

Original Research Article

GC-MS analysis of essential oil from *Anethum graveolens* L (dill) seeds extracted by supercritical carbon dioxide

Hongbo Li¹⁻³, Wei Zhou³, Yongshuai Hu⁴, Haizhen Mo³, Jinshui Wang¹, Liangbin Hu^{3*}

¹College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, ²Postdoctoral Research Base, ³School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, ⁴College of Food Science and Technology, Henan Agricultural University, Zhengzhou 450001, China

*For correspondence: **Email:** lihongbo@hist.edu.cn

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Abstract

Purpose: To conduct gas chromatography-mass spectrometric (GC-MS) analysis of the chemical compositions of dill seed essential oil (DSEO) obtained by supercritical CO₂.

Methods: The impact on extraction yield were examined by single factor test, the particle size of dill seed, extraction temperature, time, pressure, as well as CO₂ flux. The best extraction conditions were obtained by an orthogonal test. The chemical configurations of essential oil were examined by GC-MS analysis.

Results: The optimal extraction conditions included an extraction time of 120 min, particle size of 60 mesh, CO₂ flow of 25 L/h, temperature of 40 °C, and pressure of 20 MPa. Under these conditions, the yield of essential oil was 6.7 %. Out of 38 recognized compounds, the main ones were D-carvone (40.36 %), D-limonene (19.31 %), apiol (17.50 %), α-pinene (6.43 %), 9-octadecenoic acid (9.00 %) as well as 9,12-octadecadienoic acid (2.44 %).

Conclusion: A total of 38 constituents of the essential oil obtained by supercritical CO₂ were identified. The findings may provide a theoretical basis for comprehensive utilization of dill seed essential oil (DSEO) from China.

Keywords: Dill seeds, Essential oil, Supercritical CO₂ extraction, D-Carvone, D-Limonene, Apiol, α-Pinene, 9-Octadecenoic acid

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INTRODUCTION

Dill is widely cultivated in India, the Middle East, Russia, Egypt, Thailand, and China, where its leaves and seeds are used as an herb or spice for flavoring food. Matured dill seeds are rich in right-hand carvone, limonene, and apopinol and are commonly used to extract essential oil. As a

broad-spectrum antifungal agent for many kinds of food spoilage fungi, DSEO has been widely used in food preservation and pharmaceuticals [1].

The chemical composition of DSEO varies depending on different geographic origins. The main chemical composition and relative content

of essential oil, extracted from Xinjiang dill by steam distillation, are pentadecane (27.96 %), dioctyl-1,2-two-phenyl hydroxylate (25.10 %), octacosane (13.81 %), and tricosane (9.41 %). Moreover, the corresponding content of carvone and limonene are 2.12 % and 1.68 % [2]. Thirty-five components have been identified from DSEO extracted from Indian dill with the same method. The main components of DSEO are carvone (55.2 %), limonene (16.6 %), apopinol (14.4 %), as well as linalool (3.7 %) [3]. Different chemical compositions can also alter the biological activity of essential oil, therefore, it is necessary to study the DSEO from different regions.

Essential oils have been conventionally extracted using techniques for instance hydrodistillation, steam distillation, solvent extraction, cold pressing, as well as simultaneous distillation–extraction methods, among others [4]. The disadvantages of these common methods are low efficiency, longer time duration, thermal degradation, loss of volatiles, and solvent residue [5,6]. The supercritical fluid extraction technique has been studied extensively as a promising alternative to conventional methods of extraction as it offers many advantages [7]. CO₂ is usually the most preferred solvent as it is non-hazardous, easily available, inexpensive, and fire-resistant with little critical point necessities for temperature and pressure [8].

The optimization of the supercritical CO₂ process for DSEO was studied in this paper. The extraction yield of DSEO was discussed by using single factor test and orthogonal array experiments. The main compounds of DSEO were analyzed by GC-MS. This study may provide a theoretical basis for comprehensive utilization of DSEO from China.

EXPERIMENTAL

Materials and chemicals

Dill seeds were purchased from Lanzhou Baihe (Gansu Winshine Metallurgy Chemicals Co., Ltd, China) in 2016. The whole seeds (<4 mm) were using a crusher (JHF-500A, Juhongfeng, China). The crushed powder was forced through a sieve, resulting in powder with a range of particle sizes [9]. Crushed powder of various particle sizes was acquired by grinding the seeds and sieving the material attained. Carbon dioxide (purity 99.9 %) and helium (purity 99.9 %) were supplied by Zhibao Co. Ltd. (Xinxiang, China). Analytical-grade chemicals were used for all experiments.

Optimization of supercritical CO₂ extraction of DSEO

A laboratory scale supercritical fluid extraction system (Aerospace Wujiang Electromechanical Equipment Co., Ltd., Guizhou, China) was used in the experiments for extraction of DSEO. The effects on the extraction yield of DSEO under various extraction situations, such as the particle size of raw materials, the extraction pressure, temperature, CO₂ flux, and the extraction time, were discussed to find the optimum parameters [6,10]. The single factor test was used in a series of experiments by keeping other factors constant in the optimization experiments.

As per the associated literature as well as the results of initial tests [1,11], the particle size of raw materials was set to 20, 40, 60, 80, 100 mesh, the extracting temperature was 30, 35, 40, 45, and 50 °C, the extracting pressure was 20, 22, 24, 26 and 28 MPa, the extracting time was 30, 60, 90, 120 and 150 min, and the CO₂ flow rate was 15, 20, 25, 30 and 35 L/h. Then, an orthogonal design [L₁₆(4⁵)] was used to improve the extraction situations. Table 1 provides the factor-level list of the orthogonal test was shown in Table 1. The yield of oil extract was used as the evaluation index, which was defined as the mass of extracted DSEO per 100 g of wet feed material.

Table 1: Factors and levels of the orthogonal experiment

Particle size (mesh) A	Temp (°C) B	Pressure (MPa) C	Time (min) D	CO ₂ flow (L/h) E
40	35	20	30	15
60	40	22	60	20
80	45	24	90	25
100	50	26	120	30

GC-MS analysis of DSEO

Qualitative analysis of DSEO was done using GC-MS (7890B GC/5977A MSD, Agilent Technology, CA, USA), combined to a 5972 mass selective detector. The oven temperature was set at 50 °C for 2 min, augmented to 150 °C at a rate of 2 °C/min, and then augmented to 280 °C for 2 min. The split ratio was 5:1 at the GC front inlet. The carrier gas was helium with a flow rate of 1 mL/min. 1 µL of each sample was injected. For the detection of the peaks in the chromatograms, the probability-based matching algorithm was applied to find the utmost prospective match in the NIST library. The relative composition of the components present

in DSEO was calculated from the GC peak area [12].

Statistical analysis

SPSS (IBM, version 23) and Origin (9.1) were used for statistical analysis. Two-way ANOVA was utilized to assess the differences among groups [13]. The significance level was set at the 95% confidence level with $p < 0.05$.

RESULTS AND DISCUSSION

Effect of particle size on yield of oil extract

The effect of particle size on Supercritical CO₂ extraction of DSEO was tested using the ground powder of different particle sizes. Extracting temperature and pressure were kept constant at 45 °C and 24 MPa, respectively. The extraction time was 90 min. The CO₂ flow rate was fixed at 15 L/h.

The results are shown in Figure 1 A. Within a certain range, the yield of DSEO was increased with decreasing particle size. However, the particle size was so small that it clogged the screen, and the flow rate of the supercritical fluid was declined. When the particle size was < 60 mesh, the extracting ratio decreased. Hence, the best value for particle size was 60 mesh.

Effect of extraction temperature on oil yield

The extracting temperature was set at 30, 35, 40, 45 and 50 °C, with other conditions unchanged. Particle size was 60 mesh. It was concluded that as the temperature increased, the yield of DSEO also increased (Figure 1 B). However, But the upward trend flattened when the temperature was higher than 50 °C. and because of this, the higher temperature increased the vapor pressure and diffusion coefficient. Hence, the best value for the extracting temperature was 45 °C.

Effect of extraction pressure on oil yield

The extracting pressure was set at 20, 22, 24, 26 and 28 MPa, with other conditions unchanged. As shown in Figure 1 C, the yield of DSEO increased with pressure, but the upward trend flattened when the pressure increased constantly. Due to this occurrence, the main composition of DSEO is volatile oils easily soluble in supercritical CO₂. The content of the nonvolatile component was low so that the yield increased by a small amount as the pressure increased. Taking compression resistance and product cost into account, the best extracting pressures were between 24 and 26 MPa.

Effect of extraction time on oil yield

When the extraction time exceeded 90 minutes, the yield of DSEO increased slowly and there was not any substantial difference (Figure 1D). Therefore, the extraction times of 30, 60, 90, 120 min can be used as the four-levels of orthogonal test on the temperature factor.

Effect of CO₂ flow on oil yield

The CO₂ flow was set at 15, 20, 25, 30 and 35 L/h, with other conditions unchanged. As shown in Figure 1F, the yield of DSEO increased with the CO₂ flow. When extraction time exceeded 25 L/h, the yield of DSEO stayed flat. The increase in the CO₂ flow increased the mass transfer driving force and solvent ratio during the extraction process. The dissolution rate of the soluble matter was accelerated and the extraction efficiency was improved. However, a solvent flow that is too fast will only increase the consumption of carbon dioxide and energy consumption. Taking compression resistance and product cost into account, 15, 20, 25 and 30 L/h were chosen as the four-levels of the orthogonal test on the CO₂ flow factor.

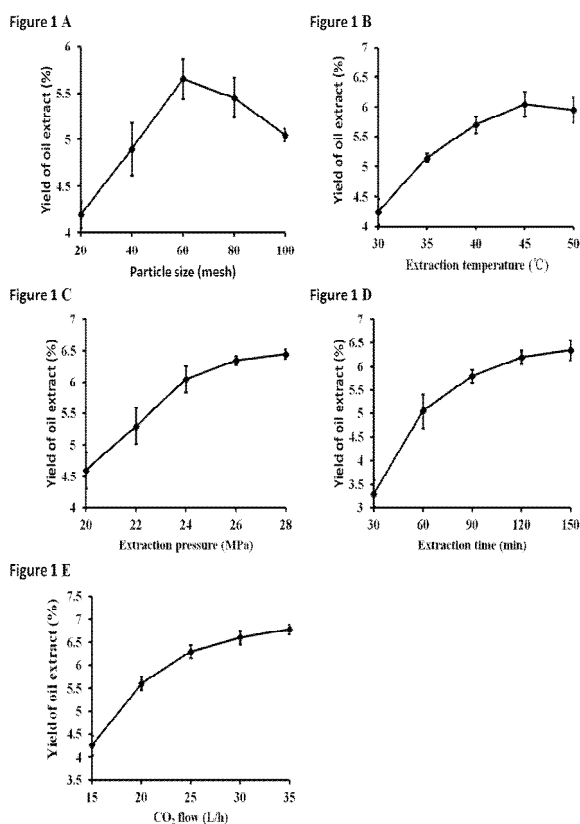


Figure 1: Effect of various operating factors on the extraction of DSEO

Orthogonal array

In a series of experiments using the single factor test, extraction conditions and levels were selected for orthogonal experiments. The experimental runs and visual analysis of the orthogonal experiments are presented in Table 2. The extraction yields were 2.9 - 6.7 %. In the current study, collaborations amongst the variables were not integrated in the matrix. We focused on the major effects of the five utmost imperative factors. The range analysis specifies that the effect order of DSEO is $D > A > E > B > C$. In other words, the supreme effect factor is the extraction time, followed by the particle size, the CO₂ flow, the extraction temperature, and lastly the extraction pressure. The data provided in Table 2 show that the sixth experimental combination gave a highest DSEO yield of 6.7 %. The best extraction conditions were an extraction time of 120 min, particle size of 60 mesh, CO₂ flow of 25 L/h, temperature of 40 °C and extraction pressure of 20 MPa.

Components of DSEO

The acquired extract samples were injected into GC-MS. Figure 2 demonstrates the GC-MS total ion chromatograms of DSEO, and the relative contents of each component are shown in Table 3. We recognized 38 components of the essential oil, which accounted for 96.84 % of the total oil. The primary composites in DSEO were D-Carvone (40.36 %), D-Limonene (19.31 %),

Apiol (17.50 %), α -pinene (6.43 %), 9-octadecenoic acid (9.00 %) as well as 9,12-Octadecadienoic acid (2.44 %) followed by n-Hexadecanoic acid (1.61 %) and cyclohexanone (1.56 %) (Table 3). Compared to previous studies, the oil showed some differences in composition [14]. These differences may be due to different parts of dill, different geographic origins, seasonal variation, treatments prior to isolation, and isolation procedures [1].

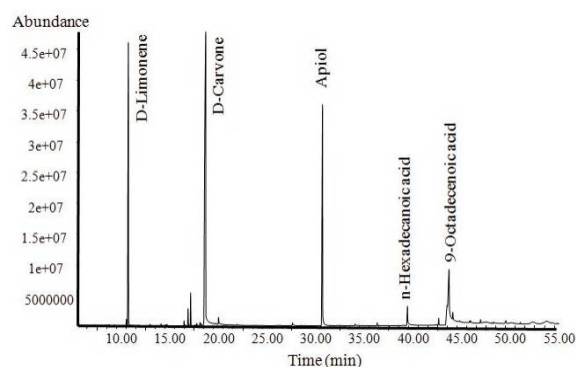


Figure 2: The GC-MS total ion chromatograms of DSEO

CONCLUSION

With the method of supercritical CO₂ extraction, the optimal conditions of extracting essential oil from dill seed are: extraction time of 120 min, particle size 60 mesh, CO₂ flow 25 L/h, temperature 40 °C, and pressure 20 MPa. Under these conditions, essential oil yield is 6.7 %.

Table 2: Visual analysis of the orthogonal experiment

Run	Particle size (mesh) A	Temperature (°C) B	Pressure (MPa) C	Time (min) D	CO ₂ flow (L/h) E	Yield of oil extract (%)
1	1	1	1	1	1	3
2	1	2	2	2	2	4.5
3	1	3	3	3	3	5.3
4	1	4	4	4	4	5.3
5	2	1	2	3	4	6.3
6	2	2	1	4	3	6.7
7	2	3	4	1	2	4.5
8	2	4	3	2	1	5.8
9	3	1	3	4	2	5.7
10	3	2	4	3	1	5.3
11	3	3	1	2	4	5.3
12	3	4	2	1	3	3.7
13	4	1	4	2	3	3.9
14	4	2	3	1	4	2.9
15	4	3	2	4	1	4.6
16	4	4	1	3	2	4.4
K1	4.525	4.725	4.850	3.525	4.675	
K2	5.825	4.850	4.775	4.875	4.775	
K3	5.000	4.925	4.925	5.325	4.900	
K4	3.950	4.800	4.750	5.575	4.950	
Range	1.875	0.200	0.175	2.050	0.275	

Table 3: Chemical composition of DSEO

No.	Retention time (min)	the relative content (%)	Library/ID
1	9.8377	0.0413	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
2	11.2281	0.0589	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
3	11.3483	0.0547	beta.-Pinene
4	11.8461	0.0536	beta.-Myrcene
5	13.0763	0.3425	p-Cymene
6	13.2823	19.3122	D-Limonene
7	14.3466	0.0398	gamma.-Terpinene
8	15.4967	0.0579	o-Isopropenyltoluene
9	17.0931	0.0502	Limonene oxide, cis-
10	17.2591	0.0778	Limonene oxide, trans-
11	19.01	0.235	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran
12	19.1244	0.0413	trans-p-mentha-1(7),8-dien-2-ol
13	19.6795	1.5608	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-
14	20.1429	0.0906	Neodihydrocarveol
15	20.3089	0.1858	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
16	20.6465	0.2637	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)-
17	20.7609	0.138	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
18	21.2301	40.3562	D-Carvone
19	22.5405	0.5993	Anethole
20	24.4173	0.1275	1,2-Cyclohexanediol,1-methyl-4-(1-methylethenyl)-
21	30.1392	0.2612	1,3-Benzodioxole,4-methoxy-6-(2-propenyl)-
22	33.2176	17.5025	Apiol
23	41.9436	1.6149	n-Hexadecanoic acid
24	44.9935	0.0458	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
25	45.1537	0.4019	6-Octadecenoic acid, methyl ester, (Z)-
26	46.0177	2.438	9,12-Octadecadienoic acid (Z,Z)-
27	46.218	9.0039	9-Octadecenoic acid, (E)-
28	46.4984	0.1573	6-Octadecenoic acid
29	46.6013	0.8815	Octadecanoic acid
30	47.3051	0.1156	9-Octadecenoic acid, (E)-
31	48.3179	0.0857	Isopropyl linoleate
32	48.4267	0.1489	2-Methyl-Z,Z-3,13-octadecadienol
33	50.6067	0.0519	9,17-Octadecadienal, (Z)-
34	50.8184	0.1129	Eicosanoic acid
35	52.598	0.0564	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
36	53.5307	0.0928	Heptadecane
37	55.8995	0.1227	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-
38	57.33	0.0624	7-Pentadecyne

The findings of this study can be used for process advancement of pilot-scale extraction of DSEO from the seed as well as from similar or related materials. A total of 38 components of the DSEO have been recognized by GC-MS. D-Carvone could not previously be evaluated by only GC-MS.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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