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Optimization of essential oil supercritical extraction from Algerian *Myrtus communis L.* leaves using response surface methodology

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ABSTRACT

The present work deals with the application of the supercritical fluid extraction process to extract essential oils from the leaves of an Algerian myrtle plant (*Myrtus communis L.*). Using the surface response methodology, an optimization of the extraction recovery was carried out, varying the pressure in the range of [10–30 MPa], the temperature within [308–323 K], a solvent flow rate fixed at 0.42 kg h^{-1} and a mean particle diameter equal to 0.5 mm or less than 0.315 mm. The maximum value of essential oil recovery relative to the initial mass of leaf powder was 4.89 wt%, and was obtained when the SC–CO_2 extraction was carried out under 313 K, 30 MPa and with a particle diameter less than 0.315 mm. A second-order polynomial expression was used to express the oil recovery. The calculated mass of recovered oil using the response surface methodology was very close to the experimental value, confirming the reliability of this technique.

1. Introduction

The extraction and/or fractionation of natural complex mixtures in order to obtain pure compounds or concentrates of interest are the subject of extensive research. For this purpose, supercritical fluid extraction (SFE) process is widely used as an alternative way to traditional techniques like among others, steam distillation (with or without vacuum) or organic solvent extraction [1].

Actually, the application of SFE is growing continuously due to its several advantages over the conventional solvent extraction processes. The most widely used supercritical fluid is CO_2 which is non-toxic, relatively inert, and non-flammable; it is considered as a GRAS (Generally Recognized As Safe) solvent and available, as a by-product of the chemical industry. Supercritical CO_2 can be rather selective and its solvent power can be improved by tuning on pressure and temperature. Moreover, CO_2 can be used to extract thermolabile compounds due to its low critical temperature (304.21 K). Finally, another interesting property is that CO_2 is gaseous at ambient conditions of temperature and pressure, allowing a spontaneous and complete separation from the extract and residue. At industrial scale, CO_2 is recycled, hence enabling a clean and compact operation [2–5].

The common myrtle (*Myrtus communis L.*) belongs to the Myrtaceae family which includes more than 5650 species that are known to be rich in essential oils [6]. It is a small wild shrub, typical of Mediterranean regions, with aromatic leaves quite resistant to both hot and cold weather. In Algeria, it is mostly present on the hills and on coastal areas from east to west of the Tell regions. Besides, the desert species, *Myrtus nivellei*, are commonly found in Hoggar and Tassili regions (south of Algeria) [7].

Leaves, flowers and roots of *M. communis L.* have been used since a long time for medicinal, food such as for flavoring meat and sauces, spice and cosmetic purposes [8]. This plant emits a pleasant odor when the leaves and flowers are crushed, mainly due to essential oil compounds which are stored in the secretor cells located in the leaves, flowers and berries [9–12]. Myrtle is now one of the main medicinal plants in Algeria, with essential oils having hypoglycemic [13], antimicrobial [14], antiseptic and anti-inflammatory [15,16], mutagenic [17] and antioxidant [18] properties. Its leaves are used to treat stomach ulcers, urinary tract inhalation of the vapors produced after leaf decoction. The large variability in the chemical composition of its essential oils is still a stimulating factor for many researchers to carry out specific studies [10,19–27], particularly on the optimization of the oil extraction yield using well developed approaches like the response surface methodology due to its efficiency and less experimental data requirement compared to the conventional methods [28,29].

Consequently, the objective of the present work is to investigate the effects of pressure, temperature, and particle diameter, on the recovered mass using supercritical CO_2 extraction of Algerian *M. communis L.* An experimental design has been used to optimize the number of experiments. Response surface methodology has been

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used to support the interpretation of experimental design results, discussed mainly in terms of recovered mass. The essential oil composition is given for some trials.

2. Materials and experimental procedure

2.1. Materials

The used myrtle was sampled from the November 2012 local harvest (in Constantine, North East of Algeria). As a first step, dried myrtle leaves were ground in a mechanical grinder for a short but sufficient period of time (15 s) to get a uniform particle size distribution. The obtained charge was sieved using a Retsch-type vibrating system. The water content in the myrtle leaves was determined as equal to 0.9 wt% by means of drying for 6 h in a vacuum oven at 378 K. The bulk density of the ground myrtle was 498 kg m^{-3}. Carbon dioxide was supplied by Air Liquide Méditerranée (France) with a purity of 99.7%. Oil extracts were collected in 99.8% pure hexane (Carlo Erba, France).

2.2. Experimental set-up

The set-up used for extractions at laboratory scale (Separex-4219) has been supplied by Separex (Champigneulles, France). This apparatus allows working with three autoclaves (5, 10 and 20 cm^3) corresponding to batches comprised between 2 and 13 g of dry leaves powder. The maximum working pressure and flow rate are 45 MPa and 0.5 kg h^{-1}, respectively.

The SC–CO_2 extraction set-up is shown on Fig. 1 and was used as follows: the autoclave is filled with the dry leaves powder and heated until the desired temperature was reached. Liquid CO_2 is cooled by a cryogenic bath at 277 K, filtered and pumped to fill the extractor until the working pressure was reached. The pressure is controlled by a pressure gauge. Then the expansion valve is opened and a flow of CO_2 goes through the leaves powder at a constant pressure, temperature and flow rate during predefined durations corresponding to the extraction times. After passing through the extractor, the CO_2 is expanded through an expansion valve. The CO_2 becomes gaseous, and extracted compounds (essential oil) are collected in a collecting vessel. The CO_2 flow rate is measured by a flow meter placed at the end of the extraction line. Oil extracts were collected in hexane and stored in a freezer (255 K).

Samples were recovered every 15 min. The recovered mass of essential oil is determined by double-weight of the extraction cell. The plotted extraction curves represent the recovered mass of essential oil as a function of extraction time (min) or as a function of solvent/biomass mass ratio (kg/kg). The dynamic extraction was pursued for 1 h, after which it was noted that the extracted mass was no more significative.

2.3. Experimental design for response surface methodology (RSM)

In the present work, the extractions were performed at temperatures in the range of 308–313 K and at pressures between 10 and 30 MPa. This experimental domain was determined based on preliminary experiments and corresponds the most frequently operating range adopted for similar system types (i.e. essential oil extraction) when CO_2 is used as supercritical solvent. For all experiments, an extraction duration of 1 h was fixed and the fluid flow rate was always equal to 0.42 kg h^{-1}.

An experimental design has been realized in order to study the influence of the parameters cited above (*P* and *T*). So, those two factors are chosen for entry values of the experimental design and for each, three levels (two values at the extremity and one in the middle) are considered. We performed a full plan composed of 3^2 experiments. Table 1 sums up the operating conditions of the conducted experiments. The response (output of the experimental design, noted as *Y*) of the experimental design is the recovered mass of essential oil.

Table 1
Factors and levels studied for the experimental design.

Parameter	Pressure, *P* (MPa)	Temperature, *T* (K)
Factor	X_1	X_2
Maximum parameter value	30	323
High level	(+1)	(+1)
Average parameter value	20	315.5
Medium level	(0)	(0)
Minimum parameter value	10	308
Low level	(−1)	(−1)

Second-order response surface models were used to express the variation of oil recovery (*Y*) as a function of the independent variables (X_1 and X_2):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

where *Y* represents the response variable, b_0 is a constant, b_i, b_{ii} and b_{ij} are the linear, quadratic and interactive coefficients, respectively. The coefficients of the response surface equation were determined by using Nemrodw software (LPRAI, Marseille, France). The agreement of model fit was evaluated using the coefficient of determination, R^2.

The different operating conditions of the experiments carried out in this study are presented in Table 2. The first set of experiments realized randomly corresponds to the experimental design plan (exp. 1–9). Some experiments were repeated twice to ensure reproducibility of the results. Additional experiments (exp. 10–16) have been performed in order to compare the experimental results obtained to the predicted RSM results.

For each experimental conditions listed in Table 2, two series of experiments have been realized. Indeed, powder with two different granulometries has been used: equal to 0.5 mm and less than 0.315 mm. The CO_2 density for those different operating conditions (see Table 2) varies from 437 to 934 kg m^{-3}.

3. Results and discussion

In this section, the results obtained from the present study are presented and discussed. First, the ability of the SC–CO_2 to extract the essential oil from the local myrtle is shown.

The observation and the comparison of the different micrographs obtained by means of a Fujitsu electronic microscope (TM3000) clearly show that the extraction process produced

Table 2
Operating extraction conditions carried out for both particle diameters: equal to 0.5 mm and less than 0.315 mm.

Exp. N°	Temperature (K)	Pressure (MPa)	CO_2 density (kg m^{-3})
1	308	10	726
2	315.5	10	578
3	323	10	437
4	308	20	859
5	315.5	20	801
6	323	20	770
7	308	30	934
8	315.5	30	889
9	323	30	863
Additional experiments			
10	308	25	904
11	313	30	911
12	313	10	652
13	313	20	818
14	313	25	870
15	315.5	25	822
16	323	25	813