THE VOLATILE COMPONENTS OF OLEORESINS AND THE ESSENTIAL OILS OF *FOENICULUM VULGARE* IN ISRAEL[1]

Uzi Ravid, Eli Putievsky, and Nava Snir

Division of Aromatic and Pharmaceutical Plants, Agricultural Research Organization,
Newe Ya'ar Regional Experiment Station Haifa Post, 31-999, Israel

ABSTRACT.—The steam-distilled essential oils from various parts of *Foeniculum vulgare* Mill. var. dulce were examined. Oleoresins were obtained by supercritical carbon dioxide, petroleum ether, and dichloromethane extractions of ripe and unripe umbels, and their volatile components were analyzed.

The important constituents of aromatic herbs and spices are their flavor and aroma components. In sweet fennel, *Foeniculum vulgare* Mill. var. dulce, the chemical composition of the volatile components is an important criterion of plant quality (1-4). The biosynthesis of fennel aroma chemicals occurs during maturation in various parts of the plant (5-7). Secondary reaction products are generated during processing and storage of essential oils. Excessive heat, ultraviolet radiation, oxygen, and such reactive reagents as acids, bases, or enzymes, modify the oil composition because of rearrangement, isomerization, oxidation, or hydrolysis reactions (8).

Essential oils obtained by steam distillation and essential oils from spice extracts differ in taste, odor, appearance, and chemical composition (9,10). A novel extraction method with supercritical fluids, such as carbon dioxide, has the advantage of using a cheap, nontoxic, and nonflammable gas with retention of the starting material's aroma and flavor (11,12).

MATERIALS AND METHODS

PLANT MATERIAL.—Commercial sweet fennel seeds were sown in Newe Ya'ar, Israel, in early winter. The cultivation practices have been described elsewhere (13). Unripe (green) and ripe (brown) umbels were separated from the plants at a late fruiting stage and extracted immediately.

EXTRACTION.—Extractions with dichloromethane and petroleum ether (boiling point, 40-60°) were carried out using a small- (250 ml) and a large- (3000 ml) scale Soxhlet apparatus. After 24 h, the resulting mixture was cooled to room temperature and filtered, and the remaining solvent was removed in a short-path still. The viscous extract was dissolved in hexane prior to injection on glc columns. Supercritical carbon dioxide extraction was carried out with a Nova-Werke semi-industrial apparatus consisting of four main parts: an extraction vessel, a pressure-reducing valve, a separator, and a compressor for fluid recycling. The umbels were extracted for 5 h under a pressure of 200 atm at 40°.

DISTILLATION.—Steam distillation of the oils from leaves, stems, and umbels was performed at early and late fruiting stages. The separated parts of plants were submitted to distillation immediately after harvesting. Essential oils were obtained from a laboratory essential oil determination apparatus (a modified Claevenger type). After distillation—a period of 2 h proved to be sufficient for an almost complete isolation—the oil was cooled to room temperature and separated from the distillation water. The oil was dried over anhydrous sodium sulfate and stored in a refrigerator at 2-6°.

ANALYSIS.—A hexane solution of oleoresin (0.1 μl) or a neat sample (0.1 μl) of essential oil was injected on the following glc columns: (A) 5% carbowax 20 M on Chromosorb W 80-100 mesh, 2.5 m × 4 mm (id) glass, 30 ml/min N_2 flow rate. Temperature was programmed at 4°/min, from 80° initial temperature to 200° (*trans*-anethole retention time = 14 min); (B) 6% OV-17 on Chromosorb W 60-80 mesh, 2.5 m × 4 mm (id) glass, flow rate and temperature programming as for column (A) (*trans*-anethole retention time = 15 min). Solid supports were acid washed and treated with dichloromethylsilane. Chromatograms and postintegration reports were obtained on the following gas-chromatographs: a Varian model 3700

with flame ionization detector, connected to a Hewlett Packard Model 3390 A reporting integrator; a Packard-Becker model 7400 with flame ionization detector, connected to a Spectra-Physics System I computing integrator.

The commercial compounds used as standards in gas chromatography were supplied as gifts from SCM Co. and CA Aromatic Co., USA.

RESULTS AND DISCUSSION

The main components of sweet fennel oil isolated from various parts of the plant (tables 1 and 2) were identified by co-injection of the oils with authentic samples on polar

TABLE 1.   Volatile components of sweet fennel oils in the early fruiting stage

Component	Plant Part (%)			
	Leaves	Stems	Unripe Umbels	Ripe Umbels
α-Pinene	1.7	2.7	1.4	0.1
Camphene	0.1	0.1	0.1	trace
β-Pinene	0.2	0.5	0.2	0.1
Myrcene + α-Phellandrene . . .	1.4	1.6	1.0	0.3
Limonene	73.9	53.6	18.7	2.8
γ-Terpinene	3.3	3.2	0.9	0.2
p-Cymene	trace	trace	trace	trace
Fenchone	1.4	0.2	11.5	8.7
Camphor	1.6	4.5	1.4	0.2
Estragole	0.2	1.0	2.8	3.3
trans-Anethole	11.4	28.6	60.2	84.2
Anisaldehyde	0.1	trace	0.5	trace
Total	95.3	96.0	98.7	99.9
Yield of oil	0.5	0.2	1.2	3.0

and nonpolar columns. The oil was distilled from leaves, stems, and unripe and ripe umbels in early and late fruiting stages. The maximum content of oil was found in ripe umbels in the early fruiting stage (3.0% v/w), and the minimum oil content was found in leaves in the late fruiting stage (0.1%). Lal and Sen (14) found that the maximum yield of oil was obtained from seeds collected before the plants were fully ripe.

TABLE 2.   Volatile components of sweet fennel oils in the late fruiting stage

Component	Plant Part (%)			
	Leaves	Stems	Unripe umbels	Ripe umbels
α-Pinene	3.1	2.7	1.2	0.3
Camphene	0.1	0.1	0.1	trace
β-Pinene	0.3	0.3	0.2	trace
Myrcene + α-Phellandrene . . .	1.4	1.7	0.8	0.1
Limonene	57.3	68.3	14.2	4.1
γ-Terpinene	2.4	4.4	0.8	0.2
p-Cymene	trace	trace	trace	trace
Fenchone	0.5	0.1	6.2	6.4
Camphor	3.5	3.6	0.6	0.2
Estragole	0.3	0.5	2.9	3.8
trans-Anethole	21.7	13.7	71.0	84.4
Anisaldehyde	0.2	0.2	trace	trace
Total	90.8	95.6	98.0	99.5
Yield of oil	0.1	0.2	1.5	1.6

Ripe umbels contained most of the oxygenated compounds (96.4%, 94.8%). Leaves from the early fruiting stage were rich (80.6%) in monoterpene hydrocarbons. *Trans*-anethole was the major component of ripe umbel oils (84.2%, 84.4%) and of unripe umbel oil (60.2%, 71.0%). In stems, the content of *trans*-anethole decreased (28.6% to 13.7%) during plant ripening. The content of limonene was high in leaves (73.9%, 57.3%) and in stems (53.6%, 68.3%). Small amounts of limonene were found in ripe umbels (2.8%, 4.1%). Von Tóth (6,7) reported that limonne content of European sweet fennel was 37-40% in leaves, 27-33% in stems, 14% in young fruits, and 5% in ripe fruits.

Fenchone content was higher in ripe umbels (8.7%, 6.4%) and unripe umbels (11.5%, 6.2%) than in leaves (1.4%, 0.5%) or stems (0.2%, 0.1%). Betts (15,16) reported that the fenchone content of European sweet fennel was 8% in developing fruits and not less than 10% in mature fruits. Karlsen, *et al.* (3) reported 2-5% fenchone in brown fruits and 11-14% in green fruits. Canadian fennel herb and fruit oils were found to contain *trans*-anethole (68.9%, 39.2%), limonene (19.8%, 8.3%), and fenchone (16.1%, 10.8%) (17).

The autooxidation reaction product of *trans*-anethole-anisaldehyde (8) was found in small quantities in the fresh oils. Acetaldehyde was not detected in the oils. Dry oils filled in bottles under a CO_2 blanket and closed tightly and stored at 2-6° did not show any significant change in their initial composition and aroma after 6 m. Addition of traces of pure anisaldehyde to the fresh oils did not cause deterioration of their aroma, whereas trace amounts of synthetic acetaldehyde caused a dramatic decrease in their quality. *Cis*-anethole was found in trace amounts in all the fresh oils. Addition of traces of *cis*-anethole to the fresh oils did not influence their quality.

Both ripe and unripe umbels were extracted by two types of solvents: dichloromethane, and petroleum ether (boiling point, 40-60°). Unripe umbels were also exracted by a supercritical fluid, carbon dioxide (table 3); CH_2Cl_2 extraction of ripe

TABLE 3. Volatile components of sweet fennel oleoresins obtained by three extraction solvents

Component	Plant Parts (%)				
Umbels Stage:	Unripe	Unripe	Unripe	Ripe	Ripe
Solvent:	CH_2Cl_2	Petroleum ether	CO_2	CH_2Cl_2	Petroleum ether
Color:	Dark green	Yellow	Yellow	Brownish yellow	Yellow
α-Pinene	2.0	0.7	1.0	1.7	0.6
Camphene	0.1	trace	trace	0.1	trace
β-Pinene	0.1	1.5	trace	0.7	0.7
Myrcene +α-Phellandrene . .	1.0	0.3	1.0	0.9	0.3
Limonene	20.1	21.6	20.4	13.2	11.5
γ-Terpinene . . .	0.3	0.6	0.7	0.9	0.4
p-Cymene	trace	trace	trace	trace	trace
Fenchone	6.0	5.7	5.2	6.9	4.1
Camphor	trace	0.5	0.8	trace	trace
Estragole	2.9	2.7	2.6	2.5	2.3
trans-Anethole . .	67.0	63.8	65.6	72.1	71.4
Anisaldehyde . . .	0.2	0.2	trace	0.5	0.4
Total	99.7	97.6	97.3	99.5	91.7
Yield of oleoresin .	2.5	1.2	2.0	3.7	1.9

umbels yielded 3.7% oleoresin, while petroleum ether extraction of unripe umbels gave the lowest yield: 1.2%. The maximal content of oxygenated compounds was found in the CH_2Cl_2 extract of ripe umbels (82.0%). *Trans*-anethole (72.1%) and fenchone (6.9%) were the main constituents of this type; limonene (13.2%) was the main monoterpene hydrocarbon in this extract, and CH_2Cl_2 extraction of unripe umbels gave more monoterpene hydrocarbons (23.6%) and fewer oxygenated compounds (76.1%).

The novel technology of high-pressure extraction with carbon dioxide has some advantages: it requires minimal heat, and the solvent is completely harmless and oxygenfree. The trace amount of anisaldehyde in the CO_2 unripe umbel extract proves that autooxidation and degradation of *trans*-anethole were very limited. Because CO_2 is inert, no free oxygen could react with any component in the extract.

The aroma quality of the extracts was closer to that of the original plant than was the aroma of the steam-distillerd essential oils.

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