

Supercritical CO₂ Reverse Microemulsion Extraction of Ginsenosides from *Panax ginseng*

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Abstract : The extraction of ginsenosides from *Panax ginseng* root using supercritical CO₂ reverse microemulsion formed by bis(2-ethylhexyl) sodium sulfosuccinate (AOT), ethanol and water was studied, which was carried out in a 1 L supercritical CO₂ extraction device. Single-factor and orthogonal array design methods were applied to optimize the extraction. The effects of 7 parameters on ginsenosides extraction were investigated by the single-factor method. A higher extraction yield of ginsenosides was achieved using ethanol as the cosurfactant in comparison with butanol and n-pentanol, with the optimal added amount of 120 ml/ 100 g ginseng. Other 5 parameters such as amount of added water, amount of added AOT, extraction time, temperature and pressure were optimized using the orthogonal array design method. The optimal levels of the 5 parameters for improved ginsenosides extraction were determined as follows: amount of added water 36 ml/ 100 g ginseng, amount of added AOT 0.06 mol/100 g ginseng, extraction time 3 h, extraction temperature 55 °C and extraction pressure 30 MPa. An extraction yield of 0.757% was achieved under the optimized conditions. Moreover a dynamic model for ginsenosides yield as a function of extraction time was developed, which was $E = 0.870 \times (1 - e^{-0.618t})$. Compared to supercritical CO₂ extraction (SCE), this extraction method yielded more ginsenosides.

Key words: supercritical CO₂ reverse microemulsion extraction; supercritical CO₂ extraction; ginsenosides

超临界 CO₂ 反相微乳萃取人参皂甙的研究

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摘 要 : 利用萃取罐体积为 1 L 的超临界 CO₂ 萃取设备, 采用琥珀酸二(2-乙基己基)酯磺酸钠(AOT)/乙醇/水/超临界 CO₂ 反相微乳对人参皂甙的萃取进行了研究。结果表明: 最优萃取参数为萃取温度 55 °C, 萃取时间 3h, 加水量 36ml/100g 人参, 萃取压力 30MPa 和 AOT 添加量 0.06mol/100g 人参, 此时人参皂甙的得率为 0.757%; $E=0.870 \times (1 - e^{-0.618t})$ 为超临界 CO₂ 反相微乳萃取人参皂甙的具体动力学模型方程; 超临界 CO₂ 反相微乳萃取与超临界 CO₂ 萃取人参皂甙相比是一种相对有效的萃取技术。

关键词: 超临界 CO₂ 反相微乳萃取; 超临界 CO₂ 萃取; 人参皂甙

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Panax ginseng is a traditional Chinese medicinal plant. Ginsenosides of ginseng have hemostatic and antioxidant functions, and can promote blood circulation and relieve pain^[1]. The traditional extraction method of ginsenosides is organic solvent extraction. Supercritical CO₂ extraction (SCE) has attracted much attention because of its friendly environmental and unique physical and chemical properties^[2]. However,

supercritical CO₂ is a kind of very poor solvent for polar components, such as saponins, flavones and alkaloids. So the applications of SCE are mainly limited to extract nonpolar or poorly nonpolar components. Furthermore, SCE has low extraction efficiency for ginsenosides from *Panax ginseng*. Therefore, improvements in the solubility and mass transfer of ginsenosides in supercritical CO₂ are required.

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To improve the solubility of polar components in supercritical CO₂, cosurfactants were introduced into supercritical CO₂ to form reverse micelles or microemulsions^[3-6]. At the same time, the mass transfer of extraction process is improved. The extraction technology is called supercritical CO₂ reverse microemulsion extraction (SCRME).

The purpose of the present work was to optimize SCRME of ginsenosides from *Panax ginseng* roots, model the extraction process and compare the extraction efficiency between SCRME and SCE.

1 Materials and Methods

1.1 Materials and reagents

Panax ginseng roots purchased from Qiping market, (Guangzhou, China) were crashed and sifted (40 mesh). Carbon dioxide (> 99.5%, V/V) was obtained from Guangzhou Regang Gas Company (Guangzhou, China). Panaxadiol standard of analytical reagent grade was provided by National Institute for the Control of Pharmaceuticals and Biologicals Products (Beijing, China). Bis (2-ethylhexyl) sodium sulphasuccinate (AOT) of analytical reagent grade was purchased from Shanghai Reagent Company (Shanghai, China). Methanol, ethanol, butanol and *n*-pentanol of analytical reagent grade were obtained from Guangzhou Chemicals Company (Guangzhou, China).

1.2 Methods

1.2.1 Preparation of standard curve of panaxadiol

Using panaxadiol as the reference substance, a standard curve was prepared to determine ginsenosides concentration in extracts of *Panax ginseng* roots. The procedure was described as follows:

A standard solution of 1 mg/ml of panaxadiol was prepared with methanol in a 10 ml measuring flask. Subsequently, 20, 40, 60, 80 and 100 µl of the standard solution were pipetted carefully into five 10 ml tubes, respectively. After the solvent was evaporated by heating at 60 °C, each of the tube was added with 0.2 ml of 5% vanillin acetic acid solution and 0.8 ml of HClO₄, hold at 60 °C in water bath 10 min, and then added with 5 ml of acetic acid again. After being cooled to room temperature, the mixture was scanned against the blank solution using UV-2102PC in the range of 400 — 800 nm, and a maximum absorption was found at 547 nm. Based on this, we read absorbance (*A*) of the test portion solution at 547 nm to determine panaxadiol concentration. The panaxadiol concentration could be calculated by the following equation:

$$C = 9.9657A - 0.0023 \quad (R^2 = 0.9981) \quad (1)$$

where *C* (mg/ml) was the concentration of panaxadiol, and *A* was the absorbance at 547 nm.

1.2.2 Analysis of ginsenosides

Extracts of *Panax ginseng* roots were dried in a vacuum desiccator and then dissolved and diluted to 250ml with methanol. Aliquots of the diluted solution were analyzed using as described previously^[7].

1.2.3 Calculation of extraction yield (EY) of ginsenosides

EY of ginsenosides was calculated by the following equation:

$$EY = \frac{(9.9657A - 0.0023)V}{m} \quad (2)$$

Where *V* was the total volume of extraction solvent (1500 ml), *m* was the mass of ginseng (mg), and *A* was the absorbance at 547 nm.

1.2.4 Optimization of SCRME

In order to optimize the extraction process of SCRME, the effects of cosurfactant type, amounts of added ethanol, water and AOT, extraction time, temperature and pressure on ginsenosides extraction were investigated by single factor experiments. On the basis of the single factor experiments, an orthogonal array design leading to a set of 16 experiments with different combinations of five factors at four levels was conducted to attain the maximum extraction yield of ginsenosides. Levels of the factors were as follows: extraction temperature ranging from 45 to 60 °C, extraction duration ranging from 3 to 6 h, amount of added water ranging from 12 to 48 ml/100 g ginseng, extraction pressure ranging from 15 to 30 MPa and amount of added AOT ranging from 0.05 to 0.08 mol/100 g ginseng. Consumption of raw material for extracting ginsenosides, separation pressure and separation temperature were kept constant at 100 g, 6 MPa and 55 °C throughout all the experiments, respectively.

1.2.5 Comparison of extraction efficiency of SCRME and SCE

Both SCRME and SCE of ginsenosides were conducted under respective optimal conditions in triplicate. The optimal conditions of SCE were extraction temperature 50 °C, extraction pressure 30 MPa, extraction time 3 h and CO₂ flow rate 2.5 L/h. After extraction, the extracts were collected and analyzed.

2 Results and Analysis

2.1 Single factor testing

2.1.1 Effects of cosurfactant type

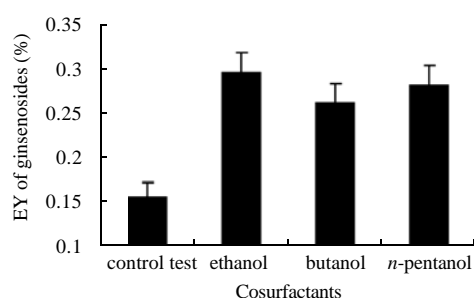


Fig.1 Effects of different cosurfactants on EY of ginsenosides

Fig.1 shows that all of the three cosurfactants could increase EY of ginsenosides. This is because that the cosurfactants could enter reverse microemulsion micelles: the short-carbon-chain alcohols, such as ethanol, can enter the inner of reverse microemulsion micelles because of their higher polarity and smaller molecular radius; the medium-carbon-chain alcohols, such as butanol and *n*-pentanol, could only enter the palisade layer of reverse microemulsion micelles because of their lower polarity and bigger molecular radius. All of the three cosurfactants can make the volume of reverse microemulsion micelles swell, and structure loose and therefore ginsenosides enter reverse microemulsion micelles more easily and quickly. Ethanol was the most beneficial cosurfactant among the three for SCRME (Fig.1). The smaller the volume of reverse microemulsion micelles is, the stronger the spatial resistance that restricts ginsenosides to enter into reverse microemulsion micelles is. In the presence of ethanol, the volume of reverse microemulsion micelles swells, and the structure loosens. We chose ethanol as the optimal cosurfactant due to the highest EY of ginsenosides.

2.1.2 Effects of ethanol concentration

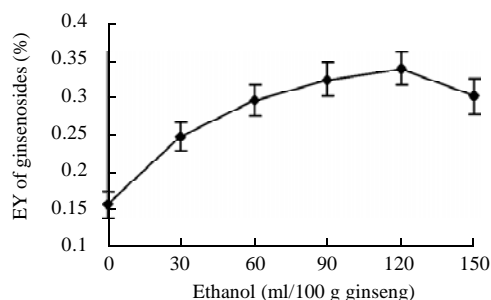


Fig.2 Effects of amount of added ethanol on EY of ginsenosides

As can be seen from Fig.2, EY of ginsenosides showed a changing trend of first increase and then decrease with the increase of amount of added ethanol. In the first stage, the growth of ethanol concentration resulted in increase of the solubility of AOT in supercritical CO₂ and therefore an increase of the amount of reverse microemulsion micelles was

found, and ethanol easily entered the palisade layer of reverse microemulsion micelles, which their volume was swelled^[8]. All of these factors were beneficial to the raise EY of ginsenosides. However, when the amount of added ethanol was excessive, the polarity of the supercritical system increased, the apply force of solvation between supercritical CO₂ and AOT rose, the driving force of formative reverse microemulsion micelles went down and AOT tended to be the existence of solvate monomer^[9]. So EY of ginsenosides fell down. Fig.2 shows that EY of ginsenosides comes to a climax when 120 ml of ethanol is added to 100 g of raw material for the extraction.

2.1.3 Effects of water concentration

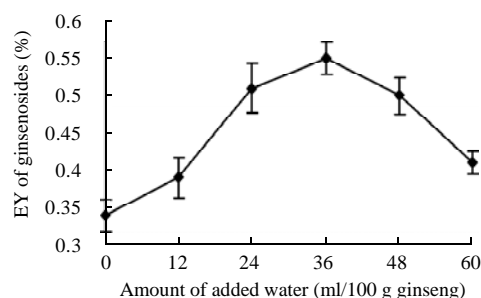


Fig.3 Effects of amount of added water on EY of ginsenosides

In the range from 0 to 36 ml/100 g ginseng, EY of ginsenosides rose with the increase of amount of water. Water in the dried ginseng mainly exists in the form of bound water which is easy to enter reverse microemulsion micelle core and form water pools. The water pools have a much higher polarity compared with that of bulk ambient water^[5]. So the amount of reverse microemulsion micelles increased and their volume enlarged. As a result, EY of ginsenosides rose. When the excessive water was added (over 36 ml/100 g ginseng), the surface of ginseng was immersed in water and therefore the diffusion of ginsenosides was inhibited. Due to this reason, EY of ginsenosides decreased. From the curve in Fig.3, it can be seen that a maximum EY of ginsenosides appeared at 36 ml/100 g ginseng.

2.1.4 Effects of amount of added AOT

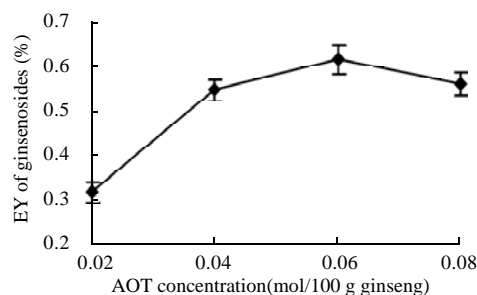


Fig.4 Effects of amount of added AOT on EY of ginsenosides

The effects of amount of added AOT on the extraction process can be explained from the following aspects: on one hand, the extraction rate increases significantly with AOT concentration, due to the increase of amount of reverse microemulsion micelles and the enlargement of their volumes; on the other hand, the increase of AOT concentration results in the increase of polarity of the system, and therefore the interaction force between dipoles is weakened, which is needed for the formation of reverse microemulsion micelles. So the amount and volume of micelles both go down. The effects of AOT concentration on EY result from two sides. As shown in Fig.4, a maximum EY of ginsenosides could be achieved when the amount of added AOT was 0.06 mol/ 100 g ginseng.

2.1.5 Effects of extraction time

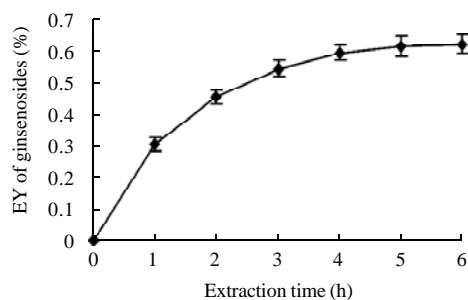


Fig.5 Effects of extraction time on EY of ginsenosides

The effects of extraction time on EY of ginsenosides are shown in Fig.5. As shown, along with extraction time prolonging, EY of ginsenosides gradually increases. At the early stage of extraction, ginsenosides can easily and quickly diffuse into the supercritical CO₂ reverse microemulsion micelles because the content of ginsenosides in ginseng is relatively high. At the stage the extraction process is mainly controlled by solubility magnitude of ginsenosides in the supercritical CO₂. After a certain period of time (4 h in this experiment), the content of ginsenosides, the amount and volume of reverse microemulsion micelles, the content of water and ethanol all are relatively lower, so the extraction process is mainly controlled by the diffusion of ginsenosides into the supercritical CO₂. In terms of EY of ginsenosides, 4 h could be considered time enough to complete the extraction.

2.1.6 Effects of extraction temperature

The effects of extraction temperature on EY of ginsenosides are very complicated. Firstly, the formation process of reverse microemulsion micelles was exothermal, So the rise of temperature results in the reduction of amount

of reverse microemulsion micelles. Secondly, the changes of ginsenoside solubility caused by varying temperature are also very complicated: when temperature rises, CO₂ density decreases, resulting in the reduction of its solubility; on the other hand, the vapor pressure of ginsenosides also increase along with extraction temperature rising, resulting in an increase of its solubility^[9-10]. Thirdly, when the temperature rises, ginsenoside diffusivity increase and the possibility of collision between CO₂ and AOT increased. As a result, the amount of reverse microemulsion micelles increases. So the effects of extraction temperature on EY of ginsenosides are the overall results of these factors. The optimal points can reflect the combined effects of extraction temperature on EY of ginsenosides. As can be seen from Fig.6, the optimal extraction temperature was 55 °C. Operations at temperatures lower or higher than the optimal value would result in lower EY.

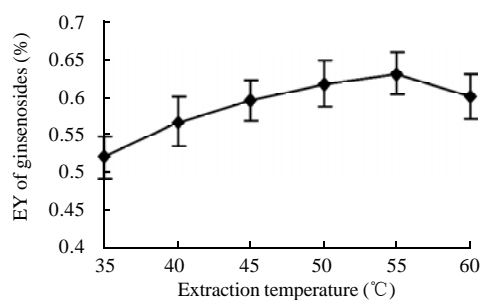


Fig.6 Effects of extraction temperature on EY of ginsenosides

2.1.7 Effects of extraction pressure

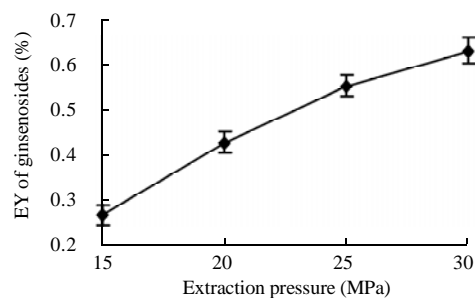


Fig.7 Effects of extraction pressure on EY of ginsenosides

The effects of extraction pressure on the extraction process mainly concern the following aspects: on one hand, the extraction rate increases significantly with extraction pressure, due to the increase of the CO₂ density. This is attributed to the increase of solubility magnitude of ginsenosides and AOT and the increase of diffusion coefficient of ginsenosides. All these are beneficial to the increase of amount and volume of reverse microemulsion micelles. As

a results, EY of ginsenosides is increased; on the other hand, the volume of reverse microemulsion micelles reduces with the increase of extraction pressure. As shown in Fig.7, between 15 MPa and 30 MPa, EY of ginsenosides increased with the rise of extraction pressure (Fig.7). This indicated that the beneficial factors were dominant. When the extraction pressure was 30 MPa, the EY of ginsenosides reached the maximum.

2.2 Orthogonal array testing

On the basis of the single factor experiments, extraction temperature, extraction time, amount of water, extraction pressure and amount of AOT were optimized by the five-factor and four-level orthogonal array design. The results of orthogonal test were listed in Table 1.

Table 1 Orthogonal array design arrangement and the experimental results

Run	Extraction temperature (°C)	Extraction time (h)	Amount of added water (ml/100g ginseng)	Extraction pressure (MPa)	Amount of added AOT (mol/100g ginseng)	EY of ginsenosides (%)
1	1(45)	1(3)	1(12)	1(15)	1(0.05)	0.331
2	1	2(4)	2(24)	2(20)	2(0.06)	0.472
3	1	3(5)	3(36)	3(25)	3(0.07)	0.586
4	1	4(6)	4(48)	4(30)	4(0.08)	0.643
5	2(50)	1	2	3	4	0.604
6	2	2	1	4	3	0.712
7	2	3	4	1	2	0.501
8	2	4	3	2	1	0.535
9	3(55)	1	3	4	2	0.757
10	3	2	4	3	1	0.639
11	3	3	1	2	4	0.453
12	3	4	2	1	3	0.467
13	4(60)	1	4	2	3	0.503
14	4	2	3	1	4	0.411
15	4	3	2	4	1	0.717
16	4	4	1	3	2	0.622
k ₁	5.08	5.49	5.30	4.28	5.55	
k ₂	5.88	5.59	5.65	4.91	5.88	
k ₃	5.79	5.64	5.72	6.13	5.67	
k ₄	5.63	5.67	5.71	7.07	5.28	
R	0.80	0.18	0.42	2.79	0.60	

As shown in Table 1, extraction pressure presented the most significant effect on the EY of ginsenosides due to the highest range range value, while other four factors had little effect (Table 1). The order of each factor that affected on EY of ginsenosides was: extraction pressure > extraction temperature > amount of added AOT > amount of added water > extraction time. The optimal conditions for the maximum extraction of ginsenosides were as follows: extraction temperature 55 °C, extraction time 3 h, amount of added water 36 ml/100 g ginseng, extraction pressure 30 MPa and amount of added AOT 0.06 mol/100 g ginseng. A 0.757% EY of

ginsenosides was found under the optimal conditions.

In order to verify these results, the extraction was carried out in triplicate under the optimal conditions. EYs of ginsenoside were 0.736%, 0.757% and 0.771%, respectively, and the relative standard deviation (RSD) was 2.47%, suggesting that the optimal results are reliable.

2.3 Kinetic modeling

The model as developed by Luo^[11] was employed to describe the experimental data of this study.

$$E = E_{\infty} \times (1 - e^{-kt})$$

where E was the specific EY of ginsenosides at " t " time t (h), E_{∞} was the E value for infinite extraction time, and k was rate constant. The adjustable parameters of the model were E_{∞} and k .

The kinetic process was modeled when the other conditions were optimal (Table 2), The specific kinetic equation was modeled by TableCurve 2D V5.01. The model described all the experimental data quite well (Fig.8 and Table 3).

Table 2 EYs of ginsenosides at different extraction time by SCRME

Extraction time (h)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
EY of ginsenosides (%)	0	0.258	0.402	0.513	0.607	0.682	0.737	0.778	0.796

Table 3 Parameter values obtained by kinetic model simulation

	E_{∞}	k	R^2	AAD
SCRME	0.870	0.618	0.998	2.08%

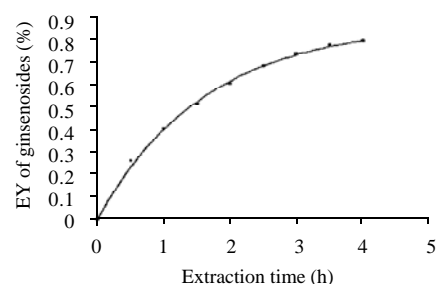


Fig.8 Time-course curve of extraction of ginsenosides by SCRME

2.4 Comparison of EYs of ginsenosides by SCRME and SCE

Table 4 Comparison of EYs of ginsenosides by different extraction techniques

Extraction techniques	EY of ginsenosides (%)
SCRME	0.757
SCE	0.259

Table 4 shows that the EYs of ginsenosides by SCRME and SCE were 0.757% and 0.259%, respectively. As shown,

SCRME is superior to SCE in terms of EY of ginsenosides. Further SCRME also is an effective technique for the extraction of polar components.

3 Conclusions

3.1 The single-factor method was adopted to investigate the effects of cosurfactant type, amounts of added ethanol, water and AOT, extraction time, extraction temperature and extraction pressure on EY of ginsenosides. The optimal cosurfactant was determined as ethanol, and the highest EY of ginsenosides could be achieved by the addition of 120 ml/100 g ginseng. The other factors were optimized by the orthogonal array design. Range analysis for the experimental results showed that the effects of the factors on EY of ginsenosides decreased in the following order: extraction pressure > extraction temperature > amount of added AOT > amount of added water > extraction time, and their optimal levels for improved extraction of ginsenosides were as follows: extraction temperature 55 °C, extraction time 3 h, amount of added water 36 ml/100 g ginseng, extraction pressure 30 MPa and amount of added AOT 0.06mol/100 g ginseng. The optimized extraction yielded 0.757% ginsenosides.

3.2 The kinetic model obtained, $E = 0.870 \times (1 - e^{-0.618t})$, gave quite a good fit to EY of ginsenosides under optimized conditions versus extraction time (AAD = 2.08%).

3.3 Only 0.258% EY of ginsenosides was achieved by SCE, much lower than 0.757% by SCRME, suggesting that SCRME is a more effective extraction technique for ginsenosides.

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