Characterization of Some Italian Types of Wild Fennel (*Foeniculum vulgare* Mill.)

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Wild samples of *Foeniculum vulgare* Mill. (fennel) were collected from thirteen Italian localities at different latitudes and grown in field trials to evaluate their morphological and agronomic characteristics and essential oil compositions. All the parameters were recorded at full bloom stage, and the essential oils (obtained by steam distillation) were characterized by gas chromatography (GC) and GC/mass spectrometry (GC/MS). The morphological characteristics showed positive relations among the number of umbels per plant, the plant weight, and the umbel weight percentage, and between these three parameters and the oil content. The essential oils evidenced five chemical groups characterized by (1) α-phellandrene, methyl chavicol, and *trans*-anethole; (2) α-pinene, limonene, and *trans*-anethole; (3) methyl chavicol and α-phellandrene; (4) methyl chavicol and α-pinene; and (5) α-phellandrene.

Keywords: *Fennel; Foeniculum vulgare Mill.; Apiaceae; wild germ plasm; morphological characteristics; essential oil composition; chemical groups*

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is a well-known aromatic plant belonging to the Apiaceae family and is typical of the Mediterranean area. It can be an annual, biennial, or perennnial herb and is divided into two subspecies: *piperitum* and *capillaceum* which includes three varieties: var. *vulgare* (Mill) Thell., var. *dulce* (Mill) Thell, and var. *azoricum* (Mill) Thell. (Verghese, 1990). The sp. *capillaceum* is the more known and studied, and is usually employed in different applications. The first two varieties are used to flavor foods and liqueurs and the third one is extensively cultivated as a vegetable. The chemical compositions of their oils have been well described, as have their biological properties (Seher and Ivanov, 1976; Marotti et al., 1994). However, the sp. *piperitum* (pepper fennel) has been less investigated. This type, also known as Italian or Sicilian fennel or "carosella", grows wild and is a perennial herb (Muenscher and Rice, 1955). Its morphological characters are variable with regard to size, habit, shape and color of leaf and fruit, and number of rays in the umbel, and, generally, it can be distinguished from *capillaceum* by smaller umbels, rigid and rather fleshy leaf-lobes, and fruit with sharp taste (Ferrazzi, 1986; Badoc et al., 1994). It is mainly used to flavor fish and meat, giving them strong aroma and taste, and it is also used as an ingredient in cosmetics (EINECS, 1990). Fennel is widespread in middle and southern Italy, and numerous wild types are traditionally employed in folk recipes. The wild fennel has been scantily studied, and investigations devoted to selecting chemotypes suitable for purposive uses or for the introduction in extensive cultivations have not been performed as has been the case for other aromatic species such as mint (Lamaison et al., 1987) and basil (Marotti et al., 1996).

Because of the interest in improving the knowledge and the exploitation of the biodiversity of aromatic plants typical for the Mediterranean area, several types of fennel were collected from wild plants at different Italian localities and grown in field trials to evaluate their morphological traits, agronomic yields, and oil compositions.

EXPERIMENTAL PROCEDURES

Plant Material. Germ plasm of thirteen fennel types, collected from different Italian localities (Figure 1), were grown in northern Italy on a farm of the University of Bologna. The germ plasm of each fennel type was obtained, at the end of summer, from several wild plants grown in a defined area of the cited localities. The trial was performed on a soil with 36% sand, 36% clay, 28% mud, and neutral pH, and without fertilization. The experimental design was a randomized block with three replications. Fennel seeds were sown and grown in a greenhouse for 6 weeks. In May, the plants were transplanted into the experimental field in plots of 6 m^2 with 1 m of distance between rows and plants, and were harvested in summer at the full bloom stage. Each fennel type was evaluated for the morphological characteristics, and a representative sample obtained from the three replications of each type was employed for essential oil extraction and characterization.

Oil Distillation. The fresh aerial parts of the plants (1 kg) were steam-distilled for 1 h in a Clevenger-type apparatus. The oils, dried over anhydrous sodium sulfate, were kept in dark glass bottles at −20 °C before analysis.

Oil Analysis. The essential oils were analyzed by using a Carlo Erba HRGC 5160 Mega gas chromatograph fitted with a FID and a Hitachi D-2000 integrator. The separation of compounds was obtained by injecting a 0.1% oil solution in ethanol into a Supelco SPB5 fused silica column (30 m × 0.32 mm, 0.25 μm film thickness) operating from 70 to 200 °C at 5 °C/min and holding the initial temperature for 18 min. The injection system was on-column, the carrier gas was helium at 1 mL/min, and the detector temperature was 250 °C.

The GC/MS analyses were performed by using a Finnigan Mat ion trap detector (ITD), model 800, set at 70 eV, coupled with a gas chromatograph operating under the same conditions as described above.

The identification of compounds was obtained by comparing the retention time of the peaks with those of standard compounds, by enhancement of peaks with pure substances,

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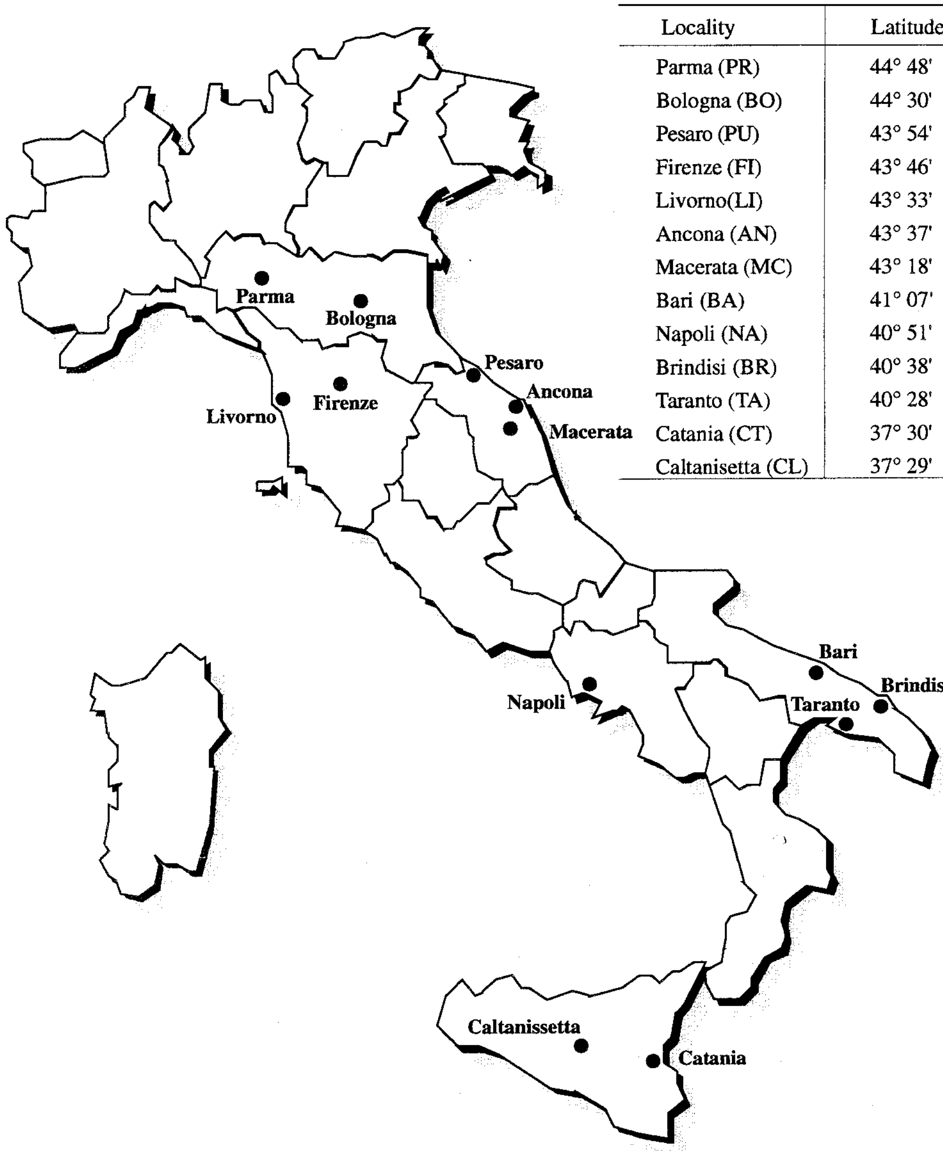

Locality	Latitude
Parma (PR)	44° 48'
Bologna (BO)	44° 30'
Pesaro (PU)	43° 54'
Firenze (FI)	43° 46'
Livorno(LI)	43° 33'
Ancona (AN)	43° 37'
Macerata (MC)	43° 18'
Bari (BA)	41° 07'
Napoli (NA)	40° 51'
Brindisi (BR)	40° 38'
Taranto (TA)	40° 28'
Catania (CT)	37° 30'
Caltanisetta (CL)	37° 29'

Figure 1. Germ plasm collection localities.

and by matching the obtained MS spectra with those reported in the ITD library and in the literature (Adams, 1988).

The quantitative data were expressed as relative percentage of the oil constituents calculated from the GC peak areas without using correction factors, and each oil was analyzed twice.

Clustering of Samples. Samples were grouped by cluster analysis (procedure CLUSTER, SAS, 1997), based on the sum of square analysis of quantitative data of essential oils (Green, 1979).

RESULTS AND DISCUSSION

The fennel types strongly differed in their morphological traits and agronomic yields (Table 1). The plant heights varied from 161 (Pesaro) to 253 cm (Livorno), the number of stems per plant ranged from 9 (Napoli) to 25 (Bari), the number of umbels ranged from 12 (Brindisi) to 340 (Firenze), and the oil content varied from 0.04 (Bologna) to 0.38% (Firenze). The plant sample from Firenze differed significantly from the others for highest number of umbels per plant mainly due to a considerable presence of secondary inflorescences. The same sample was also characterized by a higher plant weight (4.9 kg) and a higher percentage of umbels (9). Considering the weight ratio of stems, leaves, and umbels, a consistent percentage of umbels was also found in the types from Ancona and Taranto, and a very high percentage of stems was found in the types from Livorno, Napoli, and Bari which, at the same time, were scantily leafy. The fennel from Pesaro showed the significantly highest percentage of leaves and the lowest percentage of stems. A high ratio between the number of umbels and number of stems, as shown by the samples from Firenze and Macerata, can be indicative of a potential good seed yield, whereas a low ratio between the two considered characteristics, as found in the samples from Brindisi and Pesaro, can be related to a scanty plant capacity of sexual reproduction. The plant height was significantly and positively related (Table 2) to plant weight ($r = 0.50$) and negatively to leaf weight percentage ($r = -0.53$). This latter characteristic, evidently, was negatively related to stem weight percentage ($r = -0.94$). The number of umbels per plant, the plant weight, and the umbel weight percentage were significantly and positively related among them and all of these three parameters influenced the oil content ($r = 0.65$, 0.57, and 0.73 respec-

Table 1. Plant Characteristics and Yields

locality	plant height (cm)	stems per plant (no.)	umbels per plant (no.)	plant weight (kg)	% plant weight			oil content (%, v/w)	harvesting date
					stems	leaves	umbels		
Parma	176	16	104	2.8	70	26	4	0.26	06/30
Bologna	168	19	56	3.0	76	22	2	0.04	06/30
Pesaro	161	11	20	2.3	58	40	2	0.24	06/30
Firenze	224	15	340	4.9	75	16	9	0.38	07/28
Livorno	253	17	120	4.3	80	15	5	0.37	07/28
Ancona	214	16	155	3.7	68	25	7	0.31	07/28
Macerata	251	15	203	3.8	75	19	6	0.34	07/28
Napoli	241	9	49	1.6	81	17	2	0.20	07/28
Bari	178	25	73	2.3	84	14	2	0.15	07/21
Brindisi	175	9	12	2.7	72	26	2	0.24	06/30
Taranto	162	14	99	2.7	72	21	7	0.31	06/30
Caltanisetta	219	16	56	4.0	73	24	3	0.21	07/04
Catania	199	10	78	3.2	70	24	6	0.20	07/04
LSD ($p \leq 0.05$)	21	7	60	0.9	5	6	2	0.06	
LSD ($p \leq 0.01$)	29	9	82	1.2	7	8	3	0.08	

Table 2. Correlation Coefficients[a]

	stems per plant (no.)	umbels per plant (no.)	plant weight (kg)	stems weight (%)	leaves weight (%)	umbels weight (%)	oil content (% v/w)
plant height (cm)	−0.069	0.476	0.503*	0.431	−0.534*	0.330	0.497
stems per plant (no.)		0.189	0.202	0.466	−0.439	−0.030	−0.194
umbels per plant (no.)			0.733**	0.148	−0.441	0.833**	0.651**
plant weight (kg)				0.032	−0.274	0.679**	0.569*
stems weight (%)					−0.935**	−0.088	−0.124
leaves weight (%)						−0.272	−0.140
umbels weight (%)							0.727**

[a] Significant at $p \leq 0.05$ (*) and at $p \leq 0.01$(**).

Table 3. Chemical Composition (%) Of the Thirteen Types of Wild Fennel[a]

compound	Parma	Bologna	Pesaro	Firenze	Livorno	Ancona	Macerata	Napoli	Bari	Brindisi	Taranto	Caltanisetta	Catania
α-pinene	0.75	7.21	5.02	0.90	3.82	0.84	1.80	21.28	1.25	23.19	13.81	26.54	24.02
camphene	0.19	0.09	0.20	0.18	0.19	0.17	0.12	0.10	0.04	0.09	0.05	0.14	0.11
sabinene	0.23	0.25	0.26	0.31	0.24	0.27	0.43	0.44	0.39	0.37	0.34	0.38	0.47
β-pinene	0.10	1.01	0.53	0.11	0.42	0.09	0.23	2.39	0.16	2.49	1.67	2.61	2.66
myrcene	1.79	1.94	2.02	2.08	1.68	1.68	1.72	2.59	1.48	3.12	1.38	2.83	2.74
α-phellandrene	30.76	15.67	28.66	39.53	24.78	33.75	36.75	18.12	82.07	7.57	9.09	1.97	0.31
hexyl acetate	0.11	0.24	0.24	0.25	0.09	0.18	0.29	0.27	0.06	0.30	0.18	0.22	0.38
δ-3-carene	0.03	0.02	0.03	0.15	0.05	0.03	0.04	tr.	0.02	3.40	1.64	3.49	0.05
p-cymene	2.03	2.12	1.71	1.12	0.62	0.91	0.85	0.24	2.23	0.90	0.59	0.38	0.19
limonene	5.00	3.24	4.80	6.81	3.94	5.09	7.36	2.69	11.35	3.34	1.74	3.38	22.68
cis-ocimene	0.32	0.50	0.89	0.32	0.50	2.29	1.79	0.48	0.04	0.72	0.60	1.61	1.80
trans-ocimene	tr.	0.03	0.03	0.02	0.02	0.09	0.06	tr.	tr.	0.04	0.05	0.10	0.07
γ-terpinene	0.39	0.27	0.13	0.95	0.47	1.13	0.36	0.26	0.97	0.33	0.49	0.58	0.83
fenchone	10.96	8.02	11.22	7.56	9.84	10.70	2.21	2.37	1.70	3.38	2.16	4.58	1.80
camphor	0.12	0.11	0.13	0.08	0.10	0.14	tr.	0.05	0.04	tr.	0.03	0.07	0.09
terpinen-4-ol	0.09	0.06	0.09	0.06	0.08	0.07	0.11	0.10	0.07	tr.	0.08	0.09	0.11
methyl chavicol	23.98	24.45	43.10	40.75	50.52	35.02	45.47	49.35	0.15	38.96	52.17	46.98	8.78
trans-carveol	0.51	0.19	tr.	tr.	tr.	0.11	tr.	tr.	tr.	tr.	0.14	0.03	0.24
carvone	0.58	0.33	0.14	0.05	0.16	0.31	0.14	tr.	0.06	0.09	0.08	0.18	3.80
anisaldehyde	0.07	0.22	tr.	tr.	tr.	tr.	tr.	tr.	n.d.	tr.	0.13	0.05	0.13
trans-anethole	22.09	42.26	6.19	0.83	0.90	4.08	4.35	0.14	0.26	11.57	15.26	2.53	28.31

[a] tr < 0.02.

tively). It was observed that the samples with the tallest plants were those having a late flowering stage. The relations among the different characteristics evidenced that the wild fennel with high oil content could be identified with a type characterized by a tall plant with numerous umbels and late flowering stage. The morphological traits and the yields did not seem to be influenced by the latitude of the origin localities because it was not possible to evidence common characteristics in the samples from germ plasm originated at similar latitudes when grown at the same site. The differences observed can be mainly attributable to genetic factors and to some extent to environmental conditions of the growing locality as also reported for other wild species (Baldoni et al., 2000).

The samples were also very different in essential oil composition (Table 3). The oils were characterized by the presence of 21 compounds (12 hydrocarbons and 9 oxygenated) of which α-pinene, α-phellandrene, limonene, fenchone, methyl chavicol, and *trans*-anethole were the main compounds. All fennel samples, with the exception of those from Parma, Bologna, Bari, and Catania, had methyl chavicol as their main constituent.

Data from essential oil components were used for a cluster analysis to point out possible groups among the samples. The resulting dendrogram (Figure 2) showed

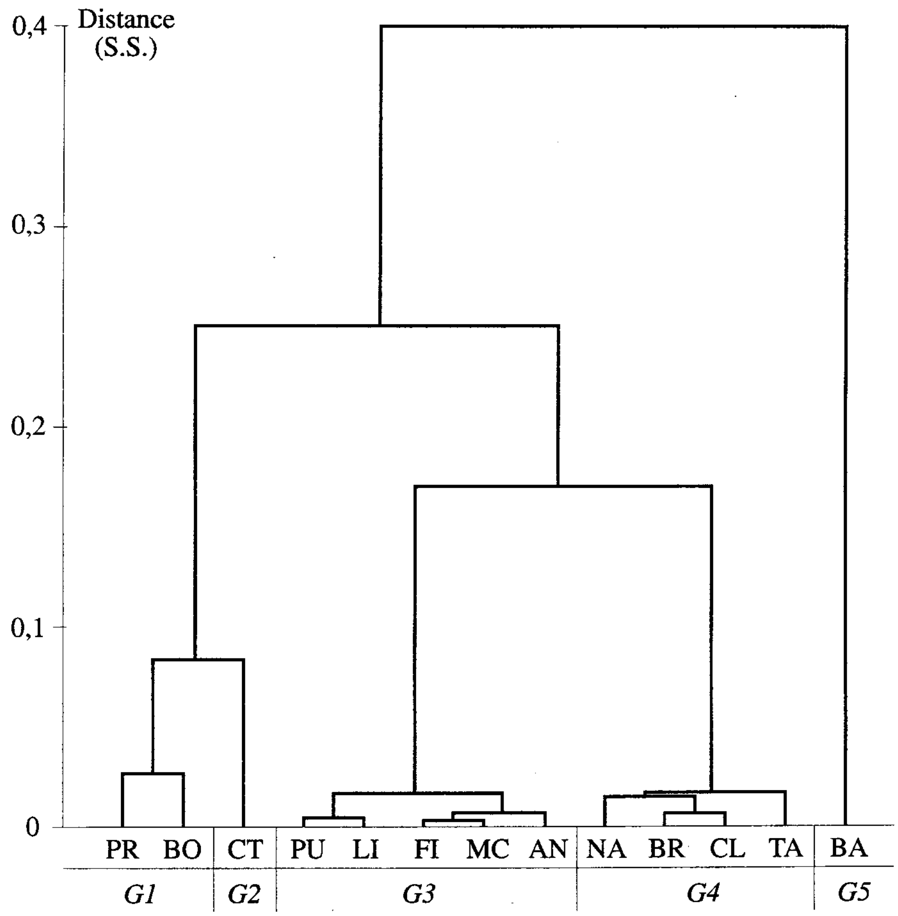

Figure 2. Clustering of fennel essential oils using sum of square analysis (SSA).

Table 4. Main Compounds (%) of Essential Oils from the Five Groups of Wild Fennel[a]

compound	group no. 1	2	3	4	5
α-pinene	4.26	24.02	2.49	20.99	1.25
α-phellandrene	23.41	0.31	32.73	9.31	82.07
limonene	4.15	22.68	5.57	3.10	11.35
fenchone	9.60	1.79	8.31	3.13	1.70
methyl chavicol	24.52	8.77	42.96	47.25	0.14
trans-anethole	30.73	28.30	3.26	7.38	0.26

[a] Average values except for groups 2 and 5.

five main groups of different size determined mainly by the contents of their main components (Table 4). As an example, two GC profiles of oils belonging to different groups are shown in Figure 3. The first group was formed by the samples from Parma and Bologna and was characterized by α-phellandrene, methyl chavicol, and *trans*-anethole as major constituents (30.8, 24.0, 22.1% and 15.7, 24.5, 42.3%, respectively). The second group was represented by a single fennel type, that from Catania, which showed three main compounds: α-pinene, limonene, and *trans*-anethole at very similar concentrations (24.0, 22.7, and 28.3%, respectively). The third group, constituted by the samples from Pesaro, Livorno, Firenze, Ancona, and Macerata, was the largest one and is characterized by fennel types rich in methyl chavicol (from 35.0 to 50.5%) and α-phellandrene (from 24.8 to 39.5%). Within this cluster, the samples from Pesaro and Livorno seemed somehow different from the other ones by having lower concentrations of α-phellandrene. The fourth group included the samples from Napoli, Brindisi, Taranto, and Caltanisetta and was characterized by methyl chavicol (from 39.0 to 52.2%) and α-pinene (from 13.8 to 26.5%). The fifth group was formed by the fennel from Bari which differed greatly from the other ones because α-phellandrene (82.1%) was the dominant compound.

Based on the peculiarity of having only one main compound, it can be supposed that the sample from Bari was relatively "pure" and probably less subjected to hybridation in the past by retaining the characteristics of the wild plant. The presence of high amounts of α-phellandrene (which confers an aromatic pungent note like pepper and a bitter taste), the lack of *trans*-anethole and methyl chavicol (known for their sweet notes), and the presence of small umbels as it is possible to deduce by the lowest umbels weight percentage with respect to a medium plant weight and umbel number, are reasons to consider the fennel from Bari as that more attributable to sp. *piperitum*. In the other samples (except that from Catania) the presence of considerable amounts of *trans*-anethole or methyl chavicol or both, typical for the sp. *capillaceum* (Marotti et al., 1993; Badoc et al., 1994), along with relatively high percentages of α-phellandrene, could suggest the hypothesis of a marked hybridization by cross pollination in the past between sp. *piperitum*, sp. *capillaceum*, and var. *azoricum* in particular, which is extensively cultivated and reproduced in the Mediterranean area. In the oils from the fennel samples belonging to groups 1, 2, 3, and 4, the simultaneous presence of terpenic and phenylpropanoid compounds suggests that two biosynthetic pathways occurred, whereas in the fennel from Bari, the presence of only terpenic compounds evidenced that only one biosynthetic pathway was operating. In fact, it is

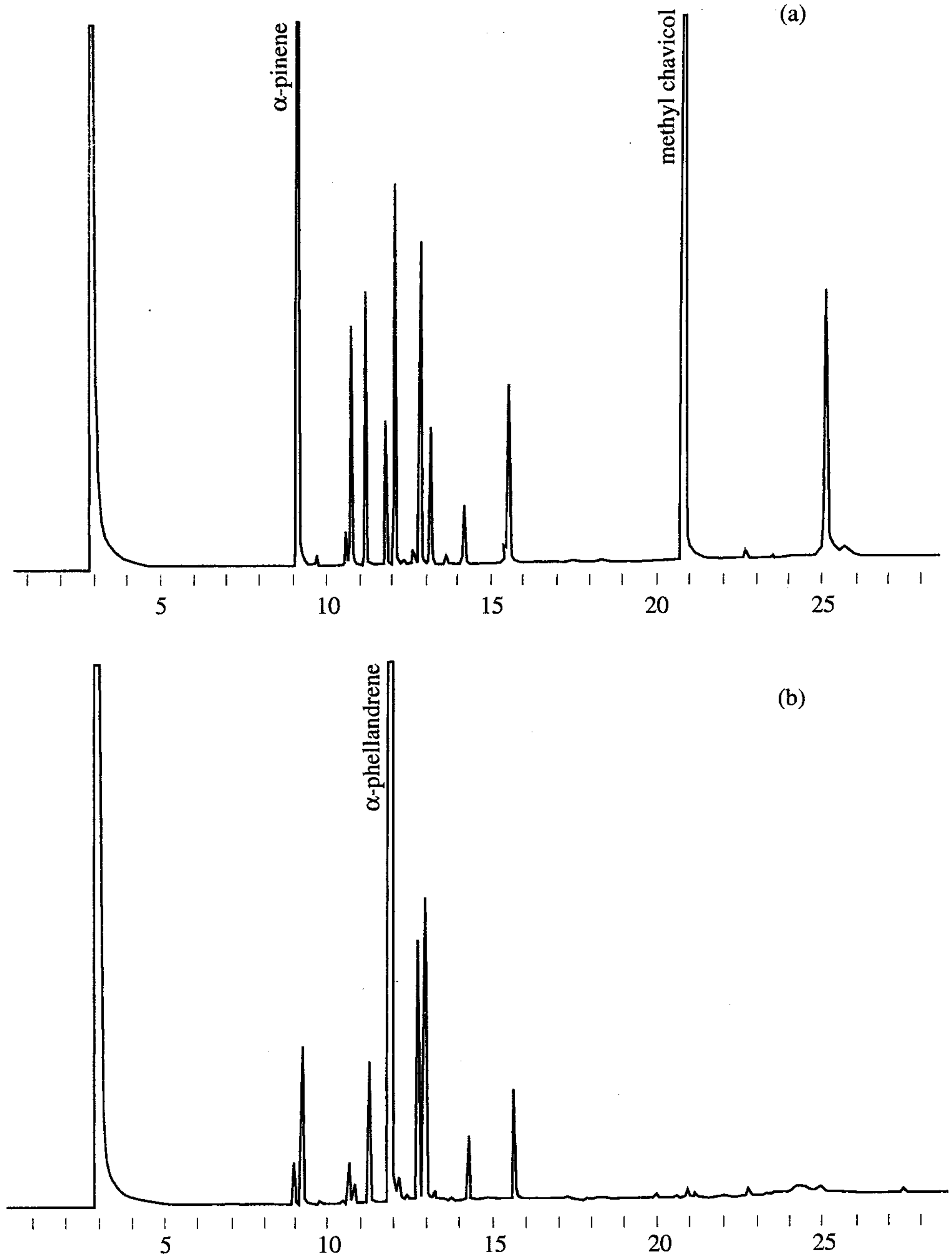

Figure 3. Typical GC profiles of two fennel oils: (a), sample from Napoli belonging to group 4; (b), sample from Bari belonging to group 5.

well-known that the phenylpropanoid compounds such as *trans*-anethole and methyl chavicol have a common biosynthesis originating from the same precursor (L-phenylalanine and cinnamic acid), whereas terpenic compounds such as α-phellandrene and limonene follow another biogenetic pathway from mevalonic acid via geranyl pyrophosphate (Nikänen, 1989). It is also interesting to note that the latitude of origin localities seemed to influence the oil compositions. In fact, the samples belonging to the same groups, as determined by the cluster analysis, were collected from localities at very similar latitudes.

With the exception of the fennel from Bari (which can be, most likely, attributable to sp. *piperitum*), on the basis of the morphological characteristics and essential oil compositions, it is questionable to assign, with certainty, which subspecies the other samples belong to. This difficulty can be attributed to the high outcrossing of the species which results in a wide number of intermediate forms with highly variable characteristics.

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