Fast analysis of principal volatile compounds in crude and processed *Atractylodes macrocephala* by an automated static headspace gas chromatography-mass spectrometry

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ABSTRACT

Objective: *Atractylodes macrocephala*, a famous herbal medicine, is used extensively in the practice of Traditional Chinese Medicine (TCM). Processing procedure is a common approach that usually occurs before *A. macrocephala* is prescribed. This paper describes a sensitive and specific assay for the determination of principal volatile compounds in crude and processed *A. macrocephala*. **Materials and Methods:** The present study concentrated on the development of a static headspace gas chromatography-mass spectrometry (SHS-GC/MS) for separating and identifying of volatile compounds from crude and processed *A. macrocephala* samples. **Results:** The results showed that the volatile oil in crude and processed *A. macrocephala* was markedly quantitatively and qualitatively different. Processing resulted in the reduction of volatile oil contents and variation of chemical compositions in *A. macrocephala*. **Conclusion:** The proposed method proved that SHS-GC/MS is rapid and specific, and should also be useful for evaluating the quality of crude and processed medicinal herbs.

Key words: *Atractylodes macrocephala*, processed, quality control, static headspace gas chromatography-mass spectrometry, volatile compounds

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INTRODUCTION

Atractylodes macrocephala (Bai Zhu in Chinese), derived from the rhizome of *A. macrocephala* Koidz, as a valuable Traditional Chinese medicine (TCM), has been used for thousands of years all over the world because of its special pharmacological activities.[1-3] The rhizome of *A. macrocephala* is an important ingredient of several chinese herbal prescriptions, and has been used in drugs for diarrhea, abdominal pain, and insufficiency of the stomach, intestine, liver, kidney, or insufficiency of the spleen with abundance of dampness. Many components, such as volatile oils, sesquiterpenoids, polysaccharides, amino acids, vitamins, resins and other ingredients have been found in *Atractylodes macrocephala* up to now.[4-8] The essential oil as the major constituent has been isolated from A. *macrocephala* and has several pharmacological functions.[9] The essential oil of the herb has anti-inflammatory and anti-ulcer properties, scavenges the CC_{l3} radical, inhibits lipid peroxidation and xanthine oxidase inhibition, inhibits the tert-butyl hydroperoxide-induced cytotoxicity and lipid peroxidation in primary culture of rat hepatocytes, and inhibits the growth of esophageal carcinoma cells and tumor cells.[10] The crude *A. macrocephala* and its processed products are used clinically for thousands of years.[11,12] A proper pharmaceutical processing methodology may significantly alter the pharmacological properties of the original crude TCM, such as the reduction of toxicity and the enhancement of pharmaceutical efficacy.[13,14]

Steam distillation and solvent extraction methods combined with gas chromatography (GC) or gas chromatography-mass spectrometry (GC/MS) are used as the routine methods for the analysis of the volatile oils of TCMs. GC/MS after steam distillation (SD) has been used for the analysis of essential oils in *A. macrocephala*.[15] However, it requires a relatively large amount of samples

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and is a time-consuming procedure. However, none has reported the development of static headspace gas chromatography-mass spectrometry (SHS-GC-MS) as a rapid and efficient analytical tool for the solvent-free fractionation of crude and processed *A. macrocephala* volatiles. Here, we present the development of an automated SHS with GC-MS based analytical method to the separation and identification of the essential oils present in the crude *A. macrocephala* and its processed products by stir-frying with wheat bran (SFWB). SHS sampling has great advantage in the analysis of highly volatiles compounds in *A. macrocephala*. In SHS, the samples are enclosed in a gas tight sealed vial and are heated at a defined temperature.[16] The developed method has been demonstrated to be a powerful tool to study the principal volatile compounds in crude and processed *A. macrocephala*.

MATERIALS AND METHODS

Plant materials

The crude *A. macrocephala* samples were collected from Zhejiang provice. Processed *A. macrocephala* of SFWB was obtained according to the Chinese Pharmacopoeia edited in 2010. These herbal samples were authenticated by Professor Jianbao Zheng (Research Center of TCM Processing Technology, Zhejiang Chinese Medical University). Voucher specimens were stored at the Research Center of TCM Processing Technology.

Sample preparation

The powder of crude and processed *A. macrocephala* samples were precisely weighed (2.0 g) and then introduced into a 20 mL headspace vial. The headspace vial was immediately sealed with a silicone septum and stored at −10°C until use. SHS equilibration was performed at 65°C for 20 min., shaking at 250 rpm. 350 μL of headspace gas were injected using a heated (85°C) gastight syringe (1 mL) in split mode 10:1.

Gas chromatograph-mass spectrometer analysis

Volatile oil analysis was carried out on a Thermo Fisher ISQ single quadrupole gas chromatograph-mass spectrometer (GC/MS). An DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm) was used for separation of the volatile compounds in the A. *macrocephala* samples. The injection temperature was set at 250°C and a splitless mode was used. The oven temperature program was as follows: Initial temperature 60°C for 1 min, increased to 160°C at 3°C/min for 0 min, then increased to 250°C at 5°C/min, 250°C was maintained for 5 min. Helium (99.999%) carrier gas had a flow-rate of 1.0 ml/min. Mass spectra were recorded in electron impact (EI) with full scan mode at 70 eV, scanning the 40-450 m/z range. Ion source temperature was 200°C. Filament emission current was 50 MA. The MS transferline temperature was set at 250°C. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA).

RESULTS AND DISCUSSION

SHS-GC/MS is a technique suitable for determining volatile compounds in herbal medicine samples. In present study, 49 volatile compounds were identified from crude and processed *A. macrocephala* samples, representing 88.59 and 87.57% of the oils, respectively, according to their elution order. The typical GC/MS chromatogram of volatile oil in crude and processed *A. macrocephala* under the same column systems are depicted in Figure 1. The chemical constituents identified by GC/MS in the essential oil and their contents are summarized in Table 1. Although the identified components were similar in the

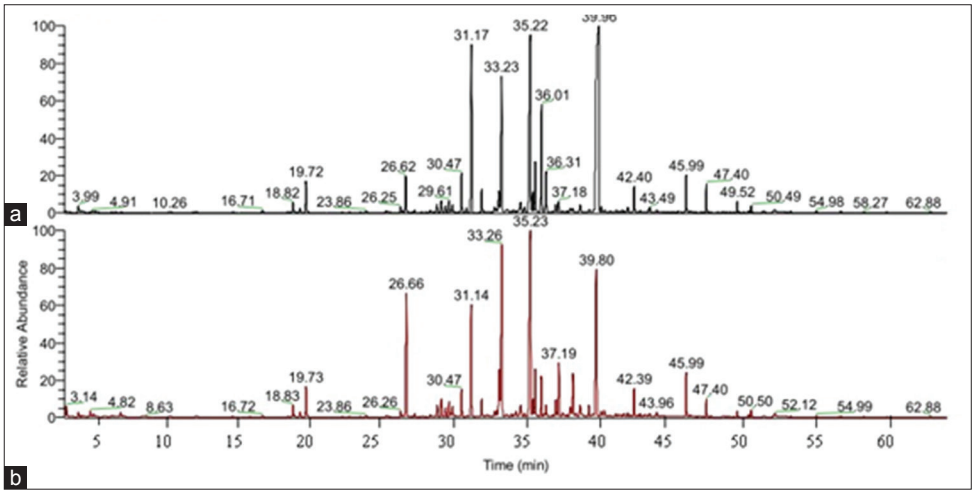

Figure 1: Typical GC/MS chromatogram of SHS analysis of volatile compounds from crude (a) and processed (b) *Atractylodes macrocephala* samples

Table 1: Chemical composition of volatile oils in crude and processed *Atractylodes macrocephala*

Compounds	T_R (min)	Library Formula	Crude sample (%)	Proprocessed samples (%)
(Z)-2-Heptenal	10.25	$C_7H_{12}O$	0.16	0.17
Nonanal	16.71	$C_9H_{18}O$	0.16	0.17
Trans-2-decenal	23.86	$C_{10}H_{18}O$	0.11	0.13
2,4-Decadienal	25.29	$C_{10}H_{16}O$	0.13	0.16
(E, E)-2,4-Deca dienal	26.25	$C_{10}H_{16}O$	1.13	0.37
7,7-Dimethyl-1-vinylbicyclo[2.2.1]heptan-2-one	26.62	$C_{11}H_{16}O$	1.79	7.57
Longifolene-(V4)	27.21	$C_{15}H_{24}$	0.2	0.17
2-Undecenal	28.29	$C_{11}H_{20}O$	0.14	0.15
β-Selinene	28.78	$C_{15}H_{24}$	0.4	0.67
2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	29.08	$C_{14}H_{20}O$	1.01	0.72
τ-Elemene	29.38	$C_{15}H24$	0.56	0.37
α-Gurjunene	29.61	$C_{15}H_{24}$	0.59	0.82
2-methylene-5-(1-methylvinyl)-8-methyl-Bicyclo[5.3.0]decane	29.86	$C_{15}H_{24}$	0.44	0.73
β-Caryophyllene	30.47	$C_{15}H_{24}$	1.89	1.47
β-Elemene	31.17	$C_{15}H_{24}$	10.28	6.18
α-Caryophyllene	31.87	$C_{15}H_{24}$	1.08	0.91
Thujapsene-13	32.74	$C_{15}H_{24}$	0.26	0.28
(3E,5E)-7-Isopropyl-8-methyl-3,5,7-nonatrien-2-one	33.06	$C_{13}H_{20}O$	1.14	2.93
Allo-aromadendrene	33.24	$C_{15}H_{24}$	7.77	12.34
α-Selinene	33.56	$C_{15}H_{24}$	0.11	0.29
α-Bergamotene	33.67	$C_{15}H_{24}$	0.15	/
Isoledene	34.55	$C_{15}H_{24}$	0.66	0.91
β-Sesquiphellandrene	34.81	$C_{15}H_{24}$	0.3	0.18
Aromadendrene	35.22	$C_{15}H_{24}$	14.28	17.48
Eudesma-3,7 (11)-diene	35.41	$C_{15}H_{24}$	0.81	0.76
β-Vatirenene	35.57	$C_{15}H_{22}$	2.45	2.5
α-Guaiene	36.01	$C_{15}H_{24}$	0.11	2.11
Dehydro-Arom adendrene	36.3	$C_{15}H_{22}$	2.17	0.75
Caryophyllene oxide	36.97	$C_{15}H_{24}O$	0.41	1.02
8-isopropylidene-Bicyclo[4.3.0]nonan-2-one	37.18	$C_{12}H_{18}O$	0.54	2.91
1,5,5,8-Tetrame thyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	37.97	$C_{15}H_{24}O$	0.2	0.54
1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	38.15	$C_{13}H_{22}$	0.54	2.59
Spathulenol	38.66	$C_{15}H_{24}O$	0.53	0.78
2-(4a, 8-Dimethyl-1,2,3,4,4a, 5,6,7-octahydronaphthalen-2-yl)-prop-2-en-1-ol	39.31	$C_{15}H_{24}O$	0.21	0.64
Atractylon	39.96	$C_{15}H_{20}O$	29.55	10.73
Bulnesol	40.14	$C_{15}H_{26}O$	0.29	0.42
β-Patchoulene	40.37	$C_{15}H_{24}O$	0.14	0.46
Juniper camphor	41.17	$C_{15}H_{26}O$	0.1	0.17
Heptadecane	41.4	$C_{17}H_{36}$	0.1	0.13
Pistane	41.61	$C_{19}H_{40}$	0.13	0.11
Spathulenol	41.96	$C_{15}H_{24}O$	0.27	0.23
Longiverbenone	42.4	$C_{15}H_{22}O$	1.18	1.39
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	43.49	$C_{15}H_{24}O$	0.33	0.24
Velleral	45.99	$C_{15}H_{20}O_2$	1.65	2.17
4,5-Dehydro-isolongifolene	47.4	$C_{15}H_{22}$	1.09	0.77
8,9-dehydro-9-formyl-Cycloisolongifolene	49.52	$C_{16}H_{22}O$	0.46	0.29
Eudesma-5,11 (13)-dien-8,12-oide	50.49	$C_{15}H_{20}O_2$	0.3	0.33
Linoleic	52.03	$C_{18}H_{32}O_2$	0.11	0.13
Oleic acid	52.12	$C_{18}H_{34}O_2$	0.18	0.23

essential oils derived from the two groups, the quantity of some components in each essential oil was significantly different.

Processing procedure had a great effect on the volatile components and the content of them in *A. macrocephala*. There were more components in the oil from the processed samples than the crude ones. In this study, one compound (α-bergamotene), which was not identified in the oils of the processed samples, was lost during the processing in the oils of *A. macrocephala*.

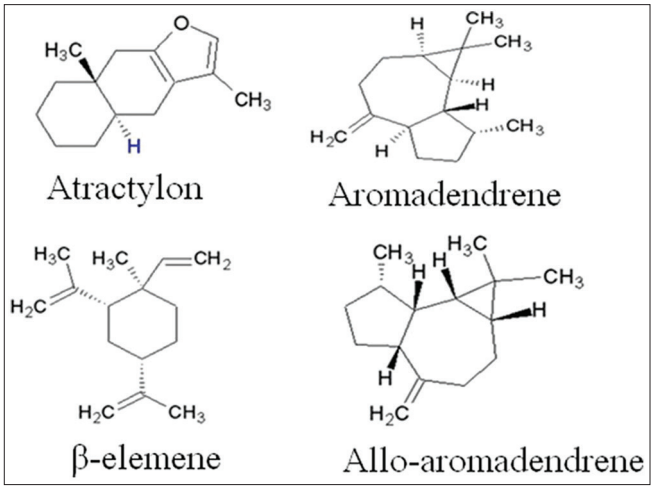

Figure 2: The structures of mian constituents in the volatile oils of crude and processed Atractylodes macrocephala

Moreover, there was a large difference in the contents of volatile composition between the crude and processed drugs. The main constituents in the essential oils of crude and processed *A. macrocephala* were atractylon, aromadendrene, τ-Elemene and allo-aromaden drene, accounting for more than 61.80% and 46.70% of the total oil from crude and processed samples, respectively. The structures and accurate mass spectra of the above four compounds were shown in Figure 2 and Figure 3. After processing, heat treatment increased relative amount of allo-aromaden drene and aromadendrene from 7.77-12.34%, 14.28-17.48%, respectively, however, the contents of τ-Elemene and atractylon in processed samples were lower than in crude ones. On the hole, except for 16 volatile compositions, other compounds from processed *A. macrocephala* were increased in various degrees. According to historical records, the processed Atractylodis rhizome of SFWB showed stronger effect of invigorating the function of the spleen than the crude ones.[17] It is presumed that the increased contents of volatile components induced by the processing procedure would be related to the effect of invigorating the function of the spleen.

CONCLUSIONS

In the present study, a fast, simple, and reliable method was developed to evaluate the quality of crude and processed

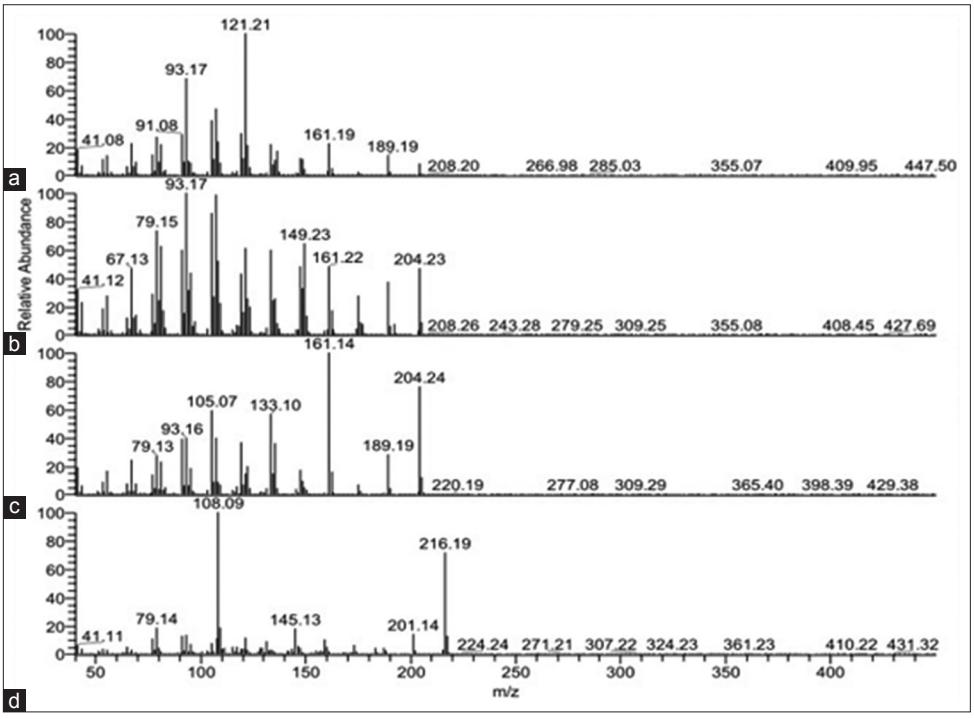

Figure 3: The accurate mass spectra of the four compounds in the volatile oils of crude and processed *Atractylodes macrocephala*. (a) β-elemene; (b) Allo-aromadendrene; (c) Aromadendrene; (d) Atractylon

A. macrocephala samples through determination of principal volatile compounds. The results demonstrate that processed procedure can reduce the volatile oil content and variation of chemical composition in *A. macrocephala* and the further pharmacological studies are necessary to determine the relationship between the variation in composition and curative effects. This study also contributes to the global quality investigation of decoctions derived from crude and processed herbal medicines, as well as safe and effective medicines for customers with great social and economic interest.

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