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Research article

Canola oil is an excellent vehicle for eliminating pesticide residues in aqueous ginseng extract

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ABSTRACT

Background: We previously reported that two-phase partition chromatography between ginseng water extract and soybean oil efficiently eliminated pesticide residues. However, an undesirable odor and an unpalatable taste unique to soybean oil were two major disadvantages of the method. This study was carried out to find an alternative vegetable oil that is cost effective, labor effective, and efficient without leaving an undesirable taste and smell.

Methods: We employed six vegetable oils that were available at a grocery store. A 1-mL sample of the corresponding oil containing a total of 32 pesticides, representing four categories, was mixed with 10% aqueous ginseng extract (20 mL) and equivalent vegetable oil (7 mL) in Falcon tubes. The final concentration of the pesticides in the mixture (28 mL) was adjusted to approximately 2 ppm. In addition, pesticides for spiking were clustered depending on the analytical equipment (GC/HPLC), detection mode (electron capture detector/nitrogen–phosphorus detector), or retention time used. Samples were harvested and subjected to quantitative analysis of the pesticides.

Results: Soybean oil demonstrated the highest efficiency in partitioning pesticide residues in the ginseng extract to the oil phase. However, canola oil gave the best result in an organoleptic test due to the lack of undesirable odor and unpalatable taste. Furthermore, the qualitative and quantitative changes of ginsenosides evaluated by TLC and HPLC, respectively, revealed no notable change before or after canola oil treatment.

Conclusion: We suggest that canola oil is an excellent vehicle with respect to its organoleptic property, cost-effectiveness and efficiency of eliminating pesticide residues in ginseng extract.

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1. Introduction

The pesticide strategy concerns *plant protection products*, which are those pesticides that are used to protect plants and plant products from pests, diseases, and weeds and to regulate the growth of plants [1]. It is generally known that crop yields will drop by more than 30% without the use of pesticides. In this case farmers have to expand their fields to compensate for the decrease in crop yield. To increase the field size, forests have to be destroyed, which can be detrimental to the environment. Pesticides help farmers

protect their crops from pests, fungi, and weeds so that people can enjoy an abundance of high quality food. However, this food must be safe to eat. Careful use of pesticides can deliver substantial benefits for society: increased availability of good quality crops; reasonably priced foodstuffs, in particular, fruits and vegetables; and clean urban environments. However, pesticides can, by their nature, be harmful to living organisms, so there are risks associated with their use. It is important that these risks are accurately assessed and that appropriate measures are taken to minimize them [2]. However, some pesticides, specifically lipophilic agents:

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are not biodegradable; accumulate in soil, at the water surface, in plants, and in the bodies of animals and shellfish; and are recycled via the food chain, which in turn harms humans. Due to their toxicity, the use of some pesticides is strictly restricted or forbidden.

Crop cultivation without pesticides seems almost impossible, particularly for ginseng because it should be cultivated for 6 years. It is well known that the loss of its root due to phytopathogens and insects accounts for a 10% crop loss every year, indicating that there is > 50% loss of ginseng crop over 6 years [3]. Chemical pesticides are environmentally unfriendly due to their physicochemical properties and recalcitrance to biodegradation. For example, chemicals, such as the dichlorodiphenyltrichloroethane (DDT) metabolite dichlorodiphenyldichloroethylene, are termed endocrine disruptors, which are known to elicit adverse effects by mimicking or antagonizing natural hormones in the body. A long-term, low-dose exposure of these pesticides has been linked to human health effects, such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer [4–6]. Therefore, DDT derivatives were forbidden to be manufactured and used almost half a century ago. However, DDT derivatives are still detected in the soil of ginseng fields. In addition, the soil environment is interconnected from country to country because of atmospheric circulation. Thus, it seems necessary to develop a health-friendly method of eliminating pesticide residues or environmentally unfriendly chemicals after harvest.

There have been many attempts to develop an elimination method for pesticide residues, including microwave decomposition, photolysis, and CO₂ supercritical extraction methods. However, most of the methods have poor effectiveness in view of the efficiency of elimination, loss of active ingredients of ginseng, labor, and, most importantly, cost. Among them, the CO₂ supercritical extraction method has a greater advantage in the efficiency of pesticide residue elimination compared to the other methods. However, importantly, it has a disadvantage in its capacity for treatment per unit time and cost-effectiveness [7,8].

We have developed a partition chromatography method for removing residues using soybean oil as a solvent. It has great advantages over the other methods with respect to cost, labor, and efficiency [9]. However, it gives an unpalatable smell and taste to the soybean oil-treated ginseng extract. Therefore, a new vegetable oil that is devoid of undesirable taste and smell but is effective in terms of cost, labor, and efficiency has to be developed. We investigated the pesticide residue elimination efficiency, change in ginsenosides profiles, and smell and taste before and after treatment of the ginseng extract with six vegetable oils that are readily available in the market.

We propose a new cost effective, labor effective, and efficient vegetable oil extraction method of removing pesticide residues from ginseng extract that is based on two-phase partition chromatography between six different oils and aqueous ginseng extract as well as organoleptic tests.

2. Materials and methods

2.1. Chemicals and materials

Pesticide standards were purchased from Chem Service (West Chester, PA, USA), and six different vegetable oils were purchased from a local grocery store. Ethanol (70%) ginseng extract devoid of pesticide residues was prepared by our laboratory. A LC-Florisil solid-phase extraction 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). The organic solvents employed for the quantitative analysis of ginsenosides were HPLC grade (Tedia,

Fairfield, OH, USA). Fourteen reference ginsenosides [Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2(S), Rg2(R), Rh1, Rh2(S), Rh2(R), Rg3(S), Rg3(R)] were kindly supplied by the Korea Ginseng Corporation (Seoul, Korea).

2.2. Equipment for chemical analysis

An Agilent 6890N gas chromatography device was used for the analysis of pesticide residues. The detector temperature was set to 300°C, the H₂ flow rate was 3.0 mL/min, the air flow rate was 60 mL/min, and there were columns in the HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm, Agilent) and DB-17MS capillary column (30 m × 0.25 mm, 0.25 μm, Agilent). The gas flow rate was 1.0 mL/min. The HPLC device used for the analysis of pesticide residue was a Hewlett Packard 1100 (Agilent, Santa Clara, CA, USA). The detection wavelength was set to 254 nm and 275 nm and the column was an InertSustain C18 column (4.6 × 250 mm, 5 μm; GL Science, Torrance, CA, USA). The mobile phase was a mixture of water and acetonitrile: 70:30 (0–5.0 min) and 15:85 (5.0–22.0 min) with a flow rate of 1.0 mL/min.

2.3. Two phase-partition chromatography between vegetable oils and aqueous ginseng extract

The collection of pesticides used for spiking into the mixture of aqueous ginseng extract and vegetable oil were grouped depending on their retention time in the GC or HPLC to avoid overlapping their fingerprint: GC/electron capture detector (ECD) group 1, pentachloronitrobenzene, pentachlorothioanizole (PCTA), pentachloroaniline (PCA), tefluthrin, chlorothalonil, dichlorodiphenyldichloroethylene, endrin, dichlorodiphenyldichloroethane, cyfluthrin, and DDT; GC/ECD group 2, α-hexachlorocyclohexane (HHC), β-HHC, γ-HHC, δ-HHC, aldrin, dieldrin, bifenthrin, prochloraz, and difenconazole; GC/nitrogen-phosphorus detector (NPD) group 1, tolclofos-methyl, diethofencarb, hexaconazole, flusilazole, and carbosulfan; and GC/NPD group 2, cyprodinil, flutolanil, buprofezin, kresoxim-methyl, tebuconazole, amitraz, and methalaxyl. HPLC analysis was carried out in one group under one condition but two different wavelengths (254 nm, 275 nm) were used for detection of acetamiprid, carbofuran, dimethomorph, fluquinconazole, pyrimethanil, cyazofamid, pyraclostrobin, and sethoxydim. A total of 1 mL of six different oils spiked with a predetermined amount of 32 pesticides (final concentration ca. 2 ppm for each) was mixed with the 10% ginseng extract (20 mL) and the corresponding oil (7 mL) in 50 mL Falcon tubes. The tubes were then vortexed and centrifuged at 3,000 rpm (1,500 × g) for 15 min. The lower aqueous layer was harvested with a Pasteur pipette, and both layers were subjected to pesticide analysis by GC or HPLC after purification by LC-Florisil column chromatography. The lower ginseng extract layer was further subjected to ginsenoside profile analysis by TLC and HPLC.

2.4. Analysis of multiresidue pesticides

Analysis of pesticide residues was carried out by the multi-residue methods described in the pesticide analytical manual [7]. All of the pesticides in the aqueous phase (25 mL) were extracted with CH₃CN (100 mL). The CH₃CN 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 being dissolved in hexane containing 20% acetone. The eluate was concentrated *in vacuo* at a temperature below 40°C, dissolved in hexane (2 mL) containing 20% acetone and subjected to GC/ECD or GC/NPD. Pesticides in the oil phase (2 mL) were dissolved in hexane (5 mL) and partitioned with hexane-saturated CH₃CN (100 mL, 3 times). The CH₃CN fraction was then washed with 30 mL of CH₃CN-

Table 1
Recovery of pesticide residues in the oil phase by six different vegetable oils

Recovery		Retention time	Soybean	Canola	Corn	Grape	Olive	Sunflower
Organophosphorus	Chlorothalonil	7.875	82.01	77.10	65.69	94.30	75.68	89.58
	Dieldrin	10.99	94.39	109.29	86.97	98.30	96.82	94.81
	Tolclofos-methyl	8.97	90.14	91.38	95.23	87.07	79.35	108.54
Average			88.85	92.59	82.63	93.22	83.95	97.64
SD			6.29	16.13	15.24	5.69	11.30	9.79
Organochlorides	PCNB	7.342	100.40	95.62	73.01	96.48	79.23	94.14
	PCTA	7.397	82.30	77.39	77.54	74.24	68.59	71.64
	PCA	7.511	99.68	88.64	80.80	100.93	84.15	81.90
	DDE	10.807	112.93	95.68	105.97	109.73	100.25	102.63
	Endrin	11.315	96.64	90.11	103.94	106.34	67.43	112.83
	DDD	11.533	112.50	107.66	94.95	125.78	78.78	103.35
	DDT	13.270	102.99	103.1	99.48	77.3	70.47	82.33
	α -HHC	6.836	96.16	108.12	94.54	96.85	100.27	102.52
	β -HHC	7.242	97.75	101.74	71.80	97.71	75.68	92.62
	γ -HHC	7.367	108.06	106.88	89.40	96.22	90.12	93.04
	δ -HHC	7.735	86.86	93.83	77.66	84.56	82.95	81.35
Aldrin	9.175	86.98	65.29	62.90	79.01	78.51	70.62	
Average			98.60	94.51	86.00	95.43	81.37	90.75
SD			9.82	12.94	13.95	14.92	10.99	13.33
Carbamates	Diethofencarb	9.495	104.11	106.38	98.29	70.92	87.70	105.45
	Carbosulfan	13.893	109.87	109.21	92.68	87.99	103.01	106.62
	Carbofuran	12.732	104.42	100.42	85.05	119.75	75.97	101.22
Average			106.13	105.34	92.01	92.89	88.89	104.43
SD			3.24	4.49	6.65	24.78	13.56	2.84
Pyrethroids	Tefluthrin	7.696	100.95	97.76	101.99	106.04	73.80	100.92
	Cyfluthrin	15.924	119.82	90.36	69.73	114.87	82.90	81.32
	Bifenthrin	13.082	103.05	97.89	85.83	86.61	98.96	106.32
Average			107.94	95.34	85.85	102.51	85.22	96.19
SD			10.34	4.31	16.13	14.46	12.74	13.15
Triazoles	Hexaconazole	11.125	96.12	90.81	85.42	86.22	84.45	89.69
	Flusilazole	11.442	79.07	77.76	76.27	72.93	74.49	75.51
	Fluquinconazole	15.925	102.38	104.58	94.64	100.73	80.00	109.53
Average			92.52	91.05	85.44	86.63	79.65	91.58
SD			12.06	13.41	9.19	13.90	4.99	17.09
Pyrimidines	Cyprodinil	10.106	106.30	97.99	60.07	87.98	66.24	76.48
	Pyrimethanil	15.263	129.89	112.77	84.09	117.14	95.77	110.93
Average			118.10	105.38	72.08	102.56	81.01	93.71
SD			16.68	10.45	16.98	20.62	20.88	24.36
Strobilurins	Kresoxim-methyl	11.472	106.94	98.31	101.63	84.92	112.03	84.89
	Pyraclostrobin	17.723	88.94	84.70	80.07	79.80	81.59	76.14
Average			97.94	91.51	90.85	82.36	96.81	80.52
SD			12.73	9.62	15.25	3.62	21.52	6.19
Morpholines	Dimethomorph	14.448	119.03	109.06	116.88	113.55	98.79	77.24
		Average	119.03	109.06	116.88	113.55	98.79	77.24
SD			27.92	25.41	28.68	10.60	17.08	20.80
Cyclohexenoxims	Sethoxydim	19.756	91.59	109.01	116.11	84.78	73.77	79.02
		Average	91.59	109.01	116.11	84.78	73.77	79.02
SD			26.97	13.26	25.32	21.94	22.56	27.12
Amides	Cyazofamid	17.015	102.75	103.57	125.98	126.16	80.72	115.64
	Methalaxyl	10.487	97.72	84.09	90.00	96.17	97.77	108.70
Average			100.24	93.83	107.99	111.17	89.25	112.17
SD			3.56	13.77	25.44	21.21	12.06	4.91
Total average			100.40	96.45	88.89	95.67	84.26	93.36
Total SD			11.47	11.57	15.75	15.27	11.79	13.67

DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HHC, hexachlorocyclohexane; PCA, pentachloroaniline; PCNB, pentachloronitrobenzene; PCTA, pentachlorothioanazole.

saturated hexane. The CH₃CN fraction was concentrated, dissolved in hexane (2 mL) and subjected to GC/ECD or NPD. The HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm, Agilent) and the DB-17MS column (30 m × 0.25 mm i.d., 0.25 μm, Agilent) and N₂ carrier gas (1.0 mL/min) were employed for the GC operation. The temperature of the column chamber was programmed as: 150°C/2 min, 150–200°C (15°C/min), 200–280°C/10 min (10°C/min), and 280–300°C/2 min. At the same time, the CH₃CN fraction was concentrated, dissolved in hexane (2 mL) and subjected to HPLC: Insert-Sustain C18 column (4.6 mm × 250 mm, 5 μm, GL Science) with an injection volume of 10.0 μL. For the selective and sensitive determination, dual wavelengths of 254 nm and 275 nm were employed. For HPLC operation, the flow rate was 1.0 mL/min (H₂O: acetonitrile

70:30) from 0 min to 5 min and 1.2 mL/min (H₂O: acetonitrile 15:85) from 5 min to 22 min.

2.5. Qualitative and quantitative analysis of ginsenosides

The ginseng extract was subjected to qualitative and quantitative analysis of ginsenosides before and after each oil treatment. A 20-mL 10% ginseng extract solution was extracted with BuOH (10 mL, 3 times). The BuOH fractions were pooled and dried *in vacuo*. The resulting extract was dissolved in MeOH, filtered through a Millipore filter (0.45 μm) and subjected to TLC and HPLC analyses. CHCl₃–MeOH–H₂O (65:35:10, v/v, lower phase) was used for TLC, and a gradient solvent system of CH₃CN (solvent A)–

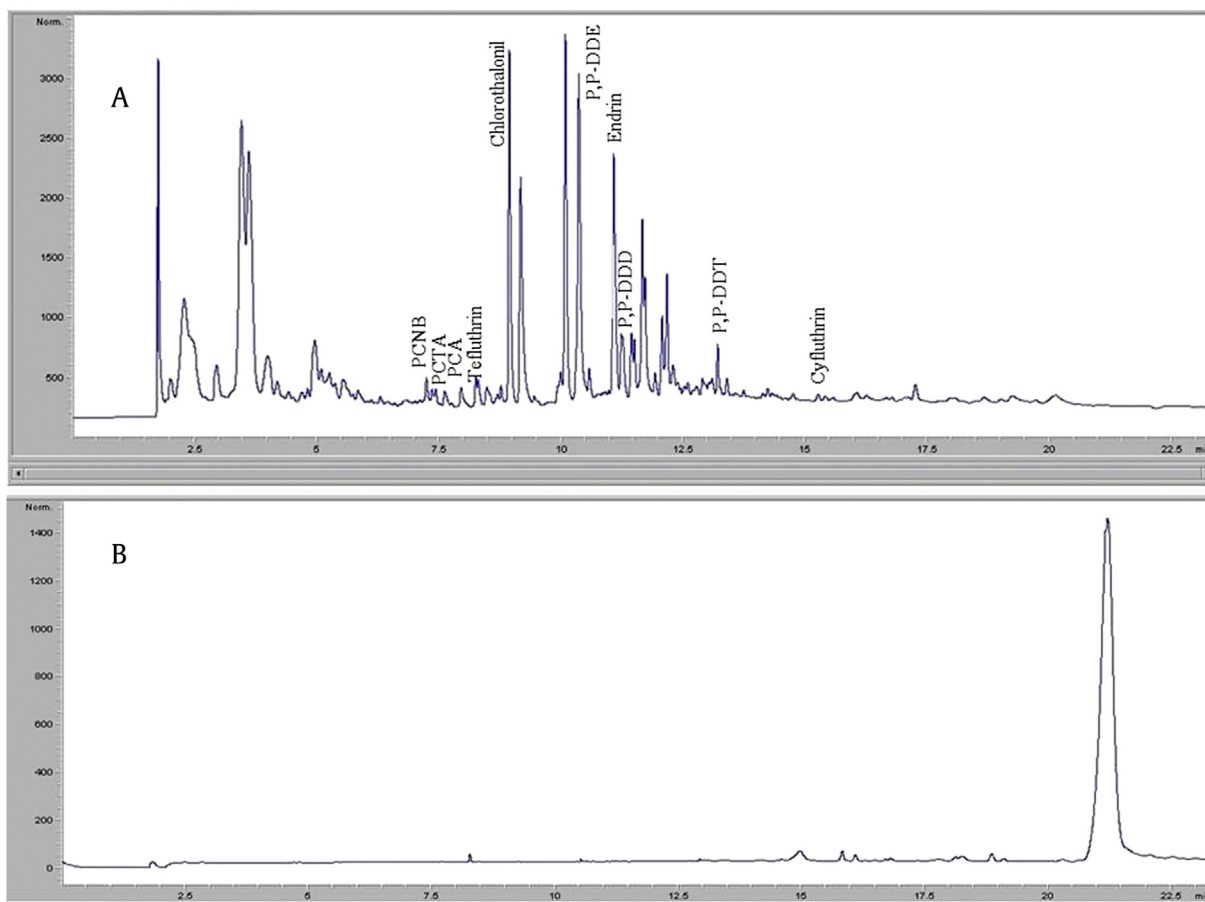


Fig. 1. (A) GC fingerprint of organochloride pesticides in the canola oil phase and (B) its corresponding aqueous ginseng extract phase. Almost all of the spiked pesticides were transferred to the oil phase and not to its corresponding aqueous ginseng extract layer, indicating a high distribution coefficient of organochloride pesticides to the canola oil phase.

H₂O (solvent B) was employed for HPLC. A:B (20:80) for 20 min, a linear gradient of A:B (20:80) to (35:65) from 20 min to 40 min, a linear gradient of A:B (35:65) to (45:55) from 40 min to 52 min, a linear gradient of A:B (45:55) to (70:30) from 52 min to 62 min, a linear gradient of A:B (70:30) to (100:0) from 62 min to 80 min and finally equilibration was performed with A:B (20:80) from 80 min to 90 min [10]. The column for the HPLC detection was the C18 column (4.6 mm × 250 mm, 5 μm; Supelco). The flow rate of the mobile phase was 1.6 mL/min, and the detector wavelength was set to 203 nm.

2.6. Organoleptic test

A total of 20 panelists (10 men and 10 women, aged 20–24 years, nonsmokers) were employed for the test. A relative score was given to each oil in terms of smell and taste. The best vehicle received a score of 6, while the poorest vehicle received a score of 1. The organoleptic test was carried out in the blind.

2.7. Value evaluation as a pesticide residue elimination vehicle

The efficiency of pesticide residue elimination was regarded as the prime factor for the candidate oil. Further, organoleptic factors of foreign smell and taste were also indispensable for the selection of a pesticide residue elimination solvent. Other factors, such as the loss of ginsenosides after oil treatment and price of the vegetative oil, were also considered in choosing the best oil

for the elimination vehicle. The oil that demonstrated the best efficiency in pesticide residue elimination was scored as 6, while the oil that exhibited poorest efficiency received a 1. In an organoleptic test, the oil with least unpalatable smell and taste was given a score of 6, while the oil with strongest odor and taste was given a score 1. In the test of ginsenosides loss after oil treatment, the oil that showed no effect on ginsenosides profile before and after treatment was graded as a 6. Likewise, the cheapest oil was scored a 6, while the most expensive oil was scored as a 1. Finally, the oil with the highest score was selected as the candidate vehicle for the elimination of pesticide residues in ginseng extract.

2.8. Effect of the solid and liquor content in ginseng extract on the loss of ginsenosides by oil treatment

Further study on the effect of the solid and liquor content in ginseng extract on the loss of ginsenosides by oil treatment was carried out with the selected candidate canola oil. The range of the solid and liquor content in the ginseng extract was adjusted to 10%, 20%, and 30%. Analysis of ginsenoside loss was performed by comparing the TLC and HPLC fingerprints of the ginseng extract layer before and after oil treatment.

2.9. Statistical analysis

All of the experiments were performed in triplicates, and the data are expressed as the mean ± standard deviation from three

independent experiments. Statistical analyses were performed using Excel (Microsoft Office Professional Plus 2010; Microsoft, Seattle, WA, USA).

3. Results

3.1. Elimination of pesticide residues in ginseng extract by six different vegetable oils

In the case of the organophosphorus group, the recovery of pesticides in the oil phase (Table 1) was shown to be 88.85% in soybean oil, 92.59% in canola oil, 82.63% in corn oil, 93.22% in grape seed oil, 83.95% in olive oil, and 97.64% in sunflower oil. In the carbamate group, soybean oil demonstrated the highest recovery of 106.13% and olive oil exhibited the lowest recovery of 88.89%. The recovery of organochloride pesticides in the oil phase was 98.60% for soybean oil, 95.43% for grape seed oil, 94.51% for canola oil, 90.75% for sunflower oil, 86.00% for corn oil, and 81.37% for olive oil. Soybean oil demonstrated the highest efficiency of pesticide residue elimination in carbamates (106.13%), organochlorides (98.60%), pyrethroids (107.94%), triazoles (92.52%), pyrimidines (118.10%), strobilurins (97.94%), and morpholines (dimethomorph, 119.03%). Sunflower exhibited the highest recovery of organophosphorus (97.64%) and amide group pesticides (112.17%). Corn oil revealed the highest efficiency of elimination in nicotinoid (acetamiprid, 119.32%) and cyclohexene (sethoxydim, 116.11%). Canola oil ranked second in the elimination of carbamates (105.34%), pyrimidines (105.38%), nicotinoids (acetamiprid, 115.72%), and cyclohexenes, 109.01%).

Fig. 1 demonstrates the GC fingerprints of the organochloride pesticides found in the canola oil layer and its corresponding aqueous ginseng extract layer. Almost all of the spiked pesticides were detected in the oil phase and not in the aqueous ginseng extract phase, indicating that the distribution coefficient of organochloride pesticides to the canola oil phase was very high. The amount of organochloride pesticides in the aqueous ginseng extract was under the detection limit.

3.2. Loss of ginsenosides to the oil phase

The key reason for using the pesticide residue elimination method on ginseng extract is to remove pesticides in a cost effective, labor effective, and efficient manner, while minimizing the loss of the active ingredients of ginseng. In addition, the method

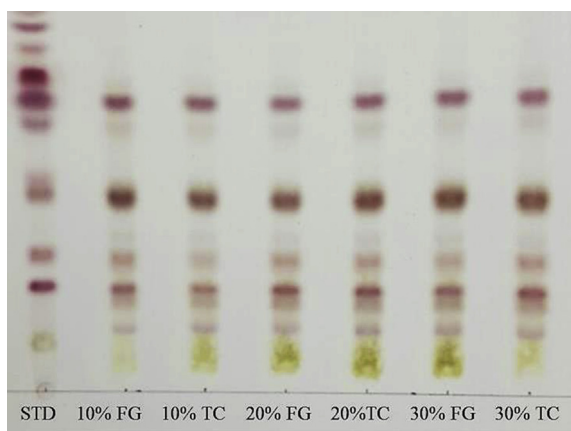


Fig. 2. TLC profiles of the ginsenosides in the aqueous ginseng extract layer before and after canola oil treatment. FG, fresh ginseng; STD, standard; TC, fresh ginseng treated canola oil.

employed should play no detrimental role to human health or the environment.

In this experiment, the loss of the ginsenosides to the oil phase was analyzed by HPLC. As shown in Fig. 2, there was no detectable difference in the HPLC profiles of the ginsenosides [Rg1, Re, Rf, Rh1, Rg2(S), Rg2(R), Rb1, Rc, Rb2, Rd, Rg3(S), Rg3(R), Rh2(S), and Rh2(R)] before and after the canola oil treatment. The content of ginsenosides Rg1, Re, Rf, Rh1, Rg2(S), Rg2(R), Rb1, Rc, Rb2, Rd, Rg3(S), Rg3(R), Rh2(S), and Rh2(R) before and after oil treatment accounted for 97.78 mg/g, 107.76 mg/g, 104.30 mg/g, 100.49 mg/g, 111.02 mg/g, 90.59 mg/g, 98.30 mg/g, 90.31 mg/g, 93.96 mg/g, 93.23 mg/g, 94.09 mg/g, 101.05 mg/g, 99.41 mg/g, 95.80 mg/g, and 98.44 mg/g, respectively. In particular, the levels of ginsenosides Rg3(S), Rg3(R), Rh2(S), and Rh2(R), which are susceptible to loss to the oil phase due to their nonpolar chemical properties, were not changed by the canola oil treatment.

3.3. Organoleptic test

All of the participating panelists gave a score of 6 to the canola oil-treated ginseng extract in terms of smell and taste, but scored the soybean oil-treated ginseng extract as a 1. Obviously, the soybean oil-treated ginseng extract revealed an unpalatable smell and taste unique to soybean. The same results were obtained from the oil itself when a blind test was performed. Sunflower seed oil also gave a good result in the organoleptic test. The smell and taste of olive oil was not unpalatable, but the ginseng extract treated with olive oil demonstrated a slightly foreign odor and taste. Sunflower seed and olive oils obtained a relatively high score in the test when compared with soybean oil.

3.4. Value evaluation of the oil as the vehicle for pesticide residue elimination

The value of the oil as the vehicle for pesticide residue elimination was evaluated with regard to: (1) the efficiency of pesticide residue elimination; (2) the organoleptic test on smell and taste; (3) the loss of ginsenosides after vegetative oil treatment; and (4) the price of the oil. As shown in Table 2, soybean oil demonstrated the best result for the efficiency of pesticide residue elimination, but received the poorest score in the organoleptic test. Canola oil ranked the second in the efficiency of pesticide residue elimination, but first in the organoleptic test, loss of ginsenosides after oil treatment, and price. It obtained a total score of 29. No oil induced a detectable change in the ginsenosides profile of the ginseng extract after oil treatment. Therefore, all of the oils obtained a score of 6 in this parameter. In terms of price, canola oil was the cheapest, obtaining a score of 6. The total score for the soybean, canola,

Table 2

Evaluation of the oils as a pesticide residue elimination vehicle

Oil	Soybean	Canola	Sunflower	Grape	Corn	Olive
Parameter						
Elimination efficiency	6	5	3	4	2	1
Organoleptic factor						
Smell	1	6	4	5	2	3
Taste	1	6	5	4	3	2
Loss of ginsenosides ¹⁾	6	6	6	6	6	6
Price	5	6	3	2	4	1
Total score	19	29	21	21	17	13

¹⁾ A score was given by evaluating the relative rank of the six oils. The oil with best results obtained a score of 6, but the lowest grade received a score of 1. A score of 6 was given to all of the oils because none influenced the loss of ginsenosides

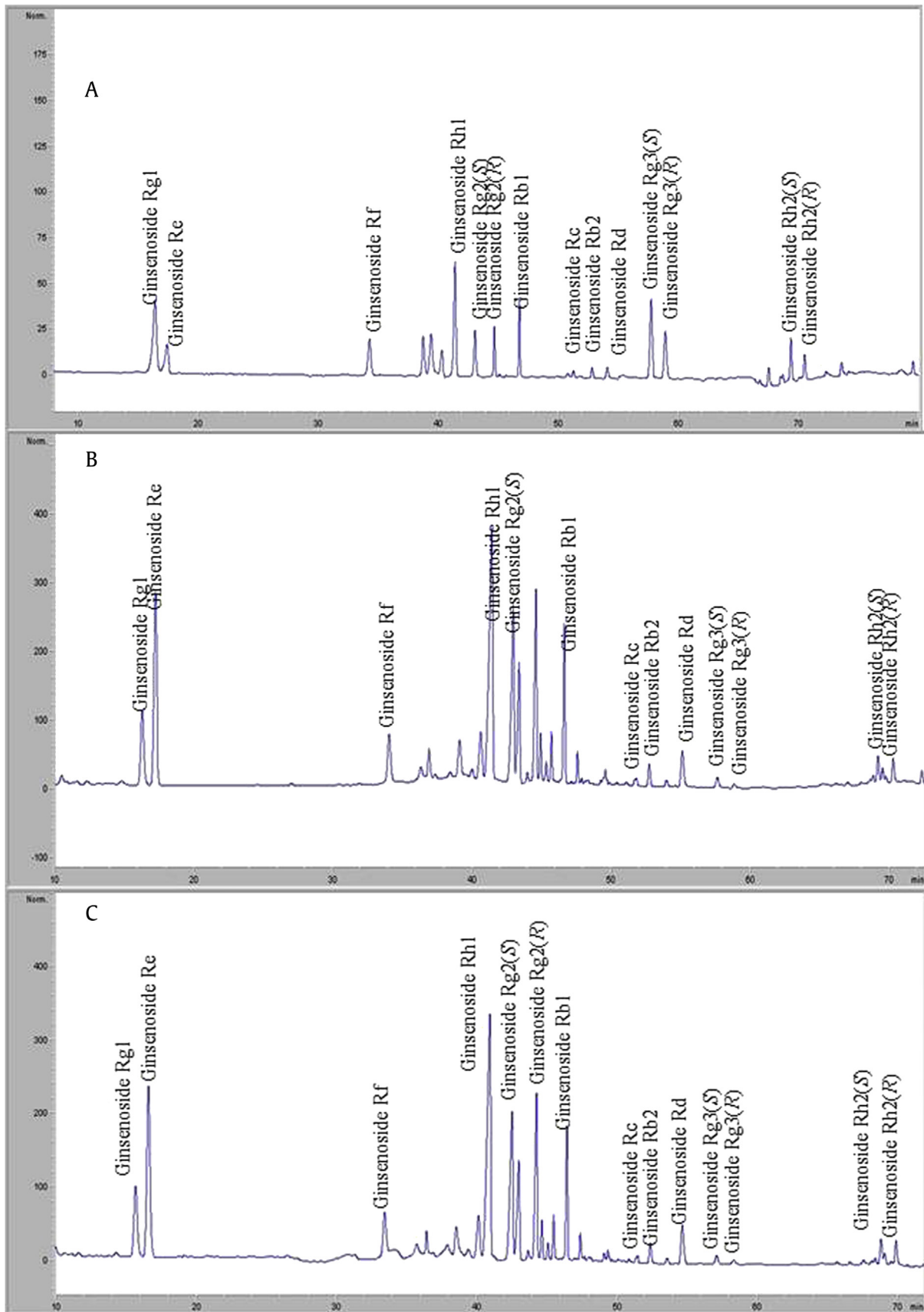


Fig. 3. HPLC ginsenoside profiles in the aqueous ginseng extract layer before and after canola oil treatment. (A) Fourteen standard ginsenosides; (B) ginsenoside profile of the ginseng extract layer before oil treatment; and (C) ginsenoside profile of the ginseng extract layer after oil treatment.

sunflower, grape, corn, and olive oils was 19, 29, 21, 21, 17, and 13, respectively.

3.5. Effect of the solid and liquor content in the ginseng extract on the loss of ginsenosides by oil treatment

We investigated the effect of the solid and liquor content on the loss of ginsenosides by oil treatment because the solid and liquor content are two important factors in the ginseng industry. The solid content of the first batch of the water extract can be higher than 10%. In addition, we use two different solvents for the preparation of the ginseng extract. As shown in Fig. 3, there was no observable change in the TLC profiles of ginsenosides in the solid range of 10–30% when the ginseng extract was treated with canola oil. Further, ginsenosides did not transfer to the canola oil phase when the liquor content in the ginseng extract was 10–30%.

4. Discussion

Pesticides are designed to be toxic to living things, so by their very nature they pose risks. The risk of a substance is a function of the substance's toxicity and the amount of exposure to that substance [11]. According to an ancient adage, the dose makes the poison. Toxic substances can enter the body through the skin, mouth, eyes, or lungs. Some lipophilic agents are not biodegradable and therefore accumulate in the soil, at the water surface, in plants, and in the bodies of animal and shellfish and are recycled via the food chain. Due to toxicity, the use of some pesticides is restricted or strictly forbidden. However, it would be extremely difficult for farmers to grow crops without synthetic pesticides. In conclusion, it can be said that pesticides are a necessary evil. Most recently, pesticide-contaminated ginseng products were frequently detected by inspection institutes and thus led us to develop a method to eliminate pesticide residues [12,13]. CO₂ critical extraction is very expensive and relatively less efficient in removing pesticide residues [14], which has a great impact on the manufacturing cost.

If pesticides are indispensable agents for the sustainable and foreseeable supply of crops, it occurred to us that we had to develop a reasonable measure that can minimize the malignant impact of pesticides to human health. Most probably, blockade of the incorporation of pesticide to the table could be the ideal measure. The elimination of pesticide residues during the manufacturing process could be one approach.

To date, the CO₂ critical extraction method, gloss oxidation method, gloss decomposition method, and microwave decomposition method were developed for the elimination of pesticide residues before or after harvest. The degradation of pesticide residues by ozone was also attempted [14–16]. However, these methodologies have a critical disadvantage in cost effectiveness, labor effectiveness, and efficiency. To complicate matters, when trying to eliminate the pesticide residue ginseng we have to keep in mind that the chemical bonds in pesticides are more stable than the O-glycosidic bond in ginseng glycosides (ginsenosides). We reported that the two-phase partition between soybean oil and aqueous ginseng could efficiently eliminate pesticide residues. The prime advantage of this method is that it is absolutely safe to human health. In addition, the loss of ginsenosides by soybean oil treatment was almost negligible. Most important, this method has advantages in cost- and labor-efficiency over the other proposed methods. However, the soybean oil-treated ginseng extract demonstrated a slightly unpalatable odor and taste peculiar to soybean oil after treatment. This led us to screen new vegetable oils that can rival soybean oil in cost and pesticide residue elimination effectiveness but have better results in an organoleptic test. We employed six different vegetable oils that are readily available at

local markets: soybean, canola, corn, grape, olive, and sunflower. The overall elimination efficiency of pesticide residues for soybean, canola, corn, grape, olive, and sunflower oils accounted for $97.91 \pm 12.02\%$, $96.55 \pm 12.21\%$, $92.14 \pm 18.31\%$, $95.87 \pm 14.99\%$, $84.25 \pm 11.55\%$, and $92.28 \pm 14.12\%$, respectively. The elimination rate of pesticides by canola oil was found to be slightly lower than that by soybean oil. However, canola oil overwhelmed soybean oil in the organoleptic test: it gave no perceptible change in ginseng odor and taste. In addition, canola oil exhibited a higher potency of pesticide elimination efficiency than that of the other four vegetable oils. In the viewpoint of cost, canola oil had the lowest price compared to the other five oils, indicating the cost-effectiveness of canola oil. The reason for the lower elimination power of canola oil compared with soybean oil could be its weaker solvent power to pesticides associated with polarity.

Generally, pesticides are lipophilic compounds that cannot readily be dispersed by rain and therefore can stay on the surface of the plant long enough to be toxic to pests. This is especially true for organochlorine pesticides, which are very nonpolar. In the case of water-soluble compounds, pesticides are used in combination with surfactants, such as Tween 80. Additionally, most of the lipophilic pesticides are recalcitrant to biodegradation. In summary, pesticide residue elimination by two-phase partition chromatography between vegetable oil and aqueous ginseng extract is an excellent approach. Particularly, canola oil was found to be the best vehicle to eliminate pesticide residues in aqueous ginseng extract with regards to the pesticide elimination efficiency, organoleptic properties, health- and environmentally friendliness, cost-effectiveness, and loss of ginsenosides.

In conclusion, we propose an alternative cost effective, labor effective, and efficient vehicle for removing pesticide residues from ginseng extract by two-phase partition chromatography between canola oil and aqueous ginseng extract.

Conflicts of interest

The authors have no conflicts of interest to declare.

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