SUPERCRITICAL FLUID EXTRACTION AND FRACTIONAL SEPARATION OF ESSENTIAL OILS

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Supercritical fluid extraction of essential oils is one of the most widely discussed applications in the supercritical fluid literature. Peppermint leaf oil was isolated by a supercritical fluid extraction using CO_2 in which the extraction was followed by a two-stage fractional separation. Chemical analysis revealed that oils extracted under different supercritical fluid extraction conditions possessed a widely different percentage composition. Oil obtained by hydrodistillation was also compared with the extracted oils. Although practically the same compounds were present in the hydrodistilled oil, its composition was similar to supercritical fluid extraction oil extracted under non-optimised conditions. The oil obtained at optimum supercritical fluid extraction conditions (p = 90 bar, T = 40°C) had a fragrance that better resembled that of the peppermint leaves used for the extraction of the oils.

Introduction

Supercritical fluid extraction (SFE) is an interesting technique for the extraction of flavouring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam distillation processes [1].

SFE allows a continuous modification of solvent power and selectivity by changing the solvent density [2]. Nevertheless, the simple SFE process, consisting of supercritical CO_2 extraction and a one-stage subcritical separation, in many cases does not allow a selective extraction because of the simultaneous extraction of many unwanted compounds. This situation is typical of CO_2 supercritical fluid extraction of essential oils from herbaceous material in which, even when the process is conducted at conditions that produce the optimum oil composition, cuticular waxes are co-extracted because of their lipophilic character and their localization on the leaf surface [3÷5]. SFE followed by fractional separation of the extract in multiple-stage separators overcomes these limitations and produces high-quality essential oil [6÷8].

Peppermint essential oil is an important raw material for flavouring confectionery, liquors, cosmetics, toothpastes and many other products. For these reasons, the extraction of this oil using SFE could have an industrial impact.

This paper deals with the SFE of peppermint essential oil using a process that involves the fractional separation of the extract in two separators in series. The oils isolated under various SFE conditions were analysed by GC-MS. Sensory analysis was used to determine the optimum oil composition that was compared with that of peppermint oil isolated by hydrodistillation.

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Experimental part

Peppermint (*Mentha piperita*, fam. *Lamiaceae*) leaves were colected from plants growing in a subcarpathian region of Romania. The leaves were air-dried and comminuted until an approximate size of 0.5 mm was obtained. The essential oil content, determined by the supplier using steam distillation, was about 3.0% by weight.

A scheme of the supercritical extraction apparatus is presented in the Fig. 1. It contains as essential parts a 350 ml extractor (3) stainless steel made with double garniture teflon rings and two separators operated in series (6), (7) with a volume of 250 ml each. A thermostated jacket (4) allows regulating the temperature in the extractor by using of an electronic device (5) with Pt thermometer control. Both separators were immersed in two 8 L Dewar vessels filled with a mixture of ethylene glycol and water.
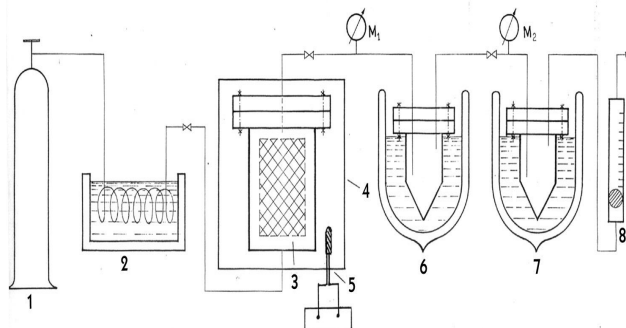

Fig. 1: *Scheme of the supercritical extraction apparatus:*
(1) CO_2 tank; (2) thermostat; (3) extractor; (4) thermostated jacket;
(5) thermometer; (6), (7) separators; (8) flow measurement device.

About 200 g of comminuted peppermint leaves was submitted to extraction in each run. A CO_2 flow rate of 1.0 kg/h and an extraction period of 120 min were used.

First the SFE process was performed at various CO_2 densities. GC-MS data and sensory analysis were used to determine the extraction conditions that minimize the co-extraction of unwanted compounds; subsequently the optimum fractionation conditions to be used in the two separators were studied. The yield of the various fractions was measured by weight with respect to the dried material charged in the extractor. The plant material was also subjected to hydrodistillation (HD) for 120 min according to the standard procedure.

GC-MS data were obtained using a Varian model 3400 gas chromatograph equipped with a fused silica DB-5 column (J&W; 30 m × 0.25 mm i.d., film thickness 0.25 μm) for the essential oil analysis. The GC apparatus was interfaced with a Finnigan-MAT 800 Ion Trap Detector (ITD, software version 4.1). GC conditions for the essential oil separation were as follows: oven temperature 50°C for 5 min, then programmed 50÷250°C at 2°C/min and

subsequently isothermal at 250°C for 15 min. The samples were injected using the splitless sampling technique.

The percentage composition of the essential oil was computed from the GC peak areas without using correction factors. The identification of the compounds was based on a comparison of retention times and mass spectra with corresponding data of components of reference oils and authentic compounds. Some mass spectra were compared with those of mass spectra libraries (NIST, version 4.0 and WILEY, version 5.0).

Extractions performed at various CO_2 densities showed that an extraction at $p = 90$ bar and $T = 40°C$ was optimum in order to minimize the co-extraction of unwanted compounds. The best parameters to perform the fractionation were $p = 90$ bar and $T = 0°C$ for the first separator and $p = 20$ bar and $T = 10°C$ for the second one. Using this procedure, cuticular waxes were selectively precipitated in the first separator, in the second one the essential oil was recovered. Small quantities of water (c. 20% of the extract collected in the second separator) were separated by centrifugation.

Results and discussion

The yield of peppermint oil at the optimum SFE conditions was 2.0% by weight, is somewhat lower than the yield found by the supplier using steam distillation. However, to obtain a yield of the SFE process closer to the reference one, it is possible to extend the extraction period and to further comminute the plant material.

Two extraction conditions were taken as reference: $p = 90$ bar and $T = 40°C$, called SFE-1, and $p = 100$ bar and $T = 50°C$, called SFE-2.

Compounds that exhibit retention times shorter than 60 min under the GC conditions applied are related to peppermint oil (see Table 1). Under these GC conditions, cuticular waxes exhibit longer retention times, beginning with about 90 min.

The percentage composition of the peppermint oil obtained under optimum SFE conditions is given in Table 1. CO_2 density increased from about 0.2 g/ml for SFE-1 to 0.4 g/ml for SFE-2 that justifies the marked difference in composition between the extracts obtained under these conditions. The oil extracted under SFE-1 conditions had a higher content of menthone, menthol, 1,8-cineole and piperitone compared with the SFE-2 conditions, and a lower content of menthyl acetate, β-caryophyllene and γ-cadinene. The compounds mainly responsible for the peppermint fragrance (oxygenated monoterpenes) amounted to 79.2% for SFE-1 compared with 74.4% at SFE-2 conditions. In contrast, sesquiterpenes were only 7.7% for SFE-1 and 11.6% for SFE-2.

Sensory analysis confirmed that the oil extracted under the SFE-1 conditions better resembled the starting material. The oil extracted under the SFE-2 conditions showed fewer top fragrance notes and more back notes, probably because it contained a lower percentage of oxygenated monoterpenes and a higher percentage of sesquiterpenes.

The SFE products were also compared with the peppermint oil isolated by hydrodistillation. The results of a detailed analysis of the hydrodistilled oil (HD) are again given in Table 1. The compounds isolated were practically the same as those extracted by SFE. The

hydrodistilled oil possessed the higher percentage of terpene acetates, 12.5% against 12.0% for SFE-1.

The organoleptic comparison of the SFE-1 oil with the hydrodistilled oil showed again that the aroma of the SFE-1-obtained oil was closer to that of the peppermint leaves.

Table 1. Percentage composition of peppermint oil isolated by supercritical CO$_2$ extraction (SFE-1 and SFE-2) and by hydrodistillation (HD), respectively; the percentages are based on GC peak areas.

Compound	Retention time (min)	SFE-1 (%)	SFE-2 (%)	HD (%)
α-Pinene	17.19	0.2	0.1	0.4
β-Pinene	20.19	0.5	0.4	0.6
β-Myrcene	21.36	0.1	0.1	0.2
Octan-3-ol	21.55	0.2	0.1	0.2
1,8-Cineole	24.22	8.1	5.1	4.0
cis-β-Ocimene	25.08	0.1	0.1	0.4
Menth-2-en-1-ol	29.44	0.5	0.4	0.3
trans-Menthone	33.39	39.3	38.8	37.6
cis-Menthone	34.22	4.6	3.8	5.0
Neomenthol	34.30	1.1	1.0	1.3
trans-Menthol	35.08	23.3	23.0	24.3
cis-Menthol	35.58	0.5	0.4	0.6
Dihydrocarveol	38.05	0.1	0.2	0.3
Pulegone	39.48	0.1	0.3	0.4
Piperitone	40.54	1.6	1.4	0.9
Linalyl acetate	41.19	0.1	0.4	0.2
Neomenthyl acetate	42.39	0.4	0.3	0.3
Bornyl acetate	43.30	0.1	0.3	0.5
trans-Menthyl acetate	43.59	10.9	11.6	11.1
cis-Menthyl acetate	44.57	0.5	0.6	0.4
α-Cubebene	49.39	0.1	0.2	0.2
β-Bourbonene	50.13	0.5	0.8	0.7
β-Elemene	50.44	0.4	1.0	1.1
β-Caryophyllene	52.30	2.5	4.2	4.4
β-Gurjunene	53.09	0.1	0.3	0.3
Humulene	54.43	0.1	0.1	0.3
β-Farnesene	54.58	0.1	0.8	0.4
γ-Muurolene	55.20	0.1	0.1	0.4
γ-Cadinene	56.30	3.8	4.1	3.2

Conclusions

A fractional separation combined with SFE yielded peppermint oil that did not contain cuticular waxes, thereby overcoming the main handicap of SFE. The oil obtained by SFE under optimum extraction conditions did show some quantitative differences in composition and possessed a superior aroma compared with the oil obtained by hydrodistillation.

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