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Supercritical CO₂ extraction of *Tetraclinis articulata*: Chemical composition, antioxidant activity and mathematical modeling

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A B S T R A C T

Operating conditions for extraction from the leaves of *Tetraclinis articulata* using supercritical carbon dioxide (SCCO₂) were studied to focus on the feasibility of obtaining volatile and nonvolatile fractions through the use of different extraction pressures (90, 280 and 1000 bar). In addition, influence of temperature, static pretreatment and dynamic extraction durations, particle size and CO₂ flow rate were investigated. All extracts were analyzed by GC-FID/MS and their antioxidant activity was measured using ABTS^{•+} and DPPH[•] methods. Conventional hydrodistillation (HD) was also performed for comparison. At high CO₂ pressure (280 and 1000 bar), the amount of phenolics in the extracts was higher (respectively 102.03 and 267.90 GAE mg/g) than for HD and supercritical fluid extraction (SFE) at 90 bar (respectively 8.89 and 9.70 GAE mg/g). Correlatively, high antioxidant activity was found for high pressure SFE. Surprisingly, for extracts obtained by SFE at 90 bar, despite very low phenolic content, significant antioxidant activity was observed, while essential oil obtained by HD, which presented also low phenolic content, exhibited low antioxidant activity.

Physical aspects were only investigated for the low pressure supercritical extraction (90 bar) process. Qualitative assessment of kinetic curves together with their modeling revealed that the extraction process was mainly limited by the thermodynamic equilibrium of easily accessible solutes but where axial dispersion was significant. From this result a simple extrapolation procedure was proposed.

Keywords:

Supercritical carbon dioxide extraction

Tetraclinis articulata

Essential oil

Antioxidant activity

Modeling

1. Introduction

In the last 20 years, research upon natural substances such as aromatic and medicinal plants has concentrated on two primary areas: identification of their biological activity (such as antioxidant activity) and development of novel extraction methods insuring better quality and eco-friendly operation. In this work, the *Tetraclinis articulata* specie will be investigated in respect to both these aspects.

T. articulata belongs to the family Cupressaceae and is a native plant of the south-western Mediterranean, mainly North Africa. It is an important element of the Maghreb vegetation (Morocco, Algeria and Tunisia). This plant is a widely distributed tree in North Africa where it covers a total area of 10,000 km² [1]. *T. articulata*

is known as a high quality health food which is commonly used in Tunisian traditional medicine. It has been found to have pharmacological effects, including antioxidant activity that may explain the benefits in treating circulatory disorders perceived from the use of this herb over the centuries. It is mainly used against childhood fevers, respiratory and intestinal infections, stomach pain, diabetes and hypertension [2]. Therefore, it is interesting to find an effective method for isolation of bioactive compounds from *T. articulata*.

It is known that the isolation of natural antioxidants is difficult because most of them are heat-sensitive and volatile in steam, and can be therefore destroyed when a nonadapted extraction process is used [3]. Traditionally, extraction of bioactive compounds from plants is performed by steam distillation, hydrodistillation (HD) or organic solvent extraction [4,5]. Using these techniques, long extraction time, toxic solvent residues, labor-intensive operation and degradation of thermo-sensitive compounds are usually observed. Such disadvantages can be avoided by using the supercritical carbon dioxide (SCCO₂) technique [6,7]. Indeed, this technique, more generally referred as supercritical fluid extraction (SFE), should improve the volatile oil quality by avoiding any

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thermal stress to the volatile component. The solvent power of SCCO₂ is directly related to its density. So, choice of the values of operating pressure and temperature is a critical step in the development of an efficient process. These operating variables determine the value of the density and consequently the extract yield and composition, and, therefore, the functional properties [8,9] of the extract. Also, other factors such as duration of the static and dynamic periods of extraction, particle size and solvent flow rate can influence the process performance [10], and must be investigated.

In the literature, to our knowledge, very few studies were performed on *T. articulata*. As an example, Rached et al. [11] presented results obtained from a plant from Algeria harvested in April 2008. But, up to now, no study on the SFE extraction of *T. articulata* from Tunisia has been reported in the literature. However, very numerous works have studied the extraction of volatile oil from other plants using SCCO₂ [6,7,12,13]. For example, extraction of the volatile oil from *Salvia officinalis* has been studied by several authors (Reverchon et al. [14], Aleksovski and Sovová [15], Glisic et al. [16]) who used the same extraction conditions to recover volatile oils (pressures between 80 and 130 bar, temperatures between 40 and 60 °C and solvent flow rates between 5 and 30 g/min approximately). Only the method proposed by Reverchon et al. [14] differed from the fact that they carried out the separation of the extracts using two separators at different conditions, which yielded absence of waxes in the collected volatile extract.

The objective of the present work was to evaluate the extracting capacity of SCCO₂ to obtain *T. articulata* volatile oil with possibly different properties and to investigate the effect of the extraction conditions. It is suspected that the volatile fraction could be selectively isolated at low pressure extraction, whereby the pressures between 100 and 1000 bar would be more favorable for obtaining fractions containing heavier compounds with high antioxidant characteristics. To check these assumptions, extractions were performed at pressures 90, 280 and 1000 bar and temperatures equal to 40 and 60 °C. CO₂ flow rate was varied between 5 and 20 g/min, and different mean particle sizes so as different extraction durations were tested. In this work comparison with extracts obtained by HD was also proposed.

On account of importance of SFE for industrial application, extrapolation of laboratory experimental data is necessary and requires extensive knowledge of the physical phenomena occurring during the course of the extraction. Then the use of mathematical models allows giving the extrapolation rules. Especially, for extrapolation purposes, it is of great interest to identify whether the extraction process is limited by mass transfer phenomena or by thermodynamics. Such information can be obtained from the study of extraction kinetics and their modeling using mathematical models proposed in the literature [17].

2. Materials and methods

2.1. Plant material

Leaves from cultivated plants of *T. articulata* were collected by hand during October 2010 (in the morning) from the same location, Korbos, located in the region of Cap Bon at 60 kilometers from Tunis (latitudes 36.82/36°49'44" and longitudes 10.59/10°35'36"), Tunisia. Harvested material was dried in the air, protected against direct sunlight, until a constant weight was achieved. The average particle size of *T. articulata*, was obtained after sieving of grinded leaves (using laboratory knife grinder). The moisture content of the air-dried plant material, determined by the Karl Fischer volumetric titration, was 9% (w/w). The efficiency of the different extraction processes is assessed using the extraction yield, defined as the mass

of collected extract divided by initial mass of wet plant material (9%, w/w moisture content).

2.2. Chemicals

All chemicals were of analytical reagent grade. All reagents were purchased from Sigma–Aldrich–Fluka (Saint-Quentin, France). Commercial carbon dioxide (99.99% purity) was purchased from Air Liquide (Bordeaux, France) for the extraction of volatile and nonvolatile components by SFE process.

2.3. Supercritical fluid extraction (SFE)

Supercritical extractions were carried out using two different devices. The first one termed SF200 extraction pilot unit, from Separex Chimie fine, (France) is described elsewhere [18,19]. Briefly, this apparatus is composed of a 113 mL (16 cm height and 3 cm internal diameter) tubular extractor (300 bar max) with a cascade of three 15-mL cyclonic separators connected to the extractor outlet.

In the present study, the 113 mL stainless steel extractor was loaded with 50 g of dried *T. articulata* leaves with an average particle size of 1.5 ± 0.3 mm. The system was operated at a temperature between 40 and 60 °C and pressure of 90 bar for volatile fraction extraction. Pure CO₂ was passed into the cell with the flow rate kept between 5 and 20 g/min in all runs.

The second apparatus, termed A21 (SFE1000 bar), from Separex Chimie fine, France, is composed of a 63 mL tubular extractor which can be operated up to 1000 bar and 200 °C. Only one separator is connected to the extractor outlet. Pressure in the extractor is adjusted by a backpressure regulator. In this work, the extractor was loaded with 28 g of dried *T. articulata* leaves with an average particle size of 1.5 ± 0.3 mm. The system was operated at 280 and 1000 bar, CO₂ flow rate of 20 g/min and two temperatures, 60 and 40 °C.

Density of the solid phase has been experimentally determined and is equal to $\rho_s = 815$ kg/m³. The bed void fraction was estimated by $\varepsilon = 1 - \rho_a/\rho_s = 0.4575$ where solid apparent density is obtained with $\rho_a = m_0/V_{\text{extr}}$ ($\rho_a = 442$ kg/m³) where m_0 is the initial mass of plant material.

2.4. Hydrodistillation

A conventional method of HD was carried out to compare the extraction performances with SFE. This set-up was composed of a 2-L boiler, a condenser and a measuring tube with a stopcock. A return tube for the aqueous part of the distillate allowed the cohobation technique to be used. Dried leaves of *T. articulata* (100 g), ground at diameter 1.5 mm, were placed in a Clevenger apparatus and mixed with distilled water at a ratio of 1/10 (plant material/water). After 180 min (corresponding to the duration when no more essential oil was obtained at the condenser), the essential oil was collected, dried over anhydrous sodium sulfate and stored at 4 °C in the dark until used.

2.5. Chemical components analysis: GC–FID and GC–MS

Quantitative and qualitative analysis of the volatile oil was carried out by gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). Gas chromatography analyses were performed on a Varian Star 3400 Cx chromatograph (Les Ullis, France) fitted with a DB-5MS fused silica capillary column (5% phenylmethylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 μm). Chromatographic conditions were a temperature rise from 60 °C to 260 °C with a gradient of 5 °C/min and then 15 min at 260 °C. A second gradient of 40 °C/min was applied to reach 340 °C. Total analysis duration was 57 min. For

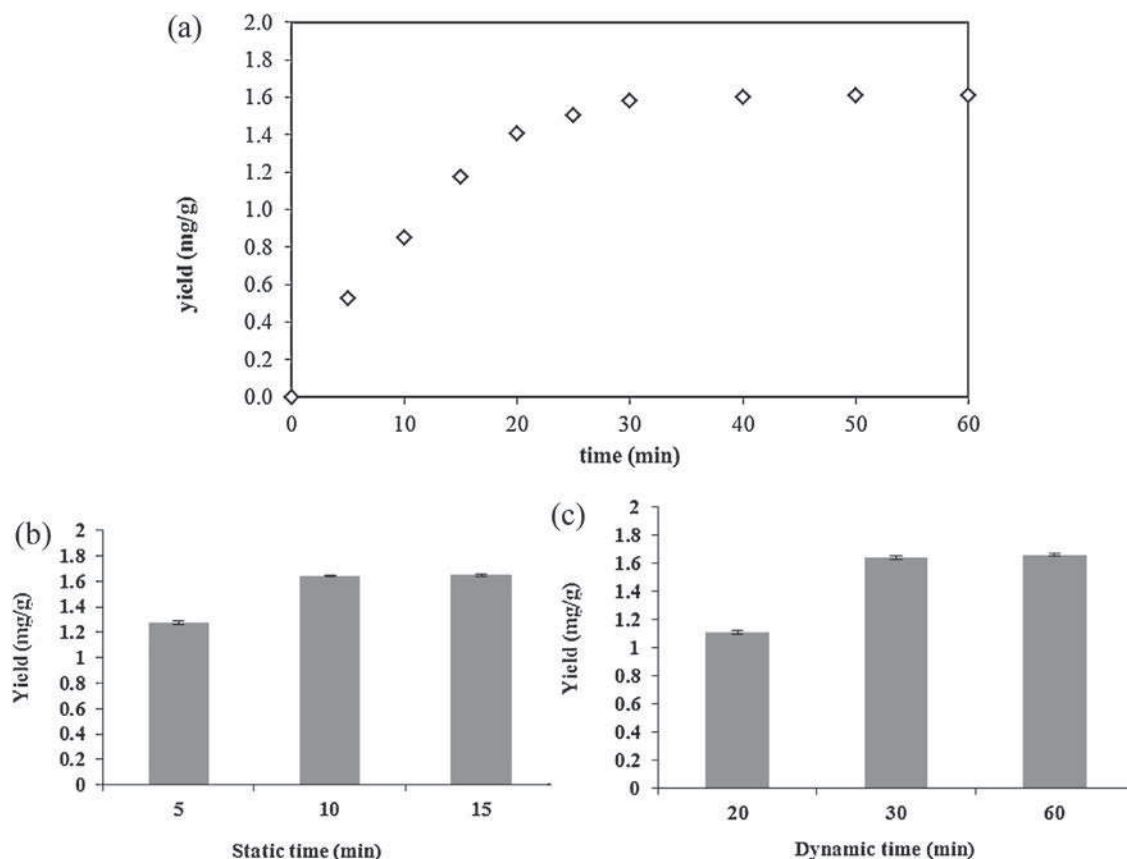


Fig. 1. SFE yield of volatile oil from *T. articulata* at $W_{CO_2} = 20$ g/min, $P=90$ bar and $T=40^\circ\text{C}$. (a) Kinetics of the extraction process, (b) influence of static period duration (followed by 30 min of dynamic extraction) and (c) influence of dynamic extraction duration (with a static period of 10 min).

analysis purposes, the volatile oil was dissolved in petroleum ether. Samples (1 μL) were injected in the split mode at a ratio of 1:10. Helium (purity 99.999%) was used as the carrier gas at 1 mL/min. The injector was operated at 200°C . The mass spectrometer (Varian Saturn GC-MS-MS 4D) was adjusted for an emission current of 10 μA and electron multiplier voltage between 1400 and 1500 V. The temperature of the trap was 150°C and that of the transfer line was 170°C . Mass scanning was from 40 to 650 amu.

Compounds were identified (i) by comparison of their retention index (RI), obtained on a nonpolar DB-5MS column relative to C_5 - C_{24} n-alkanes, with those provided in the literature, (ii) by comparison of their mass spectra with those recorded in NIST 08 (National Institute of Standards and Technology) and reported in published articles, (iii) by co-injection of available reference compounds. The samples were analyzed in duplicate. The percentage composition of the volatile oil was calculated by the normalization method from the GC peak areas, assuming identical mass response factors for all compounds. Results were calculated as mean values after two injections of volatile oil, without using correction factors. The identification is only made for the volatile compounds. Especially, for SFE extracts, some nonvolatile compounds cannot be identified. The detection limit is 0.1 mg/L while the quantification limit is 1 mg/L.

2.6. Determination of total phenolic content

The phenolic contents of extracts were determined by the Folin-Ciocalteu method [20]. A diluted solution of each extract (0.5 mL) was mixed with the Folin-Ciocalteu reagent (0.2 M, 2.5 mL). This mixture was kept at room temperature for 5 min and then a sodium carbonate solution (75 g/L in water, 2 mL) was added.

After 1 h of incubation, the absorbance was measured at 765 nm against water blank. A standard calibration curve was plotted using gallic acid (0–300 mg/L). The results were expressed as mg of Gallic Acid Equivalent (GAE)/kg of plant material.

2.7. DPPH• free radical scavenging activity

Antioxidant scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•) as described by Blois [21] with some modifications; various dilutions of the test materials (ascorbic acid or extracts, 1.5 mL) were mixed with a 0.2 mM methanolic DPPH• solution (1.5 mL). After 30 min incubation period at 25°C , the absorbance at 520 nm was recorded as $A_{(\text{sample})}$. A control experiment was also carried out by applying the same procedure to a solution without the test material and the absorbance was recorded ($A_{(\text{blank})}$). The free radical scavenging activity of each solution was then calculated as percentage inhibition according to the following equation: % inhibition = $100 \times [(A_{(\text{blank})} - A_{(\text{sample})})/A_{(\text{blank})}]$

Extract antioxidant activity was expressed as IC_{50} , defined as the concentration of the test material required to cause a 50% decrease in initial DPPH• concentration. Values were estimated using linear regression. Ascorbic acid was used as a reference.

2.8. ABTS•+ radical scavenging

The radical scavenging capacity of the samples for the ABTS•+ (2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulfonate) was determined as described by Re et al. [22]. ABTS•+ was generated by mixing a 7 mM solution of ABTS•+ at pH 7.4 (5 mM NaH_2PO_4 , 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM of potassium persulfate

(final concentration) and stored in the dark at room temperature for 16 h before use. The mixture was diluted with water to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometry. For each sample, a diluted methanol solution of the sample (100 μL) was allowed to react with fresh ABTS^{•+} solution (900 μL), and the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a reference and the free radical scavenging capacity was expressed by IC_{50} (mg/L) values, which represents the concentration required to scavenge 50% of ABTS^{•+}. The free radical scavenging capacity IC_{50} was determined using the same equation as used previously for the DPPH[•] method.

2.9. Statistical analysis

Results of antioxidant activity and total phenolic content were expressed as means \pm standard deviations of triplicate measurements. The confidence limits were set at $P < 0.05$. Data analysis procedure (ANOVA) was performed in order to assess the data.

3. Results and discussion

3.1. Influence of parameters for SFE

3.1.1. Influence of extraction duration

An example of kinetics of the extraction process at 90 bar and 40 °C is presented in Fig. 1(a). The global extraction yield increases linearly until about 30 minutes and then a plateau is reached. The initial linear shape of the curve suggests that extraction is limited by the solubility of volatile oil in CO₂ or by solid–fluid equilibrium; internal diffusion limitation seems not to be present in these operating conditions.

We used a dynamic extraction method where the extraction duration is actually constituted of a period of static pretreatment (i.e., no CO₂ flow rate) followed by a dynamic period (i.e., with constant CO₂ flow rate). The static pretreatment allows equilibration of the solid and the fluid and does not require CO₂ flow, and also allows SCCO₂ dissolving into the oil present in secretory structures of the plant tissue (cells, cavities or ducts), previously opened by grinding, as described by Stamenić et al. [23].

In the first part of this study, the effect of static pretreatment on SFE efficiency was studied. Three static pretreatment durations (5, 10 and 15 min), followed by 30 min dynamic extraction, were employed. The pressure and temperature were fixed at 90 bar and 40 °C with particle diameter 1.5 mm. Experimental global yields are presented on Fig. 1(b). It is seen that increasing the static extraction period, from 5 to 10 min, increased extraction efficiency of volatile oil by more than 40%. However, increasing the static extraction duration from 10 to 15 min had minimal effect on the extraction efficiency. Therefore, in all experiments, 10 min of static pretreatment was used.

Effect of dynamic extraction duration on extractability of the natural substances was investigated while keeping other parameters fixed. The dynamic method using three durations (20, 30, and 60 min) was tested with 10 min static pretreatment duration. The pressure, temperature and CO₂ flow rate were 90 bar, 40 °C and 20 g/min for all experiments.

Fig. 1(c) shows how extended dynamic extraction durations increase the efficiency of the process. However, the dependence between recovery and extraction duration gradually becomes flat. Therefore a dynamic method with 30 min was chosen in subsequent extractions.

3.1.2. Influence of temperature

The influence of temperature on the extraction yield of *T. articulata* at three temperature levels, 40, 50 and 60 °C is shown in Fig. 2. A high temperature is likely to improve mass transfer rates

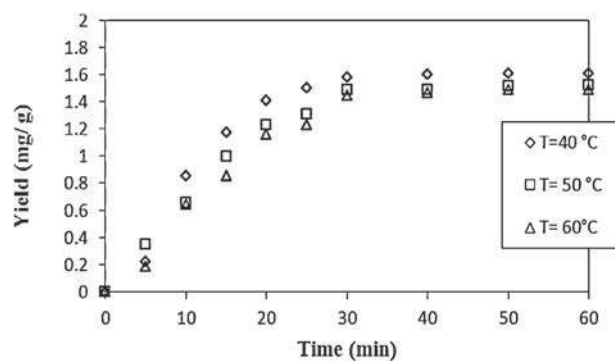


Fig. 2. Influence of the temperature on the *T. articulata* volatile oil yield for 1.5 mm particles as a function of the extraction time at $P = 90$ bar and $W_{\text{CO}_2} = 20$ g/min.

and thus kinetics of extraction. Nevertheless at low pressure, the density of CO₂ is very sensitive to temperature and decreases with increasing temperature, leading possibly to reduced solvent power of SCCO₂ depending on the competition between density of SCCO₂ and volatility of the solute (retrograde solubility effect) [24]. This might be the reason of the observed decrease of the yield (1.6–1.48 mg/g) when temperature is increased from 40 to 60 °C, as seen on Fig. 2. Such a similar result was also obtained by da Cruz Francisco et al. [25] in the case of the extraction of monoterpenes of eucalyptus oil. In addition, it is very likely that high-temperature extraction have a detrimental effect on the quality of the extract because of possible thermal degradation reactions. The possibility to perform efficient extraction of volatile compounds at low temperature is indeed one of the main advantages of the SFE technology when compared to traditional HD.

3.1.3. Influence of pressure

Fig. 3 presents the influence of pressure on the extraction yield of *T. articulata* leaves in SC-CO₂ at three pressure levels of 90, 280 and 1000 bar for 30 min extraction duration (1.6, 19.2 and 25.5 g/kg, respectively).

The SFE yield of *T. articulata* was significantly influenced by the pressure, as can be seen in Fig. 3. In fact, increasing the extraction pressure from 90 to 280 bar increased the yield more than 9 times and, from 90 to 1000 bar, for more than 16 times. The high CO₂ density at high pressure increased its solvent power and therefore, more substances were extracted. Also, it can be noticed that, especially, the increase from 90 to 280 bar is very efficient upon the yield.

High extraction pressure is likely to favor the extraction of heavy and more polar compounds such as phenolics, lipids or waxes. Therefore, in the present study, the phenolic content is expected to increase with increasing pressure (280 and 1000 bar).

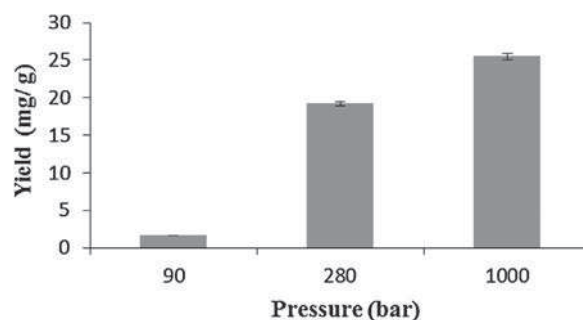


Fig. 3. Influence of the pressure on the *T. articulata* extraction yield for 1.5 mm particles, a CO₂ flow rate of 20 g/min, $T = 40$ °C and extraction duration 30 min.

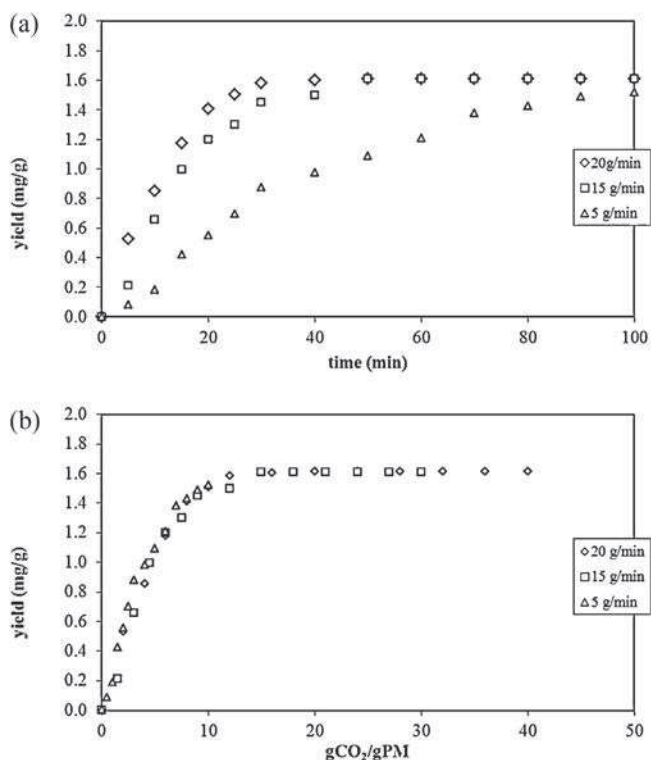


Fig. 4. *T. articulata* volatile oil yield for 1.5 mm particles at different CO₂ flow rates as a function of the extraction time (a) and a function of amount of CO₂ referred to initial amount of plant material (PM) (b) at $T=40^{\circ}\text{C}$ and $P=90\text{ bar}$.

Nevertheless, increasing the pressure obviously results in a decrease of the selectivity of the extraction [26,27].

3.1.4. Influence of CO₂ flow rate

As shown in Fig. 4(a), for extraction at 90 bar and 40 °C, the CO₂ flow rate exhibited a positive and significant effect on the *T. articulata* volatile oil extraction. This result indicates that, for CO₂ flow rate equal to 5 g/min, 60 min of extraction are not sufficient to achieve complete extraction while 30 min are sufficient at 20 g/min. Also, at all flow rates, the initial quasi-linear shape of the

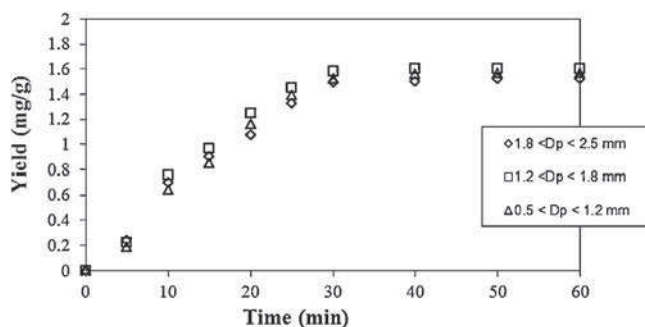


Fig. 5. *T. articulata* volatile oil yield for different mean particle sizes at a CO₂ flow rate of 20 g/min, temperature 40 °C and pressure 90 bar.

curves suggests the hypothesis that this SFE process is solubility or solid–fluid equilibrium limited.

When plotting the yield versus the amount of CO₂ (Fig. 4(b)), the curves merge and this confirms the occurrence of solubility or equilibrium limitation for the extraction process. The slope of these curves gave the solute concentration in the output fluid (C_0) at a value of $0.20 \times 10^{-2} \text{ g/gCO}_2$, a value which is below the value of solubility of usual terpenes (α -pinene for instance is $1.051 \times 10^{-2} \text{ g/g CO}_2$ at $T=40^{\circ}\text{C}$ and $P=90\text{ bar}$ [28]). When high mass transfer limitation is not suspected, this indicates very probable adsorption phenomena which limit the extraction process.

3.1.5. Influence of particle size

The effect of particle size on extraction rate is shown in Fig. 5. Despite some dispersion in the experimental points, the curves are similar, and especially the same final yield is reached after the same extraction duration (around 30 min) for all particle sizes, indicating similar kinetic behavior. This result is in accordance with the suspected predominant equilibrium limitation which predicts kinetics almost independent of internal and external mass transfer, and therefore of particle size. The low impact of particle size on extraction kinetics is typical of glandular structures corresponding to secretory ducts [23,29], as it is the case for plants of the Asteraceae, Apiaceae, Hypericaceae, Pinaceae or Coniferae family. These ducts are opened by grinding and the oil is easily accessible for CO₂. CO₂ dissolves in the early stage of extraction, leading to an increase of the volume of the oil that wets the surface of the plant. This

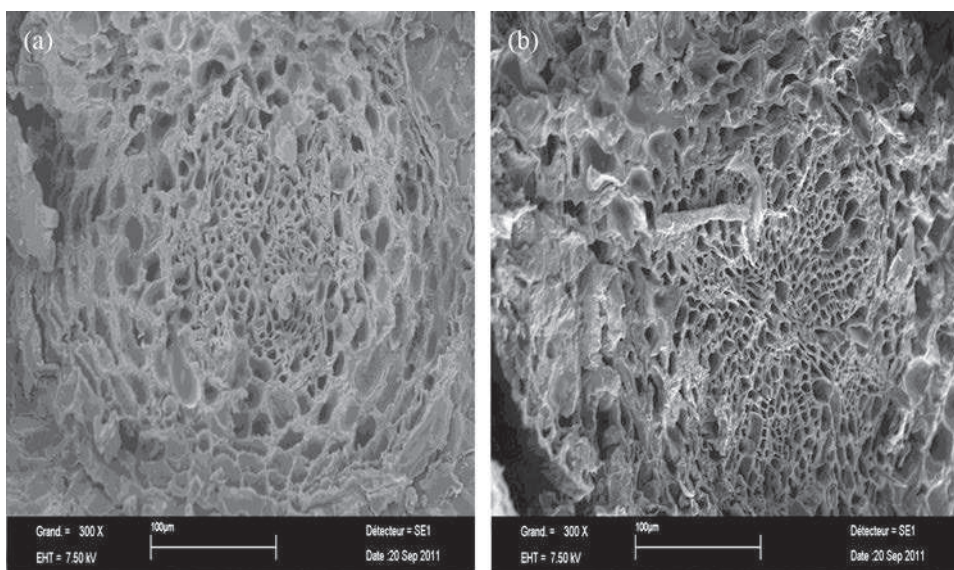


Fig. 6. SEM images of a section of *T. articulata* ground leaves before SFE (a) and leaves after SFE at 1000 bar, 40 °C (b).

Table 1Chemical composition (GC–MS) and global yield of *T. articulata* extracts obtained by SFE at different pressures and by HD.

		Type of extraction technique		
		SFE		HD
	Pressure (bar)	90	280	1000
	Temperature (°C)	40	40	40
	Flow rate (g/min)	20	20	20
	Solvent	CO ₂	CO ₂	CO ₂
	Extraction time (min)	30	30	30
	Global yield (g/kg)	1.6	19.2	25.5
RI	Compounds	%	%	%
906	Santolina triene	0.85		0.39
931	Artemesia triene	0.8		0.6
936	α-Pinene	31.32		24.9
940	Cumene	0.33		
953	α-Fenchene	0.22		0.14
958	Camphene	0.49		0.45
967	β-Thujene	2.64		1.99
973	1-Ethyl-4-methylbenzene	0.43		
990	Myrcene	0.17		0.48
1004	Pseudolimonene	0.26		0.37
1008	3-Carene	3.39		3.44
1012	α-Terpinene			0.12
990	Myrcene	0.17		0.48
1037	Limonene	0.16		0.18
1047	3-Methyl-1-decene	0.13		
1068	Isoterpinolene	0.26		0.46
1077	Ethyl maltol			0.26
1079	Artemisia alcohol	0.19		
1089	α-Terpinolene			
1108	cis-Thujone	0.22		0.35
1122	trans-2,8-Menthadienol	0.17		0.38
1128	α-Campholenal	3.44	0.1	0.43
1132	Isotujol			0.17
1148	Camphene hydrate	1.25		4.36
1157	Isoborneol			
1159	β-Terpineol	0.18		0.73
1172	3,5,5-Trimethyl-1,4-cyclohexanedione	0.09	0.14	1.41
1193	Myrtenal	0.23		0.08
1200	Z-Dihydrocarvone	0.18		0.34
1232	cis-Carveol	0.15		
1266	Linalool acetate	18.18	1.72	21.44
1279	p-sec-Butylphenol			0.58
1318	(Z)-3-Hexenyl 2-methyl-(E)-2-butenolate	0.38		
1327	Trans-Pulegone oxide [a]	0.91	0.31	1.53
1356	α-Cubebene	1.11	0.47	0.26
1370	Cyclosativene	0.72		0.16
1383	β-Bourbonene	1.74		0.44
1400	γ-Caryophyllene	4.16	2.05	2.53
1409	Aromadendrene			
1435	α-trans-Bergamotene	1.29	0.59	1.04
1444	α-Caryophyllene			0.13
1455	α-Patchoulene	0.48		0.18
1461	Alloaromadendrene	7.55	1.41	1.14
1474	γ-Murolene			0.35
1479	γ-Curcumene	0.58		0.43
1494	α-Zingiberene	2.14	2.38	0.53
1501	α-Amorphene	1.39		1.64
1516	α-Selinene	0.32		
1534	incisol	0.17		0.59
1557	Caryophyllene alcohol	1.06		
1568	Caryophyllene oxide	1.82	2.45	4.24
1588	β-Oplopenone			0.38
1594	Cedrenol	0.48	1.03	2
1613	1-epi-Cubenol	0.52	2.12	2.83
1619	trans-Isolongifolanone			0.42
1626	γ-Eudesmol	0.58		2.56
1642	Spathulenol	0.43	0.77	2.37
1660	14-Hydroxy-9-epi-(E)-caryophyllene	0.14	0.5	0.97
1673	Cedr-8-en-13-ol			0.23
1678	cis-Alpha-santalol	0.44	1.24	1.74
1717	(Z)-2-Heptadecene ^a	0.22		
1761	1,10-Dihydronootkatone		0.88	0.08
1928	16-Hexadecanolide		0.43	0.15
1962	Palmitic acid		2.98	36.19
1991	Manoyl oxide	0.13		0.26
2008	Kaur-16-ene	0.14		0.1

Table 1 (Continued)

		Type of extraction technique		
		SFE		HD
2053	abietatriene			0.32
2095	Heneicosane	0.4	3.66	0.09
2135	1R-pimaral	0.13	6.09	0.27
2143	Cubitene	0.22		
2187	Cembrene A ketone	0.25	1.37	0.14
2216	15-Ripperten-3 α -ol	0.41	2.11	0.52
2223	trans-Totarol	0.12		
2274	Larixol ^a		2.17	
2288	2-Methyltricosane		10.29	
2311	trans-Totarol		4.85	0.07
2368	androst-5-en-17-ol, 4,4 dimethyl		2.28	2.49
2383	Podocarp-7-en-3-one, 13a-methyl-13-vinyl		7.77	25.53
2473	Docosyl acetate		14.4	1.35
2922	Abietic acid			0.31
2925	ni			1.05
2930	Podocarp-8(14)-en-15al, 13a-methyl-13-vinyl			10.79
	ni (M=410)			0.73
	ni (M=552)			2.14
	ni (M=618)			8.96
	ni (M=296)		7.34	7.98
	Number of compounds	56	29	14
	Monoterpene hydrocarbons	40.73	0	0
	Monoterpene oxygenated	6.92	0.41	0.43
	Sesquiterpenes hydrocarbons	21.48	6.9	0
	Sesquiterpenes oxygenated	5.64	8.99	0
	Others	21.14	67.55	97.71
	Total	96.24	83.85	98.14

ni, not identified.

^a Tentative identification supported by good match of mass spectrum.

hypothesis could explain the predominance of equilibrium phenomena as compared to internal mass transfer in the extraction process.

3.1.6. Effect of SCCO₂ extraction on the structure of vegetal material

Optical microscope or Scanning Electron Microscope (SEM) analyses of the material were done to visualize its structure and the effect of the extraction process upon this structure. The initial structure of the ground leaves from *T. articulata*, observed by SEM, is shown in Fig. 6(a). From comparison with Fig. 6(b), it is seen that SFE had almost no effect on the structure of the plant, at least on a visual point of view. Moreover, SEM images tend to confirm the hypothesis of secretory ducts, since the plant is actually made of a core of interconnected channels surrounded by a dense bark.

3.2. Characterization of extracts and comparison with HD

3.2.1. Chemical composition of the extracts

The global yield and the results of the GC-MS analysis of volatile oil obtained from *T. articulata* by SFE and HD are presented in Table 1.

As expected, there was a significant difference in the extraction yields between the extracts obtained using HD and SFE at the three pressures. Extraction at high pressure was found to be more efficient than other conditions studied in extracting the antioxidant components present in the *T. articulata* leaves.

Although a total of 86 compounds were identified, for the sake of simplicity, the discussion has been restricted to the major components identified for each method. These major components are presented in Table 2, where a total of 28 components with a percentage higher than 2% are gathered. Results are presented in terms of relative proportion (% of area of the peak in the chromatograms) and amount in mg/kg of extract. Compounds appear in Table 2 in the order of decreasing volatility. Although this could lead to approximate results, because terpenes are the main components, it has

been considered here that palmitic acid, abietic acid and terpenes have similar response factor.

When comparing composition of essential oil (HD) and volatile oil obtained by SFE at 90 bar, no great differences were observed and α -pinene and linalool acetate are predominant in the extracts obtained with these methods. However, the compounds in extracts obtained by SFE at 280 and 1000 bar are significantly different compared to those obtained by HD or SFE at 90 bar.

It appears that the higher the pressure of SFE, the lower the number of detected compounds, which in that case corresponds to heavy compounds. For example, only 14 volatile compounds have been detected by GC-MS in the extract obtained by SFE at 1000 bar (only 10 have been reported in Table 2). Note also that, due to the gas chromatography method, only high molecular mass compounds with retention index (RI) value greater than 1900 were detected. On the other side, the essential oil (HD), volatile oil of SFE (at 90 bar) and extract from SFE at 280 bar were composed of 56, 56 and 29 compounds, respectively. This result is surprising because high pressure operation allows extracting more compounds due to the increased solvent power of SCCO₂. Indeed, this results from an "artifact" of the recovery system of the A21 (SFE1000 bar) apparatus which was not very effective or at least incorrectly used. This apparatus has only one separator (conversely to SF200 extraction which has 3 separators in series). Experiments at 280 and 1000 bar (where volatile components were not detected) were done on A21 apparatus while experiments at 90 bar were done on the SF200 extraction (where these volatile components were recovered and detected). So it is very probable that the unique separator of A21 has favored the selective loss of the volatile components that obviously were extracted at high pressure but not recovered in the low pressure recovery zone. This explains that low molecular mass compounds do not appear in the composition.

In volatile oils obtained by HD and SFE at 90 bar, the main constituents were α -pinene (24.90–31.32%), linalool acetate (21.44–18.18%), alloaromadendrene (1.14–7.55%), camphene hydrate (4.36–1.25%) and γ -caryophyllene (2.53–4.16%). The main

Table 2Chemical composition obtained by GC–MS (abundance >2%) and global yield of *T. articulata* extracts obtained by SFE at different pressures and by HD.

	Type of extraction technique								
	SFE				HD				
	Flow rate (g/min)	20	20	20	20	0.02 mL/s			
	Temperature (°C)	40	40	40	40	100			
	Pressure	90	280	1000		-			
	Solvent	CO ₂	CO ₂	CO ₂		Water			
	Extraction time (min)	30	30	30		180			
	Global yield (mg/kg)	1.63	19.2	25.9		0.61			
RI	Compounds	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
936	α-Pinene	31.32	511			24.9	152		
967	β-Thujene	2.64	43			1.99	12		
1004	Pseudolimonene	0.26	4			0.37	2		
1008	3-Carene	3.39	55			3.44	21		
1128	α-Campholenal	3.44	56	0.1	19	0.43	111	3.84	23
1148	Camphene hydrate	1.25	20			4.36	27		
1266	Linalool acetate	18.18	296	1.72	330	21.44	131		
1400	γ-Caryophyllene	4.16	68	2.05	394	2.53	15		
1461	Alloaromadendrene	7.55	123	1.41	271	1.14	7		
1494	α-Zingiberene	2.14	35	2.38	457	0.53	3		
1568	Caryophyllene oxide	1.82	30	2.45	470	4.24	26		
1613	1-epi-Cubanol	0.52	8	2.12	407	2.83	17		
1626	γ-Eudesmol	0.58	9			2.56	16		
1642	Spathulenol	0.43	7	0.77	148	2.37	14		
1962	Palmitic acid			2.98	572	36.19	9373		
2095	Heptacosane	0.4	7	3.66	703			0.09	1
2135	1R-pimaral	0.13	2	6.09	1169			0.27	2
2216	15-Ripperten-3α-ol	0.41	7	2.11	405			0.52	3
2274	Larixol ^a			2.17	417				
2288	2-Methyltricosane			10.29	1976				
2311	trans-Totarol			4.85	931	0.05	13	0.07	
2368	Androst-5-en-17-ol, 4,4 dimethyl			2.28	438	2.49	645		
2383	Podocarp-7-en-3-one, 13a-methyl-13-vinyl			7.77	1492	25.53	6612		
2473	Docosyl acetate			14.4	2765	1.35	350		
2930	Podocarp-8(14)-en-15al, 13a-methyl-13-vinyl					10.79	2795		
	ni (<i>M</i> =552)					2.14	554		
	ni (<i>M</i> =618)					8.96	2321		
	ni (<i>M</i> =296)			7.34	1409	7.98	2067		
	Total	78.62	1282	76.94	14,772	95.91	24,841	77.49	473

ni, not identified.

^a Tentative identification supported by good match of mass spectrum.

compounds of extracts obtained by SFE at 280 and 1000 bar were palmitic acid (2.98–36.19%), docosyl acetate (14.40–1.35%), podocarp-7-en-3-one, 13a-methyl-13-vinyl (7.77–25.53%), 2-methyltricosane (10.29–0%), podocarp-8(14)-en-15al, 13a-methyl-13-vinyl (0–10.79%) and 1R-pimaral (6.09–0%). Also, some compounds with high molecular mass were detected but not identified. For instance, the amount of some of these unidentified compounds was significant in SFE extract at 1000 bar (8.96% (*M*=618 g/mol) and 7.98% (*M*=296 g/mol)).

On the other hand, important difference between the compounds of each extracts can be seen in term of quantity (mg/kg). It is the case for example of α-pinene which was identified as the major compound in HD and SFE at 90 bar, where quantities determined in HD and SFE extracts, were 152 mg/kg (for 24.9% of global oil) and 511 mg/kg (for 31.31% of global oil) respectively.

It is interesting to compare these results with the few studies that have been conducted on the essential oils of *T. articulata*. Ben Hadj Ahmed et al. [30] have studied essential oil from *T. articulata* plants harvested in the region of Sned Gafsa (Tunisia) and have only reported about antioxidant activity of the essential oil and not about its chemical composition. Tekaya-Karoui et al. [31] have worked on *T. articulata* plants harvested in the region of Zaghous (Tunisia) and have shown that chemical composition was different, depending on the part of the plant. In the essential oil obtained from branches, the major compounds were camphene (43.2%), Z-murolène (29.0%) and p-cymene-8-ol (10.4%) while essential oil from the roots was richer in camphene (70.2%). A study of Barrero et al. [32] upon the chemical composition of essential oil

from leaves and wood of *T. articulata* plants harvested in the region of Amsa Téouan (Morocco) has shown that the leaves were rich in bornyl acetate (16.5%), camphor (19.1%) and borneol (9.6%) and that the essential oil from the wood was rich in cedrol (28.2%) and 1,7-di-epi-cedrol (17.9%). The results reported by Aitigri [33] have shown that essential oil from wood of *T. articulata* plants harvested in the region of Rabat (Morocco) were rich in carvacrol and α-cedrol. Another study of Zrira et al. [34] upon *T. articulata* plants from the region of Khemisset and Aoulouz (Morocco) has reported the predominant presence of carvacrol (21.3–36.4%), α-cedrene (10.1–13.1%), cedrol (1–7.3%) and terpinen-4-ol (2.8–6%). In a study from Buhagiar et al. [35], 18 compounds were identified in aerial parts of *T. articulata* plants, where α-pinene, limonene, camphor and bornyl acetate were the main compounds. From this survey, it is seen that the chemical compositions of *T. articulata* essential oils is very dependent on the harvesting place and on the specific part of the plant. To the best of our knowledge, no study upon chemical composition of SFE extracts of *T. articulata* has yet been proposed.

3.2.2. Phenolics content and antioxidant activity of the extracts

The concentration of phenolics in the extracts, expressed as Gallic Acid Equivalent (GAE) is shown in Table 3. As expected, the amount of phenolic compounds in the SFE extracts at high pressure was the highest (102.03 ± 4.57 and 267.90 ± 8.06 mg GAE/g plant material for 280 bar and 1000 bar, respectively), followed by SFE at 90 bar 9.70 ± 0.57 and HD 8.89 ± 0.16 mg GAE/g dry plant material.

The results from Table 3 show that, although the quantity of phenolics is low and almost identical for the HD essential oil and

Table 3

Total phenolics content and antioxidant activity of *T. articulata* extracts obtained by SFE at different pressures and by HD.

Samples	Phenolics (GAE mg/g)		DPPH		ABTS	
	GAE mg/g	±	IC ₅₀	±	IC ₅₀	±
SFE 90 bar	9.70	0.57	146.02	1.99	40.91	0.53
SFE 280 bar	102.03	4.57	120.21	3.65	33.55	0.40
SFE 1000 bar	267.90	8.06	108.16	3.07	29.77	1.30
HD	8.89	0.16	3681.49	69.33	324.45	14.21

GAE, Gallic Acid Equivalent.

SFE extract at 90 bar, their antioxidant activity is different. The SFE extract at 90 bar (IC₅₀ (μg/ml)=40.91 ± 0.53) is about 8 times more active than essential oil (IC₅₀ (μg/ml)=324.45 ± 14.21). These results suggest that the SFE volatile oil from *T. articulata*, is more concentrated with antioxidants and could be used as a potential source of natural antioxidants with possible applications in food systems. The presence of antioxidants in this plant is confirmed by a previous publication [11] where phenolics and antioxidant activity of Algerian *T. articulata* leaves extract were determined. These authors showed that extract of *T. articulata* from Algeria obtained by heat reflux with distilled water exhibited good antioxidant activity (IC₅₀ (μg/ml)=9.519 ± 0.521 and high total phenolic content (mg GAE g⁻¹)=206.187 ± 16.612). Although GC-MS is able to identify some of these molecules, they were not detected in our case. Therefore the high measured antioxidant activity can be explained by the presence of high molecular weight phenolics (tannins, anthocyanins, etc.) which cannot be detected using GC-MS.

Another explanation could be the occurrence of low quantities of highly active phenolic compounds. For example Harish Nayaka et al. [36] reported that, from values of IC₅₀ obtained for phenolic acid extracts of swallow roots, vanillic acid was 45 times less active than gallic acid.

3.3. Mathematical modeling

3.3.1. Preliminary analysis of the physical phenomena

Because vegetal matter is very diverse, numerous models describing supercritical extraction process are available in the literature, from the simple empirical ones to the most complex that are able to handle all physical phenomena occurring during extraction. The choice is always difficult and the most complex model might not be the most useful because it is associated with the estimation of numerous parameters, while some of them may have a negligible influence. Moreover their complexity prevents sometimes from easy understanding which is necessary to select the optimal conditions for the extraction process, especially when scale-up is envisaged. So, qualitative interpretation of kinetic curves together with the use of easy-to-implement simplified models is very useful. Especially, qualitative study of the extraction curves at different flow-rates and different particle sizes is very instructive. Such an approach has been recently recommended by Sovová [17] in a recent paper which provides a simplified method based on the use of characteristic times for all involved physical processes. Comparison of these characteristic times greatly helps for discriminating the prominent parameters. However, care must be taken to insure that, when scaling up, the choice of these parameters remains pertinent.

We have restricted our approach to volatile oil supercritical extraction and therefore the modeling is proposed for results obtained at 90 bar and 40 °C.

In the case presented here, from the overlapping of curves of Fig. 4(b), as indicated in Section 3.1.4, a limitation by the adsorption phenomenon is strongly suspected. In this case, when external mass transfer is not involved, estimation of the

adsorption coefficient is obtained from the initial slope of Fig. 4b. The hypothesis of negligible external mass transfer limitation can be assessed by computing external mass transfer characteristic time t_f [17]. External mass transfer characteristic time t_f depends on k_f (external mass transfer coefficient), λ which is the volume/surface ratio of the particle ($=R/3$ for spherical particles) and ε , the void fraction of the particle bed.

$$t_f = \frac{\varepsilon \lambda}{1 - \varepsilon k_f} \quad (1)$$

$$t_1 = \frac{G}{K_m \cdot q' \cdot (1 - \exp(-1/\theta_f))} \quad (2)$$

The extraction time t_1 corresponding to the end of the linear part of the extraction curve is thus a function of q' which is the CO₂ flow-rate referred to the mass of solid, G , the fraction of easily accessible cells, and K_m which is the solute partition coefficient (kg plant/kg solvent) defined as $C = K_m \cdot q$, where C is the concentration of volatile oil in the fluid phase (g solute/g CO₂) and q is the concentration of volatile oil in the solid phase (g solute/g plant). θ_f is the ratio of the external transfer characteristic time t_f to the residence time t_r . This latter is given by:

$$t_r = \frac{\gamma}{q'} \quad (3)$$

where γ is the ratio between initial CO₂ mass and solid mass in the extractor.

To provide a quantitative assessment of these characteristic times, extraction at the higher flow-rate, 20 g/min, for assumed spherical 1.5 mm diameter particles, is considered because this case is the most likely to exhibit influence of external mass transfer. Considering a void fraction $\varepsilon=0.457$, the characteristic time for external transfer t_f is computed at 1.05 s using the correlation of Tan et al. [37] for the evaluation of k_f (which yielded $k_f=1.61 \times 10^{-4} \text{ m s}^{-1}$) and t_r is equal to 75 s. The value of t_f being low as compared to t_r confirms that external mass transfer resistance can be neglected. This result makes it possible to use a simple equilibrium model for the linear part of the extraction curve. Such an approach was proposed by Reis-Vasco et al. [38] for the extraction of pennyroyal essential oil. Visually, from Fig. 4 (a), the value $G=0.7$ for the accessible fraction is estimated, corresponding to the end of the linear part of the curves which occurs at $t_1=750$ s.

The interpretation of the curved final part of the extraction curves is trickier. In a first approach, as suggested by Sovová, this part would correspond to an internal mass transfer limited process, concerning the extraction of the nonaccessible part of the solute. If this was true, final parts of curves of Fig. 4(b) (which relates the extraction yield to the mass of CO₂ used) should exhibit influence of the flow rate, while this is not the case because curves overlap up to the end of extraction. In addition, a confirmation of this hypothesis is found when attempting to fit the experimental data with the simplified model for plug-flow with adsorption given in Sovová's paper [17]:

$$e = x_u - (1 - G)x_u \exp\left(-\frac{t - t_1}{t_{comb,i}}\right) \quad \text{for } t \geq t_1 \quad (4)$$

where x_u is the initial solute content of the solid before extraction (it is determined from the value of the final yield of the extraction curves at a value $x_u=1.6 \times 10^{-2} \text{ g/g}$). The combined characteristic time of mass transfer $t_{comb,i}$ is obtained, as a function of t_f , K_m and t_i (internal mass transfer characteristic time) that involves the effective internal effective diffusion coefficient D_e . These characteristic times are given by:

$$t_i = \frac{R^2}{15D_e} \quad (5)$$

$$t_{comb,i} = t_i + \frac{t_f}{\gamma \cdot K_m} \quad (6)$$

When using this approach for the final part of the experimental curves obtained at different CO₂ flow-rates, parameter identification yielded different values of the internal diffusion coefficient D_e for each flow-rate, while this parameter should not be affected by the flow-rate.

All these elements indicate that the final curvature of the extraction curves corresponds more probably to the influence of axial dispersion (which is not accounted for in the simplified model). Eventually, this suggests an adsorption limited extraction process where all the solute is accessible with negligible external mass transfer limitation, but where significant axial dispersion is present. The computation of the Peclet number, $Pe = h \cdot u / (D_L \cdot \varepsilon)$, with the relation of Funakuzuri [39] for the axial dispersion coefficient, yielded $Pe = 19$ which is quite low and ascertains the hypothesis of a non-negligible axial dispersion influence (see details below in the paragraph about modeling). This makes it possible to use a simplified model which indeed corresponds to the first part of the Reis-Vasco's model, i.e., differential mass balance equations corresponding to the case of extraction limited by the solute adsorption and describing the flow pattern as a piston flow with axial dispersion [38].

3.3.2. Description of the equations of the model

Mass balance equations in that case correspond to the following assumptions: (1) the superficial velocity of SCCO₂, u , is constant during the process, (2) the process is isothermal and pressure drop is neglected, (3) volatile oil is described by a single component, the "solute" and (4) a linear equilibrium relationship is assumed defined as $C = K_m \cdot q$. The corresponding model equations, boundary and initial conditions are (Reis-Vasco et al.) [38]:

$$\rho_f \varepsilon \frac{\partial C}{\partial t} + \rho_s (1 - \varepsilon) \frac{\partial q}{\partial t} = -\rho_f u \frac{\partial C}{\partial z} + D_L \rho_f \varepsilon \frac{\partial^2 C}{\partial z^2} \quad (7)$$

$$C = K_m \cdot q \quad (8)$$

$$t = 0, \quad C = C_0 \quad \text{and} \quad x_u = q_0 + \gamma C_0 \quad z > 0 \quad (9)$$

$$z = 0, \quad \frac{u}{\varepsilon} C - D_L \frac{\partial C}{\partial z} = 0 \quad t > 0 \quad (10)$$

$$Z = L, \quad \frac{\partial C}{\partial z} = 0 \quad t > 0 \quad (11)$$

Eq. (7) can be re-written as:

$$\left(1 + \frac{(1 - \varepsilon) \rho_s}{\varepsilon \rho_f K_m} \right) \frac{\partial C}{\partial t} + \frac{u}{\varepsilon} \frac{\partial C}{\partial z} - D_L \frac{\partial^2 C}{\partial z^2} = 0 \quad (12)$$

where t is extraction time, z is the axial coordinate of the extraction bed, C_0 is the concentration of volatile oil in the fluid phase at the beginning of the extraction process which corresponds to the equilibrium with the initial solid phase concentration q_0 , q_0 and C_0 being related by Eq. (9), ε is the bed void fraction, ρ_s density of vegetable matter, and D_L is the axial dispersion coefficient (m²/s).

The system of equations was solved with the Matlab® software. Initial concentration of solute in CO₂, C_0 , was computed from the initial slope of Fig. 4(b) and, the value $C_0 = 0.2 \times 10^{-2}$ g solute/g CO₂ was obtained. Equilibrium adsorption constant $K_m (=C_0/q_0)$ was then computed at $K_m = 0.14$ kg plant/kg CO₂.

At 90 bar and 40 °C, density and viscosity of CO₂ were evaluated at $\rho_f = 448.45$ kg/m³ and $\mu_f = 40.1 \times 10^{-6}$ Pa s. The axial dispersion

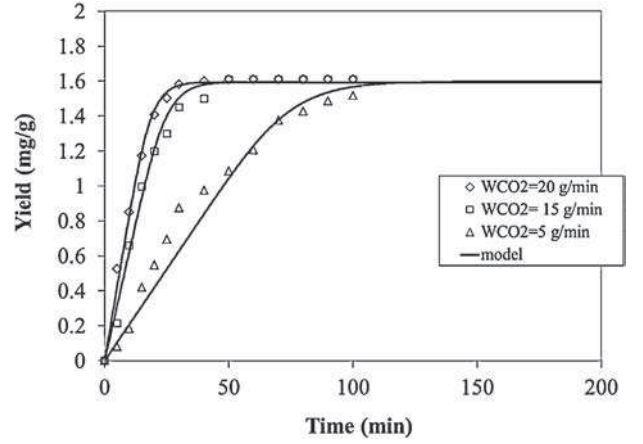


Fig. 7. *T. articulata* volatile oil yield for 1.5 mm particles size, pressure 90 bar and temperature 40 °C at different CO₂ flow rates with Reis-Vasco model (first part only).

coefficient D_L was determined using the correlation proposed by Funakuzuri [39]:

$$D_L = 1.317(ReSc)^{1.392} \left(\frac{D_{12}}{\varepsilon} \right) \quad (13)$$

where

$$Re = \frac{\rho_f u d_p}{\mu_f} \quad (14)$$

$$Sc = \frac{\mu_f}{\rho_f D_{12}} \quad (15)$$

The binary diffusion coefficient D_{12} was estimated by the Wilke-Chang equation as proposed by Funakuzuri:

$$D_{12} = 7.410^{-12} \frac{T \sqrt{\Phi M_{CO_2}}}{\mu_f V_{2bp}^{0.6}} \quad (16)$$

where T is the temperature (K), Φ is the association factor of the solvent ($\Phi = 1$ for CO₂), M is the molar mass of the solvent and V_{2bp} is the molar volume of the solute at its normal boiling point expressed in cm³/mol. As α -pinene is a compound present in all volatile extracts, its molar volume $V_{2bp} = 178.63 \times 10^{-3}$ m³/kmol was used in (15) [17]. A value of $D_{12} = 1.7 \times 10^{-8}$ m²/s was obtained for 90 bar, 40 °C. Axial dispersion coefficient was found to be equal to 1.9×10^{-5} m²/s and $Pe = 19$ in the case of CO₂ flow-rate equal to 20 g/min.

In Fig. 7, experimental curves are compared with calculated yields using this model and a good agreement is observed for the three different CO₂ flow-rates. Together with the qualitative analysis we have developed in the preliminary analysis, this result validates the choice of the proposed model.

3.3.3. Use of the modeling for scaling-up the extraction process

As we mentioned, significant axial dispersion was pointed out at laboratory scale ($Pe = 19$ for 20 g/min CO₂ flow-rate). Nevertheless its influence on the effective duration for extracting the solute is not very strong. For instance, at 20 g/min, duration for the obtaining of 90% yield is predicted at 16 min with the Sovova's simplified model (which neglects axial dispersion, t_1 obtained by Eq. (2)) while our modeling, which takes into account axial dispersion, indicates a value close to 20 min. This rather moderate difference makes it possible to propose simplified scale-up by maintaining the specific flow-rate q' and the ratio height to diameter of the extractor (5 in our case). With this extrapolation rule, for instance for a 1000 fold greater vegetal mass to extract, simple computation results in a 113 L extractor (diameter 0.3 m, height 1.6 m) percolated by

240 kg/h of CO₂ (superficial velocity 10 mm/s) in which 90% yield is obtained after 16 min. In this case, it can be computed that the Peclet number value is now 82, which is more favorable than in the laboratory scale extractor (where Pe = 19) and indeed reinforces the validity of this simple extrapolation procedure.

4. Conclusions

Recovery of bioactive compounds from *T. articulata* leaves was obtained using SCCO₂ extraction. For volatile compounds, optimal conditions were found to be 90 bar, 40 °C, and 30 min duration. For non-volatile fraction, suitable conditions were found to be 1000 bar, 40 °C and 30 min duration. Also, quantitative assessment of the extract antioxidant power and the enrichment of antioxidant at different extraction conditions were performed. Interesting selectivity for compounds with high antioxidant activity (ABTS*⁺ essay (29.77 ± 1.3, 33.55 ± 0.4 and 40.91 ± 0.53 mg/L) and phenolic content (267.90 ± 8.06, 102.03 ± 4.57 and 9.70 ± 0.57 GAE mg/g material)) were observed for SFE extracts at different pressures (1000, 280, and 90 bar), respectively. Conventional HD was tested and essential oils obtained by this technique revealed low antioxidant activity (324.5 ± 14.21 mg/L).

Also, different chemical compositions of the extracts were found, depending on the SFE pressure extraction. Physical aspects of the low pressure supercritical extraction (90 bar, 40 °C) were investigated and modeling of the extraction kinetics using a simplified form of the Reis-Vasco's mathematical model proved to be adequate. In our range of operating conditions, extraction of *T. articulata* was found to be governed by adsorption phenomenon and significant axial dispersion was pointed out. This made it possible to propose simple extrapolation procedure. It is noteworthy that productivity can be increased proportionally to CO₂ flow-rate, as long as mass transfer effects remain negligible. Also, adsorption constant can be rendered more favorable by increasing the solvent power of CO₂ using higher pressure but a correlative loss of selectivity is expected.

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