Pyrethrin extraction from pyrethrum flowers using carbon dioxide

H.K. Kiriamiti, S. Camy, C. Gourdon, J.S. Condoret *

Laboratoire de Génie Chimique, UMR CNRS 5503, ENSIACET, 118, Route de Narbonne, 31062 Toulouse Cédex 4, France

Received 18 February 2002; received in revised form 27 June 2002; accepted 10 July 2002

Abstract

Extractions of pyrethrins from ground pyrethrum flowers, using supercritical carbon dioxide as the solvent, were carried out in a semi-batch pilot plant (extraction volume: 200 ml). Extracts were very similar to those obtained by hexane Soxhlet extraction, except that the ratio of pyrethrins I to pyrethrins II was lower (1.58 instead of 1.79), and less pigments were present. At 40 °C, the amount of crude pyrethrum extract was found to be independent of pressure above 100 bar. Pyrethrin content in the crude extract was shown to be higher at 20 °C than at 40 °C and decreased with decrease in pressure. Effect of particle size was investigated and the biggest particles yielded a lower quantity of extract and contained less pyrethrin. Extract obtained from small particles, at 40 °C, contained more undesired product. We also established that the seed part of the whole flower contained more crude extract and pyrethrins than the flower part. Pre-treatment, by preliminary SC-CO2 washing of unground flowers, improved the quality of SC-CO2 extract, because a part of the undesired waxes was eliminated by this pre-treatment.

Keywords: Supercritical CO2 extraction; Pyrethrum; Pyrethrins; Natural insecticide

1. Introduction

Pyrethrum, also called chrysanthemum, is a perennial herb with white—yellow flowers that grows to a height of about 60 cm. In most cases, pyrethrin content in the flower ranges from 0.5 to 2% of the dry flower mass. In conventional processing, flowers are ground and extracted with an organic solvent to yield pyrethrum extract or insecticidal essential oils. The main objective of the extraction process is to obtain a light coloured product, with a high recovery of pyrethrin active ingredients.

The most active ingredients in pyrethrum extract are pyrethin I and pyrethin II. Others are jasmolin I, cinerin I, jasmolin II and cinerin II. Pyrethrin I alone is toxic, acting in minutes, but pyrethrin II alone stuns insects in seconds, thus it has a high knockdown effect, although insects easily metabolise pyrethrin II and recover in a few hours. Combined use of pyrethrin I and II has an outstanding effect for household pest control [1].

* Corresponding author
E-mail address: jeanstephane.condoret@ensiacet.fr (J.S. Condoret).
Facing growing solvent regulations, extraction by carbon dioxide of essential oil from pyrethrum flower is worth considering. Furthermore, it is operated at low temperature and simpler extraction purification processes are expected, together with better quality of the extract.

In their earliest works, Stahl et al. [2] observed that, between 20 and 40 °C, no decomposition of pyrethrins occurred in carbon dioxide. Marr et al. [3] identified the six active ingredients in pyrethrum extract, using high performance liquid chromatography (HPLC) and observed that the pyrethrins I were extracted faster than pyrethrins II. In 1980 Sims [4] described and patented an extraction process from pyrethrum flowers with liquid carbon dioxide, which yielded a pale, transparent, concentrated extract of pyrethrins. Wynn et al [5] described a preparative SC-CO₂ extraction process from pyrethrum flowers at 40 °C and 80 bar. Very recently, Otterbach et al. [6] compared pyrethrum extract obtained by ultrasonic extraction, Soxhlet extraction using hexane, and SC-CO₂ extraction, and observed that the SC-CO₂ process yielded better quality in terms of colour and pyrethrin content.

In this paper we have studied the effect of pressure, temperature, particle size and pre-treatment of raw material upon quality and quantity of crude extract obtained with carbon dioxide extraction.

2. Material and methods

The pyrethrum flowers were bought from local farms in Kenya. Soxhlet extraction by hexane enabled us to determine the total hexane extractable pyrethrin content in the flower. An average pyrethrin content of 1.14% w/w was obtained. This value is here referred to as total hexane extractable pyrethrins content (THEPC). Indeed, dry pyrethrum ‘whole flowers’ consist of seed part (that is mature dry ovary of floret), supported on the flower stalk and dry stigma, anthers, corolla, stamens, calyx, style, referred to as ‘flower part’. Fig. 1 presents a photograph of a whole flower indicating the so-called flower part and seed part. By conventional solvent extraction, Haunold [7] observed that seeds contain more pyrethrin than the flower part.

The CO₂ extraction was performed with a pilot plant from Separex Chimie Fine, France (series 3417 type SF 200), having an extraction capacity of 200 ml and a maximum CO₂ flow rate of 5 kg/h. The extractor is connected with three separators in series of 15 ml each. The schematic diagram of the pilot plant is shown in Fig. 2. Pressures in extractor and separators were varied as shown in Table 1. The extractor and separators are jacketed to maintain a constant temperature. The temperature in all the separators was set to 40 °C because, at lower temperatures, the extract in the separators was solid and not easily removable, while in the extractor the temperature was either 20 or 40 °C.

Analyses of the extracts were performed using HPLC Hewlett-Packard series 1050 chromatograph equipment with a 250 × 4.6 mm column Lichrosorb SI60 5 μm, following the method proposed by Marr [3]. Elution was conducted with a mixture of acetyl acetate and hexane in a ratio of 1:10 at a constant flow rate of 1.5 ml per minute, leading to a 15-min analysis. The UV-detector was set at 242 nm wavelength. A refined pyrethrin sample was bought from RdH laborech-emikalien & Co. KG (Germany) for standardisation of the analytic method. Its pyrethrin content was claimed to be 21.1%.

The flowers were ground and the particle size distribution was determined by sieve analysis. The samples were placed in the extractor cylinder, and filter mesh screens are placed at both ends of the cylinder, to prevent any unwanted escape of particles. The cylinder was then introduced into the temperature-controlled extractor. Care is taken to ensure that the air is purged before extraction is started. The CO₂ is pumped at constant flow rate

![Fig. 1. A cross-section view of a dry pyrethrum flower.](image_url)
and directed into the bottom of the extractor for upflow extraction configuration. The fluid phase from the extractor is passed through the vessels where the pressure is throttled via the three separators in series, and then the CO₂ is cooled and recycled again into the system. The extracts from all separators were collected at regular intervals as a single sample, which was weighed and analysed. When mixed together with all the preceding samples, they constituted the cumulative crude extract, which was recorded as a function of time, representing the cumulative mass extraction curves. The average mass extracted ranged from 2 to 8 g. At the end of extraction CO₂ is purged in order to weigh the residue in the extractor. Taking the difference of the mass before and after the experiment in the extractor as a reference, the actually collected mass of extracts was in an average range of 12.4% lower than this reference mass. This loss was due to traces of extract which remain on the internal surface of three 15 ml separators. In all experiments, the flow rate was kept constant at 3.02 kg/h.

3. Results

3.1. Crude extracts and total mass extracted

All experiments were performed with batches of 50.0 ± 0.3 g of unsieved ground flowers at 70, 100 and 250 bar, while temperature was maintained at 40 or 20 °C. The SC-CO₂ extract lacks undesirable colouring matter and chlorophyll pigments, the colour range being from yellow to orange. On exposure to air, the yellow colour changes to orange. It is a viscous product, which is not totally soluble in hexane, but soluble in acetone and excess methanol. It has a strong characteristic smell of pyrethrum flower. Indeed, Pyrethrum extract consists of vegetable oil, pigments, pyrethrins and waxes. Fig. 3 shows the peaks of the six major active ingredients in pyrethrum extract obtained from SC-CO₂ extraction.
Fig. 4a and b show the cumulative mass extraction curve in crude extract at different pressures and temperatures. The total mass extracted at 40 °C was observed to be higher than that at 20 °C. We obtained the highest crude extract mass at 40 °C and 250 bar. On Fig. 4a, at 20 °C, we observed that, at the beginning of extraction (up to 100 min), extraction curves are not dependent on pressure. The most probable reasons are that under these conditions (i) the variation of density with pressure is moderate (150 kg/m³ between the lowest and the highest pressure in contrast with 40 °C where the density variation is 678 kg/m³), so the solubility is almost the same, (ii) the amount of soluble undesirable product extracted is very low.

At 40 °C (Fig. 4b), cumulative mass extraction curves at 100 and 250 bar are similar throughout the extraction time, and it was noted that, at 70 bar, quantity of extract is lower due to low density.

At both temperatures and above 100 bar, the extraction curves of crude extract are almost independent of pressure.

3.2. Pyrethrin extraction

As it was mentioned previously the extracted mass includes undesired products, like waxes and triglyceride oil. Fig. 5a and b show percentage pyrethrin extracted, at 20 and 40 °C, respectively, as a percentage of the THEPC at different pressures. For both temperatures, it was observed that the amount of pyrethrin extracted decreases with pressure decrease. This is due to density effects on pyrethrin solubility. At constant pres-
sure, it was observed that more pyrethrin was extracted at 20 °C than at 40 °C. This is due to a slightly higher density of carbon dioxide. Furthermore, the solubility of undesired products being slightly lower, at low temperature the extraction of pyrethrin is favoured at the expense of these undesired products.

3.3. Crude extract quality

Additional information about the extraction mechanism may be drawn from the curves of Fig. 6. It was observed that at 20 °C the pyrethrin mass fraction of cumulative crude extract was higher than at 40 °C. Moreover, the mass fraction of pyrethrins in extracted sample always decreases with extraction time as shown in Fig. 6a and b. This indicates that at the beginning of the extraction, pyrethrins are extracted faster than undesired products. In all SC-CO2 extracts, whatever the extraction conditions, the same mass fractional percentages of different pyrethrins were obtained. They were: jasmolin I 4.5%, cinerin I 5.2%, pyrethrin I 51.5%, jasmolin II 2.6%, cinerin II 3.97% and pyrethrin II 32.2%. Thus, the ratio of pyrethrins I to pyrethrins II was 1.58, while, in our experimental hexane Soxhlet extract, it was 1.79.

3.4. Effect of particle size

A series of experiments was carried out with 50.0±0.3 g of sieved particle, separated into four classes: less than 0.2, 0.2–0.5, 0.5–0.9 and 0.9–1.4 mm. They were extracted under two different operating conditions: 20 °C–100 bar and 40 °C–250 bar.

At 100 bar–20 °C (condition 1), as shown in Fig. 7a, the mass of crude pyrethrum extract decreases with increase in particle size. At 250 bar–40 °C (condition 2, Fig. 7b), for particles <0.9 mm, there is slight difference in the mass of crude pyrethrum extract. In both conditions, the amount extracted for large particle sizes (0.9–1.4 mm) was low; this could be interpreted either by the occurrence of limited internal mass transfer, or more simply by the fact that these big particles are mainly non-seeds, and are likely to contain less extractable material (as it will be seen below).

![Fig. 6. Pyrethrin mass fraction in cumulative crude extract (a) 20 °C, (b) 40 °C. △, 70 bar; ○, 100 bar; □, 250 bar; ▲, 70 bar; ●, 100 bar; ■, 250 bar.](image)

![Fig. 7. Amount of crude extract for different particle sizes at (a) 100 bar and 20 °C; (b) 250 bar and 40 °C. ○, < 0.2 mm; △, 0.2–0.5 mm; ◆, 0.5–0.9 mm; □, 0.9–1.5 mm; ●, < 0.2 mm; ▲, 0.2–0.5 mm; ◆, 0.5–0.9 mm; ■, 0.9–1.5 mm. Initial load: 50.0±0.3 g of sieved ground flowers.](image)
Under condition 1, it was observed that the amount of pyrethrins in crude pyrethrum extract was almost the same, except for small particles less than 0.9 mm (see Fig. 8a). In the case of condition 2, the pyrethin content was higher in particles between 0.2 and 0.9 mm (see Fig. 8b). This indicates that small particles (<0.2 mm) have more undesired products, since they yielded more crude extract but contain the same amount of pyrethrins as found in particles 0.2–0.9 mm. Under both conditions, pyrethin content of big particles (between 0.9 and 1.5 mm) proved to be low and similar. In condition 2, we observed that extraction of undesired products was favoured.

Fig. 9a and b shows the variation in time of pyrethrin mass fraction in cumulative crude extract, in small particles and big particles, and for both conditions. In both cases, pyrethin content was higher at 100 bar–20 °C, and at the beginning of extraction.

From these results, it is confirmed that at 250 bar–40 °C, more undesired products are extracted, which causes low pyrethrin content in the crude extract. The bigger particles have lower pyrethrin content, the smaller particles have more undesired products and the medium size particles have more seed particles, which contain more pyrethrins.

3.5. Effects of raw material pre-treatment

Another series of experiments was carried out with 23.5±0.4 g of pre-treated material at 250 bar–40 °C.

3.5.1. Pre-treatment by segregation of the flower part

Flower parts and seed parts, as described on Fig. 1, were manually separated and each fraction was separately extracted with SC-CO₂ after grinding. It was noticed, in the case of the seed part, more crude extract (Fig. 10a), as well as a higher mass of pyrethrins (Fig. 10b), were obtained. It was observed that mass fraction of pyrethrins in cumulative crude extract from seed parts was higher than from flower parts (Fig. 11a). For both cases, pyrethrin content decreases with time,
which indicates occurrence of extraction of more undesired product, but this phenomenon is less pronounced in the case of the seed parts.

3.5.2. Pre-treatment by pre-extraction of ungrounded material

A sample of ungrounded whole flower was pre-extracted, an operation referred to here as washing, with CO$_2$ at 250 bar–40 °C, for one hour before being ground and extracted again with CO$_2$, while an identical sample of whole flower was ground and directly extracted with CO$_2$ as a control experiment. The whole flower (see Fig. 12a) yielded more crude extract than the washed whole flower. On the other hand, also the washed whole flower exhibited higher pyrethrin content in crude extract than plain whole flower (see Fig. 12b). Therefore, washing of the whole flower sample with CO$_2$, before extraction, has improved the pyrethrin content in crude extract. This is probably due to the fact that washing before grinding removed undesired products, mainly located on the surface of the flowers. Indeed, waxes are likely to be located on the surface as reported by Reverchon [8]. Fig. 10b shows mass fraction of pyrethrins in cumulative crude extract. It was observed that washed sample
extract showed a negligible change in pyrethrin mass fraction in cumulative crude extract, which means that the rate of extraction of the undesired product occurred at a constant ratio referred to rate of pyrethrin extraction.

In Fig. 11 the flower part and plain whole flower always showed a significant decrease in pyrethrin mass fraction in their respective cumulative crude extract and this indicates that the flower part and plain whole flower yield more undesired products. It was also noted that the final quality of the washed sample was better than the unwashed. This confirms that most of the pyrethrins are located in the core of the whole flower and most of the undesired products in the surface of the flower.

4. Conclusion

This study has confirmed the feasibility of SC-CO₂ extraction of pyrethrins from pyrethrum flowers. The extract is very similar to the one obtained in n-hexane, still containing waxes and oil. The effect of temperature and pressure upon the relative amounts of pyrethrins and undesired products extracted, were investigated, and extraction at 20 °C (in fact with liquid CO₂) leads to better selectivity. From the study of the particle size effects, we have proposed some attempts of pre-treatment of the raw material that could improve the quality of the extract. Nevertheless, the key to such a new process lies in the integrated purification of the extract, using the fractionation potential of supercritical fluids. Our next stage is to improve quality by carrying out a pre-fractionation by a cascade of depressurisations, after the extractor. In this case, the purification step could be simplified and such a process could compete with the conventional one, but having the great advantage of providing a ‘natural’ insecticide extracted by a clean technology.

References