



Supercritical carbon dioxide fractionation of *T. minuta* and *S. officinalis* essential oils: Experiments and process analysis

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ARTICLE INFO

Article history:

Received 25 January 2013

Received in revised form 14 March 2013

Accepted 15 March 2013

Keywords:

Supercritical fluid extraction

Fractionation

Essential oil

Biocidal agents

Tagetes minuta

Salvia officinalis

ABSTRACT

Essential oils are an important source of compounds with different degree of biocidal activity against microorganisms, insects, weeds and other pathogens. They have potential application in pharmaceutical, cosmetic and food industry, as well as for agriculture and crop protection. Supercritical fluid fractionation is an interesting technology for the selective removal of monoterpenes (MT) and the purification or enrichment of the more bioactive oxygenated terpenes (OT), resulting in more effective, stable and water soluble products. In this work, the fractionation of *Tagetes minuta* and *Salvia officinalis* essential oils with carbon dioxide is experimentally studied in a semicontinuous apparatus at 313 K and 80 bar. Successive extracts are collected and the composition determined by gas chromatography. Process behavior is modeled with the group contribution equation of state (GC-EOS), showing a good agreement between model predictions and experimental results. The GC-EOS model is applied to the simulation and analysis of two continuous fractionation schemes: a simple countercurrent column and a countercurrent column with external reflux. The influence of operation variables (temperature, pressure, solvent-to-feed ratio, thermal gradient, reflux ratio) is studied in order to maximize OT concentration and recovery in the raffinate and to minimize carbon dioxide consumption. Experimental and simulation results are discussed in terms of the relative volatilities between MT and OT fractions. Particularly in the case of *S. officinalis*, the presence of several OT of intermediate volatility and a significant amount of higher molecular weight sesquiterpenes (ST) limits the separation performance, even when external reflux is applied.

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1. Introduction

The use of supercritical fluids (SCFs) – and specially carbon dioxide – for the fractionation and purification of complex mixtures has been increasingly studied and applied to different systems during the last decades. This technique provides interesting advantages over traditional fractionation methods (e.g. vacuum distillation or liquid–liquid extraction): (i) absence of solvent residues in the final product, (ii) “fine tuning” of selectivity and solvent power with small changes in pressure and temperature, (iii) non-toxic and non-flammable solvent (CO₂) and (iv) fractionation at relatively low temperatures. One of the most studied applications is the fractionation of essential oils, particularly citrus oils. Essential oils are complex mixtures of volatile compounds occurring in many vegetable species and responsible for their characteristic smell. Essential oils are mainly composed of hydrocarbon monoterpenes (MT), oxygenated monoterpenes (OT), sesquiterpenes (ST)

and higher molecular weight compounds (di- and triterpenes, waxes, pigments, etc.). In this case, the aim of supercritical fluid fractionation is the removal of MT (“deterpenation”) in order to concentrate the oxygenated fraction (aroma), thus increasing oil quality, stability, water solubility and value.

Gerard [1] and Temelli et al. [2,3] studied in early works the deterpenation of citrus oil with supercritical CO₂ and discussed about the best operating conditions, based on the experimental and predicted solubility behavior of terpenes. Since then, several authors have investigated different ways of improving selectivity, due to the generally low content of oxygenated compounds in citrus oils. One line of investigation was the adsorption of the oil (or model mixtures) onto a polar adsorbent, like silica gel, and the selective desorption of MT with a continuous stream of CO₂ [4–6].

Other authors have studied the deterpenation in different multistage columns: continuous and semicontinuous, with external, internal or no reflux. Sato et al. [7–9] studied the fractionation of model mixtures representative of citrus oils in columns of different length and operating at different temperature, pressure and solvent-to-feed ratio conditions, with and without thermal gradient. They proposed a fractionation system with the oil fed in an intermediate stage and an external reflux in the top. The MT

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fraction is obtained as the top product, waxes and heavier compounds as bottom products and OT by a side stream. Reverchon et al. [10] and Budich et al. [11] have investigated design, scale-up and hydraulic aspects. More recently, Benvenuti et al. [12] and Gironi and Maschietti [13] have studied the semicontinuous fractionation of lemon peel oil at different pressure and temperature conditions with and without external reflux. They also modeled the system using the Peng–Robinson equation of state (PR-EOS) and simulated a continuous process. Finally, other authors have proposed different solvents, for example ethane [14], based on its lower critical pressure that could allow operation at milder conditions and with better solvent economy.

Due to the low content of OT (generally the valuable fraction) and the high flow rates of CO₂ required, an optimization of the operating conditions is needed, in order to maximize purities and recoveries and minimize solvent consumption. Espinosa [15] and Diaz et al. [16] have reported an optimization study of the deterpenation of orange and lemon peel oil, using the group contribution equation of state (GC-EOS) [17] as thermodynamic model and applying phase equilibrium engineering concepts.

On the other hand, there are comparatively few works reported in literature concerning the fractionation of herbal essential oils. In the case of these oils, fractionation is usually coupled with the extraction from the plant material, with fractions of the extract being recovered into separators operating at different pressure and/or temperature conditions. Examples of supercritical fractionation of liquid feeds, previously obtained by hydrodistillation or other conventional processes, can be found in the works of Köse et al. [18], concerning supercritical fractionation of MT and non-MT in origanum oil, and Varona et al. [19], regarding the separation of linalool and linalyl acetate in lavender oil.

Many essential oils are potential sources of bioactive compounds or extracts of interest for pharmaceutical, cosmetic and food industry, as well as for agriculture and crop protection [20,21]. Some of them show a strong biocidal or repellent activity against insects, weeds, microorganisms and other pathogens [22], constituting a natural defensive strategy of plants.

The use of natural biocidal agents has increased due to health and environmental concerns [23]. The OT and ST fractions are generally more bioactive than MT fraction in many essential oils [24]. Therefore supercritical fractionation is regarded as a suitable technology for concentrating them, thus obtaining more active extracts with better product quality and process economy. Removal of MT also increases oil stability and water solubility, which is of great interest for application and formulation purposes.

The fractionation of oils from *Tagetes minuta* and *Salvia officinalis* has been studied in this work because these common plants have recognized biocidal activity. The main oil components with biocidal activity are ocimenone and thujone, respectively. Cytotoxic activity of *S. officinalis* oil and specific compounds (such as camphor and thujone) against pathogenic bacteria and fungi has been reported [20,25]. *T. minuta* essential oil has been reported to have different degrees of toxicity and repellency against several parasites and insects, such as *Aedes aegyptii* [26,27], *Ceratitis capitata* or fruit fly and *Triatoma infestans* (vector of Chagas disease) [28], *Varroa destructor* (an acaricidal ectoparasite of bees) [29,30], *Poediculus humanus capiti* [31], among others, as well as allelopathic activity [32,33]. The effect of different fractions of *T. minuta* oil obtained by vacuum distillation and some of its pure components on aphid reproduction and mortality has also been studied [34], concluding that fractions richer in OT and ST were more effective in decreasing aphid population in greenhouse conditions.

The design and optimization of a fractionation process requires information about the phase behavior of the mixture. The phase equilibrium of these oils with CO₂ at supercritical conditions has been studied and modeled by the authors in a previous work [35],

where the influence of temperature and pressure on solubility and relative volatility of the different fractions has been discussed. Based on these facts, the specific objectives of the present work are:

- the experimental study of a semicontinuous fractionation process of *T. minuta* and *S. officinalis* oils with supercritical CO₂, in order to selectively remove the MT and purify or enrich the bioactive OT–ST fraction;
- the simulation of the semicontinuous fractionation process using the GC-EOS as thermodynamic model, comparing model predictions and experimental results;
- the simulation of a continuous countercurrent fractionation process, using the previously validated model, to analyze the influence of different operating conditions.

In forthcoming studies the biocidal activity of the purified fractions against different microorganisms and parasites will be evaluated in comparison to the raw oils.

2. Materials and methods

2.1. Materials

T. minuta and *S. officinalis* essential oils were obtained by hydrodistillation from fresh plants harvested in Sierras de Cordoba and Sierra de la Ventana (Argentina), respectively. Both oils were stored at 263 K, protected from light. Their composition was determined in a gas chromatograph coupled to a mass spectrometer (GC–MS, Perkin-Elmer, USA) with a capillary column DB5-HT (Agilent Technologies Inc., USA, 60 m length, 0.25 mm i.d., 0.25 μm film thickness). Hydrogen was used as carrier, at a flow rate of 1 cm³/min and a split ratio of 100:1. Injection temperature was fixed at 493 K and detector temperature at 543 K. The column temperature program was 323 K constant for 4 min, and 8 K/min up to 513 K. Peak identification was performed by comparison of the spectra with available databases (NIST, MassBank, Pherobase). MT, OT and ST compositions can be seen in Tables 1 and 2. Oil composition stability under storage conditions was periodically verified by chromatographic analysis.

Industrial extra-dry CO₂ supplied by Linde (Argentina) was used in the fractionation experiments and chromatographic grade hydrogen (99.999%) from Air Liquide (Argentina) was used in the GC analysis.

Ethanol (99.5%, Ciccarelli, Argentina), n-hexane (98.5%, Ciccarelli, Argentina) and acetone (99.5%, Aberkon Química, Argentina) were used as solvents for analysis and for cleaning the system.

Table 1
T. minuta essential oil composition.

Component	M (g/mole)	Peak area (%)
Hydrocarbon monoterpenes (MT)		
β-Pinene	136.24	1.32
D-Limonene	136.24	7.01
E-β-ocimene	136.24	25.97
Other (individually <1%)	–	0.80
Total MT		35.10
Oxygenated monoterpenes (OT)		
Dihydrotagetone	154.25	1.72
E-tagetone	152.23	2.25
Z-tagetone	152.23	2.33
Z-ocimenone	150.22	11.20
E-ocimenone	150.22	44.13
Other (individually <1%)	–	3.27
Total OT		64.90

Table 2
S. officinalis essential oil composition.

Component	M (g/mole)	Peak area (%)
Hydrocarbon monoterpenes (MT)		
α-Pinene	136.4	8.71
Camphene	136.4	5.54
β-Pinene	136.4	1.59
Myrcene	136.4	1.30
Other (individually <1%)	–	0.95
Total MT		18.09
Oxygenated monoterpenes (OT)		
1,8-Cineol	154.25	5.27
α-Thujone	152.23	36.31
β-Thujone	152.23	7.61
Camphor	152.23	14.66
Other (individually <1%)	–	5.47
Total OT		69.32
Sesquiterpenes (ST)		
α-Caryophyllene	204.36	4.04
β-Caryophyllene	204.36	4.18
Gurjunene	204.36	3.29
Other (individually <1%)	–	1.07
Total ST		12.58

2.2. Experimental apparatus and procedure

The experimental apparatus used in this work is a lab-scale high-pressure extraction cell (internal volume: 50 cm³) built in PLAPIQUI and schematically described in Fig. 1.

In this work, 1.5 cm³ of oil embedded on glass beads (35–60 mesh) were loaded into the cell. Semicontinuous fractionation was performed using a constant flow rate of CO₂ at 313 K and 80 bar, corresponding to a solvent density of 0.28 g/cm³. These conditions were chosen as a convenient trade-off between solubility and selectivity. A low CO₂ flow rate was used (0.1 g/min) in order to obtain saturation/equilibrium conditions. Successive extracts were collected in a refrigerated glass U-tube every 2 h. The extracts were weighed and dissolved in hexane for later analysis. The amount of CO₂ was calculated by integration of the volumetric flow rate and conversion to mass units at room temperature, pressure and humidity conditions. This procedure was repeated until reaching approximately 40–50 wt.% of oil extraction (two-fold product). At this point, the raffinate (totally or partially dewatered oil) was collected by rising the pressure up to 200 bar to achieve complete oil solubility. The same procedure was applied for both essential oils. More details about the equipment and its operation can be found in a previous work [35].

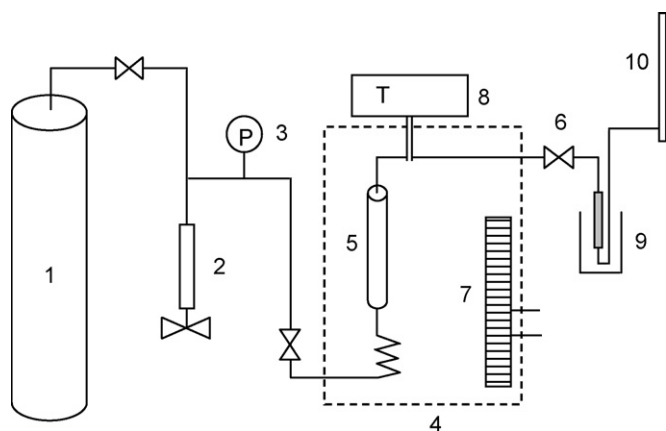


Fig. 1. Experimental apparatus. 1. CO₂ reservoir, 2. pressure generator, 3. manometer, 4. thermostatic chamber, 5. extraction column, 6. expansion valve, 7. heating resistance, 8. temperature controller, 9. collector U-tube, 10. bubble gas meter.

2.3. Analysis

Analysis of the extract and raffinate samples obtained in the fractionation assays were performed in a gas chromatograph (Varian CX3400, USA) with a flame ionization detector (GC-FID), and a capillary column DB5-HT (Agilent Technologies Inc., USA, 15 m length, 0.32 mm i.d., 0.1 μm film thickness). Hydrogen was used as carrier, at a flow rate of 1 cm³/min and a split ratio of 100:1. Injection temperature was fixed at 493 K and detector temperature at 543 K. The column temperature program was 323 K constant for 4 min, and 8 K/min up to 513 K.

Peak identification was performed by comparison with the raw oil chromatographic profiles obtained by GC–MS under similar conditions. It was assumed that the ratio between the area of each peak and the total area (without computing the solvent peak area) was equal to the mass fraction of the corresponding component in the extract on a solvent-free basis. This is a common practice for comparative analysis of essential oils, due to the difficulty of determining the response factors of the multiple components of the mixture.

3. Modeling and process simulation

3.1. Thermodynamic model

The phase equilibrium behavior of the essential oil + CO₂ systems has been already modeled by the authors [35], using the group contribution equation of state (GC-EOS). This model is particularly suitable for the representation of complex mixtures of a great number of compounds using a limited number of functional groups and binary interaction parameters. The GC-EOS has been previously used in several studies to describe the behavior of essential oils and other mixtures in supercritical processes [36].

Each oil fraction was modeled as a pseudo-component, represented by a selected key component. This approach, besides simplifying the representation of complex multicomponent mixtures, has proven to predict fairly well the system behavior at different pressure and temperature operating conditions. Thus, *T. minuta* oil was represented as a pseudo-binary mixture of 35.1% β-ocimene (MT) and 64.9% E-ocimenone (OT), while *S. officinalis* oil was represented as a pseudo-ternary mixture of 18.1% α-pinene (MT), 69.3% α-thujone (OT) and 12.6% α-caryophyllene (ST). Table 3 shows the required physico-chemical properties of the selected components used in the model (critical properties and normal boiling point). Group contribution methods were used for predicting physical properties when no experimental information was available in literature. No information from the experimental data of this work was used in the model parameter estimation; therefore the model results are completely predictive.

More details about the model, as well as the pure group and binary interaction parameters and the equilibrium data used for their correlation, can be found in the cited work [35].

Table 3
Physicochemical properties of key components.

Key component	<i>T_c</i> (K)	<i>P_c</i> (bar)	<i>NBP</i> (K)
α-Pinene	644.0 ^a	27.6 ^a	428.2 ^a
E-ocimene	643.3 ^b	24.5 ^b	447.6 ^a
α-Thujone	680.5 ^b	28.8 ^b	474.0 ^a
Z-ocimenone	640.8 ^b	26.0 ^b	505.1 ^a
β-Caryophyllene	746.3 ^b	20.1 ^b	536.0 ^a
CO ₂	304.2 ^a	72.8 ^a	–

^a Data from literature.

^b Estimated by Joback group contribution method.

3.2. Semicontinuous process simulation

The experimental semi-continuous fractionation process was modeled as a series of stages, each one corresponding to a single flash calculation at constant pressure and temperature. Results are expressed in terms of CO₂, MT, OT and ST composition in each phase. After each extraction step, the global composition of the system is recalculated by a mass balance and new relative volatilities are computed for the next flash step. In this way, the variation of the relative volatilities with composition is taken into account. For comparison purposes, calculations with constant relative volatility (corresponding to the initial equilibrium conditions) were also performed. The subroutine GCHREE [37] was used for the flash calculations. The step size was adjusted during the calculations according to the sensitivity of composition along the fractionation process.

3.3. Continuous process simulation and analysis

Semicontinuous fractionation has several limitations, although it can be useful in some particular conditions, e.g., when flexibility is needed for processing feeds with different composition or seasonal variations, or when the capacity is too small to allow continuous operation at a practical rate [38]. The main drawbacks are related to the loss of valuable compounds, as can be seen from the experimental results and practical limitations that increase operating costs, making continuous processing a generally simpler and more efficient alternative. According to this, a continuous countercurrent multistage fractionation process was also simulated, using the subroutine GCETRAC, a rigorous model for a high-pressure multistage extractor, supported by the GC-EOS model [39].

Two different arrangements were analyzed: (a) a simple countercurrent column, with oil feed in the top and CO₂ feed in the bottom, and (b) a countercurrent column with external reflux, with the objective of exploring the best conditions for producing an OT-rich raffinate with low solvent consumption and high OT recovery.

4. Results and discussion

4.1. Experimental results and model predictions for a semicontinuous fractionation process

Fig. 2(a) and (b) shows the experimental results and model predictions for the extract-phase composition of both studied systems, in a solvent-free basis, along the fractionation process. The experimental results show that the extracts are initially richer in MT than the original oils, as expected from their lower polarity and higher volatility and affinity for CO₂. Afterwards, as MT are being removed by the CO₂ stream, the extracts become increasingly richer in OT and in a lesser degree in ST, whose solubility in extract-phase is low along the whole process, as can be seen for *S. officinalis* oil.

In the case of *T. minuta* oil, the fractionation process yields an extract of around 80% MT during the first part (up to 25% of oil extraction), then MT concentration decreases rapidly until its almost complete exhaustion at around 50% of oil extraction. This means that an OT-rich raffinate can be obtained without losing a significant amount of this fraction: at the end of the experiment, the obtained two-fold raffinate yielded 95% OT concentration with a loss of 26% of OT in the extract-phase. This can be more easily seen in Fig. 3(a), which shows the accumulated extracted mass of MT and OT (as percentage relative to their initial mass) vs. CO₂ consumption. Chromatographic profiles of the feed oil, a MT-rich extract and the final deterpenated raffinate are shown in Fig. 4 as an example.

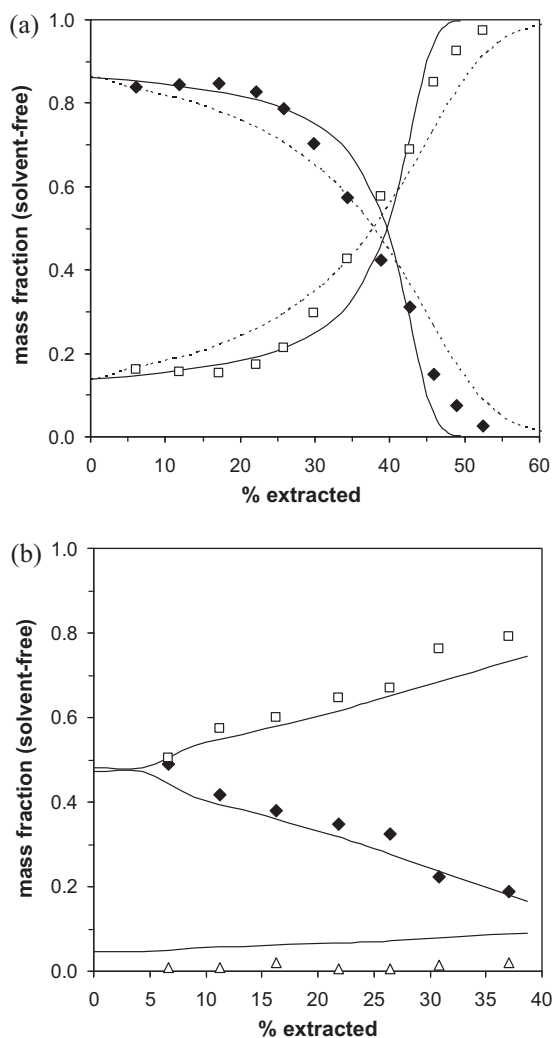


Fig. 2. Extract-phase composition (solvent-free basis) for (a) *T. minuta* oil, (b) *S. officinalis* oil at 313 K and 80 bar. (◆) MT, (□) OT, (△) ST, (—) GC-EOS prediction using variable relative volatility, (---) GC-EOS prediction using constant relative volatility.

In the case of *S. officinalis* oil, the extracts are actually richer in OT (above 50%), although their MT content is higher than in the original oil. The deterpenation by these means is possible, but the loss of OT in the extract-phase is higher: for example, a removal of 80% of MT implies the loss of 33% of OT, as can be seen in Fig. 3(b). The composition of this oil is more complex, because it contains a higher amount of ST and several OT of intermediate volatility – some of them very close to MT, like 1,8-cineol.

As can be seen in Figs. 2 and 3, in both systems the GC-EOS model predicts correctly not only the initial equilibrium composition, but also the compositions along the process, except for the ST concentration in *S. officinalis* extract, which is overpredicted. It is observed that calculations with a variable relative volatility give a better agreement with the experimental results than using a constant value, especially during the first part of the fractionation. The good agreement between experimental data and GC-EOS predictions implies that the composition-dependency of relative volatilities (and not only the temperature and pressure dependency) is correctly represented by the proposed model. This is an attractive feature when modeling separation processes where there are changes in composition like: changes with time (semicontinuous), with position along the column (steady state fractionation) or with oil feed composition. In Fig. 5(a) and (b) experimental data

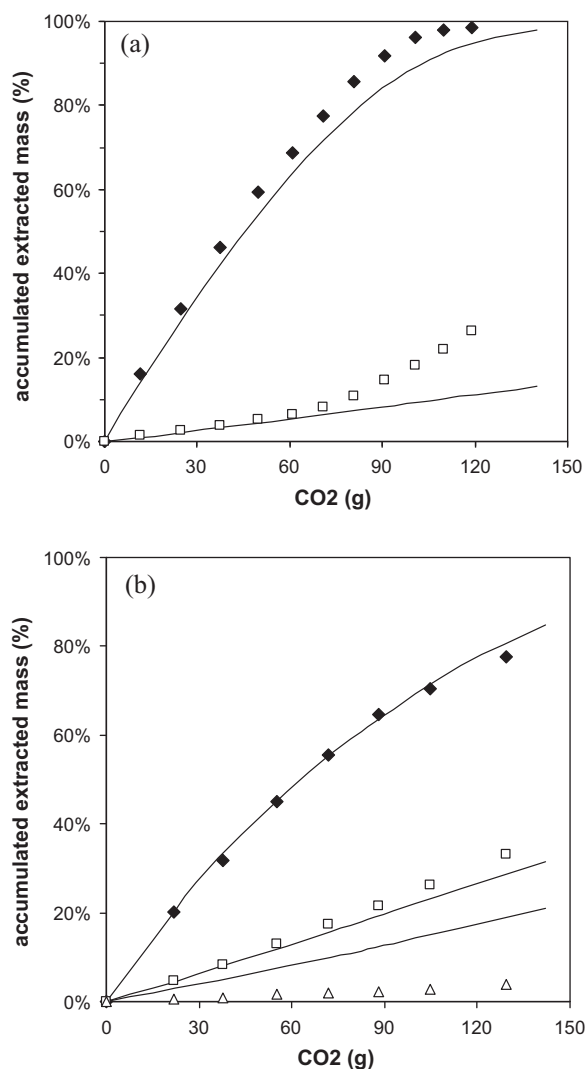


Fig. 3. Accumulated extracted mass (%) vs. solvent consumption for (a) *T. minuta* oil, (b) *S. officinalis* oil at 313 K and 80 bar. (◆) MT, (□) OT, (△) ST, (—) GC-EOS prediction.

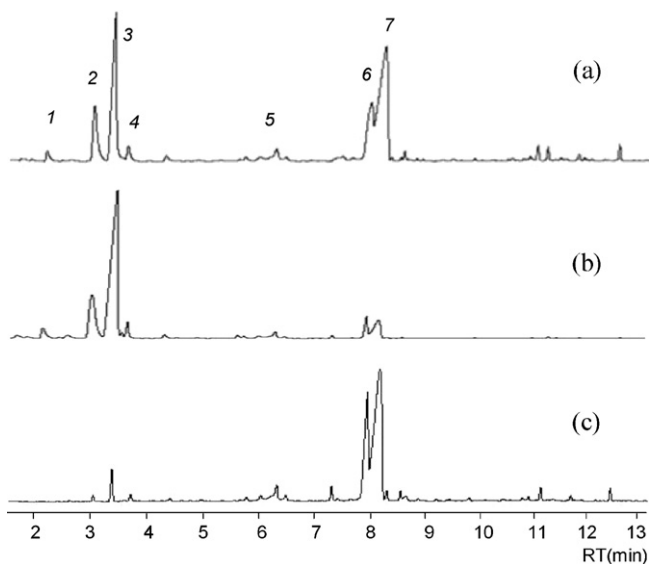


Fig. 4. Experimental GC profiles of (a) *T. minuta* oil, (b) MT-rich extract and (c) dewatered oil. Peaks: 1. β -pinene, 2. *D*-limonene, 3. *E*- β -ocimene, 4. dihydrotagetone, 5. tagetones, 6. *Z*-ocimene, 7. *E*-ocimene.

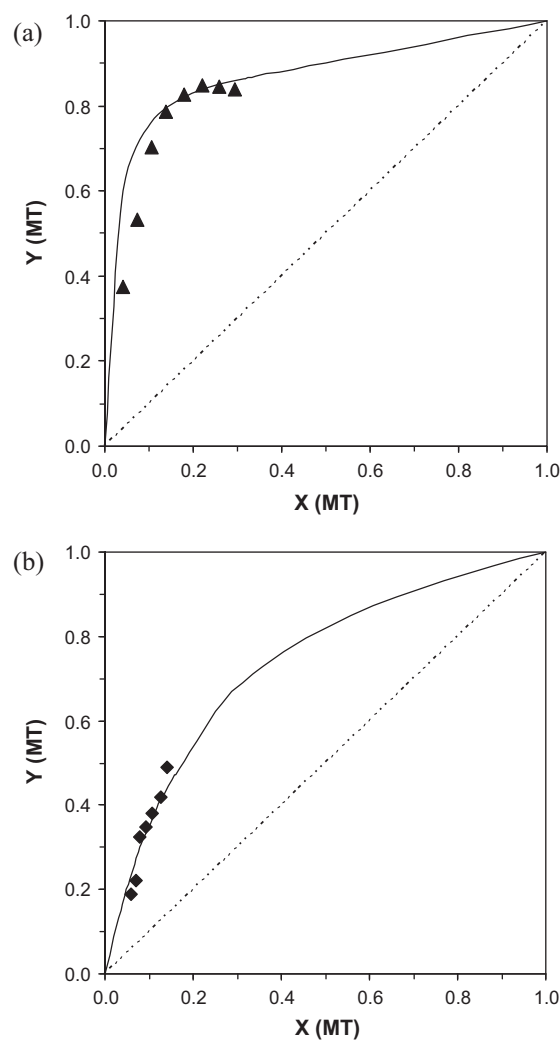


Fig. 5. Solvent-free basis extract-phase composition (Y) vs. raffinate-phase composition (X) of MT for (a) *T. minuta* oil and (b) *S. officinalis* oil at 313 K and 80 bar. Dots: experimental results, solid lines: GC-EOS prediction.

and model predictions are represented in the form of X–Y diagrams (raffinate- and extract-phase composition of MT in solvent-free basis), which are more useful for this purpose, as they show the equilibrium conditions for different liquid compositions.

4.2. Separation of oxygenated compounds and stereoisomers

The behavior of different compounds within the OT fraction of *T. minuta* has also been analyzed, and a fractionation effect has been observed among them. This fraction is composed of four main compounds, as seen in Table 1: dihydrotagetone, tagetone, *Z*-ocimene and *E*-ocimene, in order of decreasing volatility. Analyzing the extraction (or “breakthrough”) curves for each compound, different extract composition profiles can be observed, as shown in Fig. 6. After an initial similar behavior, their trends start to diverge as MT are removed. Dihydrotagetone concentration in extract-phase decreases, along with MT, being almost completely removed at the end of the experiment. Tagetone shows a maximum, followed by a continuous decrease, as a sort of “response-type” curve shifted in time with respect to dihydrotagetone. Finally, *Z*- and *E*-ocimene concentrations increase. If the process is further continued, *Z*-ocimene concentration is expected to show another peak and then start to decrease, leaving a raffinate of almost pure

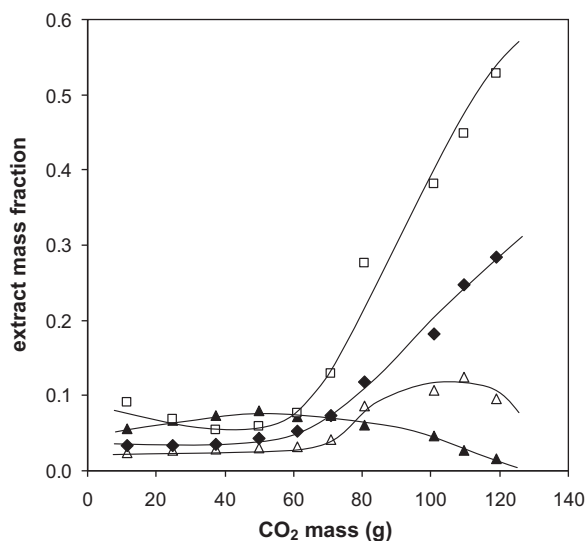


Fig. 6. Mass fraction of individual OT in the extract (solvent-free) vs. solvent consumption for *T. minuta* oil at 313 K and 80 bar. (▲) Dihydrotagetone, (△) tagetone, (◆) Z-ocimene, (◻) E-ocimene, (—) trend lines.

E-ocimene. This means that, although they are closely related compounds, small differences in their volatility make their further fractionation feasible. In particular, fractionation of ocimenes could be of interest as an example of separation of stereoisomers. This complex fractionation effect can also explain the increasing difference between experimental data and model predictions with fractionation degree, as can be seen in Fig. 3(a). In fact, the OT fraction is represented in the model by pure E-ocimene and therefore the actual behavior cannot be described accurately when MT are removed.

In the case of *S. officinalis* fractionation, the main OT compounds are 1,8-cineol, α -thujone, β -thujone and camphor, in order of decreasing volatility. As can be seen in Fig. 7, the differences in relative volatility seem to be too small to produce a significant fractionation effect within the analyzed period, not only between the optical isomers of thujone – which is expected – but also between structurally different isomers.

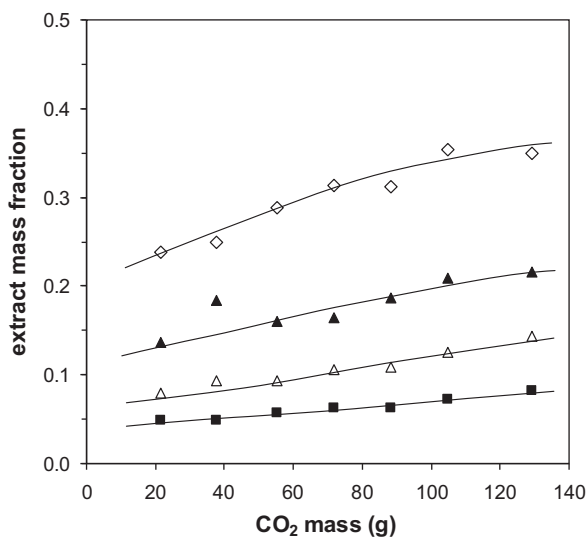


Fig. 7. Mass fraction of individual OT in the extract (solvent-free) vs. solvent consumption for *S. officinalis* oil at 313 K and 80 bar. (▲) 1,8-cineol, (◇) α -thujone, (■) β -thujone, (△) camphor, (—) trend lines.

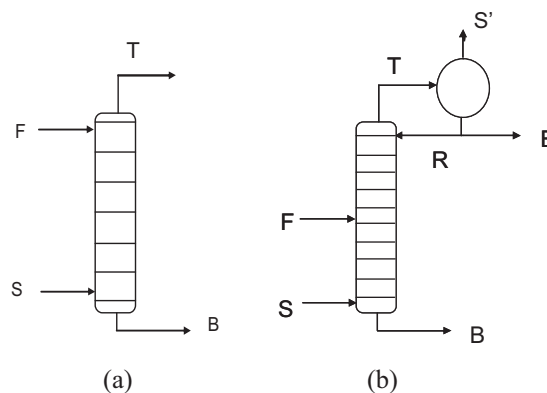


Fig. 8. Continuous multistage processing arrangements. (a) Simple countercurrent column; (b) countercurrent column with external reflux. F = oil feed, S = solvent feed, T = top extract, B = bottom raffinate, S' = recovered solvent, E = solvent-free extract, R = reflux.

4.3. Simulation and analysis of a continuous fractionation process

Fig. 8(a) and (b) shows a schematic representation of the proposed continuous fractionation systems. System (a) is composed of a multistage column, with oil feed on the top (F) and solvent feed in the bottom (S). Vapor phase (top extract, T) is removed on top and the raffinate phase (B) from the bottom. In column (b) the oil is fed in an intermediate stage, and the vapor phase is partially expanded in a separator, obtaining an almost pure solvent stream (S') and a solvent-free MT-rich liquid phase, which is partially removed as extract (E) and partially recycled to the column as a reflux stream (R). CO₂ recycling systems are not analyzed here.

4.3.1. Simple countercurrent process

Different temperature, pressure and solvent-to-feed ratio (S/F) conditions were tested in order to evaluate their effect on OT recovery and concentration in the raffinate. For *T. minuta* oil, a column of 5 theoretical stages was sufficient for achieving a good fractionation level, while for *S. officinalis* oil 10 theoretical stages were necessary. Adding more stages did not produce significant improvement.

Fig. 9 shows OT recovery and concentration in the raffinate for *T. minuta* oil for different operation conditions. Operating at higher S/F ratios decreases OT recovery, but its concentration increases. On the other hand, operation at higher pressure increases OT purity, as a consequence of the increased solvent density and solvent power, but decreases OT recovery. Temperature has a more complex effect, due to the “cross over” effect, i.e., the inversion of solubility behavior above a certain pressure, which has been discussed by the authors in a previous work [35]. As a conclusion, there is a trade-off between recovery, concentration and solvent consumption, which seems to have an optimum when operating at 90 bar, 323 K and a S/F ratio of 76 kg/kg. In this case, column (a) is capable of producing a deterpented oil with an OT purity higher than 99% and more than 90% of OT recovery.

The case of *S. officinalis* oil fractionation is more complex, due to the presence of ST that remains in the raffinate phase and modifies relative volatilities. The general effects of pressure and temperature on recovery and concentration are similar to those obtained for *T. minuta*, as expected. However, the effect of S/F ratio on OT concentration shows a maximum, after which OT concentration starts to decrease due to its higher solubilization in CO₂ and the consequent increase of ST concentration in the liquid phase. From all tested conditions, operation at 90 bar, 323 K and a S/F ratio of 57 kg/kg can produce a raffinate with almost 73% of OT recovery, 84% of ST recovery and 87% of OT concentration, very close to the maximum possible concentration (89%).

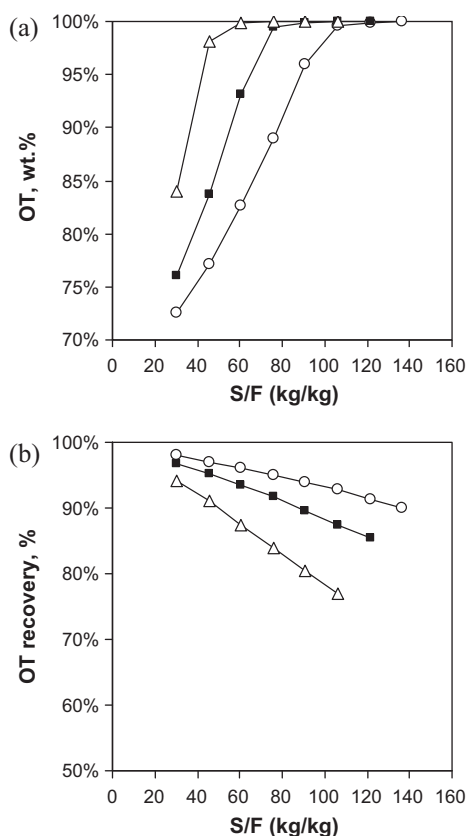


Fig. 9. Continuous countercurrent fractionation of *T. minuta* oil. Predicted OT concentration (a) and recovery (b) in raffinate phase, $n=5$, at $T=333$ K and (○) 80 bar, (■) 90 bar, (△) 100 bar.

Several authors have studied the effect of an internal reflux induced by a thermal gradient along the column. Sato et al. [8] have found that a 333–313 K gradient from top to bottom improved selectivity in the semicontinuous fractionation of citrus oils, as compared with isothermal rectification columns. Similar conclusions have been observed by Köse et al. [18] in the fractionation of origanum oil using a 328–311 K gradient. In these configurations, single phase conditions are achieved in the bottom (extraction section) and the fractionation is performed in the rectification section, with the less volatile compounds (OT) condensing and refluxing down as temperature increases. However, this approach does not necessarily improve selectivity in the case of continuous processing, as indicated by Espinosa et al. [40] from process simulation. In order to evaluate this effect, simulation of columns with thermal gradient was carried out under the operating conditions above mentioned. Results can be seen in Tables 4 and 5 compared with isothermal operation. For *T. minuta* oil, a 333–313 K

Table 4
Concentration and recovery of fractions of *T. minuta* oil in a 5-stage column with and without thermal gradient, operating at 90 bar and S/F = 76 kg/kg.

Temperature (K)	OT concentration (wt.%, CO ₂ -free)	OT recovery (%)	MT concentration (wt.%, CO ₂ -free)	MT recovery (%)
323	99.7	90.7	85.2	99.5
333	99.5	91.6	86.5	99.2
333–313	99.8	91.7	86.6	99.6

Table 5
Concentration and recovery of fractions of *S. officinalis* oil in a 10-stage column with and without thermal gradient, operating at 90 bar and S/F = 57 kg/kg.

Temperature (K)	OT concentration (wt.%, CO ₂ -free)	OT recovery (%)	MT concentration (wt.%, CO ₂ -free)	MT recovery (%)
323	87.1	72.8	45.3	97.1
338	86.9	72.7	46.2	98.0
338–320	86.7	72.8	46.0	97.1

Table 6
Continuous countercurrent fractionation with external reflux. Selected operation conditions and prediction of OT concentration and recovery in raffinate phase.

	<i>T. minuta</i>	<i>S. officinalis</i>
Column temperature (K)	323	323
Column pressure (bar)	90	90
S/F (kg/kg)	76	57
N	10	15
Feed stage	5	5
Separator temperature (K)	285	285
Separator pressure (bar)	35	35
Reflux ratio	1.00	0.67
OT concentration in raffinate, CO ₂ free (wt.%)	99.8	86.6
OT recovery in raffinate (%)	97.9	84.1
MT concentration in extract, CO ₂ free (wt.%)	96.0	55.2
MT recovery in extract (%)	95.8	80.2

gradient slightly increases raffinate purity and OT recovery and does not affect sensibly extract quality, while for *S. officinalis* oil a 338–320 K gradient does not improve separation efficiency and even reduces raffinate purity.

As pointed out by Diaz et al. [15], recoveries are thermodynamically limited by relative volatilities in simple countercurrent columns, provided the flow rates of both phases can be assumed constant:

$$\alpha_{12} = \frac{\Phi_1}{1 - \Phi_2} \quad (1)$$

where α_{12} is the relative volatility between compounds 1 and 2, and Φ_1 and Φ_2 are the corresponding limiting recoveries. When relative volatility is low, a simple countercurrent process can still produce a raffinate of high purity. However a high recovery of components 1 and 2 in the extract and raffinate phase, respectively, cannot be achieved simultaneously. The application of a thermal gradient modifies relative volatilities along the column, but does not sensibly improve efficiency. However, the use of an external reflux can overcome this limitation, increasing purities and recoveries at the same time. Moreover, it adds an extra design variable for keeping the system in the two-phase region.

4.3.2. Countercurrent process with external reflux

The use of external reflux introduces three additional operating variables: the temperature and pressure conditions in the extract separator and the reflux ratio (RR). In a first analysis, column temperature and pressure were kept constant at the previously determined values (323 K and 90 bar), with S/F ratios of 76 and 57 kg/kg for *T. minuta* and *S. officinalis* oils, respectively. Reflux ratios were selected in order to keep the system within the two-phase region. Table 6 shows the selected operation conditions for fractionation with external reflux and the improvements in comparison to the results obtained for the simple countercurrent column. It can be seen that, while OT concentration is kept

constant or slightly modified, OT recovery increase around 8% in each case when a 5-stage rectifying section with reflux is added to the column.

The main difference between both systems is the quality of the obtained extract product. In the case of *T. minuta* oil a good separation level between MT and OT can be achieved, obtaining simultaneously a high concentration of MT in the extract and of OT in the raffinate. On the other hand, for *S. officinalis* oil it is not possible to obtain a high MT concentration in the extract (in the simulation conditions it is 55.2 wt.%, in a CO₂-free basis). As pointed out before, this is consequence of the presence of several OT of intermediate volatility and the effect of a significant amount of ST on relative volatilities and general phase behavior.

5. Conclusions

Fractionation of two bioactive essential oils (*T. minuta* and *S. officinalis*) with supercritical carbon dioxide has been carried out experimentally at 313 K and 80 bar in a semicontinuous apparatus. Results were modeled with the GC-EOS as thermodynamic model. Predictions were in good agreement with the experimental results along the whole process.

In the case of *T. minuta* oil, it was found that it is possible to obtain a OT-rich raffinate by means of a semicontinuous fractionation, with acceptable recovery values. On the other hand, in the case of *S. officinalis* oil it was not possible to obtain a high level of OT purity nor recovery in the raffinate product, due to the presence of a significant amount of ST and several OT of intermediate volatility that make a difficult separation.

In order to improve process performance or overcome its limitations, two continuous fractionation schemes were simulated: a simple countercurrent column and a column with external reflux, supported by the GC-EOS as thermodynamic model. The analysis of the influence of different operation variables (temperature, pressure, solvent-to-feed ratio, thermal gradient) showed that OT purification (up to 99%) is feasible in the case of *T. minuta* oil in a simple column, and OT recovery can be significantly improved (up to 98%) when an external reflux is added. On the other hand, for *S. officinalis* oil a sharp separation is difficult even when using reflux: it is possible to obtain an almost deterpenated raffinate (composed by OT and ST), but with lower recovery due to losses in the extract phase.

Acknowledgements

This work was carried out due to the financial support of SECYT (Universidad Nacional de Córdoba), FONCYT and CONICET, Argentina. The authors thank Prof. Dr. Julio Zygadlo for providing the samples of *T. minuta* essential oil.

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