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Ultrasonics Sonochemistry 14 (2007) 219–224

Ultrasonics SONOCHEMISTRY

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Ultrasound assisted supercritical fluid extraction of oil and coixenolide from adlay seed

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Received 21 September 2005; received in revised form 23 February 2006; accepted 7 March 2006
Available online 4 May 2006

Abstract

Oil and coixenolide are important components of adlay seed (*Coix lachrymal-jobi L. var. Adlay*) with many beneficial functions to human health. In this work, a novel extraction technique—ultrasound assisted supercritical fluid extraction (USFE)—was studied. Effects of operating conditions on the extraction, including extraction temperature (T), pressure (P), time (t), CO_2 flow rate (F) and ultrasonic power (I) were investigated. There are optimum temperatures which gives the maximum extraction yields (EYs) for the supercritical fluid extractions with and without ultrasound. The effect of pressure on EYs for is similar to that of pressure on CO_2 density. Based on the yield of extraction, the favorable conditions for supercritical fluid extraction (SFE) were: T at 45 °C, P at 25 MPa, t at 4.0 h and F at 3.5 L/h. While ultrasound was applied as in USFE, the following parameters were preferred: T at 40 °C, P at 20 MPa, t at 3.5 h and F at 3.0 L/h, respectively. The results show that supercritical fluid extraction with the assistance of ultrasound could reduce the temperature, pressure, CO_2 flow rate, as well as time used in the process. Compared with SFE, USFE could give a 14% increase in the yield for extracting oil and coixenolide from adlay seed with less severe operating conditions.

Keywords: Supercritical fluid extraction; Ultrasound; Oil; Coixenolide; Adlay seed

1. Introduction

Wild *coix lachrymal-jobi L.* (Coix) is native to and extensively grown in South Asia [1]. The cultivated variety, *coix lachrymal-jobi L. var. adlay* (Adlay), is a soft-shelled seed crop cultivated in countries such as India [1], Brazil [2], Japan [3] and China [4]. Adlay has long been used as animal feed, as food for humans and in herbal medicine. Adlay oil and coixenolide are important components of adlay seed with many health benefits. Adlay oil can inhibit the growth of cancer cells with an efficiency of above 87%, and help prevent the decrease of white blood cells during chemical therapy [5]. Coixenolide has a stimulatory effect on lung, heart, striated muscle and smooth muscles with low dosage, but an inhibitory effect with high dosage. It can dilate the pulmonary veins and improve the blood circulation of the lung [6]. In addition, coixenolide has the effect of reducing inflammation, purulence and pain as well as being anti-tumor.

Adlay oil and coixenolide are normally obtained using mechanical or chemical processes. Mechanical processes often associate with low yields, while chemical extraction methods often involve the use of organic solvents which can be harmful to human health and environment [7]. Tough new regulatory requirements on the use of organic solvents have prompted active research on clean extraction technologies [8]. Supercritical fluid extraction is one of the

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doi:10.1016/j.ultsonch.2006.03.005

newly emerging clean and environmentally friendly technologies for food and pharmaceutical products [9].

Among supercritical fluids, CO_2 is the most commonly used solvent for the extraction of oils from natural products. However, the efficiency of supercritical fluid extraction (SFE) is hindered by the low solubility of the triglycerides in CO_2, and the high pressures and long extraction time required [8].

Recently, the application of ultrasound techniques in solvent extraction has attracted more attention. Sonication has been used successfully to isolate pharmaceutically active components from *Salvia officinals*, and increase the yield of xylems from corn hulls [10] and corn cobs [11]. The solvent extraction of active components from plants and seeds was found to be significantly improved by the introduction of ultrasound waves, and extensive researches on ultrasound assisted solvent extraction have been conducted. However, the application of ultrasound in supercritical fluid extraction is still in the developing era. More recently the use of power ultrasound in SFE to enhance the extraction yield of oil from almonds by 20% was reported [12].

The main objective of this work is to extract adlay oil and coixenolide using the ultrasound assisted supercritical fluid extraction (USFE). The results obtained from USFE will be compared with that obtained from the SFE to reveal the effects of ultrasound under supercritical conditions.

2. Materials and methods

2.1. Materials

Adlay seeds were bought from the local traditional Chinese medicine shop. The oil content of the adlay seed, as determined by extraction with acetone, was found to be 9.5%. CO_2 of 99.9% purity was obtained from Guangzhou Gas Company, China. All chemicals were purchased from local chemical stores and at analytical grades.

2.2. USFE system

As showed in Fig. 1, the USFE system is made up of two units: SFE unit and ultrasonic unit. The USFE extractor is designed to withstand pressures up to 32 MPa and temperatures up to 85 °C. The major components of SFE include a positive displacement liquid pump, a 1000 ml pressurized extraction vessel, a separation column and a separation vessel. The extraction vessel and two separators are equipped with water jackets and temperature controllers. In order to ensure accurate and stable supercritical CO_2 delivery, the pump head is cooled by circulating water. The temperatures in the system are controlled within ±0.1 °C and the pressures within ±0.5 MPa.

The probe with Langevin type transducer is installed in the upper part of the extractor, and driven by electrical signals from an ultrasound generator, which gives adjustable continuous power outputs at fixed frequency of 20 kHz. The transducer can stand for a maximum temperature of 105 °C. The ultrasound generator consists of a power amplifier and a special electro circuit designed to justify the power outputs at a constant level during the USFE process. The ultrasonic power outputs were set at 0, 50, 91, 97 and 110 W in this study, respectively, and kept at a constant level with minor fluctuation caused by minor oscillating static pressure and CO_2 flow rate when supercritical fluid was pumped into the extractor during the process. The electroacoustical efficiency of the ultrasonic transducer was 87%.

2.3. Experimental methods

In order to fully explore the effects of USFE, experiments were carried out at various extraction temperatures (30–55 °C), pressures (10–30 MPa), times (up to 4.5 h) and CO_2 flow rates (1.5–4 L/h). 100 g of grounded adlay seed of 0.3–0.45 mm in diameter was placed into the extractor in a typical extraction experiment. Liquid CO_2 was pumped into the extractor until the desired extraction pressure was reached. The extractor was heated to the extraction temperature, and pressure valves located downstream of the extractor were slowly opened while maintaining the pressure constant in the extractor.

2.4. Calculation of adlay oil extraction yield

The adlay oil, which precipitated under a low pressure and temperature in the separation vessel, was recovered. The adlay oil extraction yield (EY) is calculated using the following formula:

$$\mathrm{EY} = \frac{M_t}{M_0 X_0} \times 100\% \tag{1}$$

where M_t is the mass of extracted adlay oil (mg), M_0 the initial mass of adlay seed put into the extractor, X_0 the initial adlay oil content of raw adlay seed (mg/mg).

2.5. Measure of the coixenolide

1.3 g extracted adlay seed oil was placed into a 250 ml conical flask with 25 ml solution of potassium hydroxide in ethanol (0.5 mol/L), and refluxed for 30 min. After cooling, 10 ml ethanol was used to wash the wall of the condenser and the under-stopper. 1.0 ml phenolphthalein was added into the solution. To measure the remaining potassium hydroxide, hydrochloric acid (0.5 mol/L) was gradually added until the solution just turn from red to colorless. A blank was prepared in the same way without adlay oil. The coixenolide content is found by using the following equation:

$$\text{Coixenolide}(\%) = [(B - A) \times G_1 \times 10^3 / G_0] \times 100\%$$

where A is the volume of hydrochloric acid consumed by the sample (ml), B the volume of hydrochloric acid consumed by the blank (ml), G_1 the quantity of coixenolide re-

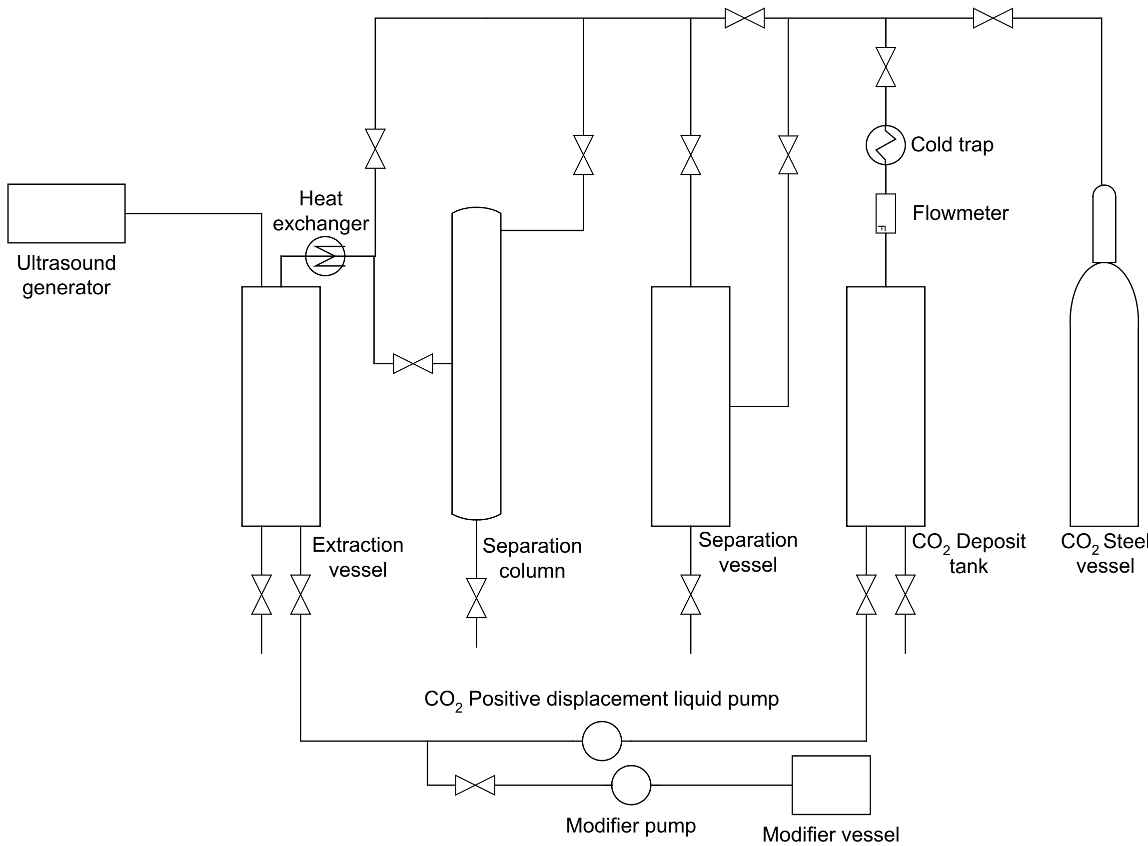

Fig. 1. USFE system.

acted with 1 ml of potassium hydroxide in ethanol solution (mg/ml), and G_0 the quantity of sample (g) [7].

3. Results and discussion

3.1. Effect of extraction temperature

The experimental conditions in the study of temperature effect for both USFE and SFE were: extraction pressure at 20 MPa, time at 3.5 h and CO_2 flow rate at 2.5 L/h. For USFE, ultrasound of 20 kHz and 110.5 W was used. When ultrasound was applied, the local heating effect may occur due to multiplication of high intensity ultrasonic waves. If the flow rate of supercritical CO_2 was too slow to carry the heat away timely, clusters of burned materials might be produced as a result of irregular outputs of ultrasound focused on a particular place within the extractor. Thus the flow rate of CO_2 in all USFE trials should be 1.5 L/h or higher. SFE without ultrasound was also conducted for comparison. All experiments were carried out in triplicate with reproducibility within ±5%.

Fig. 2 shows that the effect of temperature on the EY of both adlay oil and coixenolide are quite different for USFE and SFE. Each data point in the figure (and other figures afterward) is the mean from three experimental runs and vertical bars are the standard errors. The EY obtained by USFE is much higher than that obtained by SFE under the same conditions. This result can also be interpreted as that a much lower temperature is needed for USFE to achieve the same EY as from SFE.

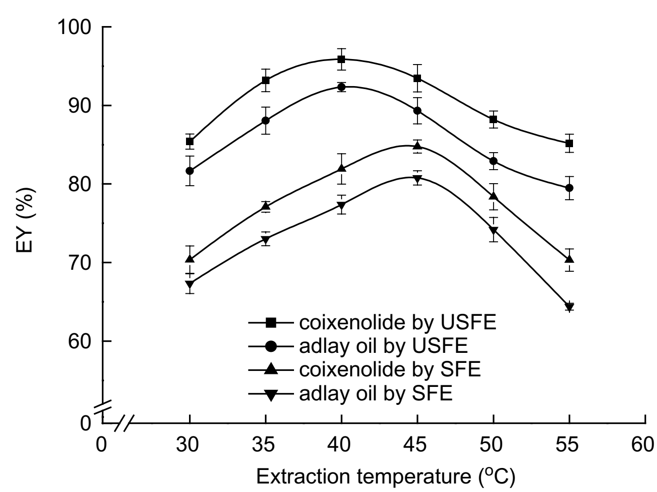

Fig. 2. Effect of extraction temperature on the yield of adlay oil and coixenolide: (●) adlay oil by USFE, (■) coixenolide by USFE, (▼) adlay oil by SFE, (▲) coixenolide by SFE. Each data point is the mean of three experimental runs and vertical bars are the standard errors (the same for all figures afterward). Other experimental parameters fixed at: P 20 MPa, t 3.5 h, F 2.5 L/h, and ultrasound 110.5 W at 20 kHz.

Also in Fig. 2, both EYs increase first when temperature changes from low (33 °C) to moderate (40 °C for USFE and 45 °C for SFE), and goes down when temperature is too high (45 °C for USFE and 50 °C for SFE). There is an optimum temperature at which maximum EY is obtained. This optimum temperature is 45 °C for SFE and 40 °C for USFE. Extraction temperature affects the extraction process by two aspects: solute diffusivity and solute solubility. When the temperature rises, solute diffusivity increases, thus the EY improves. However, the effect of solute solubility caused by temperature changes is complicated: when temperature rises, CO_2 density decreases, resulting in a reduction in its solubility; on the other hand, the vapor pressure of solute also increases with temperature, resulting in an increase of its solubility. Overall, each solute–solvent system has then a specific apparent behavior versus temperature. The optimum points for USFE and SFE reflect the combined effects of temperature on the diffusivity and solubility of solute. Operations at temperatures lower or higher than these optimum values would result in lower EYs. This result also indicates that ultrasound can increase the overall mass diffusion within the supercritical system.

3.2. Effect of extraction pressure

The experimental conditions in the study of pressure effect were the same as those pre-mentioned except that the extraction pressure was taking as variable and temperature was fixed at 45 °C for SFE and 40 °C for USFE.

As showed in Fig. 3, at pressures between 10 and 20 MPa, EY for both adlay oil and coixenolide clearly increased with the rise of extraction pressure, and the increase was diminished when pressure was higher than 20 MPa for USFE and 25 MPa for SFE. This phenomenon indicates that the solubility of the solute is closely related to the density of supercritical fluid, which exhibits very similar behavior with pressure at temperature range of 40–50 °C. Thus further increase in pressure would not result in remarkable influence on EYs. In our study, the preferred pressure for USFE is 20 MPa, at which the EYs for coixenolide and adlay oil were about 93%. In contrast, the preferred pressure for SFE is 25 MPa, at which the EYs for both components were about 81%. The results showed in Fig. 3 do not only reiterate the findings that ultrasound can increase the mass diffusion in supercritical extraction, but also indicate that ultrasonic effect is more effective at relative less severe pressure conditions. The results could also be interpreted as that it is possible to use lower extraction pressure in USFE to obtain the same EY as in SFE.

3.3. Effect of extraction time

The experimental conditions for the effect of time were: temperature at 40 °C and pressure at 20 MPa for USFE, and temperature at 45 °C and pressure at 25 MPa for SFE. The CO_2 flow rate for both experiments was fixed at 2.5 L/h. Ultrasound of 20 kHz and 110.5 W was used for USFE. The results as showed in Fig. 4 indicate that for USFE time of 3.5 h is enough to obtain the maximum EYs of adlay oil and coixenolide, while 4.0 h is needed for SFE to reach the maximum EYs. Also the EYs obtained by USFE are higher than that obtained by SFE for most time settings, indicating that the positive effect of ultrasound on mass diffusion is an instant phenomenon.

3.4. Effect of CO_2 flow rate

Fig. 5 shows the results obtained from CO_2 flow rate ranged from 1.5 to 4 L/h with other parameters fixed at previously found optimums, which were: temperature at 40 °C,

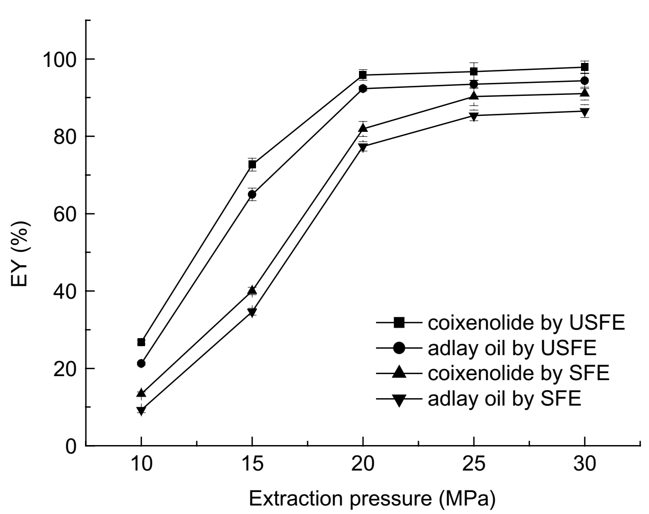

Fig. 3. Effect of extraction pressure on the yield of adlay oil and coixenolide: (●) adlay oil by USFE, (■) coixenolide by USFE, (▼) adlay oil by SFE, (▲) coixenolide by SFE. *T* at 45 °C for USFE and at 40 °C for SFE; other parameters: *t* 3.5 h, *F* 2.5 L/h, and ultrasound 110.5 W at 20 kHz.

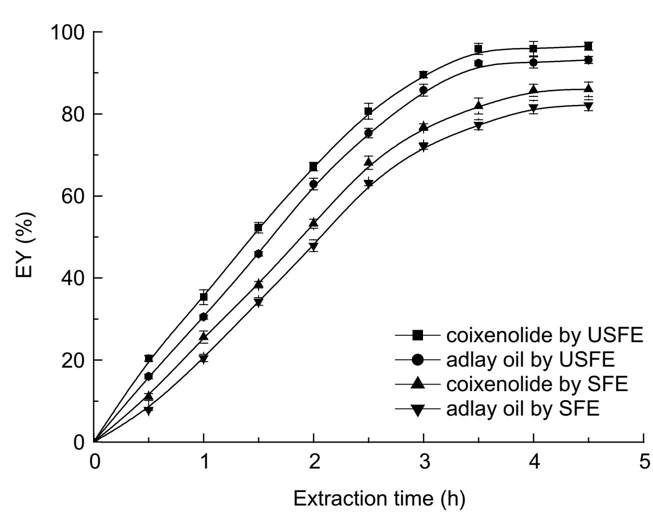

Fig. 4. Effect of extraction time on the yield of adlay oil and coixenolide: (●) adlay oil by USFE (■) coixenolide by USFE, (▼) adlay oil by SFE, (▲) coixenolide by SFE. *T* and *P* were set at 40 °C and 20 MPa for USFE, and at 45 °C and 25 MPa for SFE, respectively; other parameters: *t* 3.5 h, *F* 2.5 L/h and ultrasound 110.5 W at 20 kHz.

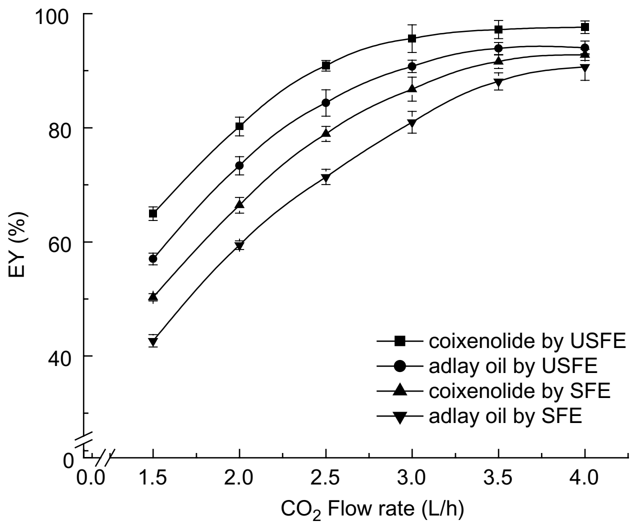

Fig. 5. Effect of CO_2 flow rate on the yield of adlay oil and coixenolide: (●) adlay oil by USFE, (■) coixenolide by USFE, (▼) adlay oil by SFE, (▲) coixenolide by SFE. *T* 40 °C, *P* 20 MPa, and *t* 3.5 h for USFE, and *T* 45 °C, *P* 25 MPa and *t* 4.0 h for SFE, respectively. Ultrasound of 20 kHz at 110.5 W was used for USFE.

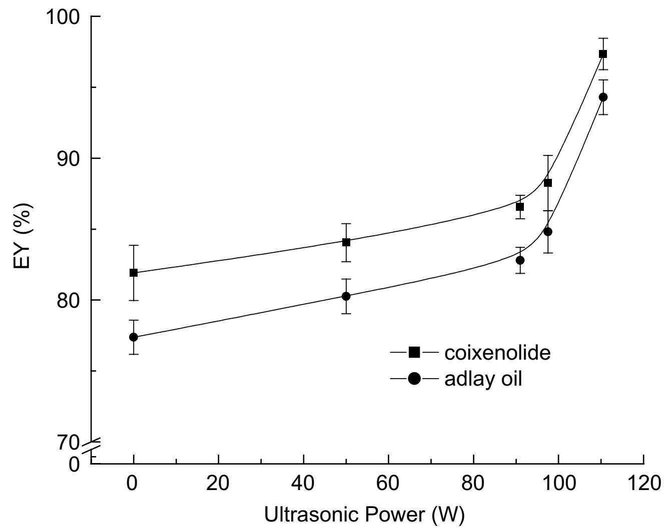

Fig. 6. Effect of ultrasonic power on the yield of adlay oil and coixenolide: (▼) adlay oil, (▲) coixenolide. *T* 40 °C, *P* 20 MPa, *t* 3.5 h, *F* 3.5 L/h and ultrasound of 20 kHz.

pressure at 20 MPa and time at 3.5 h for USFE; and temperature at 45 °C, pressure at 25 MPa and time at 4.0 h for SFE. Ultrasound of 20 kHz and 110.5 W was used. The results indicated that the effect of CO_2 flow rate is similar for both USFE and SFE. The increase in EY is more pronounced as the CO_2 flow rate increased from 1.5 to 3.0 L/h. Such increases are flattened for further flow rate increase from 3.5 to 4 L/h, for both processes. The EY obtained by USFE is higher than that obtained by SFE, indicating that with ultrasound a lower CO_2 flow rate is needed than that without it. It is to say, when ultrasound is applied, the CO_2 flow rate for USFE can be reduced in order to get the same EY as that of SFE. Based on above, the suitable CO_2 flow rate is 3.0 L/h for USFE and 3.5 L/h for SFE.

3.5. Effect of ultrasonic power

The effects of ultrasound power on the EYs of adlay oil and coixenolide are presented in Fig. 6 with other parameters fixed at previously found optimums (temperature at 40 °C, pressure at 20 MPa, time at 3.5 h and CO_2 flow rate at 3 L/h). The effect of ultrasound on extraction is due to the vibration occurred in the interfaces between solvent and solid matrix caused by ultrasound wave. For a given medium and a fixed radiation area, the vibration is proportional to the ultrasound power. The higher the ultrasound power is, the stronger the vibration to be. So the EY will increase.

Under supercritical conditions, the static pressure on the tip of ultrasound probe is usually very high. In such a system, part of ultrasound power is dissipated for overcoming the static pressure. Only when the ultrasound power was high enough, the turbulent vibration, one of the well known ultrasound phenomenon, would then be envisioned. Our result shows that both EYs of adlay oil and coixenolide increased in a gentle path when ultrasound power changed from 0 W to 90 W, indicating that this range of ultrasonic power was small regarding to the given system, thus turbulent vibration did not occur. When the ultrasound power was increased from 90 W to 110 W, the increases in EY for both components became steep, indicating that apparent sonication took effect at this ultrasound power range (at around 100 W) regarding to the given static pressure surroundings (at 20 MPa).

3.6. Comparison of SFE and USEF under the optimum conditions

Effects of operation conditions such as extraction temperature, extraction pressure, time and CO_2 flow rate with

Table 1
Comparison of SFE and USFE under their own optimum conditions

Target compounds	Extraction method	*T* (°C)	*P* (MPa)	*t* (h)	*F* (L/h)	*I* (W)	EY (%)[a] (S.D.)
Adlay oil	SFE	45	25	4	3.5	0	84.95 (1.22)
Adlay oil	USFE	40	20	3.5	3	110.5	96.36 (0.09)
Coixenolide	SFE	45	25	4	3.5	0	84.72 (2.96)
Coixenolide	USFE	40	20	3.5	3	110.5	96.55 (1.12)

[a] Difference between SFE and USFE is significant at 95% confidence interval.

and without ultrasound were studied and the optimum experiment conditions were obtained. To reconfirm the experiment results, the parallel confirmation runs were repeated twice. The results and standard deviations are listed in Table 1.

As showed in Table 1, values of all parameters were reduced when ultrasound was applied. The EYs of USFE could be increased significantly about 14% by comparing to SFE operating at its own optimum conditions with 5% significant level.

4. Conclusions

Our study indicated that ultrasonic irradiation in supercritical CO_2 fluid promotes the extraction of adlay oil and coixenolide from adlay seed, and the EYs increased around 14% with sonication. There are optimum temperatures which gives the maximum EYs in both USFE and SFE. The effect of pressure on EYs for both USFE and SFE is similar to that of pressure on CO_2 density. Furthermore, the extraction temperature and pressure can be 5 °C and 5 MPa lower with ultrasound compared to that without it. The extraction time as well as CO_2 flow rate can also be reduced at 0.5 h and 0.5 L/h with the use of ultrasound. The increase in ultrasound power helps to increase the EY of adlay oil and coixenolide. However, the effect of sonication will be more obvious when the power output is high enough to overcome the high static pressure of surroundings.

Acknowledgments

We are grateful for financial support from the Research Grants Committee of Hong Kong (Project No. PolyU5137/01E) and Research Committee of Hong Kong Polytechnic University (Grant Code G-T182). We also thank the Hong Kong Polytechnic University ASD Fund.

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