FLAVOUR AND FRAGRANCE JOURNAL, VOL. 7, 227-230 (1992)

Isolation of Rosemary Oil: Comparison between Hydrodistillation and Supercritical CO_2 Extraction

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Rosemary leaf oil was isolated by a supercritical fluid extraction (SFE) procedure coupled to a fractional separation following the extraction stage. The oil produced was compared with rosemary oil isolated by hydrodistillation. Chemical analysis revealed that, although roughly the same compounds were extracted, the two oils possessed a widely different percentage composition. Qualitative aroma testing showed that the oil obtained by SFE using CO_2 showed a fragrance that better resembled that of the rosemary leaves used for the isolation of the oil.

KEY WORDS Rosemary *Rosmarinus officinalis* L. Labiatae Essential oil Supercritical extraction Hydrodistillation

INTRODUCTION

Supercritical CO_2 extraction (SFE) can be applied using a wide range of solvent densities by modifying the extraction pressure and temperature. It is possible thereby to realize highly selective extractions and to obtain different products from the same starting material. Moreover, at low temperatures degradation of thermal labile compounds can be avoided. Solvent-free extracts can be obtained after the low-pressure separation stage.[1,2] For these reasons, the application of supercritical CO_2 extraction for the isolation of essential oils from herbaceous matrixes is very promising in principle. Unfortunately, some difficulties exist, because supercritical CO_2 shows a high affinity not only for essential oil components but also for many other classes of compounds included in the vegetable matrix, such as cuticular waxes, fatty acids, colouring matters, resins, etc. Except for cuticular waxes, the content of these unwanted compounds can be controlled by choosing appropriate extraction conditions. In a single stage extraction, the co-extraction of cuticular waxes is unavoidable, because they are soluble in supercritical CO_2 and are in a favourable position with respect to extraction. They consist essentially of C_{25}-C_{35} *n*-paraffins that are present on leaf surfaces.

Nevertheless, it is possible to produce essential oils by supercritical CO_2 extraction, adopting a more sophisticated process scheme that was successfully used for the first time by Stahl *et al.*[3] It consists of a fractional separation which is realized during the decompression of the supercritical solution coming out of the extractor. So, it is possible to selectively precipitate cuticular waxes, if suitable conditions have been chosen in the separation stages.

In this study, SFE has been applied to isolate the essential oil from rosemary leaves. Capillary gas chromatography combined with mass spectrometry was used to compare this oil with rosemary oil isolated by hydrodistillation. The influence of the different procedures on the product composition has been investigated.

EXPERIMENTAL

Plant Material

Rosemary leaves (*Rosmarinus officinalis* L., Labiatae) were collected from plants growing in

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0882-5734/92/040227-04 $07.00

Received 20 September 1991
Accepted 27 December 1991

southern Italy. The leaves were air-dried and then comminuted by milling at a controlled temperature until a mean diameter of 0.23 mm was obtained. This pretreatment shortens the diffusion time of the solvent into the vegetable matter and can improve extraction efficiency. The extraction yield can also be increased.[4] The moisture content was about 7% by weight on dry basis.

Isolation of Essential Oil

The supercritical extraction apparatus mainly consisted of a 400 ml extractor (L/D = 2.7) and two separation vessels, operated in series, with a volume of 200 ml each. CO_2 circulation was assured by a Milton Roy high-pressure diaphragm pump (Milroyal B) capable of liquid flow rates up to 5 l/h and pressures up to 500 bar. A schematic representation of the apparatus is given in Figure 1. About 200 g of comminuted rosemary leaves were submitted to extraction in each run.

Hydrodistillation was performed for 2 h according to the standard procedure described in the *European Pharmacopoeia*.[5]

Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS analysis was performed using a Varian model 3400 gas chromatograph coupled with a Finnigan Mat 800 ion trap detector (ITD). Mass spectra were also obtained by a Hewlett Packard 5890 gas chromatograph equipped with a mass selective detector (MSD 5970 HP).

GC conditions were as follows: J & W, fused-silica DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature 50°C for 5 min, then programmed 50–250°C at 2°C/min, and 250°C for 15 min.

The identification of the volatile compounds was based on a comparison of mass spectra with those of mass spectra libraries, a comparison of retention times and, whenever possible, on a comparison of mass spectra with those of reference compounds.

RESULTS AND DISCUSSION

Since a one-stage supercritical extraction does not produce a pure essential oil from a vegetable matrix, a subsequent fractional separation of the supercritical extract was performed within the separators located downstream from the extractor. In this way an essential oil is obtained in two steps which allows fine control of the extract composition.

First, the optimum extraction conditions, that minimize the co-extraction of unwanted compounds, were investigated in the range p = 80–150 bar and T = 35–50°C; they were found to be optimum at p = 100 bar and T = 40°C.

In the second stage, the optimum fractionation conditions to be used in the two separators were studied. Several experiments were carried out to determine the conditions that lead to minimum solubility of the paraffins in dense CO_2.[6] The best operating parameters to perform the fractionation found were: p = 80 bar and T = 10°C for the first

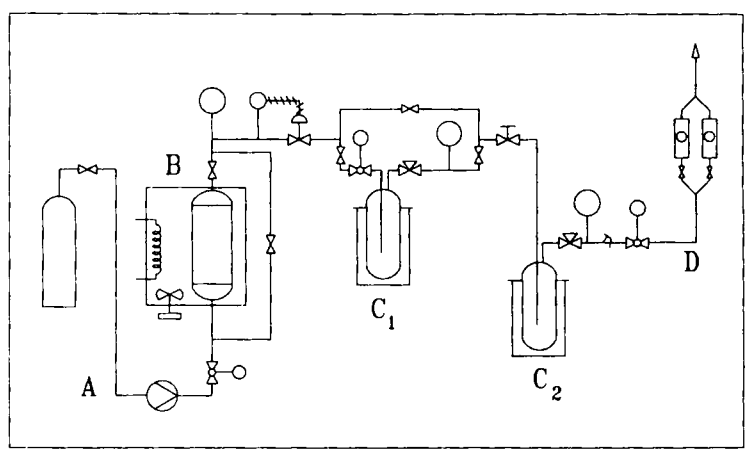

Fig. 1. Schematic diagram of the supercritical fluid extraction apparatus. A, pumping section; B, extraction section; C1, C2, fractionation section; D, flow measurement section

separator and $p = 25$ bar and $T = 0°C$ for the second one. In the first separator the cuticular waxes were selectively precipitated; in the second one the essential oil was recovered. Small quantities of water, precipitated together with the oil, were separated by centrifugation. The yields of all products were measured by direct weighing of the precipitates.

The CO_2 flow rate and the extraction time are the main parameters that contribute to optimum extraction, i.e. a maximum yield at a minimum extraction time.[7] A CO_2 flow rate of 0.8 kg/h and an extraction period of 120 min were experimentally found to be the most suitable for the SFE procedure used. At these operating conditions the yield of essential oil amounted to 1.03% (standard deviation 0.04%) of the leaf material extracted.

Figure 2 shows a comparison between the optimum extract composition after single supercritical extraction (Figure 2(a)), and after extraction followed by fractionation (Figure 2(b)). In the latter case, the selective elimination of compounds at higher retention times is evident, i.e. the cuticular waxes are no longer present.

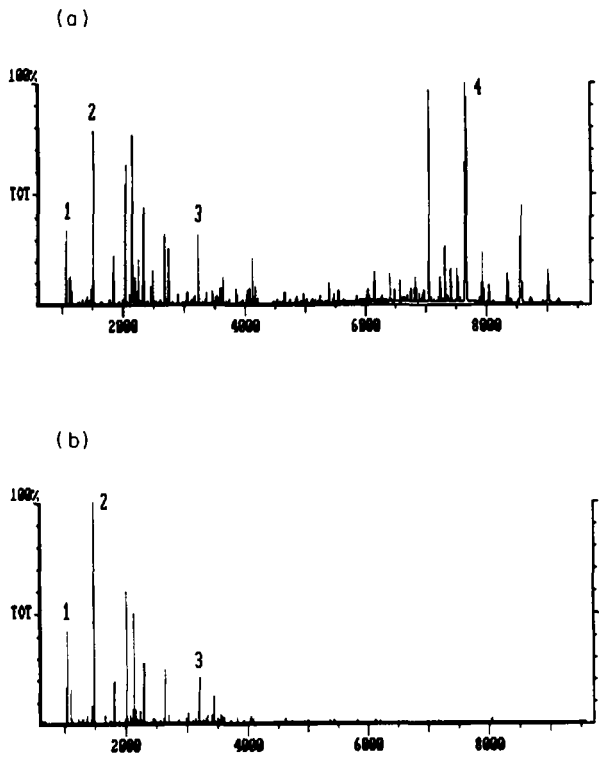

Fig. 2. (a) GC trace of rosemary leaf extract obtained by single-stage supercritical CO_2 extraction ($p = 100$ bar; $T = 40°C$). 1, α-pinene; 2, 1,8-cineole; 3, β-caryophyllene; 4, *n*-nonacosane. (b) GC trace of rosemary leaf oil obtained by supercritical CO_2 extraction followed by fractionation; extraction parameters as in (a). 1, α-pinene; 2, 1,8-cineole; 3, β-caryophyllene

Subsequently, a quantitative comparison of the constituents present in the supercritical fractional extract and in the hydrodistilled rosemary oil was made; the results are summarized in Table 1.

Both techniques yielded substantially the same main compounds, but the SFE yielded additionally traces of other oxygenated terpenes, sesquiterpenes and diterpenes. There was no evidence of specific solvent/solutes interactions; the traces were probably lost during hydrodistillation. In both cases, the oils were mainly made up of the same 14 terpenes (about 87% and 90% for SFE and hydrodistillation, respectively). These compounds roughly correspond with those indicated by Tucker and Maciarello in their study on various rosemary cultivars.[8]

Considering individual compounds, the difference in α-pinene content is noteworthy: the hydrodistilled oil contained 25% of this compound, whereas α-pinene was only 8.3% of the SFE oil. It can also be noted that, except for *p*-cymene, the monoterpene hydrocarbons were present in larger amounts in the hydrodistilled oil. The total percentage of monoterpene hydrocarbons was more than doubled (36.5% against 15.5%) in the hydrodistilled oil. Oxygenated monoterpenes showed the opposite trend. The SFE oil possessed higher contents of linalol, verbenone and isobornyl acetate; their content was almost double that of the hydrodistilled oil. The difference is equally evident in terms of the total percentage of oxygenated monoterpenes: 73.7% for the SFE oil against 59.4% for the distilled oil. The same trend was observed for the content of sesquiterpenes of the two oils (Table 2).

Thus, the hydrodistilled oil contained more monoterpene hydrocarbons. These are the less valuable compounds since they contribute only to a minor extent to the aroma and tend to oxidize because of their unsaturated character. In some cases, such as citrus oils, extraction is followed by a deterpenation stage intended to concentrate the aroma and to eliminate these labile compounds.[9,10] In contrast, the SFE oil contained higher percentages of oxygenated monoterpenes which strongly contribute to the fragrance. Therefore, the SFE oil should give better reproduction of the natural aroma of the rosemary leaves than the distilled oil.

To qualitatively validate this hypothesis based

Table 1. Percentage composition of rosemary leaf oils isolated by supercritical CO_2 extraction (SFE) and hydrodistillation (HD), respectively

Components	R_t	SFE	HD
α-Pinene	1037	8.30	25.16
Camphene	1103	3.11	5.52
Sabinene	1120	0.32	0.63
Δ^3-Carene	1223	0.39	0.41
Oct-1-en-3-ol	1251	0.18	—
Myrcene	1289	0.35	0.55
β-Pinene	1366	0.74	1.05
p-Cymene	1443	1.81	1.82
Limonene	1478	0.51	1.33
1,8-Cineole	1481	20.02	20.64
n.i.	1790	0.63	—
Linalol	1800	3.53	1.82
Camphor	2007	15.33	10.26
Pinanone	2061	0.80	1.03
Pinocarvone	2069	0.39	0.22
Borneol	2126	15.56	13.71
Nonanol	2136	1.17	0.64
Terpinen-4-ol	2157	1.00	0.71
α-Terpineol	2226	1.87	1.95
Verbenone	2280	8.36	4.76
Dihydrocarveol	2419	0.39	0.45
Linalyl acetate	2448	0.48	0.75
n.i.	2468	0.25	—
Carvone	2541	0.26	—
Isobornyl acetate	2616	4.94	2.04
Thymol	2677	0.69	1.07
n.i.	2833	0.12	0.19
α-Cadinol	2965	0.19	—
α-Cubebene	2992	0.43	0.15
Methyleugenol	3095	0.12	—
n.i.	3106	0.17	—
β-Caryophyllene	3168	2.22	0.97
α-Santalene	3308	0.41	0.19
β-Gurjunene	3386	0.38	0.14
Curcumene	3414	1.28	0.18
α-Selinene	3468	0.16	—
α-Muurolene	3477	0.20	trace
β-Bisabolene	3516	0.41	0.10
γ-Cadinene	3531	0.26	0.13
δ-Cadinene	3553	0.35	0.21
Calamenene	3564	0.15	0.10
Farnesene	3576	0.37	trace
Caryophyllene oxide	3785	0.37	0.22
n.i.	4005	0.25	—
Cedranediol	4052	0.22	0.70
n-Octadecane	4683	trace	—
Abietatriene	5324	trace	—
Totarol	6065	trace	—
n.i.	7970	0.30	—

R_t, retention time on DB-5.
n.i., not identified.
trace, < 0.10%.

Table 2. Percentage composition of rosemary leaf oils isolated by supercritical CO_2 extraction (SFE) and hydrodistillation (HD), with respect to grouped components

Grouped components	SFE	HD
Monoterpene hydrocarbons	15.5	36.5
Oxygenated monoterpenes	73.7	59.4
Sesquiterpene hydrocarbons	6.6	2.2
Oxygenated sesquiterpenes	0.8	0.9
Other components	1.3	0.7

on the analysis, organoleptic tests were performed by a standard testing panel.[11] The SFE oil was judged to have a strong fragrance of rosemary leaves. The hydrodistilled oil possessed a less intense aroma that was also considered to be somewhat different from that of the starting material.

Acknowledgements—This work was partly carried out within the framework of Progetto Finalizzato Chimica Fine II, financed by CNR (Consiglio Nazionale delle Ricerche, Rome).

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