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Technical note

Quality and characteristics of fermented ginseng seed oil based on bacterial strain and extraction method

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ABSTRACT

Background: In this study, the fermentation of ginseng seeds was hypothesized to produce useful physiologically-active substances, similar to that observed for fermented ginseng root. Ginseng seed was fermented using *Bacillus, Pediococcus, and Lactobacillus* strains to extract ginseng seed oil, and the extraction yield, color, and quantity of phenolic compounds, fatty acids, and phytosterol were then analyzed.

Methods: The ginseng seed was fermented inoculating 1% of each strain on sterilized ginseng seeds and incubating the seeds at 30°C for 24 h. Oil was extracted from the fermented ginseng seeds using compression extraction, solvent extraction, and supercritical fluid extraction.

Results and Conclusion: The color of the fermented ginseng seed oil did not differ greatly according to the fermentation or extraction method. The highest phenolic compound content recovered with the use of supercritical fluid extraction combined with fermentation using the *Bacillus subtilis* Korea Food Research Institute (KFRI) 1127 strain. The fatty acid composition did not differ greatly according to fermentation strain and extraction method. The phytosterol content of ginseng seed oil fermented with *Bacillus subtilis* KFRI 1127 and extracted using the supercritical fluid method was highest at 983.58 mg/100 g. Therefore, our results suggested that the ginseng seed oil fermented with *Bacillus subtilis* KFRI 1127 and extracted using the supercritical fluid method can yield a higher content of bioactive ingredients, such as phenolics, and phytosterols, without impacting the color or fatty acid composition of the product.

1. Introduction

The incidence of chronic diseases including hyperlipidemia, heart disease, cancer, diabetes, and obesity are rising due to imbalances caused by dietary lifestyle changes. Ginseng is an important herb that has been used as a medicinal plant to remedy such imbalances for thousands of years in Asia and eastern North America. Korean ginseng (*Panax ginseng* Meyer) root has long been used as an oriental medicine, and demand has been rising with the accumulation of scientific evidence for its pharmacological efficacy. Ginseng byproducts, such as leaf, stem, and flower extracts, have been added to cosmetics and soaps, and the plant body is used in animal feed [1–3].

Studies have employed fermentation by lactic acid bacteria to increase the yield of active compounds recovered in extracts of natural substances [4–7]. Particularly, fermentation methods have also been used to improve the bioactivity and sensory qualities of plant products including ginseng [8–10]. However, such studies have been limited to ginseng root, with fermentation of ginseng fruits and seeds seldom considered.

Phytosterols are natural constituents of plants and perform critical roles in plant cells. β-Sitosterol, campesterol, and stigmasterol are integral natural components of plant cell membranes that are abundant in vegetable oils, nuts, seeds, and grains [11,12]. Moreover phytosterols have important bioactive properties, such as cancer prevention [13,14], lowering of plasma total cholesterol levels [15,16], and other nutritive properties. Most plants contain polyphenolic compounds, which are present as free, esterified, or combined forms depending on the species. Phenolic acids are divided into benzoic acids and cinnamic acids, which are responsible for the flavor and aroma of fruits and vegetables, and have specific physiological roles [17–20]. In this study, the fermentation

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of ginseng seeds was hypothesized to produce useful physiologically-active substances, similar to that observed for fermented ginseng root. Ginseng seeds were fermented using *Bacillus subtilis, Pediococcus pentosaceus*, and *Lactobacillus gasseri* strains, and the resultant oil quality characteristics, fatty acid contents, phenolic compounds, and phytosterols were analyzed and evaluated.

2. Materials and methods

2.1. Materials

The ginseng seeds used in this study were from 4-yr-old ginseng plants grown in 2012 and obtained from the Geumsan Ginseng Market in Chungcheongnam-do (Geumsan, Korea). The ginseng seeds were dried after removing the skin and the endosperm was used for compression extraction and supercritical fluid extraction. Maltol, coumaric acid, cinnamic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, gentisic acid, β-sitosterol, campesterol, and stigmasterol were purchased from Sigma Co. (St. Louis, MO, USA). Hydroxyl benzoic acid was purchased from Junsei Co. (Tokyo, Japan).

2.2. Strains

The strains used to ferment the ginseng seeds were Gram-positive *L. gasseri* KCTC 3162, *P. pentosaceus* LY011, *B. subtilis* KFRI 1124, and *B. subtilis* KFRI 1127 obtained from Korean Collection for Type Cultures (KCTC) maintained by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) and the Korea Food Research Institute (KFRI). The *Bacillus* strains were inoculated in TS (Triptic Soy) broth, and the *Lactobacillus* and *Pediococcus* strains were inoculated in MRS (de Man, Rogosa and Sharpe) broth and incubated at 30°C for 24 h.

2.3. Fermentation

The sterilized ginseng seeds (500 g) were fermented by inoculating 1% of each strain and then incubating at 30°C for 24 h. Independent fermentations were carried out in triplicates, and fermented ginseng seeds were combined and freeze-dried for analysis.

2.4. Extraction

The fermented ginseng seed oil was extracted by compression extraction, solvent extraction, or supercritical fluid extraction. Fermented ginseng seed endosperm was pressed using a screw-type oil sampler (Hyeondae Green Industry, Seoul, Korea) for compression extraction and then centrifuged at 8,224*g* for 20 min to eliminate impurities and obtain the fermented ginseng seed oil. For solvent extraction, fermented ginseng seeds were extracted twice with *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 (N-1001S; EYELA, Tokyo, JAPAN). Supercritical fluid extraction (Greentek21 Co., Anyang, Korea) was conducted at 15 MPa and 65°C.

2.5. Color measurements

After each extraction, fermented ginseng seed oil color was determined using lightness (L), redness (a), and yellowness (b) values with a Minolta CR-200 colorimeter (Tokyo, Japan). All samples were measured five times to obtain an average value.

2.6. Phenolic compound analysis

The phenolic compounds in the ginseng seed oil were analyzed by high-performance liquid chromatography (PU-980; Jasco, Tokyo, Japan) under the following analytical conditions: Waters C-18 column (5.0μm, 4.6mm × 250mm; Milford, MA, USA), the mobile consisted of 2% acetic acid in water (Solvent A) and 50% acetonitrile with 0.5% acetic acid (Solvent B) utilizing the following gradient over a total run time of 80 min: 45% A for 70 min, 0% A for 73 min, 100% A for 78 min, and 100% A until completion of the run. The flow rate of the mobile phase was 0.8 mL/min. The sample was detected at 280 nm. 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 *n*-hexane was added to the extract to eliminate the remaining lipid constituents, and solvent in the 80% methanol layer was evaporated completely using a vacuum evaporator. The concentrated extract was dissolved in LC grade methanol (Merck, Kenilworth, New Jersey, USA) to 10 mg/mL and filtered through a 0.45-μm syringe filter (Whatman, Maidstone, England).

2.7. Fatty acid analysis

Fatty acid analysis of the ginseng seed oil was performed by gas chromatography (GC) (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA) according to an Association of Official Analytical Chemists (AOAC) official method [21]. The GC column was an HP-FFAP (polyethylene glycol–terephthalic acid; 25m× 0.32mm × 0.5μm). Column temperature was maintained at 150°C for 1 min, which was increased at 4°C/min up to 230°C, and maintained for 10 min. The injection temperature was 230°C, with the detector temperature at 250°C. The carrier gases were He at a flow rate of 1.5 mL/min, H_2 at a flow rate of 30 mL/min, and air at a flow rate of 300 mL/min. The samples were treated with a methanol–sodium hydroxide solution to form an alkaline salt, and trifluoroborane–methanol was added and heated for esterification. The fatty acid esters were dissolved in isooctane to obtain samples for the experiment. The samples (1 μL) were injected and analyzed using a flame ionization detector. The standard material for fatty acid identification was the Supelco 37 component fatty acid methyl ester mix C4–C24 (Supelco, Belfonte, PA, USA), and samples were identified by comparing retention times.

2.8. Phytosterol analysis

The samples were pretreated for the phytosterol analysis according to the plant sterol test solution preparation method (4.3.38. phytosterol) in the Health Functional Food Code, and phytosterols were analyzed by GC (M600D; Youngling, Seoul, Korea). The standard materials used for analysis were 70% β-sitosterol and 5α-cholestane. Each standard was dissolved in the internal standard solution (1–5 mg/mL dihydrocholesterol in chloroform) for analysis. The GC column was an HP-ultra-2 crosslinked 5% PHME siloxane (25m × 0.25mm × 0.33μm), and the column temperature was 285°C. The injection and detector temperatures were 300°C, and the carrier gas was N_2 (1.0 mL/min). The samples (2 μL) were analyzed using an flame ionization detector.

2.9. Statistical analysis

All expressed values are means ± standard deviations of triplicate determinations. All statistical analyses were performed using SAS Version 9.3 (Cary, North Carolina, USA) [22]. Differences were detected using Duncan's multiple range tests and one-way analysis of variance. A p value < 0.05 was considered significant.

Table 1
Ginseng seed oil yield based on extraction conditions (%)

	Extraction method		
	Compress extraction	Solvent extraction (*n*-hexane)	Supercritical fluid extraction (15 MPa, 65°C)
Control[1)]	5.2 ± 1.09[c2)]	16.68 ± 0.97[a]	4.87 ± 1.60[a]
Bacillus subtilis KFRI 1124	7.8 ± 1.12[a]	13.53 ± 1.05[b]	3.68 ± 1.84[b]
Bacillus subtilis KFRI 1127	7.7 ± 1.97[a]	13.83 ± 0.93[b]	4.11 ± 1.77[ab]
Pediococcus pentosaceus LY 011	6.5 ± 1.13[b]	14.80 ± 1.08[b]	2.71 ± 1.73[c]
Lactobacillus gasseri KCTC 3162	6.7 ± 1.83[b]	16.35 ± 1.13[a]	3.85 ± 1.87[b]

KCTC, Korean Collection for Type Cultures; KFRI, Korea Food Research Institute
1) Ginseng seed oil was not fermented
2) All values are mean ± standard deviation of triplicate determinations. Means with the same letter in each column are not significantly different at $p < 0.05$ by Duncan's multiple range tests

3. Results and discussion

3.1. Extraction yield

The yields derived from the fermented ginseng seed oil based on the extraction method are shown in Table 1. Compressed extraction resulted in a mean yield of 7.8%, and was significantly different ($p < 0.05$) to solvent extraction (13.53%) but similar to supercritical fluid extraction (3.68%) using samples fermented with *B. subtilis* KFRI 1124. A difference ($p < 0.05$) was detected between compression and solvent extraction methods when using samples fermented with *B. subtilis* KFRI 1127. Supercritical fluid extraction using *P. pentosaceus* LY 011 fermented samples resulted in a mean yield value of 2.71%, which was significantly different ($p < 0.05$) from the compression (6.5%) and solvent extraction (14.8%) methods. The values for compression (6.7%) and supercritical fluid extraction (3.85%) were different in the *L. gasseri* KCTC 3162 treated samples. In the control treatment (in which the ginseng seed oil was not fermented), compression extraction had a mean yield of 5.2%, which was significantly different ($p < 0.05$) from solvent extraction (16.68%) and supercritical fluid extraction (4.87%).

3.2. Color

The Hunter L, a, and b values are shown in Table 2. The highest lightness was 42.69 derived from *P. pentosaceus* LY 011 and solvent extracted samples, whereas the lowest lightness was observed with compression extraction without the use of a bacterial strain (39.80). These results indicate that the extraction method and microorganism strain had an effect on the ginseng lightness value. The L (lightness) value in *B. subtilis* KFRI 1127 and compression extraction had a mean value of 41.96, which was significantly different ($p < 0.05$) from all other microorganisms. The a (redness) values were from −0.19 (*B. subtilis* KFRI 1127) to 0.51 (control). Significant differences ($p < 0.05$) were detected for all microorganisms and the control. The b (yellowness) values ranged from 1.5 (*P. pentosaceus* LY 011) to 2.51 (*B. subtilis* KFRI 1124). Significant differences ($p < 0.05$) were detected for all microorganisms and the control. ΔE ranged from 55.14 (*P. pentosaceus* LY 011) to 58.04 (control). Significant differences ($p < 0.05$) were detected for all strains and the control, with the exception of *P. pentosaceus* LY 011 and *L. gasseri* KCTC 3162 (55.20) which had similar values.

3.3. Phenolic compound component

The phenolic compounds in the fermented ginseng seed oil extractions were analyzed. As shown in Table 3, compression-extracted oil contained maltol, *ρ*-coumaric acid, and *trans*-cinnamic acid, and the content varied according to the fermenting microorganism used. Phenolic compound content was lower in oils fermented with *Bacillus subtilis* than in oils fermented with *Pediococcus* or *Lactobacillus*. Solvent-extracted oil only contained *ρ*-coumaric acid and *trans*-cinnamic acid, showing that the number and yield of compounds detected were considerably lower than for those recovered using the other extraction methods. Supercritical fluid-extracted oil contained maltol, vanillic acid + caffeic acid, *ρ*-coumaric acid, and *trans*-cinnamic acid, a greater number compared with that derived from compression or solvent extraction methods. In particular, the number of phenolic compounds increased significantly in oils fermented with *B. subtilis* KFRI 1127 and *L. gasseri* KCTC 3162. *B. subtilis* KFRI 1127-fermented oils extracted with supercritical fluid contained 22.8 μg/100 g maltol, 26.7 μg/100 g vanillic acid + caffeic acid, 224.9 μg/100 g *ρ*-coumaric acid, and 28.7 μg/100 g *trans*-cinnamic acid, whereas *L. gasseri* KCTC 3162-fermented oils contained 22.7 μg/100 g maltol, 41.1 μg/100 g

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

Extraction conditions		*L*	*a*	*b*	*ΔE*
Compress extraction	Control[1)]	39.80 ± 0.05[d2)]	0.51 ± 0.05[a]	2.41 ± 0.03[b]	58.04 ± 0.05[a]
	Bacillus subtilis KFRI 1124	40.97 ± 0.07[c]	0.13 ± 0.04[b]	2.51 ± 0.00[a]	56.87 ± 0.07[b]
	Bacillus subtilis KFRI 1127	41.96 ± 0.05[b]	−0.19 ± 0.02[c]	2.26 ± 0.01[c]	55.87 ± 0.05[c]
	Pediococcus pentosaceus LY 011	42.69 ± 0.02[a]	−0.47 ± 0.01[e]	1.57 ± 0.01[e]	55.14 ± 0.02[d]
	Lactobacillus gasseri KCTC 3162	42.63 ± 0.01[a]	−0.28 ± 0.03[d]	1.79 ± 0.02[d]	55.20 ± 0.01[d]
Solvent extraction (*n*-hexane)	Control	40.92 ± 0.05[d]	−0.50 ± 0.02[a]	2.99 ± 0.01[d]	56.92 ± 0.05[a]
	Bacillus subtilis KFRI 1124	42.30 ± 0.03[a]	−0.73 ± 0.04[c]	3.93 ± 0.01[b]	55.56 ± 0.03[d]
	Bacillus subtilis KFRI 1127	42.18 ± 0.09[b]	−0.85 ± 0.02[d]	4.34 ± 0.04[a]	55.70 ± 0.09[c]
	Pediococcus pentosaceus LY 011	41.86 ± 0.05[c]	−0.56 ± 0.04[b]	2.56 ± 0.02[e]	55.97 ± 0.05[b]
	Lactobacillus gasseri KCTC 3162	42.27 ± 0.10[ab]	−0.70 ± 0.03[c]	3.39 ± 0.04[c]	55.58 ± 0.10[d]
Supercritical fluid extraction (15 MPa, 65°C)	Control	42.25 ± 0.14[a]	−0.13 ± 0.08[a]	1.86 ± 0.04[e]	55.58 ± 0.14[d]
	Bacillus subtilis KFRI 1124	40.91 ± 0.12[c]	−0.13 ± 0.03[a]	3.91 ± 0.09[a]	56.95 ± 0.12[b]
	Bacillus subtilis KFRI 1127	41.52 ± 0.27[b]	−0.12 ± 0.04[a]	2.42 ± 0.10[d]	56.31 ± 0.26[c]
	Pediococcus pentosaceus LY 011	42.13 ± 0.01[a]	−0.17 ± 0.10[a]	3.73 ± 0.04[c]	55.73 ± 0.01[d]
	Lactobacillus gasseri KCTC 3162	40.55 ± 0.07[d]	−0.12 ± 0.02[a]	3.82 ± 0.01[b]	57.31 ± 0.07[a]

a, redness; b, yellowness; KCTC, Korean Collection for Type Cultures; KFRI, Korea Food Research Institute; L, lightness
1) Ginseng seed oil was not fermented
2) All values are mean ± standard deviation of triplicate determinations. Means with the same letter in each column are not significantly different at $p < 0.05$ by Duncan's multiple range tests

Table 3
Phenolic compound contents of fermented ginseng seed oil based on extraction conditions

Phenolic compound (μg/100g)		Maltol	Vanillic acid + caffeic acid	ρ-Coumaric acid	Ferulic acid	*trans*-Cinnamic acid
Compress extraction	Control[1)]	ND[2)]	ND	ND	ND	7.0 ± 0.7
	Bacillus subtilis KFRI 1124	ND	ND	2.0 ± 2.5	ND	6.1 ± 2.1
	Bacillus subtilis KFRI 1127	ND	ND	2.0 ± 1.4	ND	7.2 ± 2.5
	Pediococcus pentosaceus LY 011	7.2 ± 1.2	ND	77.8 ± 4.9	ND	14.1 ± 0.7
	Lactobacillus gasseri KCTC 3162	5.4 ± 0.9	ND	27.9 ± 7.1	4.5 ± 2.5	9.2 ± 2.8
Solvent extraction (*n*-hexane)	Control	ND	ND	ND	ND	0.9 ± 0.7
	Bacillus subtilis KFRI 1124	ND	ND	2.5 ± 1.8	ND	1.0 ± 0.7
	Bacillus subtilis KFRI 1127	ND	ND	1.8 ± 1.4	ND	1.3 ± 0.2
	Pediococcus pentosaceus LY 011	ND	ND	4.2 ± 3.5	ND	1.3 ± 0.1
	Lactobacillus gasseri KCTC 3162	ND	ND	2.2 ± 2.8	ND	1.2 ± 0.6
Supercritical fluid extraction (15 MPa, 65°C)	Control	5.2 ± 1.7	6.2 ± 1.8	ND	ND	11.8 ± 4.2
	Bacillus subtilis KFRI 1124	5.7 ± 2.4	6.6 ± 1.2	21.9 ± 10.6	ND	5.6 ± 2.1
	Bacillus subtilis KFRI 1127	22.8 ± 3.8	26.7 ± 5.5	224.9 ± 3.5	ND	28.7 ± 2.8
	Pediococcus pentosaceus LY 011	11.7 ± 0.7	12.5 ± 3.5	9.4 ± 5.7	ND	18.2 ± 4.9
	Lactobacillus gasseri KCTC 3162	22.7 ± 2.0	41.1 ± 1.5	131.1 ± 4.1	ND	23.6 ± 2.8

KCTC, Korean Collection for Type Cultures; KFRI, Korea Food Research Institute; ND, not detected
[1)] Ginseng seed oil was not fermented
[2)] Not detected

vanillic acid+caffeic acid, 131.1 μg/100 g *ρ*-coumaric acid, and 23.6 μg/100 g *trans*-cinnamic acid. *ρ*-Coumaric acid content was the highest in samples fermented with both bacteria, and maltol content was more than four times higher in these extractions compared with control oil. The amount of vanillic acid + caffeic acid also increased compared with that in control oil, with a greater than six-fold value in oil fermented with the *L. gasseri* KCTC 3162 strain compared with control oil. *trans*-Cinnamic acid was detected in all samples regardless of extraction method or fermentation conditions.

3.4. Fatty acids

Table 4 presents the composition and content of fatty acids in the fermented ginseng seed oil based on extraction method. Fatty acid composition did not vary greatly according to the fermentation or extraction method. The fatty acid composition of fermented ginseng seed oil was > 90% unsaturated fatty acids, including 78% oleic acid (C18:1) and 18% linoleic acid (C18:2). These results are similar to those reported by Beveridge et al, [23] for which the fatty acid composition of American ginseng seed oil was similar to that of olive oil, with high monounsaturated fatty acid content, particularly oleic acid (> 87%). The fatty acid composition included approximately 2% palmitic acid (C16:0) with some palmitoleic acid (C16:1), stearic acid (C18:0), α-linolenic acid (C18:3, ω3), γ-linolenic acid (C18:3, ω6), and gadoleic acid (C20:1). These results were similar to fatty acid content values reported during the growth of ginseng seeds: oleic acid > linoleic acid > palmitic acid > stearic acid [24]. In addition, the fatty acid composition of the compression-extracted and solvent-extracted oils did not differ between treatment groups. Compared with other extraction methods, however, the supercritical fluid-extracted oil contained higher amounts of saturated fatty acids, such as palmitic and stearic acids than those in oils extracted with the other methods. In particular, the value for oil fermented with the *P. pentosaceus* LY 011 strain was highest, and oleic acid content decreased as the presence of saturated fatty acids increased.

3.5. Phytosterols

Table 5 shows the content and ratio of phytosterols, such as campesterol, stigmasterol, β-sitosterol, and sitostanol in fermented ginseng seed oil based on extraction method. The content of these phytosterols differed significantly according to the bacterial species and extraction method used. Supercritical fluid extraction resulted in the highest total phytosterol content, followed by solvent and

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

Fatty acid (g/100g)		Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	γ-Linolenic acid	α-Linolenic acid	Gadoleic acid	Total
Compress extraction	Control[1)]	2.1 ± 0.36[2)]	0.3 ± 0.01	0.3 ± 0.01	79.1 ± 1.06	17.9 ± 1.00	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	100.0
	Bacillus subtilis KFRI 1124	2.1 ± 0.26	0.3 ± 0.01	0.3 ± 0.02	79.2 ± 1.63	17.8 ± 0.76	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	100.0
	Bacillus subtilis KFRI 1127	2.1 ± 0.17	0.3 ± 0.02	0.3 ± 0.02	78.9 ± 0.34	18.1 ± 1.04	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	100.0
	Pediococcus pentosaceus LY 011	2.0 ± 0.17	0.3 ± 0.02	0.3 ± 0.01	79.3 ± 0.62	17.8 ± 0.75	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.01	100.0
	Lactobacillus gasseri KCTC 3162	2.0 ± 0.11	0.3 ± 0.03	0.3 ± 0.01	79.4 ± 0.85	17.7 ± 1.53	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.03	100.0
Solvent extraction (*n*-hexane)	Control	2.1 ± 0.25	0.3 ± 0.02	0.3 ± 0.02	78.5 ± 1.32	18.5 ± 0.83	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	100.0
	Bacillus subtilis KFRI 1124	2.0 ± 0.15	0.3 ± 0.03	0.3 ± 0.01	80.2 ± 0.57	16.9 ± 1.05	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	100.0
	Bacillus subtilis KFRI 1127	2.1 ± 0.20	0.4 ± 0.02	0.3 ± 0.02	78.7 ± 1.31	18.2 ± 0.68	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	100.0
	Pediococcus pentosaceus LY 011	2.0 ± 0.17	0.4 ± 0.02	0.3 ± 0.01	78.7 ± 1.02	18.3 ± 0.76	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	100.0
	Lactobacillus gasseri KCTC 3162	2.0 ± 0.11	0.3 ± 0.03	0.3 ± 0.01	78.5 ± 1.50	18.6 ± 0.58	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	100.0
Supercritical fluid extraction (15 MPa, 65°C)	Control	4.0 ± 0.17	0.5 ± 0.02	0.3 ± 0.01	77.1 ± 0.10	17.7 ± 0.92	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.01	100.0
	Bacillus subtilis KFRI 1124	2.5 ± 0.20	0.4 ± 0.03	0.3 ± 0.02	79.3 ± 1.19	17.2 ± 0.68	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	100.0
	Bacillus subtilis KFRI 1127	2.7 ± 0.20	0.4 ± 0.04	0.3 ± 0.01	77.9 ± 0.79	18.4 ± 0.52	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.02	100.0
	Pediococcus pentosaceus LY 011	5.8 ± 0.15	0.7 ± 0.02	0.3 ± 0.02	75.2 ± 2.55	17.6 ± 0.32	0.1 ± 0.03	0.1 ± 0.01	0.2 ± 0.01	100.0
	Lactobacillus gasseri KCTC 3162	2.1 ± 0.11	0.4 ± 0.01	0.3 ± 0.01	79.2 ± 1.04	17.7 ± 0.70	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	100.0

KCTC, Korean Collection for Type Cultures; KFRI, Korea Food Research Institute
[1)] Ginseng seed oil was not fermented
[2)] All values are mean ± standard deviation of triplicate determinations

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

Phytosterol (mg/100g)		Campesterol	Stigmasterol	β-Sitosterol	Sitostanol	Total phytosterol
Compress extraction	Control[1)]	42.70 ± 0.77^{e}[2)]	44.18 ± 4.82^{c}	45.34 ± 0.26^{c}	141.21 ± 0.97^{d}	273.43
	Bacillus subtilis KFRI 1124	54.21 ± 8.33^{b}	41.79 ± 0.30^{e}	41.72 ± 3.54^{d}	146.82 ± 16.13b	284.54
	Bacillus subtilis KFRI 1127	49.11 ± 4.86^{d}	41.84 ± 6.72^{d}	41.37 ± 1.96^{e}	138.51 ± 9.82^{e}	270.83
	Pediococcus pentosaceus LY 011	51.15 ± 4.52^{c}	45.46 ± 0.79^{b}	46.36 ± 2.97^{a}	147.80 ± 7.52^{a}	290.77
	Lactobacillus gasseri KCTC 3162	59.41 ± 9.26^{a}	49.66 ± 1.24^{a}	45.61 ± 0.15^{b}	141.92 ± 18.11^{c}	296.60
Solvent extraction (*n*-hexane)	Control	56.09 ± 0.47^{b}	55.31 ± 0.89^{d}	148.09 ± 7.00^{e}	151.90 ± 10.12^{c}	411.39
	Bacillus subtilis KFRI 1124	53.33 ± 6.88^{c}	73.76 ± 7.20^{a}	164.98 ± 3.17^{c}	113.57 ± 5.30^{e}	405.64
	Bacillus subtilis KFRI 1127	51.75 ± 7.76^{e}	60.64 ± 6.59^{c}	154.83 ± 10.08^{d}	151.68 ± 0.57^{d}	418.90
	Pediococcus pentosaceus LY 011	52.38 ± 8.61^{d}	64.69 ± 11.2b^{g}	167.48 ± 25.86^{b}	155.90 ± 4.31^{b}	440.45
	Lactobacillus gasseri KCTC 3162	56.95 ± 5.12^{a}	54.68 ± 0.23^{e}	175.77 ± 7.89^{a}	158.32 ± 5.16^{a}	445.72
Supercritical fluid extraction (15 MPa, 65°C)	Control	105.59 ± 18.45^{d}	116.76 ± 4.08^{e}	295.21 ± 1.23^{e}	228.49 ± 34.19^{e}	746.05
	Bacillus subtilis KFRI 1124	90.35 ± 5.15^{e}	156.65 ± 1.46^{b}	386.53 ± 10.44^{c}	241.04 ± 14.76^{d}	874.57
	Bacillus subtilis KFRI 1127	118.22 ± 7.82^{b}	160.72 ± 24.94^{a}	398.91 ± 24.42^{a}	305.73 ± 17.12^{a}	983.58
	Pediococcus pentosaceus LY 011	112.78 ± 2.94^{c}	126.56 ± 12.93^{d}	344.43 ± 7.26^{d}	272.80 ± 4.44^{c}	856.57
	Lactobacillus gasseri KCTC 3162	120.82 ± 3.50^{a}	140.05 ± 7.00^{c}	391.91 ± 4.95^{b}	291.65 ± 10.36^{b}	944.43

KCTC, Korean Collection for Type Cultures; KFRI, Korea Food Research Institute
[1)] Ginseng seed oil was not fermented
[2)] All values are mean ± standard deviation of triplicate determinations. Means with the same letter in each column are not significantly different at $p < 0.05$ by Duncan's multiple range tests

compression extraction. As for the difference in phytosterol content in oils according to the extraction method, campesterol content was similar in compression extracted and solvent extracted oils, and stigmasterol, β-sitosterol, and sitostanol contents were higher in solvent-extracted oils than in compression-extracted oils. Total phytosterol content was twofold higher in supercritical fluid-extracted oils than in compression-extracted or solvent-extracted oils. Stigmasterol, β-sitosterol, and sitostanol increased significantly in the order of compression extracted < solvent extracted < supercritical fluid extracted oils, and β-sitosterol increased the most. The quantities of phytosterols in the ginseng seed oils differed according to the fermenting bacterial species used. The phytosterol contents of ginseng seed oil fermented with *L. gasseri* KCTC 3162 and subjected to compression and solvent extraction were 296.6 mg/100 g and 445.75 mg/100 g, respectively, and these values were higher than those fermented with other strains. The phytosterol content of ginseng seed oil fermented with *B. subtilis* KFRI 1127 strain subjected to supercritical fluid extraction was 983.58 mg/100 g, which was the highest for the methods used. The phytosterol content was considered to be higher compared with other methods because nonpolar substances dissolve well in supercritical fluid [25]. In general, the phytosterol composition of the plant oils was 40–60% β-sitosterol, which was the highest, and 10–30% campesterol, 10–20% stigmasterol, and approximately 5% Δ^5-avenastanol [16]. These results were similar with previous results, with phytosterol content being slightly different but the β-sitosterol (including sitostanol) content was > 60%, campesterol content was 10–20%, and stigmasterol content was 12–18%, regardless of sample or extraction method.

The significance of this study is the change of bio-ingredients content in supercritical fluid extract combined with fermented ginseng oil being reported for the first time. As the results, supercritical fluid extraction combined with fermentation by *B. subtilis* KFRI 1127 strain led to increase the phenolic compound and phytosterol contents in ginseng oil. Our future study will investigate biological activities of fermented ginseng oil supercritical fluid extract based on the results of this study.

Conflicts of interest

The authors have no conflicts of interest to declare.

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