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Quality and characteristics of ginseng seed oil treated using different extraction methods

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Ginseng seed oil was prepared using compressed, solvent, and supercritical fluid extraction methods of ginseng seeds, and the extraction yield, color, phenolic compounds, fatty acid contents, and phytosterol contents of the ginseng seed oil were analyzed. Yields were different depending on the roasting pretreatment and extraction method. Among the extraction methods, the yield of ginseng seed oil from supercritical fluid extraction under the conditions of 500 bar and 65°C was the highest, at 17.48%. Color was not different based on the extraction method, but the b-value increased as the roasting time for compression extraction was increased. The b-values of ginseng seed oil following supercritical fluid extraction were 3.54 to 15.6 and those following compression extraction after roasting treatment at 200°C for 30 min, were 20.49, which was the highest value. The result of the phenolic compounds composition showed the presence of gentisic acid, vanillic acid, ferulic acid, and cinnamic acid in the ginseng seed oil. No differences were detected in phenolic acid levels in ginseng seed oil extracted by compression extraction or solvent extraction, but vanillic acid tended to decrease as extraction pressure and temperature were increased for seed oil extracted by a supercritical fluid extraction method. The fatty acid composition of ginseng seed oil was not different based on the extraction method, and unsaturated fatty acids were >90% of all fatty acids, among which, oleic acid was the highest at 80%. Phytosterol analysis showed that β-sitosterol and stigmasterol were detected. The phytosterol content of ginseng seed oil following supercritical fluid extraction was 100.4 to 135.5 mg/100 g, and the phytosterol content following compression extraction and solvent extraction was 71.8 to 80.9 mg/100 g.

Keywords: *Panax ginseng* Meyer, Ginseng seed oil, Supercritical fluid extraction, Solvent extraction, Phytosterol

INTRODUCTION

Ginseng root (*Panax ginseng* Meyer) has been used as an Oriental herbal medicine ingredient, but the aerial part of the plant has seldom been used. Ginseng contains saponins, polyphenols, polyacetylenes, alkaloids, and polysaccharides [1] and has recently been used by adding the leaf, stem, and berry extracts to cosmetics and soaps, and by adding the plant to feed [2]. Ginsenoside, which is a representative active ingredient in ginseng, has various functions including anticancer [3], immune improvement [4], blood pressure-lowering [5], antidiabetic [6], hyperlipidemia preventive [7], and antioxidant effects [8]. Ginseng root has been studied for decades, and high-quality Korean ginseng products have been used as functional foods or pharmaceuticals due to their efficacy. In contrast, studies on ginseng leaves, stems, or berries have mainly focused on skin cosmetics or antioxidant effects

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including the antioxidant and antiaging effects of ginseng berry extract [1], improvement of blood circulation [9], and prevention of skin aging [10]. Some studies have been reported regarding the ginseng seed itself, including saponin analysis of ginseng berries and seeds [2,11-13], free amino acid analysis [14], oil characteristics of ginseng species [15], and storage [16]. Studies on the phytosterol composition, content, and activity in American ginseng seeds have also been reported [17].

Plant oils contain large amounts of phytosterols and tocopherols. Tocopherol has an antioxidant effect by suppressing the lipid peroxide production associated with aging, and plays an important role in nutrition [18,19]. Additionally, phytosterols are widely distributed in plants, including fruits, vegetables, nuts, grains, corn, and legumes [20] and are also contained in the aleurone layer, endosperm, and pericarp [21]. About 250 kinds of phytosterol are known, and five of them, campesterol, β-sitosterol, stigmasterol, campestanol, and β-sitostanol, which are 4-desmethyl sterols, are of major interest for their nutritional content and functionality [20]. Several physiological activities of phytosterols have been reported, including a cholesterol-lowering effect [22], antitumor activity [23], and periodontal disease treatment [24], but all of these studies have been conducted in relation to nuts. Studies on phytosterol in American ginseng seeds have been actively conducted. However, studies on Korean ginseng seeds are insufficient, particularly for the content, composition, and physiological activity of their phytosterols, which are known active ingredients in ginseng seeds [25]. Fatty acid composition and phytosterol content analysis of ginseng seed oil [17] and fatty acid analysis of ginseng seed oil [15] have been reported, but these studies were conducted on the phytosterol content analysis or oil analysis depending on the cultivation environment of the plants. Investigations have been lacking on the antioxidant and physiological activities of ginseng seed phytosterol.

This study was performed to extract ginseng seed oil from Korean ginseng seeds using compression extraction, solvent extraction, and supercritical fluid extraction to investigate the quality characteristics of ginseng seed oil including fatty acids, phenolic compounds, and phytosterols.

MATERIALS AND METHODS

Materials

The ginseng seeds used in this study were obtained from a 4-year-old ginseng produced in 2011 at the Geumsan ginseng market in Chungcheongnam-do (South Korea). The ginseng seeds were dried and after eliminating the skin, the endosperm was used for supercritical fluid extraction. The entire ginseng seed including the skin was ground and used for compression extraction. A total of 10 standard substances were used in the phenolic compound analysis: maltol, coumaric acid, cinnamic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, gentisic acid, and caffeic acid (Sigma, St. Louis, MO, USA). Hydroxyl benzoic acid was purchased from Junsei (Tokyo, Japan). The standard materials used for phytosterol analysis were β-sitosterol, campesterol, brassicasterol, and stigmasterol from Tama Biochemical (Kanagawa, Japan).

Ginseng seed oil and ginseng seed extract preparation

The endosperm portion of the ginseng seeds was roasted at 200°C for 10, 20, or 30 min, ground, and extracted. Samples without roasting treatment were used as controls. Ginseng seed oil extraction was performed using compression extraction, solvent extraction, and supercritical fluid extraction. Each roasting-treated ginseng seed endosperm was pressed using a screw-type oil sampler (Hyeondae Green Industry, Seoul, Korea) for compression extraction and then centrifuged at 8,000 rpm for 20 min to eliminate impurities and to obtain the ginseng seed oil. Roasted and ground ginseng seed endosperm was extracted twice using n-hexane in a vacuum evaporator for 3 h per extraction and vacuum filtered. The solvent in the filtrate was eliminated using a vacuum rotary evaporator to obtain oil from the ginseng seed residue including both skin and endosperm, which were ground without roasting treatment using a grinder (Fritsch, Idar-Oberstein, Germany) for supercritical fluid extraction, and the particles were passed through a 1 mm mesh. Supercritical fluid extraction was conducted under the following conditions: 150 bar/35°C, 150 bar/65°C, 500 bar/35°C, and 500 bar/65°C.

Color measurements

After each extraction condition, the ginseng seed oil color was determined as L, a, and b values by placing a particular amount of ginseng seed oil sample on a 35×10 mm Petri dish (Falcon, San Jose, CA, USA) and measuring using a Minolta, CR-200 colorimeter (Tokyo, Japan). All samples were measured three times to obtain an average value, after which the color was ascertained following calibration with a standard white board (L: 97.83, a: -0.36, b: 1.94).

Phenolic compound analysis

The phenolic compounds in ginseng seed oil were analyzed by HPLC (PU-980; Jasco, Tokyo, Japan) under the following analytical conditions: a Waters C-18 column (5.0 μm, 4.6×250 mm; Waters, Milford, MA, USA) was used, with a mobile phase of water with 2% acetic acid (solvent A) and 50% acetonitrile with 0.5% acetic acid (solvent B) or (solvent A) with 2% acetic acid and 50% acetonitrile (solvent B) with 0.5% acetic acid; samples were developed from an initial 100% of A solvent to a 45% gradient after 70 min with a speed of 0.8 mL/min for 80 min. The sample was detected at 280 nm using a UV detector (MD-2010, Jasco) using a 20 μL sample for injection. Each 2 g sample was dissolved in 10 mL *n*-hexane, and 20 mL of 80% methanol was added to extract the phenolic compounds. Finally, 10 mL of *n*-hexane was added to the extract to eliminate the remaining lipid constituents, and the solvent in the 80% methanol layer was evaporated completely using a vacuum evaporator. The concentrated extract was dissolved in methanol to a 10 mg/mL concentration and filtered through a 0.45 μm syringe filter (Whatman, Buckinghamshire, UK) for further analysis.

Fatty acid analysis

Fatty acid analysis in ginseng seed oil was performed using GC (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA) according to an AOAC official method [26]. The GC column was an HP-FFAP (polyethylene glycol-terephthalic acid; 25 m×0.32 mm×0.5 μm). The column temperature was maintained at 150°C for 1 min, increased 4°C/min up to 230°C, and then maintained for 10 min. The injection temperature was 230°C, and the detector temperature was 250°C. The carrier gas injected was He at 1.5 mL/min, H_2 at 30 mL/min, and air at 300 mL/min. Samples were treated with a methanol-sodium hydroxide solution to make an alkaline salt and then trifluoroborane-methanol was added and heated for esterification. The fatty acid ester produced was dissolved in isooctane to obtain samples for the experiment. Samples were injected at 1 μL each and analyzed using a flame ionization detector (FID). The standard material for fatty acid identification was the Supelco 37 component fatty acid methyl ester mix C4 to C24 (Supelco, Belfonte, PA, USA), and samples were identified by comparing retention times.

Phytosterol analysis

Sample pretreatment for phytosterol analysis of the extracted ginseng seed oil was performed according to the plant sterol test solution preparation method described in the Health Functional Food Code, and phytosterols were analyzed by GC. Each standard material was dissolved in the internal standard solution (dihydrocholesterol in chloroform, concentration of 5 mg/mL to 1 mg/mL concentration) for analysis. The GC column was an HP-1 (25 m×0.32 mm×0.17 μm, 19091Z-012). Column temperature was maintained at 200°C for 1 min and then increased 5°C/min up to 300°C and maintained for 8 min. The injection temperature was 230°C, detector temperature was 250°C, and the carrier gas was He (1.0 mL/min). Samples were injected at 1 μL each, and analyzed using a FID.

Statistical analysis

All expressed values are the means of triplicate determinations. All statistical analyses were performed using the SAS ver. 9.2 (SAS Institute, Cary, NC, USA). The statistical significance of differences was determined using Duncan's multiple tests and one-way ANOVA, evaluating significant differences at $p<0.05$. All data are at the 5% significance level and are reported as means±SD.

RESULTS AND DISCUSSION

Extraction yield

The yields of ginseng seed oil following roasting treatment and compression, solvent, and supercritical fluid extraction are shown in Table 1. The yield of ginseng seed oil extracted by compression extraction (%) was higher than that by solvent extraction using *n*-hexane (%). Supercritical fluid extraction showed great differences in yield depending on extraction pressure and temperature. The ginseng seed oil yield showed that yield was higher

Table 1. Ginseng seed oil yield based on the extraction conditions

	Extraction conditions	Extraction yields (%)
Compress extraction	Control	13.90
	200°C, 10 min	16.00
	200°C, 20 min	16.53
	200°C, 30 min	16.10
Solvent extraction (*n*-hexane)	Control	10.20
	200°C, 10 min	12.00
	200°C, 20 min	13.40
	200°C, 30 min	13.70
Supercritical fluid extraction	150 bar, 35°C	4.30
	150 bar, 65°C	2.40
	500 bar, 35°C	16.59
	500 bar, 65°C	17.48

in roasted ginseng seed compared to that of unroasted ginseng seeds. The compression extraction yield was not greatly different depending on ginseng seed roasting time, and the yield from ginseng seeds roasted at 200°C for 20 min was the highest at 16.53%. The solvent extraction yield tended to slightly increase as roasting time increased, and the yield of ginseng seed oil from seeds roasted for 30 min was 13.70%. The yield of supercritical fluid-extracted seed oil at 500 bar pressure was higher than that at 150 bar, indicating that the yield of oil by supercritical fluid extraction was greatly influenced by pressure. A comparison of oil yield by the different extraction methods demonstrated that the yield was higher following supercritical fluid extraction than following compression or solvent extraction and that the yield following supercritical fluid extraction at 500 bar and 65°C was the highest at 17.48%.

Color

The color of ginseng seed oil following roasting and compression, solvent, and supercritical fluid extraction is shown in Table 2. Neither the L-value nor the a-value changed based on the ginseng seed roasting conditions. However, the b-value increased in oils extracted by compression and solvent extraction as roasting time increased. In particular, the b-value of oil following compression extraction with a roasting treatment at 200°C for 30 min was 20.49, which was the highest value compared to those following solvent and supercritical extraction. This was considered as the result of a browning reaction caused by the roasting treatment. The L-value of ginseng seed oil following supercritical fluid extraction was not different based on the extraction conditions but was slightly higher compared to that by compression and solvent extraction. The a-value was slightly different based on extraction pressure, showing 57.24 and 55.63 at 35°C and 65°C, respectively, at 150 bar, and 57.67 at 35°C and 65°C at 500 bar. The b-value was higher as extraction pressure and temperature increased, with the highest value of 15.60 at 65°C and 500 bar.

Phenolic compounds

The HPLC analysis of the phenolic compounds and ginseng seed oil content following roasting treatment and compression, solvent, and supercritical fluid extraction is shown in Table 3. Among the 10 standard substances used-maltol, coumaric acid, cinnamic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, gentisic acid, caffeic acid, and ρ-hydroxyl benzoic acid-the phenolic acids detected in ginseng seed oil were gentisic acid, vanillic acid, ferulic acid, and cinnamic acid. Ferulic and cinnamic acids were detected following compression extraction, with almost no difference in content depending on the roasting treatment. Cinnamic acid was detected at 0.007 to 0.029 μg/g, and gentisic acid was detected at 1.438 μg/g in unroasted ginseng seed oil following solvent extraction. The phenolic acids detected in ginseng seed oil following supercritical fluid extraction were vanillic and ferulic acids, and the vanillic acid content tended to decrease as pressure and temperature increased. This result was similar to the result of a study by Kim et al. [27] on changes in phenolic acid composition with different steaming times of fresh ginseng in which cinnamic acid, ρ-hydroxybenzoic acid, and vanillic acid decreased as

Table 2. Hunter color of ginseng seed oil based on the extraction conditions

Extraction conditions		L-value	a-value	b-value	ΔE
Compress extraction	Control	55.23±0.32$^{c1)}$	-2.33±0.13^{c}	10.98±0.27^{i}	60.93±0.47^{c}
	200°C, 10 min	54.67±0.18^{d}	-1.99±0.08^{c}	17.50±0.26^{c}	62.98±0.28^{a}
	200°C, 20 min	55.50±0.40^{c}	-2.20±0.08^{d}	17.91±0.24^{b}	61.96±0.57^{b}
	200°C, 30 min	55.23±0.19^{c}	-1.96±0.11^{c}	20.49±0.20^{a}	63.04±0.22^{a}
Solvent extraction (*n*-hexane)	Control	56.51±0.09^{b}	-2.70±0.06^{g}	10.87±0.16^{i}	59.11±0.15^{d}
	200°C, 10 min	57.51±0.18^{a}	-2.78±0.08^{gh}	11.58±0.34^{h}	57.38±0.30^{ef}
	200°C, 20 min	56.38±0.27^{b}	-2.54±0.05^{f}	13.02±0.17^{f}	59.65±0.40^{d}
	200°C, 30 min	56.69±0.25^{b}	-2.47±0.06^{f}	14.01±0.35^{e}	59.42±0.40^{d}
Supercritical fluid extraction	150 bar, 35°C	57.24±0.21^{a}	-0.22±0.03^{b}	3.54±0.13^{k}	57.42±0.31^{f}
	150 bar, 65°C	55.63±0.19^{c}	-0.03±0.01^{a}	5.11±0.24^{j}	59.76±0.26^{d}
	500 bar, 35°C	57.67±0.39^{a}	-2.68±0.07^{g}	12.60±0.16^{g}	57.78±0.55^{ef}
	500 bar, 65°C	57.67±0.06^{a}	-2.89±0.03^{h}	15.60±0.24^{d}	58.41±0.06^{e}

[1)]Means with the same alphabet in each column are not significantly different at $p<0.05$ using Duncan's multiple range test.

Table 3. Phenolic compounds in ginseng seed oil based on the extraction conditions

Phenolic compound (μg/g)	Compress extraction				Solvent extraction (*n*-hexane)				Supercritical fluid extraction			
	Control	200°C, 10 min	200°C, 20 min	200°C, 30 min	Control	200°C, 10 min	200°C, 20 min	200°C, 30 min	150 bar, 35°C	150 bar, 65°C	500 bar, 35°C	500 bar, 65°C
Maltol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxy benzoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gentisic acid	ND	ND	ND	ND	1.438	ND	ND	ND	ND	ND	ND	ND
Vanillic acid	ND	ND	ND	ND	ND	ND	ND	ND	1.286	1.064	0.025	0.08
Caffeic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Syringic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coumaric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ferulic acid	0.020	0.025	0.034	0.027	ND	ND	ND	ND	ND	ND	0.012	0.016
Salicylic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cinnamic acid	0.039	0.040	0.041	0.038	0.029	0.007	0.011	0.017	ND	ND	0.003	ND

ND, not detected.

Table 4. Free fatty acid contents of ginseng seed oil based on the extraction conditions

Fatty acid (g/100g)	Compress extraction				Solvent extraction (*n*-hexane)				Supercritical fluid extraction			
	Control	200°C, 10 min	20°C, 20 min	200°C, 30 min	Control	200°C, 10 min	200°C, 20 min	200°C, 30 min	150 bar, 35°C	150 bar, 65°C	500 bar, 35°C	500 bar, 65°C
Palmitic acid (C16:0)	1.9	2.0	2.0	2.1	1.9	2.0	2.0	2.0	2.5	2.9	1.9	1.9
Palmitoleic acid (C16:1)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3
Stearic acid (C18:0)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3
Oleic acid (C18:1)	80.0	79.8	79.8	79.3	79.9	79.7	79.7	79.7	79.1	79.1	79.6	79.8
Linoleic acid (C18:2)	16.1	16.2	16.2	16.6	16.2	16.3	16.3	16.3	16.4	15.9	16.5	16.3
Linolenic acid (C18:3)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Gadoleic acid (C20:1)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unknown	0.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

steaming time increased. Ferulic acid was not detected at a pressure of 150 bar during supercritical fluid extraction but was detected at 0.012 μg/g at 500 bar and 35°C and 0.016 μg/g at 500 bar and 65°C, indicating that a small amount of ferulic acid was detected at higher pressures during supercritical fluid extraction.

Fatty acids

The fatty acid composition in ginseng seed oil following roasting treatment and compression, solvent, and supercritical fluid extraction is shown in Table 4. Overall, fatty acid composition was not different based on the extraction method. Additionally, the unsaturated fatty acids content was high among fatty acids. That is, ginseng seed oil contained >95% unsaturated fatty acids: 80% oleic acid (C18:1), 16% linoleic acid (C18:2), 2% palmitic acid (C16:0), and some palmitoleic acid (C16:1), stearic acid (C18:0), linolenic acid (C18:3), and gasoleic acid (C20:1). Sung et al. [28] studied the fatty acid content in nuts such as walnut, pistachio, hazelnut, cashew nut, ginkgo, pecan, sunflower and pumpkin seeds and reported that the oleic acid content in hazelnuts was 82%, and that total unsaturated fatty acid content was 91%, which was very similar to our results. The oleic acid content of ginseng seed was more than twice that than that of soybean and sesame oil (23.5% and 45.3%, respectively).

Phytosterols

The analysis of stigmasterol, β-sitosterol, brassicasterol, and campesterol content in ginseng seed oil following roasting treatment and compression, solvent, and supercritical fluid extraction is shown in Table 5. β-Sitosterol and stigmasterol were detected, but brassicasterol and campesterol were not detected. A phytosterol content

Table 5. Phytosterol contents of ginseng seed oil based on the extraction conditions

Phytosterol (mg/100 g)	Compress extraction				Solvent extraction (*n*-hexane)				Supercritical fluid extraction			
	Control	200°C, 10 min	200°C, 20 min	200°C, 30 min	Control	200°C, 10 min	200°C, 20 min	200°C, 30 min	150 bar, 35°C	150 bar, 65°C	500 bar, 35°C	500 bar, 65°C
Stigmasterol	43.8	44.5	49.2	47.4	41.2	47.7	44.3	48.1	71.5	76.6	53.1	55.0
β-Sitosterol	28.0	29.5	31.1	30.6	26.9	30.5	28.6	32.7	55.1	59.0	47.3	48.8
Brassicasterol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Campesterol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	71.8	74.1	80.3	78.1	68.1	78.2	72.9	80.9	126.6	135.5	100.4	103.8

ND, not detected.

analysis in ginseng seed oil showed that the phytosterol content of oil following supercritical fluid extraction was 100.4 to 135.5 mg/100 g, whereas that following compression extraction and solvent extraction was 71.8 to 80.9 mg/100 g, The higher phytosterol content found following supercritical fluid extraction was expected because supercritical fluid carbon dioxide has the excellent property of dissolving non-polar substances. The phytosterol content was highest at 150 bar; that is, phytosterol content was highest at 135.5 mg% under the conditions of 150 bar and 65°C. The phytosterol composition of plant oils is generally 40% to 60% sitosterol, 10% to 30% campesterol, 10% to 20% stigmasterol, and about 5% Δ^5-avenastanol [20]. In our results, the level of stigmasterol was about 10% higher than that of β-sitosterol.

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