

Comparison of the Volatile Composition of Wild Fennel Samples (*Foeniculum vulgare* Mill.) from Central Spain

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Comparison of the volatile composition of fennel (*Foeniculum vulgare* Mill.) has been carried out using direct thermal desorption (DTD) coupled to gas chromatography–mass spectrometry. Forty-two wild fennel stem samples were collected in two different geographical areas of Central Spain. DTD allowed a high recovery of volatiles from small sample sizes without thermal decomposition. *trans*-Anethole was the main volatile compound for most cases, although a high variability was found among samples, showing clear phytochemical differences.

KEYWORDS: Volatile composition; direct thermal desorption; DTD; *Foeniculum vulgare* Mill.; fennel

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is a Mediterranean aromatic plant belonging to the Apiaceae family. Some authors (1) distinguish two subspecies, *piperitum* and *vulgare*: Most Spanish wild plants seem to correspond to subspecies *piperitum*, with bitter seeds, while subspecies *vulgare* (called by other authors *ssp. capillaceum*) has sweet seeds used as flavorings in baked goods, meat and fish dishes, ice cream, and alcoholic beverages (2), due to its characteristic anise odor, and is cultivated in Mediterranean countries. Morphological differences between these two subspecies are not always clearly defined (1). The major components of fennel seed essential oils are *trans*-anethole, fenchone, and estragole (methyl chavicol) in the case of *ssp. vulgare* and α -phellandrene in *ssp. piperitum*. The relative concentration of these compounds varies considerably depending on the phenological state and origin of the fennel (3–6). Like its seeds, its stems present also an aniselike aroma and are used as flavorings for Almagro eggplants.

The odor produced by fennel and other aromatic plants is caused by their volatile compounds. The accumulation of these compounds inside the plant is variable, appearing practically in any of its parts (roots, stems, sheets, flowers, and fruits) (4, 7).

Gas chromatography–mass spectrometry (GC-MS) is commonly used to study the volatile composition of aromatic plants; however, this technique requires a previous step of extraction and concentration of the volatile fraction. Different extraction methods can be used for this purpose. Hydrodistillation and steam distillation are two of the most used techniques; however, these methods require large amounts of sample and in general

are time-consuming (6, 8). Although simultaneous distillation–extraction (SDE) has been widely used to obtain plant volatiles from smaller sample sizes (9, 10), it is well-known that monoterpenes can also be vulnerable to chemical changes under SDE conditions. Supercritical fluid extraction can provide high-quality products (7, 10), but monoterpenes hydrocarbons are extracted at low proportions, since CO₂ depressurization can produce losses of the most volatile compounds (11). For the characterization of volatile compounds in aromatic plants, solid-phase microextraction can be used, although the recovery of this technique is only partial and depends on the compound (12, 13). Direct thermal desorption (DTD) is a rapid and solvent-free technique very useful to remove the volatile components of low-moisture samples. It requires small amounts of sample and avoids both preliminary fractionation and posterior concentration steps. It has been successfully used in the analysis of the volatile compounds in aromatic plants and seeds (14–17) and in oak woods (18). DTD reproducibility is comparable to that obtained with SDE and solvent extraction, depending on the type and amount of the compound being analyzed; RSD values are in the range of 5–9% (14). DTD thermal decomposition appears to be lower than in distillation-based techniques such as SDE.

The aim of this study was to compare the volatile composition of wild fennel samples collected at two different Central Spanish localities, in order to improve the knowledge of the biodiversity of aromatic plants typical of Mediterranean countries.

MATERIALS AND METHODS

Plant Material. The sample set included fennel stems of wild plants from two geographical locations, in the centre of the Iberian Peninsula, in Spain, 26 samples from Castilla-La Mancha, and 16 samples from Madrid. Fennel stems were dried at room temperature and ground before their introduction into the cartridges.

DTD. An automatic thermal desorption apparatus (ATD400, Perkin-Elmer, Norwalk, CT) coupled to a GC-8000 gas chromatograph (Fisons,

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Milan, Italy) equipped with a MD-800 quadrupolar mass detector (Fisons, Manchester, United Kingdom) was used to isolate the volatile components. Ground fennel stems (15 mg) were introduced into a desorption cartridge between two glass-wool plugs and heated to 180 °C under a carrier gas flow (helium) of 40 mL/min for 15 min. Then, volatile compounds were cryofocused on a cold trap at -30 °C and desorbed at 220 °C in a few seconds allowing rapid transfer to the GC capillary column. The flow rates through the system and the amount of volatiles transferred to the column were controlled by an outlet split (5 mL/min) placed after the cold trap. Other conditions are detailed in Esteban et al. (14).

GC-MS. A GC-8000 gas chromatograph (Fisons) equipped with a MD-800 quadrupolar mass detector (Fisons) in electron impact mode at 70 eV was used to carry out the GC-MS analysis. Volatile compounds were separated on a polyethyleneglycol column (50 m × 0.25 mm × 0.25 μm film thickness). The oven temperature, set at 60 °C for 2 min, was then programmed at 8 °C/min to 180 °C and maintained for 30 min. Mass detector conditions were as follows: interface temperature, 180 °C; mass acquisition range, 40–350. The carrier gas was helium.

Identification of the volatile components was performed comparing their GC retention times and mass spectra with those of authentic standards from Sigma-Aldrich. Semiquantitative data were calculated from the GC peak areas without using correction factors and were expressed as relative percentage (peak area %) of the total volatile constituent identified.

Multivariate Data Processing. Processing of semiquantitative results was carried out by using the STATISTICA for Windows program package (19). The unsupervised techniques used were multidimensional scaling, principal components analysis, and cluster analysis (tree and *K* means clustering).

RESULTS AND DISCUSSION

Tables 1 and 2 show the volatile composition (peak area %) of the fennel stems analyzed by DTD-GC-MS. Thirteen components showing high relative concentrations in the analyzed samples, and including monoterpenoids and phenylpropanoids, were selected among the identified compounds. Most samples from Castilla-La Mancha had *trans*-anethole as their main volatile compound (**Table 2**), whereas samples from Madrid (**Table 1**) presented a high variability in their composition.

Multivariate analysis was applied to the relative concentration data for the 13 selected compounds in the 42 analyzed samples, in order to point out possible trends in sample composition.

Multidimensional scaling produces bidimensional plots in which sample distance reflects dissimilarities among sample composition. **Figure 1**, obtained from the Euclidean distance matrix calculated from relative compositions, plots in a compact group most of the Castilla-La Mancha samples, which are characterized by high concentrations of *trans*-anethole; the concentration of this compound is directly related with positive values for dimension 1. Samples C1, C8, and C13 from Castilla-La Mancha are loosely distributed at the center and left of the plot, together with all samples from Madrid (M1–16).

Principal component analysis from covariance matrix shows that two components explain 91.9% of the total variance. **Figure 2**, which uses as the axis first and second principal components, shows a sample distribution similar to that plotted in **Figure 2** for multidimensional scaling. The first component (80.7%) is clearly related to *trans*-anethole; α-pinene, 1,8-cineole, and limonene have positive loadings for the second component, while fenchone and estragol present a negative contribution.

Cluster analysis was also applied to semiquantitative data in order to point out possible groups among the samples. **Figure 3** presents the dendrogram corresponding at the complete linkage mode, when using the Euclidean distance matrix. Two main groups are clearly defined.

The first group (group 1, left) includes 23 Castilla-La Mancha samples, characterized by their high relative content of *trans*-anethole. Seven of these samples (center) form a subgroup, which presents *trans*-anethole concentrations between 68 and 85%, while the values of the rest of the samples in group 1 are higher than 88%. The second group was constituted by 19 samples, three from Castilla-La Mancha and all of the Madrid samples, with a variable volatile composition: These samples appear loosely distributed in **Figures 2 and 3**. Although several subgroups could be defined in group 2, separation is not clear.

For this reason the *K* means clustering method was applied to the data. The best results were obtained with four groups: Results are summarized in **Table 3**. Group A includes the 23 samples, all from Castilla-La Mancha, which appeared as group 1 in the dendrogram of **Figure 3**; the mean concentration value for its main component, *trans*-anethole, was 89.5%, while fenchone presented a mean value of 6.7%. *trans*-Anethole

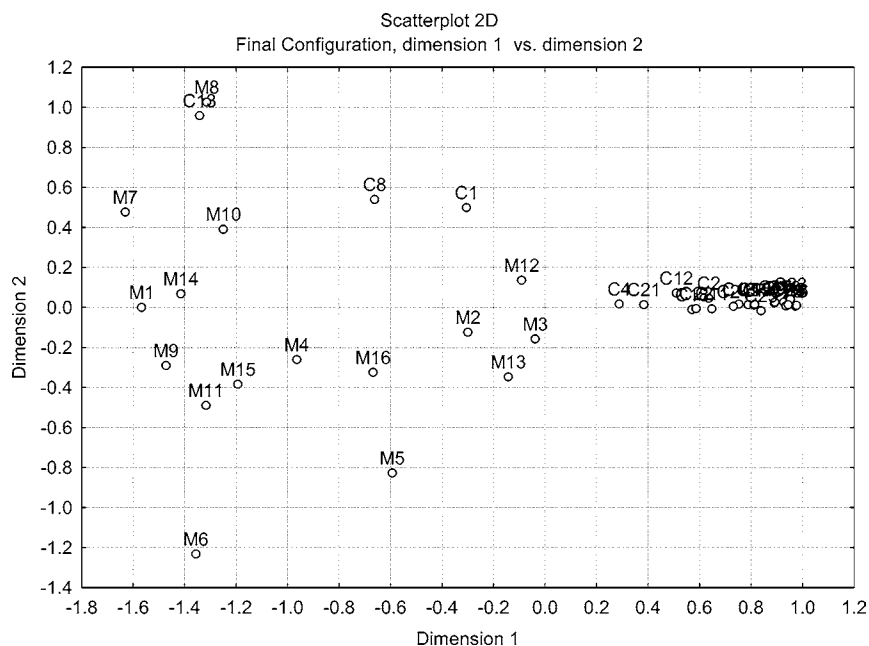


Figure 1. Bidimensional plot of fennel samples obtained by multidimensional scaling (M, Madrid; C, Castilla-La Mancha).

Table 1. Volatile Constituents (%) of Different Types of Wild Fennel from Madrid (Middle Spain) Analyzed by DTD-GC-MS^a

compound	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16
α -pinene	6.3	1.2	1.1	5.9	1.9	21.3	7.0	2.2	5.1	2.1	1.0	0.6	3.4	1.8	2.4	0.5
camphene	0.6	0.4	0.3	0.7	0.6	1.1	0.2	0.4	0.4	0.5	0.6	0.3	0.2	0.3	0.3	0.3
β -pinene	0.5	0.2	0.2	1.2	0.3	1.5	0.5	0.2	2.7	2.9	1.0	0.1	1.2	0.5	0.4	Tr
β -myrcene	3.8	1.7	2.1	4.3	1.8	7.8	4.0	1.9	2.7	2.2	5.8	2.2	2.4	3.3	4.6	2.9
α -phellandrene	21.8	3.5	0.2	24.9	0.3	0.4	14.9	0.5	39.5	0.2	31.4	8.5	Tr	26.7	25.8	33.3
1,8-cineole	7.5	11.6	13.1	8.4	41.2	46.1	0.6	2.5	8.9	21.7	27.2	3.4	21.2	8.5	23.5	6.6
limonene	3.4	4.0	5.0	3.2	1.3	12.4	5.2	0.1	0.4	0.4	Tr	0.9	7.7	4.7	6.5	0.6
β -phellandrene	Tr	Tr	0.2	0.1	0.1	0.5	1.5	Tr	Tr	Tr	Tr	0.1	0.5	0.6	1.5	0.3
<i>p</i> -cymene	6.7	4.1	0.2	2.2	1.0	0.1	6.0	0.1	2.1	0.1	1.4	0.8	Tr	1.8	1.3	0.8
fenchone	26.9	21.5	17.7	22.0	13.5	0.7	16.6	18.3	16.4	25.5	12.9	10.5	9.6	17.1	8.3	16.2
camphor	1.1	1.5	0.9	1.0	0.7	1.9	0.5	0.6	0.2	0.7	Tr	0.1	Tr	0.7	Tr	Tr
estragol	17.5	1.3	Tr	0.4	0.3	Tr	40.3	58.6	10.6	29.8	6.7	16.3	1.5	25.6	9.9	1.0
<i>trans</i> -anethole	0.7	45.9	55.9	23.1	34.9	3.6	Tr	14.2	8.2	13.6	11.9	56.3	52.0	8.5	15.5	37.3

^a Tr, trace amounts (less than 0.01% of total peak area).

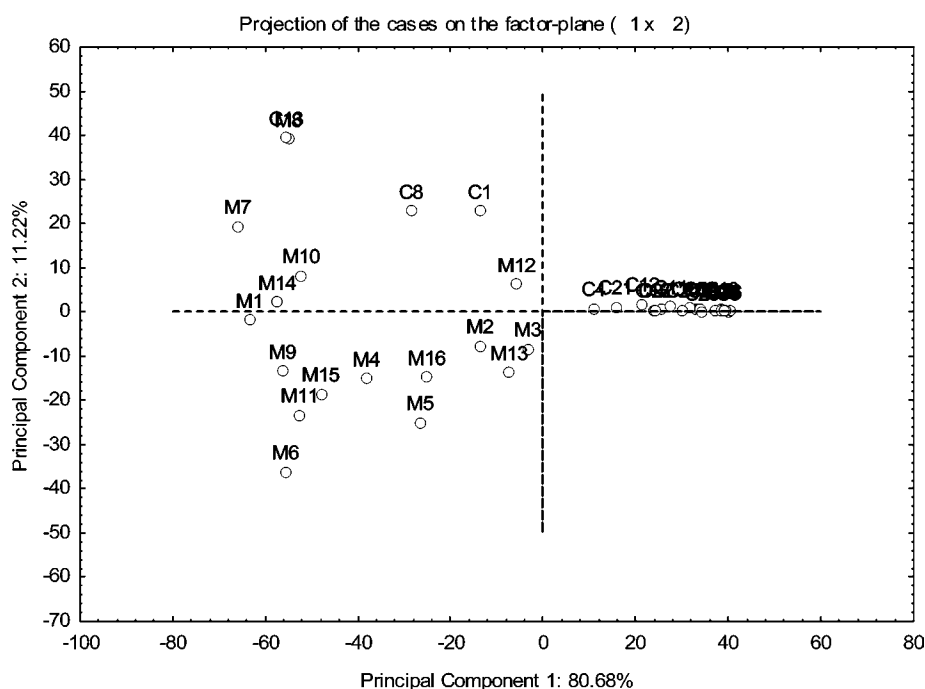
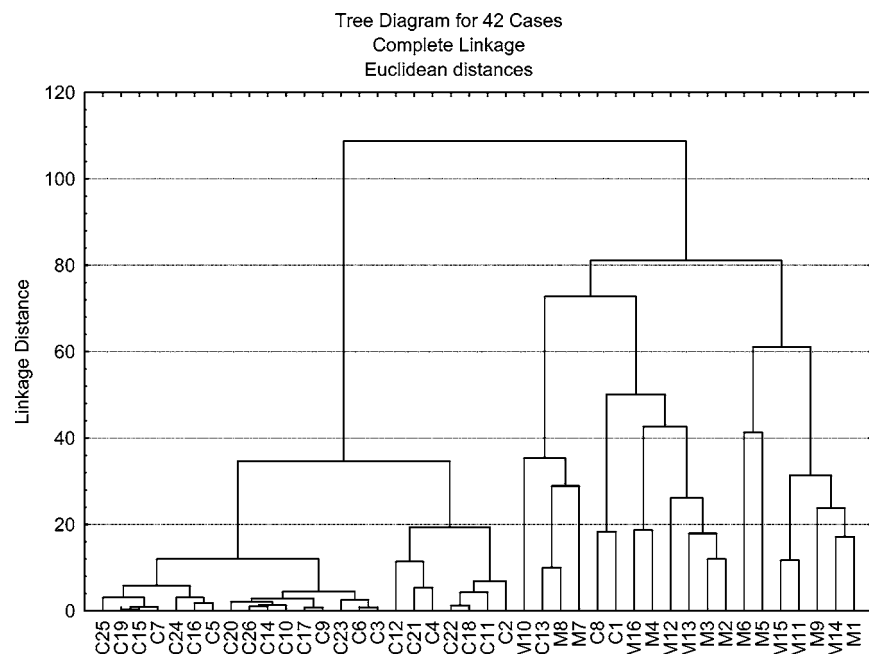
**Figure 2.** Plot of fennel samples on the coordinate grid defined by principal components 1 and 2 (M, Madrid; C, Castilla-La Mancha).**Figure 3.** Dendrogram of Spanish fennel samples using the Euclidean distance matrix.

Table 2. Volatile Constituents (%) of Different Types of Wild Fennel from Castilla-La Mancha (Middle-South Spain) Analyzed by DTD-GC-MS^a

compound	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26
α -pinene	0.03	0.16	0.01	0.51	0.08	0.16	0.02	0.11	0.01	Tr	0.01	0.03	0.85	0.08	0.62	0.28	0.04	0.34	0.36	0.02	0.06	0.40	0.06	0.24	Tr	0.01
camphene	0.02	0.09	Tr	0.09	0.02	0.03	0.01	0.12	0.01	Tr	0.01	0.01	0.06	0.03	0.01	0.01	0.03	0.05	0.05	0.01	0.05	0.06	Tr	0.03	Tr	0.02
β -pinene	0.15	0.59	Tr	0.31	0.32	0.07	0.10	0.06	0.03	0.01	0.06	0.02	0.66	0.13	0.05	0.15	0.40	0.78	0.21	0.09	0.06	0.32	0.10	0.20	0.16	0.03
β -myrcene	0.08	0.40	0.01	0.05	0.14	0.12	0.04	0.14	0.03	0.02	0.08	0.01	0.24	0.01	0.07	0.07	0.10	0.39	0.09	0.06	0.12	0.22	0.06	0.04	0.13	0.02
α -phellandrene	0.35	0.97	0.03	0.22	0.04	0.01	0.40	1.12	0.07	Tr	0.22	Tr	0.22	0.04	0.02	Tr	0.01	0.03	Tr	0.62	0.53	0.02	Tr	0.45	0.02	0.20
limonene	0.16	0.57	Tr	0.07	0.40	0.26	0.03	0.09	0.02	0.21	0.16	0.26	0.72	0.03	0.37	0.73	0.50	1.37	0.54	0.02	0.45	1.49	2.16	0.50	0.87	0.18
β -phellandrene	0.02	0.05	Tr	0.04	Tr	Tr	0.03	0.05	0.01	Tr	0.01	Tr	0.04	Tr	0.01	Tr	0.01	Tr	Tr	0.02	0.06	Tr	Tr	0.04	Tr	0.02
<i>p</i> -cymene	0.22	0.63	0.02	1.39	0.04	Tr	0.46	0.73	0.34	Tr	0.07	0.02	0.39	Tr	0.02	Tr	Tr	0.02	0.01	0.18	0.93	0.03	Tr	0.11	0.04	0.15
fenchone	15.5	6.30	0.17	19.0	7.06	0.40	5.42	27.0	3.28	2.79	12.5	18.3	27.0	2.37	5.59	8.08	3.09	12.4	5.61	0.82	18.3	11.3	0.04	6.84	2.62	1.53
camphor	0.10	Tr	Tr	0.22	0.01	Tr	0.03	0.21	0.03	0.01	0.05	0.20	0.03	0.02	0.09	0.05	0.04	Tr	0.07	Tr	0.08	0.12	Tr	0.07	Tr	0.09
estragol	31.9	1.68	0.10	0.49	0.40	0.39	0.67	31.4	0.23	0.65	0.44	0.54	55.2	0.12	0.63	0.68	0.41	0.05	0.67	0.62	0.62	0.35	0.78	0.58	0.35	0.40
<i>trans</i> -anethole	50.5	83.4	97.4	68.9	91.0	98.1	91.7	36.2	94.6	96.3	85.7	80.2	13.4	97.1	91.9	89.6	94.7	81.8	91.8	95.9	74.1	82.2	96.5	88.0	91.7	96.6

^a Tr, trace amounts (less than 0.01% of total peak area).Table 3. Mean Volatile Composition (Peak Area %) for Each *K* Means Group

variable	groups			
	group A <i>n</i> = 23	group B <i>n</i> = 6	group C <i>n</i> = 5	group D <i>n</i> = 8
α -pinene	0.15	1.35	2.43	5.53
camphene	0.03	0.30	0.26	0.58
β -pinene	0.18	0.36	0.85	0.98
β -myrcene	0.10	1.72	1.70	4.40
α -phellandrene	0.17	2.15	3.39	25.5
1,8-cineole	0.00	15.1	4.97	17.1
limonene	0.49	3.17	1.28	3.90
β -phellandrene	0.01	0.16	0.32	0.38
<i>p</i> -cymene	0.20	1.04	1.47	2.06
fenchone	6.69	14.7	22.9	15.1
camphor	0.05	0.55	0.41	0.61
estragol	0.52	8.54	43.1	8.96
<i>trans</i> -anethole	89.5	49.2	15.5	13.6

represents the characteristic anise, sweet, and liquorice notes of fennel (20) and is typical of *F. vulgare* Mill. ssp. *vulgare*, sweet chemovariety (sweet fennel) (21). Other authors have analyzed chemotypes of similar composition grown in Iran (22). Group B was formed by samples M2, M3, M5, M12, and M13 from Madrid and sample C1 from Castilla-La Mancha. The mean value for *trans*-anethole was 49.2%, and other important components were 1,8-cineole (15%) and fenchone (14.7%).

Five samples (M7, M8, M10, C8, and C13) form group C, which is characterized by a high concentration of estragol with a mean value of 43.1%; fenchone is also present in high concentration (mean value, 22.9%) followed by *trans*-anethole (15.5%). The results obtained are similar to those recently reported (4–6) for fennel seeds of an estragol chemovariety of *F. vulgare* Mill. ssp. *vulgare*. This volatile composition has also been found in wild fennel seeds from the North of Spain (3) and from Tuscany, Italy (8). Estragol and *trans*-anethole have anise and sweet odors, whereas fenchone presents camphoraceous notes (20).

Group D includes eight samples (M1, M4, M6, M9, M11, and M14–16, all from Madrid). It presents a low content of *trans*-anethole (mean value, 13.6%), and its most distinctive component is α -phellandrene, with a mean value of 25.5%. The presence of relatively high amounts of α -phellandrene agrees with the reference data for ssp. *piperitum* (pepper fennel) (6), while low concentrations of *trans*-anethole and estragol are not usual in *vulgare* subspecies (6).

In conclusion, most Castilla-La Mancha plants have a similar composition (group A in cluster results, *trans*-anethol being the main component). However, all plants collected in the Madrid area and three Castilla-La Mancha samples present a highly variable composition. Multivariate analyses graphical plots show a continuous distribution of concentrations, although some groups can be defined on the basis of their chemical composition. Compounds characteristic of these groups have been found in other *F. vulgare* subspecies or chemovarieties. A previous analytical study is then necessary, when wild plants are selected for cultivation, since the organoleptic properties of the cultivated fennel will depend on its volatile composition.

The main advantages of DTD for this study are the possibility of on-line coupling with GC-MS, the elimination of fractionation and concentration steps, the reduction of the analysis time, the small amount of plant required, the high volatile recovery, and the low production of thermal artifacts. Statistical analysis of the distribution of volatile compounds in plants is required to determine them in a high number of samples. Parameters such

as time, temperature, and flow rate can also be selected in order to optimize the extraction for a given sample. DTD, a fast technique for volatile fractionation that uses a small sample amount, is useful for this purpose and can also be used to analyze volatile compounds of different parts of plants, such as seeds, roots, and even woods.

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Received for review April 5, 2006. Revised manuscript received July 13, 2006. Accepted July 20, 2006. The Spanish Ministerio de Ciencia y Tecnología is gratefully acknowledged for financial support (Project AGL 2003-00188). This work was also financed in part by the Comunidad de Madrid, Program S-505/AGR-0312.

JF0609532