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Influence of Modifier in Supercritical CO_2 on Qualitative and Quantitative Extraction Results of *Eucalyptus* Ecential Oil

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Abstract

A supercritical CO_2 extraction behavior of *Eucalyptus* oil was investigated under different conditions of pressure, temperature and time with or without cosolvent. The pressure range was from 8 to 25 MPa, temperature from 35 to 55˚C and CO_2 flow rate from 10 to 26 g/min. For 1,8-cineole the appropriate extracting pressure was 15 MPa and temperature was 45˚C. When CO_2 flow rate was 18 g/min, it was benefit to extract the other three substances (limonene, *p*-cymene and *γ*-terpinene, respectively) except 1,8-cineole. Prolonging extraction time could not obviously increase the extract concentration, but the extract yield would increase. The results also indicated that ethanol as a modifier could improve extraction velocity and extraction concentration.

Keywords

Supercritical CO_2 Extraction Behavior, *Eucalyptus* Oil, 1,8-Cineole, Modifier

1. Introduction

Eucalyptus oil, which is widely acknowledged as an important species resource, consists of essential oil, amaroid, tannin, resin and other components [1] [2]. Most of them are terpenes. The main component is 1,8-cineole, which is widely used in food and medicine industries [3] [4] [5] [6]. Crude *Eucalyptus* oil was mainly extracted from *Eucalyptus* leaves by steam distillation. There are many methods to purify *Eucalyptus* oil, including distillation [7], crystallization [8] [9], molecular distillation [10], chemical reaction [11], silica gel column chromatography [12] and so on. Supercritical fluid extraction (SFE), which has received much attention to industrial applications, has not been used in purifying

Eucalyptus oil. Here CO_2 is a nontoxic, inexpensive, nonflammable, and non-polluting solvent for the extraction of natural products [13] [14]. Because of the low temperature and not high pressure of supercritical CO_2 fluid, CO_2-SFE process might be operated under mild conditions that could protect natural products from thermal decomposition [15]. CO_2-SFE technology has been used to extract essential oil from leaf, seed or fruit of natural plants, such as palm oil from its fruit, limonene from caraway seed and rosemary, fennel and anise essential oils from their leaves and seeds [16] [17] [18], but scarcely used to purify crude essential oil. Thus, in this work, the extraction behavior of *eucalyptus* oil with supercritical CO_2 was investigated under certain conditions of pressure, temperature, CO_2 flow rate, time and with or without entrainer to obtain some basic data for commercial application.

2. Materials and Methods

2.1. Materials

The raw material liquid was a crystallization mother liquor and its components were listed in **Table 1**, which had been distilled and frozen for extracting 1,8-cineole, and was supplied by Yunnan Emerald Essence Co. Ltd. at Kunming China. The species of *eucalyptus* used was *Eucalyptus globulus*. Ethanol (at a purity of 99.80%) selected as cosolvent was provided by Shandian Medicine Co. Ltd. at Yunnan China. CO_2 at a purity of 99.50% was obtained from Kunming Hongfa Gas Co. Ltd. at Kunming China.

2.2. Supercritical Fluid Extraction Apparatus

The supercritical fluid extraction experiments were carried out on the SFE-500 extraction system manufactured by Thar Process Inc. USA and supplied by Te-

Table 1. Results of GC-FID analysis on crude *Eucalyptus* oil.

Number	Compound name	Molecular formula	Boiling point at 101 kPa/˚C	Molecular weight	Mass fraction/%
1	*α*-pinene	$C_{10}H_{16}$	157	136.23	1.36
2	*β*-pinene	$C_{10}H_{16}$	165	136.23	0.31
3	*β*-myrcene	$C_{10}H_{16}$	167	136.23	0.75
4	*α*-phellandrene	$C_{10}H_{16}$	167	136.23	1.10
5	Limonene	$C_{10}H_{16}$	178	136.23	21.78
6	1,8-cineole	$C_{10}H_{18}O$	177	154.24	56.00
7	*γ*-terpinene	$C_{10}H_{16}$	182	136.23	5.94
8	p-cymene	$C_{10}H_{14}$	177	134.22	11.51
9	L-linalool	$C_{10}H_{18}O$	200	154.24	0.08
10	4-terpinenol	$C_{10}H_{18}O$	212	154.24	0.04
11	*α*-terpineol	$C_{10}H_{18}O$	213	154.24	0.06
	others				1.07

gent Scientific Ltd. at China. The maximum pressure and CO_2 flow rate of the extraction system can reach 60 MPa and 50 g/min, respectively. The operation temperature is in the range of 0˚C - 150˚C. The interface area between oil and CO_2 in the extraction tank was about 15 cm^2. In each test, ten grams of *eucalyptus* oil was used to investigate the interface extraction behavior.

2.3. Gas Chromatography

The composition of the extracts was analyzed by gas chromatograph (GC-2014, Shimadzu from Japan, PEG-20 M (60 m × 0.25 mm i.d., 0.5 μm of film thickness from GL Sciences Inc. Japan). The split ratio was 1:850 and the sample size was 1 μL. The carrier gas was Nitrogen (99.999%) at a flow rate of 0.67 mL/min. The temperature program was determined as follows: 70˚C for 1 min, and 8˚C/min to 120˚C, then 15˚C/min to 200˚C, followed by isothermal period for 2 to 10 min. The temperature of injector and transfer chamber were 240˚C and 280˚C, respectively. The contents were measured by hydrogen FID (flame ionization detector) and quantified by the normalization method of peak area.

3. Results and Discussion

3.1. Analysis for Crude *Eucalyptus* Oil

The components of crude *eucalyptus* oil were determined by GC-FID. As shown in Table 1, the main components were 1,8-cineole (56.00%), limonene (21.78%), *p*-cymene (11.51%) and *γ*-terpinene (5.94%), respectively. Its GC chromatogram was showed in Figure 1. Taking extraction efficiency as the aim, extraction experiments were performed at low temperature of 35˚C - 50˚C and pressures of 8 - 25 MPa. And the effects of CO_2 mass flow and extraction time on the extraction process were also investigated.

3.2. Effect of Entrainer

As the solubility of most terpenes in supercritical CO_2 fluid was too low to obtain the target product effectively, ethanol as an entrainer was introduced to improve the extraction efficiency.

As shown in Figure 2(a), the extraction yield of four main components (1,8-cineole, limonene, *p*-cymene and *γ*-terpinene) were close to zero if no entrainer was added. However, for the case of ethanol introduced, four main components were significantly detected in the extract. It had been found that ethanol could form chemical association [19] [20] with 1,8-cineole, limonene, *p*-cymene and *γ*-terpinene, respectively. The polar solvent ethanol could significantly increase the solubility of polar solute, and the extraction yield of four main components were improved obviously, especially for 1,8-cineole. It showed that in Figure 2(b), ethanol had almost no effect on the extraction contents. Compared with the extracting content, the extraction yield had an obvious improvement with entrainer existed. So, the following experiments would use ethanol as entrainer with the amount of 3% in the total CO_2 flow.

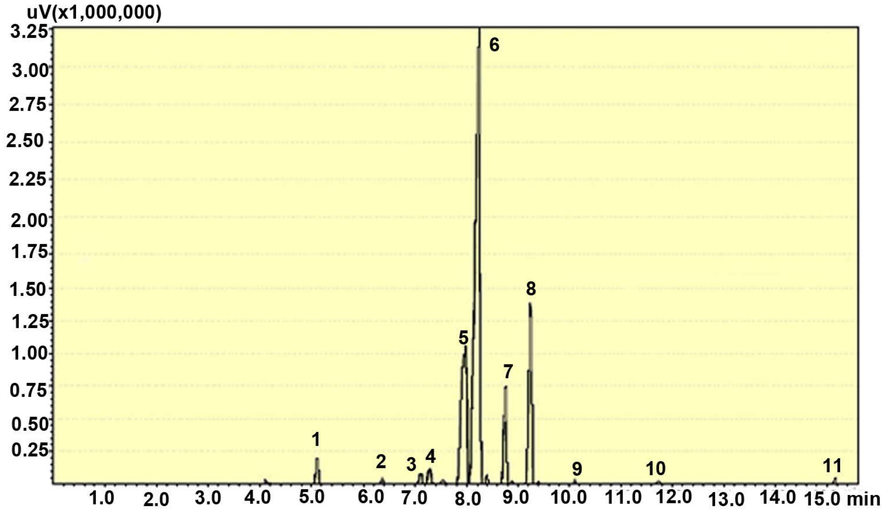

Figure 1. The GC chromatogram of crude *Eucalyptus* oil.

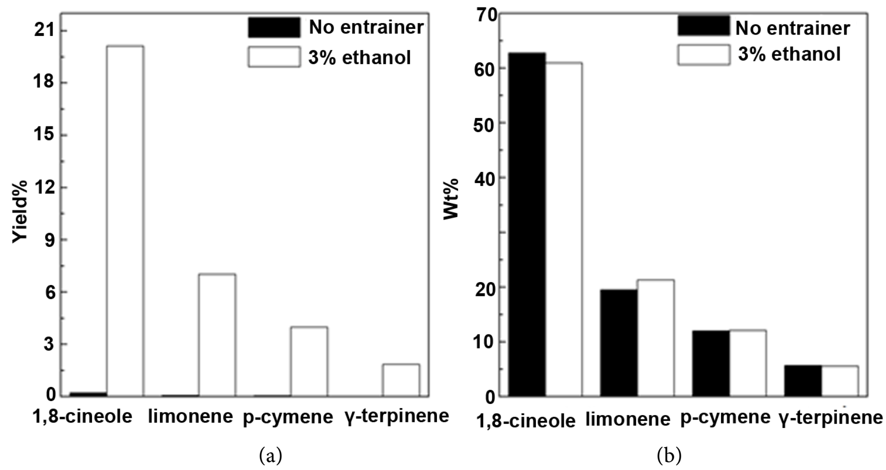

Figure 2. Effect of ethanol on extraction behavior (P = 15 MPa, T = 45˚C, q = 18 g/min, t = 1 hr).

3.3. Effect of Pressure

The effects of extraction pressure (P) on the main components of *eucalyptus* oil were shown in Figure 3. It showed that the extraction content of 1,8-cineole significantly increased with pressure going up, while the contents of limonene, *p*-cymene and *γ*-terpinene were decreasing trend. In addition to high pressure increasing dissolving capacity, the pressure might also change the polarity of the supercritical fluid. With the increase of extraction pressure, the deformability of O=C=O bond of CO_2, the polarity, the density and the solubility of polar solutes increased simultaneously, which would lead to the increase of the selectivity for polar materials. The polarity of 1,8-cineole (dielectric constant equal to 4.32) is greater than limonene (dielectric constant equal to 2.44), *p*-cymene(dielectric constant equal to 2.34) and *γ*-terpinene (dielectric constant equal to 2.65), thus the extraction content of 1,8-cineole increased with the pressure from 8 to 15 MPa. But when the extraction pressure increased further, from 15 to 25 MPa, the con-

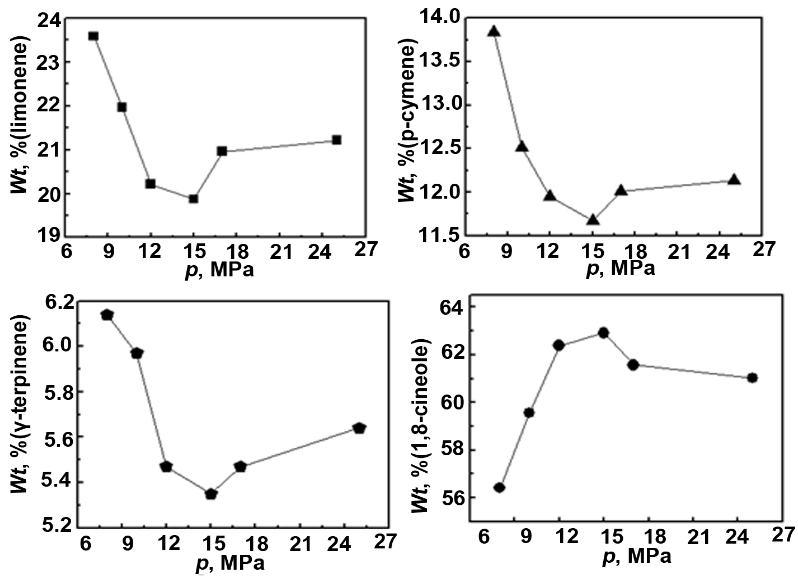

Figure 3. Pressure influence on extraction behavior (T = 45˚C, q = 15 g/min, t = 1 hr, with 3% ethanol).

tent of 1,8-cineole decreased a little, while the content of limonene, *p*-cymene and *γ*-terpinene showed slightly increasing trend. Therefore, 15 MPa was optimum for the extraction of 1, 8-cineole.

3.4. Effect of Temperature

Temperature (T) was an important parameter for supercritical CO_2 extraction process. On the one hand, with temperature increasing, the fluidity of the solute sped up in supercritical CO_2 fluid, the diffusion coefficient and the solvent vapor pressure increased. At the same time, both the volatility and solubility of the solute in CO_2 also increased, which was similar to liquid solubility [21] [22]. On the other hand, temperature increasing could also reduce the fluid density, which would decrease solvent effect and the solubility of supercritical CO_2 in oil [23]. So, the extraction temperature held a dominant role in the extraction process [24].

With temperature increasing from 35˚C to 45˚C, the mass transfer rates rose for all four main components, and the content of 1,8-cineole increased while the content of the other three components decreased as shown in **Figure 4**. The reason might be the difference in molecular structure, of which 1,8-cineole was nearly spherical, but limonene, *p*-cymene and *γ*-terpinene were all flat with double bonds, and the latter were more easily to contact and overlap with each other, which lead to strengthen intermolecular forces [25]. But when temperature changed from 45˚C to 50˚C, the content of 1,8-cineole decreased, the content of the other three components increased. Compared with the increase in the mass transfer rate, the reduction in density of supercritical CO_2 was dominant at the condition. It was also predicted that the interaction force between 1,8-cineole and supercritical CO_2 fluid dropped more than the other three compounds did.

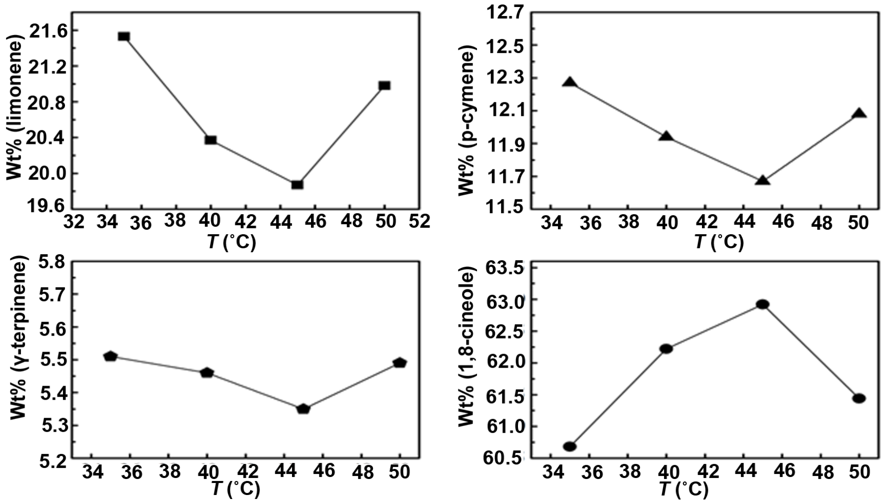

Figure 4. Temperature effect on extraction behavior (P = 15 MPa, q = 15 g/min, t = 1 hr, with 3% ethanol).

The spherical molecular structure of 1,8-cineole would make it flee from supercritical CO_2 fluid easily. Additionally, high temperature will also increase operation cost. So 45˚C was chosen as the optimum extraction temperature for 1,8-cineole.

3.5. Effect of Flow Rate of CO_2

As shown in Figure 5, when CO_2 flow rate increased from 10 to 18 g/min, the contents of limonene, *p*-cymene and *γ*-terpinene increased except 1,8-cineole. However, when CO_2 flow rate exceeded over 18 g/min, content variation trends of the four substances were reverse.

3.6. Effect of Extraction Time

Figure 6 showed the effect of time on the extraction behavior of *eucalyptus* oil. It could be found that in the initial time range of 0 - 0.5 hr, the content of 1,8-cineole increased, while both the contents of limonene and *γ*-terpinene decreased. With extraction time increasing, the percentage of the extracted material changed slightly. So prolonging extraction time could not raise the extract concentration distinctly.

With the rise of CO_2 flow rate, both the mass transfer rate and concentration difference increased, and the extraction time was shortened correspondingly [26]. The experiments under different extraction time were operated at CO_2 flow rate of 18 g/min. Different from the literature [27] the process could be divided into two stages: initial phase extraction and relatively stable extraction stage. In the first 0.5 hr the content and yield changed in a large variation. After 0.5 hr the extract contents almost had no changes and the extract yield raised gradually.

4. Conclusions

In this work, CO_2-SFE technology was used to investigate the interface extrac-

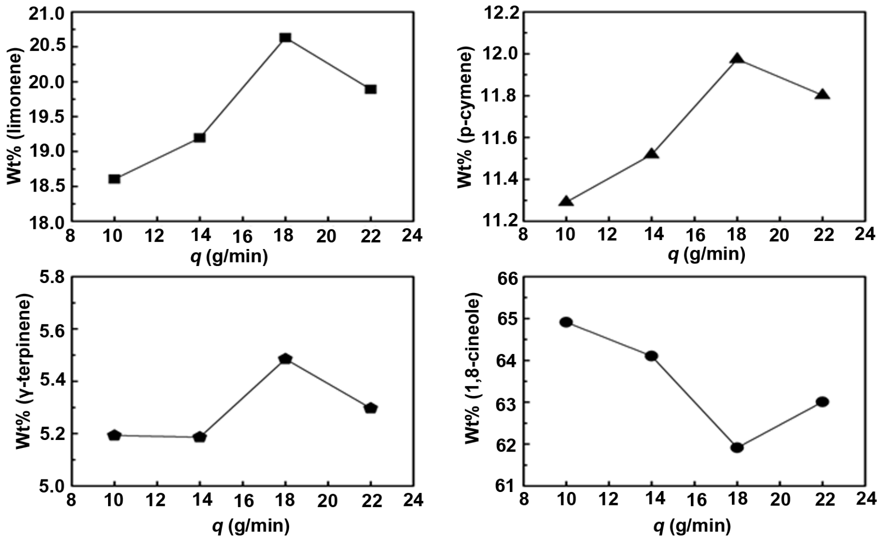

Figure 5. CO_2 flow rate effect on extraction behavior (P = 15 MPa, T = 45˚C, t = 1 hr, with 3% ethanol).

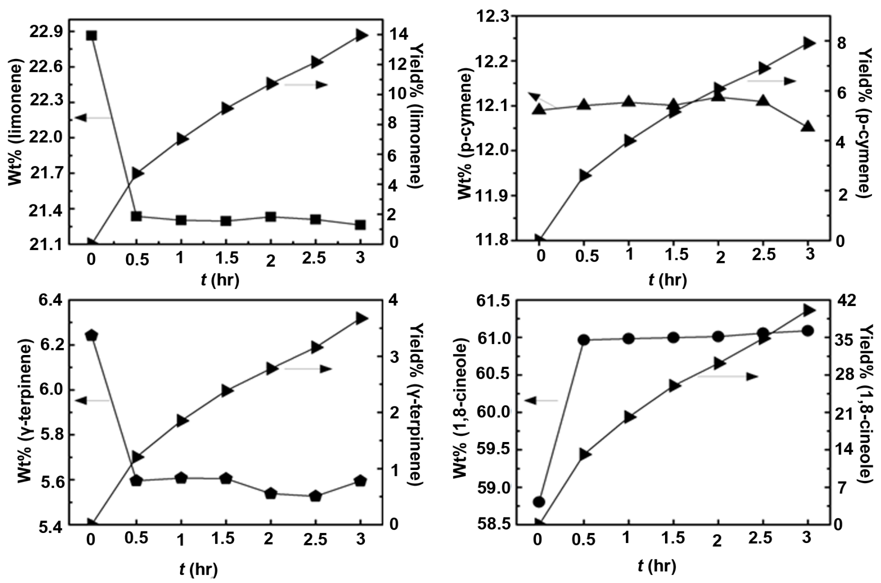

Figure 6. Time effect on extraction behavior (P = 15 MPa, T = 45˚C, CO_2 flow rate of 18 g/min, with 3% ethanol).

tion behavior of *eucalyptus* oil, and the results were summarized as follows:

Extraction pressure, temperature, CO_2 flow rate and time were the main factors for the extraction process of *eucalyptus* oil with supercritical CO_2. The effects of the above factors on 1,8-cineole, limonene, *p*-cymene and *γ*-terpinene were quite different.

Ethanol as an entrainer could significantly improve the extraction yield. For 1,8-cineole, the appropriate extraction pressure was 15 MPa and the temperature was 45˚C. Low CO_2 flow rate, not more than 10 g/min, was benefit to 1,8-cineole within the range of the experiment. Prolonging extraction time could not improve the concentration of extracts obviously, but the extract yield increased gradually.

Fund

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