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Short communication

Supercritical-carbon-dioxide extraction of lipids from a contaminated soil

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Abstract

Different classes of lipidic components have been isolated from a contaminated soil by CO₂ under supercritical conditions. The extracts were analyzed by gas chromatography–mass spectrometry and compared with those obtained by Soxhlet extraction using two different solvent mixtures. The influence of pressure and temperature on the extraction efficiency were evaluated by using a rotatable central composite design. The method is useful for optimization of the extraction of the esters of fatty acids. However for fatty acids, an empirical approach was needed. Both methods, namely supercritical extraction and Soxhlet extraction give results which are in good agreement. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Due to its well-known advantages supercritical fluid extraction (SFE) has greatly expanded its application field as alternative to conventional extraction methods in the last decade. In particular, SFE has been widely applied for the determination of organic pollutants in environmental matrices [1–8]. SFE has also been proposed as a technological solution for the remediation of soils, sediments and sludges that are contaminated with hazardous organic chemicals [9–11]. However, most of these applications do not include the screening of all extractable components, pollutants or not. But these methods are

specific for the extraction of different classes of organic pollutants, such as chlorinated benzenes and cyclohexanes [8], pesticides [12,13], polycyclic aromatic hydrocarbons [14–17], polychlorinated biphenyls [18], resin acids [19], etc.

Our approach includes the application of SFE for the screening of the different classes of lipids occurring in a contaminated soil taken from a soil barrier in a controlled landfill, which have previously been fully characterized after Soxhlet extraction [20]. These soil–refuse mixtures are appropriate to evaluate the efficiency of lipids extraction from real samples, since such a material contains a wide variety of components, including volatile fatty acids (VFAs), long-chain free fatty acids (FAs) (especially palmitic, stearic and oleic acids, short-chain esters (degradation products from higher lipids) and organic pollutants from anthropic activity.

The supercritical fluid extracts were analyzed by

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gas chromatography–mass spectrometry (GC–MS) and compared with those obtained using two different solvent mixtures by Soxhlet extraction. As many different factors may affect the extraction efficiency for this specific analytical problem, it is necessary to empirically determine the best extraction conditions. For this purpose the use of a statistical approach, to obtain reliable results in a reasonable time, has become a common practice in SFE [21,22]. In our case a computational program which have been found suitable for evaluating and optimizing response–surface curves based on central composite experimental designs has been used.

2. Experimental

2.1. Samples and reagents

A soil–refuse mixture taken at 5 m depth in a controlled landfill (Conica-Montemarta) near Seville, Southern Spain, was selected for this study. Chemical characteristics and further details on the sample were published elsewhere [20]. Before the extractions aliquots of samples were air-dried and ground to pass a 2-mm sieve, homogenized and lyophilized.

All organic solvents were for organic trace analysis quality from Merck. Purissimum grade inorganic reagents were purchased from Panreac. Soxhlet cartridges were from Albet, and diatomaceous earth (60–80 mesh) from Hewlett-Packard. SFE/SFC grade CO₂ supplied by Sociedad Española de Oxígeno (SEO) was used as extraction fluid.

2.2. Soxhlet extractions

Soil samples (20 g) were exhaustively extracted both with dichloromethane–methanol (3:1) and hexane–acetone (1:1) in a Soxhlet apparatus for 24 h. The extracts were then saponified with 10% KOH in methanol–toluene (1:1) and fractionated into neutral and acidic fractions.

2.3. Supercritical fluid extractions

All the supercritical fluid extracts were obtained by using supercritical CO₂ on a Carlo Erba SFC 300 pump instrument, controlled by an external computer

and provided with a refrigerated circulator assembly DC3-K20 (Haake-Fisons) at 4°C. This apparatus allows, in each experiment, to vary the composition of supercritical fluid (neat or mixed with modifiers) and/or pressure (gradient operations) but not temperature. To maintain the pressure of the system fused-silica capillary columns of 12 cm×50 µm I.D. were used as restrictors. They were thermostated by a heated mobile block. The flow ranged between 500 and 2500 µl/min, depending of the pressure.

The extraction thimble (Keystone Scientific, 3.47 ml) was filled with 4 g of sample. Anhydrous sodium sulphate (0.4 g) was added at the top to absorb moisture and to minimize dead volumes in the extraction cell. Glass wool was placed at both ends of the cell to avoid plugging of the restrictor with the soil sample. A static time that allows the fluid to diffuse through the soil sample is necessary. An extraction time of 30 min (static plus dynamic times) is sufficient if the flow is maintained above of 500 ml/min.

The extracts were collected into 2 ml of methanol. Sublimation of CO₂ decreases the temperature of the collect solvent, acting as a freezing-trap. So the loss of volatile compounds is minimized.

All the experiments were carried out in control pressure operation mode at pressures between 8 MPa (target pressure) and 30 MPa (maximum pressure). The extraction was carried out in static mode for 10 min after an equilibrium time of 2 min, followed by a 20 min dynamic extraction. The restrictor was heated up to 70°C by the heated mobile block.

2.4. Gas chromatography–mass spectrometry

All the extracts were analyzed by GC (Hewlett-Packard 5890) and GC–MS (Hewlett-Packard 5988A). Separation of the mixture of lipids was achieved with a 25 m×0.2 mm I.D. BP-5 (SGE). A 30 m×0.25 mm I.D. DB-FFAP (J&W Scientific) fused-silica capillary column was also used for the analyses of the acidic fractions. In both cases the oven temperature was programmed from 50 to 100°C at a rate of 30°C/min and then up to 280°C/min at a rate of 6°C/min with 20 min final hold. He was used as carrier gas at a flow of 1 ml/min and N₂ as auxiliary gas to optimize the efficiency of the detector. The injector temperature was set to 250°C

and the detector temperature was 300°C (BP-5 column) or 240°C (FFAP column). Mass spectra were acquired at 70 eV ionizing energy.

The quantitative determination of the different components in the total ion chromatograms (TICs) was performed from the areas of the chromatographic peaks, and their identification by matching with computer stored library spectra (Wiley, NBS).

2.5. Experimental optimization planning

In order to optimize the SFE procedure, the two experimental variables, namely, pressure (P) and temperature (T) were selected as controlled factors for the experimental design. The SFE recovery was chosen as the experimental response (Y). Within the realm of response–surface methodology, the output Y may be suitably modeled by using multiple linear regression algorithms where the controlled factors act as independent variables [23]. The true function which relates Y to P and T is unknown. However, a low-order polynomial like a second-order one, considering the quadratic factor contributions and two-way factor interactions can be used to approximate the true function in the region under investigation [24]. Central composite designs are useful and economic for estimating the coefficients of second-

order regression models and consequently they were applied to optimize SFE following a program (CCDOPT) developed by one of the authors [25]. In order to perform the calculations a prior task is the coding of factors. Here, the coding was done for the sake of rotatable designs with a star arm $\alpha=1.414$. The design matrix showing both uncoded and coded factor levels covering the experimental working range, as well as supplementary data, is presented in Table 1. CCDOPT searches for the coordinates within the factor space corresponding to the maximum response.

3. Results and discussion

To assess the relative efficiency of the different extraction conditions established in the experimental matrix (Table 1), the same conditions were also applied to samples consisting of diatomaceous earth spiked with a Soxhlet extract of known composition. Assuming a 100% of recovery by Soxhlet extraction to a selection of 11 components representative of those present in the lipid extract of a soil–refuse mixture, Table 2 shows that the relative recoveries gained by supercritical CO₂ changes dramatically with the different set of conditions. Thus, whereas

Table 1
Data matrix for the experimental design used to optimize SFE of lipids from a contaminated soil sample

Factor	Unit	Highest/lowest value	Centre, Z°	Radius, r	
Pressure	MPa	30/8	19	11	
Temperature	°C	120/40	80	40	
For rotational design, $\alpha = 1.414$					
Experiment No.	Coded factors (X) ^a		Uncoded factors (Z)		Density (g/ml)
	Pressure	Temperature	Pressure	Temperature	
1	-1	-1	11.2	52	0.477
2	1	-1	26.8	52	0.843
3	-1	1	11.2	108	0.207
4	1	1	26.8	108	0.578
5	-1.414	0	8.0	80	0.160
6	1.414	0	30.0	80	0.746
7	0	-1.414	19.0	40	0.835
8	0	1.414	19.0	120	0.373
9	0	0	19.0	80	0.559
10	0	0	19.0	80	0.559

^a The coded coordinates are transformed in actual Z coordinates, according to the equation: $X = (Z - Z^\circ)\alpha/r$.

Table 2
Relative SFE recoveries of selected compounds in a model mixture according to the experimental design

Compound	Experiment ^a								
	1	2	3	4	5	6	7	8	9 ^b
Diisobutyl phthalate	82.9	100.0	0	41.5	0	78.0	58.5	9.8	41.5
Ethyl palmitate	46.4	100.0	0	7.3	0	56.5	12.7	2.7	12.7
Palmitic acid	6.1	85.5	0	18.4	0	100.0	20.5	0.4	15.9
Methyl oleate	20.6	94.1	0	8.8	0	100.0	17.6	0	14.7
Ethyl oleate	100.0	94.5	0	31.3	0	81.3	81.3	3.8	82.4
Oleic acid	17.5	83.8	0	15.9	0	100.0	41.4	2.9	14.9
Stearic acid	0	93.2	0	0	0	100.0	91.5	0	0
Propyl oleate	92.0	100.0	0	34.0	0	88.0	65.3	2.3	66.7
Butyl oleate	96.4	100.0	0	37.8	0	89.2	70.3	2.7	75.7
Pentyl oleate	82.6	100.0	0	20.3	0	82.6	56.5	5.8	47.8
Diisooctylphthalate	84.0	100.0	0	25.5	0	83.2	58.0	1.7	37.0

^a The experimental conditions of the extractions are described in Table 1 and in Experimental.

^b Average of two experiments.

the SFE experiments 3 and 5 lead to very poor extracts, the conditions used in experiments 2 and 6 allow one to achieve recoveries for the selected compounds similar to those obtained by Soxhlet extraction.

Individual components were evaluated under different density conditions. No significant yields of free fatty acids were observed under conditions in which CO₂ have densities below 0.4 g/ml. For densities between 0.4–0.6 g/ml, palmitic and oleic acids are poorly extracted (15–20%) and stearic acid is not removed. All these free acids are only extracted at 100% level under the conditions of experiment 6, i.e., the pressure is at least 30 MPa. These results are in good agreement with previous findings [26,27].

Dialkyl phthalates are better extracted under the conditions of experiment 2, whereas they are practically not extracted with CO₂ at density below 0.4 g/ml. In general, the extraction behavior of these compounds is anomalous at densities above 0.4 g/ml. The same occurs with the short chain esters of palmitic, stearic and oleic acids, which are better extracted under conditions 2 and 6.

The CCDOPT program was applied for the sake of optimizing the SFE procedure. Thus, once the factors involved (P and T) were encoded (X_1 and X_2), as indicated in Table 1, the experimental responses Y (SFE recoveries) depicted in Table 2 were fitted to X_1 and X_2 according to a second-order model response surface.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_1X_2$$

The goodness of the fit may be evaluated by the correlation coefficient of the multiple linear regression procedure. Once the B coefficients have been evaluated, a previous canonical analysis is performed in order to know if the stationary point is a maximum, a minimum or a saddle point (minimax). When the stationary point is not a minimax, the coded co-ordinates of X_1 and X_2 are found by setting

$$\frac{\partial Y}{\partial X_1} = 0 \text{ and } \frac{\partial Y}{\partial X_2} = 0$$

These coded values are then uncoded to give the real values of the factors P and T corresponding to the stationary point of the response–surface.

For the ethyl, propyl and butyl oleates, the response–surface exhibits a maximum in the effective range. For the free fatty acids (palmitic and oleic acids) and methyl and pentyl oleates a minimax is obtained. Thus, the experimental conditions for the extraction of these compounds must be empirically chosen. For the phthalates, the program cannot be applied.

The results of searching the co-ordinates of the singular (or stationary) point for each compound are gathered in Table 3, indicating the goodness of fit (correlation coefficient), nature of stationary point (maximum, minimum or minimax) and the uncoded co-ordinates for maxima and minima. It is apparent that the computational program only has lead to

Table 3
Coordinates of the singular point

Compound	Correlation coefficient	Uncoded coordinates		Observations
		<i>P</i> (MPa)	<i>T</i> (°C)	
Ethyl palmitate	0.839411	10.9	88.0	Minimum
Palmitic acid	0.978269	–	–	Minimax
Methyl oleate	0.949365	–	–	Minimax
Ethyl oleate	0.941102	20.9	52.5	Maximum
Oleic acid	0.970727	–	–	Minimax
Propyl oleate	0.923432	27.0	51.6	Maximum
Butyl oleate	0.929819	23.7	55.1	Maximum
Pentyl oleate	0.903993	–	–	Minimax

indicate the correct extraction conditions of the fatty acid esters, but not of the other classes of lipid components.

When comparing conditions 1 and 2 it is observed that at the same temperature (52°C) the fatty acid:esters ratio varies from 0.38 at 11.2 MPa (density

0.477 g/ml) to 6.11 at 26.8 MPa (density 0.843 g/ml). This means that it would be theoretically possible to selectively fractionate free and esterified fatty acids by a sequential extraction ranging from low pressure to higher pressures. This selective fractionation is explained by the fact that, at high density, the solvent polarity of the supercritical CO₂ increases, as its solvent capacity and is more similar to that of polar solvents. This effect should be equivalent to that obtained using solvent mixtures of different polarity in classical Soxhlet extraction.

This theoretical equivalence could be clearly demonstrated by comparing the TICs of the extracts obtained from real soil samples using supercritical CO₂ with Soxhlet extraction using two different solvent mixtures (Fig. 1). By increasing the CO₂ density the TIC of the CO₂ extract becomes similar to that obtained by Soxhlet extraction with the mixture acetone–hexane (1:1) (Fig. 1b vs. d), which is much more effective to extract the fatty acids than

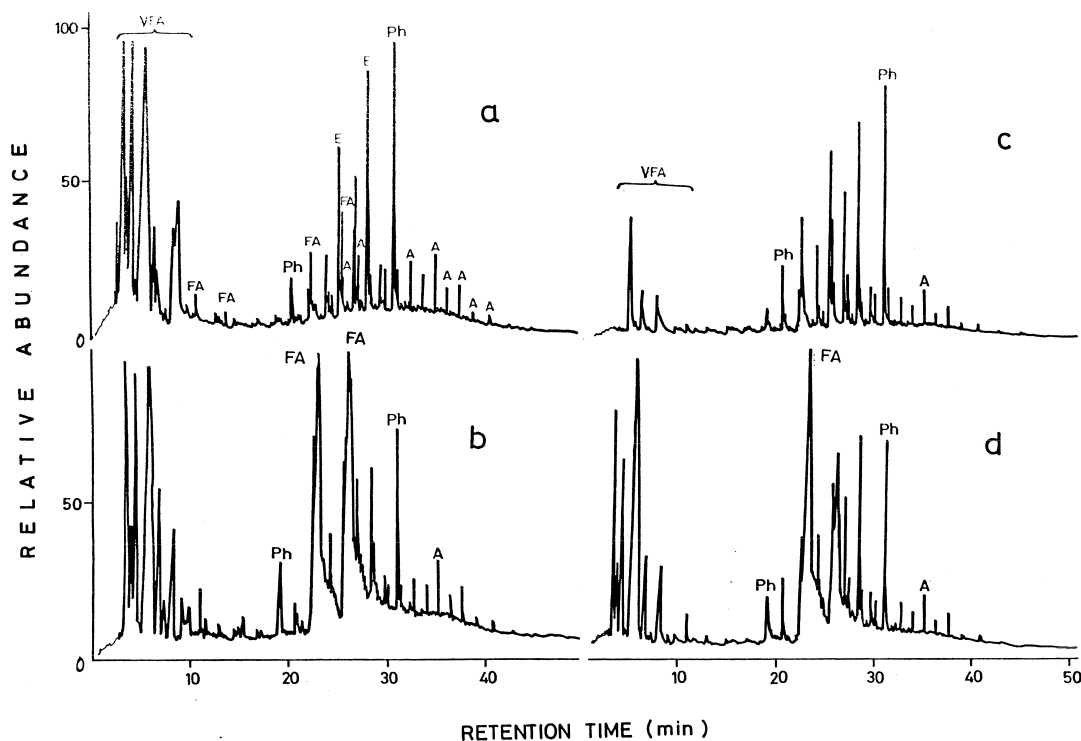


Fig. 1. Comparison of the chromatograms of the extractions by classical and SFE methods: Soxhlet extraction with dichloromethane–methanol (3:1) (a) and acetone–hexane (1:1) (b); SFE at 11.2 MPa, 52°C, $d=0.477$ g/ml (c) and 30 MPa, 80 °C, $d=0.746$ g/ml (d). Labels on the peaks correspond to volatile fatty acids (VFAs), long-chain fatty acids (FAs), dialkyl phthalates (Phs) and *n*-alkanes (A).

dichloromethane–methanol (3:1) (Fig. 1a). However, the recoveries of the other compounds were similar by both methods. In fact, the variations observed for the fatty acid:phtalate ratios do not make possible that kind of fractionation among these compounds.

The SFE of free fatty acids must be achieved using CO₂ of density 0.6 g/ml or higher. The drastic influence that small changes in the SFE conditions brought about in the recovery of fatty acids from a real soil sample is shown in Fig. 2. Fig. 2a vs. b shows the changes in composition obtained by varying the temperature, and Fig. 2c vs. d by modifying the pressure.

It is apparent that the more efficient extraction of the most VFAs (VFA: 4 to 12 atoms of C) are achieved at pressures higher than 26.8 MPa. For the C₁₄–C₁₈ homologues (mainly myristic, palmitic and oleic acids) the best results are obtained at pressure

about 30 MPa. In all cases the pressure is more important than the density to achieve the best recoveries.

4. Conclusions

Supercritical CO₂ extraction has been successfully applied to remove the lipid fraction of the organic matter from a contaminated soil sample. Similar results are obtained in the extraction of the lipid fraction of a soil when comparing with Soxhlet methods, which demonstrate the accountability of SFE as a appropriate enrichment procedure for soil lipids. The use of a suitable computational program failed to indicate the correct supercritical fluid parameters to choose for the screening of all extractable lipid components.

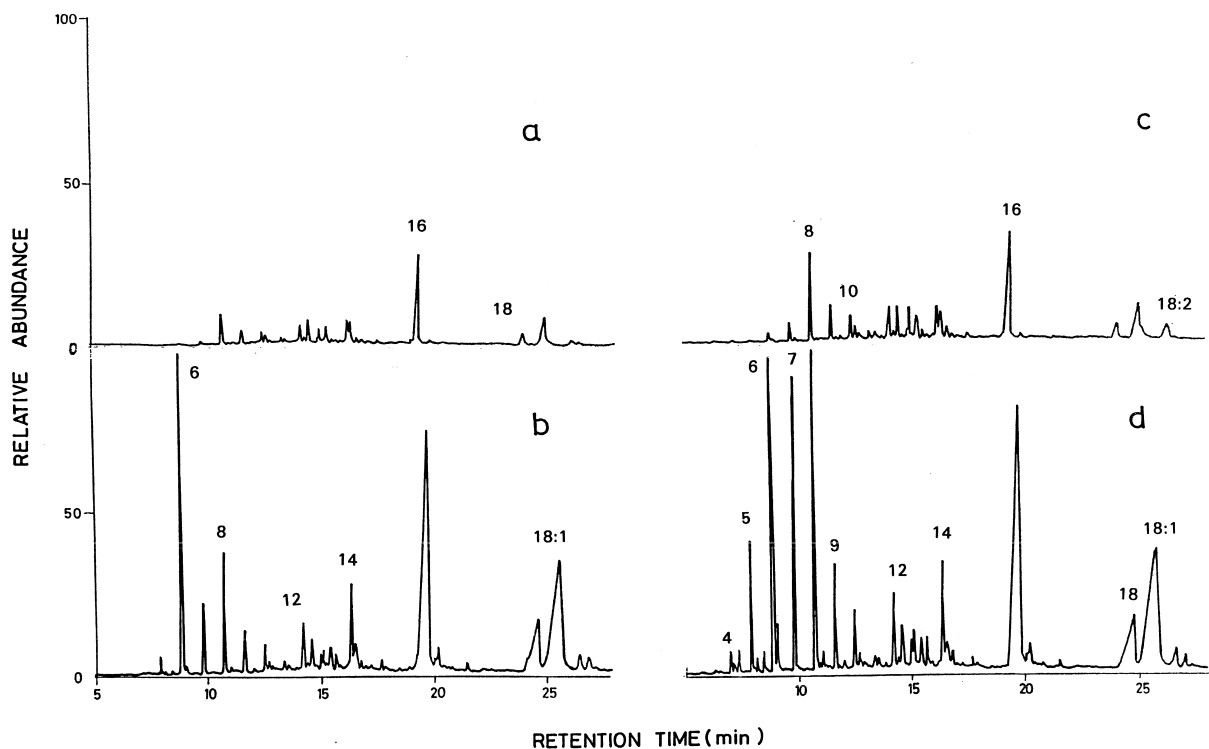


Fig. 2. Total ion chromatograms (FFAP column) of fatty acids under different SFE conditions: 19 MPa, 80°C ($d=0.559$), experiment 9 (a), 19 Mpa, 40°C ($d=0.835$), experiment 7 (b), 11.2 MPa, 52°C ($d=0.477$), experiment 1 (c), 26.8 MPa, 52 °C ($d=0.843$), experiment 2 (d). Numbers on the peaks correspond to chain length.

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