Ultrasound-assisted extraction of ginsenosides in supercritical CO_2 reverse microemulsions

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Abstract: Ultrasound-assisted extraction of ginsenosides from ginseng in supercritical CO_2 reverse microemulsions formed by bis(2-ethylhexyl) sodium sulfosuccinate (AOT) was studied. Prior to extraction the ginseng was soaked in water for 12 h. It was found that ultrasound significantly enhanced supercritical CO_2 reverse microemulsion extraction. The ginsenoside extraction yield from supercritical CO_2 reverse microemulsion with ultrasound of 20 kHz, 15.2 W cm^{-2} and 3/6 s was 2.63 times that without ultrasound at 24 MPa extraction pressure, 45 °C extraction temperature, 4 h extraction time, 5 MPa separation pressure, 55 °C separation temperature and 2 L h^{-1} CO_2 flow rate with 140 mL of 0.07 mol L^{-1} AOT/ethanol. The maximum extraction yields of ginsenosides from different concentrations of reverse microemulsions were obtained at different ultrasonic intensities. The extraction yield with 20 kHz ultrasound was higher than that with 38 kHz ultrasound at suitably low intensity; however, it was lower at high intensity. The yield improvement may be basically attributed to the mechanical and thermal effects of ultrasound.

Keywords: ultrasound; supercritical CO_2 reverse microemulsion extraction; AOT; ginsenosides

INTRODUCTION

Panax ginseng is a traditional Chinese medicinal plant. Ginsenosides contained in ginseng have haemostatic, antioxidant, blood circulation-promoting and pain-relieving activities.[1] They are commonly extracted using organic solvents. Owing to increasing environmental restrictions, supercritical CO_2 extraction (SCE) has attracted much attention because of its environmental friendliness and unique physical and chemical properties.[2–4] However, supercritical CO_2 (SC) is a very poor solvent for polar molecules such as saponins, flavones and alkaloids. Therefore applications of SCE are limited in the case of such compounds. It is also difficult to extract hydrophilic components. Furthermore, SCE has a low dynamics for ginsenosides from the ginseng matrix. Therefore improvements in polar compound solubility and mass transfer are required in SCE.

One way to improve CO_2 dissolution of polar molecules is by introducing surfactants into SC to form reverse micelles or microemulsions.[5–10] However, the mass transfer of the extraction process is not improved.

Ultrasound is a form of sound waves. When ultrasound passes through a medium, it can produce effects such as compression and rarefaction, as well as radiation pressure and streaming, to enhance mass transfer in SCE.[11–13]

The aim of this study was to combine supercritical CO_2 reverse microemulsion extraction (SCRME) and ultrasound to extract ginsenosides from ginseng. The results of SCE, SCRME and ultrasound-assisted supercritical CO_2 reverse microemulsion extraction (USCRME) were compared. The effects of various ultrasound operational conditions were also investigated.

MATERIALS AND METHODS

Materials and reagents

Panax ginseng was purchased from Guangzhou Qiping market, PRC; it was clear, normal, crushed and sifted (40 mesh). CO_2 gas (>99.5 vol%) was obtained from Guangzhou Regang Gas Company (Guangzhou, PRC). Panaxadiol standard of analytical reagent grade was provided by China National Institute for the Control of Pharmaceuticals and Biologicals (PRC). Bis(2-ethylhexyl) sodium sulfosuccinate (AOT) of analytical reagent grade was purchased from Shanghai Regent Company (Shanghai, PRC). Methanol, ethanol, perchloric acid and vanillin of analytical reagent grade were obtained from Guangzhou Chemicals Company (Guangzhou, PRC).

Apparatus

The SCE equipment (capacity 1 L) was designed by Guangzhou Light Engineering Institute (Guangzhou,

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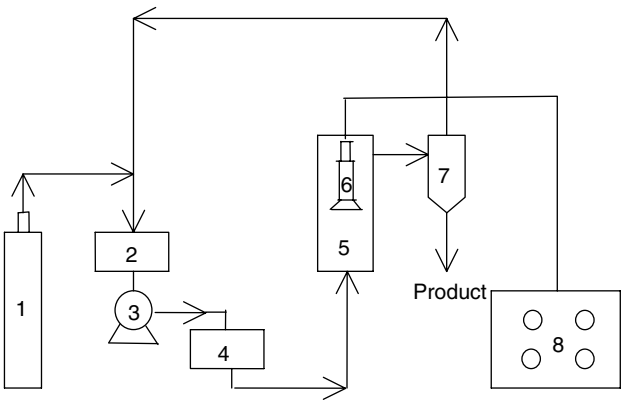

Figure 1. Experimental set-up of 1 L ultrasound-enhanced supercritical CO_2 extraction: 1, CO_2 supply; 2, cooler; 3, high-pressure pump; 4, CO_2 storage; 5, extractor; 6, ultrasonic transducer; 7, separator; 8, ultrasonic generator.

PRC). The USCRME equipment was self-designed based on the SCE equipment (Fig. 1). The ultrasonic system is composed of a piezoelectric transducer (ultrasonic intensity range 0–19 W cm^{-2}) and an ultrasonic generator. The generator comprises a power piezoelectric impedance-matching box and a power generator unit. The power generator unit consists of two parts: a power amplifier and a resonant frequency control system to maintain constant power to the transducer during the SCE process. The transducer was placed in the extractor of the SCE equipment, while the generator was located outside to control the ultrasonic intensity.

Analysis of ginsenosides

The extracted product was dried in a vacuum desiccator and then dissolved and diluted to 250 mL with methanol. An aliquot of diluted solution was analysed using the method developed by Li.[14]

Calculation of extraction yield of ginsenosides

The extraction yield of ginsenosides was calculated as

$$\text{extraction yield} = (m_t x_t / m_0) \times 100\%$$

where m_0 is the mass of material before extraction (mg), m_t is the mass of dry extract (mg) and x_t is the proportion of ginsenosides in the dry extract (mass%).

Supercritical CO_2 extraction (SCE)

Ginseng (100 g) was soaked in water (60 mL) for 12 h and placed in the extractor. The co-surfactant ethanol (140 mL) was added via the high-pressure pump. The ginseng was statically extracted for 0.5 h and circularly extracted for 3.5 h at 24 MPa extraction pressure, 45 °C extraction temperature, 5 MPa separation pressure, 55 °C separation temperature and 2 L h^{-1} CO_2 flow rate. The extract was collected and analysed. All trials were replicated twice and an average value was taken. SCRME and USCRME were also investigated for comparison.

Supercritical CO_2 reverse microemulsion extraction (SCRME)

The co-surfactant ethanol was replaced by 0.05 mol L^{-1} AOT/ethanol. The other experimental conditions were the same as for SCE.

Ultrasound-assisted supercritical CO_2 reverse microemulsion extraction (USCRME)

The co-surfactant ethanol was replaced by 0.05 mol L^{-1} AOT/ethanol. The ultrasound started to work when 0.05 mol L^{-1} AOT/ethanol was added. The other experimental conditions were the same as for SCE.

RESULTS AND DISCUSSION

Ginsenoside extraction kinetics of SCE, SCRME and USCRME

The intensity and frequency of ultrasound used in USCRME were 7.6 W cm^{-2} and 20 kHz respectively and the manner of ultrasonic irradiation was 3/6 s, i.e. the time of continuous ultrasonic irradiation was 3 s at intervals of 6 s. The ginsenoside extraction kinetics of SCE, SCRME and USCRME was studied.

The ginsenoside extraction kinetic curves of SCE, SCRME and USCRME are shown in Fig. 2. The three curves were similar but had different extraction rates. The extraction mechanisms were different for SCE and SCRME. Ginsenosides did not dissolve in SC below 40 MPa extraction pressure. Ginsenoside extraction depended mainly on the modifier ethanol in SC, because ethanol can dissolve some ginsenosides. Because CO_2 is a non-polar solvent, it is hardly polarised by ethanol. The ginsenoside extraction yield by SCE was still very low owing to very weak molecular interactions between ethanol and SC. In SCRME the ginsenosides dissolve in water pools of reverse microemulsions. The solubility of ginsenosides depends on factors such as the size, polarity and number of water pools. The manner of ginsenoside solubilisation depends on the polarity of water pools.

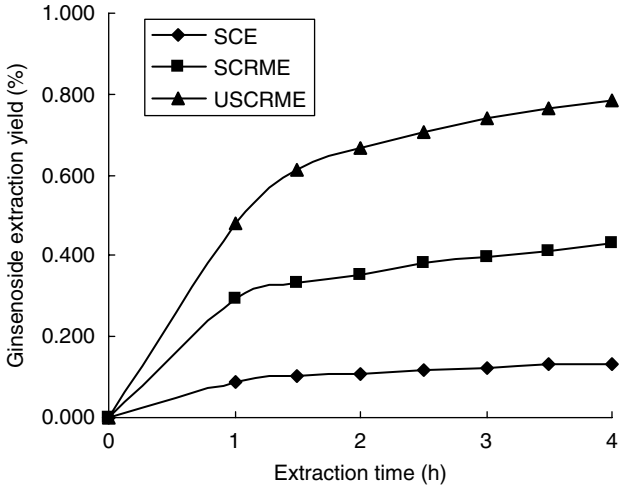

Figure 2. Ginsenoside extraction kinetics of SCE, SCRME and USCRME.

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Hutton *et al*.[15] found that the polarity of water pools in AOT/ethanol/SC reverse microemulsions was lower than that of bulk water, the value being close to that of *n*-butanol. In addition, the solubility of AOT in SC with ethanol is limited, which limits the number of reverse microemulsions formed.[16] Therefore the ginsenoside extraction yield by SCRME after 4 h extraction time was 3.23 times that by SCE; however, it was lower than the yield by Soxhlet extraction. It can also be seen in Fig. 2 that the ginsenoside extraction yield by USCRME after 4 h was 1.82 times that by SCRME. This suggests that ultrasound had an enhancing effect on ginsenoside extraction in supercritical CO_2 reverse microemulsions. Ultrasound can produce three types of effect in liquids: mechanical fluctuation, thermal and cavitation effects. Cavitation only exists in liquids. While SC is a special fluid between a liquid and a gas, there is no liquid–vapour interface in SC, hence no cavitation effect in SC with ultrasound.[17] The ultrasound-enhanced effect on SCRME may be attributed to mechanical fluctuation and thermal effects of ultrasound. Ultrasound can produce condensed and expanded phases in SC; micelles grow large in the expanded phase to dissolve more ginsenosides. On the other hand, polar or giant molecules can easily enter micelles as a result of mechanical fluctuations weakening steric hindrance, destroying plant cell walls and enhancing mass transfer.[18] The thermal effect of ultrasound accelerates molecular movement to increase material exchange and local temperatures, the latter enlarging the maximum water content of micelles to dissolve more ginsenosides.

Effect of ultrasonic intensity on ginsenoside extraction by USCRME

The ultrasonic intensities selected were 0, 5.7, 7.6, 9.5, 11.4, 13.3 and 15.2 W cm^{-2} at 20 kHz ultrasonic frequency, 3/6 s ultrasonic irradiation and 4 h extraction time. The other experimental conditions were the same as for SCE. The effect of ultrasonic intensity on ginsenoside extraction by USCRME was studied.

Figure 3 shows that the ginsenoside extraction yield increased with increasing ultrasonic intensity up to 11.4 W cm^{-2}, where it was 2.30 times the yield at 0 W cm^{-2}. With further increase in ultrasonic intensity, however, the ginsenoside extraction yield decreased. This is due to effects produced by compression/decompression, radiation pressure, streaming, etc. In addition, ultrasound acts as an agitator in SC, because the use of mechanical stirrers is not possible.[12] The extraction process comprises four steps: (1) the components for extraction leave the matrix; (2) they diffuse into the matrix–fluid interface; (3) they react with the solvent; (4) the dissolved components diffuse into the flowing solvent through the porous matrix.[19] The first and second steps are the rate-controlling steps. The effects of ultrasound below 11.4 W cm^{-2} intensity, such as turbulence, micro-disturbances and interface and spot energy effects, can enhance all four steps, especially the rate-controlling steps. For example, turbulence decreases the boundary layer and increases the mass transfer rate; micro-disturbances enhance the micropore diffusion of material cell walls; the interface effect enlarges the surface area of mass transfer; and the spot energy effect activates the separated materials. These effects increase the mass transfer coefficient and cause many ginsenosides to enter into micelles, thus enlarging the micellar size and weakening steric hindrance. However, ultrasound above 11.4 W cm^{-2} intensity can destroy the micellar structure and convert large micelles into small micelles or even single molecules, because high-intensity ultrasound can produce a fluctuation effect strong enough to destroy the structure and stability of the micellar electric double layer.[20,21]

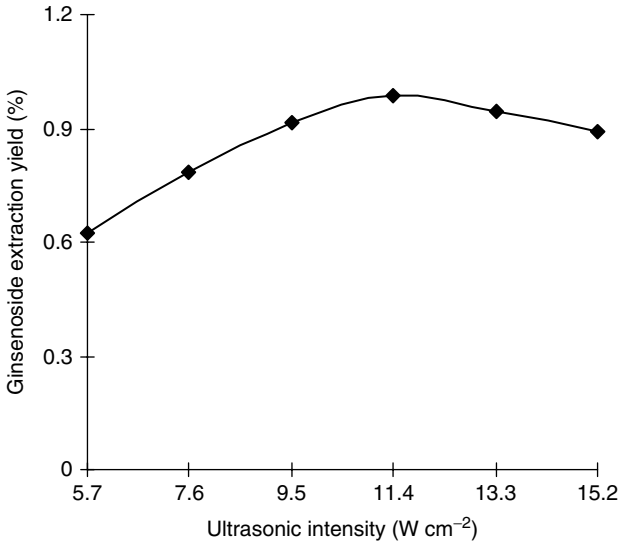

Figure 3. Effect of ultrasonic intensity on ginsenoside extraction by USCRME.

Effect of manner of ultrasonic irradiation on ginsenoside extraction by USCRME

The manners of ultrasonic irradiation selected were 3/6, 4/6, 5/6, 6/6, 8/6 and 12/6 s at 9.5 W cm^{-2} ultrasonic intensity, 20 kHz ultrasonic frequency and 4 h extraction time. The other experimental conditions were the same as for SCE. The effect of ultrasonic irradiation manner on ginsenoside extraction by USCRME was studied.

As seen in Fig. 4, the ginsenoside extraction yield by SCRME with ultrasound was much higher than that without ultrasound. It was slightly affected by the manner of ultrasonic irradiation and approached the maximum value at 6/6 s. This can be mainly attributed to the mechanical fluctuation of ultrasound, since 20 kHz of ultrasound can move a particle back and forth 20 000 times per second. Such fast fluctuation causes many ginsenosides to diffuse into the water pools of reverse microemulsions from the cell walls of ginseng, thus approaching the highest solubility. Even

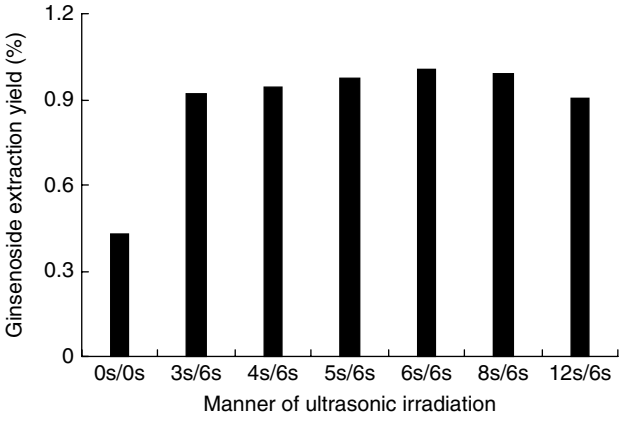

Figure 4. Effect of manner of ultrasonic irradiation on ginsenoside extraction by USCRME.

when the ultrasonic irradiation time is delayed, the extraction yield increases slowly. However, a long time of ultrasonic irradiation can destroy micellar stability, causing ginsenosides to leak out.

Effect of ultrasonic frequency on ginsenoside extraction by USCRME

The ultrasonic frequencies selected were 20 and 38 kHz at 0, 5.7, 7.6, 9.5, 11.4, 13.3, 15.2 and 17.1 W cm^{-2} ultrasonic intensities, 3/6 s ultrasonic irradiation and 4 h extraction time. The other experimental conditions were the same as for SCE. The effect of ultrasonic frequency on ginsenoside extraction by USCRME was studied.

As seen in Fig. 5, the trends of the 20 and 38 kHz curves differed in the intensity range 0–17.1 W cm^{-2}. No inflection was found in the 38 kHz curve, and the ginsenoside extraction yield at 38 kHz and 28 MPa was slightly higher than that at 20 kHz and 16 MPa. Because the maximum intensity of ultrasound available in our experiments was 19 W cm^{-2}, intensities higher than that could not be investigated, though it was expected that an inflection in the 38 kHz curve would occur at some intensity above 17.1 W cm^{-2}. The curve inflection is related to the effects of ultrasound. Ultrasound can have both advantageous and disadvantageous influences on SCRME. At suitably low intensity it was advantageous to ginsenoside extraction, whereas at higher intensity it was disadvantageous. According to the equation $W = 1/2\rho cf^2A^2V$,[22] where ρ (fluid density), c (velocity of sound) and V (fluid volume) are constants, f (ultrasonic frequency) is inversely proportional to A (amplitude of vibration) when W (ultrasonic power) is given, i.e. the smaller f is, the larger A is. Because the transducer areas for 20 and 38 kHz are the same, A of 20 kHz ultrasound is higher than that of 38 kHz ultrasound at the same intensity. In addition, the energy of high-frequency ultrasound attenuates faster than that of low-frequency ultrasound. Hence lower-frequency ultrasound is more helpful to USCRME at suitably low intensity. When the intensity is above 11.4 W cm^{-2}, 20 kHz ultrasound produces an amplitude of vibration large enough to destroy micelles and has a disadvantageous influence on SCRME. For the same distance between micelle and transducer, the intensity of 38 kHz ultrasound is weaker than that of 20 kHz ultrasound at the same transmitting intensity owing to different ultrasonic attenuation. On the other hand, the amplitude of vibration of 38 kHz ultrasound is lower than that of 20 kHz ultrasound at the same intensity.[23] Hence above 11.4 W cm^{-2} the disadvantageous influence of 20 kHz ultrasound on SCRME is stronger than that of 38 kHz ultrasound. This causes the inflection in the 20 kHz curve to occur earlier at 15.2 W cm^{-2}, whereas no inflection in the 38 kHz curve was observed. In addition, the number of vibrations per second of 38 kHz ultrasound is larger than that of 20 kHz ultrasound. Therefore higher-frequency ultrasound is more helpful to USCRME at high intensity.

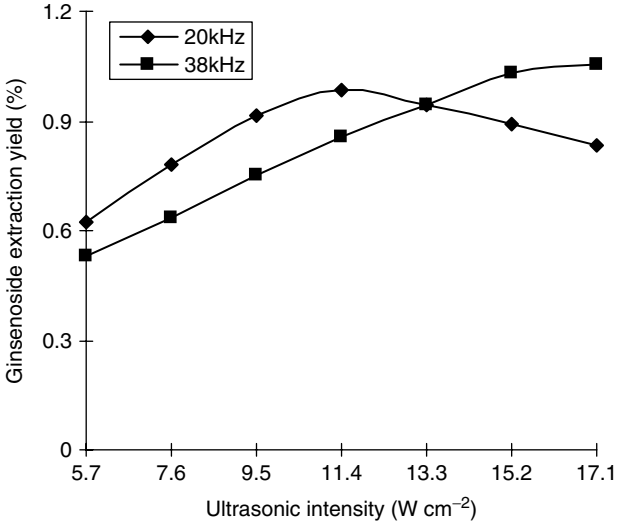

Figure 5. Effect of ultrasonic frequency on ginsenoside extraction by USCRME.

Effect of ultrasound on different concentrations of reverse microemulsions in ginsenoside extraction by USCRME

The concentrations of AOT/ethanol selected were 0.03, 0.05, 0.07 and 0.09 mol L^{-1} at 0, 5.7, 7.6, 9.5, 11.4, 13.3, 15.2 and 17.1 W cm^{-2} ultrasonic intensities, 20 kHz ultrasonic frequency, 3/6 s ultrasonic irradiation and 4 h extraction time. The other experimental conditions were the same as for SCE. The effect of ultrasound on different concentrations of reverse microemulsions in ginsenoside extraction by USCRME was studied.

As seen in Fig. 6, ultrasound enhanced ginsenoside extraction with different concentrations of AOT by USCRME. Different ultrasonic intensities had different effects depending on the concentration of AOT. The effect of suitably low ultrasonic intensity at low AOT concentration on ginsenoside extraction yield was more advantageous than that of high intensity, whereas the effect of high ultrasonic intensity at high AOT concentration on ginsenoside extraction yield

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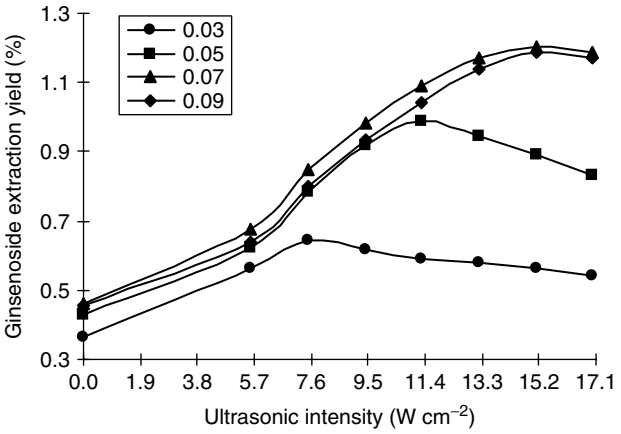

Figure 6. Effect of ultrasound on different concentrations of reverse microemulsions in ginsenoside extraction by USCRME.

was more advantageous than that of low intensity. The curve inflections for different concentrations of AOT corresponded to different ultrasonic intensities, with low concentrations corresponding to low intensities and high concentrations corresponding to high intensities. Because the aggregation number and size of micelles are proportional to the surfactant concentration, the lower the concentration of surfactant is, the lower the aggregation number of micelles is, the smaller the size of micelles is and the thinner the micellar electric double layer is. Hence, at low surfactant concentration, high-intensity ultrasound will destroy the micellar structures of reverse microemulsions, whereas suitably low-intensity ultrasound will lead to more ginsenosides dissolving in the micellar polar cores. This influence is one of the main reasons that there are different trends between low (0.03 and 0.05 mol L^{-1}) and high (0.07 and 0.09 mol L^{-1}) concentrations in Fig. 6. The ginsenoside extraction yield of 0.09 mol L^{-1} AOT reverse microemulsion was slightly lower than that of 0.07 mol L^{-1} AOT reverse microemulsion. This is because the concentration of AOT is too high to completely dissolve in the SC phase, so excess AOT deposits on the material surface and limits mass transfer.

CONCLUSIONS

The experimental results show that ultrasound significantly accelerated the kinetics of the process and improved the ginsenoside extraction yield from ginseng by SCRME. The ginsenoside extraction yield from SC reverse microemulsion with ultrasound of 20 kHz, 15.2 W cm^{-2} and 3/6 s was 2.63 times that without ultrasound at 24 MPa extraction pressure, 45 °C extraction temperature, 4 h extraction time, 5 MPa separation pressure, 55 °C separation temperature and 2 L h^{-1} CO_2 flow rate with 140 mL of 0.07 mol L^{-1} AOT/ethanol.

The maximum extraction yields of ginsenosides from different concentrations of SC reverse microemulsions were obtained at different ultrasonic intensities. High ultrasonic intensity was advantageous to USCRME at high surfactant concentration, whereas it was disadvantageous at low surfactant concentration. The extraction yield with low-frequency ultrasound was higher than that with high-frequency ultrasound at low intensity, whereas the opposite occurred at high intensity. These improvements may be basically attributed to the mechanical and thermal effects of ultrasound. The mechanism by which ultrasound affects reverse microemulsion extraction requires further experimental and theoretical study.

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