Influence of Modifier in Supercritical CO₂ on Qualitative and Quantitative Extraction Results of *Eucalyptus* Ecncial Oil

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Abstract

A supercritical CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ fluid, CO₂-SFE process might be operated under mild conditions that could protect natural products from thermal decomposition [15]. CO₂-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 CO₂ was investigated under certain conditions of pressure, temperature, CO₂ 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. CO₂ 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>
<thead>
<tr>
<th>Number</th>
<th>Compound name</th>
<th>Molecular formula</th>
<th>Boiling point at 101 kPa/°C</th>
<th>Molecular weight</th>
<th>Mass fraction/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
<td>C₁₀H₁₆</td>
<td>157</td>
<td>136.23</td>
<td>1.36</td>
</tr>
<tr>
<td>2</td>
<td>β-pinene</td>
<td>C₁₀H₁₆</td>
<td>165</td>
<td>136.23</td>
<td>0.31</td>
</tr>
<tr>
<td>3</td>
<td>β-myrcene</td>
<td>C₁₀H₁₆</td>
<td>167</td>
<td>136.23</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>α-phellandrene</td>
<td>C₁₀H₁₆</td>
<td>167</td>
<td>136.23</td>
<td>1.10</td>
</tr>
<tr>
<td>5</td>
<td>Limonene</td>
<td>C₁₀H₁₆</td>
<td>178</td>
<td>136.23</td>
<td>21.78</td>
</tr>
<tr>
<td>6</td>
<td>1,8-cineole</td>
<td>C₁₀H₁₆O</td>
<td>177</td>
<td>154.24</td>
<td>56.00</td>
</tr>
<tr>
<td>7</td>
<td>γ-terpinene</td>
<td>C₁₀H₁₆</td>
<td>182</td>
<td>136.23</td>
<td>5.94</td>
</tr>
<tr>
<td>8</td>
<td>p-cymene</td>
<td>C₁₀H₁₄</td>
<td>177</td>
<td>134.22</td>
<td>11.51</td>
</tr>
<tr>
<td>9</td>
<td>L-linalool</td>
<td>C₁₀H₁₈O</td>
<td>200</td>
<td>154.24</td>
<td>0.08</td>
</tr>
<tr>
<td>10</td>
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<td>C₁₀H₁₈O</td>
<td>212</td>
<td>154.24</td>
<td>0.04</td>
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<tr>
<td>11</td>
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<td>C₁₀H₁₈O</td>
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<td>154.24</td>
<td>0.06</td>
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<tr>
<td></td>
<td>others</td>
<td></td>
<td></td>
<td></td>
<td>1.07</td>
</tr>
</tbody>
</table>
gent Scientific Ltd. at China. The maximum pressure and CO₂ 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 CO₂ in the extraction tank was about 15 cm². 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 CO₂ 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 CO₂ 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 content. 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 CO₂ flow.
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 $\gamma$-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 $\text{O} = \text{C} = \text{O}$ bond of $\text{CO}_2$, 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 $\gamma$-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-
tent of 1,8-cineole decreased a little, while the content of limonene, $p$-cymene and $\gamma$-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 $\gamma$-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.
The spherical molecular structure of 1,8-cineole would make it flee from supercritical CO2 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 CO2

As shown in Figure 5, when CO2 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 CO2 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 CO2 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 CO2 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, CO2-SFE technology was used to investigate the interface extrac-
Extraction behavior of eucalyptus oil, and the results were summarized as follows:

Extraction pressure, temperature, CO₂ flow rate and time were the main factors for the extraction process of eucalyptus oil with supercritical CO₂. 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 CO₂ 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.

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**References**


