Semi-continuous fractionation of bioactive essential oils with supercritical carbon dioxide. Experiments, modeling and separation of stereoisomers

N. Gañan, A. Velez, P. Hegel, E.A. Brignole[*]

Planta Piloto de Ingeniería Química (PLAPIQUI – UNS – CONICET)
Camino La Carrindanga Km. 7 – CC 717, Bahía Blanca, Argentina
* Corresponding author e-mail: ebrignole@plapiqui.edu.ar

ABSTRACT

In this work we study the fractionation of two bioactive essential oils (*Salvia officinalis* and *Tagetes minuta*) with carbon dioxide in a semi-continuous single stage apparatus. Essays were performed at 313 K and 80 atm in order to selectively remove the hydrocarbon monoterpenes and enrich the oxygenated and sesquiterpene fraction. Succesive extracts were collected during the process and their composition determined by GC-MS. From these results, the feasibility of a single stage deterpenation process with high purity and recovery of products is discussed. The systems carbon dioxide + essential oil are modeled and their behavior predicted using the group contribution equation of state (GC-EOS). With this thermodynamic model, the fractionation process is simulated using the mathematical background of batch distillation (Rayleigh equation). The correlation between the simulation and the experimental data is improved when a composition-dependent relative volatility is considered instead of only a pressure and temperature-dependent one.

Separation of stereoisomers is also investigated. Although no selectivity is observed between optical isomers (α- and β-thujone in *Salvia* oil), this is not the case for the stereoisomers Z- and E-ocimenone from *Tagetes* oil. These components exhibit an important degree of separation, which is an interesting feature considering that the bioactivity of stereoisomers is usually different.

INTRODUCTION

Essential oils are complex mixtures of volatile compounds of terpenic nature obtained from different plants. Many essential with different degrees of biocidal activity oils have been reported: as antimicrobials, repellents, insecticides, pesticides, conservatives, etc. [1,2]. Health and environmental concerns are pressing towards the use of natural agents, which are generally biodegradable and have less or no effects on human health and safety. From this point of view, essential oils appear as an attractive alternative,in spite of some drawbacks: their high volatility, low water solubility and chemical instability among the most important. Some of them have been "generally recognized as safe" (GRAS) and, in a traditional way, have been used by people for centuries.

Essential oils bioactivity is generally connected with some specific compounds, although synergistic effects are likely to occur. Oxygenated monoterpenes are considered the most active fraction of the oils, so its concentration or purification is of great interest in order to obtain natural biocidal agents or raw materials for producing specific pharmaceuticals by organic synthesis.

Supercritical fluids, and specially carbon dioxide, have been investigated and applied as solvents for the extraction and fractionation of different essential oils, as well as other complex mixtures [3-6]. They have some advantages over traditional methods, like lower operation temperatures when dealing with thermolabile compounds, and the obtention of

solvent-free extracts. Their solvent power and selectivity can be tuned easily by small changes in pressure and temperature. Besides this, carbon dioxide is neither flammable nor toxic, allowing a safer operation.

In this work we study the fractionation of two essential oils (from *Salvia officinalis* and *Tagetes minuta*) with supercritical carbon dioxide in a semi-continuous single stage apparatus, in order to selectively remove the hydrocarbon monoterpenes and obtain an oxygenated and sesquiterpene enriched fraction. The bioactivity of these oils has been investigated and reported by several authors [7-11].

On the other hand, essential oils generally contain mixtures of stereoisomers (optical or enantiomers and Z-E type). Separation of stereoisomers is a very interesting feature, because bioactivity is usually connected with one of the isomers. Obtaining pure enantiomers or stereoisomers with simple and non expensive techniques is a main goal in pharmaceutical and biomedical industry.

Therefore the specific objectives of this work are: (a) to study the feasibility of a semi-continuous process for the separation of the oxygenated fraction of interest with high purity and recovery of products using supercritical carbon dioxide as solvent; (b) to model the process using the GC-EOS as thermodynamic model and the simulation of batch distillation in order to reproduce experimental data, and (c) to study the separation of stereoisomers (optical and Z-E type).

MATERIALS AND METHODS

Materials:

Salvia officinalis essential oil was obtained by hydrodistillation from fresh plant samples from Sierra de la Ventana (Argentina). *Tagetes minuta* essential oil was obtained by hydrodistillation from fresh plant samples from Sierras de Córdoba (Argentina). Their composition was determined by gas chromatography–mass spectrometry (GC-MS) and is shown in **Tables 1-2**. Compounds have been grouped into three main fractions: hydrocarbon monoterpenes (MT), oxygenated monoterpenes (OT) and sesquiterpenes (ST).

Extra-dry carbon dioxide was provided by Linde Argentina.

Experimental apparatus and procedure:

Experimental runs were performed in a single stage semi-continuous extraction apparatus shown schematically in **Figure 1**. Each oil sample was loaded into the extraction column, embedded on fine glass beads (35-60 mesh). System temperature and pressure were kept constant (313 K and 80 atm). Carbon dioxide was fed continuously to the column, at a flow rate low enough to assure its saturation with the essential oil phase. The extract was depressurized using a heated expansion valve and collected in a glass U-tube immersed into a cooling bath (ethanol at 220 K to minimize losses. Gas flow rate was measured after expansion using a bubble gas meter. In each run, successive extracts were collected until exhaustion of approximate 50% of the oil sample. More details about this apparatus can be found in a previous work [12].

Table 1. *Salvia officinalis* essential oil composition

COMPONENT	M (g/mole)	%
Hydrocarbon monoterpenes (MT)		
α-pinene	136.24	8.71
camphene	136.24	5.54
β-pinene	136.24	1.59
myrcene	136.24	1.30
other (<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 (<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 (<1%)	-	1.07
TOTAL ST		**12.58**

Table 2. *Tagetes minuta* essential oil composition

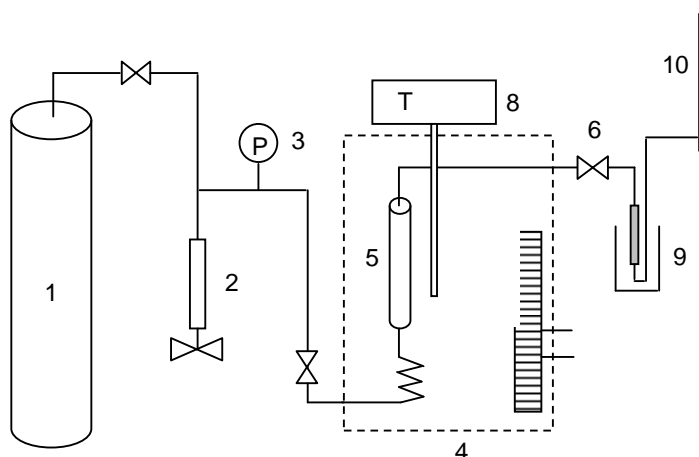| COMPONENT	M (g/mole)	%
Hydrocarbon monoterpenes (MT)		
β-pinene	136.24	1.32
d-limonene	136.24	7.01
E-ocimene	136.24	25.97
other (<1%)	-	0.80
TOTAL MT		**35.10**
Oxygenated monoterpenes (OT)		
dihydrotagetone	154.25	1.72
E-tagetone	152.23	2.25
NI	-	2.33
Z-ocimenone	150.22	11.20
E-ocimenone	150.22	44.13
other (<1%)	-	3.27
TOTAL OT		**64.90**

Figure 1. Experimental apparatus. 1. CO_2 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.

Solubility of the extract was calculated by weighing and determining volumetrically the corresponding mass of CO_2. Extract composition was determined by gas chromatography with flame ionization detector (GC-FID), after dissolution of the extract with acetone, in a Varian CX3400 chromatograph with capillary column DB5-HT (Agilent Technologies Inc., 15 m length, 0.32 mm i.d., 0.1 μm film thickness). Hydrogen was used as carrier, at a flow rate of 1 ml/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, 8 K/min up to 513 K.

MODELING

Thermodynamic model:

The phase equilibria of the [essential oil + carbon dioxide] systems was modeled using the group contribution equation of state (GC-EOS) [13]. 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. This model has been previously used in several studies to describe the behavior of essential oils and other mixtures in supercritical processes [14].
Salvia officinalis essential oil was modeled as a pseudo ternary system composed by α-pinene (MT), α-thujone (OT) and β-caryophyllene (ST), the most representative compounds of each fraction. In the same way, *Tagetes minuta* oil was modeled as a pseudo binary system of β-ocimene (MT) and ocimenone (OT). Physical properties of these compounds are shown in **Table 3**, and their molecular structures in **Figure 2**. Pure group and binary interaction parameters have been reported in a previous work [12]. No information from the experimental data of this work is included into the model, so the results are completely predictive.

Table 3. Physical properties of selected compounds
[a] Data from literature; [b] estimated by Joback group contribution method

Component	T_c (K)	P_c (bar)	NBP (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]	-

α-pinene   α-thujone   β-caryophyllene   β-ocimene   ocimenone
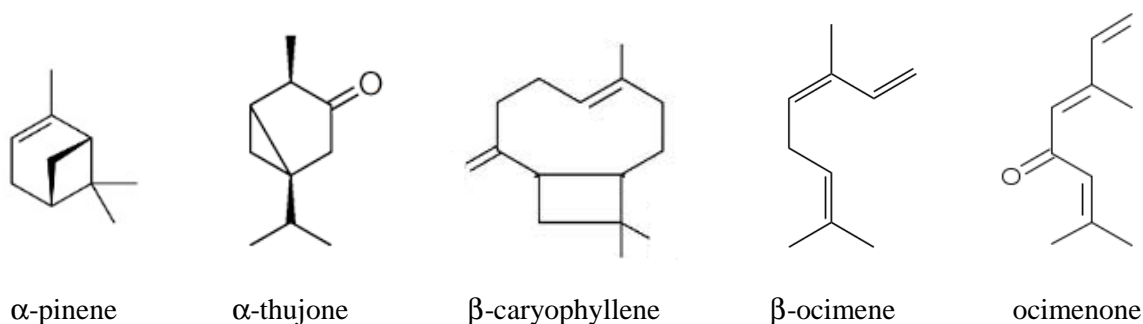
Figure 2. Chemical structure of selected key components

Process simulation:

The fractionation process was simulated using the mathematical background of single-stage batch distillation, consisting of a series of successive steps, each one corresponding to a single flash calculation. 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 composition-dependency of the relative volatilities is taken into account. The software GCTHREE [**15**] was used for the flash calculations.

RESULTS AND DISCUSSION

Semi-continuous fractionation:

Extract-phase composition during the fractionation process can be seen in **Figure 3**, for both oils in free-solvent basis. For Salvia oil, the three fractions are shown, while for Tagetes oil only the MT fraction is shown. Initially the extracts are richer in the lighter fraction (MT) than the raw oil and, as the extraction proceeds and the MT are removed, the extract-phase becomes richer in the less volatile fractions (OT and ST). For Tagetes oil, the first part of the process yields a MT-rich extract (above 80%) and then it rapidly decreases, with an almost complete deterpenation when the extraction degree reaches 50%. For Salvia

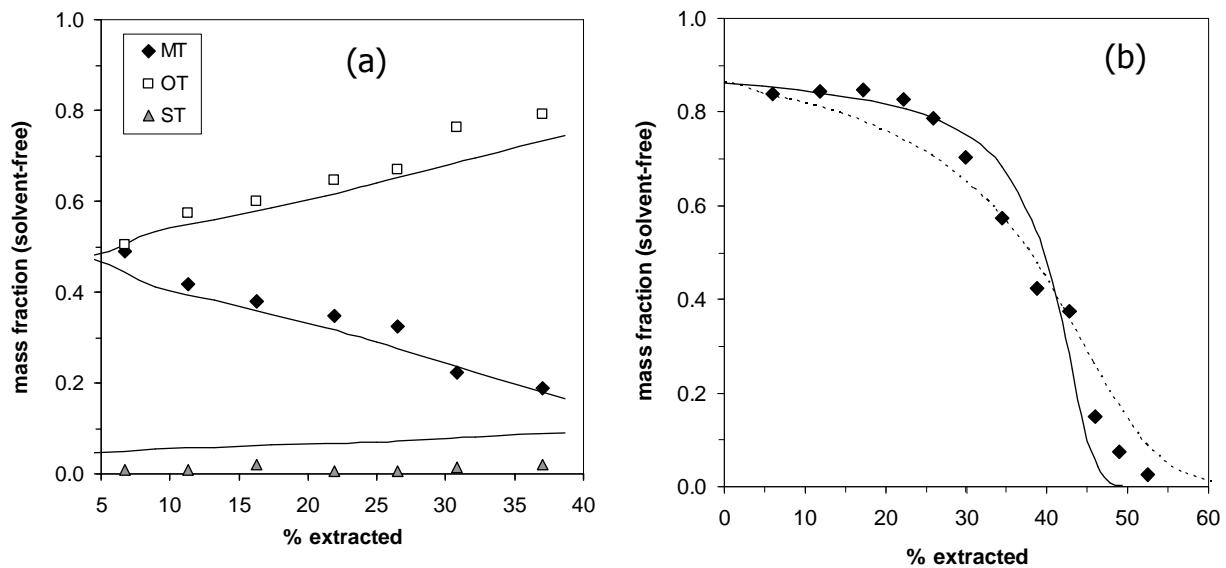oil the separation degree between MT and OT is lower, thus yielding extracts actually richer in OT.

Figure 3. Extract-phase compositon (solvent-free basis).
(a) Salvia oil; (b) Tagetes oil. Dots: experimental data; (–) GC-EOS prediction using *variable* relative volatility, (--) GC-EOS prediction using *constant* relative volatility

Model predictions are shown together with experimental data. As can be seen, the simulation describes correctly the system behaviour in both cases. For Tagetes oil the model prediction using a constant relative volatility is also shown for comparison purposes. This relative volatility is calculated from the initial system composition at the operation temperature and pressure, and it is assumed constant during the process. It is observed that a better prediction is achieved with a variable relative volatility, specially in the first part of the process.
Figure 4 shows the accumulated extracted mass of each fraction as a function of the mass of solvent (breakthrough curves). It can be seen that the MT fraction for Tagetes oil tends to a maximum value corresponding to its depleting in the liquid phase. In this curves, the initial slope is a measure of the solubility of each fraction in the multicomponent system. In the case of Salvia oil, MT and OT exhibit practically the same solubility. In binary systems with carbon dioxde, MT are actually more soluble than the polar OT, but this shows that the solubility behaviour of MT is different when they are in a multicomponent system, and therefore predictions based only in binary information can be inaccurate. This has been discussed previously [**12**]. For Tagetes oil, as already pointed, the separation degree between MT and OT is much higher. Model predictions are also shown, and are in good agreement with the experimental data.
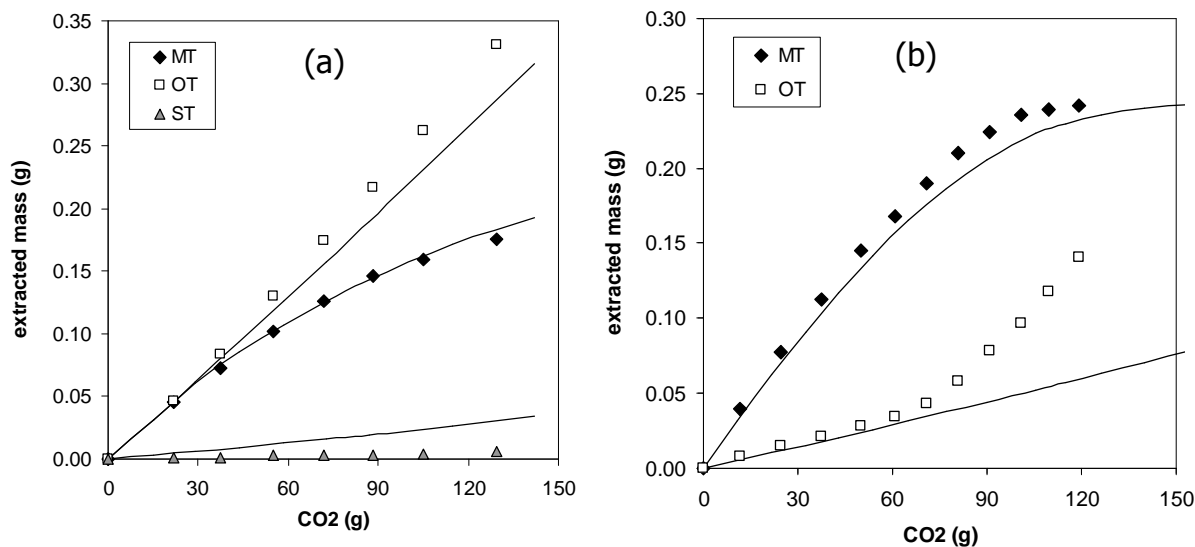
Figure 4. Extracted mass vs. mass of solvent
(a) Salvia oil; (b) Tagetes oil. Dots: experimental data; (–) GC-EOS prediction

Separation of stereoisomers:

As shown in **Table 1**, each oil fraction is composed by several structurally related compounds, presenting in many cases different kinds of isomery. Salvia oil OT fraction contains (in order of decreasing volatility): 1,8-cineol, the enantiomers α- and β-thujone and camphor. Tagetes oil OT fraction is composed by four closely related ketones: dihydrotagetone, tagetone and the stereoisomers Z- and E-ocimenone.

Runs were performed in the same semi-continuous apparatus. Temperature was kept constant (313 K) and samples from the extract-phase were taken at different pressure conditions, in order to analyze the effect on relative volatility. Carbon dioxide flow rate was low, in order to obtain equilibrium conditions (gas saturated method). Samples were analyzed by GC-FID and relative volatilities were calculated by estimating liquid phase composition by mass balances. Besides Salvia and Tagetes oil, experiments were also carried on with lemon peel oil, which contains the isomers Z- and E-citral (or neral and geranial, respectively).

Results are shown in **Figure 5(a-c)**. In all cases relative volatilities decrease with pressure, due to the higher solvent power of carbon dioxide. They tend to the limiting value of 1 as the system approaches complete miscibility (homogeneity) conditions. In the case of Salvia oil, the enantiomers α- and β-thujone have no difference in volatility, so its separation by near critical fractionation is not feasible. Camphor and 1,8-cineol can be more easily separated, specially operating at lower pressures. In the case of Tagetes and lemon peel oil, Z- and E-isomers can be separated at lower pressure, as indicated by their relative volatility higher than 1. The experimental results show, for example, that Z-ocimenone volatility is twice that of E-ocimenone at 80 bar (and approximately the same for neral and geranial around 75 bar), thus allowing their purification provided an adequate system (like a fractionation column with reflux). This different volatility of Z- and E- configurations can be attributed to small differences in dipolar moment, affecting the interaction strength between molecules. Among terpenes, it is often observed that the position of a double bond can modify the boiling point of a compound within a range of 10 K or even more.
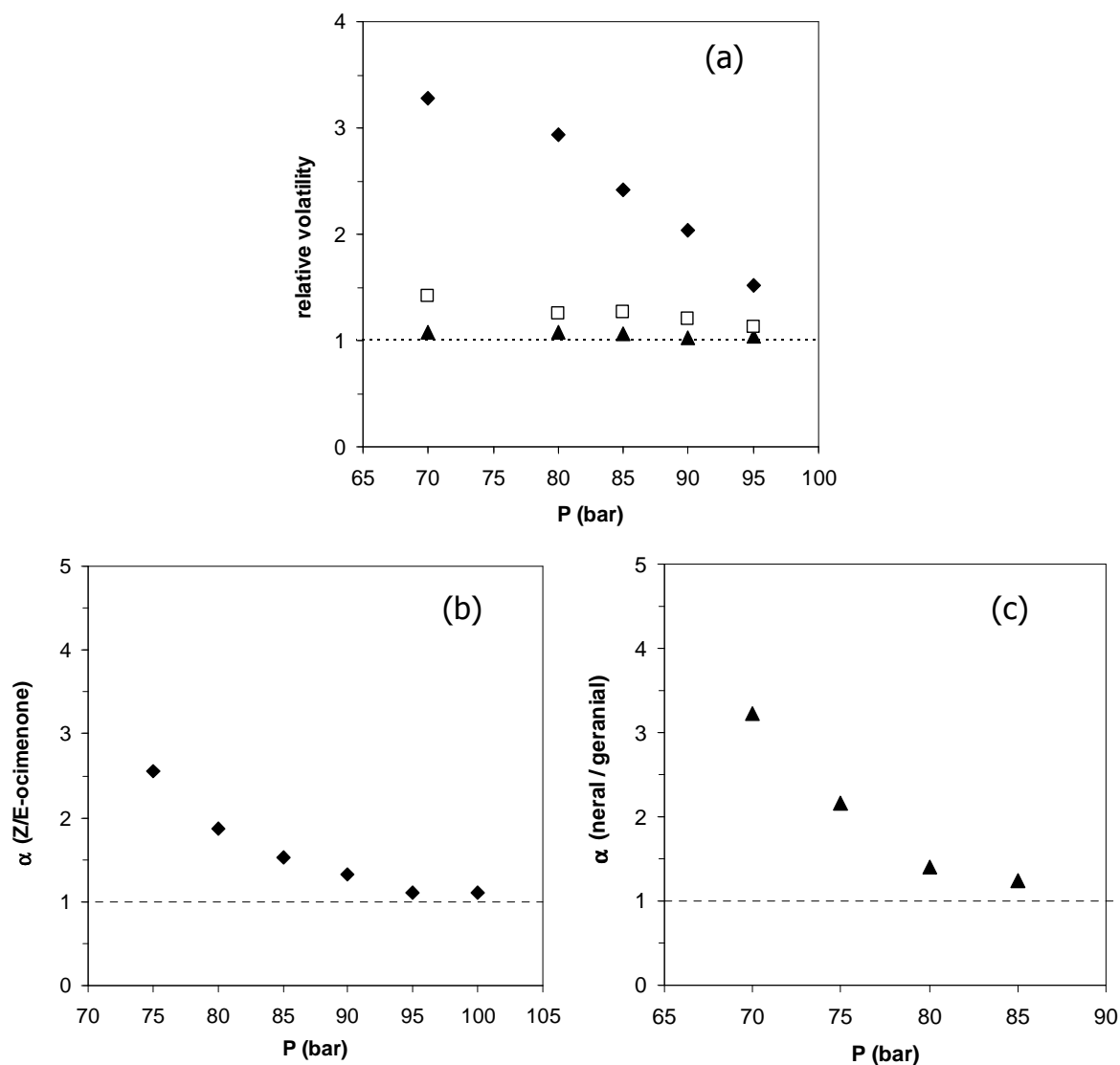
Figure 5. Relative volatility vs pressure (T=313 K).
(a) Salvia oil: (◆) 1,8-cineol/thujones, (□) α-/β-thujone, (▲) thujones/camphor;
(b) Tagetes oil; (c) Lemon peel oil.

CONCLUSION

Supercritical carbon dioxide fractionation of two essential oils (Salvia officinalis and Tagetes minuta) was studied in order to concentrate or purify the bioactive oxygenated fraction. Experiments were carried out in a semicontinuous apparatus operating at 313 K and 80 bar and samples of the extract-phase were collected and analyzed by gas chromatography. In the case of Tagetes oil, a MT-rich extract (above 80%) and a OT-rich raffinate (above 95%) could be obtained; but in the case of Salvia oil, the separation performance of the semicontinuous process was lower.

Fractionation process was simulated using the GC-EOS as thermodynamic model for the essential oil + carbon dioxide systems. Model predictions were in good agreement with the experimental data.

Separation of stereoisomers was studied for Salvia, Tagetes and lemon peel oil. Extract-phase samples were collected at different pressure conditions (T=313 K) and analyzed. No separation of enantiomers was observed (α- and β-thujone in Salvia oil), but Z-E type stereoisomers exhibited some degree of separation, possibly due to differences in volatility associated with the molecular arrangement.

ACKNOWLEDGMENTS

REFERENCES

[1] ZYGADLO, J.A., JULIANI, H.R., Current Topics in Phytochemistry, 3, **2000**, p. 203.
[2] NERIO, L.S., OLIVERO-VERBEL, J., STASHENKO, E., Bioresource Technology, 101, **2010**, p. 372.
[3] REVERCHON, E., Journal of Supercritical Fluids 10, **1997**, p. 1.
[4] REVERCHON, E., DE MARCO, I., Journal of Supercritical Fluids 38, **2006**, p. 146.
[5] DIAZ, S., ESPINOSA, S., BRIGNOLE, E.A., Journal of Supercritical Fluids 35, **2005**, p. 49.
[6] DIAZ, M.S., BRIGNOLE, E.A., Journal of Supercritical Fluids, 47, **2009**, pp. 611.
[7] LONGARAY DELAMARE, A.P., MOSCHEN-PISTORELLO, I., ARTICO, L., ATTI-SERAFINI, L., ECHEVERRIGARAY, S., Food Chemistry, 100, **2007**, p. 603.
[8] GILLIJ, Y.G., GLEISER, R.M., ZYGADLO, J.A., Bioresource Technology, 99, **2008**, p. 2507.
[9] SCRIVANTI, L.R., ZUNINO, M.P., ZYGADLO, J.A., Biochemical Systematics and Ecology, 31, **2003**, p. 563.
[10] LOPEZ, M.L., BONZANI, N.E., ZYGADLO, J.A., Biochemical Systematics and Ecology, 36, **2009**, p. 882.
[11] VASUDEVAN, P., KASHYAP, S., SHARMA, S., Bioresource Technology, 62, **1997**, p. 29.
[12] GAÑAN, N., BRIGNOLE, E.A., Journal of Supercritical Fluids, 58, **2011**, p. 58.
[13] SKJOLD-JORGENSEN, S., Industrial and Engineering Chemistry Research, 27, **1988**, p. 110.
[14] ESPINOSA, S., BOTTINI, S.B., BRIGNOLE, E.A., Industrial and Engineering Chemistry Research, 39, **2000**, p. 3024.
[15] MICHELSEN, M.L., Fluid Phase Equilibria, 9, **1982**, p. 21.