Influence of Extraction Mode and Temperature in the Supercritical Fluid Extraction of *Citrus sinensis* (Osbeck)

Silvia R. Sargenti and **Fernando M. Lanças***

Universidade de São Paulo, Instituto de Química de São Carlos, Caixa Postal 780, 13560-970, São Carlos, SP, Brazil

Abstract

This work describes the influence of extraction modes and temperature on the supercritical fluid extraction of peels of the baiana variety of *Citrus sinensis* (Osbeck). The dynamic, static, and conjugated (static following by dynamic extraction) modes with different modifiers added to supercritical CO_2 as well as two different temperatures are studied. The principal compounds in the extracts are analyzed by capillary gas chromatography and gas chromatography–mass spectrometry. Different chromatographic profiles are obtained when either the modifier, extraction mode, or temperature are changed. Five compounds are identified in the extracts: β-myrcene, 1,8-cineole, *d*-limonene, dihydrocarveole, and caryophylene.

Introduction

The experimental techniques for measuring the solubility of a heavy liquid or a solid in a supercritical fluid (SF) are either dynamic or static. In the dynamic mode, the solute is continually swept with fresh SF, mainly in fractionation or sequential studies. In the static mode, both the solute and solvent are loaded together into the extraction cell and held static for some fixed time (1,2).

For some vegetable oil extraction procedures, an exhaustive extraction of the compounds is desirable. In these cases, the solubilizing power of the solvent should be maximized to achieve extraction of compounds with lower solubility, such as triglycerides. The solubilizing power is a function of the supercritical fluid density and depends on both the pressure and temperature. The capability to control the density, temperature, and fluid composition allows fractionation of the sample analytes to be selectively removed in a series of steps. The polarity of the SF can be changed by the addition of small amounts of organic solvents (modifiers) to supercritical CO_2 (3). Changes in the type of modifier for supercritical CO_2 allow the selective removal of analytes from the matrix in more than one fraction. This proce-

dure is also a fractional technique and is called "sequential extraction," "multi-step extraction," or, "multiple extraction." In summary, when the same modifier is used and only the quantity of modifier is changed, the procedure is called, "fractional extraction;" when different modifiers are used to modify the fluid composition, the procedure is called, "sequential" (4,5).

In partial extraction procedures, such as the extraction of essential oils (6,7), the initial CO_2 cannot have the maximum solubilizating power due to the risk of solubilizing undesirable compounds. In these procedures, selectivity is the most important factor, and the indicated regions of pressure and temperature are those near the critical pressure and temperature (8). The solvating power of an SF achieved with changes in the fluid density when near its critical points can be better explored by fractional extraction procedures. In this way, the analytes can be removed selectively from the matrix in a series of fractions by changing the conditions of pressure, temperature, and fluid polarity (9).

Selectivity for supercritical extractions means that by using fractional procedures, it is possible to fractionate a sample into different compound classes such as essential oils, triterpenes, fatty acids, resins, pigments, etc.

Sequential extraction seems to be the most interesting system for samples showing large quantities of nonvolatile products. However, few studies of fractional or sequential extractions with SFs and modifiers have been reported (4,5,10–13).

In this work, plants (peels of the baiana variety of the Brazilian citrus *Citrus sinensis* [Osbeck]) were sequentially extracted with supercritical CO_2 that had been modified with organic solvents (*n*-hexane, ethyl ether, and acetone). A comparison among different extraction modes (static, dynamic, or coupled mode) using different extraction temperatures was also performed. The major goals were a comparison of the chromatographic profiles obtained and a study of selectivity in the combined extraction modes, varying both modifiers and extraction temperatures. Some identification of the major extracted compounds was done by gas chromatography–mass spectrometry (GC–MS).

---

* Author to whom correspondence should be addressed.

Experimental

Plant material

Fresh peels of the baiana variety of *Citrus sinensis* (Osbeck) were collected in the city of São Carlos, Brazil. The test material was sliced into pieces measuring approximately 1 cm^2 in area and stored in plastic bags in a freezer until the time of analysis.

Classical extraction methods

The extraction of *C. sinensis* compounds was performed using steam in a conventional distillation system with 45 g of plant material and 1000 mL of distilled water for over 4 h.

The Soxhlet extractions were performed placing 45 g of plant material in 300 mL of each solvent (i.e., hexane and acetone) in a Soxhlet apparatus. Extraction was performed for 5 h prior to analysis.

Sonication was performed using 45 g of plant material and 300 mL of each solvent (i.e., hexane, ethyl ether, and acetone) for 1 h for each extraction.

Prior to GC and GC–MS analyses, the extracts were subjected to a cleanup step (i.e., removal of undesirable compounds such as chlorophyll, fatty acids, and waxes) using a silica gel column (100 mg, 5 × 100 mm) and ethanol as a solvent.

SF extraction

Samples (200 g) of plant material, were extracted in a previously described SF extraction (SFE) system (4,5). The first extraction step was carried out with pure supercritical CO_2 followed by supercritical CO_2 with 10% hexane, then supercritical CO_2 with 10% ethyl ether, and finally supercritical CO_2 with 10% acetone. These four steps were performed in separate experiments, first in the static extraction mode (90 min), then in the dynamic extraction mode (90 min), and finally in the coupled mode (10 min in the static mode followed by 80 min in the dynamic mode). Two different extraction temperatures were tested for each set of experiments: 60 and 75°C. The SFE conditions were as follows: the pressure was 90 atm, and 500 mL of SF were used. Each extraction step lasted 90 min. These extracts were analyzed first by GC and later by GC–MS to identify the major compounds.

GC

Extracts were analyzed using an HP model 5890/Series II GC (Hewlett-Packard Brazil, Barueri, Brazil) equipped with a 50-m × 0.2-mm column (0.33-µm film of LM-5, crosslinked 5% phenyl-methylpolysiloxane) (L&M, São Carlos,

Table I. Extraction Yields as a Function of Time and Extraction Type for *C. sinensis* (Osbeck)

Extraction type	Extraction time (h)	Yield* (%)
Steam		
Steam distillation	4	0.0800
Soxhlet		
Hexane	5	0.1800
Acetone	5	5.440
Sonication		
Hexane	1	0.2600
Ethyl ether	1	0.0400
Acetone	1	3.900

			Yield* (%)	
SFE	Extraction mode	Extraction time (h)	60°C	75°C
(CO$_2$)	dynamic	1.5	0.0556	0.1994
(CO$_2$–10% hexane)	dynamic	1.5	0.0235	0.1709
(CO$_2$–10% ethyl ether)	dynamic	1.5	0.0753	0.3082
(CO$_2$–10% acetone)	dynamic	1.5	0.2383	1.902
(CO$_2$)	conjugated	1.5	0.0096	0.0808
(CO$_2$–10% hexane)	conjugated	1.5	0.0272	0.3909
(CO$_2$–10% ethyl ether)	conjugated	1.5	0.0449	0.4551
(CO$_2$–10% acetone)	conjugated	1.5	2.487	2.523
(CO$_2$)	static	1.5	0.0247	0.4453
(CO$_2$–10% hexane)	static	1.5	0.0624	0.1661
(CO$_2$–10% ethyl ether)	static	1.5	0.8441	0.2561
(CO$_2$–10% acetone)	static	1.5	1.0899	1.1103

* Grams of extract achieved per 100 g of fresh plant material.

Table II. Tentative Identification of Selected Compounds Extracted from *C. sinensis* (Osbeck) by GC–MS

Compound	Peak	M+	Principal fragments (*m/z* [%])
C$_8$H$_{15}$	1	111	75 (100), 73 (38), 58 (17), 61 (4), 89 (2), 91 (3)
β-Mircene	2	136	93 (100), 69 (83), 79 (18), 53 (14), 121 (5), 136 (5)
1,8-Cineole	3	154	81 (100), 93 (93), 68 (91), 71 (74), 108 (72), 139 (48)
d-Limonene	4	136	68 (100), 93 (73), 79 (37), 67 (70), 53 (26), 107 (21)
Dihydrocarveol	5	136	68 (100), 67 (86), 93 (69), 94 (33), 79 (34), 107 (24)
Caryophylene	6	204	161 (100), 105 (59), 119 (52), 133 (43), 93 (55), 79 (53)
C$_{13}$H$_{26}$O	7	198	183 (100), 51 (34), 91 (31), 78 (28), 165 (21), 153 (13)
C$_{13}$H$_{26}$O	8	198	183 (100), 165 (25), 91 (16), 77 (18), 55 (10), 153 (12)
C$_{21}$H$_{34}$O	9	302	287 (100), 209 (53), 91 (52), 105 (50), 78 (47), 197 (25)
C$_{21}$H$_{34}$O	10	302	287 (100), 105 (52), 91 (33), 77 (31), 209 (31), 197 (23)
C$_{30}$H$_{30}$O	11	406	391 (100), 105 (96), 78 (93), 91 (84)
C$_{28}$H$_{34}$O$_2$	12	402	387 (100), 182 (11), 344 (11), 91 (9)
C$_{29}$H$_{26}$O$_2$	13	406	105 (100), 391 (69), 91 (49), 78 (37), 301 (14)

Brazil) and a flame ionization detector (FID). The carrier gas was hydrogen at a linear velocity of 34.5 cm/s (measured at 40°C). The samples were solubilized in absolute ethanol at a concentration of 8 mg/mL and 1.5 mL injected using a split mode (1:30). The temperature was held for 5 min at 40°C, programmed to increase at 8°C/min to 160°C, then at 6°C/min to 180°C, then at 8°C/min to 300°C, and held for 20 min.

GC–MS

The mass spectra of the extracts were obtained on an HP model 5890 GC interfaced to an HP model 5970 MS. A 25-m × 0.2-mm (0.33 mm HP-1, crosslinked dimethylpolysiloxane) column was used. The carrier gas was hydrogen at a linear velocity of 37 cm/s (measured at 250°C), and the electron-impact ionization voltage was set at 70 eV. The samples were solubilized in absolute

ethanol at a concentration of 25 mg/mL, and 1.0 mL was injected using a 1:30 split. The injector and interface temperatures were 280°C, and the temperature program contained the following sequence: held at 100°C for 2 min, then increased at 3°C/min to 150°C, then at 6°C/min to 280°C, and held for 20 min.

Results and Discussion

The yields of the extracts obtained from *C. sinensis* (Osbeck) by type, mode, and time of extraction are summarized in Table I. Table II lists the compounds tentatively identified by GC–MS. These compounds were determined by comparison of the spectra fragmentation with data from the literature (14,15).
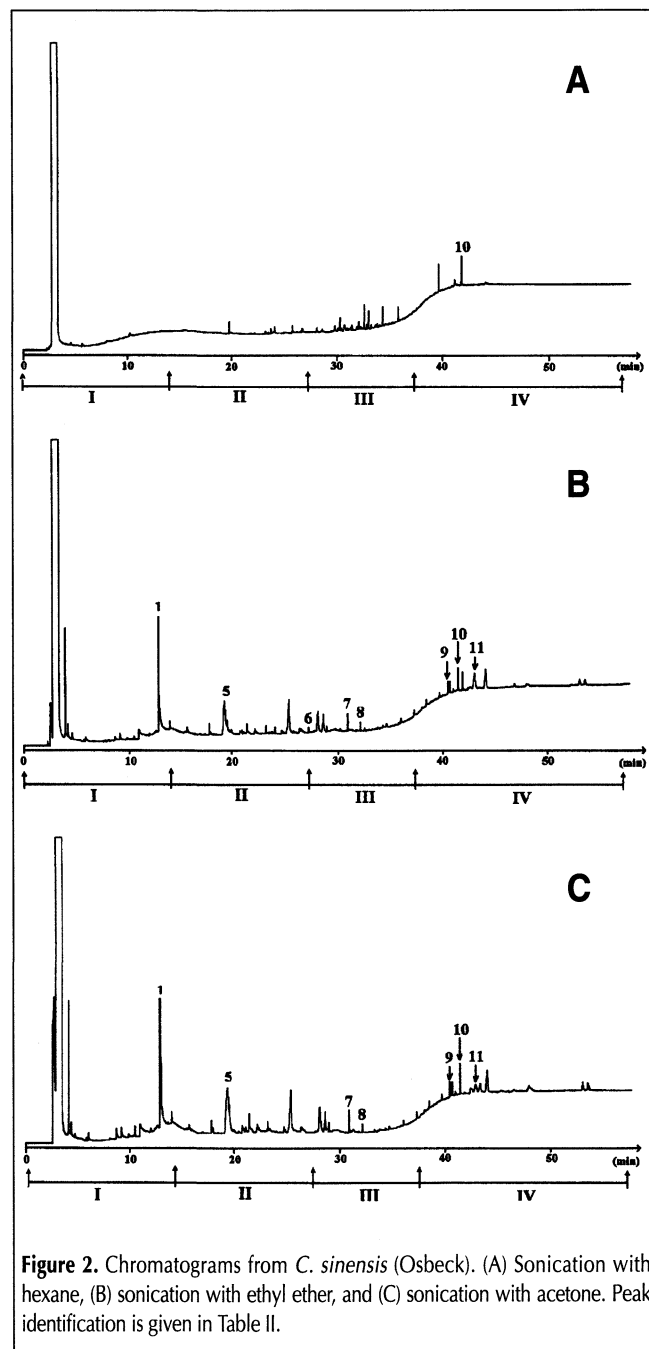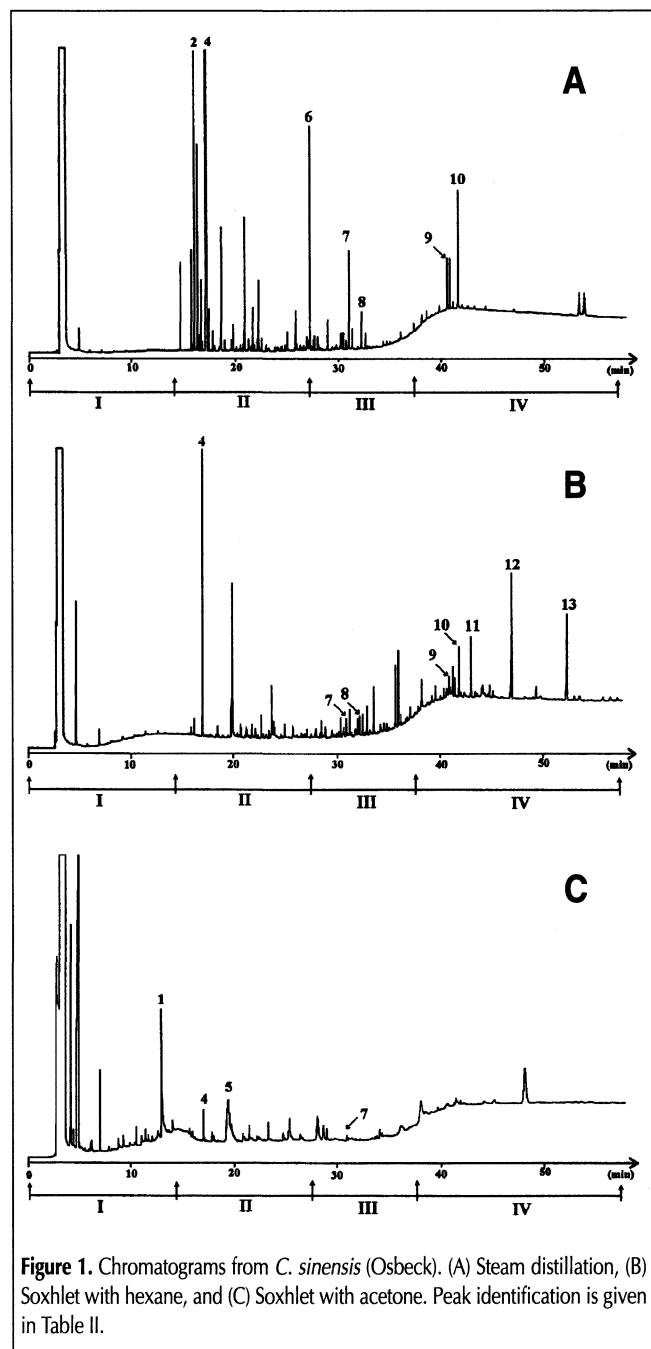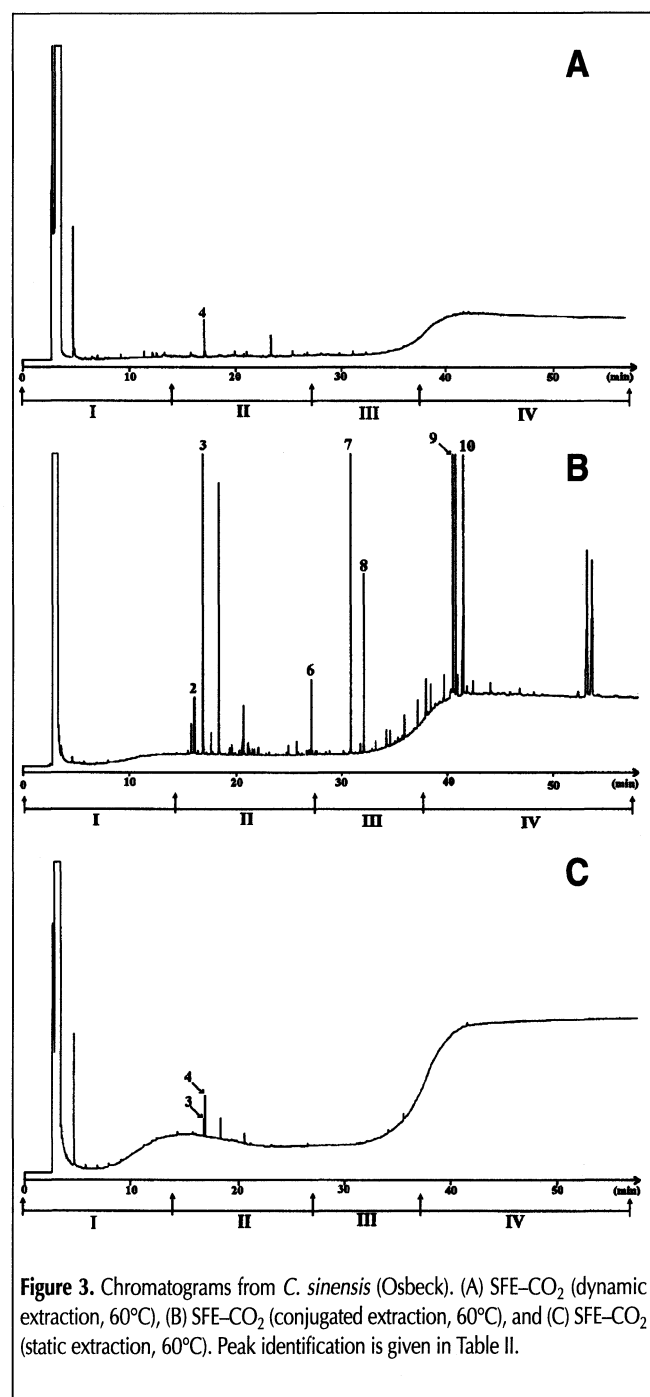

Figure 1. Chromatograms from *C. sinensis* (Osbeck). (A) Steam distillation, (B) Soxhlet with hexane, and (C) Soxhlet with acetone. Peak identification is given in Table II.

Figure 2. Chromatograms from *C. sinensis* (Osbeck). (A) Sonication with hexane, (B) sonication with ethyl ether, and (C) sonication with acetone. Peak identification is given in Table II.

In order to simplify the data evaluation, the chromatograms for extractions performed at 75°C were omitted. Figures 1–6 show the chromatogram profiles for the extractions discussed in this paper; to make it easier to understand, the chromatograms were divided into four regions (i.e., 0–13 min, 13–27 min, 27–37 min, and 37–58 min were named regions I–IV, respectively).
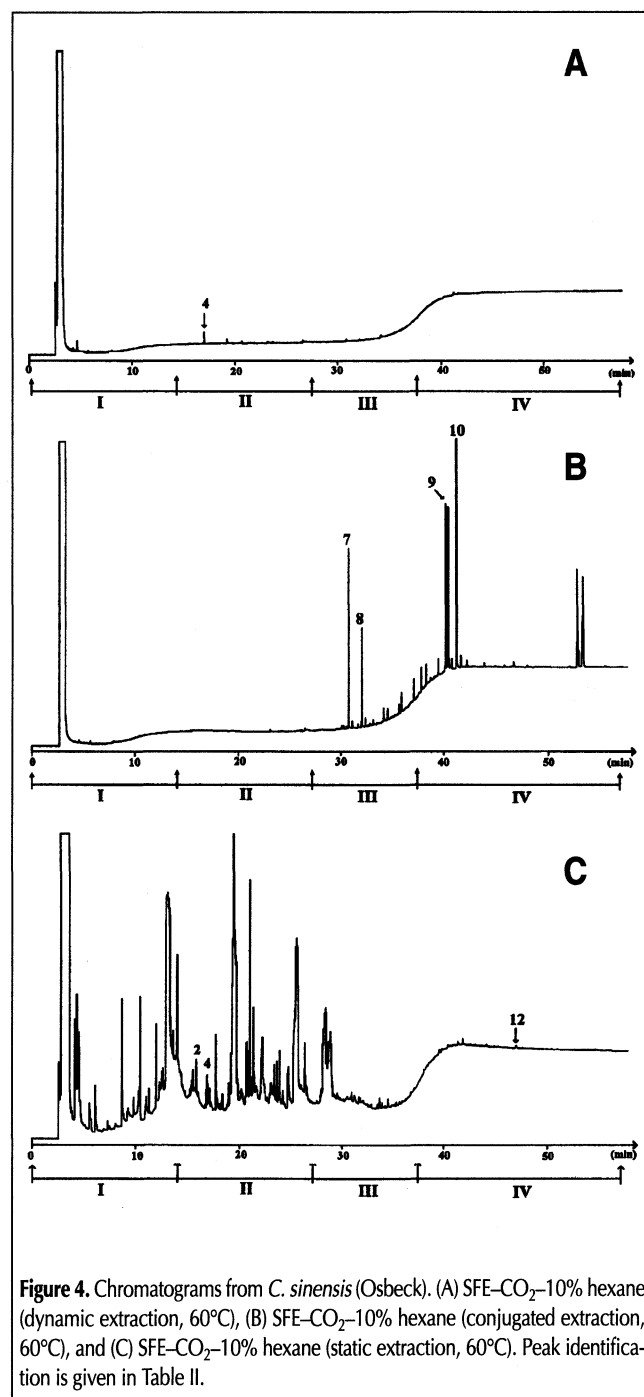
Comparing the yields by the extraction technique, SF extracts had higher yields than those obtained by steam distillation but lower yields than those produced by Soxhlet and sonication extraction (Table I).

Based on the chromatograms obtained with all extracts at the same concentration (8 mg/mL), the extracted amounts of certain compounds in some of the SFE extracts such as peaks 1, 3, 5, and 7–10 (see Figures 3B, 4B, and 6C) increased. In addition, 1,8-cineole (Figure 3B, peak 3) was extracted only by SFE, as shown by the analysis of the MS spectra.
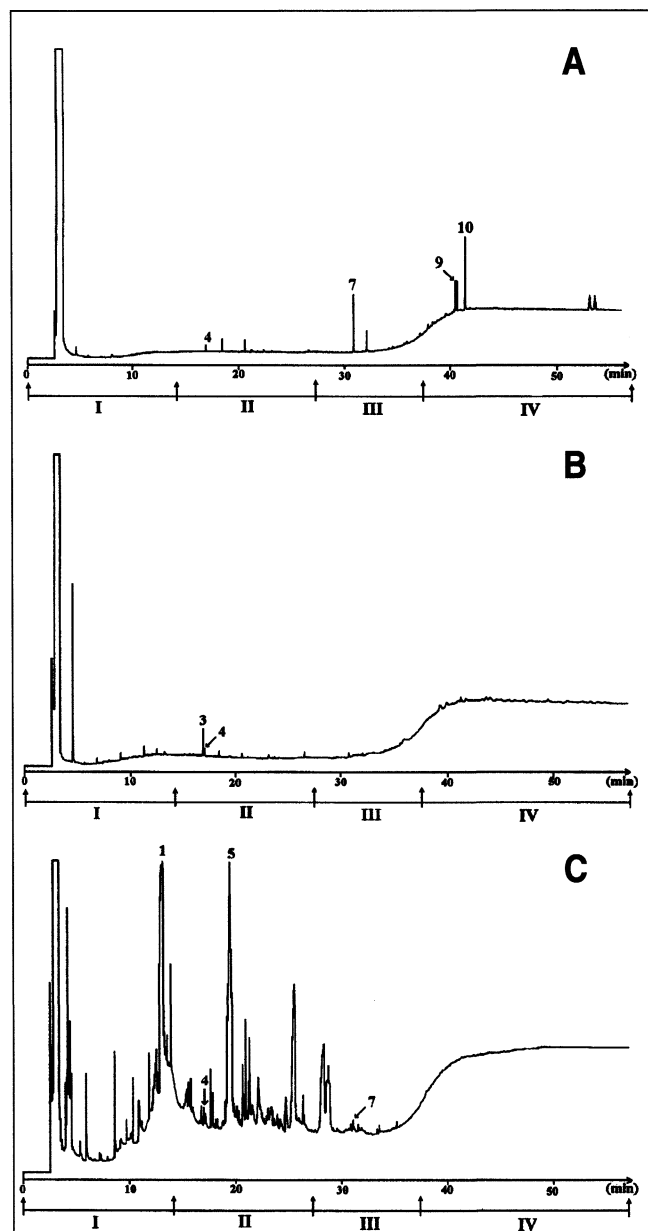
Figures 4B, 4C, 5B, 5C, 6B, and 6C show that SFE in either the conjugated or static modes in the presence of modifiers produced the highest yields among the supercritical extractions (Table I). This agrees with the fact that the recoveries obtained by using modifiers into the pressurization vessel were generally lower than those obtained by adding the modifier directly to the matrix (16). In this case, the modifier was introduced with the CO_2 into the pressurization vessel and held in the static extraction mode. Thus the contact of the matrix with the modifier was more effective and allowed better contact of the matrix with the extracting solvent. Also, the amount extracted was enhanced with the increase of the modifier polarity.

Figure 3. Chromatograms from *C. sinensis* (Osbeck). (A) SFE–CO₂ (dynamic extraction, 60°C), (B) SFE–CO₂ (conjugated extraction, 60°C), and (C) SFE–CO₂ (static extraction, 60°C). Peak identification is given in Table II.

Figure 4. Chromatograms from *C. sinensis* (Osbeck). (A) SFE–CO₂–10% hexane (dynamic extraction, 60°C), (B) SFE–CO₂–10% hexane (conjugated extraction, 60°C), and (C) SFE–CO₂–10% hexane (static extraction, 60°C). Peak identification is given in Table II.

The temperature of 75°C increased the extraction yields (Table I), and better selectivity was also observed when the extraction modes and temperature were changed.
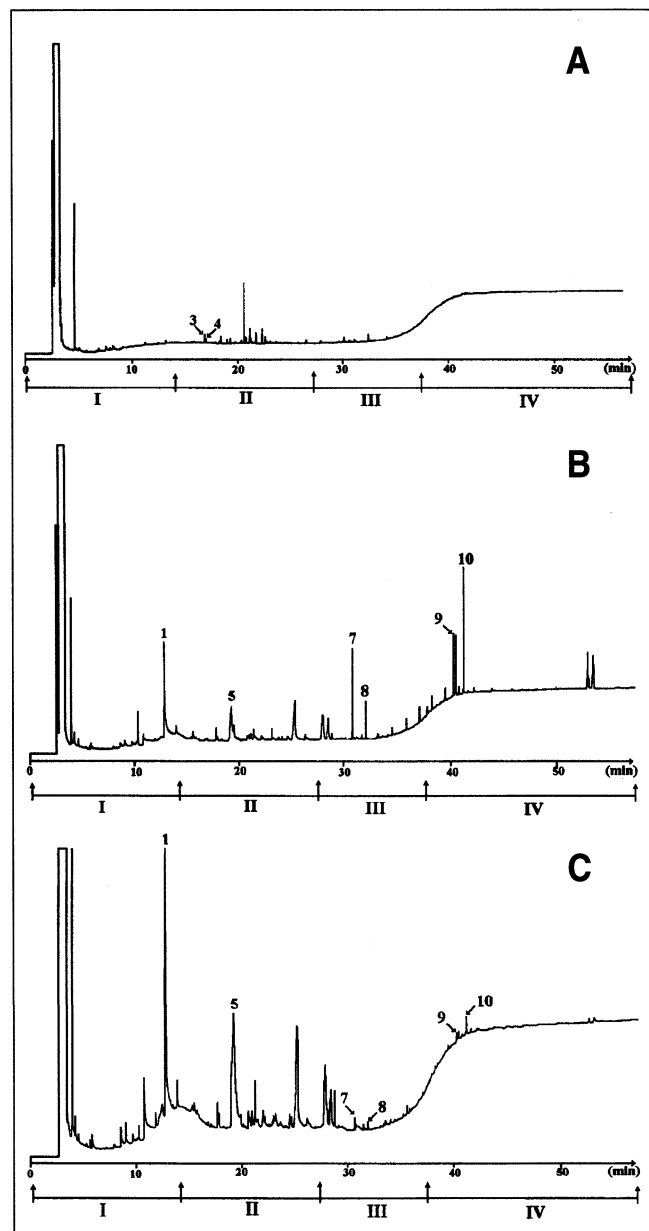
SFE at 60°C did not allow the extraction of some of the compounds obtained by steam distillation (region II, Figures 1A and 3–6). For instance, compound 1 (hydrocarbon C_8H_{15}) could be extracted only using acetone alone or as a modifier for the supercritical CO_2, which means that the extraction of this compound required a solvent with better solvating power.

When using pure CO_2 for SFE in the conjugated mode, the essential oils and compounds of medium and high polarity were extracted (i.e., Figure 3B, regions II–IV), but CO_2 modified with 10% hexane extracted only medium- to low-polarity compounds

(Figure 4B, regions III and IV), and CO_2 modified with 10% acetone extracted compounds in all regions of the chromatograms. However, for the static mode, practically only the low-polarity compounds (lower hydrocarbons and essential oils) were extracted with the use of modifiers (regions I and II, Figures 4C, 5C, and 6C). The dynamic extraction mode did not work well for this matrix. Therefore, this work showed the selectivity achieved with changes on fluid composition, temperature, and extraction mode. This study also agreed with other work that found the conjugated (combined or coupled) extraction mode to work the best with SFE (9). Five major compounds were identified: β-myrcene (2), 1,8-cineole (3), d-limonene (4), dihydrocarveole (5), and caryophylene (6).

Figure 5. Chromatograms from *C. sinensis* (Osbeck). (A) SFE–CO_2–10% ethyl ether (dynamic extraction, 60°C), (B) SFE–CO_2–10% ethyl ether (conjugated extraction, 60°C), and (C) SFE–CO_2–10% ethyl ether (static extraction, 60°C). Peak identification is given in Table II.

Figure 6. Chromatograms from *C. sinensis* (Osbeck). (A) SFE–CO_2–10% acetone (dynamic extraction, 60°C), (B) SFE–CO_2–10% acetone (conjugated extraction, 60°C), and (C) SFE–CO_2–10% acetone (static extraction, 60°C). Peak identification is given in Table II.

Conclusion

In the comparison of SFE modes, the conjugated extraction mode produced the largest yields and better selectivity, the dynamic mode was not efficient for this matrix, and the static mode was not selective, even when the modifier was changed. An increase in the temperature from 60 to 75°C enhanced the total amount of extract in all cases. Regarding the extraction mode, the static extraction at 75°C increased the number of compounds extracted. In the conjugated extraction mode, pure CO_2 extracted only the essential oils, whereas supercritical CO_2 modified with hexane produced medium to polar compounds, and supercritical CO_2 modified with acetone was not a selective fluid.

In summary, this work showed that it is possible to combine temperature, modifier, and extraction mode to tune the extraction for the desired product, as was the case for C_8H_{15} (Figure 6C) and 1,8-cineole (Figure 3B).

Acknowledgment

References

1. M.A. McHugh and V.J. Krukonis. *Supercritical Fluid Extraction, Principles and Practice.* Butterworths, Boston, MA, 1986.
2. C. White and V. Berry. A primer on supercritical fluids. *LC–GC* **8:** 734–38 (1990).
3. C.R. Blatt and R. Ciola. Analysis of vetiver essential oil by supercritical fluid extraction and on-line capillary gas chromatography. *J. High Res.Chromatogr.* **14:** 775–77 (1991).
4. S.R. Sargenti. Extração com fluido supercrítico: projeto e construção de um novo sistema e sua aplicação em produtos naturais. Ph.D. dissertation, USP, São Carlos, Brazil, 1994.
5. S.R. Sargenti and F.M. Lanças. Design and construction of a simple supercritical fluid extraction system with semi-preparative and preparative capabilities for application to natural products. *J. Chromatogr.* **667:** 213–18 (1994).
6. S.P.M. Germer. Extração de óleo essencial de Cravo-da-India em leito fixo com dióxido de carbono líquido subcrítico. MS dissertation, UNICAMP, Campinas, Brazil, 1989.
7. E.Stahl and D.Gerard. High-pressure extraction of natural substances with supercritical and liquefied gases. 9. Methods for obtaining essential oils under mild conditions. *Parfuem. Kosmet.* **63:** 117–25 (1982).
8. C.D. Bevan and P.S. Marshall. The use of supercritical fluids in the isolation of natural products. *Nat. Prod. Rep.* **11:** 451–66 (1994).
9. J.L. Hedrick, L.J. Mulcahey, and L.T. Taylor. Fundamental review: Supercritical fluid extraction. *Mikrochim. Acta* **108:** 115–32 (1992).
10. S.R. Sargenti and F.M. Lanças. Comparative study of vegetal extraction. Classical extraction methods vs supercritical fluid extraction. *Proc.15th Int. Symposium on Capillary Chromatography,* Rival del Garda, Italy, 1993, p. 1651.
11. S.B. Hawthorne, J.J. Langenfeld, and D.J. Miller. Comparison of hydrodistillation and supercritical fluid extraction for the determination of essential oils in aromatic plants. *J. Chromatogr.* **634:** 297–308 (1993).
12. E. Reverchon and F. Senatore. Supercritical carbon dioxide extraction of chamomile essential oil and its analysis by gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **42:** 154–58 (1994).
13. Y. Yang, A. Gharaibeh, S.B. Hawthorne, and D.J. Miller. Combined temperature/modifier effects on supercritical CO_2 extraction efficiences of polycyclic aromatic hydrocarbons from environmental samples. *Anal. Chem.* **67:** 641–46 (1995).
14. *Eight Peak Index of Mass Spectra,* 3rd ed., Vol. 1–3, Unwin Brothers, Nottingham, UK, 1986.
15. A.A. Swigar and R.M. Silverstein. *Monoterpenes – infrared, mass, ^1H NMR, and ^{13}C NMR spectra, and Kóvats indices.* Aldrich Chemical, Milwaukee, WI, 1981, p. 130.
16. C.R. Knipe, D.R. Gere, and M.E. McNally. Supercritical fluid extraction: The exotericisms of developing a "turnkey" method. *ACS Symposium Series Reprint,* 1991, p. 16.