ISOLATION OF *Tagetes minuta* L. OIL USING SUPERCRITICAL CO_2 EXTRACTION

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Abstract

Supercritical CO_2 extraction of *Tagetes minuta* L. oil was performed at P = 80 bar and T = 40°C using a semicontinuous extraction procedure and a two-stage separation technique. The co-extracted waxes were precipitated in the first separator at P = 80 bar and T = -15°C. The oil was isolated in the second separator operated at P = 15 bar and T = 20°C. The yield was 0,91% by weight after 840 min.

Limonene (15,1%), cis-Ocimene (19,3%), Dihydrotagetone (5,2%), cis-Ocimenone (13,3%) and trans-Anetol (9,9%) were the main components identified in the extract obtained at 60 min; trans-Anetol was identified in the hydrodistilled oil, too. This compound has not been found in this kind of plant in previous publications.

1. Introduction

Essential oils isolation can be improved with supercritical fluid extraction (SFE) using CO_2 as a solvent. In fact, traditional techniques can produce thermal degradation of the product (steam distillation) or its pollution by organic solvents (solvent extraction). Unfortunately, the single-step extraction and separation by liquid or supercritical CO_2 produce the co-extraction of nonvolatile compounds, mainly cuticular waxes: a quasi-solid extract is obtained (Reverchon *et al.*, 1994). However, the possibility of fine tuning the solvent and the selectivity of the extraction process can help in overcoming this problem. For this purpose, it is necessary to select adequate pressure and temperature conditions that can produce the selective supersaturation and precipitation of solutes.

There are no previous studies on the SFE of *Tagetes minuta* L. The essential oil obtained by steam distillation is used in perfumery and, to a limited extent, for flavoring beverages, candies and condiments (Fenaroli, 1971). It has a high content of acyclic ketones (Daghero *et al.*, 1996) and it tends to polymerize probably due to the high degree of unsaturation of these compounds (Hétheléyi *et al.*, 1986).

The aim of this work was to apply supercritical CO_2 extraction and the fractional separation process to isolate essential oil of *Tagetes minuta* L. The product was then compared to the oil obtained by hydrodistillation.

2. Materials and methods

2.1. Plant Material

Flowering tops of *Tagetes minuta* L. collected in the full flowering stage in the region of Río Cuarto (Córdoba, Argentina) were air dried (final humidity = 12%) and milled (mean diameter of particles = 0,4 mm).

2.2. Experimental Apparatus

The laboratory unit (Figure 1) consisted of: extraction vessel (400 cm^3), high-pressure pump (Milton Roy) and two separators operating in series (200 cm^3 each) to fractionate the extract. More details were given in previous work (Reverchon *et al.*, 1994).

Extraction was performed at 80 bar, 40°C. Separation was carried out at 80 bar, -15°C and at 15 bar, 15°C in the first and second separator, respectively. At these conditions, optimum results were obtained with other vegetable matrixes (Reverchon, 1997). To verify the exhausting of the essential oil, the extraction was then continued at 300 bar and 40°C, 1st and 2nd separator were operated at 100 bar, -15°C and 15 bar, 15°C, respectively. Material charged in the extractor was 200 grams producing a high-density packing to avoid the channeling of CO_2 through the sample. CO_2 flow rate was 0,8 kg/h.

Hydrodistillation was performed for 3 hours according to the standard procedure described in the European Pharmacopeia (1975).

2.3. Analytical procedure

The fractions extracted were analyzed using a GC-MS apparatus (Varian model 3400 gas chromatograph coupled to a Finnigan Mat ion trap detector). A fused silica DB-5 column, 30 m x 0.25 mm i.d., with 0.25 μm film thickness was used. GC conditions were: 40°C (5 min), 40°C to 250°C (rate = 2°C/min), 250°C (60 min). The extract composition was computed from GC peak areas without using any correction factor. The identification of compounds was based on the comparison of obtained mass spectra with NIST and WILEY5 mass spectra libraries.

3. Results and discussion

A moderate working condition (80 bar, 40°C) was selected to avoid the degradation of unstable compounds (acyclic ketones). Fractional separation of the extract was achieved by reducing temperature in the first separator with respect to the operating parameters used during supercritical extraction. Lowering of the temperature is particularly effective in producing fractionation between paraffins and terpene compounds. Indeed, these two compounds families have opposite solubility behavior in CO_2 at temperature lower than 20°C (Stahl *et al.*, 1988). To assure the release of volatile compounds from the gaseous CO_2, the second separator was operated at 15 bar and 15°C.

Essential oil yield in a period of 840min was 0,91% by weight of the material charged in the extractor. The extract composition after 60 min. extraction time is shown in Table 1.

A further extraction at high pressure (300 bar, 40°C) was used to verify complete extraction of essential oil. At this condition, the asymptotic yield was 3,85%, but high molecular weight compounds were identified by GC-MS. These compounds are not related with flavour. This fact shows that moderate pressure must be used to avoid the co-extraction of undesirable compounds.

In the first separator, 8,7 gr. of material were collected (4,4% by weight of the material charged in the extractor). Paraffins, fatty acid methyl esters and high molecular weight ketones were detected (see Table 1, WAXES column).

The hydrodistillation yield was 0,24%, lower than the values reported in literature. The hydrodistilled oil shows a similar composition as compared to the SFE extract (see Table 1, column HD). An important amount of *trans*-anethol was detected in both extracts. No previous reference was found about the presence of this compound in *Tagetes minuta* L.

4. References

Daghero J. and M. Mattea, 1996. "Aceite esencial de *Tagetes minuta* L. de la zona de Río Cuarto. Cambios en su composición química en los distintos estadios de crecimiento", X Congreso Nacional de Recursos Naturales Aromáticos y Medicinales, La Plata, 1996.

European Pharmacopeia, 1975. Maisonneuve SA, Sainte Ruff, Vol. 3: 68-71.

Fenaroli's Handbook of Flavor Ingredients, 1971. Chemical Rubber Co., p. 204.

Hétheléyi E., B. Dános and P. Tétényi, 1986. "GC/MS Análisis of the Essential Oils of Four Tagetes Species and the Anti-microbial Activity of Tagetes minuta". Flav. & Fragr. Journal, 1: 169-173.

Reverchon E. and F. Senatore, 1994. "Supercritical Carbon Dioxide Extraction of Chamomile Essential Oil and its Analysis by Gas Chromatography-Mass Spectrometry". J. Agric. Food Chem., 42: 154-158.

Reverchon E., 1997. "Isolation and Fractional Separation of Natural Products by Supercritical CO_2" Proceedings of the 4th International Symposium on Supercritical Fluids, Sendai (Japan) Ed. K. Arai, Vol C: 839-844.

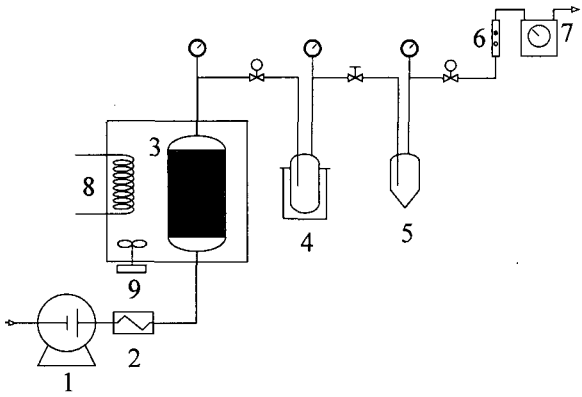
Stahl E, W. Quirin & D.Gerard, 1988. *"Dense Gases for Extraction and Refining"*. Springer Verlag, Berlin: 160-163.

Table 1 - Identification and quantitation of compounds found in *Tagetes minuta* L oil extracted by SFE and by hydrodistillation[a]

Compound	RT (min)	SFE %	Waxes %	HD %
β-Myrcene	16,5	0,24	—	0,20
para-cymene	18,6	1,12	—	tr.
Limonene	**19,2**	**15,14**	—	**12,19**
***cis*-Ocimene**	**20,3**	**19,33**	—	**20,93**
***Trans*-Ocimene**	**20,6**	**0,30**	—	**0,09**
Dihidro tagetone	**21,2**	**5,19**	—	**3,84**
γ-Terpinene	21,3	0,26	—	0,14
cis-Linalool oxide	22,2	0,43	—	0,14
Dimethyl stirene	23,3	0,22	—	—
Trans-p-ara-Menth-2-en-1 ol	24,2	0,12	—	—
Linalool	24,4	1,03	—	1,01
α-Thujone	25,4	0,24	—	0,27
cis-Sabinene hydrate	26,5	3,68	—	4,63
Camphor	27,2	0,11	—	0,12
***trans*-Tagetone**	**27,5**	**2,81**	—	**9,43**
***cis*-Tagetone**	**28,2**	**0,34**	—	**3,18**
cis-Pinanone	29,1	0,30	—	0,24
4-Terpineol	29,5	0,46	—	1,88
α-Terpineol	30,6	0,50	—	0,10
cis-Dihydro carvone	31,1	0,12	—	—
Elsholtzia ketone	31,5	0,15	—	0,17
Verbenone	32,1	0,05	—	0,86
Citronellol	32,5	0,61	—	—
cis-Carveol	33,1	0,23	—	0,91
***cis*-Ocimenone**	**34,1**	**13,26**	—	**4,34**
***trans*-Ocimenone**	**34,3**	**0,65**	—	**2,24**
para-Anis aldehyde	35,2	2,10	—	—
Linalyl acetate	35,5	0,47	—	—
Methyl citronellate	36,1	0,39	—	—
***Trans*-Anethole**	**37,5**	**9,98**	—	**3,08**
Dehydro elsholtzia ketone	38,5	1,51	—	2,54
Tridecane	39,3	0,12	—	—
δ-Elemene	41,2	3,00	—	—
Neryl acetate	41,5	0,19	—	0,38
Trans-Isosafrole	42,0	0,32	—	—
β-Patchoulene	43,3	0,10	—	—

Compound	RT (min)	SFE %	Waxes %	HD %
Geranyl acetate	44,3	0,28	—	—
Tetradecane	45,3	0,16	—	—
Methyl eugenol	45,4	0,24	—	0,47
Caryophyllene	46,2	3,44	—	4,73
Aromadendrene	47,4	0,33	—	0,34
α-Humulene	48,3	2,14	—	2,65
γ-Ionone 6 methyl	48,5	0,22	—	—
Valencene	51,1	0,57	—	0,95
α-Muurolene	51,3	0,29	—	—
n-Pentadecane	51,5	0,13	—	—
β-Bisabolene	52,1	0,08	—	0,75
γ-Cadinene	52,2	0,12	—	0,15
δ-Cadinene	52,6	0,06	—	—
Eugenyl acetate	53,1	0,15	—	—
γ-Elemene	54,3	0,11	—	0,64
Ledol	55,3	0,29	—	—
Spathulenol	**56,1**	**2,42**	—	**16,4**
Guaiol	56,3	0,28	—	—
Alchenenotiol	71,0	3,56	—	—
Methyl linoleate	**82,2**	—	**23,29**	—
Methyl stearate	86,5	—	2,11	—
Docosan-7-9-dione	**94,1**	—	**24,57**	—
Pentacosane	102,2	—	2,69	—
Heptacosane	106,2	—	0,28	—
Methyl heptacosane	108,3	—	0,45	—
Octacosane	110,0	—	0,64	—
Methyl nonacosane	112,4	—	2,84	—
Nonacosane	114,1	—	0,62	—
Triacontane	120,3	—	2,35	—
Hentriacontane	127,1	—	1,24	—
Dotriacontane	129,3	—	2,03	—
Tritriacontane	**135,4**	—	**16,33**	—
Pentatriacontane	146,3	—	3,32	—
Heptatriacontane	**160,2**	—	**17,24**	—

[a]**RT** = Retention time; **SFE**= Supercritical fluid extraction at 80 bar, 40°C, 60 min ; **%** = percentages calculated by GC peak area integration; **HD** = hydrodistillated; **tr** = traces, percentages lower than 0,05%.



gure 1 - Schematic representation of the laboratory apparatus: 1, pump; 2, heater; 3, extraction vessel; 4, 1st parator; 5, 2nd separator; 6, flowmeter; 7, flow totalizer; 8, electrical heater; 9, fan