Smashing Tissue Extraction and GC Analysis of Active Fatty Acids from Oil Cake of Perilla Seeds

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Abstract: **Objective** To optimize the extraction technology of perilla seeds oil from the oil cake of perilla seeds (OCPS) by using the contents of active fatty acids as evaluation standard. **Methods** The fatty acids were extracted from OCPS, the residue of perilla seeds after cold-press, by smashing tissue extraction (STE), the new technology selected through comparing with classical leaching extraction (LE), Soxhlet extraction (SE), ultrasonic extraction (UE), and supercritical-CO_2 fluid extraction (SFE). For optimized condition of STE, orthogonal test was designed and completed. The contents of five fatty acids in extracted oil and OCPS were determined by GC. **Results** The optimized extraction parameters were smashing for 1.5 min under extraction power of 150 W and 1:6 of the material/solvent ratio. The contents of five fatty acids in the oils extracted by five techniques from OCPS and determined by GC were as follows: α-linolenic acid (41.12%－51.81%), linoleic acid (15.38%－16.43%), oleic acid (18.93%－27.28%), stearic acid (2.56%－4.01%), and palmitic acid (7.38%－10.77%). **Conclusion** The results show that STE is the most efficient technology with the highest yield (LE: 0.57%; SE: 1.03%; UE: 0.61%; SFE: 0.80%; STE: 1.17%) and shortest time (LE: 720 min; SE: 360 min; UE: 30 min; SFE: 120 min; STE: 1.5 min) among five tested extraction technologies. It is first reported using STE to extract herbal oil enriched with active fatty acids.

Key words: fatty acids; flash extraction; GC analysis; oil cake of perilla seeds; *Perilla frutescens*; smashing tissue extraction

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Introduction

Perilla is the common name for *Perilla frutescens* (L.) Britt., a member of the Labiatae family. Its leaves, stems, and seeds have been used as Chinese materia medica (CMM) independently with a long history. The seeds of perilla, called perilla seeds (*Zisuzi* in Chinese), are used not only as CMM for the treatment of thick spit, cough, asthma, constipation, and intestinal dryness, but also used as a good source of dietary oil with excellent nutrition effect (Lertprasertsuke *et al*, 2008; Kopecky *et al*, 2009; Russo, 2009), at approximately 40% oil content. Perilla seeds oil (PSO) contains rich α-linolenic acid (ALA), one of the ω-3 fatty acids at 60%－70% (Shin and Kim, 1994), which is higher than that in flaxseed oil, and therefore it has been widely used for both the medicinal and healthy purpose with the function of heptaprotection, lowering blood lipid, improving memory and vision, reducing allergy reaction, and anti-aging. A nationally registered new medicine, Suzi Oil Soft Capsula, made from PSO is used clinically for the treatment of high blood lipid syndrome with the symptoms of head weight as wrap, chest tightness, spitting, and numbness and seriousness of four limbs, *etc*. Because of the importance and commercial value of PSO, high extraction yield and good quality are very significant.

The oil cake of perilla seeds (OCPS) is the solid residue after the PSO was extracted with mechanical press at low temperature. As an important byproduct

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with many potential uses, currently it is mainly used as animal feed and it's thought that the highly nutritional value of OCPS is related to the residual PSO. Simultaneously, as a de-oil product, it can be used as an appropriate raw material to develop other healthy or nutritional products or their ingredients. Many other new uses have also been proposed (Liu and Yu, 2008). So, for further development of OCPS, it is necessary to investigate the composition of OCPS, especially active fatty acids. There is no report or related study about the composition of OCPS till present.

Extraction is the first essential step to investigate bioactive compounds of plant and any related materials. As a classical and common technique, mechanical press at room temperature to obtain PSO was used. Because of the disadvantage of this technique, quite amount of residual oil containing bioactive fatty acids could be obvious and so became our first concern. Besides the mechanical press, other currently common used techniques to obtain vegetable oil mainly include solvent extraction (Jiao *et al*, 2008), ultrasonic extract (UE) (Feng and Gong, 2007), and supercritical-CO_2 fluid extraction (SFE-CO_2) (Ma, Zhuang, and Zhang, 2008). Smashing tissue extraction (STE) is a new extraction technology which combines all advantages of smashing, stirring, and vibration together with proper solvent (Liu, Yuan, and Ji, 1993; Liu *et al*, 2009). Because of its fastness countable with seconds to minutes to finish an extraction, it is called flash extraction. In our previous study, we compared the yield and composition of PSO from perilla seeds by using STE with those of SFE-CO_2, UE, and conventional methods. The result showed that STE had significant effect on oil quality. In this study, STE was used again to extract bioactive fatty acids contained in OCPS by comparing with leaching (LE), Soxhlet extraction (SE), UE, and SFE-CO_2 methods. The composition and amount of fatty acids were analyzed by qualitative and quantitative GC chromatography.

Reagents and materials

OCPS was provided by Jiashi Nutritional Plant Oil Development Co., Ltd. α-Linolenic acid methyl ester (purity > 99%, C18:3), linoleic acid methyl ester (purity > 99%, C18:2), oleic acid methyl ester (purity > 99%, C18:1), stearic acid methyl ester (purity > 99%, C18:0), and palmitic acid methyl ester (purity > 99%, C16:0) were purchased from Sigma-Aldrich (Helsinki, Finland). All other chemicals and solvents used were of analytical grade. The dried OCPS was crashed to small pieces and ground to crude powder based on extraction requirement, and then stored in dark bags. Petroleum ether (60−90 ℃) was used as solvent for the extraction of fatty oil (except for SFE-CO_2).

Instruments

KQ3200DB Ultrasonic cleaning bath (Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu, China) was used for UE. A GC (Agilent GC122) equipped with and FID detector, Agilent Cerity QA/QC Station, and Thermon—600 T fused-silica (30 m × 0.25 μm) capillary column was used to analyze the extracts. JHBE—20A Herbal Blitzkrieg Extractor (Henan Jinnai Sci-Tech Development Ltd.) was selected for STE.

Methods and results

Extraction

LE: OCPS (10 g) was immersed in 60 mL of petroleum ether in a flask for 12 h, and the extract was obtained by filtration. Then the residue was re-extracted for twice more with fresh solvent. Finally the combined extract was evaporated at 40 ℃ under reduced pressure to constant weight and the crude oil was obtained.

SE: OCPS (10 g) was extracted with 150 mL of petroleum ether in SE at 80 ℃ for 6 h, and the petroleum ether was removed under reduced pressure with a rotary vacuum evaporator.

UE: OCPS (10 g) was extracted with 70 mL of petroleum ether in KQ3200DB Ultrasonic cleaning bath for 30 min at 500 W of power.

SFE-CO_2: SFE-CO_2 extraction was carried out by using supercritical fluid extraction system. The following extraction parameters were used: extracting pressure 20 MPa, extracting temperature 40 ℃, flow rate of carbon dioxide 30 L/h, and extraction time 2 h.

STE: The orthogonal test was designed to optimize the extractive process parameters, based on the employed characteristic of herbal blitzkrieg extractor (HBE) (Liu, 2007) and referring to previous application examples (Zhou, Liu, and Zhao, 2009), and the main parameters were solid/liquid ratio, STE time, and extract power. The orthogonal test $L_9(3^4)$ was formulated to study the effect of these parameters with

three different levels to study their effects. The total oil yield was employed to optimize the extraction process. OCPS (10 g) was put into the HBE extraction tank under designed condition; turn on the HBE for STE; the solution was filtrated through a filter paper; concentrated under vacuum to constant weight for the calculation of yield. The analysis of variance was conducted and the results showed that the power played an important role in the extraction of PSO from OCPS, followed by the solid/liquid ratio, and then STE time. The optimum combination of variables was $A_2B_3C_1$, that is STE for 1.5 min at an extraction power of 150 W and 1:6 of the solid/liquid ratio (Tables 1－3).

The extraction yield of total oil in samples was defined as yield = mass of extracted oil/mass of the material × 100%. As seen in Table 4, the maximal oil yield was 1.17% by STE, and the minimal was 0.57% by LE.

Tabel 1 Independent variable values of the process and their corresponding levels

Levels	Factors		
	Extraction time / s	Power / W	Solid/liquid
1	60	50	1:6
2	90	100	1:8
3	120	150	1:10

Table 2 $L_9(3^4)$ orthogonal test for the extraction of total oil with STE

No.	A	B	C	D	Total oil yield / %
1	1	1	1	1	0.81
2	1	2	2	2	0.71
3	1	3	3	3	1.11
4	2	1	2	3	0.72
5	2	2	3	1	0.92
6	2	3	1	2	1.15
7	3	1	3	2	0.59
8	3	2	1	1	0.88
9	3	3	2	3	1.06
K_1	0.87	0.71	0.94	0.93	
K_2	0.93	0.83	0.83	0.92	
K_3	0.84	1.10	0.87	0.90	
R	0.09	0.29	0.11	0.03	

Gas chromatography analysis

The chemical compositions in the oils extracted from perilla seeds were identified by the peaks corresponding of GC to the retention times of standard fatty acids. A GC (Agilent GC122) equipped with a FID detector and Agilent Cerity QA/QC Station was used to analyze the extracts. Thermon—600 T fused-silica (30 m × 0.25 μm) capillary column was used. The carrier gas was nitrogen. The injection temperature was set at 210 ℃ and the detector temperature was set at 280 ℃. The flow rates of air, nitrogen (carrier gas), and hydrogen were 350, 30, and 30 mL/min, respectively. Split rate was set at 1/20. Sample solution (1 μL) was injected into the GC system. All of the oil samples obtained by different methods and different materials were derivatized to fatty acid methyl esters (FAME) before injected into GC for analysis (Phippen, Isbell, and Mary, 2006).

Table 3 Analysis of variance in the ANOVA model

Source	Sum of square	Degree of freedom	Mean square	*F*-ratio
time	0.0115	2	0.0558	0.0028
power	0.1723	2	0.0861	0.0431
solvent	0.2011	2	0.1006	0.0503
error	0.0083	2	0.0042	
total	0.2467	8		

Table 4 Comparison of five extraction techniques

Methods	Time / min	Solid / liquid	Oil yield / %
LE	720	1:6	0.57
SE	360	1:15	1.03
UE	30	1:7	0.61
SFE-CO_2	120		0.80
STE	1.5	1:6	1.17

All the five standard FAMEs were analyzed alone and together respectively by GC. Under this condition, every FAME can be sufficiently separated (Fig. 1). The reproducibility of retention time (t_R) and peak areas were satisfactory.

The main components of OCPS were identified as palmitic acid (4.702 min), stearic acid (6.741 min), oleic acid (7.034 min), linoleic acid (7.716 min), and α-linolenic acid (8.847 min) by matching their t_R to standards of five FAMEs under identical condition. The GC of five FAMEs in OCPS extracted with STE was shown in Fig. 2. The qualitative calculation of every fatty acid was carried out by comparing the peak areas with the corresponding peaks of standards based on established procedures.

As presented in Table 5, five main fatty acids obtained from the OCPS by five extraction methods were identified as α-linolenic acid (41.12%－51.81%), linoleic acid (15.38%－16.43%), oleic acid (18.93%－27.28%), stearic acid (2.56%－4.01%), and palmitic

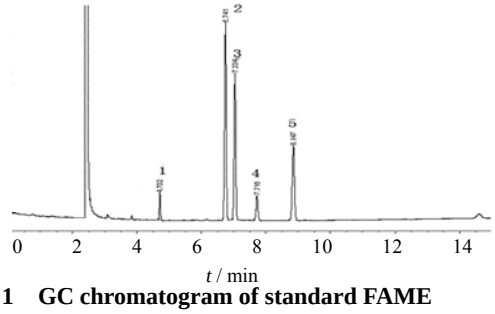

Fig. 1 GC chromatogram of standard FAME

1: C16:0; 2: C18:0; 3: C18:1; 4: C18:2; 5: C18:3

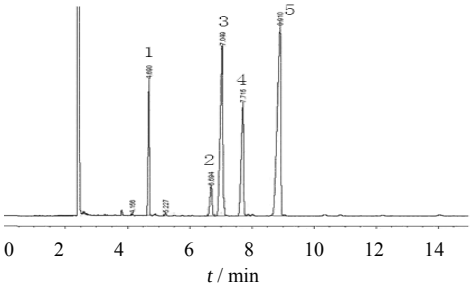

Fig. 2 GC chromatogram of FAMEs in OCPS with STE

Table 5 Contents of five fatty acids in OCPS by five extraction techniques

Methods	fatty acids / %				
	C16:0	C18:0	C18:1	C18:2	C18:3
LE	10.77	4.01	27.28	16.43	41.12
SE	8.72	3.21	22.69	16.34	48.82
UE	9.59	3.53	23.34	16.17	47.14
SFE	7.38	2.56	18.93	15.38	51.81
STE	8.51	3.12	21.30	15.87	50.17

acid (7.38%—10.77%). From Table 5 it can be clearly seen that different extraction techniques had significant effects on the content of every fatty acids. For unsaturated fatty acid, STE extract contained higher amount of α-linolenic acid (50.17%) near the highest one in SFE (51.81%), 15.87% of linoleic acid, and 21.30% of oleic acid, higher than SFE. For LE extract, the percent of oleic acid was the highest (16.43%) and obviously surpassed other four methods.

Discussion

There are several different technologies or processes to produce PSO in vegetable oil industries. The content and composition of oil contained in residual material after industry extraction, such as SE and mechanical press are significantly variable up to more than 10%. For the material used in this study, the oil content is relatively low but important for either medicinal or nutritional purpose. Because of its hardy texture, an available extraction technique to analyze and recover the residual oil in OCPS is very important. By comparing with current traditional and modern extraction techniques including LE, SE, UE, and SFE, it is proved that STE combining with smashing, soaking, stirring, and vibration together is an effective and practicable extraction technology from the yield and composition of the extract. This study provides a useful reference to the future for quick and component-safe extraction of vegetable oil and bioactive fatty acid.

Because of the new finding that palmitic acid is an effective inhibitor of HIV-1 to enter CD4 T cell through binding to its receptor, the PSO with significant amount of palmitic acid could be beneficial for the people who works in the high-risk area of HIV-1 infection (Lee *et al*, 2009).

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