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Supercritical fluid extraction of Z-sabinene hydrate-rich essential oils from Romanian *Mentha* hybrids*

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Abstract: The green solvent SC–CO_2 was used to extract the volatile part, rich in thermolabile compounds, Z-sabinene hydrate and its acetate, from a Romanian mint hybrid. The best yield, 2.26%, was obtained at 92–94 bar, 50 °C (SFE 2). Kinetic study of SC–CO_2 extraction and hydrodistillation and monitoring by gas chromatography (GC) and gas chromatography mass spectrometry (GC–MS) analysis established optimal conditions for SC–CO_2 extracts with a high content of Z-sabinene hydrate (43.5%) and its acetate (19.15%) and flavor similar to plant material (P = 90–100 bar, T = 50 °C). Only at the beginning of hydrodistillation was it possible to obtain a high content of Z-sabinene hydrate (37.3%, after 70 min), but a high content of its acetate never could be reached. This study suggests a way to obtain Z-sabinene hydrate from a natural source and reveals the importance of mild conditions to keep what is valuable in nature.

INTRODUCTION

Z-sabinene hydrate (1R,2S,5R)-2-methyl-5-(methyl-ethyl) bicyclo [3.1.0] hexan-2-ol was identified as a component of the odor bouquet emitted by several bark beetle species and also in sunflower aroma, being one of the components stimulating the honeybee workers' antennal receptors; its precise role in chemical communication systems is yet to be investigated [1]. The use of hydrodistillation for the extraction of essential oils rich in sabinene hydrates favors isomerizations and hydrolysis because of the prolonged water contact time at high temperature and the decrease of pH during the hydrodistillation [2]. SC–CO2 avoids this thermal degradation and solvent pollution [3]. Partial results of some intrinsic and extrinsic factors' influence on essential oil composition [4] and the influence of process parameters, such as particle size, extraction time, flow rate, charge, extraction, and separation parameters have been previously reported [5,6]. The aim of our work is the evaluation of SC–CO_2 extraction/hydrodistillation parameters to obtain an extract/essential oil rich in Z-sabinene hydrate and its acetate, with a flavor similar to plant material.

MATERIALS AND METHODS

Plant material

Mentha hybrid, used in this work, is the result of a complex hybridization and selection work, starting from *M. x piperita* L., *M. aquatica* L., *M. viridis* L., and *M. spicata* Huds. The hybrid's code is H 616.

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The hybrid, caryologically characterized, was cultivated at Grid (county of Brasov), dried, vacuum-packed, and stored in a cool, dry place.

Extraction methods

Hydrodistillation

Neo Clevenger apparatus was used according to Romanian-European Pharmacopoeia.

Supercritical CO_2 extraction, quasi batch mode

To avoid solid plugs on pipes, special attention was given to extraction and separation parameters and CO_2 flow rate. Particle size of plant material was chosen to maximize the supercritical fluid contact surface and to shorten the time until maximum yield was reached for the whole process to be practically advantageous. It was noticed that particle dimensions smaller than 0.18 mm determine the passage of vegetal material through porous plates from the ends of the extractor, together with supercritical fluid, creating plugs on the pipes (Fig. 1).

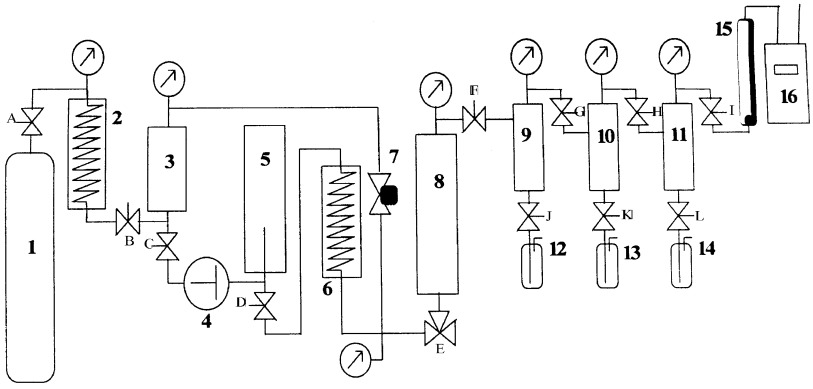

Fig. 1 Schematic diagram of SF–CO_2 extraction apparatus (quasi batch mode, ENSIC, Nancy, France): 1. CO_2 cylinder; 2. heat exchanger; 3. liquid CO_2 reservoir; 4. high-pressure pump; 5. pulsations dampener; 6. heat exchanger; 7. pressure regulation valve; 8. extraction column; 9–11. precipitation cyclones; 12–14. solute recovery vials; 15. flowmeter; 16. gas totalizer.

The monitoring of the extraction process was done by periodically collecting samples of extract from the second separator, weighing, and organoleptic and gas-chromatographic analysis immediately after extraction.

The control of cuticular waxes' separation from the first separator was made only at the end of the extraction process. Most of the time, cuticular waxes were not isolated from the first separator owing to the small quantities of vegetal material that were processed.

Supercritical fluid extraction from only 10–20 g of vegetal material was, upon our knowledge, not mentioned in the literature. Usual quantities are, for preparative scale, around hundreds of grams, and for analytical scale are around hundreds of milligrams.

Analysis

GC–FID; GC–MS; (Table 1). Kovats indices on DB 5: $I = 100\ (n + \lg t_x - t_n / \lg t_{n+1} - \lg t_n)$.

Table 1 Chromatographic systems used in essential oils and supercritical CO_2 extracts analysis.

Chromatograph type	Detector	Column/ Stationary Phase	Carrier Gas	Temp. Injector Detector °C	Injection Split Ratio	Temp. Program	Identification	Quantitative Analysis
Hewlet Packard 5890 II, 5972	MSD	HP-5MS 30 m × 0,53 mm × 0,25 µm	He 3,0 psi	250	1 : 100	60–240 °C, 3 °C/min	Comparison with SM from Wiley 6 Kovats indices	% Peak area without correction
Varian 3400	Finigan MAT ITD	Fused silica DB-5;30 m × 0,25 mm × 0,25 µm	He	250	1:30	45–250 °C, 4 °C/min	Comparison of SM with those from Wiley 6, Nist 5 Kovats indices compared with literature	% Peak area without correction
Chrompack CP 9002	FID/ Catoro meter	CP-SIL 5 CB 10 m × 0,25 mm	He 0,36 ml/min	250/ 200	0,4–1 µl in hexane	50 °C (5 min), 50–250 °C 8 °C/min, 250 °C (5 min)	Retention time of etalons	% Peak area without correction

RESULTS AND DISCUSSION

The parameters of SC–CO_2 extraction are presented in Table 2.

Yield of extraction is presented in Fig. 2.

Table 2 Experimental results of CO_2 supercritical fluid extraction in quasi-batch mode.

Plant material (Moisture %) (η HD m/m%)	Extraction Code	Charge Parameters Particle Size (mm); quantity (g)	Extraction Parameters P(bar); T(K) Time (min)	Separation Parameters P(bar); T(K) Time (min)	CO_2 Parameters m_{CO2}/mcharge g/g $m_{CO2}/m_{extract\ SI}$ Separator II Flow Rate (kg .h^{-1})	Yield SFE
Mentha H 616 16.08.97 (dried leaves) (9.47%; 2.45%)	SFE 1	0.5; 13.346	90; 50; 430	I 80; 1.5 II 35; 3.9 250	55.57; 5267.73; 0.178	1.055
-"-	SFE 2	0.7; 10.0439	92–94 50 220	I 80; 1.8–1.9 II 40; 4 160	107.44 4758.01 0.4	2.26
-"-	SFE 3	0.6; 11.958	90; 50; 270	I 80; 1.8–1.9 II 40; 4 180	84.23 9206.96 0.33	0.92
-"-	SFE 4	0.7; 15.9710	100; 50; 580	I 80; 1.5 II 38; 3.9 360	19.49 1470.6 0.052	1.325

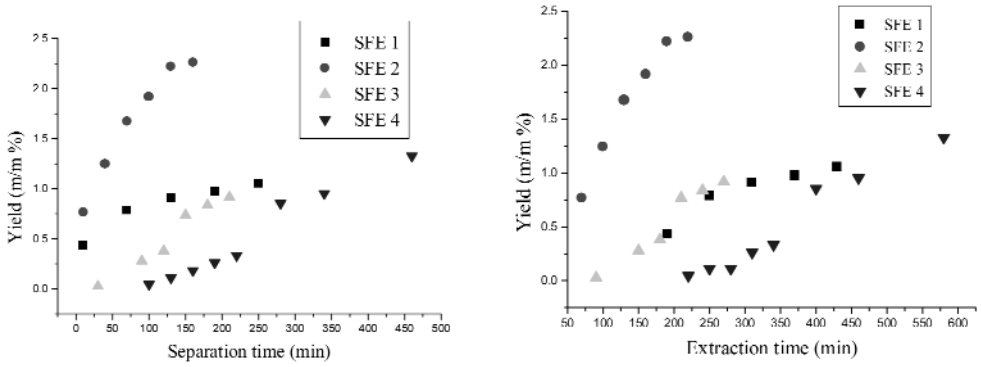

Fig. 2 Yield oil versus time for CO_2-SFE from H 616.

The conditions of SFE 2 were the best ones, giving the maximum yield in the shortest time. The first part of extraction is governed by solubility phenomena. Owing to the high solubility of terpenoids in operating conditions, a long contact time in batch mode is neither necessary, nor desired.

Composition of H 616 supercritical CO_2 extracts

Supercritical fluid extracts from H 616 are characterized by a high content of Z-sabinene hydrate and its acetate and a low content of 4-terpineol, confirming thus, its artifact of hydrodistillation origin (Figs. 3 and 4).

High concentrations of sabinene hydrates and their acetates in SFE extracts with flavor similar to plant material suggests that these components may be considered key compounds for the quality of H 616 extracts. H 616 SC–CO_2 extracts vs. essential oil quality is presented in Fig. 3.

The comparative kinetic study of hydrodistillation and SFE 2 from H 616 showed that it is possible to obtain an essential oil with a high content of Z-sabinene hydrate, after an hour of hydrodistillation, at pH = 6–7, but a high content of its acetate never could be reached.

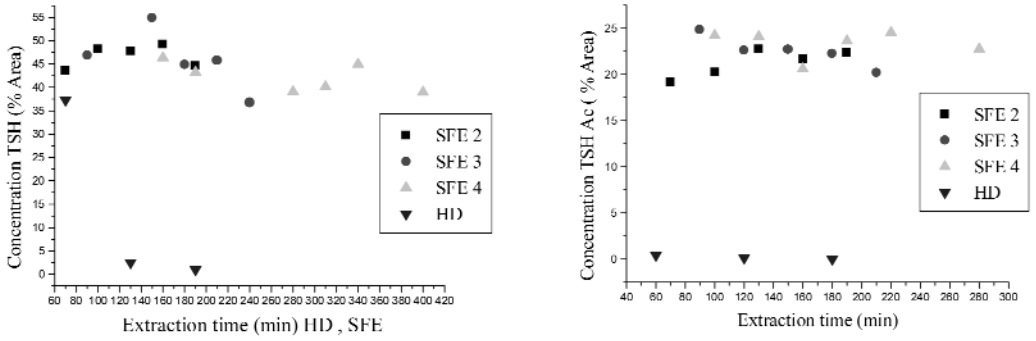

Fig. 3 H 616 SC–CO_2 extracts vs. essential oil quality expressed as Z-sabinene hydrate (TSH) and its acetate (TSHAc) essential oil content.

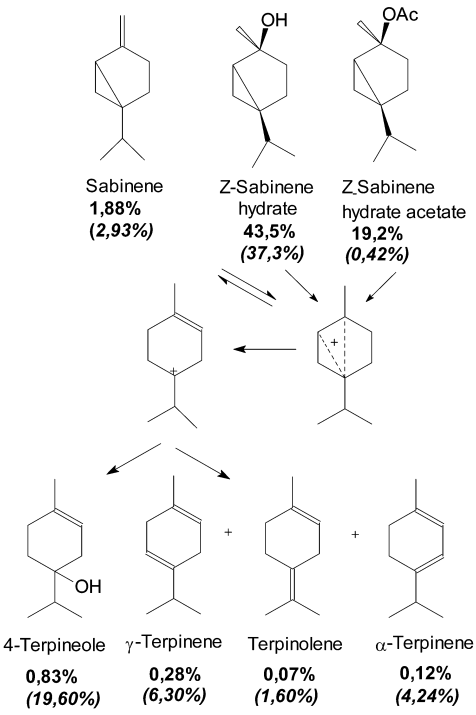

Fig. 4 Distribution of some monoterpenes in supercritical CO_2 extraction / *(Hydrodistillation)* of H 616 mint hybrid (adapted from Fischer *et al*., 1988, in ref. 7).

CONCLUSION

Comparison between hydrodistillation and SC–CO_2 extraction reveals the superiority of the green solvent, SC–CO_2, to obtain a good yield and a flavor similar to plant material and to keep the native composition present in the genuine plant. This study contributes to a better characterization of the volatile part of H 616, and suggests a way to obtain Z-sabinene hydrate in high yield.

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