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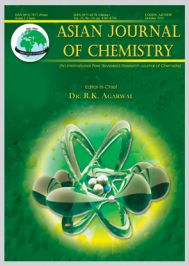

Ultrasound-Enhanced Supercritical CO_2 Extraction of Masson Pine Wood Essential Oil

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Ultrasound-enhanced supercritical CO_2 was used to extract the essential oil of masson pine. The extraction technology was optimized through orthogonal experiments. The results showed that a good outcome can be achieved with an extraction pressure of 18 MPa, a temperature of 60 ºC, a time of 90 min, a CO_2 flow rate of 12 kg h^{-1}, an ultrasonic power of 250 W and an ultrasonic frequency of 22 KHz. Using these parameters, the average yield of essential oil was 88.12 %. GC-MS analysis showed that the ingredient with the highest content of 44.42 % was longifolene in the essential oil of masson pine wood, followed by α-pinene and caryophyllene, with contents of 28.17 and 6.03 %, respectively.

Key Words: Masson pine wood essential oil, Supercritical CO_2 extraction, Ultrasound-enhanced.

INTRODUCTION

Masson pine (*Pinus massoniana* Lamb) is a Pinaceae evergreen tree that is widely distributed in southern China. The main volatile substance in extracts of masson pine wood is essential oil, which has broad applications and is an important raw material for the food, pharmaceutical and chemical industries.

At present, the commonly used extraction methods for essential oil include solvent extraction, steam distillation and simultaneous distillation/extraction[1,2]. However, these methods display some disadvantages, such as high energy consumption and low yield. In contrast, supercritical CO_2 extraction has multiple unique advantages[3,4], *i.e.*, high yield, low temperature, easy control, capability to retain the active ingredients of biamoss material and no enrichment process. It is particularly appropriate for the refinement and separation of unstable products and active substances. During extraction, ultrasound technology can play a strengthening effect on supercritical fluids[5]. Many reports described the ultrasound-enhanced supercritical fluid extraction (USFW), but extraction of the masson pine wood essential oil using ultrasound-enhanced supercritical fluid extraction has not been reported yet.

In this study, ultrasound-enhanced supercritical fluid extraction was used to extract the masson pine wood essential oil and the impact of temperature, pressure, time, CO_2 flow rate, ultrasonic power, ultrasonic frequency *etc.* was investigated. Orthogonal analysis was used to optimize the extraction technology to obtain the best results.

EXPERIMENTAL

The materials and equipment used in this study include the following: sanding masson pine wood powder taken from a furniture factory; CO_2 gas from industrial products (purity ≥ 99.0 %); 1 L (liter) USFE device customized to generate pressure of up to 30 MPa (China); gas chromatography (Shimadzu, GC-7A) and gas chromatography-mass spectrometer (GC-MS; Shimadzu, GC-MS-QP20l0).

Method: Completely dry pine powder with 40 grit was weighed and put into the extractor and the supercritical extraction system was started with the appropriate settings for temperature, pressure and fluid flow. When the supercritical fluid extraction system became stable, ultrasound was set to working power, frequency and time. After extraction, the whole apparatus was gradually stopped and the essential oil was removed from the tube. The yield was calculated according to formula 1.

$$\text{Essential oil yield} = \frac{\text{Actual amount of essential oil}}{\text{Theoretical amount of essential oil}} \times 100\ \% \quad (1)$$

GC-MS analysis: For chromatography, SE-30 silica capillary column (0.25 mm × 0.52 μm × 50 m) was used and the temperature was increased as programmed. Column

temperature was kept at 70 ºC for 4 min, then increased to 230 ºC by 4 °C min^{-1} and finally kept for 8 min. The sample injector temperature was 250 ºC, with high-purity helium as carrier gas, column flow of 1 mL min^{-1}, split ratio at 50:1 and injection of 0.6 µL.

Gas chromatography-mass spectrometry used OV-101 fused silica capillary column (0.25 mm × 0.25 µm × 30 m) and the injector temperature was 250 ºC. At working condition, the column temperature was kept at 70 ºC for 4 min, then increased to 250 ºC by 4 ºC min^{-1} and finally kept for 10 min. Constant linear speed was 26.9 cm s^{-1} and the column flow was set to 1.0 mL min^{-1}, split ratio at 2:1 and injection at 0.2 µL.

RESULTS AND DISCUSSION

Single factor test: According to the literature[6], the experimental results are affected by extraction temperature, pressure, time, CO_2 flow rate, ultrasonic power and frequency. Single factor test was performed based on the preliminary experiments.

Impact of extraction pressure: Extraction pressure significantly affects the yield and is influenced by both extraction efficiency and the choice of extraction conditions[7]. At constant extraction temperature, elevated extraction pressure increases supercritical CO_2 fluid density. This results in augmented solubility and higher yield, but they are not in linear relationship with each other.

When the pressure reaches a certain level, the solubility growth of CO_2 becomes slow. At an extraction temperature of 50 ºC, an extraction time of 80 min, CO_2 flow rate of 5 kg h^{-1} and ultrasonic power of 250 W, the impact of extraction pressure and ultrasonic frequency on the yield is shown in Fig. 1. With increasing extraction pressure, the yield of the essential oil is elevated. In the presence of ultrasound and over 18 MPa of pressure, the yield growth rate declines with increasing pressure. However, in the absence of ultrasound, the yield improves with increasing pressure, but it is lower than the ultrasound sample. Considering the safety and cost, the appropriate extraction pressure is 15-21 MPa.

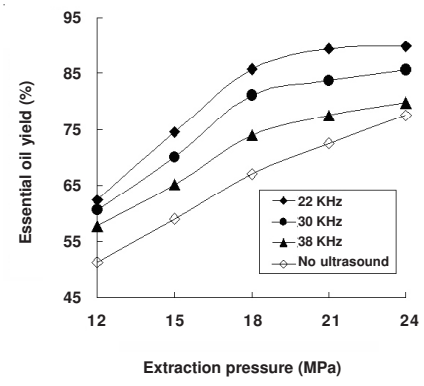

Fig. 1. Effect of extraction pressure on essential oil yields

Impact of extraction temperature: Temperature is an important factor in supercritical fluid extraction. Under certain pressure, increasing the pressure enlarges the molecular distance between extractant CO_2 and decreases the intermolecular force and the supercritical fluid density. Thus, this causes the corresponding decline in solubility and extraction yield. At the same time, under certain pressure, rising temperature augments the volatility of extracted solute and the thermal motion of molecules increases the opportunity for molecular association that can improve the yield[8]. Therefore, the yield depends on the dominant state of extraction system. At an extraction pressure of 18 MPa, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1} and ultrasonic power of 250 W, the impact of temperature and ultrasonic frequency on yield of masson pine wood essential oil is shown in Fig. 2. In the ultrasonic field, the yield of essential oil rises with increasing extraction temperature; however, under the same conditions, higher yield is achieved at 22 KHz ultrasound.

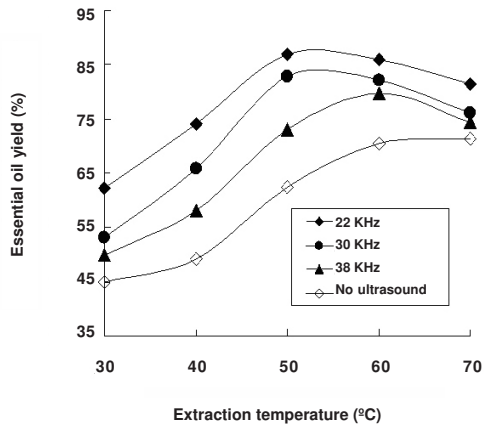

Fig. 2. Effect of extraction temperature on essential oil yields

Impact of extraction time: The impact of extraction time on the yield is shown in Fig. 3. At an extraction pressure of 18 MPa, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1} and ultrasonic power of 250 W, the yield rapidly increases when the extraction time also increases, but is less than 80 min. After 80 min of extraction time, the yield grows slowly as time is increased. The yield curve almost becomes stable with further increasing time. This is because at the beginning of extraction, the supercritical CO_2 is not in close contact with the solute and the yield is relatively low. When the extraction time is increased, the mass transfer achieves a good status and the extraction amount is elevated in unit time. However, with extended extraction time, the amount of solute that can be dissolved by CO_2 fluid gets saturated and the yield will not increase.

Impact of CO_2 flow: The impact of CO_2 flow on essential oil yield depends on two factors. When the CO_2 flow increases, the residence time for fluid in the extraction tank decreases and the fluid and solute contact time is reduced. This means that the CO_2-extract contact time declines and is not conducive for extraction. However, enhancing the CO_2 flow increases the collision probability of the solute and the supercritical CO_2 molecule, thereby promoting the mass transfer[9], which is conducive for improving extraction rate and yield. As shown in Fig. 4, at an extraction pressure of 18 MPa, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1} and ultrasonic power of 250 W, increasing CO_2 flow causes the yield of the essential oil to improve. When the CO_2 flow exceeds 12 kg h^{-1}, the yield growth is slowed as CO_2 flow is increased. However, at very high flow rates, energy consumption is elevated. Considering

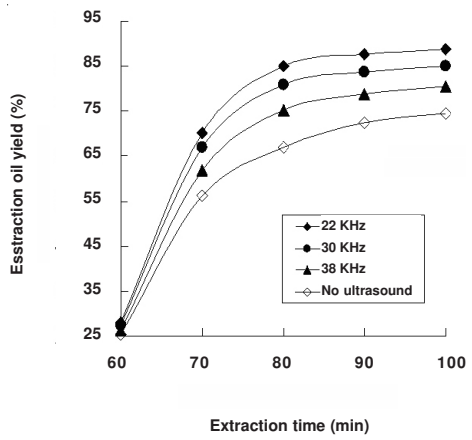

Fig. 3. Effect of extraction time on essential oil yields

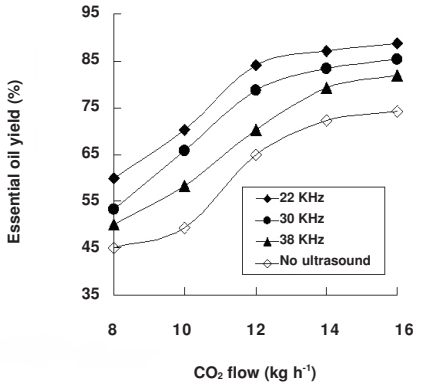

Fig. 4. Effect of extraction CO_2 flow on essential oil yields

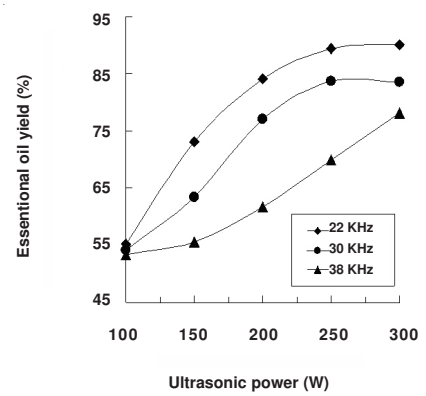

Fig. 5. Effect of ultrasonic power on essential of yields

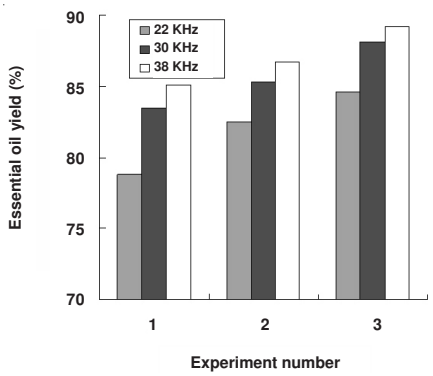

Fig. 6. Effect of ultrasonic frequency on essential oil yields

comprehensive factors in these experimental conditions, the CO_2 flow rate is set to 12 kg h^{-1}.

Impact of ultrasonic power: Ultrasound has multi-level physical effects, such as cavitation, mechanical vibration, micro jets and micro-acoustic streaming. It can promote the breaking or deformation of plant tissue[10], thereby enhancing the yield of the essential oil. However, when the ultrasonic power increases to a certain level, the formation of cavitation bubbles will be unprecedentedly high, which strengthens the energy dissipation between cavitation bubbles and leads to its insufficient collapse, causing reduced energy transfer efficiency and essential oil yield. At an extraction pressure of 18 MPa, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1}, the correlation of ultrasonic power and frequency with the yield of essential oil is shown in Fig. 5. The yield growth is slowed at 250 W of ultrasonic power and the maximum ultrasound-enhanced yield is detected at 22 KHz frequency.

Impact of ultrasonic frequency: At an extraction pressure of 18 MPa, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1} and ultrasonic power of 250 W, the correlation of ultrasonic power with the yield of essential oil is shown in Fig. 6. The yield is maximum at 22 KHz frequency, minimum at 38 KHz frequency and in the middle at 30 KHz frequency. The reason is that the large amplitude of low frequency makes solid materials and fluid uniformly mixed, leading to a higher yield. However, the frequency cannot be too low because low frequency has high energy consumption and requires a lot from the equipment.

Orthogonal analysis: To optimize the extraction technology, L_{16} (4^5) multi-factor orthogonal analysis is used to test the five important factors (extraction pressure, ultrasonic frequency, ultrasonic power, extraction time and extraction temperature) based on the single factor experiment. The orthogonal experimental design is shown in Table-1.

As shown in the orthogonal results in Table-1, the order of influence on essential oil yield is A > B > C > E > D and the optimal combination of technology is $A_2B_4C_4E_4D_4$. Under these optimal conditions, three experiments are performed and the average essential oil yield is 88.12 %. Using the same conditions, the yields of steam distillation and simultaneous distillation/extraction approaches are only 50.16 and 58.87 %, respectively. By comparison, USFE process improves the yield by 37.96 and 29.25 %, respectively.

Ingredient analysis of essential oil: The essential oil of the masson pine wood extracted under the conditions mentioned above is analyzed by GC-MS analysis and the chromatogram is shown in Fig. 7. The highest contents are 1, 11 and 12.

The results are compared with the NIST standard spectral database and 16 major ingredients are identified. As shown in Table-2, the highest content (44.42 %) is longifolene, which corresponds to peak 11; following this are α-pinene (28.17 %) and caryophyllene (6.03 %), which correspond to peak 1 and 12, respectively.

Conclusion

Masson pine essential oil was extracted by ultrasound-enhanced supercritical CO_2. Extraction pressure, ultrasonic frequency, ultrasonic power, extraction temperature and

TABLE-1
ORTHOGONAL EXPERIMENTAL RESULTS OF THE ESSENTIAL OIL EXTRACTION BY USFE

Number	Extraction pressure A (MPa)	Ultrasonic frequency B (KHz)	Ultrasonic power C (W)	Extraction time D (min)	Extraction temperature E (°C)	Yield (%)
1	1(15)	1 (No ultrasonic)	1(100)	1 (60)	1 (30)	51.22
2	1	2(38)	2 (150)	2 (70)	2 (40)	69.57
3	1	3(30)	3 (200)	3 (80)	3 (50)	74.26
4	1	4(22)	4 (250)	4 (90)	4 (60)	85.62
5	2(18)	1	2	3	4	76.83
6	2	2	1	4	3	82.02
7	2	3	4	1	2	83.11
8	2	4	3	2	1	85.85
9	3(21)	1	3	4	2	69.64
10	3	2	4	3	1	81.97
11	3	3	1	2	4	82.08
12	3	4	2	1	3	81.71
13	4(24)	1	4	2	3	83.16
14	4	2	3	1	4	86.43
15	4	3	2	4	1	86.60
16	4	4	1	3	2	87.88
K_{1i}	70.17	65.21	70.80	70.62	51.41	–
K_{2i}	81.95	75.00	73.68	75.17	52.55	–
K_{31i}	78.85	76.51	74.05	75.24	55.29	–
K_{4i}	66.02	80.27	78.47	75.97	57.74	–
R_i	15.93	15.06	7.67	5.35	6.33	–

TABLE-2
THE CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM MASSON PINE

Number	Compound name	m.f.	Content (%)	Number	Compound name	m.f.	Content (%)
1	α-Pinene	$C_{10}H_{16}$	28.17	9	α-Germacrene	$C_{15}H_{24}$	0.64
2	Camphene	$C_{10}H_{16}$	0.61	10	Sativene	$C_{15}H_{24}$	1.23
3	β-Pinene	$C_{10}H_{16}$	1.82	11	Longifolene	$C_{15}H_{24}$	44.42
4	β-Mycrene	$C_{10}H_{16}$	0.60	12	Caryophyllene	$C_{15}H_{24}$	6.03
5	D-Limonene	$C_{10}H_{16}$	1.81	13	Thujopsene	$C_{15}H_{24}$	1.28
6	α-Terpineol	$C_{10}H_{16}O$	0.13	14	Caryophyllene oxide	$C_{15}H_{24}O$	1.38
7	Terpineol acetate	$C_{12}H_{20}O_2$	0.45	15	Pulegol	$C_{15}H_{26}O$	0.45
8	α-Longipinene	$C_{15}H_{24}$	1.93	16	Bisabolene epoxide	$C_{15}H_{24}O$	0.66

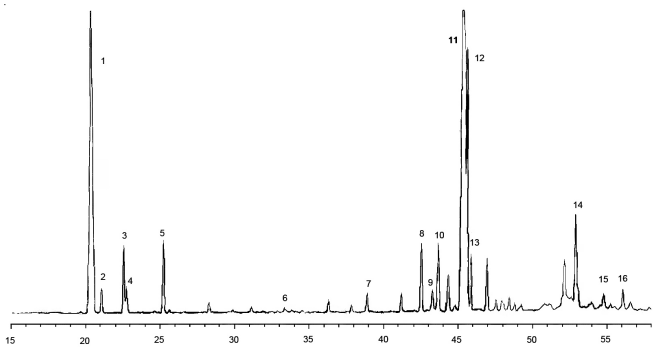

Fig. 7. Chromatogram of the essential oil from masson pine

extraction time showed a large impact on the yield of essential oil. The optimal condition was found at an extraction pressure of 18 MPa, extraction temperature of 50 ºC, extraction time of 80 min, CO_2 flow rate of 12 kg h^{-1}, ultrasonic power of 250 W and ultrasonic frequency of 22 KHz. Using these parameters, the average yield of essential oil was 88.12 %. The yield had increased by 37.96 and 29.25 %, respectively, compared with the steam distillation and simultaneous distillation/extraction. GC-MS analysis of masson pine wood essential oil identified 16 major ingredients, in which longifolene (with a content of 44.42 %) was the most abundant one, followed by α-pinene and caryophyllene (with contents of 28.17 and 6.03 %, respectively).

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