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FAST EXTRACTION OF BIOACTIVE FATTY ACIDS FROM THE PERILLA SEEDS BY SMASH TISSUE EXTRACTION

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Abstract

In this study, smashing tissue extraction (STE) was successfully applied to extract bioactive fatty acids from the Perilla seeds. As control experiment, four conventional extraction methods including leaching, Soxhlet, ultrasonic-assisted, and supercritical-CO_2 fluid extraction have been comparatively investigated. The content of bioactive fatty acids from each extract were quantified with the help of GC-FID in order to further optimize the extraction process of STE. The result shows that STE is an efficient extraction method to obtain fatty acids due to its simple, rapid preconcentration of required components and the highest oil yield among those methods tested. The optimized parameters of STE obtained through orthogonal design were the ratio of liquid to solid material at 8:1, the power of extraction at 50w, and extracting time 30 second.

Introduction

Perilla frutescence (L.) Britt, as an aromatic herb traditionally cultivated in Asia and now throughout world-wide, is one of the most important oil crops used as edible oil, nutraceutical, cosmetic, and medicinal purposes. Perilla seeds, the seeds of *Perilla frutescence* (L.) Britt had been reported to contain abundant amount of bioactive oil, especially with high yield of α-linolenic acid (Shin & Kim, 1994). As the precursors of n-6 and n-3 polyunsaturated fatty acid (PUFA) families, linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) are finally transformed to EPA and DHA *in vivo*. Many literatures had described that bioactive fatty acids exhibited a number of interesting biological activities, such as regulating the metabolism of fat (Kopecky *et al*., 2009), preventing cardiovascular diseases (Russo, 2009), resisting the carcinogenesis (Lertprasertsuke *et al*., 1991; Huang, 2008; Trombetta *et al*., 2007) and obesity, and improving the cognitive function (Willis *et al*., 2009).

Traditionally, the major techniques to obtain vegetable oils, such as steam distillation, Soxhlet extraction (Sew *et al*., 2010; Siddiqi *et al*., 2012), ultrasonic extraction (Jiao *et al*., 2008), SFE-CO_2 extraction (Feng & Gong, 2007; Lu & Dou, 2003; Ma *et al*., 2008) and others (Chemat *et al*.,2006; Fayyaz-ul-Hassan *et al*., 2012; Qayum *et al*., 2012), spend long time, usually several hours to days. Other main disadvantages of those technologies are increased solvent cost and the potential to contaminate the environment with more solvent residue.

Smashing Tissue Extraction (STE) was firstly proposed as an extract method in 1993 (Liu *et al*., 1993). The principle, instrumentation, evaluation of advantage and disadvantage, and application with real cases to extract various active ingredients including tannins, polyphenols, flavonoids and saponins etc (Liu, 2007; Yoshida *et al*., 1993; Liu *et al*., 2003; Wang *et al*., 2009) from herbal materials were extensively and deeply analyzed. The process of STE combined most current technological superiorities, such as smash, soak, stir, and vibration under high power and the existence of proper solvent to realize extraction purpose within seconds to minutes in one system. Another significant advantage is that the instrument is operated at room temperature and so the possible decomposition of heat-sensitive ingredients could be effectively avoided. Previously we reported (Wang *et al*., 2009) STE and purification process of total saponins from the basal parts of stem and hair roots of *Panax notoginseng*. In this present study, STE was successfully applied to extract bioactive fatty acids from Perilla seeds for the first time.

Materials and Methods

Reagents and materials: The perilla seeds were obtained from the local market in Jilin city, Jilin province, China and were authorized by Prof. Qishi Sun.The specimen was deposited in the laboratory of Food Pharmacy, College of Chinese Materia Medica, Shenyang Pharmaceutical University. The methylate of α-linolenic acid, linoleic acid, oleic acid, tuberculostearic acid, and palmitic acid (purity ≥ 99%) were purchased from Sigma-Aldrich (Helsinki, Finland). All other chemicals and solvents used were of analytical grade.

Apparatus: STE was performed by JHBE-50S Herbal Blitzkrieg Extractor (Henan Jinnai Sci-Tech Development Ltd.). The apparatus consists of 5 parts: tissue crushing head, high speed motor, lifting system, integrated control system, and extraction tank. The design of tissue crushing head adopted the advantage of homogenizer used for tissue homogenization. In the working state, high-speed dynamic system was generated through strong vortex of tissue crushing head driven by high speed motor. The extracted materials were thoroughly crushed, solvent

quickly permeated into the material tissue and took extracted components out through the concentration difference of solute between interior of material and solvent in a fast-changing system and eventually achieved complete smash to realize perfect extraction. The motor was controlled by integrated control system.

STE procedure: Based on the characteristics of JHBE (Liu *et al*., 1993) and previous application cases (Liu *et al*., 2003), the main parameters were designed as liquid/solid ratio, STE time, and extraction power. The orthogonal experiment L_9 (3^4) with three different levels was formulated to study the effect of these parameters (Table 1). The total oil yield was employed to optimize the extraction process and fatty acid composition was analyzed by GC. 10 g of perilla seeds were put into the extraction tank under designed condition, turn on the JHBE for STE. The crushed homogenate like material was filtrated through a filter paper, concentrated under vacuum to constant weight for the calculation of yield. The extraction yield of total oil in samples was defined as Yield (%) = mass of extracted oil/ mass of the material ×100%. A part of oil was used for GC analysis to evaluate the composition representing the quality of oil. All extraction experiments were repeated three times to get average value.

Table 1. Independent variable values of the process and their corresponding lever.

Levels	Factors		
	[A] Extraction time (s)	[B] Power (w)	[C] Solid/Liquid ratio
1	30	50	6
2	60	100	8
3	90	150	10

Extraction: The dried perilla seeds and their powder (a particle size less than 0.5 mm) were stored in dark bags. Petroleum ether was used as solvent for the extraction of fatty oil (except for SFE-CO_2). Leaching extraction (LE) 10g of dried powder of perilla seeds was immersed in 60mL of petroleum ether (60-90°C) in a flask for 12h, and the extract was obtained by filtration. Then the residue was repeated for another two times by using fresh solvent. Finally the combined extract was evaporated at 40°C under reduced pressure to constant weight and the crude oil was obtained (Zhou *et al*., 2009).

Soxhlet extraction (SE) 10g of dried powder of perilla seeds was extracted with 150mL of petroleum ether (60-90°C) in Soxhlet extractor at 80°C for 6 h, and the petroleum ether was removed under reduced pressure with a rotary vacuum evaporator (Zhou *et al*., 2009).

Ultrasonic extraction (UE) 10g of dried powder of perilla seeds were extracted with 70ml of same solvent as above in a KQ3200DB Ultrasonic cleaning bath (Kunshan Ultrasonic Instrument Co., Ltd. Kunshan, Jiangsu, China) for 30 min at 500 W of power (Jiao *et al*., 2008). SFE-CO_2 extraction was carried out by using supercritical fluid extraction system (Lu & Dou, 2003). The following extraction parameters were used: extracting pressure at 20Mpa, extracting temperature at 40°C, flow rate of carbon dioxide at 30L/h and extraction time at 2h. The material used for test was also powdered perilla seeds described above.

Optimized experimental condition for STE obtained by orthogonal experiment to extract the oil from perilla seeds (without powdered pretreatment). The extract oil yield and the content of bioactive fatty acids were used for comparing the efficiency besides significant advantage described previously with above four methods: LE, SE, UE and SFE-CO_2.

Gas chromatographic analysis: The chemical compositions in the oils extracted from perilla seeds were identified by the peaks of GC corresponding 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 × 0.25μm i.d.) capillary column was used. The carrier gas was nitrogen. The injection temperature was set at 210°C and the detector temperature was set at 280°C. The flow rates of air, nitrogen (carrier gas), and hydrogen were 350ml/min, 30 ml/min, and 30 ml/min, respectively. Split rate was set at 1/20. 1μL of sample solutions were injected into the GC system. All of the oil samples obtained by different methods were derivatized to fatty acid methylate (FAM) before injecting into GC for analysis (Zhuang & Ma, 2008).

Results and Discussion

Optimization of extraction conditions: The experimental results are summarized in Table 2 and 3. The tables showed the analysis of variance results for the calculated models which reveals the relationships between the variables and their influence on the extraction yield and was used to identify high yields trends for the extraction process. The F-test and the *p*-value results showed that the extraction power [B] affects significantly the oil extraction yields followed by the liquid/solid ratio [C] and then the extraction time [A]. The optimum combination of variables was $A_1B_1C_2$, i.e. extraction power 50W, solid/liquid ratio 1:8 and extraction time 30s. In our study, the bioactive fatty acid composition was also concerned to decide the extraction conditions. It can be seen from the Table 2 that there was no obvious influence for the contents of five main fatty acids among different variables combinations in experiment design.

Experimental verification: According to the optimized extraction parameters, 6 replicated perilla seeds samples were extracted. The experimental mean value of the total oil content was 59.3% with the relative standard deviation (RSD) was 2.1%, which showed that this method had good reproducibility for real sample analysis.

Validation of the analytical method for GC–FID: The FAM standard solutions were prepared by diluting the commercial standard references with petroleum ether to obtain the solution of 0.32mg/ml as a known concentration, and the mixture of the 5 standard solutions was injected into the GC system. The results showed that every fatty acid could be sufficiently separated and the

reproducibility of retention times and peak areas were satisfactory (Fig. 1).

Table 2. Orthogonal experiment (l9 (3^4)) for the STE of total oils.

No.	[A]	[B]	[C]	Percentage of fatty acid (%)					Total oil yield (%)
				C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3	
1	1	1	1	5.44	1.51	11.72	13.22	67.81	56.5
2	1	2	2	5.41	1.52	11.67	13.21	67.65	58.9
3	1	3	3	5.59	1.51	11.69	13.32	67.62	53.4
4	2	1	2	5.50	1.50	11.60	13.46	67.65	58.8
5	2	2	3	5.47	1.48	11.53	13.26	67.90	48.4
6	2	3	1	5.51	1.49	11.59	13.39	67.53	50.2
7	3	1	3	5.51	1.51	11.65	13.53	67.84	57.2
8	3	2	1	5.49	1.50	11.65	13.46	67.54	55.3
9	3	3	2	5.52	1.49	11.53	13.38	67.74	54.4

Table 3. Summary of analysis of variance in the ANOVA model.

Source of variance	Sum of square	Degree of freedom	Mean square	F-ratio
Time	49.899	2	24.949	10.597
Power	73.436	2	36.718	15.596
Solid/Liquid ratio	62.789	2	31.394	13.335
Error	25.898	11	2.354	
Total	212.021	17		

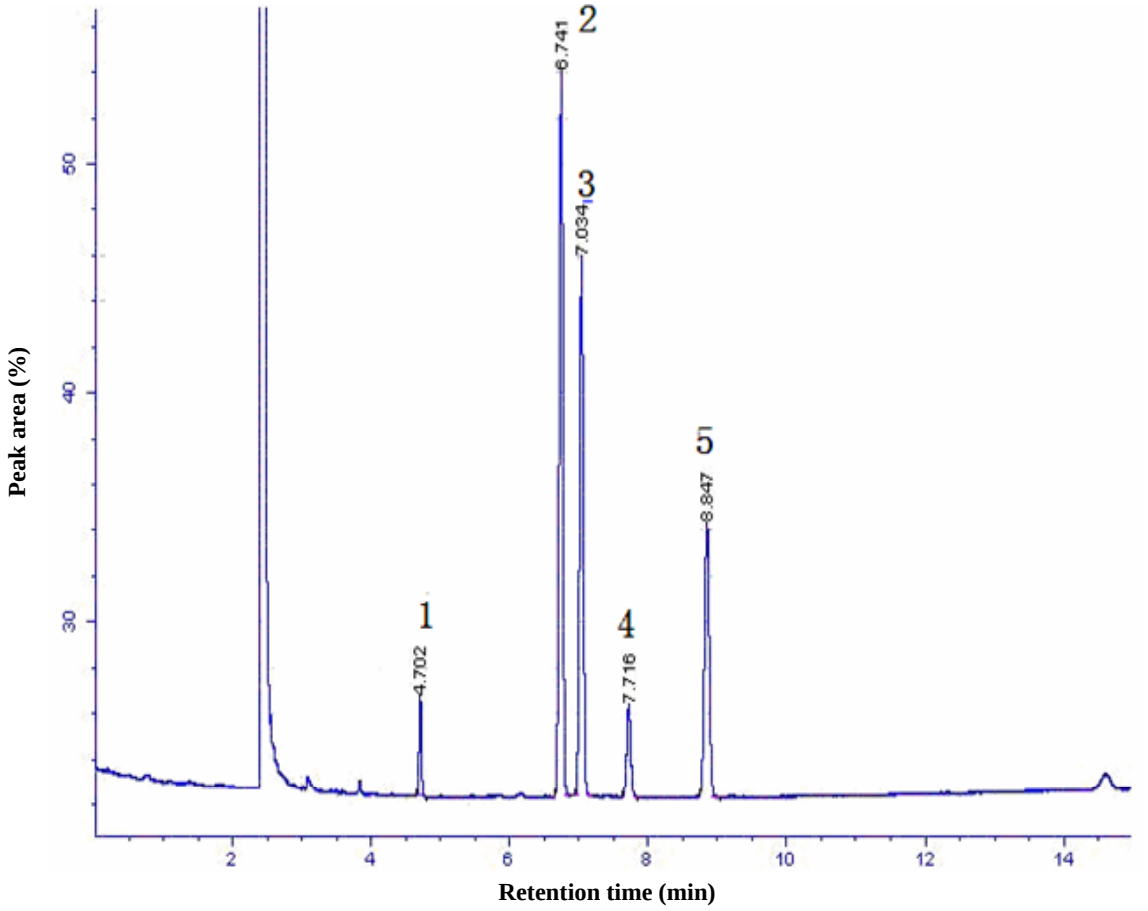

Fig. 1. GC chromatogram of standard fatty acid methyl ester mixture: (1) C16:0, (2) C18:0, (3) C18:1, (4) C18:2, and (5) C18:3

Comparison of extraction methods: It can be seen from Fig. 2 that a maximal total oil yield of 59.3% was produced by STE while a minimal yield of 22.9% given from UE method. The difference between them was very significant, suggested that the extraction method have an important influence on the total oil yield. The main components of the perilla seeds oil were identified as palmitic acid, tuberculostearic acid, oleic acid, linoleic acid and α-linolenic acid by matching their retention times to standards of 5 fatty acid methylate under identical condition. The quantitative 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 Fig. 3, different fatty acids in the perilla seeds obtained by 5 extraction methods were identified and assayed as α-linolenic acid (67.58-68.0%), linoleic acid (3.10-13.46%), oleic acid (10.85-11.59%), tuberculostearic acid (5.55-5.69%), and palmitic acid (1.42%-1.55%),respectively. The results showed that the extraction methods had no significant difference on the content of 5 individual fatty acids in crude oil, while the content of α-linolenic acid obtained by STE was near to SFE-CO_2 and slightly higher than other three methods. Table 4 summarized the extraction conditions and the results of five different extraction methods presented above in order to give an overall evaluation of these different methods. It can be seen from Table 4 that STE had a remarkable advantage on the extraction time which greatly improved the extraction efficiency and could be used as a rapid way to obtain the bioactive fatty acid from the Perilla seed.

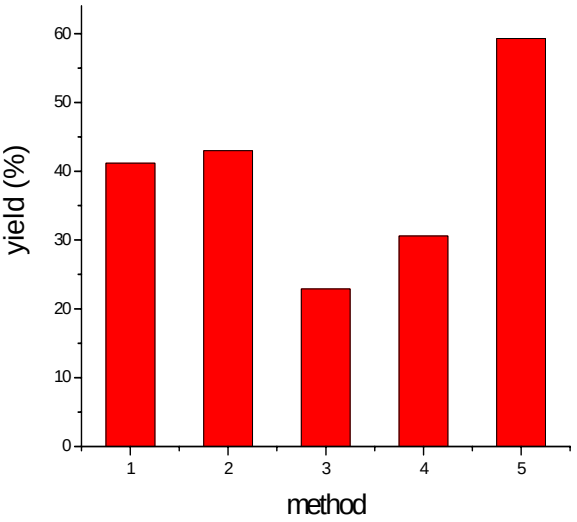

Fig. 2. The yields of Perilla seeds oil by different methods : (1) Leaching method, (2) Soxhlet extraction, (3) Ultrasonic extraction, (4) SFE-CO_2, and (5) STE.

Table 4. Comparison of five extract methods.

Method	Time	Solid/Liquid (w/v)	Oil yield (%)		C18:3 (%)	
			w/w	RSD	Mean value	RSD
LE	12h	1 : 6	41.2	2.4	67.7	1.4
SE	6h	1 : 15	43	3.1	67.6	2.0
UE	30min	1 : 7	22.9	1.8	67.9	1.1
SFE-CO_2	2h		40	14.3	68.0	3.7
STE	30s	1 : 8	59.3	2.1	67.9	0.5

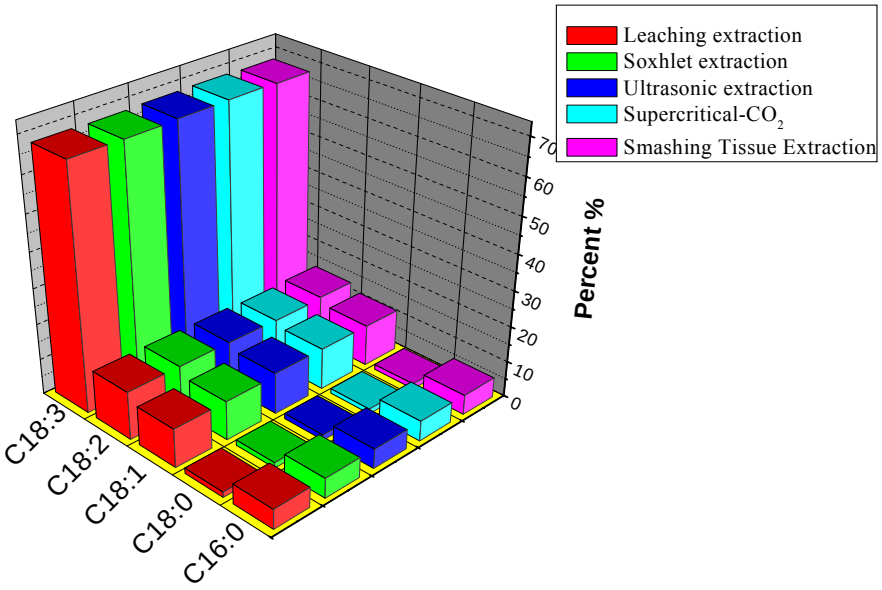

Fig. 3. The contents of five fatty acids in Perilla seeds oils by different extraction methods.

Conclusions

In this study, an efficient technology named STE, which combine the process of smash, stir, and vibration process together was employed to extract the bioactive fatty acids from Perilla seeds for the first time. The result showed that the yield of Perilla seeds oil can reach to 59.3% in tested condition, which is splendiferous and regarded as a big development in improving the total yield of vegetable oil, compared to those reported in present literatures (Feng & Gong, 2007; Jiao *et al*., 2008; Siriamornpun *et al*., 2006). Additionally, it should be emphasized that the STE technology spends very short time to complete a high-performance extraction within 30 seconds with just simply pulverization before extraction. What'more, the contents of main fatty acids obtained by STE were also equal or higher than that by conventional methods and determined as α-linolenic acid (67.91%), linoleic acid (13.49%), oleic acid (10.85%), tuberculostearic acid (1.50%) and palmitic acid (5.55%).

Therefore, the results shows that the biologically active oil of Perilla seeds can be extracted with favorable yield by STE which possess the main benefits of component-safely, extraction-quickly and environment-friendly extract of natural active ingredients applicable for medicinal purpose. In addition, this research could provide a scientific reference for the development of scale-up and industrialization production of vegetable oil.

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