 18 mesh (1 mm) testing sieve. The ground seed (100 g) was mixed with 500 mL *n*-hexane at room temperature for 2 h, followed by filtering through a Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, England). Solvent was removed from the filtrate using a rotary evaporator (Eyela N-1000, Tokyo Rikakikai, Tokyo, Japan) at 50°C . The extracted oils were stored at -70°C after flushing with nitrogen until further analysis. The oils from the FGS1, FGS2, and MGS were designated as FGSO1, FGSO2, and MGSO, respectively. Yield of the ginseng seed oil was calculated as follows:

$$\text{Yield (\%)} = (W_1/W_0) \times 100,$$

where W_0 is weight of used seed powder (g, dry basis) and W_1 is weight of extracted oil (g).

DSM were left in a hood for 2 days for removal of remaining solvent. The DSM (8 g) was twice refluxed with 160 mL 70% (v/v) ethanol for 2 h at 85°C . The solvent mixture was filtered through a Whatman No. 2 filter paper. Solvent was removed from the filtrate using the rotary evaporator. The residue was dried in the freeze dryer for 5 days, which was then stored at -70°C until analyzed. The DSM extracts from the FGS1, FGS2, and MGS were designated as FGME1, FGME2, and MGME, respectively. Yield of the ginseng DSM extract was calculated as follows:

$$\text{Yield (\%)} = (W_1/W_0) \times 100,$$

where W_0 is weight of used DSM (g, dry basis) and W_1 is weight of freeze-dried extract (g).

Analysis of proximate composition of ginseng seeds

Crude lipids, crude proteins, and ash in the dried seeds were performed according to AOAC Official Method [12] 963.15, 970.22, and 972.15, respectively.

Analysis of fatty acid compositions of ginseng seed oils

The seed oils were converted to methyl esters using BF_3 -methanol by AOCS Official Method [13] Ce 2-66. The fatty acid methyl esters were separated by an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a DB-23 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm , J&W Scientific, Folsom, CA, USA). Split ratio was 1:50. Injector and detector temperatures were set at 240 and 280°C , respectively. Flow rate of carrier gas, helium, was 1.3 mL/min. Oven was programmed at 50°C held for 2 min, from 50 to 175°C at $25^{\circ}\text{C}/\text{min}$, to 230°C at $4^{\circ}\text{C}/\text{min}$ held for 5 min, and to 250°C at $25^{\circ}\text{C}/\text{min}$ held for 3 min [8]. Peaks of fatty acid methyl esters were identified by matching with those of corresponding standards.

Analysis of color of ginseng seed oils

Color of the oils was measured using a colorimeter (CM-5, Konica Minolta Co., Tokyo, Japan). Color values are given as lightness (L), redness (a), and yellowness (b).

Analysis of carotenoids in ginseng seed oils

Carotenoid contents of the oils were evaluated using AOAC Official Method [12] 958.05 with some

modification. Absorbance was determined at 440 nm using a microplate reader (SpectraMax 190, Molecular devices, Sunnyvale, CA, USA) and converted to carotenoid content using a calibration curve of β -carotene solutions in *n*-hexane.

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

Each oil (60 mg) was mixed with 1 mL 2-propanol, which was then filtered through a 0.2 μ m hydrophobic syringe filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) before analyzing tocals [14]. Tocols were identified by an HPLC (Agilent 1260 infinity, Agilent Technologies) equipped with a single quadrupole mass spectrometer (6130, Agilent Technologies) with an APCI interface. Five microliters of each sample were injected to an Eclipse XDB-C18 column (5 μ m, 4.6 mm $\times$ 150 mm, Agilent Technologies) at 40 °C. Mobile phases were deionized water (solvent A) and methanol (solvent B) with a flow rate of 0.7 mL/min. Gradient elution was programmed at 95% B to 38 min, with linear gradient from 95 to 100% B from 38 min to 43 min, at 95% B from 43 min to 43.5 min, and reconditioning for 1.5 min. The mass spectroscopy was operated in positive mode. Full mass scan was m/z 100–620 (0.6 s/scan) and target ions generated by tocals 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). The following mass parameters were applied: vaporizer temperature: 325 °C; capillary voltage: 4000 V; corona current: 4 μ A; nebulizing pressure (N₂): 30 psi; gas temperature (N₂): 350 °C; gas flow (N₂): 8 L/min; and fragmentor: 150 V. Tocals were quantified by an HPLC (Ultimate 3000, Thermo Scientific Dionex, Waltham, MA, USA). Twenty microliters of each sample were injected to a ZORBAX Eclipse Plus C18 column (5 μ m, 4.6 mm $\times$ 150 mm, Agilent Technologies). Mobile phase was methanol and acetonitrile (1:1, v/v) and flow rate was 1.5 mL/min. A fluorescence detector was set at excitation and emission wavelengths of 295 and 325 nm, respectively [14]. Quantification of tocals was conducted comparing those of corresponding standards.

Preparation of unsaponifiable fraction of ginseng seed oils

The seed oils were saponified using a previously described method [7] with some modification before analysis of squalene and phytosterols. Each oil (1 g) was mixed with 1 mL 0.02% (w/v) 5 α -cholestane (internal standard) in *tert*-butyl methyl ether. The mixture was saponified with 20 mL 1 M methanolic KOH solution by stirring overnight, to which 40 mL water was then added. This solution

was transferred to a separatory funnel with 30 mL *tert*-butyl methyl ether to collect the upper layer, which was repeated 3 times. The collected upper layer was washed with 40 mL water. The washing process was repeated until the wash water appeared pink no longer after dripping 1% (w/v) phenolphthalein in 95% (v/v) ethanol. The *tert*-butyl methyl ether extract was passed through sodium sulfate for removal of water. The solvents were eliminated using a speed vacuum concentrator (Scanvac, LaboGene, Lyngø, Denmark) at 2000 rpm and 30 °C for 4 h. The residue was mixed with 2 mL *n*-hexane, which was then filtered through a 0.2 μ m hydrophobic syringe filter.

Analysis of squalene and phytosterols in ginseng seed oils

Squalene and phytosterols in the oils were identified by a QP2010 Plus gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a mass selective detector and a DB-5 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, J&W Scientific). Injection volume was 1 μ L with a split ratio of 1:10. Transfer line from GC to mass selective detector was set at 300 °C. Injector and detector temperatures were set at 280 and 300 °C, respectively. The mass spectrometer was performed at electron impact ionization mode with an electron energy of 70 eV. Mass range was 50–600 amu. Flow rate of carrier gas, helium, was 1 mL/min. Oven was programmed at 100 °C held for 3 min, from 100 to 275 °C at 15 °C/min held for 25 min, and from 275 to 280 °C at 3 °C/min held for 3 min [7]. Mass peaks of squalene and phytosterols were identified by comparing mass spectrum and similarity indices of the National Institute of Standards and Technology (NIST) library. Squalene and phytosterols were quantified by the Agilent 6890 gas chromatograph equipped with the FID and DB-5 column. 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. Quantification of squalene and phytosterols was conducted comparing those of corresponding standards.

Determination of total phenolics and flavonoids in ginseng DSM extracts

The freeze-dried DSM extracts (10 mg) were diluted with 1 mL 70% (v/v) ethanol before analysis of total phenolics and flavonoids. Total phenolics were evaluated according to the method described by Singleton et al. [15]. Water (1.58 mL) and 100 μ L Folin–Ciocalteu reagent were added to 20 μ L of the diluted extract. After 5 min, 300 μ L 20% (w/v) sodium carbonate was added to the mixture, which was then incubated at 40 °C for 30 min. Absorbance was determined at 765 nm. Total phenolics were expressed as

gallic acid equivalent (GAE) calibrated. Total flavonoids were measured according to the method described by Jiang et al. [16]. The diluted extract (1 mL) was dissolved in water to make its final volume 2 mL, which was then reacted with 1 mL 1% (w/v) 2-aminoethyl-diphenylborate in methanol. Absorbance was determined at 404 nm. Total flavonoids were expressed as quercetin equivalent (QE) calibrated.

Anti-oxidant activities of ginseng DSM extracts

The freeze-dried DSM extracts were diluted with 70% (v/v) ethanol to proper concentrations before analyzing anti-oxidant activities. DPPH free radical scavenging activities were determined by the method of Brand-Williams et al. [17]. One hundred microliters of 0.2 mM DPPH in 70% (v/v) ethanol were added to 50 μ L of the diluted sample. This mixture was kept in the dark at room temperature for 30 min. Absorbance was determined at 517 nm. ABTS free radical scavenging activities were measured according to the method suggested by Re et al. [18]. ABTS solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate at ratio of 1:1 (v/v) and kept overnight in the dark at room temperature before use. The ABTS solution was diluted with 70% (v/v) ethanol to have absorbance of less than 0.70 at 734 nm. The diluted ABTS solution (180 μ L) was added to 20 μ L of the diluted sample. This mixture was kept at room temperature for 5 min. Absorbance was determined at 734 nm. DPPH or ABTS free radical scavenging activity was calculated as follows:

$$\text{DPPH or ABTS free radical scavenging activity (\%)} = (1 - A_s/A_0) \times 100,$$

where A_0 is absorbance of control and A_s is absorbance of the sample. DPPH IC_{50} or ABTS IC_{50} value is the concentration of the sample (expressed on the weight basis of the freeze-dried extracts) required to scavenge 50% of the DPPH or ABTS free radical.

Statistical analysis

All the data except for color values of the oils were obtained in triplicate and expressed as means $\pm$ standard deviations. One-way analysis of variance (ANOVA) was conducted using SPSS 23 software (SPSS Inc., Chicago, IL, USA). If significant in ANOVA, differences among the samples were analyzed by Duncan's multiple range test ($p < 0.05$).

Results and discussion

Proximate composition of ginseng seeds

Crude lipids, crude proteins, and ash in the tested ginseng seeds were 17.9–22.1% (w/w, dry basis), 11.5–15.2%, and 1.4–1.7%, respectively (Table 1). A similar result was also reported by Kim et al. [4], who found 3- and 4-year-old FG seeds harvested in Geumsan (Korea) contained 19.1–19.3% (dry basis) crude lipids, 13.7–14.3% crude proteins, and 2.2–8.7% ash. The lipids, proteins, and ash in the MGS and FGS2 were significantly higher than those in the FGS1 ($p < 0.05$). It was reported that 4-, 5-, and 6-year-old FG roots harvested in Gwacheon (Korea) had more crude fat, crude protein, and ash as their cultivation duration was longer [5].

Yields of oils and DSM extracts from ginseng seeds

Oils obtained from the ginseng seeds were 16.3–19.0% (w/w, dry basis) with no significant difference among them ($p > 0.05$) (Table 2). Other researchers reported that they obtained 15.0–26.6% (dry basis) oils from ginseng seeds [6, 7]. DSM extracts from the ginseng seeds were 6.0–7.4% (w/w, dry basis) with the MGME the lowest (Table 6).

Fatty acid compositions of ginseng seed oils

All the tested oils had more than 95% (w/w) unsaturated fatty acids (Table 2). Oleic acid (77.9–78.5%) was the predominant fatty acid in the tested oils, followed by linoleic acid (16.6–17.4%). This fatty acid composition was similar to that of ginseng seed oils reported previously [4, 6, 8].

Table 1 Proximate composition of ginseng seeds (Unit: %, w/w, dry basis)

	FGS1 ¹⁾	FGS2 ²⁾	MGS ³⁾
Crude lipid	17.9 $\pm$ 0.8 ^{4)b5)}	21.4 $\pm$ 0.2 ^a	22.1 $\pm$ 0.4 ^a
Crude protein	11.5 $\pm$ 0.6 ^b	14.5 $\pm$ 0.5 ^a	15.2 $\pm$ 0.5 ^a
Crude ash	1.4 $\pm$ 0.1 ^b	1.7 $\pm$ 0.1 ^a	1.5 $\pm$ 0.1 ^{ab}

¹⁾FGS1, farm-cultivated ginseng seeds (4 years grown)

²⁾FGS2, farm-cultivated ginseng seeds (5–6 years grown)

³⁾MGS, mountain-cultivated ginseng seeds (7–13 years grown)

⁴⁾Means $\pm$ 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)

Table 2 Yields and fatty acid compositions of ginseng seed oils

	FGSO1 ¹⁾	FGSO2 ²⁾	MGSO ³⁾
Yields (% w/w)	16.3 ± 1.9 ⁷⁾	18.1 ± 0.5	19.0 ± 1.2
Fatty acid (% w/w)			
C16:0 (palmitic acid)	1.9 ± 0.01 ^{a8)}	1.9 ± 0.00 ^a	1.9 ± 0.00 ^b
C16:1 (palmitoleic acid)	0.3 ± 0.00 ^a	0.3 ± 0.00 ^a	0.2 ± 0.00 ^b
C18:1 (oleic acid)	78.0 ± 0.03 ^b	78.5 ± 0.12 ^a	77.9 ± 0.01 ^b
C18:2 (linoleic acid)	16.8 ± 0.26 ^b	16.6 ± 0.03 ^b	17.4 ± 0.08 ^a
C18:3 (γ -linolenic acid)	0.2 ± 0.00 ^b	0.2 ± 0.00 ^a	0.1 ± 0.00 ^c
C18:3 (α -linolenic acid)	0.1 ± 0.00 ^b	0.1 ± 0.00 ^b	0.1 ± 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)	0.1 ± 0.00 ^a	0.1 ± 0.00 ^b	0.1 ± 0.00 ^c
SFA ⁴⁾	2.0 ± 0.01 ^a	2.0 ± 0.00 ^b	1.9 ± 0.00 ^c
MUFA ⁵⁾	78.3 ± 0.03 ^b	78.9 ± 0.12 ^a	78.2 ± 0.02 ^c
PUFA ⁶⁾	17.0 ± 0.26 ^b	16.8 ± 0.03 ^b	17.7 ± 0.08 ^a

¹⁾FGSO1, farm-cultivated ginseng seed (4 years grown) oil²⁾FGSO2, farm-cultivated ginseng seed (5–6 years grown) oil³⁾MGSO, mountain-cultivated ginseng seed (7–13 years grown) oil⁴⁾SFA, saturated fatty acids⁵⁾MUFA, monounsaturated fatty acids⁶⁾PUFA, polyunsaturated fatty acids⁷⁾Means ± standard deviations of three determinations⁸⁾Different small letters indicate significant differences among the samples ($p < 0.05$; one-way ANOVA and Duncan's multiple range test)

Color and carotenoid contents of ginseng seed oils

The MGSO exhibited significantly the highest Hunter b value, indicating that it is more yellow than the other oils ($p < 0.05$) (Table 3). Carotenoids are present in seeds as natural pigments which contribute to yellow color [19]. The MGSO had significantly more carotenoids than the others ($p < 0.05$) (Table 3). The carotenoid contents in the FGSO1 and FGSO2 were not significantly different ($p > 0.05$). It was reported that sweet potatoes [20] and olive oils [21] with more carotenoid contents had higher Hunter b or CIE b* values. A previous study also reported that a significantly lower Hunter b value of used sunflower

oil may be due to reduction of carotenoids [22]. Therefore, the carotenoid content may affect the degree of yellowness of the ginseng seed oils.

Tocols in ginseng seed oils

α -, ($\beta + \gamma$)-, and δ -Tocotrienols in the tested ginseng seed oils were 20.8–24.9, 0.4–3.7, and 0.02–0.23 mg/100 g oil, respectively (Table 4). However, tocopherols were not detected in the tested oils. Kim et al. [8] reported that only γ -tocopherol was found in seed oils from 4-year-old FG. The MGSO contained significantly the highest ($\beta + \gamma$)- and δ -tocotrienols and the lowest α -tocotrienol among the

Table 3 Color values and carotenoid contents of ginseng seed oils

	FGSO1 ¹⁾	FGSO2 ²⁾	MGSO ³⁾
L	97.8 ± 1.1 ^{4)a5)}	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 (μ g β -carotene equivalent/g oil)	0.9 ± 0.1 ^{6)b}	0.7 ± 0.1 ^b	4.3 ± 1.0 ^a

¹⁾FGSO1, farm-cultivated ginseng seed (4 years grown) oil²⁾FGSO2, farm-cultivated ginseng seed (5–6 years grown) oil³⁾MGSO, mountain-cultivated ginseng seed (7–13 years grown) oil⁴⁾Means ± standard deviations of two determinations⁵⁾Different small letters indicate significant differences among the samples ($p < 0.05$; one-way ANOVA and Duncan's multiple range test)⁶⁾Means ± standard deviations of three determinations

Table 4 Tocols in ginseng seed oils (Unit: mg/100 g oil)

	FGSO1 ¹⁾	FGSO2 ²⁾	MGSO ³⁾
α -Tocotrienol	24.4 $\pm$ 0.4 ^{4)a5)}	24.9 $\pm$ 0.5 ^a	20.8 $\pm$ 0.3 ^b
(β + γ)-Tocotrienol	0.4 $\pm$ 0.01 ^b	0.4 $\pm$ 0.01 ^b	3.7 $\pm$ 0.06 ^a
δ -Tocotrienol	0.04 $\pm$ 0.00 ^b	0.02 $\pm$ 0.01 ^b	0.23 $\pm$ 0.01 ^a
Tocotrienols	24.8 $\pm$ 0.4	25.4 $\pm$ 0.5	24.7 $\pm$ 0.4
Tocopherols	ND ⁶⁾	ND	ND

¹⁾FGSO1, farm-cultivated ginseng seed (4 years grown) oil²⁾FGSO2, farm-cultivated ginseng seed (5–6 years grown) oil³⁾MGSO, mountain-cultivated ginseng seed (7–13 years grown) oil⁴⁾Means $\pm$ standard deviations of three determinations⁵⁾Different small letters indicate significant differences among the samples ($p < 0.05$; one-way ANOVA and Duncan's multiple range test)⁶⁾ND, not detected**Table 5** Squalene and phytosterols in ginseng seed oils (Unit: mg/100 g oil)

	FGSO1 ¹⁾	FGSO2 ²⁾	MGSO ³⁾
Squalene	328 $\pm$ 17.0 ^{4)b5)}	299 $\pm$ 22.5 ^b	405 $\pm$ 19.0 ^a
Campesterol	8.1 $\pm$ 0.4 ^a	5.9 $\pm$ 0.3 ^b	7.5 $\pm$ 0.2 ^a
Stigmasterol	39.0 $\pm$ 2.8 ^b	32.9 $\pm$ 4.0 ^c	46.6 $\pm$ 1.6 ^a
β -Sitosterol	83.5 $\pm$ 5.3 ^a	48.1 $\pm$ 2.0 ^b	83.6 $\pm$ 2.4 ^a

¹⁾FGSO1, farm-cultivated ginseng seed (4 years grown) oil²⁾FGSO2, farm-cultivated ginseng seed (5–6 years grown) oil³⁾MGSO, mountain-cultivated ginseng seed (7–13 years grown) oil⁴⁾Means $\pm$ standard deviations of three determinations⁵⁾Different small letters indicate significant differences among the samples ($p < 0.05$; one-way ANOVA and Duncan's multiple range test)**Table 6** Yields, anti-oxidant contents, and anti-oxidant activities of defatted ginseng seed meal extracts

	FGME1 ¹⁾	FGME2 ²⁾	MGME ³⁾
Yields (% w/w)	7.4 $\pm$ 0.5 ^{9a10}	7.0 $\pm$ 0.4 ^a	6.0 $\pm$ 0.3 ^b
Total phenolics (mg GAE ^{4)/g} ⁸⁾	19.8 $\pm$ 1.0 ^b	20.3 $\pm$ 0.4 ^b	28.1 $\pm$ 1.2 ^a
Total flavonoids (mg QE ^{5)/g}	3.8 $\pm$ 0.3 ^b	3.9 $\pm$ 0.3 ^b	5.5 $\pm$ 0.3 ^a
DPPH IC ₅₀ ⁶⁾ (mg/mL)	1.2 $\pm$ 0.2 ^a	1.0 $\pm$ 0.1 ^{ab}	0.8 $\pm$ 0.2 ^b
ABTS IC ₅₀ ⁷⁾ (mg/mL)	1.7 $\pm$ 0.03 ^a	1.5 $\pm$ 0.1 ^b	1.0 $\pm$ 0.1 ^c

¹⁾FGME1, defatted farm-cultivated ginseng seed (4 years grown) meal extract²⁾FGME2, defatted farm-cultivated ginseng seed (5–6 years grown) meal extract³⁾MGME, defatted mountain-cultivated ginseng seed (7–13 years grown) meal extract⁴⁾GAE, gallic acid equivalent⁵⁾QE, quercetin equivalent⁶⁾DPPH IC₅₀, concentration of the extract required to scavenge 50% of DPPH radical⁷⁾ABTS IC₅₀, concentration of the extract required to scavenge 50% of ABTS radical⁸⁾Based on the freeze-dried extract⁹⁾Means $\pm$ standard deviations of three determinations¹⁰⁾Different small letters indicate significant differences among the samples ($p < 0.05$; one-way ANOVA and Duncan's multiple range test)

tested oils ($p < 0.05$). Tocotrienol contents in the FGSO1 and FGSO2 were not significantly different ($p > 0.05$). Previous studies reported that tocotrienol contents in plants

may be affected by environment, duration, and system (utilization of fertilizer and pesticide) of cultivation [23–25].

Squalene and phytosterols in ginseng seed oils

Squalene in the tested ginseng seed oils was 299–405 mg/100 g oil (Table 5). Commercial squalene has been mostly obtained from shark liver oil [26]. However, vegetable oils have been considered to be another source of squalene since shark fishery has declined [7]. Olive oil, pumpkin seed oil, and amaranth oil have been suggested to be useful sources of squalene with squalene contents of 0.3–0.7, 0.09, and 0.1–0.4% (w/w), respectively [27, 28]. Seed oil from farm-cultivated American ginseng (*Panax quinquefolium* L.) harvested in British Columbia (Canada) has been also regarded as a useful source of squalene since it contains a large amount of squalene (0.5–0.6%) [7]. Squalene accounted for 0.3–0.4% of the tested oils with significantly the most abundant in the MGSO ($p < 0.05$). Thus, ginseng seed oil, especially MGSO, may be suggested to be a potential desirable source of squalene. Campesterol, stigmasterol, and β -sitosterol in the tested ginseng seed oils were 5.9–8.1, 32.9–46.6, and 48.1–83.6 mg/100 g oil, respectively (Table 5). Seed oil from farm-cultivated American ginseng harvested in British Columbia (Canada) contained 8.5–12.4 mg campesterol/100 g oil, 93.2–113.2 mg stigmasterol/100 g oil, and 116.6–186.4 mg β -sitosterol/100 g oil [7]. A possible reason for the differences of phytosterol contents was reported to be species of plants [29–31]. The differences in phytosterol contents between the tested oils in the present study and farm-cultivated American ginseng seed oil might be influenced by their cultivation origins. Phytosterols were significantly more in the MGSO and FGSO1 than those in the FGSO2 ($p < 0.05$). β -Sitosterol was the most prevalent phytosterol in the tested oils.

Total phenolics, flavonoids, and anti-oxidant activities of ginseng DSM extracts

The MGME contained significantly the most total phenolics and flavonoids among the tested ginseng DSM extracts ($p < 0.05$) (Table 6). Total phenolics and flavonoids in the FGME1 and FGME2 were not significantly different ($p > 0.05$). The MGME exhibited significantly lower DPPH IC_{50} and ABTS IC_{50} values than the others ($p < 0.05$), indicating the MGME possessed higher anti-oxidant activities than the others (Table 6). The more total phenolics and flavonoids of the MGME than those of the others may contribute to their higher anti-oxidant activities.

To summarize, the MGSO had more phytochemicals such as carotenoids, squalene, and phytosterols than the FGSO. The MGME had more anti-oxidant substances and higher anti-oxidant capacities than the FGME. These results suggest that MG seeds may be a novel source of functional foods.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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