THE INFLUENCE OF BED GEOMETRY IN OBTAINING VOLATILE COMPOUNDS FROM ROSEMARY BY SFE

Giovani L. Zabot, Moyses N. Moraes, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), Rua Monteiro Lobato, 80; 13083-862 Campinas, São Paulo, BRAZIL; phone: +55.19.3521.4033, fax: +55.19.3521.4027, Email: maameireles@gmail.com; meireles@fea.unicamp.br

ABSTRACT

The aim of this study was to evaluate the use of supercritical CO_2 extraction in obtaining target compounds from rosemary (*Rosmarinus officinalis*) using a laboratory system containing 2 extractors of 1 L each with different height (H_B) to bed diameter (D_B) ratios. The objective was to compare the kinetic parameters of the extraction curves obtained for both two geometries (E-1: H_B/D_B = 7.1; E-2: H_B/D_B = 2.7) maintaining the solvent mass to feed mass (S/F) ratio equal for the 2 beds. The other process variables constant the bed porosity ($\varepsilon = 0.65$), apparent and true densities of the raw material ($\rho_a = 0.48$ g/cm^3 and $\rho_r = 1.36$ g/cm^3, respectively), particle average size ($d_p = 0.66$ mm), temperature (40°C), pressure (30 MPa) and time of extraction (360 min) were maintained. It was observed that the bed E-2 presented global yields slightly superior whether compared to E-1. The mass transfer rates in the constant extraction rate (CER) period were 0.24±0.01 g of extract/min for E-1 and 0.32±0.01 g of extract/min for E-2. Likewise, the yields in the CER period were 41±5 g of extract/100 g of extractable for E-1 and 51±1 g of extract/100 g of extractable for E-2. The kinetics of extraction of oxygenated monoterpenes (i.e., 1,8-cineole, camphor, α-terpineol and borneol) and sesquiterpenes (i.e., trans-caryophyllene) were different for both bed geometries. These differences are associated to the characteristics of the raw material/extracts (i) and to the strong compaction of the vegetal matrix in the bed (ii). In the first case (i), where the solute is attached to the cellular structure, the mass diffusion phenomenon cannot be neglected and it is responsible for longer extraction time. Thus, the bed geometry presented a pronounced influence in the mass transport properties and the criterion used (equal S/F and constant extraction time) for the geometry shift was not appropriate for this system. In the second case (ii) we concluded that the lowest yield in E-1 was also influenced by strong compaction of the bed and CO_2 channeling, resulting in large axial dispersion of the solvent-solute mixture. These phenomena were most likely small in E-2.

INTRODUCTION

The advances that supercritical technology is reaching in the past years are related to the increase of activities linked to scientific research and technological development, which focus on inserting novel processes in some sectors as pharmaceutical, food, chemical and cosmetics. Although there is some available information about extracting bioactive compounds from natural resources using supercritical fluids, there is a need of scientific studies emphasizing the evaluation of the influence of process variables on the kinetic profiles of obtaining target compounds industrially useful in the cited sectors.

In mid-2004, Carvalho *et al*. [1] and Moura *et al.* [2] performed studies about the influence of the bed height (H_B) to the diameter (D_B) ratio on the extraction kinetics of rosemary (*Rosmarinus officinalis*) and fennel (*Foeniculum vulgare*) compounds, respectively. The authors proposed correlations contemplating the bed geometry (in terms of H_B/D_B) and two process variables (amount of feed raw material and solvent flow rate) to obtain similar kinetic behaviors of the extracting curves. The results were satisfactory when fennel was used, because the behaviors were similar for the kinetics of extraction yield between the tested H_B/D_B ratios. Notwithstanding, the kinetic behaviors for rosemary extracts were different. The overall extraction curves (OEC) presented some differences between the tested H_B/D_B ratios by using their own proposed correlations for the geometry shift. Furthermore, the experimental studies were restricted to extractors of small volumes (0.22 L and 0.30 L).

Then, the homemade multipurpose unit (SFE-2×1L) [3] containing 2 extractors of 1 L each with different H_B/D_B ratios was used in this study for acquiring more information about the magnitude of the influence of the bed geometry in obtaining bioactive compounds from rosemary along the extraction time. The objective consisted in knowing whether the behavior of the OEC shown by Carvalho *et al*. [1] is reproduced in this multipurpose unit, evaluating the kinetic results from the total extract and volatile compounds yields.

MATERIAL AND METHODS

Raw material characterization

Rosemary (*Rosmarinus officinalis*) leaves were obtained from the Municipal Market of Campinas, Brazil. The raw material at -18°C was comminuted in a knife mill (Marconi, MA-340, Piracicaba, Brazil) and the particle size distribution was determined using a vibratory system (Bertel, 1868, Caieiras, Brazil) with sieves of mesh sizes 8–80 (Tyler series, Wheeling, USA). The comminuted samples were packed in air impermeable bags and stored again at -18°C (HC-4, Metalfrio, São Paulo, Brazil). The mean particle diameter (d_p) was determined according to the ASAE standards [4]. The moisture (U) content of the comminuted samples was determined in duplicate using the xylene distillation method [5]. The true density of the particles (ρ_r) was measured by picnometry with helium gas at the Central Analytical Laboratory of the Institute of Chemistry – UNICAMP (Campinas, Brazil). The apparent density of the bed (ρ_a) was calculated by dividing the sample feed mass by the extractor volume. The total porosity of the bed (ε) was calculated as: $\varepsilon = 1 - (\rho_a/\rho_r)$.

Obtaining rosemary extract by LPSE

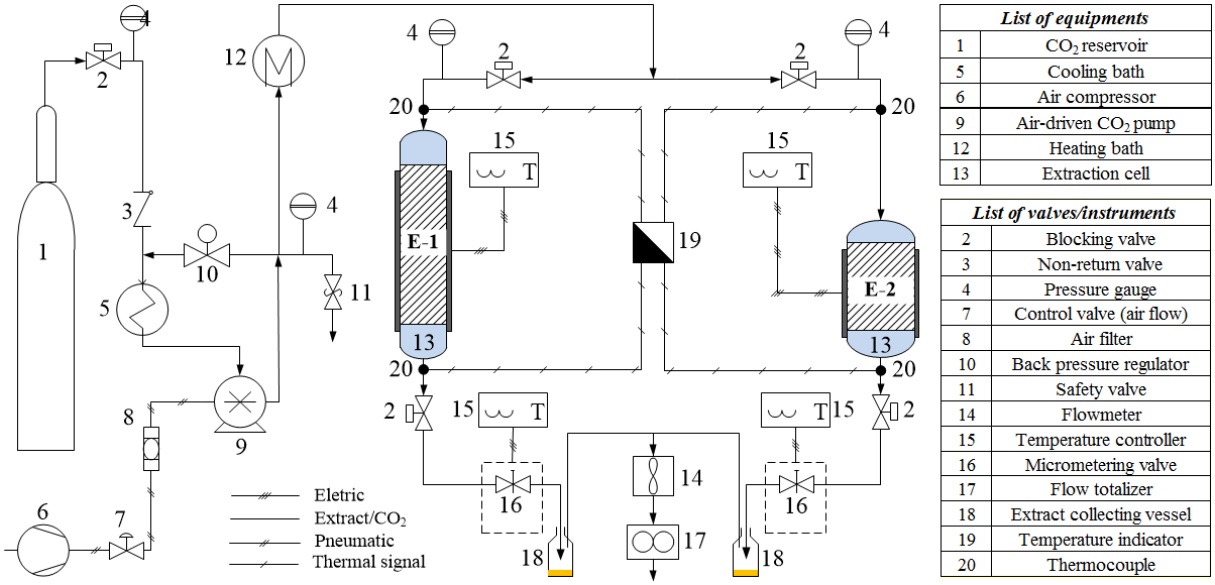
Rosemary extract was obtained using low-pressure solvent extraction (LPSE) to compare the yield and chemical composition with those of the samples obtained using supercritical CO_2 extraction (SFE-CO_2). Milled rosemary (10 g) was wrapped in filter paper and placed in a Soxhlet apparatus connected to a solvent flask with 300 mL of ethanol (Chemco, Hortolândia, Brazil). The system was refluxed for 6 h; afterwards it was removed from the extracted mixture using a rotary vacuum evaporator (Logen Scientific, LSCS-1/52C, Diadema, Brazil) at 40°C. The extract mass was determined with an analytical balance (Radwag, AS200/C/2, Radom, Poland). The assays were replicated 3 times.

Obtaining rosemary extract with supercritical CO_2

SFE-CO_2 of rosemary extract was performed at 40°C and 30 MPa. These conditions were obtained on literature, according to Carvalho *et al*. [1]. CO_2 (99.0% purity, Air Liquide,

Campinas, Brazil) was used as the solvent. Firstly, the global yield of extract (X_0) was obtained in duplicate using the commercial Spe-ed unit (Applied Separations, 7071, Allentown-USA). The extraction was done at solvent mass to feed mass (S/F) ratio of 210. The 5 mL extractor was filled completely with 2.4 g of comminuted leaves of rosemary and the CO_2 flow rate was maintained constant at 2.2 g/min. It is important to mention that X_0 consists of the maximum amount of solute that is extractable from a botanic matrix at fixed conditions of temperature and pressure for an established S/F ratio.

The experimental assays for evaluating the influence of the bed geometry on the extraction kinetics were done in a homemade multipurpose unit (Figure 1), as described by Zabot *et al*. [3]. The referenced unit contains 2 extractors of 1 L each with different bed geometry (E-1: H_B/D_B = 7.1; E-2: H_B/D_B = 2.7). The beds were filled completely with 475 g of comminuted leaves of rosemary for the runs. Baskets of 80 mesh with the same diameters as the internal diameters of the extractors (D_{E-1} = 5.7 cm and D_{E-2} = 7.8 cm) were utilized to facilitate charging/discharging the vegetal matrix. For each trial, nineteen samples of extract were collected in 0.1L glass flasks (separation at ambient pressure) in gradual intervals. The experimental runs were replicated 2 times. The extract mass was determined with an analytical balance (Radwag, AS200/C/2, Radom, Poland). The relative yields of extract (Y; g of extract/100 g of extractable) were calculated using Equation 1. The word "extractable" used in this text expresses the amount of extract obtained in the X_0 experiment.



List of equipments	
1	CO_2 reservoir
5	Cooling bath
6	Air compressor
9	Air-driven CO_2 pump
12	Heating bath
13	Extraction cell

List of valves/instruments	
2	Blocking valve
3	Non-return valve
4	Pressure gauge
7	Control valve (air flow)
8	Air filter
10	Back pressure regulator
11	Safety valve
14	Flowmeter
15	Temperature controller
16	Micrometering valve
17	Flow totalizer
18	Extract collecting vessel
19	Temperature indicator
20	Thermocouple

Figure 1: Flow diagram of the homemade SFE-2×1L unit [3].

$$Y = \frac{mass_{extract}(S/F)}{X_0\left\{mass_{extract}(S/F=210)\right\}}\cdot 100 \qquad (1)$$

The experimental OEC data were fitted to a spline with 3 straight lines [6] using SAS 9.2® to estimate: the length of the constant extraction rate period – CER (t_{CER}); mass transfer rate for the CER period (M_{CER}); yield for the CER period (R_{CER}); length of the falling extraction rate period – FER (t_{FER}); mass transfer rate for the FER period (M_{FER}); yield for the FER period (R_{FER}). These parameters were determined for each experimental replicate, which allowed us to compare the curves for the extraction beds.

The same S/F ratio in both beds (E-1 and E-2) at a fixed extraction time was the adopted criterion to this study for the scale-up. The extraction time was fixed in 360 min and

two levels of S/F ratio were studied: I = 14.3 and II = 5.0. Once the bed volumes and extraction time were equal in both cases (I and II), the CO_2 mass flow rates were also equal for each S/F ratio, resulting in 17.3 g/min for S/F I and 6.0 g/min for S/F II. An experimental randomized block design was carried out to evaluate the influence of the bed geometry (E-1 and E-2) on each block (S/F I and S/F II ratios). The order of the extractions was sorted and the statistical data analysis was performed using Minitab 16®.

Chemical analysis of extracts

The compositions of the volatile substances present in the rosemary extracts were determined using gas chromatography with flame ionization detector (Shimadzu, CG17A, Kyoto, Japan) equipped with a capillary column of fused silica DB-5 (J&W Scientific, 30 m×0.25 mm×0.25 μm, Folsom, USA).

Chemical analysis, based on Ibañez *et al.* [7], consisted of injecting 3 μL of each sample diluted to 500 ppm (w/w) in acetone (Êxodo Científica, Hortolândia, Brasil) and filtered using nylon membrane (0.45 μm). The sample split ratio was 1:20. The carrier gas (Helium, 99.9% purity, White Martins, Campinas, Brazil) at flow rate of 0.79 mL/min. The injector and the detector temperatures were 200 and 280°C, respectively. The column was programmed to 40°C during 10 min, then was heated from 40°C to 240°C at 5°C/min and after from 240°C to 280°C at 20°C/min. The final temperature (280°C) was maintained for 5 min.

The compounds present in the rosemary extracts were identified by comparing the retention indices of the samples and external standards. The standards used were: 1,8-cineole ($C_{10}H_{18}O$ - CAS 470-82-6 – Sigma Aldrich, St. Louis, USA); camphor ($C_{10}H_{16}O$ - CAS 76-22-2 – Sigma Aldrich, St. Louis, USA); trans-caryophyllene ($C_{15}H_{24}$ - CAS 87-44-5 – Sigma Aldrich, St. Louis, USA); α-terpineol ($C_{10}H_{18}O$ - CAS 98-55-5 – Sigma Aldrich, St. Louis, USA); α-pinene ($C_{10}H_{16}$ - CAS 2437-95-8 – Aldrich, Milwaukee, USA); borneol ($C_{10}H_{18}O$ - CAS 507-70-0 – Aldrich, Milwaukee, USA); camphene ($C_{10}H_{16}$ - CAS 79-92-5 – Sigma Aldrich, St. Louis, USA); p-cimene ($C_{10}H_{14}$ - CAS 99-87-6 – Aldrich, Milwaukee, USA); terpinen-4-ol ($C_{10}H_{18}O$ - CAS 562-74-3 – Acros Organics, New Jersey, USA); linalool ($C_{10}H_{18}O$ - CAS 78-70-6 – Sigma Aldrich, St. Louis, USA); and limonene (C10H16 - CAS 138-86-3 – Sigma Aldrich, St. Louis, USA). Quantification was performed using external standard calibration curves.

RESULTS

Extract yields

Extraction beds, E-1 and E-2 of SFE-2×1L (Figure 1), were loaded with 476±2 g of rosemary, thus showing $\rho_a = 0.48 \pm 0.01$ g/cm^3. The results of rosemary characterization were: $\rho_r = 1.36 \pm 0.01$ g/cm^3, $\varepsilon = 0.65 \pm 0.01$ and $d_p = 0.66 \pm 0.01$ mm. The total content of extract obtained by Soxhlet was 27.6±0.7 g/100 g of rosemary (dry basis). X_0 value was 5.13±0.03 g/100 g of rosemary.

The kinetic curves presenting the averages and standard deviation of Y on each bed for the two studied S/F ratios are shown in Figure 2. There was difference in the extraction kinetics between the beds. We obtained M_{CER} and M_{FER} for E-2 larger than for E-1 for both S/F ratios. M_{CER} for S/F I – E-1 was 0.24±0.01 g extract/min, while M_{CER} for S/F I – E-2 was 0.32±0.01 g extract/min. Furthermore, M_{FER} values were 0.11±0.01 g extract/min and 0.15±0.01 g extract/min for S/F I – E-1 and S/F I – E-2, respectively.

Likewise, the relative yields at CER period for assays corresponding to S/F I were 41±5 g extract/100 g of extractable for E-1 and 51±1 g extract/100 g of extractable for E-2. Thus, considering both S/F ratios (I and II), we observed difference between the bed geometries by comparing: M_{CER} I (p-value = 0.005); M_{FER} I (p-value = 0.018); R_{CER} I (p-value = 0.119); R_{FER} I (p-value = 0.066); M_{CER} II (p-value = 0.033); M_{FER} II (p-value = 0.009); R_{CER} II (p-value = 0.006); and R_{FER} II (p-value = 0.013).

The difference on the mass transfer rates between E-1 and E-2 was even more pronounced at S/F II ratio (6 g/min). M_{CER} value at S/F II – E-1 assay was 0.08±0.01 g extract/min, while M_{CER} value at S/F II – E-2 assay was 0.15±0.02 g extract/min. These responses indicate that the mass transfer rates during CER period are 1.4 to 2.4-fold higher for E-2 compared to E-1.

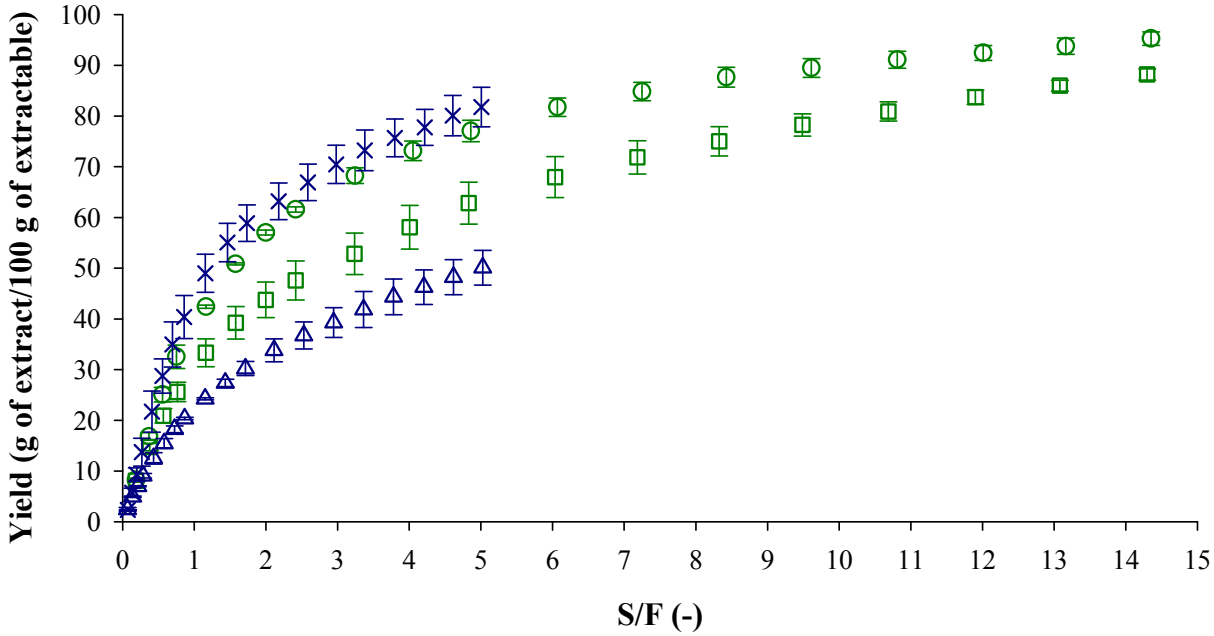


Figure 2: OEC for the system CO_2 + rosemary: (□) E-1, flow of 17.3 g CO_2/min; (○) E-2, flow of 17.3 g CO_2/min; (Δ) E-1, flow of 6.0 g CO_2/min; (×) E-2, flow of 6.0 g CO_2/min.

The differences on the kinetic curves (Figure 2) are linked to the phenomena occurring along the extraction process. The vegetal matrix/solute/solvent system is composed of two phases: one solid phase, which is the vegetal matrix from where the solute is extracted; and one fluid (supercritical) phase, which is the solvent containing the solubilized solute. As soon as the contact between the phases exists, the transport of components occurs by: convection and dispersion in the fluid phase, mass transfer in the solid-fluid interface and diffusion of the solute-solvent mixture in the solid phase [8]. These phenomena are influenced by the fluid dynamic of the flow and the fluid dynamic is influenced by the particles characteristics as shape, size and distribution inside the bed. Brunner [8] considers that the unsteady or non-stable dynamic flow and/or non-uniform distribution of the solvent viscosity due to concentration gradients of solubilized solute cause the axial dispersion of the fluid phase, that tends to increase by increasing the extractor height. This fact explains why lower yields at E-1 were obtained. The behavior in E-1 is associated to the magnitude of the axial dispersion of the fluid phase and to the presence of preferential pathways inside the bed. Excessive compaction at specific points of the bed tends to cause non-uniform distribution of porosity. Indeed, obtaining large yields in E-2 can be linked to the presence of these phenomena most

likely small in E-2 than in E-1 for this raw material. Zabot *et al.* [3] presented photographic images of bed slices in equal axial positions of the extractors to accurately assess the bed characteristics after extracting clove oil with SFE-CO_2. The authors found out that excessive compaction caused heterogeneous flow and residual extract remained mainly at E-1.

Moreover, local temperatures on each bed should have been different due to the difference in geometry, even maintaining the average temperature equal for both extraction beds. Thereafter, these non-uniformities of temperature can have changed the phase equilibrium, influencing the selectivity of obtaining bioactive compounds along the time.

We verified that the adopted criterion for geometry shift was not appropriate when rosemary compounds are extracted. This inference was based on the evaluation of OEC from Figure 2 together with the kinetic parameters obtained on each bed. Looking at the responses of this study we observed differences in the OEC profiles between the beds E-1 and E-2. Carvalho *et al.* [1] also shown different behaviors of the kinetic curves obtained in beds presenting different H_B/D_B ratios. Therefore, these results show the need of establishing other suitable criteria geometry shift and scale up when the processes using supercritical fluids involve groups of vegetal matrices presenting characteristics similar to those of rosemary. These characteristics are especially related to the amount of solute, classes of compounds present in the solute, morphology of the cellular structure and composition of the inert material (starch, fibers, etc.).

Dealing with clove (*Eugenia caryophyllus*), Prado *et al*. [9] and Zabot *et al.* [3] obtained similar kinetic profiles of extraction when they studied the behavior of OEC in different bed geometries using the same criterion applied in this study (equal S/F and constant extraction time). For these raw materials, this criterion was valid for clove because of the accessibility in obtaining, in short time and small S/F ratio, extract rich in volatile oil (mainly composed by eugenol, eugenyl acetate, β-caryophyllene and α-humulene). Nevertheless, rosemary extract contains lower quantity of volatile oil than clove, and rosemary volatile oil is composed by several substances. Thus, as discussed above, the influence of the bed geometry on the transport phenomena also interfered on the kinetic composition of volatile oil, as presented in the following section.

Obtaining volatile compounds

The influence of the bed geometry on the extraction kinetics of the major compounds quantified in the rosemary extract is exhibited in Figure 3. This figure shows the kinetic behavior of two compounds quantified by gas chromatography, 1,8-cineole and camphor, obtained by SFE-CO_2 in the unit SFE-2×1L. These volatile compounds are preferentially extracted at the beginning of the process. In the same way, there is difference in composition of the extracts obtained in E-1 and E-2. Camphor and 1,8-cineole are practically depleted at S/F = 4 in E-2. However, the depletion in E-1 is slower than E-2, bringing up the need of S/F ratios higher than 4. Such behavior is linked to the OEC (Figure 2): the mass transfer rates are small for E-1 due to the influence of the medium (bed geometry) on the intensity of driven forces that act on transport phenomena. Thus, for S/F > 4 (Figure 2), the yields (with respect to the accumulated extract mass per 100 g of raw material) presented an increment larger for E-1 (slope for straight line: tg $\alpha = 0.13$) than E-2 (slope for straight line: tg $\alpha = 0.09$). These results indicate that the diffusional period has not been totally reached yet, especially in E-1.

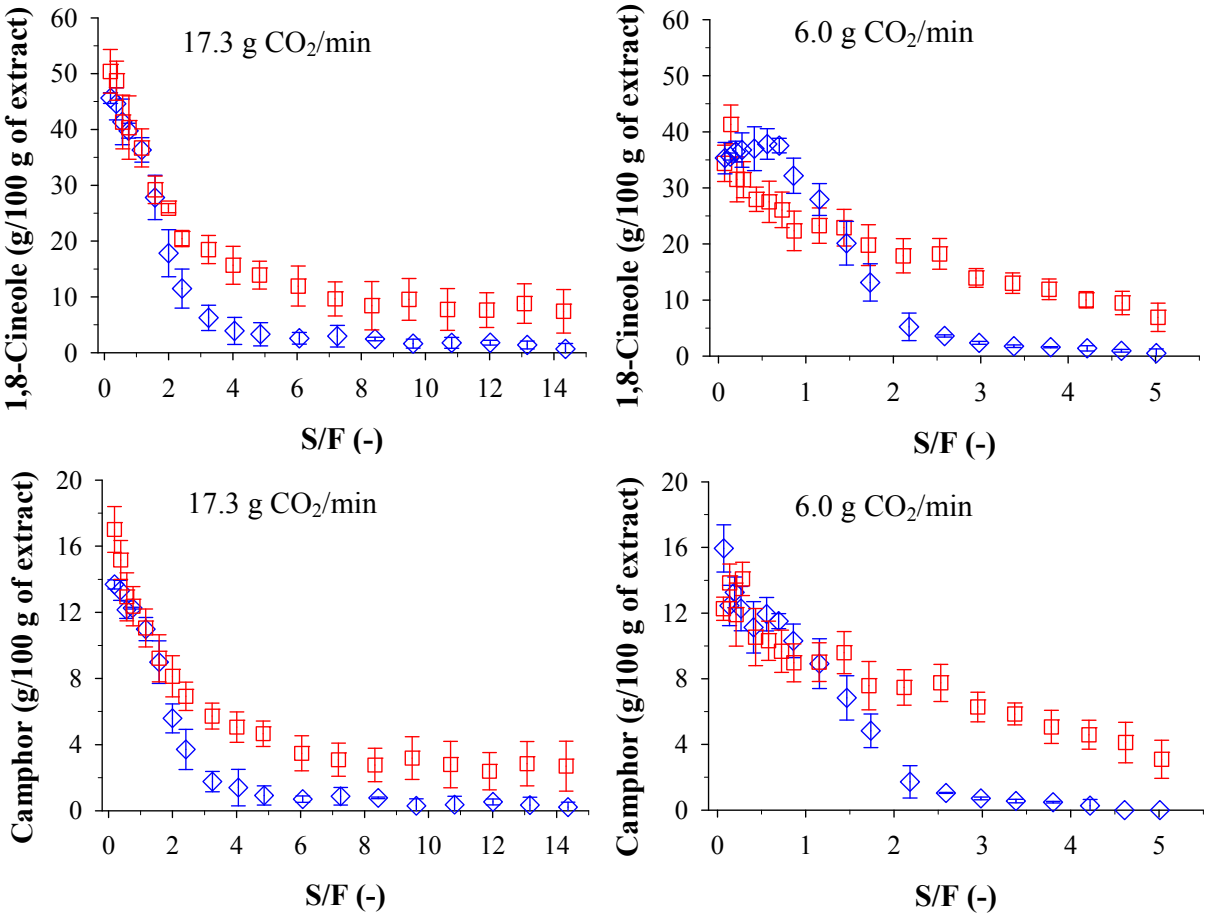


Figure 3: **Major volatile compounds of rosemary in the time-collected extract obtained by SFE-CO_2: (□) E-1; (◊) E-2.**

When comparing Figure 2 with Figure 3, we understand that the extension of the extraction can be finished in S/F = 4 whether the goal is to attain rosemary extracts rich in volatile substances, when the bed E-2 is used. Nonetheless, if the objective is to obtain total extract, we need to continue the extraction up to S/F ratios upto 15. Figure 4 shows the kinetic profile of the other compounds, expressed as accumulated area.

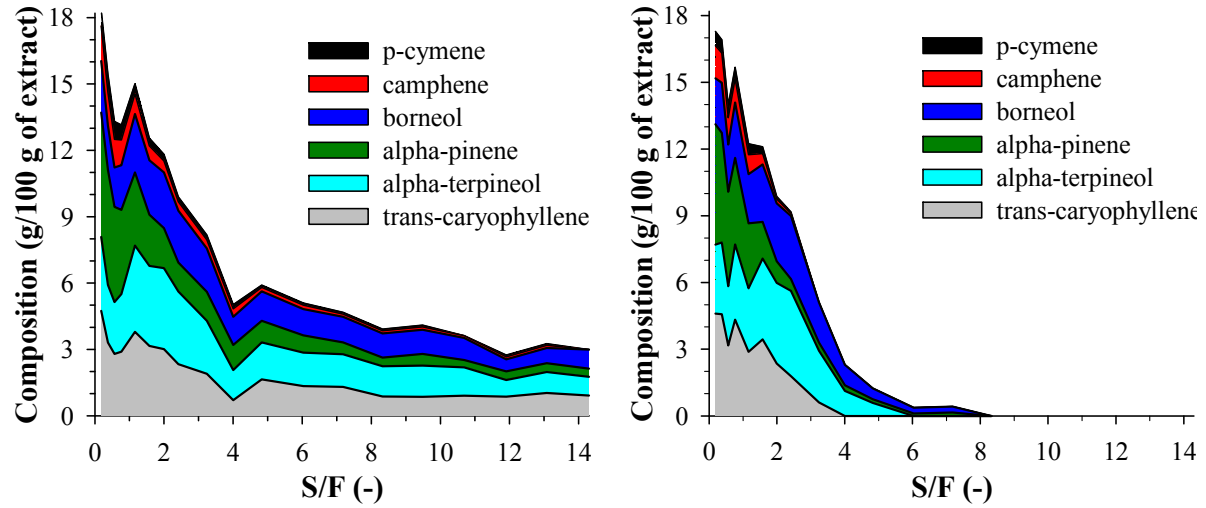


Figure 4: **Minor volatile compounds of rosemary in the time-collected extract obtained by SFE-CO_2: E-1 (Left); E-2 (Right).**

Observing Figure 4 we saw compounds depletion faster in E-2 than in E-1. The identified compounds in the rosemary extract are in agreement with the compounds identified by Ibañez *et al.* [7] and Vicente *et al.* [10]. Linalool (data not presented in Figure 4) was detected in the extracts presenting average concentration lower than 0.2 g/100 g extract and limonene was not detected in any sample. In the Soxhlet extract only two volatile compounds were identified: 1,8-cineole (1.3±0.2 g/100 g extract) and camphor (0.5±0.1 g/100 g extract). Therefore, supercritical technology promotes the selectivity extraction of bioactive substances with higher concentration in the extracts whether compared to Soxhlet.

CONCLUSION

Main conclusions obtained in this study are:

1) Yields for E-2 are a slight greater than those for E-1, even maintaining the same values for the process variables, including temperature, pressure, porosity, S/F and extraction time;
2) The studied criterion of geometry shift and scale up was not suitable for rosemary was, in spite of being valid for clove. These results are linked to the morphology of the cellular structure and to the amount of the solute in each raw material (RM), that was different (clove contains ±18 g/100 g RM; rosemary contains ±2,5 g/100 g RM);
3) Selecting either E-2 or E-1 likewise depends on evaluating the kinetic extraction of target compounds.

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