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Analysis of *Origanum vulgare* volatiles by direct thermal desorption coupled to gas chromatography–mass spectrometry ☆

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Abstract

Volatile components of samples of a population of Spanish *Origanum vulgare* have been analyzed by direct thermal desorption coupled to GC–MS. The method is fast and reliable and requires a low amount of sample, allowing analysis of leaves and flowers from a single individual plant. Volatile yield is highly variable among individual plants and concentration also presents a high variation for most *Origanum* volatile compounds, linalool being the main component in most samples. Statistical analyses are applied in order to find patterns in composition data. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Thermal desorption; Plant materials; *Origanum vulgare*; Principal component analysis; Volatile organic compounds; Aroma compounds

1. Introduction

The names ''oregano'' and ''origanum'' are commonly applied to plant species belonging to *Corydothymus*, *Origanum* and *Lippia* genera [1–4] that are used in different countries as culinary herbs. Characterization of oregano samples from the concentration of their low-molecular-mass components has been used as an aid in their taxonomic classification [4,5], and also to estimate their flavor properties, since most of oregano compounds with organoleptic properties are included in its volatile fraction. Analytical studies of oregano have been revised by Lawrence [6–8].

Plants belonging to the same species can show a different volatile composition: when the difference cannot be related to their environmental characteristics, these plants can be assigned to different chemical types (chemotypes). The study of chemotypes requires analyzing a number of samples large enough to draw statistically significant conclusions from the concentration values used to characterize each sample. The method of choice in the analysis of plant volatile components is gas chromatography (GC), which is frequently coupled to mass spectrometry (MS) in order to obtain better qualitative information. Since non-volatile compounds cannot be injected into a GC column, a previous step is required to remove the non-volatile matrix, a process

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that can be time-consuming. Direct thermal desorption (DTD) allows analysing on-line with GC–MS plant volatiles without prior sample preparation and significantly reduces total analysis time [9–11].

Many studies on *Origanum vulgare* species have pointed out the presence of intra-species variations in their volatile composition, and the existence of some chemotypes has been proposed [4].

The objective of this work is to apply the DTD–GC–MS technique to the study of the volatile components present in flowers (inflorescences) and leaves of individual samples of *Origanum vulgare* collected in a Central Spain location. Multivariate statistical analysis has been applied to composition data in order to find possible sample patterns.

2. Experimental

2.1. Samples

Origanum vulgare subsp. *virens* (Hoffmanns & Link) Bonnier & Layens samples were collected at the flowering stage near El Escorial (Madrid, Central Spain). The collection area was a strip about 2 km long, covered by oak (*Quercus pyrenaica*) trees. Thirty-seven individual plants were selected: samples were taken from both flowers and leaves, and left to dry at room temperature. Five leaf samples were also selected from a single individual plant.

2.2. Direct thermal desorption

DTD was carried out in an ATD 400 unit (Perkin-Elmer, Norwalk, CT, USA). Dry samples (2–15 mg) were introduced into a PTFE tube (52 mm×4 mm I.D.) which was then introduced into a stainless steel tube (desorption cartridge, 89 mm×4.5 mm I.D.×6.5 mm O.D.), and desorbed under a helium flow at 180°C for 15 min. Volatile compounds were cryofocused on a Tenax GC (60–80 mesh, Supelco, Bellefonte, PA, USA) trap at −30°C, which after 4 min was rapidly (~40°C/s) heated to 320°C. The desorbed volatiles were transferred to the GC column through a heated fused-silica line at 225°C. Other operation details are described elsewhere [9].

2.3. Gas chromatography–mass spectrometry

The DTD system was connected to a GC-8000 gas chromatograph (Fisons, Milan, Italy) coupled to an MD800 mass detector (Fisons, Manchester, UK). Helium was used as carrier gas. The OV-1 fused-silica capillary column (50 m×0.25 mm, 0.2 μm) was temperature programmed from 70 to 250°C (hold time 10 min) at 4°C/min.

Mass spectra were recorded in the electron impact ionization (EI) mode at 70 eV.

2.4. Qualitative and quantitative analysis

Chromatographic peaks (total ion current trace, TIC) were identified from their retention and mass spectra, using standard compounds when available, or NIST and Wiley mass spectral data libraries. An internal standard (2-pentadecanone) was added (1.51 μg) to the cartridge before desorption. Semi-quantitative results were calculated from TIC peak areas: response factor was not taken into account in their determination.

2.5. Data processing

Quantitative data were processed by using the 4M (Factor Analysis) and 7M (Stepwise Discriminant Analysis) programs in the BMDP software for personal computers [12].

3. Results and discussion

The use of an internal standard (2-pentadecanone) allows an estimation of the total volatile yield for each sample: their values ranged between 0.8 and 12.5 μg/g (mean value 8.3 μg/g) for leaves, while flower sample yields ranged between 6.4 and 44.5 μg/g (mean value 15.1 μg/g). When total volatile yield was calculated for five leaf samples taken from the same plant, values were found between 2.1 and 7.3 μg/g (mean value 4.6 μg/g). Since relative standard deviation (RSD) values for concentrations of individual compounds in homogeneous plant samples have been found to average about 0.06 [9], the high yield dispersion among individual plants appears to be mainly caused by their different

growing conditions (humidity, nutrients available). For a single plant, the characteristics of the sample (leaf selected, presence of stems) seem also to affect total yield in a higher degree than analytical method variations.

For these reasons, quantitative results are presented as percent values of total volatile composition. DTD–GC–MS data are summarized in Table 1. The first column lists the 32 compounds determined in the 37 flower and leaf samples analyzed. Mean (percent values) and RSD values (expressed as the ratio between standard deviation and mean value) are also shown in Table 1. Leaf and flower samples have the same major compounds. RSD values are high for most compounds: even those having relatively low values, as linalool, *cis*-ocimene, β-caryophyllene and D- and B-germacrenes, present a high variability. For instance, minimum and maximum values for linalool are 0 and 54.3% in leaves and 12.9 and 60.3% in flower samples.

The statistical analysis has been carried out using the matrix of the percent concentration values of the 32 sample components listed in Table 1 in leaf samples (matrix L, 37×32) and that corresponding to the same compounds present in flower samples (matrix F, 37×32).

Table 1
Concentration (percent values of total volatile composition and relative standard deviation) of volatile compounds identified in leaves and flowers of *Origanum vulgare* samples

Component		Sample			
No.	Name	Leaf		Flower	
		Mean value (%)	RSD	Mean value (%)	RSD
1	α-Thujene	0.15	1.55	0.20	0.60
2	α-Pinene	0.54	1.06	0.46	0.90
3	Camphene	0.12	1.73	0.15	0.83
4	3-Octanone	0.14	1.74	0.02	4.79
5	Sabinene	7.30	0.98	3.53	1.00
6	β-Pinene	0.97	1.09	0.50	1.20
7	Myrcene	1.02	0.46	0.70	0.42
8	α-Phellandrene	0.07	3.37	0.08	1.00
9	α-Terpinene	0.15	1.87	0.38	0.93
10	*p*-Cymene	0.33	3.48	0.32	0.65
11	*cis*-Ocimene	15.38	0.27	4.70	0.52
12	*trans*-Ocimene	4.29	0.64	5.92	0.86
13	γ-Terpinene	0.77	2.31	0.88	0.54
14	Sabinene hydrate	1.50	1.21	1.49	1.02
15	Linalool oxide	0.09	5.54	0.04	1.36
16	Linalool oxide	0.62	1.03	0.46	0.62
17	Terpinolene	0.04	2.70	0.15	1.48
18	Linalool	26.29	0.48	38.20	0.36
19	Menthatriene	0.22	1.82	0.01	4.77
20	α-Terpineol	1.30	0.99	1.68	0.99
21	Menthadienol	0.12	0.99	0.10	0.71
22	δ-Elemene	0.38	0.71	0.22	0.55
23	β-Bourbonene	0.35	0.94	0.17	1.31
24	β-Elemene	0.11	1.43	0.20	0.91
25	β-Caryophyllene	8.87	0.38	8.81	0.27
26	Germacrene-D	7.40	0.42	7.27	0.29
27	Germacrene-B	8.53	0.41	6.28	0.32
28	β-Bisabolene	1.64	1.43	3.46	0.78
29	γ-Cadinene	0.81	1.95	0.61	1.71
30	δ-Cadinene	0.71	1.54	0.79	1.38
31	α-Bisabolene	0.16	1.61	0.25	0.91
32	Spatulenol	2.62	1.06	1.46	1.35

In a first step, data were used without normalization, in order to weight positively high concentration components. In order to show graphically the possible trends in the dispersion values shown in Table 1, we have applied principal component analysis (PCA) (BMDP program 4M) to matrix L and matrix F. In the L matrix, first principal component, which represents an average sample concentration, explains 85.6% of total variance, being mainly related to linalool, *cis*-ocimene, cariophyllene and germacrenes. Second principal component (9.5% of variance) is positively related to sabinene, *cis*-ocimene and *trans*-ocimene and negatively to linalool. Variance explained by third principal component decreases to 1.9%: β-caryophyllene and germacrene-D and -B loadings are positive, while those of linalool and sabinene are negative. The rest of principal components explain a percent variance lower than 1. Sample scores for second and third components are represented in Fig. 1A: no clear trends are observed in sample dispersion.

When PCA was applied to data in matrix F, the first principal component (93.2% of total variance) was mainly related (40.5) to the linalool concentration, this compound being the most important in flowers (Table 1). Second principal component is negatively (−2.8) related with linalool and positively (between 2.1 and 5.7) with *cis*- and *trans*-ocimene, D- and B-germacrenes, sabinene and caryophyllene: it explains a 5.1% of variance. Third principal component (0.8% of variance) is positively related with the concentration of the sesquiterpene hydrocarbons. Fig. 1B plots sample scores for second and third components. Two clear groups (16 and 21 samples each) are observed, distinguished by the value of the second principal component. In each group, values of second and third component seem to be related.

A division into the same two groups also appears when correlation instead of covariance is used in PCA calculations in order to normalize the variables and assign all of them the same statistical weight (variable autoscaling). In matrix L, first principal component explains 28.5% of variance and is positively (>0.78) related to α- and β-pinenes and to sabinene, while it presents −0.77 as coefficient for linalool. Second principal component (15.6% of variance) depends mainly (>0.65) of myrcene,

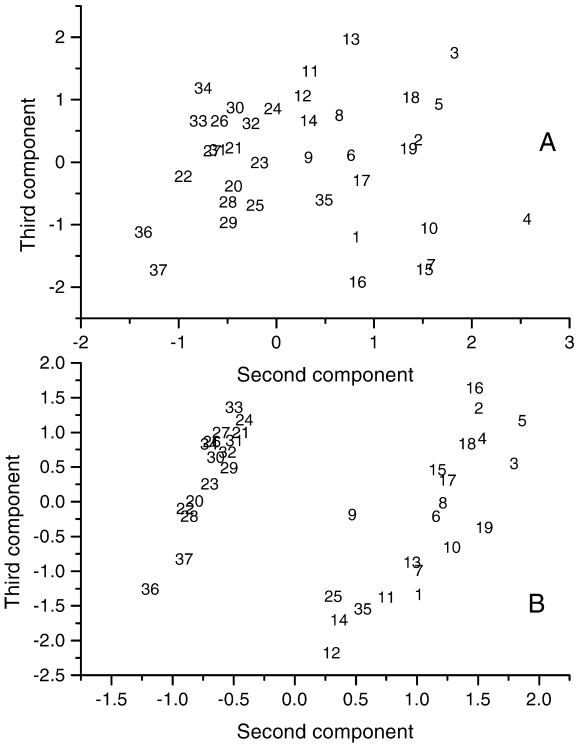

Fig. 1. Principal component plot of *Origanum vulgare* samples (original concentration values). (A) Leaf samples; (B) flower samples.

ocimene and δ-elemene. When matrix F is processed in the same way, compounds which most positively (>0.85) contribute to the first principal component (32.7% of variance) are α-pinene, *cis*- and *trans*-ocimenes, α-terpineol and sabinene hydrate, while second principal component (16.4% of variance) is related positively with monoterpene hydrocarbons and negatively with sesquiterpene hydrocarbons. Fig. 2 presents the sample scores using the first and the second component for matrix L (A) and matrix F (B).

Stepwise discriminant analysis (BMDP program 7M) was used in order to find if volatile compound concentrations could distinguish between flower and leaf samples. The highest *F*-value (a measure of the discriminating power) corresponds to *cis*-ocimene. The concentration of this compound is higher than 10% in leaf samples and lower in flower samples,

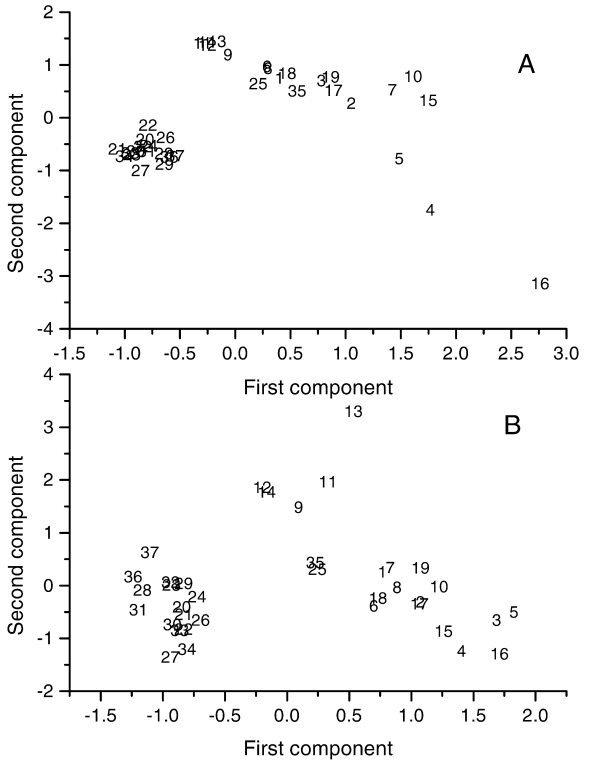

Fig. 2. Principal component plot of *Origanum vulgare* samples (normalized concentration values). (A) Leaf samples; (B) flower samples.

excepting three borderline cases. High *F*-values (always >8.0) are found for linalool, myrcene, germacrene-B and δ-elemene; some combinations of two of these compounds and *cis*-ocimene allow a correct classification of all leaf and flower samples.

Since plant samples were collected in the same area, the effect of environmental variables on their volatile composition is reduced. Figs. 1 and 2 show the existence of some trends in the highly variable composition of the samples studied. The presence of two groups appears in both flower and leaf plots. Group 1 is shown at the right side of Figs. 1 and 2, and includes samples 1 to 19 and also samples 25 and 35, while the rest of samples forms group 2. Sample numbering was not random, since they were numbered according to their collection order along a narrow area. The two groups which appear in Figs. 1 and 2 seem then to correspond roughly to two parts of this area, with the two sample exceptions before mentioned. Samples in group 1 presents a higher concentration in both flowers and leaves of sabinene, sabinene hydrate, ocimenes and α-pinene than those in group 2, comparatively richer in linalool.

The high variability of *Origanum* volatile sample component concentrations is explained in part by the existence of these two groups. *Origanum vulgare* volatile composition seems also to be easily affected by local variables, which probably cause the intra-group continuous dispersion. On the other hand, the discontinuous inter-group variability seems to correspond to the existence of a basic difference in the volatile components production of the two groups.

None of the studied samples corresponds to the ''aromatic'' *Origanum* chemotypes characterized by the major presence of thymol or carvacrol [4,13,14], being more similar to *Origanum vulgare* samples of North India characterized by the main presence of linalool, myrcene, caryophyllene and germacrene-D [15]. A recent study of *Origanum* volatile composition [16] groups samples from different species in four broad categories; composition of the analysed samples could be included in the ''acyclic monoterpenoid'' group. However, the relatively high presence of sabinene and related compounds in group 1 seems to indicate the presence in these plants of the sabinyl pathway, responsible of the high concentration of sabinyl compounds in several *Origanum* species.

It is worth noting the importance in chemotaxonomic work of studying individual plants and of comparing the composition of flowers and leaves in order to confirm the results (Figs. 1 and 2). The use of the concentration of plant volatile components with these purposes requires a fast and reproducible analytical method, which uses only a small sample amount in order to be applied to individual plants or to plant parts, and the DTD–GC–MS technique seems to be a useful tool for chemotaxonomic studies.

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