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Tentative analysis of virgin olive oil aroma by supercritical fluid extraction–high-resolution gas chromatography–mass spectrometry

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Abstract

Dramatic increase in demand for virgin olive oil over the past few years can be attributed not only to its potential health benefits, but also to reports of its fragrant flavour. Supercritical fluid extraction (SFE) was applied to the extraction of volatile compounds of virgin olive oil and olive fruit samples. The volatiles extracted were concentrated in Tenax TA traps attached to the venting valve of the supercritical fluid extractor. The traps were desorbed onto a GC column by thermal desorption with cryofocusing and then analysed by high-resolution GC–MS. Volatile compounds were identified and compared with those obtained by applying a dynamic headspace procedure. The presence of semivolatile compounds was higher in the extracts obtained by SFE. Different profiles of volatile compounds, from flavours to off-flavours were obtained changing SFE experimental parameters. Volatiles were then characterised by sensory descriptors in order to evaluate the effect of this extraction technique on the virgin olive oil flavour.

Keywords: Food analysis; Olive oil; Oils; Fatty acids; Volatile organic compounds

1. Introduction

Supercritical fluids have been utilised as extraction media on the industrial scale for many years. Their use as chromatographic mobile phases in supercritical fluid chromatography (SFC) and as extraction media for sample preparation on the analytical scale in supercritical fluid extraction (SFE) has only recently attracted interest from analytical laboratories. By virtue of their unique physical properties, the use of supercritical fluids offers a number of advantages over normal organic solvent extraction media including: more rapid extraction times, higher efficiency and the ability to directly link the technique to methods of final analysis, i.e., gas chromatography (SFE–GC), supercritical fluid chromatography (SFE–SFC), high-performance liquid chromatography (SFE–HPLC) and mass spectrometry (SFE–MS). A number of books dealing with SFC and SFE have been published [1–3] in addition to several notable reviews that address the fundamental theories, recent advances and applications of the technique [4–8]. The applications of SFE in the food industry have been centred mainly on triglyceride extraction (oil recovery), deodorization of animal fats and brewer's yeast and the decaffination of tea and coffee. More specific applications include the isolation of α- and β-tocopherols from wheat germ [9], the analysis of free fatty acids in fresh and rancid milk products [10], and the analysis of aromas and fragrances in aromatic herbs [11].

One of the most desirable characteristics of a

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supercritical fluid is that it can possess a range of solvent strengths at different densities or pressures. If a sample can be extracted at a predetermined density, at which the solubility of the target analytes maximises whilst that of potential coextractants minimises, then compound class selectivity could be achieved. Taking this a step further, class selective extractions could be performed by extracting the same sample at different pressures using the same fluid. Hawthorne and Miller [12] utilised this approach to specifically extract alkanes and aromatics from diesel exhaust particulates with supercritical carbon dioxide using sequential extractions of 75 atm (fraction 1) and 300 atm (fraction 2) (1 atm= 101 325 Pa). Fraction 1 contained 85% of the alkanes present in the sample, while more than 90% of the aromatics (with the exception of phenanthrene) were found in fraction 2, indicating that SFE could provide 85–90% selectivities for hydrocarbon classes.

Complete class selectivity or class fractionation from a wide range of matrices cannot be achieved purely by solubility discrimination at different densities. In many cases however, selectivity can be enhanced by performing extractions on samples which have been previously adsorbed onto solid-phase sorbents (i.e., silica-, alumina- or octadecyl-bonded silica).

Different analytical procedures have been applied to study the volatile components of the complex matrix of virgin olive oil [13–15]. Headspace methods have been the most applied over previous years as they represent the volatile fraction, that reaching the olfactory receptors of the nose, gives rise to a wide panoply of sensory perceptions [16]. Dynamic headspace techniques purge or sweep the surface of the oil with an inert gas to isolate the volatile compounds and then concentrate them on cryogenic or sorbent material traps [17].

Since the application of supercritical fluids to isolate volatile compounds from virgin olive oils has not been reported until now, this work was instigated in order to assess the performance of SFE using supercritical carbon dioxide for the selective extraction of volatile/semi-volatile compounds directly from olive fruit and virgin olive oil that had been adsorbed on to silica gel.

2. Experimental

2.1. Chemicals

Volatile compounds used as standard for recovery studies, identification and quantification were purchased from Aldrich (Milwaukee, WI, USA), excepting (*Z*)-3-hexenal that was synthesized according to Hatanaka et al. [19]. Tenax TA traps were obtained from Chrompack (Middelburg, Netherlands). Silica gel used as support for olive oil samples was obtained from Merck (Darmstadt, Germany). High-purity (99.99%) carbon dioxide used as extraction media was obtained from Air Products (Paris, France).

2.2. Sample preparation

2.2.1. Olive oil samples

As oil is a liquid matrix, it was necessary to use a support material to carry out the analysis. Two different support materials were evaluated in a preliminary study: filter paper and silica gel. The latter support was found to be more adequate than the former as leaks of the matrix during the trials were detected when filter paper was employed.

Silica gel 60–120 (13–25 mm) was activated in vacuo for 16 h at 130°C. Virgin olive oil was accurately weighed and added to the activated silica in a 250-ml round bottomed flask. The mixture was then agitated for 30 min to ensure a uniform coating of oil onto the silica. The resultant dry ''loaded'' silica was extracted immediately.

The chemical composition of the oil was analysed following the European Union (EU) regulation [18]. Table 1 shows the chemical composition, sensory profile and overall grading (Panel test) of the oil.

SFE was applied on aliquots of the oil using different extraction conditions to evaluate the performance of the method. Table 2 shows the conditions applied to virgin olive oil samples. The conditions applied on samples 1 and 2 were softer in order to obtain volatile compounds representatives of the virgin olive oil flavour. Samples 3 and 4 were subjected to more drastic conditions in order to evaluate the profile of volatile compounds achieved and the presence of off-flavours. In order to de-

Table 1
Chemical composition and sensory profile of virgin olive oil sample

Free acidity (%)	0.40
Peroxide value (mequiv. O_2/kg)	4.3
UV absoption at 232 nm	1.27
UV absorption at 270 nm	0.095
Total diglycerides (%)	2.1
Fatty acids (%)	
C16:0	11.82
C16:1	0.43
C17:0	Tr
C17:1	0.02
C18:0	2.35
C18:1	79.30
C18:2	4.2
C18:3	0.65
C20:0	0.27
C20:1	0.22
Panel test	7

termine the ability of the SFE–high-resolution (HR) GC technique to yield quantitative results, a sample of freshly refined olive oil (volatiles-free) was spiked with approximately 1 mg/g of different volatile compounds. The selection of the volatiles was carried out based on their mean content in virgin olive oil, their contribution to flavour and their retention time in the chromatogram. Hexanal, (*E*)-2-hexenal, hexan-1-ol and (*Z*)-3-hexen-1-ol were the volatile compounds selected, Table 3, as aldehydes and alcohols are major compounds of the virgin olive oil aroma. They appear in two important zones of the chromatogram [14], are major compounds, and contribute to valuable sensory attributes of smelling and tasting perceptions, aroma – sweet and green – and tasting – bitter and undesirable [16]. Replicate samples (4 g) were extracted and analysed.

2.2.2. Olive fruit

Fresh ripe olives *Olea europea* L. cv. Picual of good quality were collected by handling. Olive fruits were cut into small pieces and placed inside the extractor vessel. Both olive flesh and stone were extracted together. Samples 5 and 6 of Table 2 describe the conditions for olive fruits.

2.3. Sample extraction

''Loaded'' silica or chopped olives were placed into a stainless steel SFE extraction cell (7 cm length) of a HP76080T (Hewlett-Packard, Palo Alto, CA, USA) SFE system. SFE was performed using high-purity (99.99%) carbon dioxide as the extraction medium. Samples were dynamically extracted

Table 2
Supercritical fluid extraction conditions applied to virgin olive oil (samples 1, 2, 3 and 4) and olive fruit samples (samples 5 and 6)

Extraction conditions	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Density (g/ml)	0.25	0.25	0.75	0.75	0.7	0.6
Pressure (bar)	81	77	260	260	115	98
Temperature (°C)	45	40	80	80	40	40
Static time (min)	1	5	1	1	5	30
Dynamic time (min)	30	30	30	30	30	40
Flow-rate (ml/min)	1	1	1	1	1	1
Oil/silica ratio	4.2/50	4.2/50	4.2/50	6/48	–	–

Table 3
Concentration, recovery, R.S.D., retention time and sensory perception of volatile compounds

Volatile compound	Concentration (mg/100 g)	Recovery (%)	R.S.D. (%)	t_R (min)	Sensory perception
Hexanal	1.10	105	6	20.8	Sweet
(*E*)-2-Hexenal	1.45	97	8	29.3	Bitter
Hexan-1-ol	1.24	95	10	37.3	Undesirable
(*Z*)-3-Hexen-1-ol	1.37	97	7	39.3	Green

Recoveries and relative standard deviations were based on five replicate analysis of the refined olive oil.

after an initial static period. Table 2 lists the values of the experimental extraction parameters.

In order to collect the volatile/semi-volatile components of these samples, the in-built extract trap of the SFE system was by-passed. Instead, the SFE extract, together with the total volume of venting carbon dioxide, was purged through a removable Tenax TA trap.

2.4. HRGC–MS

On completion of the extraction stage, the Tenax trap was removed and analysed by thermal desorption HRGC–MS. A Chrompack thermal desorption cold trap injector (TCT) was employed to carry out the thermal desorption of the trapped volatiles by heating at 220°C for 5 min. The volatiles were then condensed on to a fused-silica trap cooled at −110°C with liquid nitrogen for 5 min just before injection which was carried out by flash heating of the cold trap at 170°C where it was held for 5 min. The volatiles were transferred on to a fused-silica J&W DB-WAX column (60 m×0.25 mm I.D., 0.25 μm film thickness). The oven temperature was held at 40°C for 6 min and programmed to rise at 2°C/min to a final temperature of 200°C where it was held for 10 min. A Fisons Mass Detector coupled to a GC 8000 series was employed for identification. Masslab version 1.-3 was the software used. Sample components were verified by comparison of mass spectral data and retention times with those of authentic reference compounds.

2.5. Dynamic headspace

A dynamic headspace (DHS) technique previously reported [14] was applied to concentrate the volatile compounds of the sample, quantification was carried out by HRGC–flame ionization detection (FID) using isobutyl acetate as internal standard, identification was performed by HRGC–MS and the aroma notes of the volatiles were assigned by HRGC–olfactometry. Thermal desorption and HRGC analysis was carried out under the same conditions described in SFE.

3. Results and discussion

Olive oil sample was analysed according to EU regulation [18] to elucidate the olive oil category where the sample should be classified. The initial chemical and sensory profile of the sample was considered important to evaluate the presence of volatile compounds obtained in further analysis. Table 1 shows the chemical composition and the sensory profile of the sample. The evaluation of the oxidation level was of great importance as the study was aimed to evaluate the content of volatile compounds responsible for olive oil flavours and off-flavours. Free acidity and peroxide values are measures of auto-oxidation, which produces off-flavors and generally makes the oil unacceptable. As rancidity is usually accompanied by free fatty acid (FFA) formation, the determination of FFAs is a general indication of the condition and edibility of the oil. The acidity of the oil becomes noticeable when the FFA value is 0.5–1.5%, expressed as oleic acid. Peroxides are formed by free radicals during oxidation. Fresh oils generally have peroxide values below 10 mequiv./kg. The oil tested had a lower value and no off-flavour was noticed by the trained sensory panel.

Measurements of absorbance at specific wavelengths in the UV region are used to provide information on the quality of olive oil. Virgin olive oil is required to have a extinction coefficient at 270 nm less than 0.25. Fatty acids composition and diglycerides were also determined. Based on the results the chemical composition and panel test of the sample allowed its classification inside the extra-virgin olive oil category according to EU regulation [18].

The extra-virgin olive oil sample was also analysed by dynamic headspace. Table 4 shows the approximate concentration (μg/kg) of the major volatile compounds quantified by DHS-HRGC–FID and their aroma notes assigned by DHS-HRGC–olfactometry. In accordance with previous results [16,20] the sample showed a profile of volatiles corresponding to an extra-virgin olive oil sample, the presence of volatile compounds produced by biogenic pathways was noticeable and the presence of off-flavors was not detected.

Table 4
Volatile compounds approximate concentration of virgin olive oil sample quantified by DHS-HRGC–FID, sensory notes assigned by DHS-HRGC–olfactometry

Volatile compound	Concentration (μg/kg)	Sensory note
Ethyl acetate	18	Aromatic
3-Methyl butanal	145	Fruity
Ethyl furan	142	Sweet
Ethyl propanoate	110	Fruity
1-Penten-3-one	493	Sweet
Butyl acetate	228	Green
Hexanal	430	Apple
(*Z*)-3-Hexenal	649	Cut grass
1-Penten-3-ol	101	Undesirable
(*E*)-2-Hexenal	10 715	Green almonds
Hexyl acetate	309	Fruity
(*Z*)-3-Hexenyl acetate	331	Green fruity
2-Penten-1-ol	477	Fruity
Hexan-1-ol	337	Aromatic
(*Z*)-3-Hexen-1-ol	794	Green grass
(*E*)-2-Hexen-1-ol	332	Green

SFE was applied to isolate and concentrate the volatile compounds. Fig. 1 shows the major volatile compounds identified in virgin olive oil samples after isolation by SFE and concentration on Tenax TA traps. Sample 1 showed presence of major volatile compounds usually found in virgin olive oil samples [13,14,21,22]. All the major volatiles responsible for virgin olive oil flavour [23] were identified. The volatile compounds produced by biogenesis through lipoxygenase pathway [24] from linoleic and linolenic acids were detected: hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 3-hexenyl acetate, hexan-1-ol, (*Z*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol. C_5 compounds produced from 13-hydroperoxide of linolenic acid by chain cleavage mediated by lipoxygenase [25] and branched-chain aldehydes and alcohols 3-methylbutanal and 3-methylbutanol produced from amino acids [26,27].

Sample 2 showed similar composition to sample 1, regarding to these compounds, but some interesting differences were found. Although qualitative differences were not clearly detected, their profiles were quite different, mainly at the end of the chromatogram. Most volatile compounds were obtained at higher concentrations in sample 1 while sample 2 showed greater amounts of less volatile compounds. Just because the main difference, in their extraction conditions, was the static time although there was also a slight increase in temperature.

A simple comparison between DHS and SFE

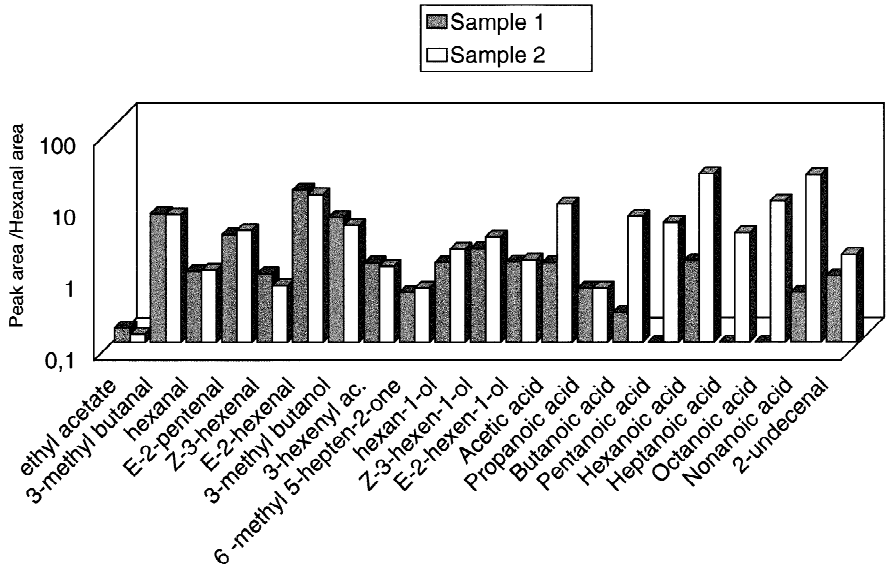

Fig. 1. Major volatile compounds identified in virgin olive oil. Samples 1 and 2. *y*-Axis=peak area/hexanal area (logarithmic scale). *x*-Axis=volatile compounds.

techniques showed that sample 1 was the most similar to DHS taking into consideration the whole experiment. Fig. 2 shows the chromatogram of SFE of sample 1. All the major compounds quantified by DHS-HRGC–FID were identified in the samples analysed by SFE–HRGC. The minor compounds identified by DHS were not all identified using SFE, with the presence of minor esters, which contribute to the fruity aroma of the oil noticeable, was greater in DHS analysis. The possible explanation could be in the necessity to optimise the SFE method to achieve better results.

The conditions applied to sample 1 were used to extract refined olive oil spiked with hexanal, (*E*)-2-hexenal, hexan-1-ol and (*Z*)-3-hexen-1-ol. Table 3 shows that the recovery of spiked compounds was essentially quantitative for each compound. The percentage of recovery was inside the range 85–110, taking into account the percentage of relative standard deviation (R.S.D.), which is adequate enough in comparison with other results [28]. This means that the percentage of recovery does not seem to be affected by the molecular mass of volatile compounds. In terms of solubility, it is well-known that lower aliphatic aldehydes, such as pentanal and hexanal, are all supercritical CO_2-miscible while unsaturations in aliphatic aldehydes, such as (*E*)-2-hexenal and (*Z*)-3-hexenal, are fully miscible with

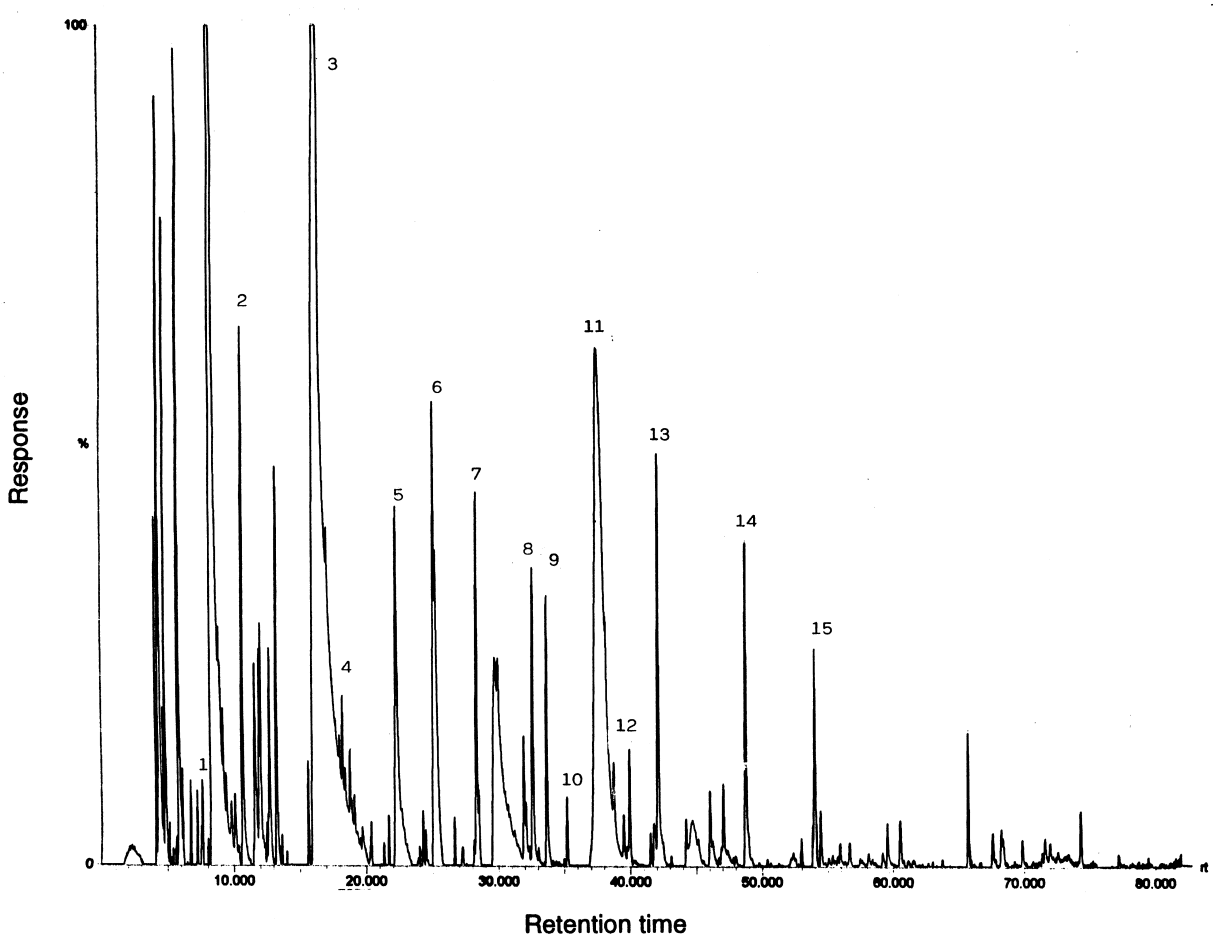

Fig. 2. Chromatogram corresponding to sample 1. Peaks identified: 1=ethyl acetate, 2=3-methyl butanal, 3=hexanal, 4=(*E*)-2-pentenal, 5=(*Z*)-3-hexenal, 6=(*E*)-2-hexenal, 7=3-methyl butanol, 8=(*Z*)-3-hexenyl acetate, 9=5-hepten-2-one-6-methyl, 10=hexan-1-ol, 11=(*Z*)-3-hexen-1-ol, 12=(*E*)-2-hexen-1-ol, 13=heptanol, 14=octanol, 15=undecenal.

supercritical CO_2 [29]. Concerning alcohols, the solubility of primary alcohols decreases keenly with increase in the length of the carbon chain above six atoms but virgin olive oil contains mostly C_6 primary alcohols such as, hexan-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol and (*E*)-3-hexen-1-ol [14,29].

Harder conditions, higher temperature and pressure, were applied to virgin olive oil samples 3 and 4 to evaluate the changes produced in the volatile composition. Samples 3 and 4 showed similar profiles but very different to those of samples 1 and 2. The higher chamber temperature (80°C) and density (0.7 g/ml) oxidised the olive oil sample producing a higher number of volatile compounds that appear at the end of the chromatogram. Most of the volatiles identified were related to oxidation of linoleic, linolenic and oleic acids [30]. They were mainly aldehydes and acids, although hydrocarbons and ketones were also identified (Table 5). These compounds had been previously found in virgin olive oil samples subjected to oxidation [31,32]. When a virgin olive oil is oxidised its profile of volatile compounds changes. The initial volatiles, mainly produced by biogenic pathways and responsible for desirable flavors, drastically change and less volatile compounds appear. The latter volatile compounds are responsible for virgin olive oil off-flavors as their aroma notes are unpleasant and their odor threshold are usually low as it was stated in a previous work [32]. Table 5 lists the major volatile compounds identified in samples 3 and 4, their retention times, sensory properties and area percentage. The presence of these volatile compounds was slightly higher in sample 4 that was prepared with a higher oil/silica ratio.

The major volatiles identified in samples 5 and 6 (olive fruits) were 3-methyl butanal, 2-butanone-3-methyl, hexanal, (*E*)-2-pentenal, (*E*)-2-hexenal, pentan-1-ol, octanal, hexyl acetate, (*Z*)-3-hexenyl acetate, 5-hepten-2-one-6-methyl, (*E*)-3-hexen-1-ol, heptanol, 3-hydroxybutanoic ethyl ester, 1,3-butanediol, 2-undecenal and phenyl ethyl alcohol. Many of them have also been identified in virgin olive oil. The most important of them from a sensory point of view are produced by the biogenic pathway of the lipoxygenase. They are C_6 aldehydes, alcohols and acetates that are responsible for green flavour [8].

The values of volatile compounds area/hexanal area were different enough in olives and virgin olive oil, Table 6. In cut olives higher values were found for (*Z*)-3-hexenal and (*E*)-2-hexenal, that are the first metabolites of the linolenic acid by the lipoxygenase pathway. (*Z*)-3-hexen-1-ol was also found to be highly produced while esters were the less produced in olive fruits. In virgin olive oil the production of volatiles was different enough. The influence of the technological process of virgin olive oil obtention on

Table 5
Volatile compounds identified in virgin olive oil samples after applying harder SFE conditions

Volatile compound	t_R (min)	Sensory note	Sample 3 (% area)	Sample 4 (% area)
Nonanal	40.4	Soapy	3.63	5.88
2,4-Heptadienal	44.8	Nutty	0.32	0.61
Decanal	47.1	Soapy	1.37	2.46
1,4-Heptadiene	49.0	–	0.89	1.05
2-Nonenal	49.8	Paper	0.10	0.19
2,5-Octadien-2-one	51.2	Fatty	0.04	0.07
Butanoic acid	51.8	Rancid	0.04	0.04
2,6-Nonadienal	54.9	Waxy	0.72	0.75
2,4-Nonadienal	57.0	Soapy	0.09	0.14
Pentanoic acid	58.6	Sweaty	0.13	0.14
(*E*)-2-Undecenal	60.6	Green	0.07	0.12
2,4-Decadienal	63.3	Fried	0.06	0.08
Hexanoic acid	64.9	Sweat	0.60	0.60
Heptanoic acid	71.0	Rancid	0.11	0.18
Phenol	74.1	–	0.03	0.04
Octanoic acid	76.9	Fatty	0.20	0.27
Nonanoic acid	82.5	Cheese	0.26	0.26

Table 6
Volatile compounds produced by lipoxygenase pathway identified in olive fruits

Volatile compound	Volatile area/hexanal area	
	Olive fruit	Virgin olive oil
(*Z*)-3-Hexenal	4.45	1.51
(*E*)-2-Hexenal	3.02	24.9
Hexyl acetate	0.35	0.72
(*Z*)-3-Hexenyl acetate	0.42	0.77
Hexan-1-ol	1.07	0.68
(*Z*)-3-Hexen-1-ol	4.60	1.61
(*E*)-2-Hexen-1-ol	1.16	0.78

Volatile compounds area/hexanal area in olives and virgin olive oil.

the volatile compounds formation was neatly stated. The formation of (*Z*)-3-hexenal was drastically reduced to form its more stable isomer (*E*)-2-hexenal, the major volatile compound in virgin olive oil, and esters formation is also favoured in the oil. The technological process helps to the formation of the last volatile compounds produced through the lipoxygenase pathway, that are responsible for a desirable fruity aroma.

The results of this tentative study show that SFE is a promising technique to isolate volatile compounds from virgin olive oil and olive fruit samples. Although a further concentration step is even required in order to enhance the sensitivity of the method. SFE could be a powerful tool for the analysis of the virgin olive oil flavour once the extraction conditions had been properly optimised.

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References

[1] B.A. Charpentier, M.R. Sevenants, Supercritical Fluid Extraction and Chromatography, American Chemical Society, Washington, DC, 1988.
[2] R.M. Smith, Supercritical Fluid Chromatography, Royal Society of Chemistry, London, 1988.
[3] M.L. Lee, K.E. Markides, Analytical Supercritical Fluid Chromatography and Extraction, Chromatography Conferences, Provo, UT, USA, 1990.
[4] J.W. King, J. Chromatogr. Sci. 27 (1989) 355.
[5] T.L. Chester, J.D. Pinkston, Anal Chem. 62 (1990) 394.
[6] S.B. Hawthorn, D.J. Miller, J.J. Langenfeld, in: K. Dinno (Ed.), Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (Journal of Chromatography Library, Vol. 53), Elsevier, Amsterdam, 1992, Ch. 12, p. 225.
[7] J.L. Hedrick, L.J. Mulcahey, L.T. Taylor, Microchim. Acta 108 (1992) 115.
[8] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 68 (1996) 487.
[9] M. Saito, Y. Yamauchi, K. Inomuta, W. Kottamp, J. Chromatogr. Sci. 27 (1989) 79.
[10] W. Gmur, J. Bosset, E. Platter, Mitt, Gebiete Lebensm. Hyg. 78 (1987) 21.
[11] S.B. Hawthorn, D.J. Miller, M.S. Krieger, J. High Resolut. Chromatogr. 12 (1989) 714.
[12] S.B. Hawthorn, D.J. Miller, J. Chromatogr. Sci. 24 (1986) 258.
[13] R.A. Flath, R.R. Forrey, D.G. Guadagni, J. Agric. Food Chem. 21 (1973) 948.
[14] M.T. Morales, R. Aparicio, J.J. Rios, J. Chromatogr. A 668 (1994) 455.
[15] M.T. Morales, R. Aparicio, Anal. Chim. Acta 282 (1993) 423.
[16] R. Aparicio, M.T. Morales, V. Alonso, J. Am. Oil Chem. Soc. 73 (1996) 1253.
[17] A.J. Nuñez, L.F. González, J. Janak, J. Chromatogr. 300 (1984) 127.
[18] Official Journal of the Commission of the European Communities, 28 March 1995, Regulation No. 656/95.
[19] A. Hatanaka, T. Kajiwara, H. Horino, K. Inokuchi, Z. Naturforsch. C 47 (1992) 183.
[20] R. Aparicio, M.T. Morales, V. Alonso, J. Agric. Food Chem. 45 (1997) 1076.
[21] M. Solinas, F. Angerosa, V. Marsilio, Riv. Ital. Sost. Grasse 65 (1988) 361.
[22] R. Gutiérrez, J.M. Olías, F. Gutiérrez, J. Cabrera, A. Del Barrio, Grasas Aceites 26 (1975) 21.
[23] M.T. Morales, J.J. Calvente, R. Aparicio, Flavour Fragr. J. 11 (1996) 171.
[24] B.A. Vick, D.C. Zimmerman, in: P.K. Stumpf, E.E. Conn (Eds.), The Biochemistry of Plants: A Comprehensive Treatise, Vol. 9, Academic Press, New York, 1987, pp. 53–90.
[25] Y.P. Salch, M.J. Grove, H. Takamura, H.W. Gardner, Plant Physiol. 108 (1995) 1211.
[26] R. Tressl, F. Drawert, J. Agric. Food Chem. 21 (1973) 560.
[27] H.T.W.M. Van der Hijden, I.J. Bom, in: A.J. Taylor, D.S. Mottram (Eds.), Flavor Science – Recent Developments, Royal Society of Chemistry, Cambridge, 1996, pp. 130–133.
[28] S.B. Hawthorne, M.S. Krieger, D.J. Miller, Anal. Chem. 60 (1988) 472.

[29] M.D. Luque de Castro, M. Valcarcel, M. Tena, Analytical Supercritical Fluid Extraction, Springer-Verlag, Berlin, 1994.
[30] E.N. Frankel, in: D.B. Min, T.H. Smouse (Eds.), Flavor Chemistry of Fats and Oils, American Oil Chemists' Society, Champaign, 1985, pp. 1–37.
[31] M.C. Dobarganes, J.J. Rios, M.C. Pérez-Camino, Grasas Aceites 37 (1986) 61.
[32] M.T. Morales, J.J. Rios, R. Aparicio, J. Agric. Food Chem. 45 (1997) 2666.