SEPARATION OF PARTHENOLIDE FROM *TANACETUM PARTHENIUM*

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In present work the extraction of *Tanacetum parthenium* flower heads was performed using supercritical carbon dioxide at pressures from 200 bar to 800 bar and at temperatures 40 °C, 60 °C and 80 °C. For comparison, the conventional extractions with organic solvents and water have been made. Dried *Tanacetum parthenium* flower heads were used as starting material.

Supercritical fluid extraction was performed using a semicontinuous flow apparatus. In order to fractionate the waxy and active components the separation was performed in two steps. The results showed that generally the parthenolide yield increases with increasing pressure and is in the range from approximately 100 to 330 mg/100 g dry material.

The extraction yields obtained by conventional solvent extractions of dried material are similar or even higher than those resulting from supercritical fluid extractions. The highest parthenolide yield using conventional extractions was achieved using methanol as solvent and was 763.6 mg/100 g dry material.

INTRODUCTION

Tanacetum parthenium contains a range of compounds known as sesquiterpene lactones. Over 85 % of these are an active compound parthenolide. Its pharmacological action is similar to that of aspirin. Parthenolide helps prevent excessive clumping of platelets and inhibits the release of certain chemicals, including serotonin and some inflammatory mediators [1, 2].

Extraction with dense gasses in sub and supercritical region is often used for the extraction of natural compounds when applications lie in food, cosmetic, or pharmaceutical industry. Several advantages of supercritical fluid extraction are known. Varying the operating conditions can change the selectivity of the solvent and obtained extracts are of high quality and also free of organic solvents. During the past decades a lot of research work has been done concerning *Tanacetum parthenium* and its main active compound parthenolide. Since the herb's medicinal effects on healing different kinds of pains are known for centuries, nowadays several commercial preparations of feverfew are available on pharmaceutical and health food market. There is a wide variation in the amounts of parthenolide in different material and herb parts. The highest content of parthenolide was found in flower heads (1,38 %) followed by leaves (0,95 %) and with only 0,08 % in stalks and 0,01 % in roots [3]. In present work the parthenolide content in herb *Tanacetum parthenium* and the efficiency of supercritical fluid extraction was investigated. Carbon dioxide was used as solvent and extractions were performed at pressures from 200 to 800 bar and temperatures from 40 to 80 °C. The separation of high pressure extracts obtained was performed in a single step or in two steps and the separation of parthenolide and waxy compound was studied. The conventional extractions with different organic solvents were carried out and results obtained were compared.

I - MATERIALS AND METHODS

Materials

The *Tanacetum parthenium* flower heads were supplied from Droga (Portorož, Slovenia). All chemicals used for analysis were purchased from Merck (Darmstadt, Germany). CO_2 (purity 99.5 %) was obtained from Messer (Ruše, Slovenia).

Methods

The *Tanacetum parthenium* flower heads were ground in small quantities and heating effect due to grinding raw material was minimal. The density of solid material was measured with helium pycnometer (multi volume pycnometer 1305, Micrometrics, USA). Moisture content of plant material was determined using Karl Fisher Titrator (Mettler Toledo DL31).

The extraction experiments with dense CO_2 at pressures 200 and 300 bar were performed on a semicontinuous lab-scale apparatus [8]. The apparatus was home built for a maximum pressure of 500 bar and a temperature of 100°C.

Approximately 20 g of ground material was charged into the extractor (V=60 mL). The temperature in the water bath was regulated and maintained at constant level (± 0.5°C; LAUDA DR.R. WOBSER GmbH & Co. KG, Lauda Königshofen, Germany). The apparatus was purged first with nitrogen and later with the gas used for extraction. In the next step, liquefied CO_2 was continuously pumped with a high pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebrasca, P_{max}= 350 bar) through the preheating coil and over the bed of sample in the extractor. The solvent flowrate was measured with a flow-meter (ELSTER HANDEL GmbH, Mainz, Germany). In the first set of experiments, the separation was performed in a single step at 1 bar and 20 °C and total yield and amount of isolated parthenolide was observed. In the second set of experiments, the two-step separation was carried out and the separation of parthenolide and waxy compounds was studied. The separation in first separator (S1) was performed at pressures 45 and 100 bar and temperatures 44 and 60 °C. The conditions in second separator (S2) were held constant at 1 bar and 20 °C. The product collected in the separators was weighed (± 0.1 mg) and analysed.

The extractions at 600 and 800 bar were performed on semicontinuous apparatus (NOVA SWISS), designed for maximal operating pressure 1000 bar and temperature 100 °C. The apparatus is equipped with heat exchangers and extractor (V= 4 L) and separator with heating jackets. The principle of the extracting process is the same as described above.

The extraction experiments with conventional solvents were performed in a batch extractor, composed of a 250 mL round-bottomed flask. The flask was filled with solvent and extracting material and the content was heated to desired temperature and mixed. Temperature in the flask was controlled with a thermometer and was 20 °C. After half an hour of extraction, the extraction mixture was filtered, solvent evaporated and the product obtained was weighted and analysed.

The yield of extraction was calculated by the formula:

$$\text{Yield}(\%) = \frac{m_{\text{extract}}}{m_{\text{raw material}}} \cdot 100\%, \qquad (1)$$

where m_{extract} is mass of extract and $m_{\text{raw material}}$ is mass of raw material (flower heads of *Tanacetum parthenium*) extracted. For quantitative determination of the parthenolide content in the extracts *HPLC* analytical method (Chrom-Circle, 1998) was used.

Analysis
The extracts were dissolved in acetonitrile and filtered through a VariDiskTeflon 0.45 μm mbrn filtr. The HPLC system consisted of a pump (constaMetric 3000, solvent delivery system, Milton Roy, USA) and a diode array detector (spectroMonitor 3100, variable wavelenght detector, Milton Roy, USA). A Symmetry Shield RP-8 250 x 4.6 mm column (Waters) with 5 μm particle size was used. The mobile phase consisted of acetonitrile:water = 55:45 (v/v) and the flow rate was 1 ml/min. The detection was performed at 210 nm. The quantification was made with multipoint calibration curve of external standard.

CONCLUSION

The moisture content in plant material was 5.60 %. Solid density of dry material was 1.480 g/cm^3 and the median particle size was 0.299 mm. The conditions of supercritical (SC) CO_2 extraction of *Tanacetum parthenium* followed by single step separation at 1 bar and 20 °C and results obtained are presented in **Table 1** and **Figures 1** and **2**. The results show that generally the yield of extraction increases with pressure at constant temperature. The best result was obtained at 600 bar and 80 °C, where yield of extraction was 9.11 %.

Table 1: Operating parameters and results of the SC CO_2 extraction of *Tanacetum parthenium*: single-step separation at T_S= 20 °C and P_S= 1 bar.

T_E (^{0}C)	P_E (bar)	ρ (kg/m^3)	Yield (%)	Parthenolide yield (mg parthenolide/100 g dry material)
40	200	840.19	2.78	286.1
40	300	910.50	2.74	208.1
60	200	723.19	2.78	208.4
60	300	829.97	3.07	60.1
60	600	968.24	8.06	328.8
60	800	1021.40	7.67	289.4
80	200	594.16	2.08	145.1
80	300	745.54	2.60	70.0
80	600	915.86	9.11	216.7
80	800	976.27	8.79	308.1

Results presented on **Figure 1** show that with increasing density from 600 to 800 kg/m^3 extraction yield increases slowly from 2 to 3 % and is independent of temperature. Rapid increase of yield is observed in density range from 800 to 1000 kg/m^3 and temperatures 60 and 80 °C.

On **Figure 2** parthenolide yield vs. solvent density is presented. It is clear that solvent density has a great impact on parthenolide obtained in *Tanacetum parthenium* extract. With increase of temperature at constant pressure the density of CO_2 decreases. Consequently, the viscosity decreases, what also reduces the solvent power. However, at very high pressures (600 and 800 bar) in spite of high temperature (60 and 80 °C), the density and viscosity of CO_2 are high enough to achieve good solubilizing capacity. The highest parthenolide yield is obtained at 600 bar and 60 °C and is 328.75 mg parthenolide/100 g material.

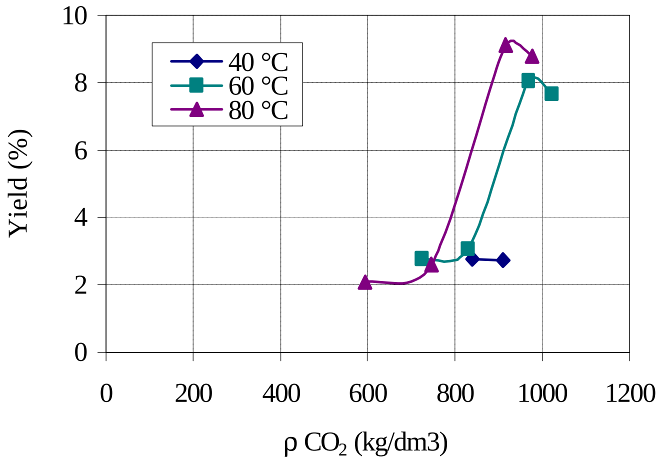

Figure 1: Semi continuous extraction of flower heads of *Tanacetum parthenium* with SC CO_2: extraction yield vs. solvent density.

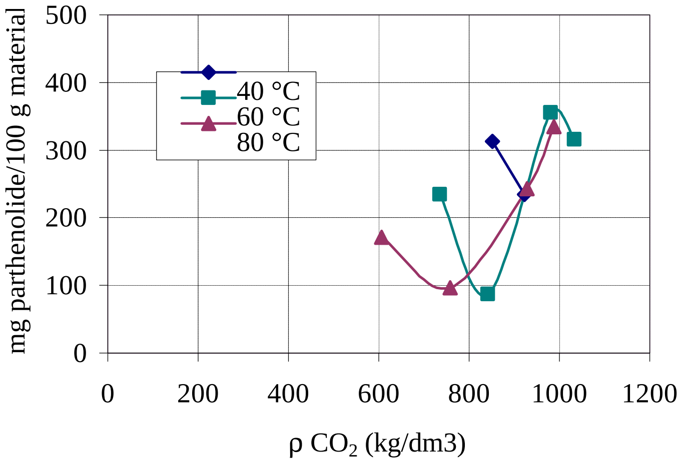

Figure 2: Semicontinuous extraction of flower heads of *Tanacetum parthenium* with SC CO_2: parthenolide yield vs. solvent density.

The operating conditions and results of SC CO_2 extraction experiments of *Tanacetum parthenium* followed by two-step separation are presented in **Table 2** and **Figure 3**. In order to observe the separation of active and waxy components the distribution coefficient of parthenolide was calculated:

$$K = \frac{(mg\ parthenolide/100g\ extract)_{S2}}{(mg\ parthenolide/100g\ extract)_{S1}}. \qquad (2)$$

Table 2: Operating parameters and results of the SC CO_2 extraction of *Tanacetum parthenium*: two-step separation, where T_{S2}= 20 °C and P_{S2}= 1 bar.

T_E (^{0}C)	P_E (bar)	T_S (^{0}C)	P_S (bar)	Yield S1 (%)	Yield S2 (%)	Parthenolide yield in S1 (mg parthenolide/100 g dry material)	Parthenolide yield in S2 (mg parthenolide/100 g dry material)	K
60	300	60	100	2.80	0.21	96.2	7.3	1.01
60	300	44	100	2.10	1.03	79.3	134.2	3.45
60	300	60	45	3.35	0.07	402.1	11.3	1.33
80	300	60	100	2.20	0.19	88.8	19.5	2.55
80	300	44	100	2.30	1.26	49.2	181.6	6.72
80	300	60	45	3.65	1.80	116.5	3.5	0.62

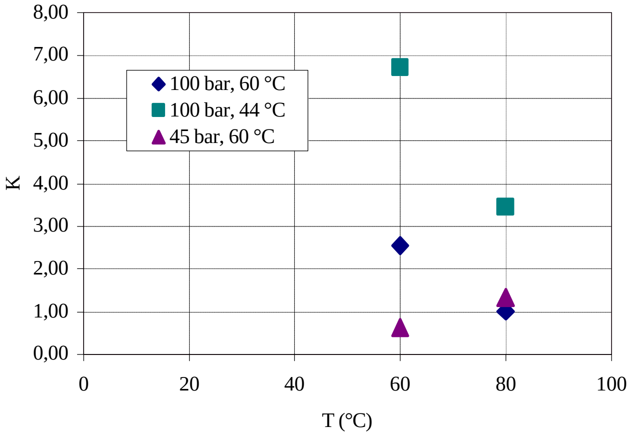

Figure 3: Distribution coefficient as function of extraction temperature at constant separation conditions.

Results show that best separation of parthenolide and waxy components under the conditions investigated was achieved when the pressure and temperature in the first separator (S1) was 100 bar and 44 °C. The yield of parthenolide obtained in second separator (S2) was in this case 181.6 mg /100 g dry material.

The results of conventional extractions of *Tanacetum parthenium* are shown in **Table 3**. The highest parthenolide content was obtained in methanol extract and was 763.6 mg/100 g dry material.

Table 3: Results of conventional extractions.

Solvent	Yield (%)	Parthenolide yield (mg parthenolide/100 g dry material)
Acetonitrile	2.40	319.1
Ethanol	4.53	167.3
Methanol	18.54	763.2
Water	18.35	0.0
Hexane	1.09	2.9

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