Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Ping-ping Huang[a]; Ri-fu Yang[b]; Tai-qiu Qiu[a]; Wei Zhang[a]; Chun-mei Li[c]
[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, Guangdong, China [b] College of Physics Sciences and Technology, South China University of Technology, Guangzhou, Guangdong, China [c] Department of Biochemistry & Molecular Biology, Guangdong Pharmaceutical College, Guangzhou, Guangdong, China

Online publication date: 15 June 2010

To cite this Article Huang, Ping-ping , Yang, Ri-fu , Qiu, Tai-qiu , Zhang, Wei and Li, Chun-mei(2010) 'Ultrasound-Enhanced Subcritical Water Extraction of Volatile Oil from *Lithospermum erythrorhizon*', Separation Science and Technology, 45: 10, 1433 — 1439
To link to this Article: DOI: 10.1080/01496391003775972
URL: http://dx.doi.org/10.1080/01496391003775972

Separation Science and Technology, 45: 1433–1439, 2010

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496391003775972

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Ping-ping Huang,[1] Ri-fu Yang,[2] Tai-qiu Qiu,[1] Wei Zhang,[1] and Chun-mei Li[3]

[1]*College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, Guangdong, China*
[2]*College of Physics Sciences and Technology, South China University of Technology, Guangzhou, Guangdong, China*
[3]*Department of Biochemistry & Molecular Biology, Guangdong Pharmaceutical College, Guangzhou, Guangdong, China*

An ultrasonic probe was introduced into the kettle of subcritical water extraction device to extract volatile oil from *Lithospermum erythrorhizon*. The effect of temperature, pressure, ultrasonic power, and frequency on the extraction yield was studied. Gas chromatography-mass spectrometry (GC-MS) was applied to the analysis of the compositions of volatile oil. The mechanism of ultrasonic enhanced subcritical water extraction (USWE) was discussed. The results showed that the ultrasound-assisted enhancement effect of 20 KHz was better than that of 36 KHz and increased with output power (0 ~ 250 W). The subcritical water extraction yield increased from 1.87% to 2.39% via ultrasonic oscillation (250 W, 20 KHz) at a temperature of 160°C and a pressure of 5 MPa in the 25-minute extraction. Nineteen components were identified chiefly consisting of 18 carbon unsaturated fatty acid methyl esters, hexadecanoic acid methyl ester and pentadecane. Mechanism of USWE was cavitation and mechanical effect.

Keywords cavitation effect; *Lithospermum erythrorhizon*; mechanical effect; mechanism; subcritical water extraction; ultrasonic enhancement effect; volatile oil

INTRODUCTION

Subcritical water extraction (SWE) is a promising technique of separating bioactive components from plants, such as polyphenolic compounds (1), essential oils (2,3), flavonoids (4), anthraquinone (5), kava lactones (6), and is proved to have advantages in environment protection and good selectivity because no organic solvent is needed (7) and the dielectric constant of water decreases with the increase in temperature (8). Nevertheless, some heat-sensitive components are prone to change at a high temperature during SWE process (9) and the yield by SWE of certain substances is not as high as by other extraction methods (10), which limits its application and development seriously. Small quantities of organic solvents and surfactants were added into subcritical water to lower extraction temperature and raise extraction efficiency (11), but this also aroused troubles to reagent residues and separation of target components. It is necessary to study a convenient and effective mass transfer enhancement technology of SWE to increase extraction yield, to lower extraction temperature and to reduce the loss of heat-sensitive components.

Ultrasound has been applied to the enhancement of solvent (12) and supercritical carbon dioxide (13) extraction which has advantages in increasing extraction yield, shortening equilibration time, saving organic solvent and lowering extraction temperature. USWE equipment was recently reported (14), but the application of USWE technology to the extraction of natural products has not been reported. The mechanism of ultrasonically enhanced mass transfer is related to cavitation, thermal and mechanical effect produced by ultrasonic oscillation in propagation medium (15,16). Ultrasonic cavitation is the dynamic process of tiny bubbles including oscillation, growth, shrinkage, and collapse in liquid due to ultrasonic oscillation (15). Cavitation intensity can be detected according to fluorescence, electrochemistry, and iodometric release methods (17,18). The minimum sound pressure needed to produce ultrasonic cavitation (cavitation threshold) in subcritical water was calculated, which showed that it increased with pressure and decreased with increasing temperature (14).

Lithospermum erythrorhizon is a kind of Chinese herbal medicine which possesses a wide spectrum of wound healing, antitumor, anti-HIV and contraceptive bioactivities (19). Many previous studies paid attention to the isolation and bioactivity of shikonin and its derivatives in this plant (20,21). However, the volatile oil in it was little reported,

Received 24 October 2009; accepted 8 February 2010.
Address correspondence to Tai-qiu Qiu, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, Guangdong 510640, China. Tel.: 86-020-87113308; Fax: 86-020-87113308. E-mail: tqqiu@yahoo.cn

but it displays certain anti-inflammatory activity (22). Thus, volatile oil in *Lithospermum erythrorhizon* is chosen as an investigated project to study the ultrasound-enhanced mass transfer effect in SWE, which is beneficial to improving the utilization of this Chinese herbal medicine.

The present study is concerned with experimental investigation on the ultrasound-enhanced mass transfer effect on SWE by testing the extraction yield of volatile oil in *Lithospermum erythrorhizon*. Mechanism of USWE is discussed by measuring the cavitation intensity and analyzing physical parameters related to mechanical effect. Thermal effect is negligible because the USWE is a homothermal system.

MATERIALS AND METHODS

Materials and Equipment

Lithospermum erythrorhizon was purchased from Qingping Medicine Market (Guangzhou, Guangdong Province, China). Medicinal materials were dried for 36 h at 40°C and crushed to pieces with diameters of 0.2–0.3 mm. Triply-distilled water was prepared in lab. N-hexane, anhydrous sodium sulfate and potassium iodide were used as analytical pure reagents, UV spectrophotometer from Shanghai Mapada instruments Co., Ltd., (Shanghai, China), RE-52A vacuum rotary evaporator from the Shanghai Yarong biochemical instrument factory (Shanghai, China) and 6890 N/5975 GC-MS, Agilent's (U.S.). USWE equipment was the same as that reported in the literature (14).

USWE of Volatile Oil from *Lithospermum erythrorhizon*

Fifty g of dried *Lithospermum erythrorhizon* particles were put into the extraction kettle of SWE equipment. Ultrasonic oscillation was started after extraction temperature and pressure reached the fixed values. The maximum ultrasonic output power was 250 W. The ultrasonic frequency was adjusted between 20 KHz and 36 KHz. The extraction solution was cooled and gathered. N-hexane was applied to the separation of volatile oil from aqueous extract. Anhydrous sodium sulfate was applied to the adsorption of the residual moisture of organic phase. Volatile oil was obtained and a vacuum rotary evaporator was applied to the recovery of the solvent. The quality of N-hexane extract (total volatile oil) was measured and the extraction yield equaled to the quality percentage of volatile oil accounting for *Lithospermum erythrorhizon* materials (w/w, %). The above steps were repeated at least three times.

Analysis of Volatile Oil Components

The compositions of volatile oil were identified by GC-MS and the analysis conditions followed (22) with some modifications. HP-5MS elastic quartz capillary column ($30\,m \times 0.25\,mm \times 0.25\,\mu m$) was applied to separating compositions. The oven temperature was programmed from 80°C to 260°C at a rate of $4°C.min^{-1}$ and held at 260°C for 5 min. Split injector with a temperature of 310°C and with a split ratio 1:10. The injection volume was 1 µl. The flow rate of the carrier gas (He) was $1.4\,ml.min^{-1}$. The quality scan ranges from 39 to 450 aum (m/z) at a rate of 352 $aum.s^{-1}$. NIST05a.L spectrum gallery was applied to the identification of compositions.

Ultrasonic Cavitation Intensity in Subcritical Water

Iodimetry (18) was applied to the determination of the ultrasonic cavitation intensity in subcritical water with some modifications. 40 g of potassium iodide was put into the extraction kettle and the ultrasonic oscillation started when temperature and pressure reached the fixed values. The solution was cooled and released after 25 min, with the absorbance at 345 nm (the maximum absorption wavelength of iodine) was measured. Potassium iodide solution which did not deal with ultrasonic oscillation was used as the control. The greater the absorbance, the stronger the cavitation effect. The experiment procedure was repeated at least three times.

RESULTS AND DISCUSSIONS

Effect of USWE Conditions on Extraction Yield of Volatile Oil

The investigated factors were temperature, pressure, extraction time, ultrasonic output power, and frequency. Figure 1 showed that the extraction yield increased with temperature. 160°C was the optimum extraction temperature, at which the maximum SWE yield was 1.87%. On the one hand, the dielectric constant of water decreased with increasing temperature which increased the solubility of weak polar volatile oil in subcritical water, and on the

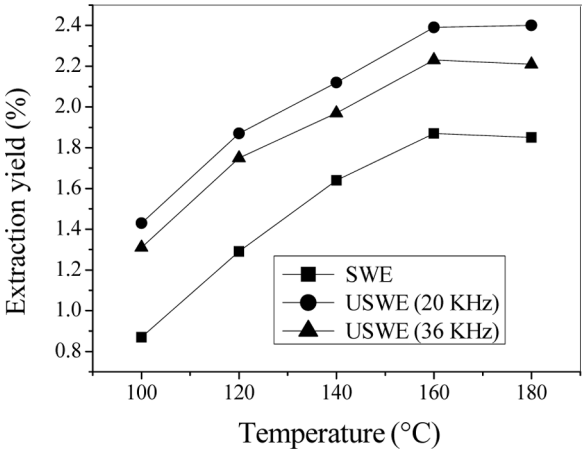

FIG. 1. Effect of extraction temperature on extraction yield of volatile oil (5 MPa, 250 W, 25 min).

other, the molecular diffusion of solute in subcritical water was accelerated, which speeded up the extraction equilibrium. The SWE yield was obviously increased by ultrasonic oscillation and the enhancement effect of 20 KHz was better than that of 36 KHz. The increased extraction yield by ultrasound with 36 KHz and 20 KHz were respectively 19.25% and 27.81% at a temperature of 160°C. The lower the frequency, the longer the ultrasonic cycle. Accordingly, the cavitation bubbles had sufficient time to grow and collapse, which contributed to the intensification of the ultrasonic cavitation. The ultrasonic frequency was also closely related to the acoustic attenuation. The acoustic absorptivity of medium increased rapidly with the increase of frequency, which reduced the ultrasonic enhancement effect. Besides, the cavitation threshold decreased with increasing temperature, which was conducive to the production of the cavitation phenomenon.

Pressure had no obvious effect on the extraction yield of subcritical water (Fig. 2), and the experimental results were consistent with the conclusion reported in reference (8). However, the USWE yield decreased with increasing pressure. The ultrasonic cavitation threshold increased with pressure and the cavitation bubbles must conquer greater resistance to collapse, which reduced the cavitation effect. Taking the enhancement by the ultrasound of 20 KHz for example, the extraction yield decreased by 9.66% as the pressure increased from 1 MPa to 20 MPa. Therefore, the low pressure was more appropriate for USWE. 5 MPa was the optimum extraction pressure at which the extraction yield was high and the subcritical water could maintain the liquid state at a high temperature of 160°C.

Figure 3 showed that the extraction yield increased with extraction time and the output power of ultrasound. The extraction yield increased by 14.35% as the output power

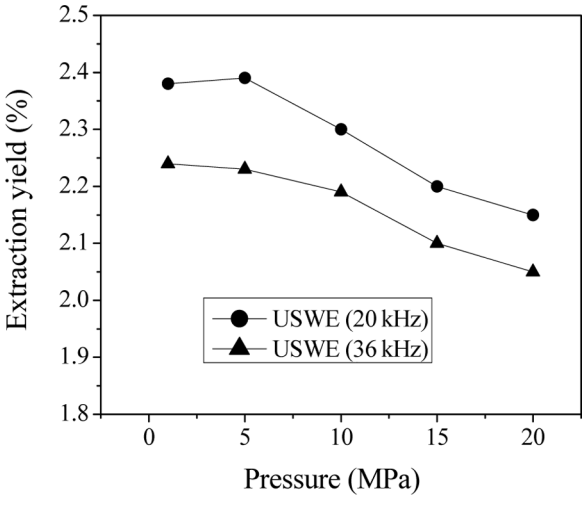

FIG. 2. Effect of extraction pressure on extraction yield of volatile oil (160°C, 250 W, 25 min).

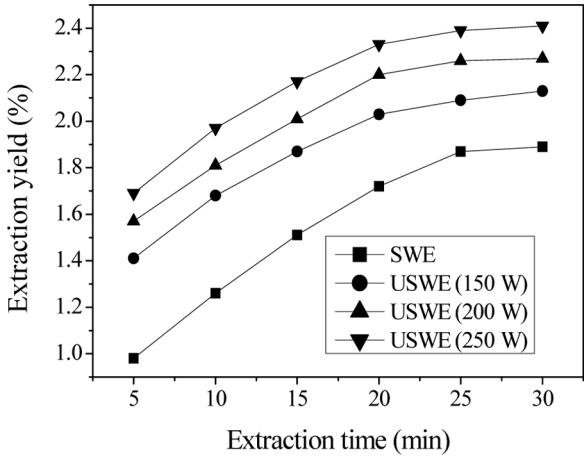

FIG. 3. Effect of extraction time on extraction yield of volatile oil (160°C, 5 MPa, 20 KHz).

increased from 150 W to 250 W. Accordingly the cavitation and mechanical effect increased with ultrasonic output power. The extraction equilibrium time of subcritical water was 5 minutes ahead of time under ultrasonic oscillation, which indicated that the ultrasonic enhancement technology could improve not only the extraction yield but the extraction rate. The ultrasonic enhancement effect was influenced not only by output power and frequency but also by swelling and compaction of vegetal matrix and solubility of target compounds in subcritical water. The maximum extraction yield of volatile oil was poorly increased from 1.87% to 2.39% in the best case (160°C, 5 MPa,250 W, 20 kHz, 25 min) possibly because of the compacted structure of *Lithospermum erythrorhizon* granules and the low solubility of volatile oil in subcritical water.

The relative standard deviation of data was $\leq 5\%$. The analysis of variance with confidence level $\alpha = 0.05$ indicated that the test pressures did not have significant effect on SWE yield, while other factors all had significant effect on extraction yield of volatile oil.

COMPOSITION ANALYSIS OF VOLATILE OIL

The ultrasound-enhanced subcritical water extract obtained under the optimum condition was light-pink volatile oil, whose gas-phase chromatogram was shown in Fig. 4. A total of 19 kinds of components were identified and their names were listed in Table 1. Eight major components were detected including 8, 11-octadecadienoic acid, methyl ester, 7-octadecenoic acid, methyl ester, hexadecanoic acid, methyl ester, pentadecane, octadecatrienoic acid, methyl ester, 9-octadecenoic acid (Z)-, methyl ester, 2-propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester, and alpha. Cubebene, thus representing the major components of volatile oil.

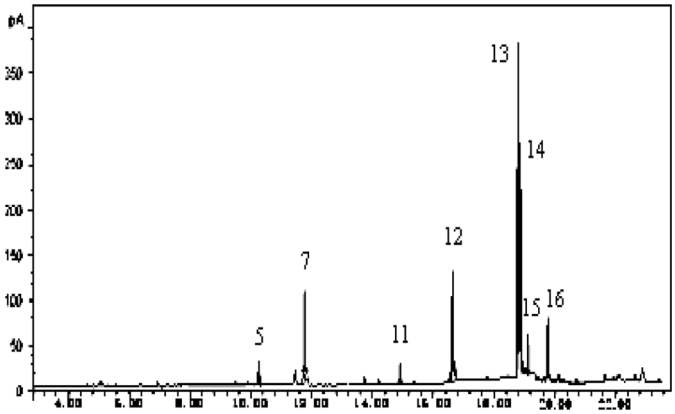

FIG. 4. Gas-phase chromatogram of volatile oil by USWE from *Lithospermum erythrorhizon*.

Mechanism of USWE

Ultrasonic cavitation was the dynamic process of oscillation, growth, shrinkage and collapse of tiny bubbles in liquid via ultrasonic oscillation. The collapse transient of cavitation bubbles could lead to a temperature of above 5000 K, pressure of 50 Mpa, and micro-jet with a speed of 400 $Km.h^{-1}$ in a very small space (14). Such strong physical effects could destroy the cell wall of plants (23) to reduce diffusion resistance and improved the mass transfer process effectively. Scanning electronic microscope (SEM) images of dried *Lithospermum erythrorhizon* granules before and after ultrasonic vibration at 20 kHz in subcritical water were shown in Fig. 5. The medicine granules had many significant bumps (a1) on the surface where there were many tiny irregularly-shaped particles and fragments (b1). The surface was completely uniform compact and relatively smooth. The ultrasonic vibration at 20 kHz had significant impact on the structures of medicine tissue. The micro-jet generated by ultrasonic cavitation impacted and destroyed the cellular structures around and produced more irregular potholes (a2, b2) on the surface.

Cavitation intensity was closely related to the ultrasonic cavitation threshold, vibration form of cavitation bubbles and energy generated by the collapse of cavitation bubbles (15). The formula for calculating ultrasonic cavitation threshold was derived in reference (14). The temperature and pressure generated by transient collapse of cavitation bubbles could be respectively estimated (15) by

$$T_{\max} = T_{\min}\left[\frac{(P_h + P_a)(\gamma - 1)}{P_g}\right] \quad (1)$$

$$P_{\max} = P_g\left[\frac{(P_h + P_a)(\gamma - 1)}{P_g}\right]^{\gamma/\gamma - 1} \quad (2)$$

where $T_{\max}$ and $P_{\max}$ are respectively the maximum temperature and pressure generated by transient collapse

TABLE 1
Components of volatile oil in *Lithospermum erythrorhizon* by USWE

No.	Retention time (min)	Compound	Peak area%
1	4.800	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	0.140
2	6.753	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1 R)-	0.235
3	7.085	Borneol	0.370
4	8.931	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	0.183
5	10.248	alpha.-Cubebene	0.897
6	10.665	Benzene, 1,2,3-trimethoxy-5-methyl-	0.199
7	11.754	Pentadecane	6.662
8	11.930	Phenol, 2,6-bis (1,1-dimethylethyl)-	0.536
9	12.055	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	0.146
10	12.158	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.236
11	14.921	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	0.941
12	16.625	Hexadecanoic acid, methyl ester	12.976
13	18.755	8,11-Octadecadienoic acid, methyl ester	39.663
14	18.829	7-Octadecenoic acid, methyl ester	27.219
15	18.884	9-Octadecenoic acid (Z)-, methyl ester	2.943
16	19.147	Octadecatrienoic acid, methyl ester	5.384
17	21.905	Eicosanoic acid, methyl ester	0.204
18	22.887	Tetracosane	0.737
19	24.737	Docosanoic acid, methyl ester	0.329

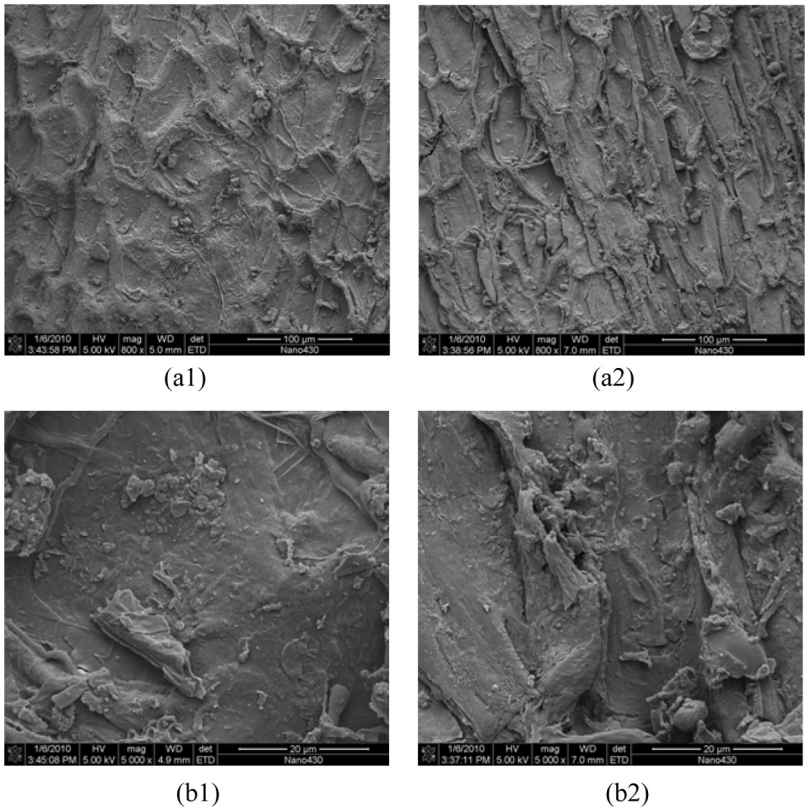

FIG. 5. SEM images of dried *Lithospermum erythrorhizon* granules. Note: (a1) and (b1) are SEM images of granules by SWE respectively 800 and 5000 times zoomed; (a2) and (b2) are SEM images of granules by USWE respectively 800 and 5000 times zoomed.

of cavitation bubbles, $T_{\min}$ is the ambient temperature, P_g is the vapor pressure, P_h is hydrostatic pressure, P_a is ultrasonic pressure, and γ is the specific heat ratio of gas. The higher the $P_{\max}$ and $T_{\max}$, the greater the cavitation intensity was (cancelled).

The cavitation intensity decreased with increasing pressure, as shown in Fig. 6. On the one hand, the cavitation threshold increased with pressure (14) to make the occurrence of cavitation phenomenon more difficult, and on the other, the greater the pressure of SWE, the greater the P_g, which made $T_{\max}$ and $P_{\max}$ smaller and further weakened the ultrasonic cavitation intensity, as shown in Formulas (1) and (2). The cavitation intensity of 20 KHz was greater than that of 36 KHz because the cavitation bubbles had a longer time to grow and collapse under ultrasonic oscillation with lower frequency, which was helpful to the intension of cavitation effect.

The cavitation intensity increased with temperature, as shown in Fig. 7. The viscosity and interfacial tension of liquid decreased with increasing temperature, which reduced the resistance to the production of ultrasonic cavitation bubbles in subcritical water. Besides, for the cavitation threshold to decrease indicated that it is easier for ultrasound to produce a cavitation phenomenon at a higher temperature (14). $T_{\max}$ increased with temperature (1) of subcritical water, thus intensifying the cavitation effect effectively. The relative standard deviation of data was $\leq 5\%$. The analysis of variance with confidence level $\alpha = 0.05$ indicated that the temperatures and pressures of subcritical water had significant effect on cavitation intensity.

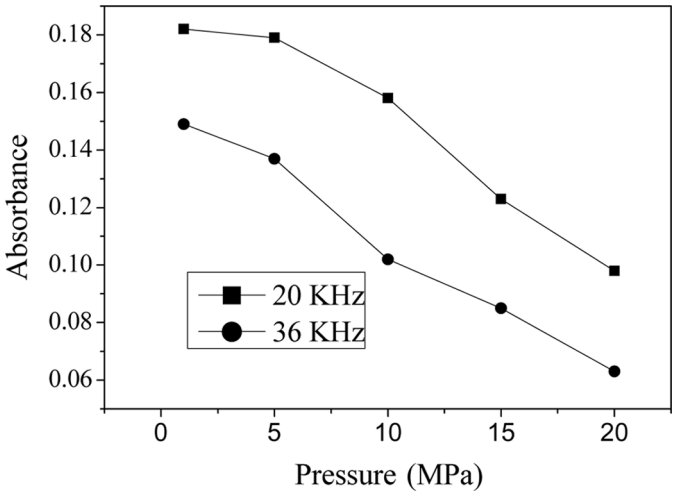

FIG. 6. Effect of pressure on cavitation intensity (160°C, 250 W, 25 min).

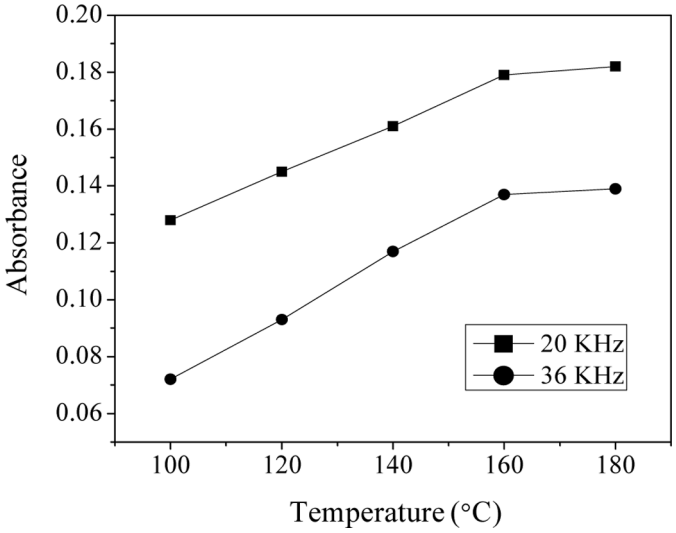

FIG. 7. Effect of temperature on cavitation intensity (5 MPa, 250 W, 25 min).

The mechanical effect of ultrasound was related to particle vibration caused by the spread of ultrasound in the medium. Taking the spread of ultrasound with frequency of 20 KHz (f) and intensity of 19.29 W.cm^{-2} (I) in subcritical water at a temperature of 160°C and a pressure of 5 MPa for example, the fluid density (ρ) was 910 Kg.m^{-3} and the ultrasonic velocity (c) was 1455 m.s^{-1}. The corresponding ultrasonic pressure amplitude was $P_A = (2\rho cI)^{1/2} = 714.72$ KPa, the maximum particle vibration velocity was $V_0 = P_A/\rho c = 0.54$ m.s^{-1}, the maximum particle acceleration was $a_0 = 2\pi f\ V_0 = 6.78 \times 10^4$ m.s^{-2} which was 6918 times the acceleration of gravity. Such drastic and rapid changes contributed a lot to the effect of ultrasound on enhancing subcritical water mass transfer process. Therefore, the mechanism of USWE was cavitation and a mechanical effect.

CONCLUSION

1. Ultrasonic oscillation could enhance mass transfer process of subcritical water and speed up the extraction equilibrium effectively. The greater the output power, the lower the frequency and the better the ultrasonic enhancement effect. The extraction yield increased by 27.81% and the extraction equilibrium was 5 min ahead of time under ultrasonic oscillation (20 KHz, 250 W) at a temperature of 160°C and a pressure of 5 MPa.
2. Nineteen components of volatile oil in Lithospermum erythrorhizon were identified by GC-MS. The major components were unsaturated fatty acid methyl esters, hexadecanoic acid methyl ester, and pentadecane.
3. The mechanism of USWE was cavitation and mechanical effect. The cavitation intensity increased with temperature and decreased with increasing pressure of subcritical water.

ACKNOWLEDGEMENTS

This study is sponsored by a grant from the Chinese National Science Foundation (20776047) and the authors are thankful to Mr. Xu Haihua, (engineer of Hangzhou Huali pump industry Co., Ltd., China), for debugging the USWE equipment.

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