

Research Article

Supercritical Fluid Extraction of Flavonoids from Dandelion

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Abstract: In this study, the total flavonoids from dandelion was extracted by supercritical CO₂ and the total flavonoids content in the extract was investigated by the sodium nitrite-aluminum nitrate method with Rutin as a standard product. Single-factor experiments were carried out to map the effects of extraction pressure, temperature, time and entrainer amount on the yield of flavonoids. The orthogonal experiments on the optimum technology parameters demonstrated that the influence of the experimental conditions over the yield from high to low was: (a) pressure, (b) temperature, (c) entrainer amount, (d) time. The optimization result showed that under the conditions of 50°C, 35 MPa, 80 min and 4.0 mL/g entrainer amount, the yield of the preparative supercritical fluid extraction was 4.974%.

Keywords: Dandelion, orthogonal design, Supercritical Fluid Extraction (SFE), total flavonoids

INTRODUCTION

Dandelion (*Taraxacum officinale*) is a member of the Asteraceae/Compositae family. It is a perennial herb, native throughout the Northern hemisphere. Traditionally, dandelion has been used as an herbal medicine due to its antidiabetic, choleric, antirheumatic and diuretic properties (Rice-Evans and Packer, 1997). By far, scientists have identified that this plant contains several active constituents such as sesquiterpene lactones, taraxacoside, triterpenes, phytosterols, henolic acids and flavonoids (Simándi *et al.*, 2002).

The existing extraction methods of flavonoids can be divided into two types: organic solvent extraction for crude extract and improved organic solvent extraction for refined extract. But these methods have some defects, such as low yield and recovery, toxic solvents and instable quality of final product.

Supercritical fluid extraction has been extensively applied in the food and medical industries in recent years. Supercritical carbon dioxide is the most commonly-used solvent and it can avoid the above defects since it is physiologically harmless, environmentally safe, non-explosive, readily available and easily removable (Mauricio, 2002; Kou-Lung *et al.*, 2002; Chun-Ming *et al.*, 2007). In recent years, supercritical fluid extraction has already exhibited promising prospects in the extraction of flavonoids in plants like liquorice, ginkgo leaf, cordate houttuynia, bamboo leaves asparagus and wheatgerm (Song, 2008).

But its application in the extraction of flavonoids from dandelion hasn't been reported by any literature yet. So, further chemical research and discovery in this field is necessary for exploitation of new medical products and pharmacological tests.

The aim of this study was to investigate the optimum conditions for the supercritical extraction of total flavones from dandelion. For this purpose, UV spectrophotometer quantitatively examined the total flavonoids in the extract. Single factor experiments investigated the effects of extraction pressure, temperature, time and entrainer on the yield of flavonoids. In the end, orthogonal design determined the optimum technology parameters for the extraction of flavonoids.

MATERIALS AND METHODS

Materials and reagents: Dandelion herb was purchased from a natural drug outlet in Yancheng, Jiangsu Province; Rutin standards (92.5%) was from National Institutes for Food and Drug Control of China; Anhydrous alcohol, sodium nitrite, sodium hydroxide, aluminum nitrate were all analytical-grade (99%), obtained from the market.

Main instruments: Double-beam UV-Vis spectrophotometer and High-speed grinder were purchased from Shanghai Precision Scientific Instrument Co., Ltd; Ultrasonic cleaner was obtained from Kunshan ultrasonic instruments Co. Ltd.; the

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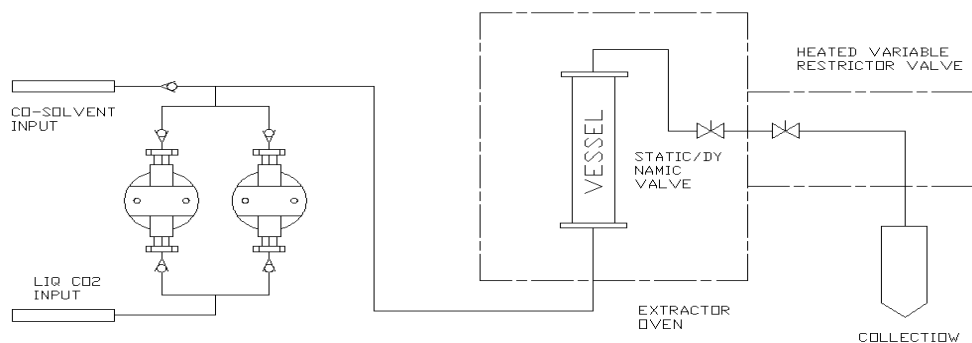


Fig. 1: Schematic diagram of supercritical fluid extraction apparatus

Table 1: Factors and levels of orthogonal test

Level	Factor			
	A: pressure (MPa)	B: temperature (°C)	C: time (min)	D: entrainer amount (mL/g)
1	25	45	60	3.0
2	30	50	80	3.5
3	35	55	100	4.0

Supercritical fluid extraction device was from SepTech Limited Company. A more detailed description of the equipment is in Fig. 1.

Methods: Dried dandelion (baked at 45°C for 12 h) was pulverized in a high-speed mixer-grinder and the powders were sieved over a 60-mesh screen. The 2 L extractor in Fig. 1 was packed with the dried dandelion powders. CO₂ was chosen as the solvent. Nitrite-aluminum nitrate method determined the flavonoids content in the extract. Rutin was used to prepare a standard curve. Flavonoids in the raw material were extracted after the less polar material essential oil was extracted by CO₂ in the system. The single factor experiments and the orthogonal experiments in the following context employed the above procedure and investigated the extraction condition sets (temperature, pressure and time and entrainer amount) were determined by the specific requirement of each experiment.

Single factor experiments: In these experiments, the extraction pressure, temperature, time and entrainer amount were generally considered to be the most important factors. The effects of the four factors were studied at 40, 45, 50, 55, 60°C, 20, 25, 30, 35, 40 MPa, 40, 60, 80, 100, 120 min and 2.5, 3.0, 3.5, 4.0, 4.5 mL/g, respectively by four designed experiments.

Orthogonal experiments: Based on the results of single factor experiments, all selected factors were examined using an orthogonal L₉ (3)⁴ orthogonal array. The detailed description of the test is listed in Table 1.

Methodology examination: Figure 2 presents the UV spectra of the rutin standards and the flavonoids, from which an intense and broad band can be found on both

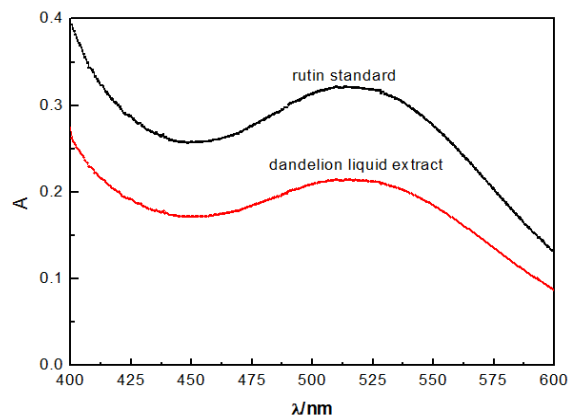


Fig. 2: Selection of the best wavelength

spectra. According to the characteristics of the band, we set the wavelength of flavonoids at 510 nm and used it to measure the concentration of flavonoids.

Rutin samples of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 mL, respectively were added to 25 mL volumetric flasks and diluted with 1 mL 5% NaNO₂, 1 mL 10% AlCl₃, 10 mL 4% NaOH and proper amount of water. Parallel sample which was also diluted with the above chemicals but contained no rutin was used for comparison.

Both samples were measured at 510 nm and the linear relation between the concentration of rutin (y) and the absorbance (x) was obtained and depicted as follows:

$$y = 8.8746x - 0.0003 \quad (R^2 = 0.9996)$$

The concentration and the yield of flavonoids can be calculated by the following equation:

$$\text{yield (mass of extract/mass of dry leaves, \%)} = c \times v/m \times 100$$

where,

c (g/mL): The mass concentration of flavonoids

m (g) : The mass of dandelion

v (mL) : The volume of the extract

RESULTS AND ANALYSIS

Single factor experiments:

Effect of extraction pressure: As one of the most important parameters and the main factors affecting the solubility of flavonoids in supercritical fluid extraction, the effect of pressures (20, 25, 30, 35, 40 MPa, respectively) on the yield of total flavonoids was determined under 40°C and 3.0 mL/g entrainer amount.

Figure 3 shows that the yield increased with the increase of pressure, especially in the pressure from 20 to 30 MPa, the yield increasing more significantly. However, when the pressure was higher than 30 MPa, the yield went up slowly and when the pressure exceeded 35 MPa, the yield even slightly decreased. The cause of this phenomenon is that the supercritical CO₂ become denser at higher pressure, which increases the mass transfer efficiency and the solubility of the flavonoids and thus leads to the yield improvement. But with the increase of the pressure, the increasing flow rate shortens the mass transfer time and the dandelion powders are further compacted, which are unfavorable to the solubility and the yield of flavonoids. Moreover, high pressure imposes a stricter requirement on the equipment and also need more electricity. Therefore, considering the operating pressure, cost and safety, we selected 25, 30, 35 MPa, respectively to conduct orthogonal experiments.

Effect of extraction temperature: Figure 4 shows the effect of temperatures (35, 40, 45, 50, 55°C, respectively) on the yield of flavonoids under 30 MPa, 60 min and 3.0 mL/g entrainer amount.

It is shown that when the temperature was below 50°C, the yield increased with the rising temperature, however, when the temperature was higher than 50°C, the yield slightly declined. There are several reasons behind this phenomenon:

- The increase of the temperature improves the solubility of flavonoids in CO₂, but decreases the density of CO₂, causing lower solubility and yield of flavonoids.
- The instable structure of flavonoids is decomposed at high temperature and hence its content is reduced.
- The rising temperature accelerates the molecular thermal motion of flavanoids which is the key to the volatility and therefore has a great influence on the yield.

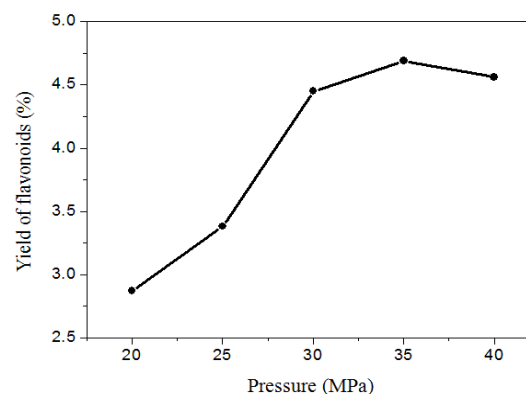


Fig. 3: Effect of extraction pressure on the yield of flavonoids

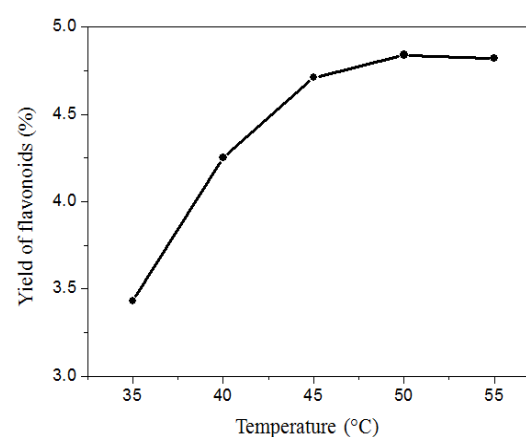


Fig. 4: Effect of extraction temperature on the yield of flavonoids

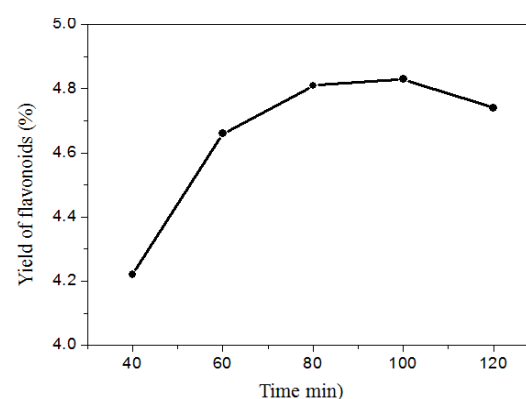


Fig. 5: Effect of extraction time on the yield of flavonoids

- The increasing temperature reduces the density of CO₂ and thus causes the decrease of solvent power and the yield of flavonoids.

The above reasons indicate that the effect, either positive or negative, brought by the temperature rise is dependent on the competition between the declining density and the rising diffusivity. Therefore, it is advisable to find a proper extraction temperature which can bring the most beneficial effect on the yield of

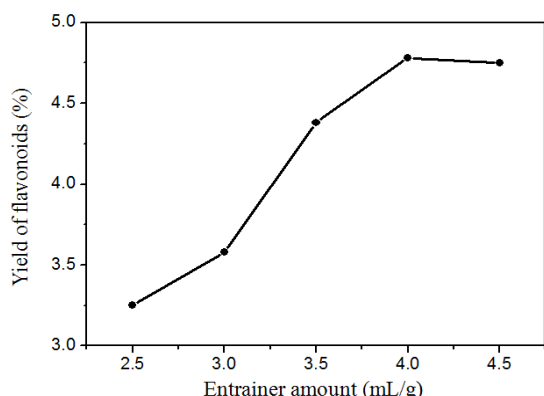


Fig. 6: Effect of entrainer amount on the yield of flavonoids

flavonoids. Regarding the conditions of this experiment, 45, 50 and 55°C, respectively were used in orthogonal experiments.

Effect of time: Figure 5 indicates that from 0 to 80 min, the yield of flavonoids increased with time, nevertheless when the extraction time was longer than 80 min, the increase of yield slowed down significantly. This is because the long extraction time results in better interaction between CO₂ and dandelion. But too long extraction time breaks the structure of flavonoids and consumes more energy in the experiment. Hence, we should guarantee that the extraction time in application could create high yield and low cost at the same time. Considering this, extraction time of 60, 80, 100 min, respectively were applied in orthogonal experiments.

Effect of entrainer amount: For strong polar molecules such as flavonoids, entrainer is essential to

the extraction of them in supercritical CO₂. In order to promote the yield, anhydrous ethanol was used as the entrainer in this experiment. And the effect of entrainer amount (2.5, 3.0, 3.5, 4.0, 4.5 mL/g, respectively) on the yield of flavonoids was investigated under 30 MPa, 45°C and 80 min.

Shown in Fig. 6, the increase of entrainer resulted in higher yield and the increase began to slow down when the entrainer was beyond 4.0 mL/g. On the basis of single factor experiments, 3.0, 3.5 and 4.0 mL/g, respectively were employed for further orthogonal experiments.

Results and analysis of orthogonal experiments:

Yield of flavonoids at different sets of temperature, pressure, time and entrainer amount were examined under orthogonal design in Table 2.

Table 2 shows that the optimum conditions for the extraction was 35 MPa, 50°C, 80 min and 4.0 mL/g and the influence of the experiment conditions from high to low was as follows:

- Pressure
- Temperature
- Entrainer
- Time

The result of variance analysis in Table 3 shows that pressure was the most important determinant of the yield and temperature also had significant influence, while entrainer amount and time didn't possess strong influence. This is consistent with the result of range analysis in Table 2. But it is worth mentioning that

Table 2: Results of orthogonal experiment and data analysis

Run	Pressure (MPa)	Temperature (°C)	Time (min)	Entrainer amount (mL/g)	Yield of flavonoids (%)
1	25	45	60	3.00	2.70
2	25	50	80	3.50	3.45
3	25	55	100	4.00	3.45
4	30	45	80	4.00	3.93
5	30	50	100	3.00	4.03
6	30	55	60	3.50	3.98
7	35	45	100	3.50	4.45
8	35	50	60	4.00	4.93
9	35	55	80	3.00	4.88
K ₁	9.60	11.08	11.61	11.61	
K ₂	11.94	12.41	12.26	11.88	
K ₃	14.26	12.31	11.93	12.31	
k ₁	3.20	3.69	3.87	3.87	
k ₂	3.98	4.14	4.09	3.96	
k ₃	4.75	4.10	3.98	4.10	
R	1.55	0.45	0.22	0.23	

Table 3: Analysis of variance for the response surface quadratic model for the flavonoid yield of dandelion

Source	Degrees of freedom	M.S.	F-value	Significance
A	2	1.8099	180.99	**
B	2	0.1931	19.31	*
C	2	0.0010	1.00	
D	2	0.0418	4.18	
Error	2	0.0000		

M.S.: Mean square

although entrainer amount don't have significant influence, the existence of it is essential to the rise of yield.

Optimum extraction conditions: Under the optimum extraction conditions, three repetitive SFE experiments was carried out and the results showed that the average yield of flavonoids was 4.974%, higher than all yields in Table 2.

CONCLUSION AND DISCUSSION

As a convenient and safe way to achieve high extraction yield, supercritical carbon dioxide has been successfully employed as a solvent for the extraction of flavonoids from dandelion. Single factor experiments revealed that the yield was affected by the experiment conditions and the conditions of 50°C, 35 MPa, 80 min, 4.0 mL/g entrainer amount were critical in determining the yield. The range and variance analysis of orthogonal experiments indicate that the influence of the experiment conditions from high to low was:

- Pressure
- Temperature
- Entrainer amount
- Time

The optimum extraction conditions, jointly determined by single factor experiments and orthogonal

experiments, had good reproducibility to reach the desired yield. The result of the study demonstrates that supercritical fluid extraction is a very good technique to extract flavonoids from dandelion and this study can provide valuable reference for further researches in this field.

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