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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

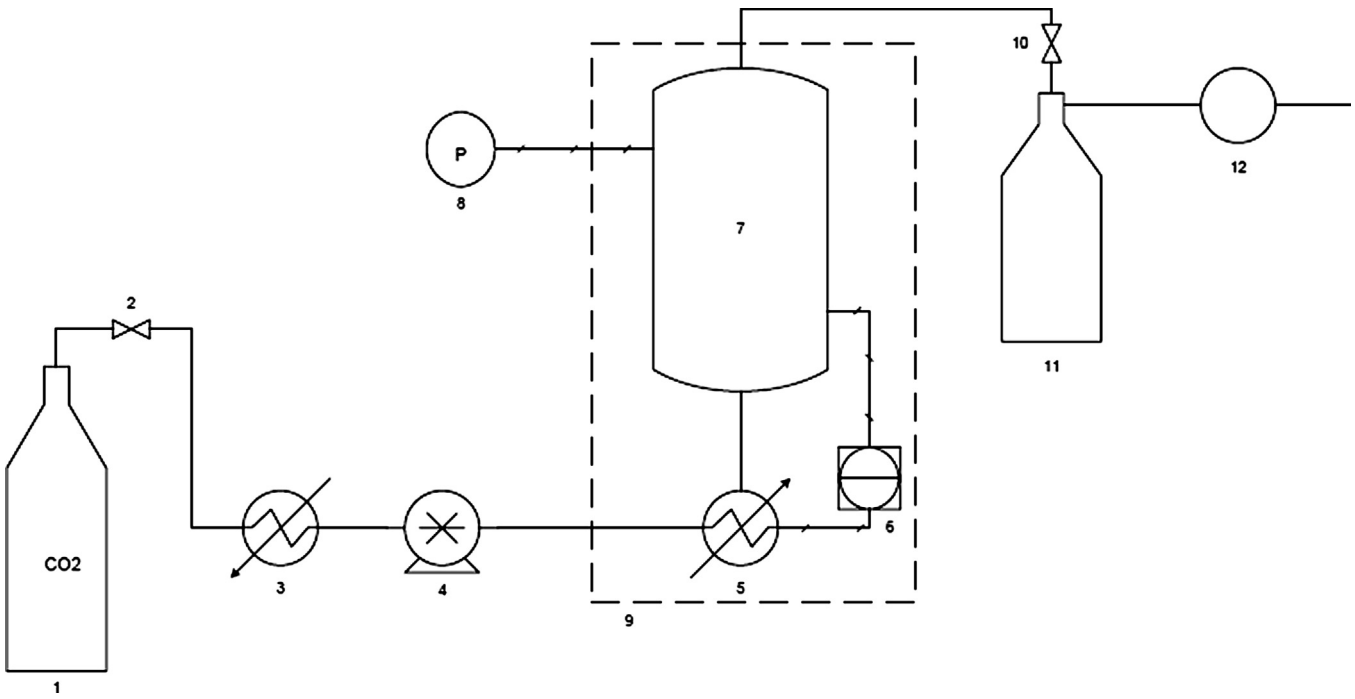

Fig. 1. Laboratory scale extraction set-up: 1. CO_2 tank; 2. Valve; 3. Cooler; 4. High pressure volumetric pump; 5. Heat exchanger; 6. Temperature regulator; 7. Extraction cell; 8. Pressure gauge; 9. Thermo regulated area; 10. Expansion valve; 11. Collector; 12. Flow meter.

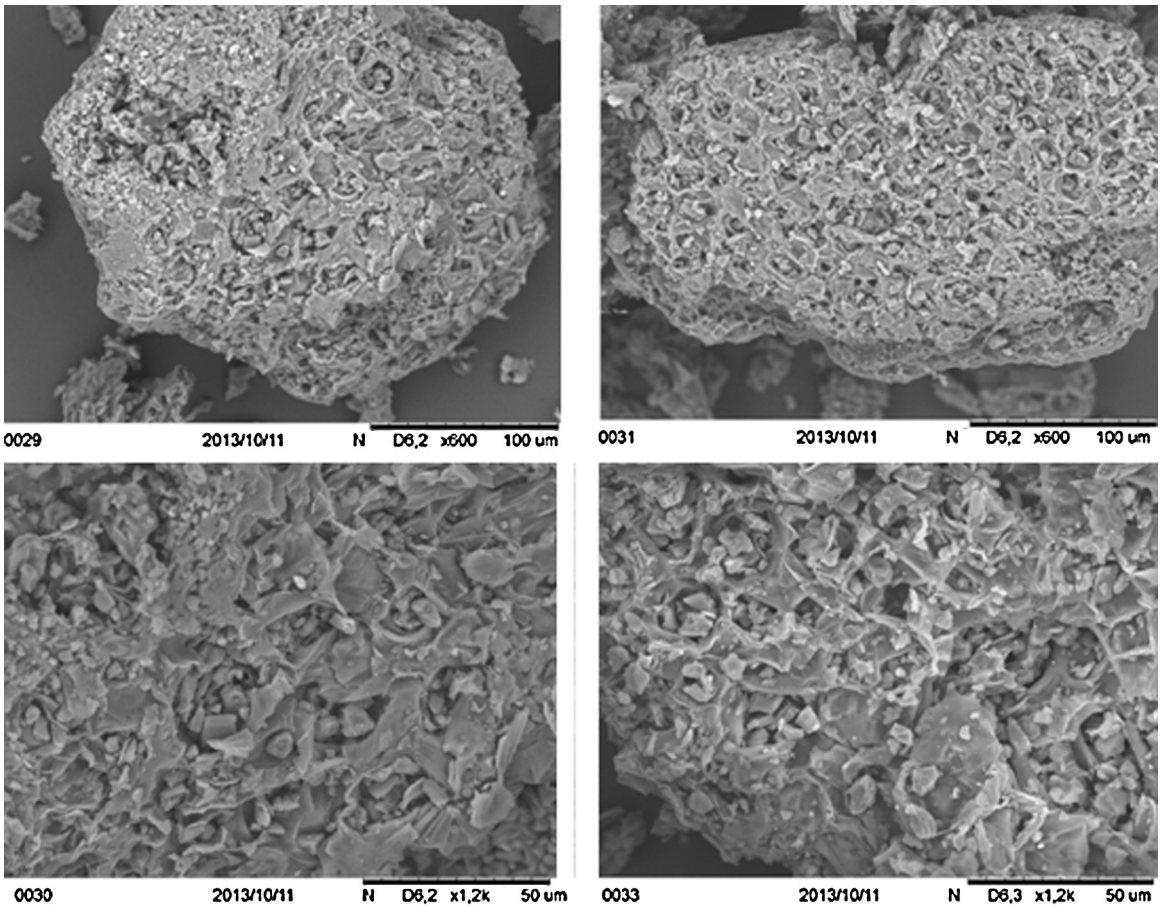

Fig. 2. Scanning electron microscope images of the surface of myrtle: (a) biomass before extraction, (b) "smashed" biomass after extraction.

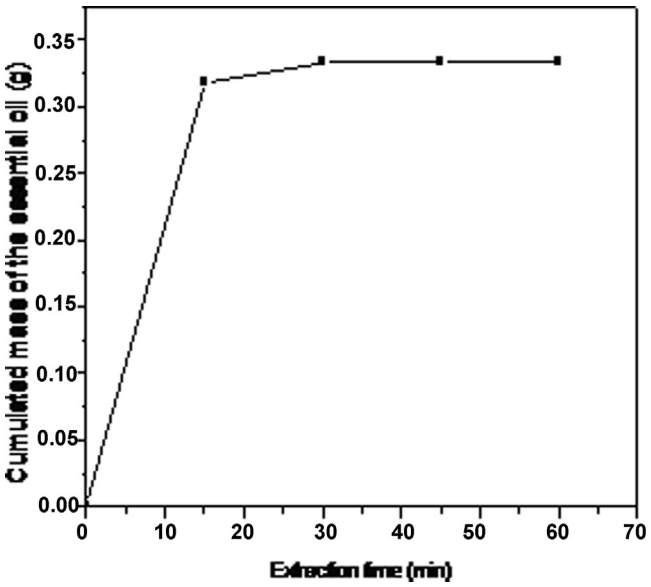

Fig. 3. Variation of the cumulated mass of the essential oil of Myrtle, extracted at 308 K, 10 MPa and for a mean particle diameter Dp < 0.315 mm, as a function of extraction time.

certain structural changes in the myrtle solid support. Fig. 2 presents two micrographs of leaves powder before treatment (a) and after supercritical extraction (b).

The initial myrtle leaves, before extraction, show glandular trichomes mainly of peltate type, which are filled with essential oil. The leaves powder undergoes damage during the SFE process and trichomes may be destroyed. The whole structure seems more porous after extraction.

A complete extraction curve obtained at 308 K, 10 MPa and for a mean particle diameter, Dp less than 0.315 mm has been plotted (see Fig. 3). The cumulated recovered mass of essential oil is plotted as a function of time. The CO_2 mass flow rate being constant and equals to 0.42 kg h^{-1}, an extraction duration of 60 min corresponds to a massic ratio CO_2/biomass of 400. It is worth noting that the extraction is complete after 30 min of extraction. The mass of essential oil extracted was calculated equal to 3.24 wt% (g oil/g dry myrtle × 100) with respect to the dry-mass fraction of the myrtle. The shape of this extraction curve illustrates that the extraction is limiting by the solubility of solutes in SC–CO_2. No mass transfer limitation by diffusion through the matrix is observed.

Table 3
Experimental results of the effects of temperature and pressure on the extraction yield of myrtle.

Exp. N°	Temperature (K)	Pressure (MPa)	Oil recovery (wt%) Dp = 0.5 mm	Oil recovery (wt%) Dp < 0.315 mm
1	308	10	2.72	3.24
2	315.5	10	2.69	2.89
3	323	10	2.17	2.76
4	308	20	3.15	3.80
5	315.5	20	3.27	3.84
6	323	20	2.82	3.54
7	308	30	3.87	4.27
8	315.5	30	3.79	4.52
9	323	30	3.24	4.13
10	308	25	3.17	4.21
11	313	30	4.02	4.89
12	313	10	2.95	3.45
13	313	20	3.41	4.05
14	313	25	3.52	4.47
15	315.5	25	3.71	4.11
16	323	25	2.99	3.87

Table 4
Coefficients of the response surface equation (Dp = 0.5 mm).

Coefficient	Value	Standard error	Significance %
b_0	3.250	0.066	<0.0[c]
b_1	−0.252	0.036	0.606[b]
b_2	0.553	0.036	0.0603[c]
b_{11}	−0.255	0.063	2.66[a]
b_{22}	−0.000	0.063	100.0
b_{12}	−0.020	0.044	68.2

[a] $\alpha < 0.05$.
[b] $\alpha < 0.01$.
[c] $\alpha < 0.001$.

Table 5
Coefficients of the response surface equation (Dp < 0.315 mm).

Coefficient	Value	Standard error	Significance %
b_0	3.811	0.109	<0.01[b]
b_1	−0.147	0.060	9.1
b_2	0.672	0.060	0.151[a]
b_{11}	−0.127	0.104	30.9
b_{22}	−0.092	0.104	44.1
b_{12}	0.085	0.073	33.0

[a] $\alpha < 0.01$.
[b] $\alpha < 0.001$.

3.1. *Effect of temperature and pressure on the myrtle essential oil extraction*

Table 3 shows the experimental results concerning the influence of the temperature and pressure on the essential oil recovery for a fixed CO_2 mass flow rate of 0.42 kg h^{-1} and for a given particle size of 0.5 mm and less than 0.315.

The oil recovery varies from 2.76 to 4.89 wt% for the smallest particles (Dp < 0.315 mm) and from 2.17 to 4.02 wt% for a mean particle diameter of 0.5 mm. It is worth noting that even if the extraction is rather limited by the solubility of extracted compounds in SC–CO_2, the oil recovery is significantly higher for the smallest particles. Up to 25% oil recovery less is observed for the biggest particles.

For all the temperature conditions tested, an increase in pressure leads, as expected, to an increase in oil recovery. When the pressure increases, the solubilities of the extracted compounds increase leading to a more important oil recovery [30,31].

Furthermore, whatever the pressure, the highest temperature (323 K) leads to smaller values of oil recovery (2.17 wt% at 10 MPa, 2.82 wt% at 20 MPa and 3.24 wt% at 30 MPa) whatever the particle size.

Thus, concerning the effects of these two parameters, i.e. pressure and temperature, it should be noted that the oil solubility is controlled by a balance between the solvent density and the solute vapor pressure, as reported in [32].

Table 6
Predicted oil yields and experimental yields (Dp = 0.5 mm).

Exp. no.	Yield cal. (%)	Yield exp. (%)	Deviation
1	2.673	2.720	0.047
2	2.697	2.690	0.007
3	2.210	2.170	0.040
4	3.247	3.150	0.097
5	3.250	3.270	0.020
6	2.743	2.820	0.077
7	3.820	3.870	0.050
8	3.803	3.790	0.013
9	3.277	3.240	0.037

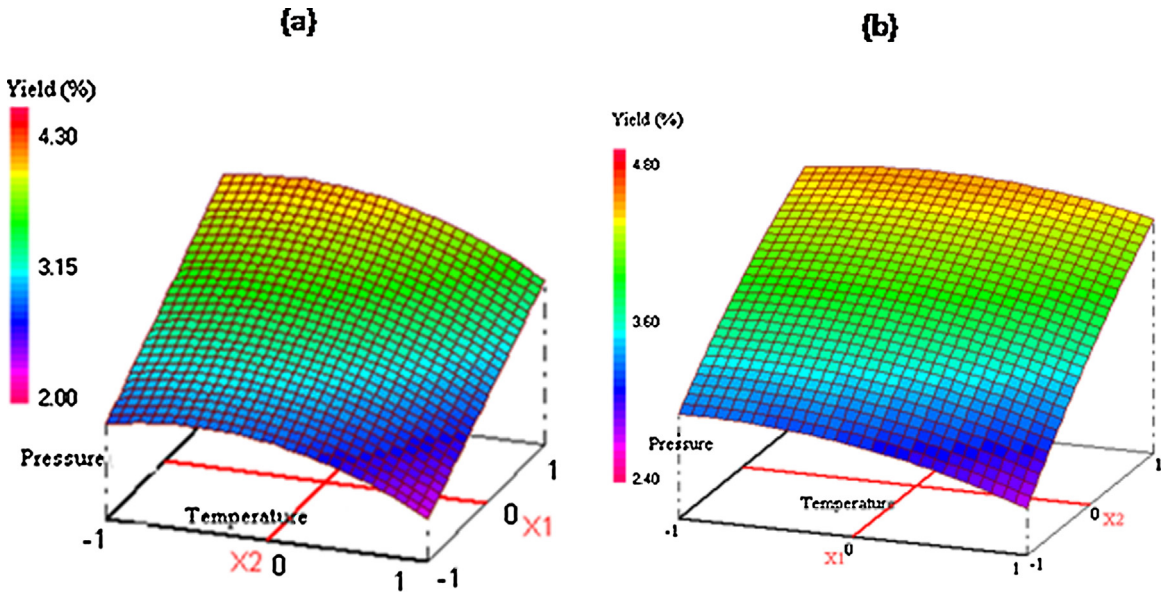

Fig. 4. Response surface for the oil yield as related to temperature and pressure at a fixed particle diameter: (a) Dp = 0.5 mm, (b) Dp < 0.315 mm.

3.2. Response surface methodology analysis

The results of the response surface methodology analysis, introduced in Section 2.3, are shown in Tables 4 and 5. Experimental oil yields were used to determine the coefficients of the response surface equation (Eq. (1)). Estimated coefficients are given in Table 4 for Dp = 0.5 mm and in Table 5 for Dp < 0.315 mm.

Obtained second-order polynomial equation was found to represent well the experimental data ($R^2 = 0.990$).

$$Y(\%) = 3.250 - 0.252X_1 + 0.553X_2 - 0.255X_1^2 - 0.020X_1X_2 \quad (2)$$

Table 7
Predicted oil yields and experimental yields (Dp < 0.315 mm).

Exp. no.	Yield cal. (%)	Yield exp. (%)	Deviation
1	3.153	3.240	0.087
2	3.048	2.890	0.158
3	2.689	2.760	0.071
4	3.831	3.800	0.031
5	3.811	3.840	0.029
6	3.538	3.540	0.002
7	4.326	4.270	0.056
8	4.391	4.520	0.129
9	4.203	4.130	0.073

Obtained second-order polynomial equation was found to represent quite well the experimental data ($R^2 = 0.972$).

$$Y(\%) = 3.811 - 0.147X_1 + 0.672X_2 - 0.127X_1^2 - 0.092X_2^2 + 0.085X_1X_2 \quad (3)$$

In these equations, X_1 is the factor used for the temperature and X_2 the factor used for the pressure. The best way for expressing the effect of any parameter on the yield within the experimental space under investigation was to generate response surface plots of the Eqs. (2) and (3).

Fig. 4 illustrates the response surface (a) for particles size equal 0.5 mm and (b) for particles size Dp < 0.315 mm, representing the influence of temperature and pressure on the yield of myrtle oil. For both particle sizes, the influence of the variation of pressure and of temperature is similar.

Whatever the temperature, an increase in pressure leads to an increase in oil recovery as mentioned above. On the contrary, an increase in temperature (at a constant pressure) leads to a decrease in oil recovery on the experimental domain corresponding to the highest values of temperature. This means that the experimental conditions of pressure studied correspond to the retrograde zone.

Table 8
Composition of some extracts.

Composition of extract recovered at 308 K and 10 MPa Yield of 2.72 wt%	Composition of extract recovered at 308 K and 30 MPa Yield of 3.87 wt%	Composition of extract recovered at 315.5 K and 20 MPa Yield of 3.27 wt%	Composition of extract recovered at 323 K and 30 MPa Yield of 3.24 wt%
10 dimethyl squalene	1,8 cineole	1,8 cineole	1,8 cineole
Squalene	10 dimethyl squalene	Oleic acid	10 dimethyl squalene
Palmitic acid	Geranyl acetate	10 dimethyl squalene	Alpha pinene
Nonadecane	Alpha pinene	Alpha pinene	Squalene
1,8 cineole	Alpha terpineol	Nonadecane	Nonadecane
Oleic acid	Palmitic acid	Squalene	Alpha terpineol
Caryophyllene oxide	Beta caryophyllene	Palmitic acid	Beta caryophyllene
Beta caryophyllene	Squalene	Beta caryophyllene	Linalool
Geranyl acetate	Caryophyllene oxide	Alpha terpineol	Linalyl acetate
Alpha terpineol	Methyl eugenol	Linalyl acetate	Palmitic acid
Linalyl acetate	Linalyl acetate	Geranyl acetate	Oleic acid
Alpha pinene	Oleic acid	Caryophyllene oxide	Geranyl acetate
Methyl eugenol	Linalool	Methyl eugenol	Caryophyllene oxide
Linalool	Nonadecane		Methyl eugenol

Indeed, in the retrograde zone, the solubilities of solutes decrease when the temperature increase.

Tables 6 and 7 show a comparison between the experimental extraction yield values and the calculated ones by means of the surface response method, and this for both particle diameter values. The deviation varies from 0.002 to 0.158 which correspond to satisfactory results.

Table 8 presents the composition of extracts obtained in different operating conditions. Extracts have been analyzed by Gas Chromatography coupled with Mass Spectrometry column: (HP5MS, 30 m × 0.25 mm × 0.25 μm; inj. split 1 μL).

Since some compounds were unidentified, the compounds are listed from the most concentrated to the less concentrated in the extract. The most concentrated compound is the 10 dimethyl squalene for the extract recovered at 308 K and 10 MPa, while the most concentrated compound in the extract is 1,8 cineole for the three other extracts listed in Table 8. At 308 K and 10 MPa, the extract is richer in apolar compounds while for the other conditions, the most concentrated compound is a polar compound.

4. Conclusion

In this study, the effect of different parameters such as temperature and pressure at fixed flow rate of solvent and two sizes of particles diameters was investigated for the supercritical fluid carbon dioxide extraction from Algerian Myrtle. Oil extractions from myrtle leaves by SC–CO_2 were performed at temperature and pressure ranges of 308–323 K, 10–30 MPa respectively, and a solvent flow rate of 0.42 kg h^{-1} (particle diameters of 0.5 mm and inferior than 0.315 mm) for 1 h of extraction. The maximum oil recovery (4.89 wt% relative to the initial mass of dry biomass) has been obtained for an extraction under 313 K, 30 MPa and the lowest mean particle diameter. The results were analyzed by a response surface methodology in this experimental range and very good concordance was observed between RSM and experimental oil yields.

References

[1] A.R.C. Duarte, Current Trends of the Supercritical Fluid Technology in the Pharmaceutical, Nutraceutical and Food Processing Industries, Bentham Science Publishers, New York, 2009.

[2] M.S. Diaz, E.A. Brignole, Modeling and optimization of supercritical fluid processes (review), J. Supercritical Fluids 47 (2009) 611–618.

[3] G. Brunner, Gas extraction: an introduction to fundamentals of supercritical fluids and the application to separation processes, in: H. Baumgurtel, E.U. Franck (Eds.), Topic in Physical Chemistry, Springer, New York, 1994.

[4] Q. Lang, C.M. Wai, Supercritical fluid extraction in herbal and natural product studies – a practical review, Talanta 53 (2001) 771–782.

[5] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, J. Supercritical Fluids 38 (2006) 146–166.

[6] R. Govaerts, E. Lucas, World Checklist of Myrtaceae, Xv, Royal Botanic Gardens, Kew, 2008, pp. 455.

[7] P. Quezel, S. Santa, Nouvelle flore de l'Algérie et des régions désertiques méridionales, Tome II-ed., CNRS, Paris, 1963.

[8] I. Jerkovic, A. Radonic, I. Borcic, Comparative study of leaf, fruit and flower essential oils from Croatian *Myrtus communis* L. during a one-year vegetative cycle, J. Essential Oil Research 14 (2000) 266–270.

[9] M.H. Boelens, R. Jimenez, The chemical composition of Spanish myrtle oils. Part II, J. Essential Oil Research 4 (1992) 349.

[10] U. Asllani, Chemical composition of Albanian myrtle oil, J. Essential Oil Research 12 (2000) 140.

[11] G. Mazza, GCMS investigation of volatile components of myrtle berries, J. Chromatography 264 (1983) 304.

[12] G. Flamini, P.L. Cioni, I. Morelli, S. Maccioni, R. Baldini, Physiochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory (East Liguria, Italy), Food Chemistry 85 (2004) 599.

[13] A. Sepici, I. Gürbüz, C. Cevik, E. Yesilada, Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits, J. Ethnopharmacology 93 (2004) 311.

[14] D. Yadegarinia, L. Gachkar, M.B. Rezaei, M. Taghizadeh, S.A. Astaneh, I. Rasooli, Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils, Phytochemistry 67 (2006) 1249.

[15] A.M. Diaz, A. Abeger, *Myrtus communis*: composicion quimica y actividad biologica de sus extractos, Una revision, Fitoterapia 58 (1987) 167.

[16] M.K. Al-Hindawi, I.H. Al-Deen, M.H. Nabi, M.A. Ismail, Anti-inflammatory activity of some Ieaqi plants using intact rats, J. Ethnopharmacology 26 (1989) 163.

[17] H. Hayder, I. Skandrani, S. Kilani, I. Bouhlel, A. Abdelwahed, R. Ben Ammar, A. Mahmoud, K. Ghedira, L. Chekir-Ghedira, Antimutagenic activity of *Myrtus communis* L. using the Salmonella microsome assay, South African Journal of Botany 74 (2008) 121.

[18] C. Gardeli, V. Papageorgiou, A. Mallouchos, K. Theodosis, M. Komaitis, Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methalonic extracts, Food Chemistry 107 (2008) 1120.

[19] B.M. Lawrence, J.W. Hogg, S.J. Terhune, Les Huiles Essentielles et Leurs Constituants. IV. Quelques Nouveaux Constituants ä l'Etat de Traces dans l'Huile Essentielle de Salvia officinalis L, Parfum. Cosmétique. Savons France 1 (1971) 256.

[20] M. Vanhaelen, R. Vanhaelen-Fastré, Constituents of essential oil of *Myrtus communis*, Planta Medica 39 (1980) 164.

[21] M.H. Boelens, R. Jimenez, The chemical composition of Spanish myrtle leaf oils. Part I, J. Essential Oils Research 3 (1991) 173.

[22] P. Weyerstahl, H. Marshall, A. Rustaiyan, Constituents of the essential oil of *Myrtus communis* L. from Iran, Flavour and Fragrance J. 9 (1994) 333.

[23] G. Pirisino, A. Mulè, M.D.L. Moretti, M. Satta, Yield and chemical composition of essential oil from self-sown *Myrtus communis* L. from Cuglieri (Sardinia Riv. Ital.), EPPOS 19 (1996) 159.

[24] P. Bradesi, F. Tomi, J. Casanova, J. Costa, A.F. Bernardini, Chemical composition of myrtle leaf essential oil from Corsica (France), J. Essential Oils Research 9 (1997) 283.

[25] P.K. Koukos, K.I. Papadopoulou, A.D. Papagiannopoulos, Chemicals from Greek forestry biomass- constituents of the leaf oil of *Myrtus communis* L. grown in Greece, J. Essential Oils Research 13 (2001) 245.

[26] C. Messaoud, Y. Zaouali, A. Ben Salah, M.L. Khoudja, M. Boussaid, *Myrtus communis* in Tunisia: variability of the essential oil composition in natural populations, Flavour and Fragrance J. 20 (2005) 577.

[27] W. Aidi Wannes, B. Mhamdi, B. Marzouk, GC analysis of leaf essential oil from two myrtle varieties at different phenological stages, Chromatographia 69 (2009) 145.

[28] M. Giovanni, Response surface methodology and product optimization, Food Technology 37 (1983) 41–45.

[29] G. Began, B. Manohar, K.U. Sankar, A.G.A. Rao, Response surfaces for solubility of crude soy lecithin lipid in supercritical carbon dioxide, European Food Research and Technology 210 (2000) 209–212.

[30] N. Raul, J.E. Carvalho, S. Lucinewton, S. Moura, P.T.V. Rosa, M.A.A. Meireles, Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): kinetic data, extract's global yield, composition, and antioxidant activity, J. Supercritical Fluids 35 (2005) 197–204.

[31] H. Sovova, M. Sajfrtova, Experiments, Modeling and Scale-up for Supercritical Extraction from Plants in 10th European Meeting on Supercritical Fluids, Colmar (France), 2005, pp. 55.

[32] B. Roy, M. Goto, T. Hirose, Extraction of ginger oil with supercritical carbon dioxide: experiments and modeling, Industrial and Engineering Chemical Research 35 (1996) 607.