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Supercritical CO_2 extraction of triterpenes from rosemary leaves: Kinetics and modelling

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ABSTRACT

The pentacyclic triterpene α,β-amyrin is a promising bioactive natural product. Supercritical fluid extraction and fractionation are used to obtain pentacyclic triterpenes compounds from dried rosemary leaves. Experiments were conducted at 50°C and 180 bar, and the fractionation of the extract was accomplished in two separators. The shift to a diffusion-controlled regime occurred when 65% of the total oil was extracted. The moments analysis of kinetic curves indicated that the contribution of the axial dispersion, the external transfer and the internal transfer were 9.16%, 80.24% and 10.58%, respectively. The overall extraction curve was represented using three mathematical models.

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Kinetics modelling; pentacyclic triterpene; rosemary; supercritical fluid extraction

Introduction

The pentacyclic triterpenes compounds are characterized by their wide range of pharmacological activities.[1] The pentacyclic triterpene α,β-amyrin has been identified in several plants and the pure compounds have shown anti-inflammatory,[2–3] anti-microbial,[4] antifungal,[5] antinoci ceptive,[6] hepatoprotective and anti-allergic,[7] and neuropathic and inflammatory pain,[8] and other interesting biological activities. Amyrins are also involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides.[9] The development of biotransformation systems to convert amyrins into these or other compounds would open new ways for using α- and β-amyrins as a source of bioactive plant secondary metabolites more scarcely distributed in the plant kingdom.[9] Recent studies have demonstrated that the anti-inflammatory and anti-nociceptive properties of α- and β-amyrin are owing to its ability to interact with the cannabinoid system.[8,10–11]

Rosemary (*Rosmarinus officinalis* L.) is a perennial herb from the Lamiaceae family, typical of the Mediterranean region, which is widely spread in Algeria. Amyrins have been reported as constituents of this genus, and were isolated for the first time by Sendra *et al.*[12] from *Rosmarinus officinalis* L. However, there are few studies reporting the isolation of amyrin from rosemary.[13,14] On the other hand, the occurrence of betulinic, oleanolic and ursolic acids and betulin and rofficeronein rosemary leaves is well documented.[15–18] There are other triterpenoids that are also present in rosemary leaves and should be investigated. Recently, Zhang *et al.*[19] reported the isolation of two new triterpenoids glycosides from the aerial parts of rosemary, named as officino-terpenosides and whose molecular formulas are $C_{36}H_{58}O_{11}$and $C_{42}H_{68}O_{15}$.

The extraction of triterpenes is traditionally performed by maceration and soxhlet extraction. These techniques require a long time and large volumes of organic solvents. In the last few years, alternative techniques such as supercritical fluid extraction and fractionation, and ultrasound- and microwave-assisted extraction, have increased the efficiency of the extraction processes.

The SC-CO_2 extraction and fractionation of rosemary leaves to produce phenolic diterpenes compounds have been widely studied and reported in the literature.[20–24] However, the SC-CO_2 extraction and fractionation of rosemary leaves to recover α- and β- amyrin have not been reported. It has been reported that the higher pressure and/or higher CO_2 density (above 500 kg/m^3) were favourable for the isolation of high molecular compounds like diterpenes, triterpenes, esters and waxes.[25–27]

To design an extraction plant, it is necessary to have reliable mass-transfer models that will allow the determination of optimum operating conditions.[28] Many studies have been published and were intended for modelling supercritical extraction using the empirical

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kinetic equations.[29,30] Heat transfer analogy of a single sphere cooled in a fluid medium.[31] The extraction process was also modelled by integrating the differential mass balances in the solid and fluid phases.[32,33] To facilitate the choice of model for supercritical fluid extraction (SFE), Sovova[34] proposed the characteristic times of four single extraction steps: internal diffusion, external mass transfer, hypothetic equilibrium extraction without mass transfer resistance and displacement of the solution from the extractor. Povh *et al.*[35] proposed another approach to simplify the evaluation of experimental extraction curves by dividing the curves of several straight sections and calculated the mass transfer coefficients and equilibrium concentrations from their slopes and endpoints.

In this work, we reported SC-CO_2 extraction and online fractionation in a two-step depressurization of rosemary oil to recover the pentacyclic terpenene. In addition, the contribution of different mass transfer resistances was investigated and three models were employed to interpret the experimental data.

Materials and methods

Material

Aerial parts of rosemary were obtained from plant culture of the Biology Institute, Algiers. They were air-dried at ambient temperature (22°C) for 15 days and stored in dark at room temperature. Dry solid weight was determined by the oven drying method at 105°C for 24 h; the water content was 9.63%. The dried plants were ground by a coffee grinder and sieved. The mean particle average size was 437.5μm, and the particle void fraction was 0.53.

Supercritical extraction and online fractionation procedure

The experiments were carried out in a supercritical fluid extractor described previously.[36] The extraction column was filled with 7.4 g of plant particles placed between layers of glass beads. Fractionation was achieved in two different separators (S1 and S2), with independent control of temperature and pressure. The extractions were performed at a pressure constant of 180 bar and a temperature constant of 50°C. Fractionation of the extract was accomplished maintaining S1 at 40 bar and 0°C and S2 at 25 bar and 20°C. Further, the CO_2 flow rate was set to 1 g/min. The major fraction was recovered in S1 and was solid and pasty. On the other hand, a small amount of oily liquid was recovered in the second separator. The extracts were collected at any time by a valve located at the base of the separator; oleoresin extracted was weighed immediately after collection. Bulk density of the milled rosemary was 300 kg/m^3, and the bed void fraction was 0.52. Extractions were performed at least in triplicate and the results shown are the average of all the extractions.

GC-MS analysis

Oleoresin content was determined using a Hewlett–Packard HP 5973 MSD mass spectrometer interfaced with an HP-6890 gas chromatograph with an HP-5 MS capillary column (30 m x 0.32 mm I.D., film thickness 0.25 μm). Carrier gas: helium; injector and detector temperatures, 250°C. Oven temperature programming was 60°C for 8 min increased at 2°C/min to 280°C and held at 280°C for 30 min; sample injections (1 μL) were performed in splitless mode (1:30); ionization energy, 70 eV; temperatures for the transference line and the ionic source were set at 280°C and 230°C, respectively. The mass spectrometer was used in the total ion current (TIC) mode. Identification of compounds was then performed by comparison of mass spectra and retention indices obtained with those of mass spectra libraries (Wiley 7N. and NIST 02.) or with those reported in the literature and using authentic standards: α-pinene, 1,8 cineole, camphor and borneol (Aldrich Algérie) when it was possible. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with the literature data.[37] The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

HPLC analysis

The analysis of the sample was performed using a high performance liquid chromatography (HPLC) instrument (Agilent 1100 Series, Agilent Technologies) with a diode-array detector (DAD) and a Zorbax SB C18 (3.5 μm, 4.6 x150 mm Agilent) column. The mobile phases used were methanol with 0.1% phosphoric acid (solvent A) and water with 0.1% phosphoric acid (solvent B). The following linear gradient was applied: 0 min, 40% B; 10 min, 50% B; 15 min, 60% B; 25 min; 60% B; 30 min, 40% B; this last composition was kept until the end of the chromatographic development. The injection volume was 5 μL and the column temperature was 30°C. The detection was accomplished by using a DAD system Varian storing the signal at wavelengths of 240, 280, 285 and 330 nm. Among the main compounds present in rosemary extract, carnosic acid was identified with regard to pure standard (Biochim Algeria); the other compounds have been identified on the basis of references in the literature.[38]

Mathematical modelling

Characteristic times of extraction steps

The distribution of the solute within the solid and the analysis of characteristics time can help in understanding where mass transfer resistances are located. The extractable substances may be free on the surface of the solid material or inside the structure of the material itself. Thus, mass transfer resistances due to the specific location of the compounds to be extracted are different (external or internal).

The different characteristic times are determined as follows[34]:

The characteristic time of displacement of the fluid from the extractor is the residence time (t_r)

$$t_r = \frac{\gamma}{q'} \tag{1}$$

where q' is the specific flow rate (kg (kg plant)$^{-1}$ s^{-1}) and γ is the solvent-to-solid mass ratio in the extractor and is given by the following expression:

$$\gamma = \frac{\rho_f \varepsilon_l}{\rho_s (1-\varepsilon_l)} \tag{2}$$

where ρ_f is the solvent density (kg m^{-3}), ρ_s is the apparent particle density (kg m^{-3}) and ε_l *is* the bed void fraction.

The characteristic time of external mass transfer (t_{EMT}) is

$$t_{EMT} = \frac{\varepsilon_l}{k_f a_p} = \frac{\varepsilon_l}{1-\varepsilon_l}\frac{\lambda}{k_f} \tag{3}$$

where k_f is the mass transfer coefficient in the fluid phase (ms^{-1}), a_p is the specific surface area (m^{-1}) and λ is the volume/surface ratio of the particle (=$R/3$ for spherical particles).

The characteristic time of internal mass transfer (t_{IMT}) is

$$t_{IMT} = \frac{1-\varepsilon_l}{k_i a_p} = \frac{\lambda}{k_i} \tag{4}$$

where k_i is the mass transfer coefficient in the solid phase (ms^{-1}). For a spherical particle of radius R (k_i = $5D_e/R$), D_e is the effective internal diffusivity (m^{2}s^{-1}).

For predicting the external mass transfer coefficient k_f, we used the correlation equation of Mongkholkha jornsilp *et al.*,[39] which is valid over an Re range from 0.1689 to 1.2913 and Sc from 6 to 25:

$$Sh = 0.135 Re^{0.5} Sc^{0.33} \tag{5}$$

where *Sh* is the Sherwood number, *Sc* is the Schmidt number and *Re* is the Reynolds number.

The binary diffusion coefficient D_{12} (m^{2}s^{-1}) of the extract in the solvent was estimated according to Sun and Chen[40]:

$$D_{12} = 1.2310^{-14}\frac{T}{\mu_{solvent}^{0.796} V_{C_{solute}}^{0.503}} \tag{6}$$

where $\mu_{solvent}$ is the CO_2 viscosity and the critical volume (V_C) was estimated by Joback's method,[41] simplifying the extract as consisting of amyrin (pseudo-solute).

The effective internal diffusivity was estimated by the correlation proposed by Wakao and Smith[42]:

$$D_e = \varepsilon_p^2 D_{12} \tag{7}$$

where ε_p is the particle void fraction.

Contribution of mass transfer resistances

The approximate additivity of the effects of axial dispersion and mass transfer resistance was first deduced by Van Deemter *et al.*[43] According to Ruthven[44] the same conclusion may be reached in a simple way from moments analysis and lead to Eq. (8) as the definition of an overall effective rate coefficient (k) incorporating the effects of both axial dispersion and mass transfer resistances:

$$\frac{1}{k} = \frac{D_{ax}}{u_i}((1-\varepsilon_l)/\varepsilon_l) + \frac{R}{3k_f} + \frac{R^2}{15\varepsilon_p D_e} \tag{8}$$

where D_{ax} is the axial dispersion (m^{2}s^{-1}). For predicting the axial dispersion coefficient, we used the correlation equation of Funazukuryi,[45] which is valid for ReSc >0.3:

$$D_{ax} = (1.317)(\varepsilon_l ReSc)^{1.392}\left(\frac{D_{12}}{\varepsilon_l}\right) \tag{9}$$

Modelling

Mathematical models available in the literature were used in the modelling of the extraction curves of rosemary oil. The experimental data were correlated using three models based on differential mass balance equations: (1) the Sovova model,[33] (2) the logistic model presented by Martınez *et al.*[46] and (3) the desorption model proposed by Tan and Liou.[47]

The broken and intact cells (BIC) model developed by Sovova assumes that the extractible oil content (x_0) is divided into accessible oil (x_p), or free oil from the broken solid particles, and inaccessible oil (x_k), oil content trapped inside the un-ruptured solid structure. The model parameters were used to determine the duration of constant extraction rate (t_{CER}), the hardly accessible solute (x_k), and the volume of the mass transfer coefficients in the fluid phase ($k_f a_p$) and solid phase ($k_i a_p$).

Martinez's model was originally conceived to consider the SFE extracts as mixtures of multiple compounds, to deal with the fact that each solute can have its own

interactions with the solvent and then be extracted at different times. The particularity of the logistic model was the definition of the interfacial mass-transfer term, which was described by one of the solutions from the logistic equation. The logistic model has two adjustable parameters b_i and t_m. No physical meaning was attributed to the first one (b_i), while the second one (t_m) was described as the time in which the extraction rate reaches its maximum value.[46]

The Desorption model of Tan and Liou[47] assumes that the interfacial mass transfer of the extraction is well described by a first-order kinetic expression, whose parameter k_d is the desorption constant and the adjustable parameter.

The goodness of these models fitting to experimental data was assessed considering the percent average absolute relative deviation (AARD (%)), calculated as follows:

$$AARD\ (\%) = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{y_i - y_{mod,i}}{y_i}\right|100 \qquad (10)$$

where n is the number of experimental points, y_i is the yield determined by experimental point i and $y_{mod,i}$ is the yield obtained by the model in point i.

Results and discussions

Analysis of rosemary extract

The GC/MS analysis of the rosemary extract (S1+S2) (Table 1) led to the separation and identification of the majority of the constituents; overall, 27 compounds were identified belonging to five representative classes of constituents: monoterpenes, sesquiterpenes, diterpenes, waxes and pentacyclic triterpenes. The extract contains a complex mixture of 21.84% of monoterpenes, 7.56% of sesquiterpenes, 18.29% of diterpenes, 0.41% of waxes, 2.65% of tocopherol and 46.48% of triterpenes. It was found to be composed mainly of pentacyclic triterpenes (45.22%), followed by oxygenated monoterpenes (21.26%), diterpene (18.29%) and sesquiterpenes hydrocarbons (4.33%). The major compounds that were identified were β-amyrin (25.83%), α-amyrin (19.39%), carnosol derivative (18.29%), verbenone (6.24%), camphor (5.11%), borneol (4.33%) and trans-caryophyllene (3.01%).

The results showed that the fractionation leads to recovering in the separator 1 the compounds amyrin and carnosol derivative from rosemary leaves. To our knowledge, no study upon the isolation and quantification of amyrins of SFE extracts of rosemary has yet been proposed. On the other hand, in this study carnosol derivative was identified instead of carnosic acid and carnosol, which are commonly found in rosemary extract due to their thermal conversion during gas chromatography analysis.[48,49] Indeed, the HPLC analysis of the SFE extract gave a total of 10 peaks, where only four peaks have been identified: vanillic acid, caffeic acid, carnosol and carnosic acid. Carnosic acid represents 40% and the carnosol 18% of the total extract area. This composition showed no great difference with the ones reported for a supercritical rosemary extract.[50] Genena *et al.*[51] reported that isocarnosol was the main diterpene (31.73%) identified by GC/MS in SFE of rosemary at 200 bar and 50°C. We should note that under these conditions and without fractionation no pentacyclic triterpenes were extracted from rosemary. It can also be noted that the oxygenated monoterpenes were present in larger amounts in this extract; as reported by several authors, the supercritical extraction gives an extract rich in oxygenated monoterpenes compounds.

Table 1. Results of GC-MS analysis of the chemical composition of rosemary fraction (S1+S2) isolated at 180 bar and 50°C.

Compound	Retention time (min)	Percentage area (%)
Monoterpenes hydrcarbons		
α-Pinene	3.86	0.47
β-Ocimene	4.89	0.11
Oxygenated monoterpenes		
1,8-Cineole	6.50	1.94
α-Thujone	9.51	1.08
3-Octaanone	10.01	0.16
Chrysanthenone	10.50	0.28
Camphor	11.28	5.11
Borneol	12.31	4.33
3-Pinanone	12.77	0.59
α-Terpineol	13.80	0.05
Vebenone	14.82	6.25
Endo-bornyl acetate	18.86	1.47
Sesquiterpenes hydrocarbons		
α-Farnesene	23.48	0.15
Trans- caryophyllene	25.92	3.01
α-Humulene	27.89	0.82
Valencene	29.50	0.15
α-Copaene	32.31	0.20
Oxygenated sesquiterpenes		
Caryophyllene-oxide	35.41	0.46
α-Bisabolol	35.90	2.77
Diterpenes		
Carnosol derivative*	58.93	18.29
Waxes		
1-Octadecanol	82.17	0.22
Octadecane,1-(ethenyloxy)-	89.17	0.19
Triterpenes		
Squalene	89.35	1.26
β-amyrin	103.40	25.83
α-amyrin	104.65	19.39
Others		
α-Tocopherol	99.06	2.65
Not identified components		2.79
Identified components		97.22

*Tentative identification.

Analysis of kinetic data

Figure 1 shows the kinetic curves presenting the average and the standard deviation of the mass of the

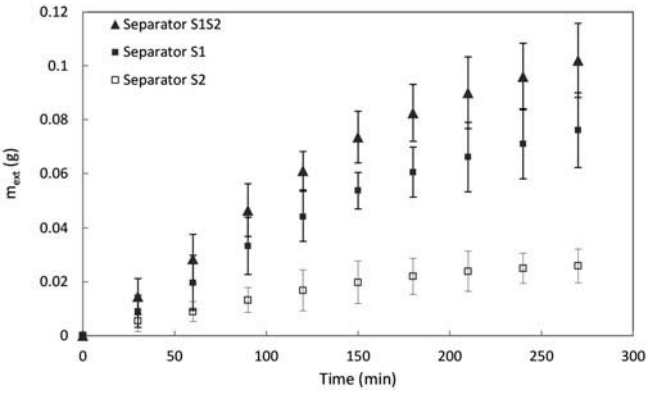


Figure 1. Kinetic SFE data from rosemary leaves at 180 bar and 50°C.

extract in each separator (S1 and S2) and the mass of the mixture of extracts S1 and S2. The total extraction yield obtained in the two separators is 1.38% (wt), of which 74.60% (wt) is recovered in the first separator and 25.39% (wt) in the second separator.

As can be deduced from Fig. 1, the rosemary plant material was not completely exhausted during the 4.5 h of extraction. Fornani *et al.*[52] reported that an extraction time of 4.5 h was insufficient to extract all extractable material present in rosemary leaves.

In addition, this figure indicates two different extraction rate periods. The first part of the curve is a straight line and, therefore, corresponds to a constant extraction rate period, this part of the process can be due to the desorption of the solute compounds which are readily available at the interface solid/fluid and are easily extracted in the earlier stages. The second part of the curve is around 3 h, which corresponds to the transition period; in this phase the solute inside the solid starts to be extracted and the readily accessible solute continues to be extracted. Besides, the observation of the experimental curve indicates the absence of the third period, corresponding to the disappearance of the accessible solute and the extraction of the hardly accessible solute. In this case the applied extraction time might have been insufficient to reach the diffusion-controlled period.

Experimental results also suggest that about 50% of the available oil is adsorbed on the surface. Indeed, the leaves of aromatic herbs of the Lamiaceae family, such as rosemary (*Rosmarinus officinalis*), produce superficial oils that are stored in abundant secretory cells called glandular trichomes or glands.[53,54]

The analysis of different characteristics times shows that the value of t_{EMT} (25.70 s) being important as compared to t_r (79.04 s) confirms that external mass transfer resistance cannot be neglected. On the other hand, the characteristic time of internal mass transfer (t_{IMT}) is equal to 1.66 s, which confirms that internal diffusion limitation seems not to be present in these operating conditions. The moments analysis provides a better appreciation of the contribution of each mass transfer resistance in the SFE of rosemary; thus, from Eq. (8), the contribution of the axial dispersion, the external transfer and the internal transfer were found to be 9.16%, 80.24% and 10.58%, respectively. Therefore, the axial dispersion and the internal mass transfer have a minor influence compared to the external mass transfer. This observation has an important implication on the choice of the model for modelling the supercritical extraction.

Modelling of the extraction curve

The experimental and modelled curves are illustrated in Fig. 2. The adjustable parameters and the AARD calculated for the applied models are presented in Table 2.

The analysis of the AARD values, as well as the visual observation of Fig. 2, indicated that all models apparently perform similarly, and they represent the data adequately. However, the model of Sovova provided the best fit with the experimental data shown by the AARD of 2.68% (Table 2) and it is more convenient because it provides a realistic description of the vegetable structure.

In the model of Sovova the pseudo-solubility of the extract in SC-CO_2 was estimated as the slope of the first part of the extraction curve. The initial mass of the extractable solute in the solid substratum was considered as the asymptotic value for large extraction times.

The adjusted value of the hardly accessible solute (x_K) obtained represents half of the initial mass of the extractable solute; this result denotes that 50% of the solute remained outside the solid particle and the other 50%

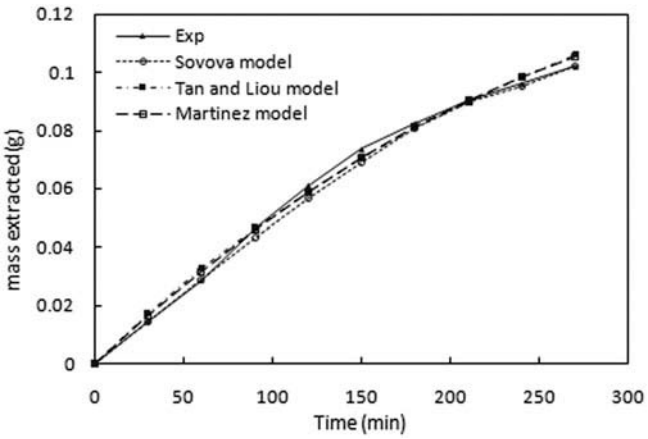


Figure 2. Comparison of experimental results and fitted OEC for SFE from rosemary leaves using the Sovova, Tan and Liou and Martínez models.

Table 2. Kinetic models parameters.

SFE Conditions	BIC model	Desorption model	Logistic model
P = 180 bar	t_{CER} = 2194.59 s	$k_d = 5.66 \times 10^{-5}$ s^{-1}	$b = 1.00 \times 10^{-4}$
T = 50°C	$k_{fap} = 3.6 \times 10^{-3} s^{-1}$	AARD = 4.63%	t_m = −5695.00 s
T_{Sep1} = 0°C	$k_{sap} = 3.37 \times 10^{-5} s^{-1}$		AARD = 4.32 %
T_{Sep2} = 20°C	x_k = 0.0099 g/g		
	AARD = 2.68 %		

remained inside the solid. The CER period was short (t_{CER} = 6235.6 s) compared with the falling extraction rate (FER) period (t_{FER} = 13600 s). Similar results were obtained by García-Risco *et al.*[55] The time at which the solute was extracted from the ruptured cells was completed at around 3.5 h. The observation of the experimental curve shape confirms these results.

The overall extraction curve (OEC) is characterized by the convective mass transfer between the solid material surface and the fluid phase in the first linear portion (CER period) and by both convective mass transfer and diffusion in the solid in the transition phase (FER period). However, the shift to a diffusion-controlled regime occurred when 65% of the total oil was extracted, this value east of 44% in the study conducted by Coello *et al.*[56] The adjusted values of the fluid-phase mass transfer coefficient ($k_f a_p$) obtained by Sovova's model are higher than the ($k_i a_p$) values, indicating that the effect of the convection mechanism is higher than the diffusion mechanism. García-Risco *et al.*[55] have simulated rosemary SC-CO_2 extraction carried out in a pilot-scale plant of 2 L capacity and processing 0.6 kg of milled rosemary leaves. Their results show that the $k_f a_p$ value (3.5×10^{-3} s^{-1}) and the $k_i a_p$ value (3.0×10^{-5} s^{-1}) are quite in agreement with the values for the low-scale case reported in this work and the previous one;[36] however, the $k_f a_p$ value reported by Carvalho *et al.*[57] is one order of magnitude lower.

The value of k_f calculated from the estimated parameter $k_f a_p$ (fitting parameter) was five times lower than predicted by Eq. (5). This may be due to the fact that the k_f estimated value does not take into account the resistances to mass transfer within the solid. In addition, the specific interfacial area (a_p) is often considered as the geometrical surface area ($a_p = 6(1-\varepsilon_l)/d_p$); in our view this overestimation is valid if all parts of the surface of the particles are exposed to mass transfer. Or if this is not the case, this effect can be represented by defining the effective surface area per unit volume by using a_e instead of a_p and establishing a surface area factor F ($a_e = Fa_p$) in the expression of the overall mass transfer coefficient $k_f a$; in this work, the value of the surface area factor F was 0.18. Marrone *et al.* (1998)[58] reported that an assumption that the solid particles were spherical was often used, and although spheres have the smallest surface area-to-volume ratio among regular geometric shapes, not all of the external surface of the particles is fully available for extraction due to tight packing in low-porosity beds. Also, Del Valle *et al.*[59] reported that the smaller best-fit k_f values than the predictions of the literature correlations are probably due to a combination of several factors, including underestimation of the contribution of internal (solid phase) mechanisms to the total resistance to mass transfer, overestimation of the mass transfer area, underestimation of solvent flow heterogeneity effects and underestimation of natural convection effects.

The t_m parameter for the logistic model was defined as the time when the extraction rate reaches its maximum value. In our experiments, the t_m value is negative; this result indicates that in this case this parameter has no physical meaning, although Martinez *et al.*[60] reported that the negative value indicates that the extraction rate decreases with the maximum value at the initial time of the extraction. Also, according to Domingues *et al.*[61] the negative value may also indicate that the extraction rate was maximum at t = 0. When applying this model to the usual OEC shapes, many authors[61–63] have obtained negative values for t_m and therefore no physical meaning could be associated with this parameter. Although the logistic model may give good fits in some particular cases, it does not provide any phenomenological information regarding the SFE process; thus the absence of physical meaning turned it into an empirical model.

The model developed by Tan and Liou presents a good fit to experimental data. This model assumes that the desorption process takes place at the solid–fluid interface and that it can be described using a first-order reaction kinetics. Therefore, it should be used with vegetable material with relatively high desorption constants; this is not the case for SFE from rosemary leaves (Table 2). In addition, the desorption model does not include a factor describing the different mass transfer resistances, which makes its applications limited.

Conclusions

The results indicated that the $SCCO_2$ fractionation employed to extract bioactive triterpene compounds from rosemary leaves was useful as a selective method

to obtain the triterpenes compounds as α and β amyrin. Data showed that the selectivity recovery of pentacyclic triterpene can be optimized by adjusting the pressure and temperature in the separators. Analysis of kinetic data indicated the presence of two different extraction rate periods, and the absence of the third period corresponding to the diffusion-controlled step. The characteristic times of extraction steps and the moments analysis have shown that axial dispersion and internal mass transfer have a minor influence compared to the external mass transfer in SFE of rosemary oil. The overall extraction curves were fitted to the models of Sovova, Martínez and Tan and Liou. The logistic model led to a negative value of the time (t_m) in which the extraction rate reaches its maximum value and therefore no physical meaning could be associated with this parameter. Also, the model developed by Tan and Liou presented a good fit to experimental data. However, it should be used with vegetable material with relatively high desorption constants; this is not the case for SFE from rosemary leaves. Finally, Sovova's model provided the best fit and a good physical description of the mass transfer phenomena in SFE of rosemary oil.

References

[1] Soldi, C.; Pizzolatti, M.G.; Luiz, A.P.; Marcon, R.; Meotti, F.C.; Mioto, L.A.; Santos, A.R.S. (2008) Synthetic derivatives of the α-and β-amyrin triterpenes and their antinociceptive properties. *Bioorganic & Medicinal Chemistry*, 16: 3377–3386.

[2] Aragão, G.F.; Pinheiro, M.C.; Nogueira Bandeira, P.; Gomes Lemos, T.L.; de Barros Viana G.S. (2007) Analgesic and anti-inflammatory activities of the isomeric mixture of alpha- and beta-amyrin from Protium heptaphyllum (Aubl.). *Journal of Herbal Pharmacothe rapy*, 7: 31–47.

[3] Wu, C.; Hseu, Y.; Lien, J.; Lin, L.; Lin, Y.; Ching, H. (2011) Triterpenoid contents and anti-Inflammatory properties of the methanol extracts of *Ligustrum* species leaves. *Molecules Journal*, 16: 1–15.

[4] El-Hagrassi, A.M.; Ali, M.M.; Osman, A.F.; Shaaban, M. (2011) Phytochemical investigation and biological studies of *Bombax malabaricum* flowers. *Natural Products Research*, 25: 141–151.

[5] Jabeen, K.; Javaid, A.; Ahmad, E.; Athar, M. (2011) Antifungal compounds from *Melia azederach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. *Natural Products Research*, 25: 264–276.

[6] Michel Otuki, F.; Ferreira, J.; Lima, F.V.; Meyre-Silva, C.; Malheiros, A.; Muller, L.A.; Cani, G.S.; Santos, A.R.S.; Yunes, R.A.; Calixto, J.B. (2005) Antinociceptive properties of mixture of α-Amyrin and β-Amyrin triterpenes: Evidence for participation of protein kinase C and protein kinase a pathways. *Journal of Pharmacology and Experimental Therapeutics*, 313: 310–318.

[7] Oliveira, F.A.; Lima-Junior, R.C., Cordeiro, W.M.; Vieira-Junior, G.M., Chaves, M.H.; Almeida, F.R.; Silva, R.M.; Santos, F.A.; Rao, V.S. (2004) Pentacyclic triterpenoids, α, β-amyrins, suppress the scratching behavior in a mouse model of pruritus and Pharmacology. *Biochemistry and Behavior*, 78: 719–725.

[8] Simão da Silva, K.A.B.; Paszcuk, A.F.; Passos, G.F.; Silva E.S.; Bento, A.F.; Meotti, F.C.; Calixto, J.B. (2011) Activation of cannabinoid receptors by the pentacyclic triterpene alpha,beta-amyrin inhibits inflammatory and neuropathic persistent pain in mice. *Pain*, 152: 1872–1887.

[9] Hernandez-Vázquez, L.; Mangas, S.; Palazón, J.; Navarro-Ocaña, A. (2010) Valuable medicinal plants and resins: Commercial phytochemicals with bioactive properties. *Industrial Crops and Products*, 31: 476–480.

[10] Chicca, A.; Marazzi, J.; Gertsch, J. (2012) The antinociceptive triterpene beta-amyrin inhibits 2-arachidonoyl glycerol (2-AG) hydrolysis without directly targeting CB receptors. *British Journal of Pharmacology*, 1671: 596–1608.

[11] Matos, I.; Bento, A.F.; Marcon, R.; Claudino, R.F.; João Calixto, B. (2013) Preventive and therapeutic oral administration of the pentacyclic triterpene amyrin ameliorates dextran sulfate sodium induced colitis in mice the relevance of cannabinoid system. *Molecular Immunology*, 54: 482–492.

[12] Sendra, J.; Seidl, O.; Miedzobrodzka, J.; Zieba, J. (1969) Chromatographic analysis of flavonoids and triterpenes in folium Rosmarini. *Dissert Pharmacy Pharmacology*, 21: 185–191.

[13] Qunfang, Z.; Zhanhui, X.; Pengfei, T.; Gansun, L.; Hongming, C. (2000) A New Triterpene from Rosemary (*Rosmarinus officinalis*). *Journal of Chinese Pharmaceutical Sciences*, 9: 131–136.

[14] European Food Safety Authority (EFSA). (2008) Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food on a request from the Commission on the use of rosemary extracts as a food additive. The European *Food Safety Authority Journal*, 72: 11–29.

[15] Razborsek, M.I.; Voncina,; D.B.; Dolecek, V.; Voncina, E. (2008) Determination of oleanolic, betulinic and ursolic acid in Lamiaceae and mass spectral fragmentation of their trimethyl silylated derivatives. *Chromatographia*, 67: 433–440.

[16] Razboršek, M.I.; Vončina, D.B.; Doleček, V.; Vončina, E. (2007) Determination of Major Phenolic Acids, Phenolic Diterpenes and Triterpenes in Rosemary *(Rosmarinus officinalis* L.) by Gas Chromatography and Mass Spectrometry. *Acta Chimica Slovenica*, 54: 60–67.

[17] Ganeva, Y.; Tsankova, E.; Simova, S.; Apostolova. B.; Zaharieva Rofficerone, E. (1993) A new triterpenoid from *Rosmarinus officinalis*. *Planta Medica*, 59: 276–277.

[18] Kontogianni, V.G.; Tomic, G.; Nikolic, I.; Nerantzaki, A. A.; Sayyad, N.; Stosic-Grujicic, S.; Stojanovic, I.; Gerothanassis, I.P.; Tzakos, A.G. (2013) Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and antiproliferative activity. *Food Chemistry*, 136: 120–129.

[19] Zhang, Y.; Adelakun, T.A.; Qub, L.; Li, X.; Li, J.; Han, L.; Wang, T. (2014) New terpenoid glycosides obtained

from *Rosmarinus officinalis* L. aerial parts. *Fitoterapia*, 99: 78–85.

[20] Chang, C.H.; Chyau, C.C.; Hsieh, C.L.; Wu, Y.Y.; Ker, Y.B.; Tsen, H.Y.; Peng, R.Y. (2008) Relevance of phenolic diterpene constituents to antioxidant activity of supercritical CO_2 extract from the leaves of rosemary. *Natural Products Research*, 22: 76–90.

[21] Visentín, A.; Cismondi, M.; Maestri, D. (2011) Supercritical CO_2 fractionation of rosemary ethanolic oleoresins as a method to improve carnosic acid recovery. *Innovative Food Science and Emerging Technologies*, 12: 142–145.

[22] Celiktas, O.Y.; Bedir, E.; Vardar Sukan, F. (2007) In vitro antioxidant activities of *Rosmarinus officinalis* extracts treated with supercritical carbon dioxide. *Food Chemistry*, 101: 1457–1464.

[23] Vicente, G.; García-Risco, M.R.; Fornari, T.; Reglero, G. (2012) Supercritical fractionation of rosemary extracts to improve the antioxidant activity. *Chemical Engineering & Technology*, 35: 176–182.

[24] Vicente, G.; Molina, S.; González-Vallinas, M.; García-Risco, M.R.; Fornari, T.; Reglero, G.; Ramírez de Molina, A. (2013) Supercritical rosemary extracts, their antioxidant activity and effect on hepatic tumor progression. *Journal of Supercritical Fluids*, 79: 101–108.

[25] Ivanovic, J.; Djilas, S.; Jadranin, M.; Vajs, V;. Babovic, N.; Petrovic, S.; Žižović, I. (2009) Supercritical carbon dioxide extraction of antioxidants from rosemary (*Rosmarinus officinalis* L.) and Sage (*Salvia officinalis* L.). *Journal of Serbian Chemical Society*, 74: 717–732.

[26] Nguyen, U.; Frankman, G.; Evans, D.A. (1991) Process for extracting antioxidants from Labiatae herbs, US patents 5, 017,397.

[27] Reverchon, E.; Taddeo, R. (1995) Extraction of sage oil by supercritical CO_2: influence of some process parameters. *Journal of Supercritical Fluids*, 8: 302–309.

[28] Ghonasgi, D.; Gupta, S.; Dooley, K.M.; Knop, F.C. (1991) Measurement and modeling of supercritical carbon dioxide extraction of phenol from water. *Journal of Supercritical Fluids*, 4: 53–59.

[29] Naik, S.N.; Lentz, H.; Maheshawari, R.C. (1989) Extraction of perfumes and flavours from plant materials with liquid carbon dioxide under liquid–vapour equilibrium conditions. *Fluid Phase Equilibria*, 49: 115–126.

[30] Spiro, M.; Kandiah, M. (1990) Extraction of ginger rhizome: partition constants and other equilibrium properties in organic solvents and in supercritical carbon dioxide. *International Journal of Food Science and Technology*, 25: 566–575.

[31] Reverchon, E.; Donsi, G.; Osseo, L.S. (1993) Modeling of supercritical fluid extraction from herbaceous matrices. *International Journal of Food Sciences and Technology*, 32: 2721–2726.

[32] Goto, M.; Roy, B.C.; Hirose, T. (1996) Shrinking-core leaching model for supercritical fluid extraction. *Journal of Supercritical Fluids*, 9: 128–133.

[33] Sovova, H. (1994) Rate of the vegetable oil extraction with supercritical CO_2—I. Modelling of extraction curves. *Chemical Engineering Science*, 49: 409–414.

[34] Sovová, H. (2012) Steps of supercritical fluid extraction of natural products and their characteristic times. *Journal of Supercritical Fluids*, 66: 73–79.

[35] Povh, N.P.; Marques, M.O.M.; Meireles, M.A.A. (2001) Supercritical CO_2 extraction of essential oil and oleoresin from chamomile (*Chamomilla recutita* [L.] *Rauschert*). *Journal of Supercritical Fluids*, 21: 245–256

[36] Bensebia, O.; Barth, D.; Bensebia, B.; Dahmani, A. (2009) Supercritical CO_2 extraction of rosemary: Effect of extraction parameters and modelling. *Journal of Supercritical Fluids*, 49: 161–166.

[37] Adams, R.P. (2007) Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrome try, 4th Ed.; Allured Publishing, Carol Stream, Illinois.

[38] Almela, L.; Sánchez-Muñoz, B.; Fernández-López, J.A.; Roca, M.J.; Rabe, V. (2006) Liquid chromatograpic-mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *Journal of Chromatography A*, 1120: 221–229.

[39] Mongkholkhajornsilp, D.; Doulas, S.; Douglas, P.L.; Elkamel, A.; Teppaitoon, W.; Pongamphai, S. (2005) Supercritical CO2 extraction of nimbin from neem seeds: a modeling study. *Journal of Food Engineering*, 71: 331–340.

[40] Sun, C.K.; Chen, H. (1986) Tracer diffusion in dense ethanol: a generalized correlation for nonpolar and hydrogen-bonded solvents. *American Institute of Chemical Engineers Journal*, 32: 1367–1371.

[41] Joback, K.G.; Reid, R.C (1987) Estimation of pure-component properties from group-contributions. *Chemical Engineering Communications*, 57: 233–243.

[42] Wakao, N.; Smith, J.M (1962) Diffusion in catalyst pellets. *Chemical Engineering Science*, 17: 825–834.

[43] Van Deemter, J.J.; Zuiderweg, F.J.; Klinkenberg, A.; (1956) Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography. *Chemical Engineering Science*, 5: 271–280.

[44] Ruthven, D.M. (1984) Principles of Adsorption and Adsorption Processes; Wiley (ed), U.S.A.

[45] Funazukuri, T.; Kong, C.; Kagei, S. (1998) Effective axial dispersion coefficients in packed beds under supercritical conditions. *Journal of Supercritical Fluids*, 13: 169–175.

[46] Martínez, J., Monteiro, A.R., Rosa, P.T.V., Marques, M. O.M., Meireles, M.A.A. (2003) Multicomponent model to describe extraction of ginger oleoresin with supercritical carbon dioxide. *Industrial and Engineering Chemical Research*, 42: 1057–1063.

[47] Tan, C., Liou, D. (1989) Modeling of desorption at supercritical conditions. *American Institute of Chemical Engineers Journal*, 35: 1029–1031.

[48] Thorsen, M.A.; Hildebrandt, K.S. (2003) Quantitative determination of phenolic diterpenes in rosemary extracts-aspects of accurate quantification. *Journal Chromatography A*, 995: 119–125.

[49] Glisic, S.; Ivanovica, J.; Ristic, M.; Skalaa, D. (2010) Extraction of sage (*Salvia officinalis L.*) by supercritical CO2: Kinetic data, chemical composition and selectivity of diterpenes. *Journal of Supercritical Fluids*, 52: 62–70.

[50] Ramírez, P.; García-Risco, M.R.; Santoyo, S.; Javier Señoráns, F.; Ibáñez, E.; Reglero, G. (2006) Isolation of functional ingredients from rosemary by preparative-supercritical fluid chromatography (Prep-SFC). *Journal of Pharmaceutical and Biomedical Analysis*, 41: 1606–1613.

[51] Genena, A.K.; Hense, H.; Smânia Junior, A.; Machado de Souza, S. (2008) Rosemary (*Rosmarinus officinalis*) – a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. *Ciência Tecnologia Alimentos Campinas*, 28: 463–469.

[52] Fornari, T.; Ruiz-Rodriguez, A.; Vicente, G.; Vazquez, E.; Garcia-Risco, M.R.; Reglero, G. (2012) Kinetic study of the supercritical CO_2 extraction of different plants from Lamiaceae family. *Journal of Supercritical Fluids*, 64: 1–8.

[53] Zizovic, I.; Stamenic´, M.; Orlovic´, A.; Skala, D. (2005) Supercritical carbon dioxide essential oil extraction of Lamiaceae family species: Mathematical modelling on the micro-scale and process optimization. *Chemical Engineering Science*, 60: 6747–6756.

[54] Stamenic´, M. Zizovic, I. Orlovic´, A. Skala, D. (2008) Mathematical modelling of essential SFE on the micro-scale. Classification of plant material. *Journal of Supercritical Fluids*, 46: 285–292.

[55] Mónica García-Risco, R.; Elvis Hernández, J.; Vicente, G.; Fornari, T.; Francisco Sênoráns, J. Reglero, G. (2011) Kinetic study of pilot-scale supercritical CO_2 extraction of rosemary (*Rosmarinus officinalis*) leaves. *Journal of Supercritical Fluids*, 55: 971–976.

[56] Coelho, J.A.P.; Mendes, R.L. Provost, M.C. Cabral, J.M. S. Novais, J.M.; Palavra, A.M.F. (1997) Supercritical carbon dioxide extraction of volatile compounds from rosemary. In: Abraham MA, Sunol AK (eds) Supercritical fluids. Extraction and pollution prevention. American Chemical Society, Washington, DC, 101–109.

[57] Carvalho Jr. R.N.; Moura, L.S.; Rosa, P.T.V.; Meireles, M.A.A. (2005) Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): kinetic data, extract's global yield, composition, and antioxidant activity. *Journal of Supercritical Fluids*, 35: 197–204.

[58] Marrone, C.; Poletto, M.; Reverchon, E.; Stassi, A. (1998) Almond oil extraction by supercritical CO2: Experiments and modelling. *Chemical Engineering Science*, 53: 3711–3718.

[59] del Valle, J.M.; de la Fuente, J.C.; Uquiche, E.; Zetzl, C.; Brunner, G. (2011) Mass transfer and equilibrium parameters on high-pressure CO_2 extraction of plant essential oils. In: J.M. Aguilera et al. (Eds). Food Engineering Interfaces. Springer Science & Business Media.

[60] Martínez, J., Rosa, P.T.V.; Menut, C.; Leydet, A.; Brat, P.; Pallet, D.; Meireles, M.A.A. (2004) Valorization of brazilian vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) oil. *Journal of Agricultural and Food Chemistry*, 52: 6578–6584.

[61] Rui Domingues, M.A.; Marcelo de Melo, M.R.; Neto Carlos, P.; Silvestre Armando, J.D.; Silva, C.M. (2012) Measurement and modeling of supercritical fluid extraction curves of Eucalyptus globulus bark: Influence of the operating conditions upon yields and extract composition. *Journal of Supercritical Fluids*, 72: 176–185.

[62] Kitzberger, C.S.G.; Lomonaco, R.H.; Michielin, E.M.Z.; Danielski, L., Correia, J.; Ferreira, S.R.S. (2009) Supercritical fluid extraction of shiitake oil: curve modeling and extract composition. *Journal of Food Engineering*, 90: 35–43.

[63] Mezzomo, N.; Martínez, J.; Ferreira, S.R.S. (2009) Supercritical fluid extraction of peach (Prunuspersica) almond oil: kinetics, mathematical modeling and scale-up. *Journal of Supercritical Fluids*, 51: 10–16.