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Supercritical CO_2 processing strategies for pyrethrins selective extraction

Lucia Baldino[a], Giovanna Della Porta[b], Ernesto Reverchon[a,*,1]

[a] Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II, 132, 84084, Fisciano, SA, Italy
[b] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Via Salvatore Allende, 1, 84081, Baronissi, SA, Italy

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ABSTRACT

The extraction of pyrethrins from chrysanthemum flowers has been performed using supercritical CO_2 (SC-CO_2) extraction coupled to fractional separation, with the aim of producing complete and selective extraction of these insecticidal principles. 90 bar, 40 °C were selected as the operative conditions for a first step of extraction, to work at moderate SC-CO_2 density; two separators in series operating at −10 °C and at a pressure equal to that of extraction, and 25 °C, 15 bar, were used for fractional separation, respectively. A second SC-extraction step, performed at 200 bar 40 °C, demonstrated that many undesired compounds were co-extracted at these process conditions. The comparison with liquid extraction, using petroleum ether, showed that using SC-CO_2 and an exhaustive processing, 30% more pyrethrins were extracted. Using the fractional separation, the produced extracts contained about 99% w/w pyrethrins if the process was stopped after about 80 min. Another suggested duration of the extraction process can be set at about 250 min, at which about 63% w/w pyrethrins were contained in the extracted material. The adopted process strategy could be readily extended to the supercritical processing of several other materials and to the industrial scale.

1. Introduction

Supercritical CO_2 (SC-CO_2) extraction is the green alternative to the traditional organic solvents extraction from vegetable matter, since it avoids pollution of environment, residues and products related to the use of toxic organic solvents. It can be successfully applied to produce solvent-less, selective extracts for food, nutraceutical, pharmaceutical and biomedical applications [1–3]. Some applications have already been developed up to a wide industrial scale, such as for example for caffeine extraction from green coffee beans and tea leaves [4–6], and hop extraction for beer industry [7,8]. SC-CO_2 processing has also been proposed in several other fields: micronization [9], membranes formation [10,11] and biomedical applications [12,13].

One possible industrial application of SC-CO_2 processing, can be the extraction of natural pesticides from vegetable matter, though a relatively small number of scientific papers have been published on the extraction of biopesticides using this technique [14]. For example, the extraction of azadirachtin from neem seeds [15,16] and of rotenone from *Derris elliptica* [17] have been proposed. Another natural pesticide are pyrethrins, extracted from *Chrysanthemum cinerariaefolium,* using hexane [18] or petroleum ether [19,20] as the extracting agent. This process is characterized by a relatively low selectivity; indeed, many undesired compounds are co-extracted, mainly coloring matter, chlorophyll, waxes and resins. The obtained extract cannot be used as a pesticide in the cultivation of biological products due to its contamination with the organic solvent residues. For these reasons, SC-CO_2 extraction has been proposed by some authors as an alternative to traditional pyrethrins processing. Stahl and Schütz [21] tested SC-CO_2 extraction from chrysanthemum flowers in a wide range of pressures and temperatures, showing that the decomposition of the active principles occurs at temperatures larger than 60 °C and the most appropriate range could be between 20 °C and 40 °C. However, a viscous extract was obtained due to the co-extraction of waxes and other compounds. Pan et al. [22] also extracted by SC-CO_2 pyrethrins from chrysanthemum flowers; they determined the most efficient extraction period, that was the first 180 min of processing; but, waxes were not separated. Marongiu et al. [23] first extracted a volatile fraction operating at 90 bar, 50 °C; then, pyrethrins were extracted together with several higher molecular weight compounds at 300 bar, 40 °C. Kiriamiti et al. [24] performed the SC-CO_2 extraction with no fractionation at 40 °C. The unresolved problem was the co-extraction of waxes at all process conditions, that can/could be resolved by washing unground flowers with SC-CO_2, followed by grinding as a pretreatment prior to SC-CO_2 extraction of the material. However, also part of pyrethrins can be co-extracted. In another work, Kiriamiti et al. [25], analyzed a different extraction strategy to obtain refined extracts from

* Corresponding author:
E-mail address: ereverchon@unisa.it (E. Reverchon).
[1] Website: www.supercriticalfluidgroup.unisa.it.

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pyrethrum flowers: to use hexane to obtain crude pyrethrins extract and, then, processing the extract by SC- or liquid-CO_2 at 100 bar, 40 °C or 80 bar, 29 °C, respectively. This process is sometimes described in the literature as Supercritical Antisolvent Extraction (SAE) and it is particularly successful when a solution of an organic solvent contains compounds that are not soluble in SC-CO_2 or shows relatively low solubilities in it [26]. In the case of the hexane extract of chrysanthemum flowers, this strategy could be only relatively successful, since pyrethrins are highly soluble in liquid CO_2 (due to its high density) and show a relatively high solubility also in SC-CO_2; therefore, the selective elimination of hexane from the crude extract cannot be obtained. Gallo et al. [27] compared SC-CO_2 extraction with traditional processes based on organic solvents. They produced an oleoresin (extracting at 40 °C, 400 bar) containing all SC-CO_2 extractable products and authors concluded that it could be useful to perform a post solvent extraction process, to eliminate organic solvent residues. However, this indication does not take into account the fact that, since pyrethrins are soluble in SC-CO_2, they will be at least partly co-extracted together with solvent residues.

Summarizing, the analysis of the previous literature confirms the feasibility of extracting pyrethrins from chrysanthemum flowers and shows some attempts at the processing of the vegetable material at different pressures and/or temperatures; but, limited selectivity was obtained due to the co-extraction of other compounds contained in the natural matrix: at low SC-CO_2 densities, they are mainly waxes that cover all vegetable structures like flowers, leaves and seeds [22,27]; when the processing pressure is increased, also higher molecular weight compounds are extracted. Nevertheless, high density SC-CO_2 extraction was performed in several of the papers previously discussed and in all cases the authors never attempted to use more selective process strategies.

To perform a successful SC-CO_2 extraction, it is relevant to apply the right processing strategy. It is possible to perform fractional separation of the extracts with the final scope of improving process selectivity and obtaining concentrate extracts, eliminating unwanted co-extracted products, that are present in the starting material [28–30]. The fractional separation of SC-CO_2 extracts has been successfully used by Reverchon and coworkers in the extraction of volatile compounds from some vegetable structures [28,29]. Indeed, vegetable waxes (paraffins) covering natural structures, show a relatively low solubility in SC-CO_2 with respect to terpenes, sesquiterpenes, diterpenes and other low molecular weight compounds; but, they are already extracted, because they are located on the surface of the vegetable matter, where they are readily accessed by SC-CO_2. The compounds of interest, instead, even when they are largely soluble in SC-CO_2, are, as a rule, located inside the vegetable structure: therefore, the extracting solvent has to overcome a relevant mass transfer resistance to solubilize these compounds [3,30]. To demonstrate the potential efficiency of smart supercritical fluid extraction process strategies to obtain the selective separation of active compounds, in this work, pyrethrins extraction by SC-CO_2 followed by two separations in series performed at different operating conditions is used. In the first cooled separator, selective precipitation of waxes is attempted, to produce a more concentrate pyrethrins extract, that is, then, collected in the second separator. Processing in a multi-step extraction mode is also performed, to evidence the completeness of the pyrethrins extraction process and to show the co-extraction of other undesired compounds. Comparison with petroleum ether extraction is also performed.

2. Apparatus, materials and methods

Supercritical CO_2 extraction was performed in a laboratory apparatus equipped with a 490 cm^3 internal volume extractor. The fractions extracted were recovered using two separation vessels of 200 cm^3 each, operated in series. The cooling of the first separator was achieved using a thermostated bath. The second separator allowed the continuous discharge of the product. A high-pressure membrane pump (Lewa, mod. LDB1 M210S), pumped liquid CO_2 at the desired flow rate. CO_2 was then heated to the extraction temperature in a thermostated oven. The extraction was carried using a continuous flow of the solvent. CO_2 flow was monitored by a calibrated rotameter (ASA, mod. N.5-2500, Serval 115022) located after the last separator. Temperatures and pressures along the extraction apparatus were measured by thermocouples and test gauges, respectively. Pressure was controlled manually by high pressure valves. More details about the apparatus were given elsewhere [31].

Dried flowers of *C. cinerariaefolium* were supplied by Aboca. They were grounded down to a mean particle size of 110 μm (measured by mechanical sieving) that should represent a good compromise to avoid problems of channeling or caking in the extraction bed (too small particles) and duration of the extraction process (too large particles). When received, the vegetable matter had a water content of about 10%, that is an usual percentage for industrial dried material and does not worsen the extraction process [30]. Pyrethrins are a group of six compounds that share a $C_{18}H_{22}O_3$ core having different substitutes in two positions at the two end of the molecule: Pyrethrin I (m.w. 328.5 Da, $C_{21}H_{28}O_3$), Cinerin I (m.w. 316.4 Da, $C_{20}H_{28}O_3$), Jasmolin I (m.w. 330.5 Da, $C_{21}H_{30}O_3$), Pyrethrin II (m.w. 372.5 Da, $C_{22}H_{28}O_5$), Cinerin II (m.w. 360.4 Da, $C_{21}H_{28}O_5$), Jasmolin II (m.w. 374.5 Da, $C_{22}H_{30}O_5$). A mixture of these compounds was used as the external standard to calculate the gas chromatographic detector response factor; they were supplied by Sigma Aldrich. Petroleum ether (purity 98%) was used to obtain a solvent extract and was supplied by Fluka. CO_2 (purity 99.9%) was supplied by Morlando Group S.R.L. (Sant'Antimo, NA – Italy).

In each extraction test, the 490 cm^3 extractor was charged with about 200 g of chrysanthemum flower particles. CO_2 flow rate was generally set at 0.8 kg/h. Before charging the extractor, the powder was mixed with 100 g of glass beads of 2 mm diameter, to avoid bed caking and channelling of SC-CO_2 in the extractor. All the experiments were performed in duplicates.

The traditional extraction by petroleum ether was performed using a 1:9 ratio between the vegetable matter and the organic solvent. After one day of maceration, the extract was dried by heating at vacuum conditions.

The gas chromatographic-mass spectrometric (GC–MS) apparatus was a Varian (mod. Saturn 2100T, San Fernando, CA). Separation was achieved using a fused-silica capillary column (mod. DB-5, J & W, Folsom, CA) 30 m length, 0.25 mm of internal diameter, 0.25 μm film thickness. GC conditions were: oven temperature of 40 °C for 5 min, then, programmed heating from 40 to 250 °C at 2 °C/min and subsequent holding at 250 °C for 60 min. The injector was maintained at 190 °C (splitless 20 cm^3/min) and helium was used as the carrier gas (1 cm^3/min). Samples were run in dicloromethane with a dilution factor of 0.05% w/w.

Pyrethrins content in the extracts was converted into an absolute value using the mass spectrometer relative response factors. The response factor was calculated using the mixture of the six pyrethrins as an external standard. The calibration curve was obtained injecting five solutions with different content (measured in ppm: solute mg/solution kg) of the six pyrethrins and measuring the intensity of the mass spectrometer response. The resulting equation was $y = 1.098*10^{-4}x$.

Waxes recovered in the first separator were identified by matching their mass spectra and retention times with those of pure paraffins.

3. Results and discussion

Based on previous experiences of our research group in the SC-CO_2 extraction of volatile and medium molecular weight compounds [29,31], on a preliminary (conference paper) study on pyrethrins extraction [32] and on the work of Stahl and Schütz [21], operative