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Scale-up and Economic Analysis of a Supercritical CO_2 Plant for Antimalarial Active Compounds Extraction

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Artemisia annua L. is characterized by a strong antimalarial activity due to the presence in its aerial parts of artemisinin and its derivative compounds: dehydroartemisinin and artemisin. These compounds are also antiulcerogenic, antifibroric and antitumoral against P-388 (murine lymphocytic leukemia), A-549 (human lung carcinoma) and HT-29 (human colon adenocarcinoma) cells.
A. annua extract is generally obtained by *N*-hexane extraction; but, this process is not selective, can induce extract degradation and requires post-processing to eliminate the organic solvent used.
In this work, supercritical CO_2 (SC-CO_2) extraction coupled to fractional separation of a solvent extract is proposed as an eco-friendly alternative to overcome these limitations. In particular, a semi-solid, waxy product (i.e., concrete) from ground *A. annua* leaves is obtained by hexane extraction; then, it is treated by SC-CO_2 selective fractionation and extraction at 90 bar and 50 °C, using a CO_2 flow rate of 0.8 kg/h.
Operating in this manner, storage and transportation costs can be reduced since the simpler parts of the process (organic solvent extraction and drying) are performed in the area where *A. annua* is cultivated and the intermediate product (i.e., *A. annua* concrete) is subsequently delivered to the SC-CO_2 plant. Moreover, the volume of the high-pressure extractor is 20 times smaller with respect to the one used to treat the equivalent quantity of vegetable matter by direct SC-CO_2 extraction since, in this case, the feed is the organic solvent extract. Therefore, the high-pressure pilot plant will be about 7 times less expensive in terms of equipment involved, if compared with the direct supercritical processing of the ground vegetable material.

1. Introduction

The extraction of active principles from vegetable matter is one of the major research fields in the scientific literature, due to the industrial interest for this kind of natural compounds to be used in medicine, cosmetic and food industry (Azmir et al., 2013; Wijngaard et al., 2012).
Generally speaking, the extraction process from vegetable matrix is performed using organic solvents, such as ethanol, methanol, ethyl acetate and hexane (Chemat et al., 2012; Ratheesh et al., 2009). But, these solvents can be dangerous for the final product and the environment. Moreover, the traditional extraction process is not selective with respect to the compound/s of interest; as a consequence, a large amount of organic solvent is required to extract the soluble compounds from the vegetable matrix; but, many of them show no activity.
In order to overcome these problems, a different processing has been proposed in the literature and, in some cases, up to the industrial scale (Aliev et al., 2015; Barros et al., 2017; Della Porta et al., 1998; Garcí-Abarrio et al., 2012; Kohler et al., 1997; Reverchon et al., 1994a; Reverchon et al., 1994b; Reverchon et al., 2001): the extraction process assisted by supercritical CO_2 (SC-CO_2).
This technology is attracting growing attention since it is eco-friendly and solvent-less. SC-CO_2 is defined as a GRAS (generally recognized as safe) solvent since it is inert and its critical conditions (p≈74 bar, T≈31 °C) are easy to be reached with respect to other substances (e.g., water) (Kitada et al., 2009). Thanks to these properties, several processes assisted by SC-CO_2 have been developed, also for the production of micro and nano-particles, membranes and aerogels (Baldino et al., 2015; Baldino et al., 2016; Cardea et al., 2014; Prosapio et al., 2015; Reverchon and Antonacci, 2006; Reverchon and Cardea, 2012). Moreover, changing the operative pressure and temperature, it is possible to modulate the SC-CO_2 solvent power; i.e., the process

selectivity can be modified in dependence on the compounds of interest. Operating in this manner, the final extract will contain a larger concentration of active principles with respect to the extract obtained using organic solvents (Baldino et al., 2017; Goto et al., 1996; Peterson et al., 2006; Reverchon et al., 1994a). Moreover, the concept of fractional separation, particularly applied in the separation of cuticular waxes (paraffins) from the extract, thanks to their reduced solubility in CO_2 at low temperatures (<10 °C), is one of the successful key steps of this processing (Baldino et al., 2017).
Artemisia annua L. contains artemisinin, artemisin and dehydroartemisinin that are characterized by an antimalaric activity against drug resistant strains of *Plasmodium falciparum*. It has been also demonstrated that its extracts are antifibrotic (Wang et al., 2012) and have a cytotoxic activity against P-388 (murine lymphocytic leukemia), A-549 (human lung carcinoma) and HT-29 (human colon adenocarcinoma) cells (Zheng et al., 2012).
A. annua extraction is generally performed using *N*-hexane (Bilia et al., 2006; ElSohly et al., 1987; Ahmad et al., 1994), since it has been demonstrated that other organic solvents, such as trichloromethane and petroleum ether, produce an extract characterized by a lower artemisinin concentration (Hao et al., 2002).
In order to resolve the process limitations previously described, Kohler et al. (1997) proposed the extraction of active principles from *A. annua* by SC-CO_2 plus 3% methanol, operating at a flow-rate of 2 mL/min, 50 °C and 150 bar, for about 20 min. Tzeng et al. (2007) performed a SC-CO_2 extraction adding 16.25% ethanol as co-solvent to obtain scopoletin and artemisinin from *Artemisia annua* L. These authors demonstrated that two hours ethanol modified SC-CO_2 extraction were superior to 16 h Soxhlet *N*-hexane extraction in producing more pure artemisinin and scopoletin and the amount of the extracts increased with the density of SC-CO_2. Martinez-Correa et al. (2017) performed a two-step extraction from *A. annua*; in the first one, SC-CO_2 at 400 bar, 60 °C was used to produce a solid residue that was, then, treated with ethanol or water. The supercritical and ethanol extracts obtained in a single step showed the largest yield of artemisinin and were active against *Plasmodium falciparum*. On the other hand, the aqueous and ethanol extracts from the second extraction step were free of artemisinin. Baldino et al. (2017) proposed a supercritical fractional extraction to process *A. annua*. Extracts enriched in active antimalarial principles were produced operating at 100 bar, 40 °C; whereas, waxes were selectively recovered in the first separator, confirming the efficiency of the fractional cooling separation. A concentration of 35% w/w of active compounds was obtained in the second separator. Moreover, these authors tested different SC-CO_2 flow rates; but, the CO_2 flow rate increase from 0.8 to 1.2 kg/h did not determine appreciable variations of the extraction rate of the various compounds, indicating that internal mass transfer resistance mainly controlled the extraction process.
In this work, scale-up and economic analysis of a SC-CO_2 plant for antimalarial active compounds extraction were proposed taking into account the literature results. An hybrid strategy for the extraction process was adopted: in the first step, *N*-hexane extraction from *A. annua* was performed; then, the dried solvent extract was treated by SC-CO_2 extraction to selectively obtain a product concentrated in the active compounds. These results, in terms of SC-CO_2 extraction apparatus and costs, were compared with the ones required for a SC-CO_2 extraction of active compounds starting from ground vegetable material.

2. Materials and methods

2.1 Materials

Artemisia annua L. leaves were supplied by Erbe di Mauro (MC, Italy). They were ground up to a mean particle size of about 200 μm; humidity content was 12% w/w. Artemisin (m.w. 282.3 daltons, $C_{15}H_{22}O_5$), Artemisinin (≥98.0%, m.w. 282.3 daltons, $C_{15}H_{22}O_5$), Dehydroartemisinin (m.w. 284.3 daltons, $C_{15}H_{24}O_5$), used as external standards, and *N*-hexane (anhydrous, 95%) were supplied by Sigma Aldrich. CO_2 (purity 99.9%) was bought by Morlando Group S.R.L. (Sant'Antimo, NA, Italy).

2.2 Extraction using hexane

400 g of ground *A. annua* were immersed in 5.5 L of hexane at room temperature. After 3 days of maceration, the solution was filtered and dried using a rotavapor (Buchi® R-210 Rotavapor® Evaporators), obtaining a so-called concrete.

2.3 SC-CO_2 extraction apparatus

Supercritical CO_2 extraction was performed in a home-made laboratory apparatus equipped with a 50 mL internal volume extractor, in which the extract obtained by hexane processing was loaded in each experiment after mixing with 3 mm glass beads. The extracts by SC-CO_2 were recovered using two separation vessels with an internal volume of 200 cm^3 each. Cooling of the first separator was achieved using a thermostated bath (Julabo, mod. F38-EH). The second separator allowed the continuous discharge of the product. A membrane high-pressure pump (Lewa, mod. LDB1 M210S), pumped liquid CO_2 at the selected flow rate. CO_2

was heated to the extraction temperature in a thermostatic oven. CO_2 flow rate was monitored by a calibrated rotameter (ASA, mod. N.5-2500) located after the last separator. Temperatures and pressures along the extraction apparatus were measured by thermocouples and test gauges, respectively. Pressure was manually controlled by a high pressure valve.

2.4 Characterization of the extracts

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 50 °C for 5 min; programmed heating from 50 to 250 °C at 2 °C/min and subsequent holding at 250 °C for 60 min. The injector was maintained at 280 °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 0.25% w/w.
The content of artemisinin and its derivatives in the extracts was calculated from the gas chromatographic area traces and converted into absolute values using the ion trap relative response factors, that were calculated using the external standards. Other components of the extract (waxes and essential oil components) were identified by matching their mass spectra and retention times with those of pure compounds.

2.5 Scale-up procedure

Scale-up procedure was implemented considering some constrains derived from the laboratory plant experiments:
- maintaining the same extraction process scheme since it was already optimized in terms of equipment position;
- operating at similar residence times of SC-CO_2 in the extractor vessel (about 20 min);
- adding 30% plant costs for automation, as general rule applied for industrial plants;
- using reference plants previously presented in the literature.

3. Results and discussion

Ground *A. annua* leaves were immersed in hexane, according to the procedure described in Materials and Methods. The maceration was performed in an extractor at atmospheric pressure and at room temperature for 3 days. An extract yield of about 3.0% w/w with respect to the starting vegetable material was measured. The hexane solution was, then, filtered and dried. The obtained *A. annua* concrete is shown in Figure 1.

Figure 1: Picture of A. annua concrete.

This intermediate product (12.0 g) was treated by supercritical processing, operating at 0.8 kg/h CO_2 flow rate, 90 bar and 50 °C (CO_2 density of 0.287 g/cm^3). These mild operative conditions were selected since, in this case, it was hypothesized that internal mass transfer was not the major controlling step of the extraction process, because the starting material was the hexane extract and not the untreated vegetable matter. Moreover, the material itself can be modelled as an inert core formed by glass beads, covered by a relatively thin layer of waxy extract (concrete).

The first separator operated at -7 °C and at the same extraction pressure; whereas, the second separator worked at 15 °C and 20 bar. Using this processing scheme, cuticular waxes, mainly formed by odd carbon number paraffins such as *n*-Entriacontane, *n*-Tetracosane and *n*-Nonacosane, were precipitated in the first separator since at low temperature their solubility in CO_2 was drastically reduced.
Operating in this way, after about 900 min processing, the total supercritical extract was 4.5 g, with a final active principles concentration of about 70% w/w (corresponding to 3.0 g).
Once the advantages to treat an intermediate product (i.e., concrete) by supercritical processing instead of the ground vegetable matter was verified, the plant scale-up was performed, considering the constrains described in Materials and Methods. In particular, a pilot extraction plant, organized according to the layout shown in Figure 2, was design using a factor of 1000 (with respect the 50 mL extractor used in this experimentation) and selecting an opportune residence time between the material and the supercritical fluid (about 20 min). It means that to obtain a final extract containing 3.0 kg active compounds, starting from about 12 kg of *A. annua* concrete, an extractor with an internal volume of 50 L should be selected. This kind of extraction process works in batch; therefore, this concrete amount will be feed at each cycle.
In a previous work of our research group (Baldino et al., 2017), it was demonstrated that to process an equivalent quantity of *A. annua* leaves by direct SC-CO_2 extraction, an extractor of about 20 times larger has to be adopted, achieving a final active principles concentration of about 35% w/w. Operating in this manner, instead, storage and transportation costs can be reduced since the simpler parts of the process, namely solvent extraction and drying, are performed in the area where *A. annua* is cultivated and the intermediate product is subsequently delivered to the supercritical plant.

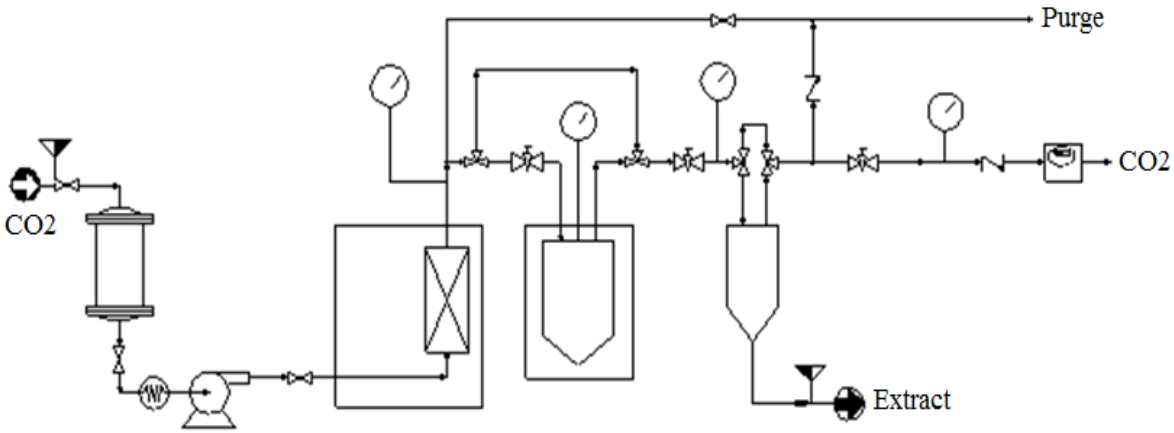

Figure 2: Example of a SC-CO_2 extraction plant layout.

The experience of our research group in the development of laboratory pilot plants and industrial plants based on SC-CO_2 extraction, suggests that laboratory plant with an extraction volume between 0.05 and 0.50 L can have very similar plant costs since, except the extraction volume, all the other parts and utilities of the plant are very similar (e.g., high-pressure valves, high-pressure pumps, pressure and temperature indicators, heating and cooling systems).
Therefore, assuming that a base cost of a 0.50 L SC-CO_2 extraction plant can range between 20000 and 28000 Euro and that the scale-up factor for the plant we want to use is 100, the costs related to the larger plant will be about 300000 Euro, taking also into account the cost addition due to the automation devices (about 30%). The scale-up results are summarized in Table 1.
It is worth to note that the cost of an industrial plant is not linearly dependent on the ratio of its volume with respect to the reference one. Therefore, the cost of a plant having a volume 20 times larger is not 20 times higher. As a result of the application of scale-up cost rules, a 1000 L SC-CO_2 extraction plant to treat ground *A. annua* leaves, will cost about 2000000 Euro; i.e., approximately 7 times more.

Table 1: Summary of the supercritical extraction plant scale-up costs

Extractor volume, L	*A. annua* concrete, kg	*A. annua* active principles, kg	Plant costs, Euro
0.05÷0.50	0.012÷0.120	0.003÷0.030	20000÷28000
50.00	12.00	3.00	≈300000

4. Conclusions

SC-CO_2 extraction coupled to fractional separation has been proposed as an eco-friendly alternative to overcome the limitations of the traditional extraction process using organic solvents.
An extract containing an active principles concentration of 70% w/w was obtained, when the *A. annua* concrete was treated by SC-CO_2.
Operating in this manner, the volume of the extractor was 20 times smaller with respect to the extractor used to treat the equivalent quantity of vegetable matter by direct SC-CO_2 and, correspondingly, it was about 7 times less expensive with respect to the equipment involved, if compared with the direct supercritical processing of the ground vegetable material.
This hybrid process scheme responds to requisites of process intensification and can be also applied to other vegetable matrices.

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