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Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil

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Abstract

Dynamic headspace sampling methods prior to capillary gas chromatography are especially suitable in the determination of volatile compounds at a wide range of concentrations, and numerous methods have been developed and applied to very different kinds of samples. In this work, a simple and rapid dynamic headspace technique was developed to determine volatiles present in virgin olive oil samples. Headspace components were swept from 0.5 g of sample at low temperature (40°C) and concentrated on Tenax TA, thermally desorbed and subsequently trapped in a fused-silica cold trap previously cooled to −110°C. They then passed to the capillary column. This system was connected to a mass spectrometer to identify the most important compounds and a comparative study of the main volatiles identified in virgin olive oil samples using other methods was carried out. Sniffing of the components eluted from the chromatographic column was also performed. Different virgin olive oil samples showing different chromatographic profiles were analysed. The differences were mainly quantitative because most compounds were present in all oils analysed, and only the proportions in which these compounds are present varied. Discriminant analysis of these compounds allowed the origin of each sample to be determined with a probability of greater than 90%.

1. Introduction

The flavour of a sample is normally very complex and the volatile components are responsible for this complex sensation. The concentration range in which these compounds are present is very wide. It used to be difficult to determine all of them using the same method because in many instances the methods used lack sensitivity and those volatiles present at trace levels are not detected.

Dynamic headspace (DHS) methods have been widely used in the determination of the volatile compounds present in foods and they have the great advantage of concentrating the sample so that it is possible to detect compounds present at low concentrations that sometimes contribute significantly to the flavour [1]. DHS–GC methods including thermal desorption are powerful tools for the efficient concentration of numerous flavour and fragance volatiles.

Virgin olive oil is the most highly flavoured of vegetable oils and for this reason it is greatly appreciated by consumers. The volatile components responsible for this flavour have been studied for years and a great number of compounds have been identified [2–4]. Different methods have been used [5]. Most studies used dynamic headspace techniques with solvent desorption, large amounts of samples, long analysis times and, in many instances, packed columns.

The first objective of this work was the identi-

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fication of the volatile compounds obtained by a new, previously optimized DHS method [6] that allows the determination of the wide range of compounds differing in polarity, volatility, concentration and molecular size that contribute to the complex flavour of virgin olive oil. The method involves the use of Tenax TA as adsorbent material, thermal desorption and cryofocusing prior to capillary GC to avoid undesirable peak broadening. The results were compared with those obtained using other techniques.

The characterization of olive oils by their non-volatile compounds has also been widely studied [7,8], but their characterization by their volatile compounds has scarcely been reported [9], despite the fact that volatiles are related to sensory notes [10] and hence to olive oil quality [11]. Hence the second objective of this work was the characterization of virgin olive oil samples on the basis of their origin.

2. Experimental

2.1. Samples

Thirty-two samples of virgin olive oil collected from Spain, Italy and Greece and corresponding to three stages of maturity were analysed in duplicate. Seven representative samples of the whole set were selected, either on the basis of their profile or the quantitative values of their peaks, for analysis by GC–MS. In this way we ensured that the most important peaks were properly identified. The most suitable sample was selected to be used in odour port assessment (sniffing).

2.2. Dynamic headspace sampling

The isolation of volatiles was carried out using a previously proposed system [6]. A 0.5-g amount of virgin olive oil sample with 3.33 ppm (w/w) of isobutyl acetate added as an internal standard was placed in a vessel, stirred and heated at 40°C to facilitate the removal of the volatiles. The sample surface was swept with nitrogen and volatiles were passed through a reflux condenser kept at 5–10°C to prevent subsequent interference from water. The volatiles were then trapped in a Tenax TA trap (Chrompack) at room temperature. The flow-rate was maintained at 200 ml/min using a rotameter and sampling was performed for 15 min. Adsorbent traps were conditioned prior to use by heating them at 300°C for several hours and again at 220°C with passage of the carrier gas. Blank runs were carried out periodically during the study.

2.3. Desorption method

The desorption of volatiles trapped in the Tenax TA trap was carried out in the opposite direction to adsorption by using a Chrompack thermal desorption cold trap injector (TCT) [12]. The temperatures and time of injection were controlled by an injector control unit. Desorption was carried out by heating the trap at 220°C for 5 min. Volatiles were then transported by the carrier gas with a desorption flow-rate of 7 ml/min to a fused-silica cold trap previously cooled to −110°C with liquid nitrogen for 5 min, where they condensed. Finally, the samples were injected into the capillary GC system by flash heating the cold trap at 170°C. The TCT system was flushed after each run by heating at 300 and 240°C.

2.4. GC analysis

A Hewlett-Packard Model 5890 Series II gas chromatograph equipped with a flame ionization detector and a Model 3396B integrator was employed for quantitative analysis. Helium (99.999%, 103 kPa inlet pressure) was used as the carrier gas and nitrogen as make-up gas. A fused-silica Supelcowax 10 column (60 m × 0.32 mm I.D., 0.5 μm film thickness) was used. The oven temperature was held at 40°C for 4 min, then increased at 4°C/min to 240°C, where it was held for 10 min; the injector temperature was 175°C and the detector temperature was 275°C. The integrator was linked to a Model 80386

computer where chromatograms were held in a relational database.

2.5. GC–MS analysis

The TCT was installed in the GC–MS system. Volatiles were obtained as described above. In order to achieve a more concentrated sample, 25 g of virgin olive oil were placed in the vessel extractor bottle and swept with nitrogen for 30 min. A Hewlett-Packard Model 5890 Series II gas chromatograph coupled with an MS 30/70 mass spectrometer (VG Analytical, Manchester, UK) and a VG Model 11/250 data system was used for mass spectrometric analyses. A J&W DB-WAX fused-silica capillary column (60 m × 0.25 mm I.D., 0.25 μm film thickness) was employed. The column temperature was held at 40°C for 15 min, then increased to 220°C at 1°C/min. The carrier gas (helium) flow-rate was 1 ml/min. The end of the fused-silica column was inserted directly into the ion source block. The spectra were recorded at an ionization voltage of 70 eV and an ion source temperature of 200°C.

Sample components were verified by comparison of the mass spectral data with those of authentic reference compounds. For some compounds, standard samples were not available to confirm positive identifications. In these cases, the sample components were tentatively identified by mass spectrum matching using the NBS mass spectral library collection. In the absence of suitable reference spectra, samples of suspect components were obtained or synthetized and their mass spectra acquired.

2.6. Sniffing

The separated components of the isolate were assessed sensorially at the outlet of the capillary column. Aromas were described by two assessors with more than 10 years experience and by two assessors who, although not experienced, were habitual consumers of virgin olive oil.

3. Results and discussion

3.1. Analysis by GC–MS and sniffing

A gas chromatogram of virgin olive oil is shown in Fig. 1, indicating the presence of over 100 components, 56 of which were identified in this work. Table 1 gives the names, the identification methods and the corresponding odour

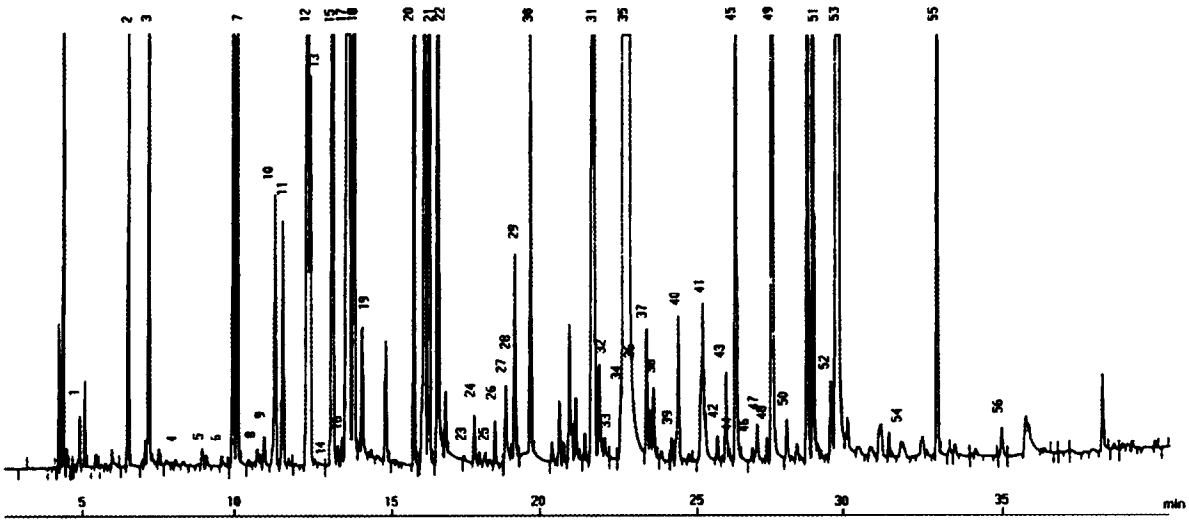

Fig. 1. Chromatogram showing identified peaks. Sample quality level, extra-virgin olive oil; nationality, Spain; variety, Arbequina; ripeness, unripe; extraction system, centrifugation. For peak identification, see Table 1.

Table 1
Volatile compounds identified in virgin olive oil

Peak No.[a]	Compound	Identification method	Sniffing	Ref.
1	Hexene	MS		
2	Acetone	GC, MS		13
3	Methyl acetate	GC, MS		
4	Octene	MS	Solvent-like	14
5	Ethyl acetate	GC, MS	Sweet, aromatic	2, 4, 13–15
6	2-Butanone	MS	Fragrant, pleasant	
7	3-Methylbutanal	MS	Sweet, fruity	2, 16
8	1,3-Hexadien-5-yne	MS		
9	An alcohol		Sweet, apple	
10	Ethylfuran	MS	Rancid	
11	Ethyl propanoate	MS	Sweet, strawberry, apple	2
12	An alcohol + hydrocarbon		Pungent, acid	
13	3-Pentanone	MS	Sweet	2, 4, 13
14	4-Methylpentan-2-one	MS	Sweet	
15	Pent-1-en-3-one	MS	Sweet, strawberry	
16	2-Methylbut-2-enal	MS	Solvent-like	
17	Isobutyl acetate[b]	GC, MS		
18	A hydrocarbon		Sweet, apple	
19	Methylbenzene	MS	Glue, solvent-like	4, 14
20	Butyl acetate	MS	Green, pungent	14, 15
21	Hexanal	GC, MS	Green, apple	2–4, 13, 16
22	A hydrocarbon		Sweet, aromatic	
23	2-Methylbutyl propanoate	MS	Aromatic, ketonic	
24	2-Methyl-1-propanol	GC, MS	Ethyl acetate-like	2, 13
25	(*E*)-2-Pentenal	MS	Green apple	2
26	An alcohol		Grassy	
27	(*Z*)-2-Pentenal	MS	Green, pleasant	2
28	Ethylbenzene	GC, MS	Strong	14, 15
29	An aldehyde		Artichoke, green, flowers	
30	3-Hexenal[c]	GC, MS	Green, green leaves, grassy	14–16
31	1-Penten-3-ol	GC, MS	Wet earth	2, 4, 14
32	3-Methylbutyl acetate	GC, O[d]	Banana	2, 4, 14
33	Heptan-2-one	MS	Fruity	14, 15
34	(*E*)-2-Hexenal	MS	Bitter almonds	2, 4, 14–16
35	(*Z*)-2-Hexenal	GC, MS	Green, fruity, sweet	2, 4, 14, 15
36	2-Methylbutan-1-ol	MS	Fish oil	2, 4
37	3-Methyl-2-butenyl acetate	MS	Putty-like unpleasant	
38	Dodecene or methylundecene	MS		
39	Pentan-1-ol	GC, MS	Pungent	4, 14
40	Ethenylbenzene	MS		4, 14, 15
41	Hexyl acetate	GC, MS	Sweet, fruity	4, 14, 15
42	A C_8 ketone	MS	Fruity, mushroom-like	
43	Octan-2-one	GC, MS	Mouldy	2, 4, 14, 15
44	3-(4-Methyl-3-pentenyl)furan	MS	Paint-like strong	
45	3-Hexenyl acetate	GC, MS	Green, green banana, green leaves	2, 4, 14–16
46	2-Penten-1-ol	GC, MS	Banana	
47	6-Methyl-5-hepten-2-one	MS	Fruity	
48	Nonan-2-one	MS	Fruity	2, 14
49	Hexan-1-ol	GC, MS	Fruity, aromatic, soft	2, 4, 14
50	(*E*)-3-Hexen-1-ol	GC, MS		2, 4, 13, 14
51	(*Z*)-3-Hexen-1-ol	GC, MS	Banana	4, 14, 16

Table 1 *(continued)*

Peak No.[a]	Compound	Identification method	Sniffing	Ref.
52	2,4-Hexadienal	MS		2
53	(*E*)-2-Hexen-1-ol	MS	Green, grassy	4, 14
54	Acetic acid	GC, MS		4, 16
55	Methyl nonanoate	GC, MS	Sweet, floral	
56	Methyl decanoate	MS	Fresh	

[a] Peak numbers refer to the chromatogram in Fig. 1.
[b] Internal standard.
[c] Compound synthesized to verify identification.
[d] Identified by odour quality perceived at the sniffing port.

descriptions. Most of them had been reported in virgin olive oil in previous works as cited, but the proposed method probably gives a greater number of aldehydes, ketones and compounds with fewer carbon atoms than other methods. The volatiles clearly identified corresponded to different chemical families, these being seven hydrocarbons, nine alcohols, nine aldehydes, nine ketones, one acid, twelve esters and two furans.

In flavour and fragance analysis, the identification of all peaks is no longer the main goal, the aim now being to separate and to investigate only those parts of the chromatogram with interesting organoleptic properties [17]. This may be possible by sniffing the effluent, a technique used for determining which components of a complex mixture of volatiles have odour and for evaluating the significance of their aroma.

In other methods [2,15] it is necessary to prefractionate the concentrate prior to GC–MS analysis because the non-polar fraction (hydrocarbons) interferes with the results. In our case the hydrocarbons, which have less significance in the aroma [2], basically appear in the first part of the chromatogram where the aroma compounds are at very low concentrations. For this reason the study of this zone is more difficult. In the rest of the chromatogram direct olfaction of the different compounds is relatively easy because this problem does not exist.

It is well known that the balanced flavour of virgin olive oil is attained by an adequate equilibrium between "green" and "fruity" notes [14], which change with ripeness. To assess the presence of the compounds responsible for these characteristic notes, sniffing of the effluent was carried out. Such a procedure identified a great number of odour descriptions, the most commonly used terms being "green', "fruity", "pleasant", "sweet" and, less commonly, "unpleasant notes". This is logical because the analysed samples correspond to virgin olive oil [18]. All of these odour descriptions have been previously described in virgin olive oil and in the direct olfaction of its isolate [4] and are present in the main sensory attributes that are usually studied in virgin olive oil [11].

The "green" notes formed a group of odour descriptions, as was expected [14]. These corresponded to typically green odour compounds [16] such as aliphatic C_6 compounds and corresponding hexyl esters: hexanal, 3-hexenal, 3-hexenyl acetate, hexyl acetate and 3-hexen-1-ol. It should be noted that 2-hexenal has been previously described as strong, green, bitter almonds [14] and as green, fruity, pleasant [15]. In our case the different *Z* and *E* isomers each showed different descriptions. This seems to be due to the fact that each isomer could be responsible for a different sensation. However, both (*E*)- and (*Z*)-2-pentenal showed green notes in sniffing.

Another large group of odour descriptions was constituted by the "fruity", "sweet" and "floral" notes, esters being mainly responsible for this sensation. Some ketones, especially C_5 ketones, are responsible for the "sweet" notes and C_{7-9}

ketones elicited a "fruity" description. Some alcohols and hydrocarbons also gave this kind of description.

A third, less important, group constituted by unpleasant notes such as "solvent-like", "paint" and "putty" was also obtained in the sniffing of the effluent. Various compounds seem to be responsible for these odour descriptions.

3.2. Characterizing samples on the basis of their origin

Data processing was performed with the BMDP package [19]. All the variables have an almost normal distribution, so that no transformation had to be applied to the data set of 56 volatiles. Analysis of variance (ANOVA) [20] was applied, using countries, Spain, Italy and Greece, as factor variables. Seventeen volatiles (Table 2), showed significant differences among countries at a significance of $F > 95\%$ ($p < 0.050$), six of them with a significance of $F < 0.001$. The seventeen volatiles are basically related to "sweet", "greens" and "fruity" sensory perceptions perceived by sniffing (Table 1), but there is not a definite preponderance of a particular series of compounds among those identified by GC–MS.

Stepwise linear discriminant analysis (SLDA) [20] was applied to the seventeen selected volatiles in order to discriminate the samples on the basis of their origin, and 90.5% of correct classifications were obtained by only three volatiles: 1,3-hexadien-5-yne, 2-methyl-1-propanol and 3-hexenyl acetate (Nos. 8, 24 and 45).

Fig. 2 shows the plot based on the two canonical equations. Three decision rules allow the samples to be characterized: If the first canonical variable has negative values, then the sample was collected in Greece; if the first canonical variable has positive values and the second canonical variable has positive values,

Table 2
Results for the volatile compounds showing significant differences between countries

Peak No.[b]	Peak-area ratio[a]			F	Significance of F
	Spain	Italy	Greece		
8	0.01 ± 0.01	0.28 ± 0.24	2.27 ± 0.69	38.655	<0.001
9	3.27 ± 1.94	0.53 ± 0.18	0.60 ± 0.49	6.417	0.005
10	1.35 ± 0.57	0.33 ± 0.27	1.74 ± 0.45	9.706	0.001
15	6.01 ± 2.43	1.32 ± 0.90	7.95 ± 2.00	11.692	<0.001
18	3.47 ± 1.37	2.51 ± 0.86	8.23 ± 3.08	9.907	0.001
20	2.89 ± 1.06	1.28 ± 0.80	3.82 ± 0.94	7.100	0.003
21	9.21 ± 4.10	2.37 ± 0.92	11.77 ± 3.53	8.436	0.001
23	tr[c] –	0.34 ± 0.22	0.10 ± 0.10	6.801	0.004
24	0.76 ± 0.29	0.40 ± 0.31	1.69 ± 0.57	11.168	<0.001
30	5.66 ± 1.92	7.93 ± 2.65	16.89 ± 2.61	25.816	<0.001
35	127.78 ± 63.79	472.70 ± 236.68	165.54 ± 47.92	7.784	0.002
44	0.68 ± 0.31	1.01 ± 0.41	7.54 ± 2.69	27.616	<0.001
45	0.73 ± 0.73	6.08 ± 3.86	6.44 ± 3.37	5.725	0.008
46	9.95 ± 5.39	6.50 ± 2.88	48.82 ± 22.46	13.659	<0.001
47	tr[c] –	tr[c] –	0.30 ± 0.24	7.154	0.003
51	11.29 ± 3.28	20.81 ± 6.45	18.49 ± 4.08	4.993	0.014
56	0.15 ± 0.10	0.56 ± 0.20	0.76 ± 0.43	5.890	0.007

[a] Peak numbers refer to the chromatogram in Fig. 1.
[b] Ratio of the compound peak area to the internal standard peak area, multiplied by 100. Mean values ±95% confidence interval.
[c] Trace (<0.01).

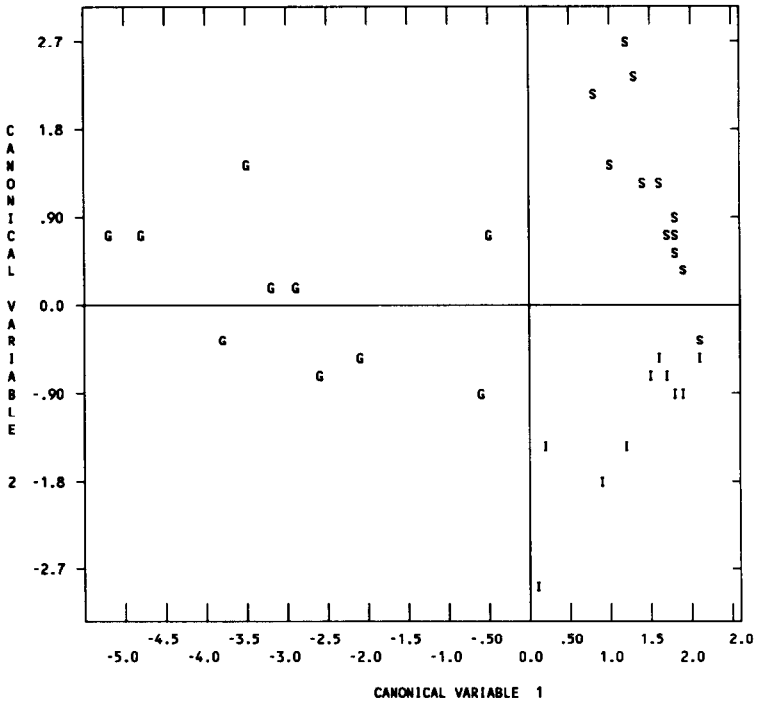

Fig. 2. Two-dimensional plot showing the discrimination obtained by means SLDA of the peaks that showed greater significant differences between samples.

then the sample was collected in Spain; if the first canonical variable has positive values and the second canonical variable has negative values, then the sample was collected in Italy.

4. Conclusions

The system proposed in this work allows the analysis of a large number of polar volatile compounds responsible for the aroma of virgin olive oil without carrying out prefractionation of the sample. Several compounds had not been previously described in this kind of sample and short-chain aliphatic compounds were especially well obtained. The main differences between samples from different origins were well established and a correct characterization was obtained.

5. Acknowledgements

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