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Two-phase partition chromatography using soybean oil eliminates pesticide residues in aqueous ginseng extract

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Abstract

This study was carried out to develop a cost-, labor- and efficiency-effective elimination method of pesticide residues in ginseng extract. A two-phase partition method between soybean oil and distilled water or aqueous ginseng extract was employed for the elimination of pesticide residues. Content of the pesticides was determined by gas chromatography with electron capture or nitrogen phosphorus detector. A total of 15 pesticides representing four categories (organochlorine, organophosphorus, carbamate, pyrethroid) were spiked (ca. 2 ppm) to 2 ml soybean oil in a test tube and the oil was mixed with 6 ml distilled water or 10% aqueous ginseng extract. The test tubes were then vortexed (2 min) and centrifuged at 3000 rpm for 15 min to separate the oil and aqueous layers. Each layer was harvested and subjected to quantitative analysis of pesticides. The average distribution ratio of the pesticides to the oil layer was $94.4 \pm 6.7\%$ in the mixture of the oil and distilled water, and $105.5 \pm 6.6\%$ in the mixture of the oil and ginseng extract. No significant qualitative and quantitative change of ginsenosides, the active ingredients of *Panax ginseng*, was observed in the ginseng extract before and after the oil treatment. These results suggest that two-phase partition chromatography between soybean oil and the aqueous phase is a cost-, labor- and efficiency-effective reliable method for the elimination of pesticide residues in ginseng extract.

Keywords: Oils; Ginseng extract; Aqueous two-phase systems; Pesticides

1. Introduction

Pesticides, such as fungicides, insecticides and herbicides are being actively used in agriculture for crop protection. A great portion of the chemical agents is highly toxic and some of them like dioxins and DDT have been known to disturb endocrine system [1]. In addition, some lipophilic agents are not biodegradable, thereby accumulate in soil, water surface, plants, bodies of animal and shell-fish, and are recycled via food chain, which in turn harms human located on the top of the chain. Due to the toxicity, the use of some pesticides is strictly restricted or forbidden. However, it would be extremely difficult for farmers to grow crops without synthetic pesticides.

Ginseng plant is highly susceptible to phytopathogens. However, farmers have to grow the plant for 4–6 years until harvest. Ohh has quantified losses of ginseng root crop from diseases in Korea as follows: anthracnose (20–47%), damping off (5–50%), root rot (1–60%), alternaria blight (10–20%) [2]. Disease control still remains the central problem in commercial cultivation of ginseng globally. Quintozene, tolclofosmethyl, endosulfan and BHC are four of the most commonly detected pesticide residues in ginseng products.

Several methods of removing pesticide residues or undesirable lipophilic contaminants have been developed. However, almost all the methods are not cost-, labor- or efficiency-effective. Therefore, those methods have limited applicability to pesticide residue elimination in ginseng root before or after harvest. For example, the supercritical carbon dioxide fluid extraction method has advantage of being

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able to treat solid material, especially powered ginseng root but is not cost-effective. In addition, it has a disadvantage in its capacity of treatment per unit time [3–5]. It was also suggested that photolysis of the residual pesticides was accelerated by spraying a photosensitive agent to the field before harvest [6,7]. But it is impossible to degrade the pesticide residues beneath the soil surface because photosensitive agents are not accessible to the pesticides in roots when sprayed on the plant. In addition, photosensitive agents themselves have toxicity. Degradation of pesticides in the field by microorganisms has also been attempted [8]. However, the method takes too long period of time for the pesticides to be degraded. Moreover, applicability of the method is limited to DDT and the benzene hexachloride (BHC) family. Attempt to remove pesticides by using a microwave-assisted device with organic solvent has also been made [9,10]. But, it can cause loss of active ingredients to the organic solvent phase, because hexane or ethanol is used as the extraction solvent. Furthermore, toxicity of the organic solvent itself is another disadvantage of this method.

For this reason, this study was carried out to find a cost-, labor-, and efficiency-effective method of eliminating lipophilic pesticide residues from aqueous ginseng extract.

2. Experimental

2.1. Chemicals and materials

Pesticide standards were purchased from Chem Service (West Chester, PA, USA) and soybean oil from a local grocery store. Ethanol (50%) ginseng extract devoid of pesticide residues was prepared by our laboratory. An LC-Florisil solid-phase extraction (SPE) tube for pesticide purification was purchased from Supelco (St. Louis, MO, USA). Silica gel TLC employed for the qualitative analysis of ginseng saponin was procured from Merck (Darmstadt, Germany). Organic solvents employed for the quantitative analysis of ginsenosides were HPLC grade (TEDIA, Fairfield, OH, USA). Reference ginsenosides were isolated in our laboratory.

2.2. Equipment for chemical analysis

A gas chromatograph (HP 6890 series, Agilent Technologies, USA) with electron-capture (ECD) or nitrogen-phosphorous detection (NPD) was employed for the analysis of pesticide residues. Quantitative analysis of ginsenosides was carried out by HPLC (Shimadzu, Kyoto, Japan) with refractive index (RI) detector. GC-electron impact ionization (EI) MS (MD 800, VG Masslab Fisons Instruments, UK) was employed for the chemical analysis of the oil phase after treating the aqueous ginseng extract to determine loss of active ingredients of ginseng. An Isoperibol calorimeter (1261 series, Parr, Moline, IL, USA) was used for the analysis of fuel value in ginseng extract before and after the oil treatment.

2.3. Two-phase partition chromatography between soybean oil and aqueous phases

Pesticide residues were eliminated by two-phase distribution between soybean oil and distilled water or ginseng extract containing 10% solid matter. Two milliliters of soybean oil spiked of a predetermined amount of 15 pesticides (ca. 2–3 ppm for each) was mixed with the ultra pure distilled water (D/W) (6 ml) or 10% ginseng extract in test tubes. The tubes were then vortexed and centrifuged at 3000 rpm for 15 min. The lower aqueous layer was harvested with Pasteur pipette and both layers were then subjected to pesticide analysis by gas chromatography after purification in LC–Florisil column chromatography.

2.4. Analysis of multiresidue pesticides in ginseng extract

Analysis of pesticide residues was carried out by the multiresidue methods described in pesticide analytical manual [11]. Cypermethrin and tolchlofosmethyl in the aqueous phase (6 ml) were extracted with CH_3CN (20 ml). The CH_3CN layer was harvested 1 h after NaCl (10–15 g) addition. The acetonitrile fraction was then dried in vacuo and passed through an LC-Florisil SPE tube after dissolved in hexane containing 20% acetone. The eluate was concentrated in vacuo at temperature below 40 °C, dissolved in hexane (2 ml) containing 20% acetone and subjected to GC-ECD.

BHC and other pesticides in the aqueous phase (6 ml) were extracted with CH_3CN (12 ml). The extract was partitioned with light petroleum (50 ml), saturated NaCl solution (approximately 10 ml) and D/W (300 ml). The light petroleum fraction was harvested and dehydrated with anhydrous Na_2SO_4 and dried in vacuo after filtration. The resultant was dissolved in hexane (approximately 2 ml) to be purified in an LC-Florisil SPE tube and eluted with hexane containing 15% diethyl ether. The eluate was dried by N_2 gas purging and dissolved in hexane (2 ml) for GC analysis with ECD or NPD.

On the other hand, pesticides in the oil phase (2 ml) were dissolved in hexane (5 ml) and partitioned with hexane-saturated CH_3CN (100 ml, three times). The CH_3CN fraction was then washed with 30 ml CH_3CN-saturated hexane and the CH_3CN fraction was concentrated and dissolved in hexane (2 ml) to be subjected to GC with ECD or NPD.

HP-1701 (14% cyanopropyl methyl siloxane, 30 m × 0.25 mm, 0.25 μm) column and N_2 carrier gas (1.5 ml/min) were employed for GC operation. The temperature of the column chamber was programmed as: 150 °C/2 min, 150–240 °C (8 °C/min), 240 °C/2 min, 240–270 °C (15 °C/min), 270 °C/11 min.

2.5. Qualitative and quantitative analysis of ginsenosides

The ginseng extract was subjected to qualitative and quantitative analysis of ginsenosides before and after oil

treatment. A 30 ml 10% ginseng extract solution was extracted with BuOH (20 ml, three times). The BuOH fractions were pooled and dried in vacuo. The resulting extract was dissolved in methanol and filtered through millipore (0.45 μm) and subjected to TLC and HPLC. Chloroform–methanol–water (65:35:10, v/v, lower phase) was used for TLC and CH_3CN–water–BuOH (80:20:10, v/v) for HPLC analyses [12]. The column for HPLC-RI detection was LiChrosorb-NH_2 (250 mm × 4.6 mm i.d., 5 μm) and flow rate of the mobile phase was 1.0 ml/min.

2.6. Chemical analysis of oil phase after treating ginseng extract

The sample for gas chromatography analysis of pesticide residues was directly subjected to GC–EI–MS (VG Masslab Fisons Instruments) to determine the active ginseng ingredients leaking into the oil phase. The used column was SPB-1 fused silica capillary (30 m × 0.25 mm i.d., 0.5 μm) and the temperature of the column chamber was programmed to increase from 220 to 280 °C at 2 °C/min. Helium was used as a carrier gas at the flow rate of 1.0 ml/min. Ion pressure and ionization voltage were 2.4×10^{-3} Pa and 70 eV, respectively.

2.7. Analysis of fuel value and crude fat in ginseng extract

Ginseng extract was freeze-dried before fuel value analysis. One gram of the freeze-dried ginseng extract was used for the analysis of fuel value. Analysis of crude fat content was performed with the ginseng extract before and after soybean oil treatment. A 100 ml 10% ginseng extract was mixed with 50 ml diethyl ether in a centrifuge tube and mixed thoroughly by violent shaking. The tube was then centrifuged at 3000 rpm for 15 min. The lower aqueous layer was harvested and subjected to the ether extraction two more times. The ether layer was pooled, concentrated in vacuo, and dried in an oven (105 °C/3 h) to determine the crude fat content.

2.8. Analysis of fatty acid in ginseng extract

At first, 10% ginseng extract (500 ml) and diethyl ether (500 ml) were mixed and shaken for oil extraction. Diethyl ether layer was harvested and dried in vaccuo. The resultant was dissolved in 2 ml hexane and evaporated in vaccuo to obtain oil fraction. The resulting oil was further purified by BF_3 method described in AOAC Official Method 969.33 (16th edition). HP-FFAP (nitroterephthalic acid modified polyethylene glycol, 25 m × 0.32 mm i.d., 0.25 μm) column and He carrier gas (1.5 ml/min) were employed for GC operation. Temperature of the column chamber was programmed as to increase from 180 to 220 °C at the rate of 4 °C/min and maintained at 220 °C for 9 min.

2.9. Statistical analysis

All the experiments were performed in triplicates and the data in the table were expressed in mean ± S.D.

3. Results and discussion

3.1. Elimination of pesticide residues in the mixture of soybean oil and D/W

At the beginning, an attempt was made to test the possibility of removing pesticide residue by two-phase distribution method, wherein a predetermined amount of pesticides was spiked into the matrix containing distilled water and soybean oil. The soybean oil was found to be free of pesticides when analyzed by GC. As shown in Table 1 and Fig. 1, the removal rate of pesticides in the mixture to the oil phase was found to be variable depending upon the pesticide, ranging from 80.3 ± 7.1% in BHC to 107.4 ± 6.9% in parathion, average 94.4 ± 6.7%. In general, the recovery rate of pesticides ranges from 70 to 110% [13]. In this experiment, the recovery rate of pesticide residues in the mixture was within this range, thus demonstrating that our method was accurate and it would be possible to remove pesticide residues from the aqueous ginseng phase by this method.

3.2. Effect of solid matter content on pesticide removal in ginseng extract

Ginseng extracts of 5, 10, 20, 30 and 40% solid matter were prepared and treated with soybean oil spiked of procymidone at the concentration of 2.0 ppm. Distribution of

Table 1
Distribution of spiked pesticides to soybean oil and D/W layers

Pesticide name	Spiked concentration of pesticide (ppm)	Recovery (%)
Procymidone[a]	2.0	102.8 ± 9.5
Chlorpyrifos[b]	2.8	99.8 ± 7.9
Chlorothalonil[a]	2.2	91.2 ± 8.0
Ethoprophos[b]	2.2	96.4 ± 5.7
Vinclozolin[a]	2.3	96.5 ± 7.9
Carbofuran[b]	2.0	93.3 ± 4.6
Diazinon[b]	2.9	101.8 ± 6.1
Prothiofos[b]	2.2	98.2 ± 3.8
BHC[a]	2.6	80.3 ± 7.1
DDT[a]	2.4	82.1 ± 7.4
Endosulfan[a]	2.7	95.2 ± 7.3
Quintozene[a]	2.2	90.4 ± 8.3
Tolclofosmethyl[a]	2.3	95.5 ± 5.0
Cypermethrin[a]	3.0	85.7 ± 5.2
Parathion[a]	2.7	107.4 ± 6.9
Average		94.4 ± 6.7

Pesticide was not detected in the aqueous layer. All experiments were performed in triplicate and data were expressed in mean ± S.D.
[a] Detected with ECD.
[b] NPD.

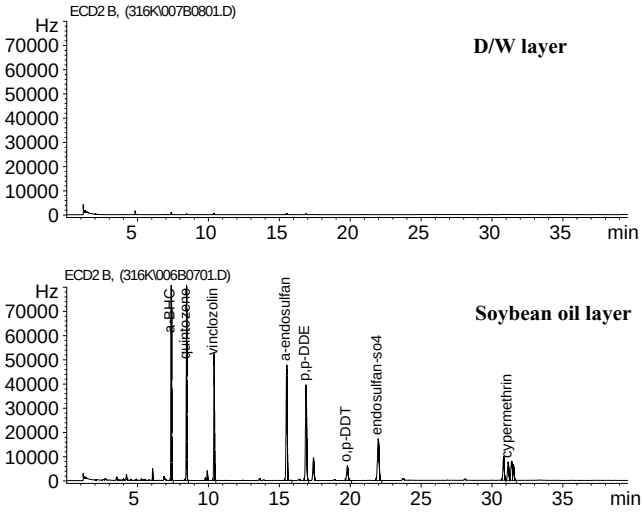

Fig. 1. Gas chromatograms of soybean oil and D/W layers after treating D/W spiked of pesticides. Column: HP-1701 (30 m × 0.25 mm i.d., 0.25 μm), carrier gas: N_2 (1.5 ml/min). Temperature: 150 °C/2 min, 150–240 °C (8 °C/min), 240 °C/2 min, 240–270 °C (15 °C/min), 270 °C/11 min, detection: ECD.

Table 2
Distribution of spiked pesticides to soybean oil and aqueous ginseng extract layers

Pesticide name	Spiked concentration of pesticide (ppm)	Recovery (%)
Procymidone[a]	2.0	113.4 ± 13.7
Chlorpyrifos[b]	2.8	109.2 ± 5.4
Chlorothalonil[a]	2.2	106.8 ± 5.7
Ethoprophos[b]	2.2	103.9 ± 9.8
Vinclozolin[a]	2.3	107.8 ± 5.6
Carbofuran[b]	2.0	104.5 ± 3.8
Diazinon[b]	2.9	112.8 ± 7.9
Prothiofos[b]	2.2	108.7 ± 4.8
BHC[a]	2.6	106.2 ± 6.8
DDT[a]	2.4	83.5 ± 6.4
Endosulfan[a]	2.7	107.4 ± 3.1
Quintozene[a]	2.2	103.9 ± 7.5
Tolclofosmethyl[a]	2.3	105.3 ± 6.5
Cypermethrin[a]	3.0	96.7 ± 8.2
Parathion[a]	2.7	112.5 ± 3.2
Average		105.5 ± 6.6

[a] Detected with ECD.
[b] NPD.

procymidone to the oil phase decreased slightly but not significantly as the solid matter increased. However, the content of solid matter in ginseng extract in the factory before concentration accounts for less than 10%, thus leading us to carry out pesticide elimination test with ginseng extract containing 10% solid matter.

3.3. Elimination of pesticide residues in the mixture of soybean oil and ginseng extract

The distribution ratio of pesticide residues to the oil phase demonstrated slightly higher value in the mixture of ginseng extract and soybean oil than that in the mixture of D/W and the oil (Table 2). Pesticides spiked into the matrix were not detected in the oil-treated aqueous ginseng extract layer (Fig. 2). Recovery of DDT to the oil phase showed the lowest value of 83.5 ± 6.4%. Average removal rate of pesticide residues to the oil phase was 105.5 ± 6.6%. This result supports that the 15 pesticide residues employed in this experiment can be removed by the two-phase distribution between soybean oil and an aqueous ginseng extract.

3.4. Loss of ginseng's active ingredients to the oil phase

Saponin in ginseng, also referred to as ginsenoside (ginseng glycoside), is known to be the main active ingredient of ginseng. A total of 34 ginsenosides were isolated from Korean red ginseng and their chemical structures were elucidated [14]. Physical and chemical properties of each ginsenoside are different depending on the structure, especially by the aglycone or number of sugar moiety bonded to C3, C6 and/or C20. The ginseng saponin has been named as ginsenosides Rx (x = o, a, b, c, d, e, f, g, h) according to the sequence of R_F value of the spots on the TLC from the bottom to the top [15]. TLC has mainly used in qualitative analysis of ginsenosides for quality control in the factory [16,17].

The key point of the pesticide residues elimination method in ginseng extract should be placed on (1) cost-, labor- and efficiency-effective removal of pesticide residues, and (2) minimizing the loss of active ingredients of ginseng. In addition, the method employed should play no detrimental role in human health and environment.

In this experiment, loss of the ginsenoside(s) to the oil phase was analyzed by silica gel TLC and HPLC. As shown in Figs. 3 and 4, there was no detectable difference in the TLC and HPLC profiles of the major seven (from Rb_1 to Rg_1) and minor ginsenosides (the other spots than the seven

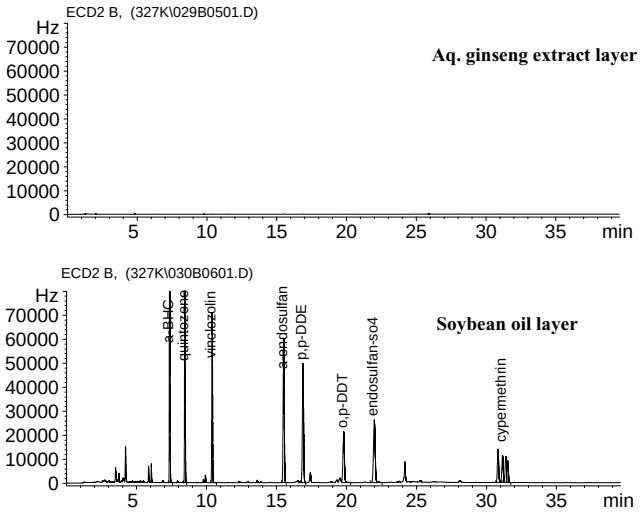

Fig. 2. Gas chromatograms of aqueous ginseng extract and soybean oil layers. Conditions as in Fig. 1.

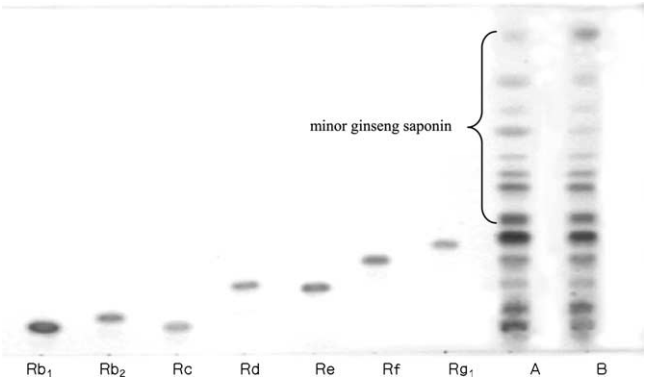

Fig. 3. TLC profile of saponin in soybean oil-untreated (A) and -treated (B) ginseng extracts. Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg_1 indicate seven major ginseng saponins. TLC plate: silica gel 60. Mobile phase: $CHCl_3$–MeOH–water (65:35:10, v/v, lower phase), sprayed with 20% aqueous H_2SO_4 and heated at 105 °C for 10 min.

references) before and after oil treatment. Content of ginsenoside Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 demonstrated 44.5 ± 0.9, 15.0 ± 0.2, 20.5 ± 0.5, 3.6 ± 0.2, 13.8 ± 0.1, 8.9 ± 0.4, 24.0 ± 0.8 mg/g, respectively. GC/MS analy-

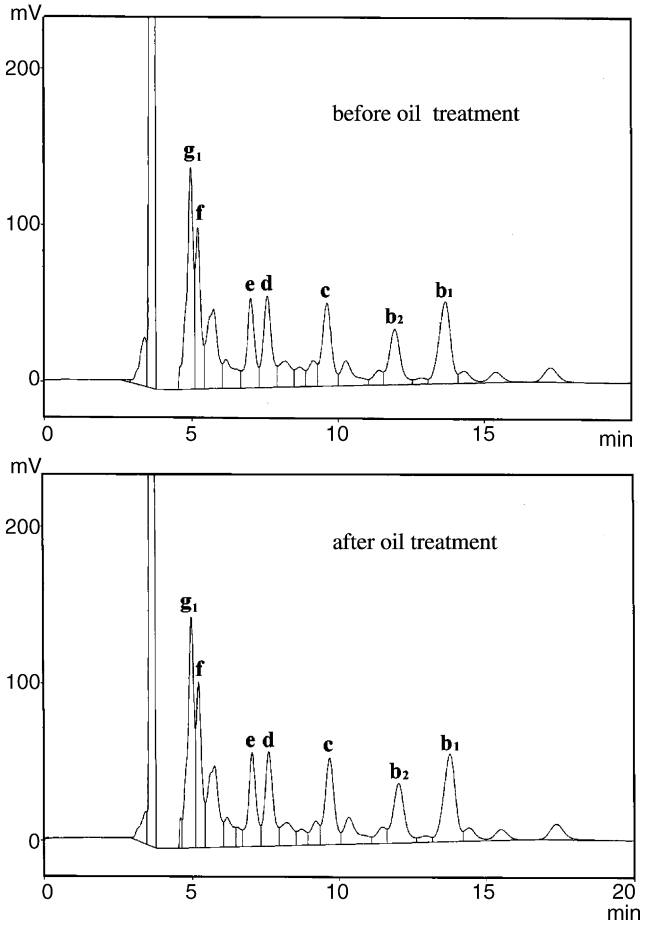

Fig. 4. HPLC profiles of ginsenosides in ginseng extract before and after oil treatment. Column: LiChrosorb-NH_2 (250 mm × 4.6 mm i.d., 5 μm). Mobile phase; CH_3CN–water–BuOH (80:20:10, v/v). Flow rate: 1.0 ml/min. Detection: RI.

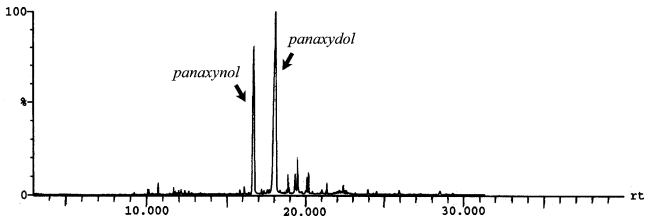

Fig. 5. Gas chromatogram of soybean oil layer harvested from two-phase partition chromatography between soybean oil and aqueous ginseng extract. Column: SPB-1 fused silica capillary (30 m × 0.25 mm i.d., 0.5 μm). Carrier gas: He (1.0 ml/min). Temperature; 220–280 °C (2 °C/min). Ion pressure: 2.4×10^{-3} Pa. Ionization voltage: 70 eV.

sis of the oil phase after treating ginseng extract revealed that panaxynol and panaxydol, two mayor polyacetylenic compounds of ginseng, were found to be lost to the oil phase (Fig. 5). Absolute amount of panaxynol and panaxydol in the ginseng extract accounted for 432.5 ± 10.5 and 479.1 ± 18.3 μg/g, respectively. Panaxynol and panaxydol have been reported to have cytotoxic effect in some cancer cell line [18]. However, it may also show cytotoxicity against a normal cell. Therefore, loss of those compounds to the oil phase will not negatively influence the biological activity of ginseng.

Supercritical carbon dioxide (CO_2) extraction method is in use in some German and Korean ginseng factories. However, the equipment itself is pretty expensive. It is also money-consuming to operate the equipment, thus increasing the cost of the products. In addition, it is limited in its capacity to remove pesticide residues in a given time. In short, this method is not cost-, labor- and efficiency-effective, therefore can be applicable to a restricted extent.

3.5. Change in fuel value and crude fat content of ginseng extract before and after oil treatment

Fuel value of oil-treated ginseng extract increased by 144.3 cal/g, indicating 3.6% increases after oil treatment (Table 3) (1 cal = 4.184 J). From this result, it can be said that very tiny amount of soybean oil is transferred to the ginseng extract phase by the treatment. Thus, content of crude fat in the ginseng extract was compared before and after the oil treatment. There was 1.9% increase in crude fat content of ginseng extract after the oil treatment. However, the increase in fuel value of ginseng extract after oil treatment is negligible in light of recommended daily food calorie intake for adults (2000–3000 kcal).

Table 3
Changes in fuel value and crude fat content of ginseng extract before and after soybean oil treatment

	Oil non-treated	Oil treated
Fuel value (cal/g)	3984.2 ± 4.3	4128.5 ± 4.0
Crude fat (mg/g)	10.7 ± 1.2	10.9 ± 1.1

Experiments carried out in triplicate and data expressed in mean ± S.D.

Table 4
Comparison of fatty acid content in ginseng extract before and after soybean oil treatment

General name	Oil non-treated (μg/g)	Oil treated (μg/g)
Palmitic acid (16:0)	1657.0 ± 355.0	1555.2 ± 315.8
Palmitoleic acid ($16:1^{\Delta 9}$)	65.2 ± 10.4	37.9 ± 19.1
Stearic acid (18:0)	214.1 ± 52.2	350.9 ± 28.7
Oleic acid ($18:1^{\Delta 9}$)	688.9 ± 31.3	1460.4 ± 162.7
Linoleic acid ($18:2^{\Delta 9,12}$)	5790.2 ± 595.1	5338.9 ± 535.9
Linolenic acid ($18:3^{\Delta 9,12,15}$)	754.0 ± 73.1	635.4 ± 47.9
Arachidic acid ($20:4^{\Delta 5,8,11,14}$)	139.6 ± 41.8	104.3 ± 19.1
Total	9309.0 ± 1158.9	9483.0 ± 1129.3

Experiments were carried out in triplicate and data were expressed in mean ± S.D. Column: HP-FFAP (nitroterephthalic acid modified polyethylene glycol, 25 m × 0.32 mm i.d., 0.5 μm). Carrier gas: He (1.5 ml/min). Temperature; 180–220 °C (4 °C/min), 220 °C/9 min. Detection: FID.

3.6. Comparison of fatty acid content in the ginseng extract before and after soybean oil treatment

Content of fatty acid in the ginseng extract was compared before and after oil treatment (Table 4). There was not a fundamental change in fatty acid content and composition of the ginseng extract before and after the oil treatment. However, content of palmitoleic acid decreased to 58% but that of oleic acid increased two-folds. Fatty acids are not the active ingredients of ginseng, therefore it appeared to us that the change in fatty acid content will not play a detrimental role in quality of ginseng.

4. Conclusions

Our newly suggested method of removing pesticide residues by two-phase distribution method between the soybean oil and an aqueous ginseng extract is neither toxic nor expensive. The process does not require inflammable organic solvents or plays no detrimental role in human health and environment. In addition, the removal rate of pesticide residues is high enough to satisfy the allowable pesticide limitation and the process is very simple. Advantage of this method is cost-, labor- and efficiency-effective. In addition, this method can be operated in continuous and cleaning in pipe (CIP) system.

In conclusion, we suggest a new cost-, labor- and efficiency-effective method of removing pesticide residues in ginseng extract by combining a two-phase partition chromatography between soybean oil and aqueous ginseng extract.

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