

Bioactivity and Kinetic Study of *Jatropha curcas* Essential Oil Extraction Using Supercritical CO₂

Laura Catalina Soto-Armenta¹, Julio C. Sacramento Rivero², Claudia Araceli Ruiz-Mercado², Azucena González-Coloma³, José Antonio Rocha-Uribe^{4*}

¹Universidad del Valle de México, Mérida, Yucatán, México

²Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Yucatán, México

³Instituto de Ciencias Agrarias, CSIC, Madrid, España

⁴Colegio de Bachilleres de Chiapas, Tuxtla Gtz., Chiapas, México

Email: *antonio.rocha@cobach.edu.mx

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Abstract

This study reports the extraction of *Jatropha curcas* leaves using supercritical CO₂. Experiments were performed varying the pressure (13 and 20 MPa) and the temperature (50 °C and 60 °C). The model of Sovová for supercritical fluid extraction was fitted to the experimental kinetic extraction curves. Two cell sizes were used and scale up equations compared. GC analysis showed phytol, carvacrol, and hexahydrofarnesyl acetone as major compounds in all the experiments. A maximum yield of 0.95% dry-weight basis was obtained. It was observed a maximum yield (0.95% dry-weight basis) extract obtained at 20 MPa and 50 °C. The results indicated that the mass yield increased with the increase of pressure. The bioassays showed that the extract of *J. curcas* possessed toxicity against *Hyalomma lusitanicum*.

Keywords

Jatropha curcas Leaves, Supercritical Fluid, Scale-Up, Acaricidal Activity

1. Introduction

Jatropha curcas belongs to the Euphorbiaceae family. It adapts to places with low fertility and alkaline soils [1] [2]. Biological activity of the leaf extracts has been reported on aqueous and methanolic extracts for insecticidal activity against *Anopheles arabiensis* [3], *Culex quinquefasciatus* [4], and for avoiding the hatching of *Rhipicephalus* (Boophilus) *annulatus* eggs [5], and for acetonic extracts against the *Aedes aegypti* [6]. Hexane extracts proved to be a potential and reliable option for the application of pesticides against *Musca domestica* [7], and

an effective and long-lasting repellent against *Aedes aegypti* when mixed with aqueous extracts of *Citrus lemon* and *Ocimum americanum* [8] (Kazembe, 2012).

Actually, infestation from tick is an important problem for human and veterinary health, because they can transmit a wide variety of diseases, the repetitive use and traditional commercial acaricides to provoked resistances. Due to this, biological control is a promising alternative in the control of this ectoparasite [5] [9].

In supercritical fluid extraction (SFE), research has shown that are pressure and temperature that mainly influence significantly the extraction yield, which in turn is determinant in the economics and profitability of the process [10].

The study reported here, is similar to the reported by Soto-Armenta [11], but in the last report the plant material was *Lippia graveolens* (oreganum), and only one cell size was used.

For these reasons, the present study aims to evaluate and characterize the effect of SFE operational parameters on the yield and bioactivity of *J. curcas* leaf extract. No previous reports were found of oil extraction from *J. curcas* leaves harvested in Mexico.

2. Materials and Methods

2.1. *Jatropha curcas* Leaves

Leaves of *J. curcas* were taken from pruning in the period from November to June, and were left to dry on an extended surface at room temperature, in a room with ventilation and roofing, for three weeks until their moisture content was approximately 10%. The leaves were manually pulverised. Particle size was within the range of 1.4 to 2.4 mm, and thickness of about 0.5 mm.

2.2. Extraction Gas

Industrial grade CO₂ was provided by Praxair, Merida-Mexico, with a purity of 99.9% and extra-dry air with a purity of 99.99%.

2.3. Supercritical Fluid Extraction (SFE)

Extractions were performed in a scale supercritical fluid extractor SFT-150 Technologies Inc., Newark, USA **Figure 1**. The diameter of the extraction cell is 0.029 m and the height of the packing is 0.15 m.

The extraction cell was loaded with about 15 g of previously dried and ground leaves; leaves had a moisture content of $9.88\% \pm 0.87$. The bed porosity was 0.90. The temperature of the equipment was raised, and the CO₂ was fed in the extractor up to the indicated extraction pressure. Constant temperature and pressure values were maintained, for a time of 10 minutes, during the dynamic extraction, a flow rate of 4.8 ± 0.009 g/min was maintained throughout the dynamic extraction stage. Two levels of temperature were handled (50°C and 60°C) and two levels of pressure (13 and 20 MPa), based on this information on other research referring to the SFE of *Jatropha curcas* [12] [13]. The extractions were

made in duplicate and the results were analyzed with an analysis of variance (ANOVA). Statistically significant factors were further analyzed by a media comparison using a Tukey test. Statistical analyses were analyzed using the software STATGRAPHICS [14].

2.4. Adjusted Mathematical Model

Mathematical model of Sovová [15] was fitted by the method of minimum squares, shown in Table 1 [16]. A student's t-test was used to determine significant differences between the experimental and simulated data. The model describes



Figure 1. Supercritical fluid equipment with a capacity of 0.1 L and 1.0 L (photo by author).

Table 1. Summary of Sovová's model.

Period	Equation
Period I. (Constant extraction rate)	$e = y_r q [1 - \exp(-Z)] \quad (a)$
	$Z = \frac{k_f a_0 \rho}{(1 - \varepsilon) \dot{q} \rho_s} \quad (b)$
Period II. (Decay of extraction)	$e = y_r [q - q_m \exp(Z_w - Z)] \quad (c)$
	$q_m = x_0 - \frac{x_k}{y_r Z} \quad (d)$
	$\frac{Z_w}{Z} = \frac{y_r}{W x_0} \ln \frac{x_0 \exp\left[\frac{W(q - q_m)}{x_0 - x_k}\right] - x_k}{x_0 - x_k} \quad (e)$
Period III. (Extraction controlled by diffusion)	$e = x_0 - \frac{y_r}{W} \ln \left\{ 1 + \frac{\left[\exp\left(\frac{W x_0}{y_r}\right) - 1 \right] \exp[W(q - q_m)] x_k}{x_0} \right\} \quad (f)$
	$W = \frac{k_f a_0}{\dot{q}(1 - \varepsilon)} \quad (g)$

the supercritical fluid extraction in three periods: I) The constant extraction rate (CER) period, where the extraction of the easily accessible solute occurs. II) Falling extraction rate (FER) period, consisting of a decrease in the accessible solute. III Diffusion controlled extraction rate (DCR) period, when the extraction of hardly accessible solute occurs.

2.5. Scale-Up Experiment

An experimental run was performed in a cell of 1.0 liter of capacity with a diameter of 0.07 m and a bed height of 0.21 m. The mass of leaves was 0.090 kg and the mass flow rate of CO₂ was 27.6 g/min. The bed porosity was the same that for the small column, $\epsilon = 0.90$.

The CO₂ mass flow rate was set trying to get an experimental oil yield similar to the one measured in the small column. Several criteria have been suggested for this:

Equal effective velocity of CO₂ in both columns:

$$Ur_2 = Ur_1, \text{ or } \frac{q_{vol-2}(\epsilon_2)}{A_2} = \frac{q_{vol-1}(\epsilon_1)}{A_1}$$

$$\frac{(q_{CO_2-2})(\epsilon_2)}{(den_{CO_2-2})(A_2)} = \frac{(q_{CO_2-1})(\epsilon_1)}{(den_{CO_2-1})(A_1)} \text{ then}$$

$$q_{CO_2-2} = \left(\frac{den_{CO_2-2}}{den_{CO_2-1}} \right) \left(\frac{A_2}{A_1} \right) \left(\frac{\epsilon_1}{\epsilon_2} \right) q_{CO_2-1} \quad (1)$$

Equal residence time:

$$t_{R1} = t_{R2}, \text{ or, } t_{R2} = \frac{(Vol_2)(\epsilon_2)}{q_{vol2}} = \frac{(Vol_1)(\epsilon_1)(den_{CO_2-2})}{q_{CO_2-2}} \quad (2)$$

$$\text{or } q_{CO_2-2} = \left(\frac{Vol_2}{Vol_1} \right) \left(\frac{\epsilon_2}{\epsilon_1} \right) \left(\frac{den_{CO_2-2}}{den_{CO_2-1}} \right) q_{CO_2-1} \quad (3)$$

Carvalho *et al.* [17], E-1:

$$q_{CO_2-2} = \left(\frac{F_2}{F_1} \right)^2 \left(\frac{H_{b1}}{H_{b2}} \right) \left(\frac{D_{B1}}{D_{B2}} \right) q_{CO_2-1} \quad (4)$$

Carvalho *et al.* [17], E-2:

$$q_{CO_2-2} = \left(\frac{F_2}{F_1} \right)^2 \left(\frac{H_{b1}}{H_{b2}} \right) \left(\frac{D_{B1}}{D_{B2}} \right)^3 q_{CO_2-1} \quad (5)$$

2.6. Extracts Composition

The extracts were analyzed by gas chromatography-mass spectrometry (GC-MS), using an Agilent Technologies (model 6890N) coupled to an Agilent 5973N mass selective detector (MSD) with an electron impact ionization of 70 eV.). An HP-5 MS (30 m × 250 μm × 0.25 μm) column was used. The oven operating

conditions were: initial temperature 50 °C for 3 min, then ramping from 50 °C to 250 °C at 20 °C/min, in the end, the temperature remained at 250 °C for 10 minutes before post run (280 °C for 5 min). The injection and transfer line temperature were 150 °C and 280 °C, respectively. The injection volume was 2 µL, with a 10:1 split ratio [18].

2.7. Tick Activity

In this case, mortality trials against larvae of *Hyalomma lusitanicum* were evaluated, according to the methodology described by González *et al.* [19]. The extracts and essential oils were added at a concentration of 20 µg/µL, the absolute negative control was cellulose, the negative control was cellulose with the solvent (water), and finally the positive control was a commercial acaricide. 20 larvae of *Hyalomma lusitanicum* were placed in test tubes carefully covered with a piece of cotton. The conditions for maintaining ticks were at 24 °C and a relative humidity greater than 70%. Subsequently, the product to be tested was poured into a cellulose matrix in the vials where the ticks were located. Each vial was covered with hydrophilic cotton, then it was shaken to verify that there was contact of the product to be evaluated and the ticks. After 24 h after treatment, dead specimens were counted. The percentage of mortality was determined through the following equation:

$$\%M_T = \left(\frac{\text{Number of dead ticks}}{\text{Total number of ticks}} \right) * 100 \quad (6)$$

$$\%M_T \text{ corrected} = (\%M_T - \%M_C) / \%M_C \quad (7)$$

where M_T refers to the percentage of mortality caused by treatment and M_C is the percentage of mortality due to control. Data were analyzed using a probit analysis, to statistically determine the differences in mortality obtained.

3. Results

3.1. Experimental Data

Table 2 shows the average essential oil yields on a dry basis obtained in the experiments are reported. Experiments showed that at a temperature of 50 °C and

Table 2. Comparison of yields (%) and statistics, obtained from leaves of *J. curcas* by supercritical extraction.

Experiment	Pressure (MPa)	Temperature (°C)	Y (SD) (%)	ρ -CO ₂ (kg/m ³)
ESJ1	13	50	0.57 (0.028) ^b	573.6
ESJ2	13	60	0.31 (0.057) ^a	478.3
ESJ3	20	50	0.95 (0.010) ^c	784.4
ESJ4	20	60	0.86 (0.024) ^c	723.8

a. Mean values followed by the same lowercase letter in the same column do not statistically differ from each other at $\alpha = 0.05$, by the Tukey test. Y: Experimental yield obtained at the end of the process (% average), SD: Standard deviation, ρ -CO₂: Density of CO₂.

at pressure at 20 MPa was obtained the highest yield. The ANOVA showed that both temperature, pressure, and interaction have a statistically significant effect on the yield. Even though no references were found reporting extraction yields from leaves of *J. curcas* by supercritical extraction, there are reports of gallic acid production by supercritical extraction from dried leaves of *J. curcas* [12] [13]. Pressure influences the extraction efficiency, because the fluid density is affected by increasing pressure, which means a higher solubility of the solid in the extraction fluid. Temperature is another parameter that influences the extraction; normally at higher temperature the extraction yield increases, however, the effect of temperature depends on the nature of the compound. If it is a non-volatile solute, a high temperature will decrease yield extraction as it decreases solubility in the gas [20] [21] [22].

The effect of temperature and pressure on yield extraction is observed (Figure 2). For 13 and 20 MPa it is observed that increasing the pressure increases the yield. Nevertheless, in this case, increasing the temperature, the yield decreases. The same effect is reported by Pormortartazavi *et al.* [20], in the study of Gaspar *et al.* [23], They studied the solubility of echium, lunaria and borage oils in compressed CO₂, they used lower pressures of between 60 and 100 bar, where they observed that by increasing temperature, density and solubility decreased. While the opposite is observed by increasing the pressure, specifically at a pressure of 300 bar.

3.2. Modeling the Overall Extraction Curves

Shown in Figure 3, the experimental and modeled curves, where lower (13 MPa, 60 °C) and higher (20 MPa, 50 °C) yields are analyzed, the extraction yield percentage was plotted as function of time. The T_{CER} is directly proportional to the extraction yield. Constant parameters used to fit the model: density of the solid $\rho_s = 1450 \text{ kg/m}^3$, measured by pycnometer with helium gas according to Zabot *et al.* [24], at the Institute of Ceramics and Glass of the CSIC, (Madrid, Spain); mass of solid load $N = 0.015 \text{ kg}$; bed porosity $\varepsilon = 0.90$ and flow rate of solvent; $\dot{q} = 0.8 \times 10^4 \text{ kg/s}$.

Fitting the Sovová model [15] to the experimental data provided the kinetic parameters and the time of the CER period shown in Table 3. The value of the minimized error coefficient (Ce) is small enough to consider that the model fits

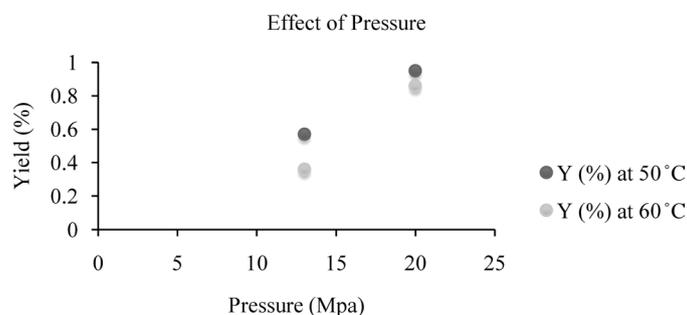


Figure 2. Effect on pressure at different temperatures on the experimental yield.

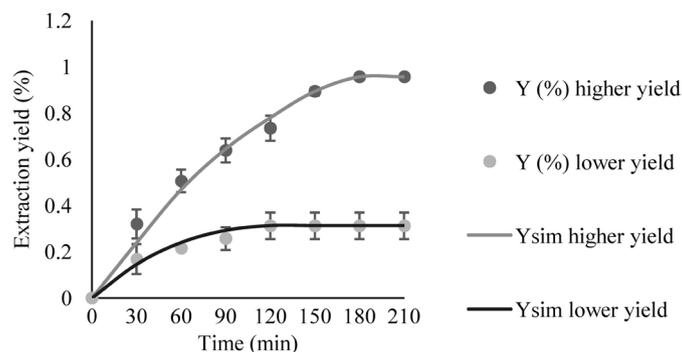


Figure 3. Experimental and modeled curves for higher (20 MPa, 50°C) and lower (13 MPa, 60°C) yields, with their respective constant extraction period time T_{CER} (-----).

Table 3. Adjustment of the Sovová model for *J. curcas*, according to the experimental data obtained.

Experiment	Y_{exp}	Y_{sim} (Sovová, 1994) (C_e)	p	k_{ra0} (s^{-1})	k_{sa0} (s^{-1})	Z	T_{CER} (s)	Solubility y in CO ₂ (kg/kg)
<i>J. curcas</i> 0.1 L								
ESJ1	0.57	0.57 (9.27×10^{-7})	0.99	0.0054 ^b	3.3×10^{-5c}	2.30	1114 ^b	0.0003
ESJ2	0.31	0.31 (1.88×10^{-2})	0.94	0.0050 ^a	3.1×10^{-5b}	1.32	489 ^a	0.0002
ESJ3	0.95	0.95 (1.99×10^{-6})	0.99	0.0021 ^c	4.7×10^{-5a}	5.48	1721 ^c	0.0005
ESJ4	0.86	0.86 (2.78×10^{-6})	0.99	0.0055 ^b	3.6×10^{-5c}	4.30	1561 ^c	0.0005
Average of C_e		3.51×10^{-4}						

a. Mean values followed by the same lowercase letter in the same column do not statistically differ from each other at $\alpha = 0.05$, by the Tukey test. Y_{exp} : Experimental yield obtained at the end of the process (% average), Y_{sim} : Simulated yield through the Sovová model, Value of p : Student's t -test at $\alpha = 0.05$, C_e : Error coefficient, k_{ra0} : Coefficient of mass transfer in fluid phase by the specific surface area per unit volume of the bed, k_{sa0} : Coefficient of solid phase mass transfer by the specific surface area per unit volume of the bed, Z : Parameter of the Sovová's model for the CER period, T_{CER} : Constant extraction period time.

appropriately to the experimental data. Additionally, the Student's t test indicates that there is no statistically significant difference between the experimental and the modelled yields. In addition, the volumetric mass transfer coefficient in the solid phase k_{sa0} increased as the pressure and temperature increased [25]. Rodrigues *et al.* [26], mentions in his research that high values of Z correspond to lower yields of extract. It was observed that higher yields were obtained when the CER period was longer. ANOVA shown, there was statistically significant difference among the kinetics between treatments.

3.3. Scale up Criteria

Figure 4 shows the extraction yield of both runs. The goal of getting the same yield was almost obtained. In this case the 1.0 liter column, the extraction yield

was 0.33%, whereas in the small column it was 0.31%. The total extraction time for both columns were about 150 minutes.

Figure 5 shows that in this case, the scale-up criterion was keeping constant the interstitial velocity of the solvent. If the criterion of constant residence time were to be chosen, a mass flow rate of 0.000653 kg/s of CO₂ would have been, and a higher yield would be obtained. The residence time for both runs from E 9 is $tR1 = 533 \text{ s} = 8.8 \text{ min}$, and $tR2 = 756 \text{ s} = 12.6 \text{ min}$.

3.4. Extracts Composition

As shown in **Table 4**, phytol, carvacrol and hexahydrofarnesyl acetone, were identified as the three majoritarian compounds in the extracts of the ESJ1, ESJ2 and ESJP1 experiments. In ESJ3, the main compounds were phytol, hexahydrofarnesyl acetone and farnesyl acetone, while in ESJ4 they were phytol, dihydroactinidiolide, and hexahydrofarnesyl acetone. In the experiment where co-solvent was applied, the majoritarian compounds were phytol, dibutyl sebacate, and palmitic acid. Reports have been found about obtaining oil from *J. curcas* leaves by supercritical extraction, such as Wang *et al.* [13], under conditions of 30 MPa and 50°C, 12 MPa and 55°C, 6 MPa and 30°C, identified 22,23-dihydro-stigmasterol, α -tocopherol and β -amirin as the main compounds, which do not match with the compounds identified in this work; Manpong *et al.* [12] handles different conditions, between 20 to 40 MPa and temperatures between 40°C to 60°C, of which only gallic acid was identified, since it was the compound of interest in their investigation. Phytol has been reported as an important component of the essential oil of *Salvia splendens*, shown a larvicidal activity against *Aedes albopictus* [27]. Phytol might be one of the responsible metabolites as an insecticidal agent [28].

3.5. Bioactivity against *Hyalomma lusitanicum*

Table 5 illustrates that the essential oil obtained by supercritical extraction (ESJ2) has a 100% efficacy against *Hyalomma lusitanicum*, Juliet *et al.* [5], report that ethanolic extracts from leaves of *J. Curcas* have a blocking effect on the hatching of *Rhipicephalus* (*Boophilus*) *annulatus*, which confirms to the leaves of *J. curcas* as a tick biocontroller.

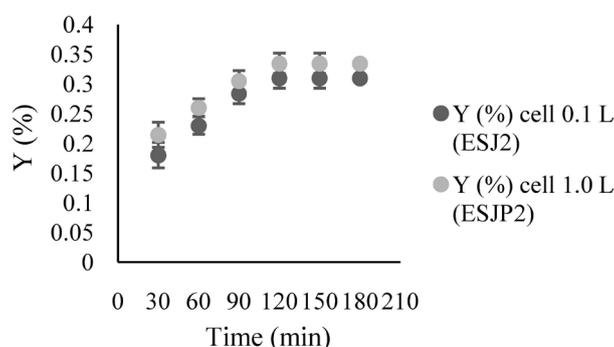


Figure 4. Extraction yield for extraction of *Jatropa curcas* with SC CO₂ at two cell sizes.

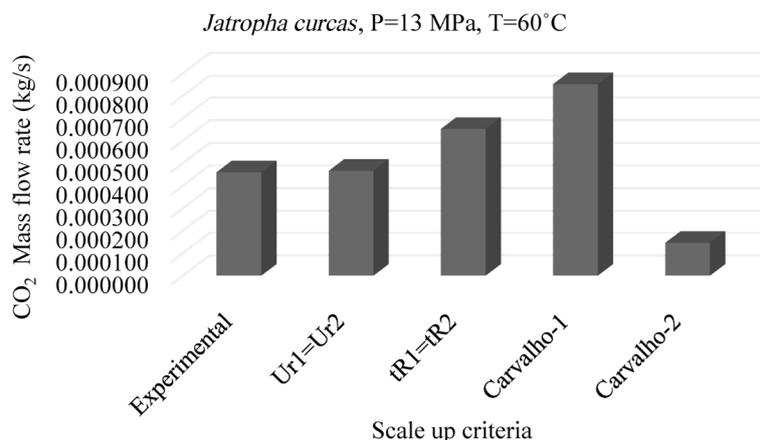


Figure 5. Comparison of CO₂ mass flow rate used and predicted with several criteria.

Table 4. Preliminary identification of the majority compounds of the essential oil of *Jatropha curcas*.

COMPOUND	% AREA Experiment			
	ESJ1	ESJ2	ESJ3	ESJ4
Phytol	82.29	80.87	83.11	85.62
Carvacrol	6.65	6.89	3.22	-
Hexahydrofarnesyl acetone	3.27	4.23	3.70	3.44
Farnesyl Acetone	2.55	3.04	3.19	2.32
1-Heptatriacotanol	1.57	1.69	-	-
Dihydroactinidiolide	1.41	-	-	4.62
Butyl octyl phthalate	-	-	1.92	-
Geranyl acetone	-	-	-	2.23

Table 5. Biological activity against *Hyalomma lusitanicum*.

Experiment	<i>H. lusitanicum</i> ^a
ESJ1	5.0 (5.00)
ESJ2	100.0 (00.00)*
ESJ3	32.5 (30.60)
ESJ4	0.0 (00.00)

^aPercentage of mortality of ticks with respect to control; the reported values correspond to the average of a total of three replicas. *Significant through the analysis of PROBIT.

4. Conclusion

The supercritical fluid extraction of *J. curcas* leaves was studied to investigate the effect of pressure and pressure temperature on yield, the scaling-up criteria and biological activity, where it was observed that the essential oil yield increased with increasing pressure and decreasing temperature. The temperature effect may be due to the non-volatile nature of the solute. Phytol was the main constituent in the essential oil extracted using various values of pressure and temperature.

This study only reports a preliminary composition and therefore it is suggested to focus more research on the analysis of the composition of the essential oil. The model of Sovova [15] fitted well to the kinetics of *J. curcas* SFE extraction, in which three phases are presented: a constant extraction stage, an extraction fall and finally, a controlled diffusion. For scaling-up the extraction operation, the criterion of maintaining constant the interstitial velocity of CO₂ was adequate. The extract obtained from the ESJ2 experiment in the supercritical extraction was bioactive against a possible tick strain. It is proposed to continue studying the effects in different doses and to elucidate the compounds responsible for said biological activity against *H. lusitanicum*, to obtain a potential product as a bio-controller.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Nomenclature

D_B	Diameter of cell, m
e	Yield = $100 \times$ mass of oil extracted/mass of solid phase (dimensionless)
H_B	Height of cell, m
N	Mass of the solute, kg
T_{CER}	Constant extraction period time, s
$k_f a_0$	Volumetric mass transfer coefficient in the fluid phase, 1/s
$k_s a_0$	Volumetric mass transfer coefficient in the solid phase, 1/s
\dot{q}	Mass flowrate of solvent per unit mass of solid phase, 1/s
q	specific amount of solvent = Q/N (dimensionless)
q_m	Specific amount of solvent at the start of the extraction that occurs inside the trichomes
q_{vol}	Volumetric flowrate of CO_2 , m^3/s
Q	Amount of solvent used, kg
V	Volume of cell, m^3
W	Parameter of slow-extraction period (dimensionless)
x_t	Easily accessible concentration of a solute in solid substrate, kg/kg
x_0	Global yield, kg/kg
y_r	Solubility, kg/kg
Z	Parameter of the Sovová's model for the CER period

Greek letters

ε	Void fraction
ρ	Density of the solvent, kg/m^3
ρ_s	Density of the solid phase, kg/m^3
