

Optimization of Supercritical CO₂ Fluid Extraction Conditions of Flavonoids from Spina Gleditsiae

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Abstract. In this work, supercritical CO₂ fluid extraction method was used to extract the flavonoids from spina gleditsiae. we investigated the influence of four factors, namely entrainment dosage, extractive temperature, extractive pressure, and extractive time on the extraction of flavonoids. The optimum process conditions for the extraction were as follows: entrainment dosage of 37.5mL, extractive temperature of 45°C, extractive pressure of 40Mpa, extractive time of 1.0h. The actual yield of flavonoids was 1.112%, which was close to the theoretical value of 1.116%.

1. Introduction

Spina gleditsiae is a dry acacia of the legumes, which is effectively and widely used in clinical application, and listed as one of the anti-cancer Chinese herbs. The components in spina gleditsiae that have anticancer activity are mainly flavonoids. The research on the spina gleditsiae is mainly used for the identification and clinical application of Chinese medicine at present, and there is little research on the effective components.

In this study, supercritical CO₂ fluid extraction technology was applied to the extraction process of spina gleditsiae for the first time, and extractive conditions of flavonoids in which was optimized. We established a safe, simple and efficient new clean technology of flavonoids extraction in spina gleditsiae, and provided scientific experimental basis for the comprehensive development and utilization.

2. Materials and Methods

Spina gleditsiae was dried 2h at 105°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 100mL extraction tank. Finally, supercritical CO₂ fluid extraction was carried out under certain extractive temperature, extractive time, extractive pressure, and entrainment dosage. After filtration, the absorbance of flavonoids in extract was determined by ultraviolet spectrophotometry and the extraction field was calculated.

3. Results and Discussion

3.1. Effect of extractive temperature on the yield of flavonoids.

The effect of temperature on supercritical CO₂ fluid extraction process is shown on two aspects. For one thing, effective constituents in supercritical CO₂ 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 CO₂, 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. Supercritical CO₂ fluid extraction was performed under the conditions: entrainment dosage was 25mL, extractive pressure was 30MPa, extractive time was 1.5h, extractive temperature was 30°C, 35°C, 40°C, 45°C, and 50°C. Results are shown in Figure1.

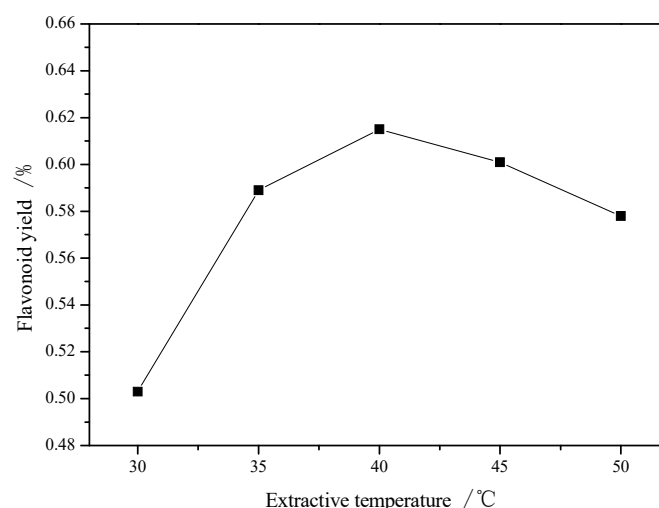


Figure 1. Extractive temperature on flavonoid yield

As shown in Figure1, flavonoids yield from spina gleditsiae is significantly increased with the extractive temperature when other conditions are controlled. When the temperature is 40°C, the extraction process achieve the best. Flavonoids yield begin to decline when the temperature more than 40°C. Therefore, the extraction of flavonoids from spina temperature 40°C is better, and orthogonal test temperature of is 35°C, 40°C, and 45°C.

3.2. Effect of extractive time on the yield of flavonoids.

The supercritical extractive time is determined by the amount of material added. Due to the poor contact between the supercritical CO₂ fluid and the solute in the initial extraction, the extraction amount is very low. With the extension of extractive time, the mass transfer state reach a good level, and the extraction volume of unit time increas. Until the maximum, the extraction amount will be reduced by the decrease in the content of the separated components. Supercritical CO₂ fluid extraction was performed under the conditions: entrainment dosage was 25mL, extractive temperature was 40°C, extractive pressure was 30MPa, extractive time was 0.5h, 1.0h, 1.5h, 2.0h and 2.5h. Results are shown in Figure2.

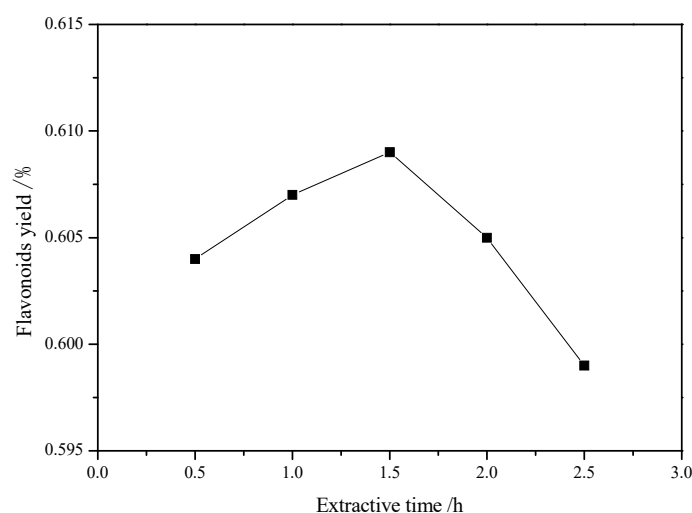


Figure 2. Extractive time on flavonoid yield

As shown in the Figure2, the effect of extractive time on flavonoid yield is not obvious. With the increase of the extractive time, the yield of flavonoids in spina gleditsiae is slowly rised and reached the maximum at 1.5h. Therefore, the orthogonal test was performed with 1.0h, 1.5h and 2.0h.

3.3. Effect of extractive pressure on the yield of flavonoids.

Extraction pressure is an important parameter to change the solubility in supercritical fluid. Changing the pressure can change the density of the supercritical fluid, thus increasing or decreasing the solubility of the material. The solubility in supercritical fluid of flavonoids is small because of the large polarity, so the extractive pressure of flavonoids is higher than other substances. Supercritical CO₂ fluid extraction was performed under the conditions: entrainment dosage was 25mL, extractive temperature was 40°C, extractive time was 1.5h, extractive pressure was 10MPa, 20MPa, 30MPa, 40MPa, and 50MPa. Results are shown in Figure3.

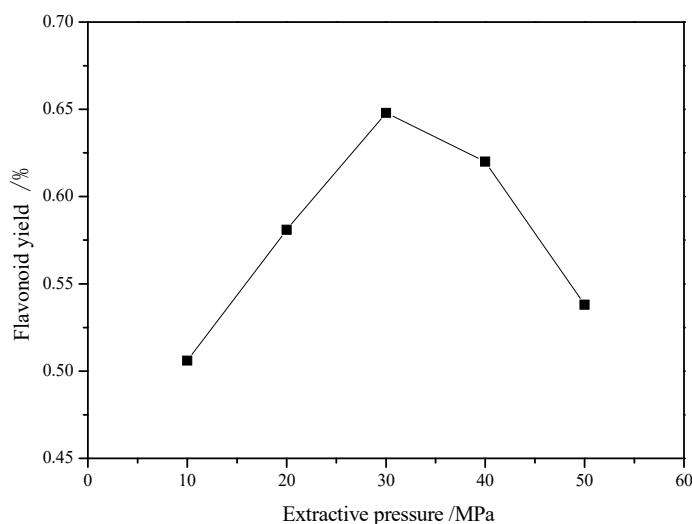


Figure 3. Extractive pressure on flavonoid yield

As shown in Figure3, when other conditions are controlled, the yield of flavonoids of spina gleditsiae is rise with the increase of pressure in the initial stage. The yield is the highest when the pressure is 30MPa, and which begins to decline when continue to increase the pressure. Therefore, the orthogonal test conditions are: 20MPa, 30MPa and 40MPa.

3.4. Effect of entrainment dosage on the yield of flavonoids.

Pure supercritical CO₂ extraction can only obtain a small amount of volatile small molecules, so the yield of flavonoids is extremely low. Using entrainment in the process of supercritical CO₂ extraction can influence solubility and selectivity of solute from two respects: the CO₂ density, and the interaction between solute and entrainment which is the main factors. In general, entrainment can enhance the solubility and selectivity of supercritical CO₂ fluid, but the dosage should be controlled. Supercritical CO₂ fluid extraction was performed under the conditions: extractive temperature was 40°C, extractive pressure was 30MPa, extractive time was 1.5h, entrainment dosage was 12.5mL, 25mL, 37.5mL, 50mL, and 62.5mL. Results are shown in Figure4.

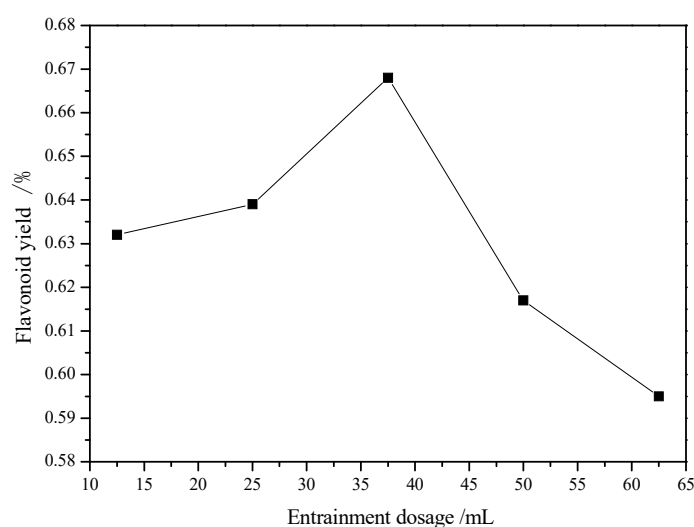


Figure 4. Entrainment dosage on flavonoid yield

As shown in Figure4, flavonoids yield increases gradually with the increase of the amount of 50% ethanol entrainment, and reach the maximum when entrainment dosage is 37.5 mL, and then decline. In the orthogonal experiment, entrainment dosage was 25mL, 37.5mL and 50mL respectively.

3.5. Orthogonal experimental design and significance verification.

On the basis of the three levels from single factor experiments, the interaction of each factor is considered. The extractive temperature, extractive pressure, time of extractive, and entrainment dosage were used to carry out the orthogonal test of the three levels of the flavonoids. With the index of flavonoids, orthogonal table L₉(3⁴) was used to determine the appropriate extraction conditions, which was shown in Table1.

Table 1. Variables and experimental design levels

Level	Factor			
	A Entrainment dosage /mL	B Extractive temperature /°C	C Extractive pressure /MPa	D Extractive time /h
1	25	35	20	1.0
2	37.5	40	30	1.5
3	50	45	40	2.0

The orthogonal test was conducted according to the design of Table1, and the results were shown in Table2.

Table 2. Orthogonal test results

Number	Factor				Yield (%)
	A Entrainment dosage	B Extractive temperature	C Extractive pressure	D Extractive time	
1	1	1	1	1	0.585
2	1	2	2	2	0.603
3	1	3	3	3	0.867
4	2	1	2	3	0.505
5	2	2	3	1	0.802
6	2	3	1	2	0.785
7	3	1	3	2	0.561
8	3	2	1	3	0.617
9	3	3	2	1	0.625
K ₁	2.055	1.651	1.987	2.012	
K ₂	2.092	2.022	1.733	1.949	
K ₃	1.803	2.277	2.230	1.989	
Optimum		A ₂ >B ₃ >C ₃ >D ₁			
R	0.289	0.626	0.497	0.063	
Prioritize		B>C>A>D			

From the orthogonal test analysis, the influence of the four factors on the extraction of flavonoids from *spina gleditsiae* was B>C>A>D. The extractive temperature had a significant influence on the yield of flavonoids, and the extractive pressure and entrainment dosage were the second, while the extractive time had the least effect on the extraction yield of flavonoids. According to the significance test, the optimum process conditions for the extraction is A₂B₃C₃D₁, which means entrainment dosage of 37.5mL, extractive temperature of 45°C, extractive pressure of 40Mpa, extractive time of 1.0h. The theoretical yield of flavonoids in *spina gleditsiae* was 1.116%. The significance verification was performed three times. The actual field of flavonoids was 1.112% repeatability, which was close to the theoretical value of 1.116%.

4. Conclusion

In this study, the *spina gleditsiae* were used as raw materials. Flavonoids was extracted by supercritical CO₂ fluid extraction. The extraction process conditions were optimized experimentally, which provide scientific experimental basis for the comprehensive development and utilization of *spina gleditsiae*.

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