Optimization of Supercritical CO$_2$ Fluid Extraction Conditions of Saponins from Spina Gleditsiae

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Abstract. In this work, saponins were extracted from spina gleditsiae by supercritical CO$_2$ fluid extraction. The effects of extraction pressure, extraction temperature, extraction time, entrainment dosage and entrainment concentration on extraction process were studied. The optimum conditions for extraction were as follows: extraction pressure as 35MPa, extraction temperature as 45$^\circ$C, extraction time as 2.5h, the ratio of materials/entrainment (g/mL) as 1:3.0, entrainment concentration as 70%. Under these conditions, saponins yield was 0.975%.

1. Introduction

Spina gleditsiae has a long history of drug use. It was first published in the book of herbal scriptures of ShenLong, and has the functions of removing phlegm, removing swelling, removing wind and killing insects. Saponins, as the symbol active components of spina gleditsiae, have the function of improving cardiovascular and hematopoietic system and regulating central nervous system.

In this study, supercritical CO$_2$ fluid extraction technology was applied to the extraction of saponins from spina gleditsiae for the first time, which provided scientific experimental basis and guided significance for the development and utilization of spina gleditsiae.

2. Materials and Methods

Spina gleditsiae was dried 2h at 105$^\circ$C in oven thermostat firstly. Then it was crushed into granules, and the 60-80 mesh particles were selected for study by standard sieve. 5.0g spina gleditsiae granules above were added to 10mL 50% ethanol for 20min at 20kHz ultrasonic frequency and then transferred to 100mL extraction tank. Finally, supercritical CO$_2$ fluid extraction was carried out under certain extraction pressure, extraction temperature, extraction time, entrainment dosage and entrainment concentration. At last, the total yield of acanthic acid and oleanolic acid in spina gleditsiae was used to determine the content of saponins in extract and the yield of saponins was calculated.

3. Results and Discussion

3.1. Effect of extractive pressure.
3.2. Effect of extractive temperature.

The solubility of effective components in supercritical fluid is closely related to extraction pressure. The change of effective pressure can change the density of the supercritical fluid, thus increasing or decreasing the solubility of the material. The increase of extraction pressure not only increases the density of supercritical CO$_2$, but also reduces the distance between molecules and increase the mass transfer efficiency. As shown in Figure 1, in the process of extraction pressure rising from 20MPa to 35MPa, the yield of saponins increased with the increase of extraction pressure. Since 35MPa was the maximum allowable pressure set in test, the best extraction pressure was 35MPa.

The effect of temperature on supercritical CO$_2$ fluid extraction process is shown on two aspects. For one thing, effective constituents in supercritical CO$_2$ increase as the temperature rises. The higher temperature increases the diffusion coefficient of the active component, and the mass transfer speed is accelerated, thus accelerating the extraction yield of the active components. On the other hand, the increase in temperature reduces the density of CO$_2$, which results decrease of the ability to carry material, thus reducing the extraction yield of active components. Therefore, there is a optimum extraction temperature that balances the above two aspects. As shown in Figure 2, saponins yield from spina gleditsiae increased with extraction temperature increase, and then reached the maximum at 45°C, after which the saponins yield declined. Therefore, the best extraction temperature was 45°C.

3.3. Effect of extractive time.
2.0 2.5 3.0 3.5 4.0

0.40 0.45 0.50 0.55 0.60 0.65 0.70

Saponins yield /%

Extraction time /h

Figure 3 Extraction time on saponins yield
As shown in Figure 3, saponins yield increased with the extension of extraction time, and finally remained constant. It was because, the effective components were on the surface of the materials particles, with low internal diffusion resistance and high content of effective components, so the extraction efficiency was relatively high at the initial stage. As the extraction progressed, the internal diffusion resistance gradually increased, and the content of effective components also decreased, so the extraction efficiency decreased. In the extraction time of 2.0-3.0h, the yield of saponins increased with the increase of extraction time. When the extraction time was over 3.0h, there was almost no extractor. Therefore, the optimal extraction time was 3.0h.

3.4. Effect of entrainment dosage.

Using entrainment in the process of supercritical CO₂ extraction could enhance the ability of supercritical fluid to saponins. As shown in Figure 4, saponins yield increased with the increase of the entrainment dosage, which indicated that the addition of the entrainment increased the dissolution capacity of supercritical CO₂ to saponins. But when the amount of entrainment was too much, the material was completely difficult to mix with supercritical CO₂, which affected the critical state of supercritical CO₂, and the cost of subsequent entrainment separation also increased. When the ratio of material/entrainment (g/mL) was greater than 1:3.5, the yield of saponins increased with the increase of entrainment dosage. However, when the ratio of material/entrainment (g/mL) was less than 1:3.5, the yield of saponins unchanged with the increase of the entrainment dosage. Therefore, the best ratio of material/entrainment (g/mL) was 1:3.5.

3.5. Effect of entrainment concentration.
Figure 5 Entrainment concentration on saponins yield

Entrainment concentration affected saponins yield, which achieved the highest when the entrainment was 70% ethanol. The force between solvent molecules and entrainment was mainly carried out by hydrogen bonds. 70% ethanol and supercritical CO₂ had the strongest intermolecular forces, and the solubility of saponins was strong at the same time, so the extraction efficiency was the highest. However, the intermolecular forces of the entrainment and supercritical CO₂ weakened with the decrease of the ethanol concentration. The entrainment with saponins dissolved could not be carried into the separation tank by supercritical CO₂ fluid and remained in the raw material, which reduced the extraction efficiency. At the same time, when the entrainment concentration was too high, the hydrogen bond between the entrainment and the saponins weakened, so the extraction efficiency would be affected. Therefore, the best entrainment concentration was 70%.

3.6. Orthogonal experimental design and significance verification.

On the basis of single factor experiments, the interaction of each factor was considered. The extraction pressure, extraction temperature, extraction time, entrainment dosage and entrainment concentration were used to carry out the orthogonal test of the four levels of saponins. With the index of saponins, orthogonal table L₁₆(⁴⁵) was used to determine the appropriate extraction conditions, which was shown in Table 1.

Table 1. Variables and experimental design levels

| Number | Factor | Yield (%) |
|--------|--------|-----------|
|        | A      | B         | C         | D         | E         | Yield (%) |
| 1      | 1      | 1         | 1         | 1         | 1         | 0.315     |
| 2      | 1      | 2         | 2         | 2         | 2         | 0.423     |
| 3      | 1      | 3         | 3         | 3         | 3         | 0.363     |
| 4      | 1      | 4         | 4         | 4         | 4         | 0.297     |
| 5      | 2      | 1         | 2         | 3         | 4         | 0.399     |
| 6      | 2      | 2         | 1         | 4         | 3         | 0.465     |
| 7      | 2      | 3         | 4         | 1         | 2         | 0.594     |
| 8      | 2      | 4         | 3         | 2         | 1         | 0.477     |
| 9      | 3      | 1         | 3         | 4         | 2         | 0.429     |
| 10     | 3      | 2         | 4         | 3         | 1         | 0.564     |
| 11     | 3      | 3         | 1         | 2         | 4         | 0.480     |
| 12     | 3      | 4         | 2         | 1         | 3         | 0.571     |
| 13     | 4      | 1         | 4         | 2         | 3         | 0.465     |
| 14     | 4      | 2         | 3         | 1         | 4         | 0.588     |
| 15     | 4      | 3         | 2         | 4         | 1         | 0.645     |
| 16     | 4      | 4         | 1         | 3         | 2         | 0.609     |
| K₁     | 0.349  | 0.402     | 0.467     | 0.517     | 0.500     | Optimum A₄B₃C₁D₁E₂ |

Optimum A₄B₃C₁D₁E₂
From the orthogonal test analysis, the influence of the five factors on the extraction of saponins from spina gleditsiae was A>B>E>D>C. The extractive pressure had a significant influence on the yield of saponins, and the extractive temperature and entrainment were the second, while the extractive time had the least effect on the extraction yield of saponins. According to the significance test, the optimum process conditions for the extraction is A4B3C2D1E2, which means extraction pressure as 35MPa, extraction temperature as 45℃, extraction time as 2.5h, the ratio of materials/entrainment (g/mL) as 1:3.0, entrainment concentration as 70%. The significance verification was performed three times. The actual field of saponins was 0.975% repeatability.

4. Conclusion
In this study, the spina gleditsiae was used as raw materials. Saponins were extracted by supercritical \( \text{CO}_2 \) fluid extraction. The extraction process conditions were optimized experimentally, which provide scientific experimental basis for the comprehensive development and utilization of spina gleditsiae.

Acknowledgements
This work was financially sponsored by Qing Lan Project of Jiangsu Province (2017), Basic Research for Application Projects(KH17022) of Xuzhou, and Natural Science Research Projects of Xuzhou Pharmaceutical Vocational College.

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