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Review

Gas and liquid chromatography of hydrocarbons in edible vegetable oils

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Abstract

Hydrocarbons, an important part of the minor constituents belonging to vegetable oils are reviewed. Their importance, origin, characterization and detection in edible vegetable oils are considered. The determination of some of them as a means of establishing oil quality and genuineness is also highlighted. The official methodologies, as well as the most commonly procedures used for isolation and analysis are reviewed. Furthermore, novel procedures applying new techniques for determining those compounds are also presented.

Keywords: Reviews; Vegetable oils; Food analysis; Hydrocarbons; Polynuclear aromatic hydrocarbons; Terpenes; Squalene; Carotenes; Steroids

Contents

1. Introduction

Hydrocarbons are the least polar compounds of the unsaponifiable matter of vegetable oils. The presence of these compounds was detected in the 1940s, when Jasperson and Jones [1] encountered in the deodorisation distillates from several vegetable oils large amounts of terpenic hydrocarbons accompanied by smaller amounts of *n*-alkanes. In the same decade Ryohei and Tomotaro [2] found that the hydrocarbon fraction of rice-bran oil was constituted mainly of squalene and traces of *n*-alkanes.

Later studies confirmed that crude vegetable oils contain elements of the *n*-alkane series from C_{10} to C_{35}, the odd numbered elements being the most abundant. Besides, low amounts of other hydrocarbons were detected, such as *n*-alkenes, sesquiter-

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penic (α-farnesene), terpenic (kaurene), carotenes, low-molecular-mass aromatics (from benzene to tetramethylbenzene, including styrene), and polycyclic aromatic hydrocarbons (mainly those of low molecular mass).

The crude vegetable oils show qualitative and quantitative differences in the hydrocarbon fraction and such differences have been used to characterize the plant origin of the oils.

In refined vegetable oils, new hydrocarbons are formed as a consequence of the reactions occurring during the refining process. These hydrocarbons are steroidal, arising from the dehydration of sterols, terpenic from terpenic alcohols, and other compounds deriving from squalene isomerisation. The presence of these degradation products is the basis of the official analytical methods for detecting refined oils in crude oils.

2. Aliphatic and terpenic hydrocarbons

The hydrocarbon composition of edible oils has been widely studied and the odd numbered carbon long chain predominance is well documented [3–5]. The *n*-alkanes are thought to be endogenous to a plant, they are formed as a result of the decarboxylation of long-chain fatty acids [6,7].

The *n*-alkane fraction is mainly constituted of *n*-alkanes in the range C_8–C_{35}, those most abundant being between C_{21} and C_{35} [5,8,9]. Table 1 shows the concentration of *n*-alkanes encountered in several edible vegetable oils.

Branched saturated hydrocarbons were tentatively identified by comparing gas chromatography (GC) retention times against standards [3,10,11], but this was never confirmed thereafter [5].

In virgin olive oils low amounts of sesquiterpenic hydrocarbons such as farnesene, *allo*-farnesenes, copaene, calarene, muurolene and eremophylene have also been described [8]. Table 2 shows the concentration ranges of terpenic hydrocarbons in virgin olive oil. Likewise, alkenes from C_{23} to C_{27} with the double bond at the C_9 (*n*-9) carbon atom, *n*-heptadecene with the double bond at the C_8 (*n*-8) and 6,10-dimethyl-1-undecene have been reported [8]. Recently, *n*-1 alkenes of C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22} and C_{24} carbon atoms have also been detected [12]. The position of the double bonds in all of those alkenes have been determined by mass spectrometry

Table 1
Concentration of *n*-alkanes in several edible vegetable oils[a]

Carbon No.	Concentration (mg/kg)				
	Sunflower	Virgin olive	Sesame	Peanut	Safflower
nC_{15}	0.17	0.16	0.16	0.38	0.41
nC_{16}	0.13	0.06	0.11	0.16	0.48
nC_{17}	0.16	0.12	0.15	0.15	0.52
nC_{18}	0.90	0.08	0.09	0.18	0.52
nC_{19}	0.12	0.13	0.10	0.17	0.55
nC_{20}	0.02	0.08	0.07	0.17	0.70
nC_{21}	0.04	0.81	0.23	0.18	0.72
nC_{22}	0.04	1.24	0.14	0.19	6.40
nC_{23}	0.15	18.54	0.54	0.20	2.92
nC_{24}	0.17	9.54	0.30	0.35	1.15
nC_{25}	1.52	17.98	1.08	0.77	2.78
nC_{26}	0.41	2.04	0.59	0.37	1.22
nC_{27}	11.19	15.72	6.29	3.35	13.52
nC_{28}	2.38	1.84	1.63	0.77	2.10
nC_{29}	49.63	12.38	18.45	12.67	27.05
nC_{30}	5.52	1.70	n.i.	0.81	1.98
nC_{31}	47.96	9.41	14.19	6.20	15.20
nC_{32}	1.79	1.54	1.44	0.39	0.89
nC_{33}	3.60	5.66	6.60	0.85	1.54

[a] Ref. [9].

Table 2
Concentration ranges of terpenic hydrocarbons in virgin olive oils[a]

Hydrocarbon	mg/kg
6,10-Dimethyl-1-undecene	n.d.[b]–7.67
α-Copaene	0.12–4.77
Calarene	n.d.–0.26
Eremophylene	n.d.–2.60
Muurolene	n.d.–1.51
α-Farnesene	n.d.–32.59
allo-Farnesene (Z2, E4, E6)	n.d.–0.15
allo-Farnesene (E2, E4, E6)	n.d.–2.44

[a] Ref. [8].
[b] n.d., Not detected.

analysis of the derivatives obtained by reactions with dimethyl disulfide.

In refined olive oils, new unsaturated hydrocarbons such as pentadecene, octadecene, pentadecadiene, octadecadiene, neophytadienes and *n*-hexacosadiene appear during the refining process. The latter one has also been detected in refined palm oil [13].

The aliphatic hydrocarbons show specific patterns and it is possible to distinguish between crude and refined oils of different plant origin, especially for crude oils taking into consideration its composition and individual *n*-alkanes [9]. Additionally, it is possible to use the aliphatic hydrocarbons profile to determine the authenticity of high-priced edible oils and whether they are admixed with cheaper ones [14]. The hydrocarbon profile can also be used to distinguish between different olive cultivars [15].

Other sources of hydrocarbons are those arising from contamination, either from packaging or from mineral oils used in the lubrication of extraction plants [16]. The jute bags are used in international transport and storage of seeds. These jute bags are softened by treating the fibers with a high-boiling fraction of mineral oil [17]. Contaminating alkanes are easily distinguish from natural ones because the predominance in the latter of the even-numbered carbon-chain. Contamination of crude palm kernel oil by hydrocarbons throughout the process of kernel extraction has been studied by Tan and Kuntom [18].

In vegetable oils, the isolation of the hydrocarbon fraction is usually performed by means of alkaline hydrolysis of the oil sample [19]. The unsaponifiable residue is fractioned by means of a silica gel low-pressure column using light petroleum as eluent [20]. The first fraction contains saturated and unsaturated aliphatic, and cyclic sesquiterpenes hydrocarbons. The second one contains α-farnesene and *allo*-farnesenes [8].

Thin-layer chromatography (TLC), both in analytical and preparative versions, is also used for the isolation of hydrocarbons from other lipids; this technique allows the knowledge of the approximate composition of the sample. Separation of the hydrocarbons takes place most satisfactorily with non-polar solvents [21], mainly in silica gel with calcium sulfate as fixing agent [5,22]. A disadvantage of TLC compared to column chromatography is the limited possibility of understanding quantitative analysis since exhaustive recovery of the separate spot is not sufficiently precise. On the other hand, an advantage over column chromatography is the possibility of using derivatization reagents [23], facilitating the determination of the structure of the substance separated.

Furthermore, isolation of the *n*-alkane fraction from other aromatic components was achieved using high-performance liquid chromatography (HPLC) with a silica gel column and hexane as eluent [9,14].

GC is certainly the most efficient method used for the quantitative determination of hydrocarbons, enabling separations of the individual members of the homologous series; furthermore, it provides very precise quantitative data on the amounts of the compounds separated. All variants of GC are used for the analysis of hydrocarbons, mainly analytical in capillary columns. Separations may be performed isothermally or in gradient temperature systems, on low-polar and non-polar stationary phases using flame ionization detection (FID).

In crude vegetable oils, the fractions isolated by column chromatography are analyzed by GC on a fused-silica capillary column coated with dimethylpolysiloxane, using *n*-eneicosane (C_{20}) as internal standard [5,8]. Fig. 1 shows a typical hydrocarbon fraction profile, obtained by GC on an SPB5 (5% diphenyl–95% dimethylpolysiloxane) stationary phase from a virgin olive oil sample. The fraction was obtained by fractioning the unsaponifiable matter on a silica gel column, eluting with 80 ml of hexane. In the chromatogram, saturated, unsaturated and terpenic hydrocarbons are shown.

A very powerful aid in the identification of

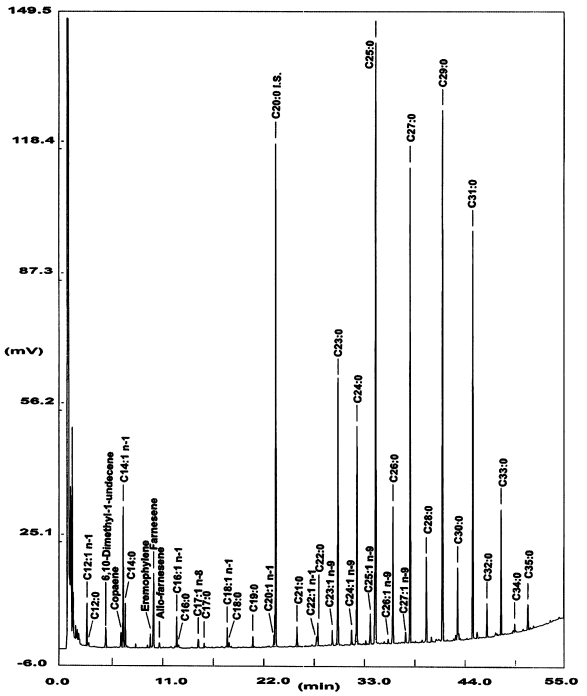

Fig. 1. Hydrocarbon fraction profile from a virgin olive oil obtained by capillary GC on a SPB5 (5% diphenyl–95% dimethylpolysiloxane) stationary phase (25 m×0.25 mm I.D., 0.25 μm film thickness), with a temperature program of 120°C for 4 min and to 310°C at 4°C/min and a hydrogen flow-rate of 1 ml/min.

hydrocarbons is the coupling of GC and mass spectrometry (MS), since GC can separate practically all hydrocarbons and their mass spectra are easily distinguished from other lipid components [5,8,9]. Hydrogenation with Adams–Shriner catalyst, oxidation with potassium permanganate [24] or reaction with dimethyl disulfide [25] are used in the identification of *n*-alkenes [8].

Finally, the analysis of the hydrocarbon fraction can be carried out by on-line HPLC–GC equipped with a HPLC silica gel column to isolate the corresponding fraction and an interface, which transfers it to the GC system equipped with an apolar column (100% methylpolysiloxane). This technique has been used in the study of the hydrocarbon fraction arising from contamination [16].

3. Squalene and derivatives

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14, 18,22-tetracosahexaene) is a naturally occurring terpenoid hydrocarbon in fish liver oils [26] and is also an important constituent of the unsaponifiable matter of numerous edible vegetable oils, in which stands out the olive (approx. 0.5%) and pumpkin oils [5,8].

The distilled fraction obtained from the deodorization step included in the refining process also contains squalene [27]. During the refining process, the squalene isomerizes [28] yielding a number of components with molecular mass of 410. Isoprenoid alkenes of molecular mass 408 have also been characterized [5,8] and attributed to dehydration products of oxidized squalene [29].

Squalene is normally used in its natural or hydrogenated form (squalane) as a moisturizing or emollient agent in cosmetic preparations [30]. Further interest arises because it is a precursor in cholesterol biosynthesis [31,32] and is a potential oxidation inhibitor contributing to the stability of different vegetable oils [33].

The isolation of the squalene is carried out in a way similar to that for the aliphatic hydrocarbons. The unsaponifiable matter is fractioned by column chromatography on silica gel, using hexane as eluent. The fraction eluted after the α-farnesene and *allo*-farnesenes contains the squalene [8]. The separation of squalene has also been achieved by TLC of the unsaponifiable matter [27,34].

Those methods are laborious and result in a significant loss of squalene during saponification. To overcome such inconveniences Lanzón et al. [35] developed a new method to isolate the squalene fraction performing a cold alkaline methylation of the oil. Recently a new method has been developed to isolate the squalene fraction without any chemical treatment. An oil solution is fractioned with a silica gel solid-phase cartridge (1 g), using 13 ml of hexane as eluent and squalane as internal standard [36]. In Fig. 2, the GC profile of the squalene fraction of a virgin olive oil can be seen.

In vegetable oils with high concentrations of squalene (olive and pumpkin seed oils), the interferences from other hydrocarbons in the GC analysis are negligible. Therefore, the European Union offi-

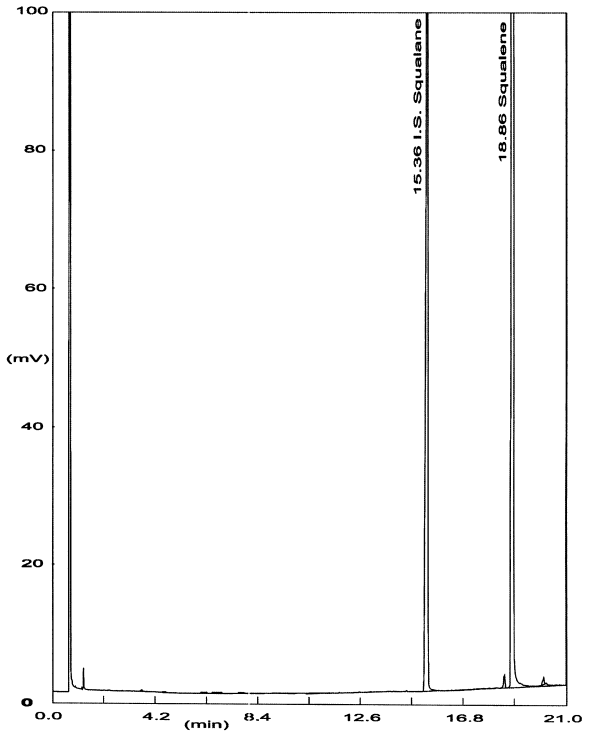

Fig. 2. GC profile of the squalene fraction of a virgin olive oil. The fraction was injected into a polar GC column (35% dimethyl–65% phenylpolysiloxane) (30 m×0.25 mm I.D., 0.1 μm film thickness), with a temperature program of 160°C for 10 min and then to 300°C at 10°C/min and a hydrogen flow-rate of 1.5 ml/min.

cial method for wax determination [37], allows the simultaneous determination of squalene and waxes using a short capillary column coated with a low-polarity phase if squalane is used as internal standard.

In vegetable oils with low squalene concentrations, the use of a normal length (25 m) capillary column (5% diphenyl–95% dimethylpolysiloxane) is advisable in order to prevent possible overlapping with saturated hydrocarbons [35,38]. In refined vegetable oils containing significant amounts of isoprenoid polyalkenes, the use of a more polar stationary phase (50% phenyl–50% methylpolysiloxane) in the GC analysis is necessary to avoid peak overlapping [5,34].

On-line HPLC–GC provides a very interesting approach to integrate the sample preparation into the chromatographic procedure. The use of normal phases in the LC separation requires the back-flushing of the column after each analysis, in order to eliminate the retained polar compounds. The application of reversed phases avoids this step [39].

4. Volatile hydrocarbons

Flavor and aroma of edible vegetable oils are generated by a number of volatile constituents that are present at extremely low concentrations. These compounds include hydrocarbons, alcohols, aldehydes, ketones, esters and acids. The hydrocarbon fraction comprises saturated, unsaturated, aromatic and sesquiterpenic compounds [40].

These fractions have been profusely studied in crude vegetable oils, since most edible vegetable oils undergo refining and the majority of the volatile components are lost along the process [41]. Volatile compounds in crude vegetable oils such as α-pinene, calarene and kaurene have been identified [41]. In addition, during oxidation of crude and refined oils various compounds responsible for undesirable flavor are formed. The analysis of pentane has been proposed to evaluate the oxidation rate [42].

In olive oils, the influence of maturation, cultivar, agronomical practices and extraction procedures have been studied by Kiritsakis [43], as well as the effect of the olive alteration [44,45].

Isolation methods, all using GC–FID, include direct injection, static headspace, dynamic headspace, high vacuum distillation and on-line LC–GC.

In the direct injection method, the oil diluted with hexane is injected splitless into a GC injector provided with a special glass liner, and a 2 m×0.32 mm pre-column. The injector is maintained at 200°C and the components of low and intermediate boiling points are volatilized and transferred to the column. This method has two major drawbacks, the interference of fat degradation products [41], and the detection of only major components due to the use of low amounts of sample (1 μl).

Dynamic headspace has been the most used concentration technique. The volatile compounds

from the sample were purged by means of an inert gas and then trapped on a solid adsorbent. Dobarganes et al. [46] optimized the trapping of the volatile compounds on activated charcoal and desorbing with carbon disulfide. The hydrocarbon fraction was obtained by fractioning the carbon disulfide extract on a silica gel column eluting with pentane [44]. Other authors desorbed the volatiles trapped on charcoal with diethyl ether [47]. The diethyl ether extract was analyzed by static headspace GC yielding a total volatile profile.

The most commonly used method consists of the adsorption of the volatiles on a porous polymer (Tenax TA), followed by thermal desorption and cryofocusing of the volatiles into a short fused-silica capillary column attached to the head of the GC column. The volatiles then are introduced into the analytical column (polyethylene glycol, 60 m×0.32 mm, 5 μm) by ballistic heating and quantified by GC–MS. The method has been used in the analysis of the volatile fraction in olive oil [48].

The isolation and analysis of the volatile fraction of vegetable oils has been achieved using on-line HPLC–GC on silica gel columns, the system was equipped with a loop type interface [41].

To identify minor components of the volatile fraction, large oil samples (400–1000 g) were subject to the steam distillation–solvent extraction (SDSE) procedure. A solution of the oil in diethyl ether was distilled at low temperature (34°C) under vacuum [49]. The distillate was condensed and the solvent evaporated. The residue was fractionated on a silica gel column [50] or by HPLC [51]. When quantification was needed, a stable-isotope dilution technique was applied [52].

5. Low-molecular-mass aromatic hydrocarbons

The presence of volatile aromatic hydrocarbons (VAHs) in the volatile fraction of edible vegetable oils has caused some public concern. In olive oil low concentrations of benzene, toluene and styrene have been found. The origin of these aromatic hydrocarbons is largely unknown. Biedermann et al. [53] carried out experiments to assess whether the VAHs arise from air contamination or from biosynthetic pathways taking place during maturation of the olive fruits. They concluded that the concentration of VAHs encountered were higher that those expected via equilibrium with the air. Therefore, the origin of VAHs could possibly arise from both endogenous and exogenous pathways [54]. Olías-Jiménez et al. [40] encountered the whole series of aromatic hydrocarbons from benzene to trimethylbenzene including xylene and styrene when studying the volatile fraction in the aroma of virgin olive oils obtained from olive fruits at different maturation stages.

The low levels of VAHs present at μg/kg levels and the presence of other volatile components requires not only methodologies allowing their enrichment, but also a previous separation of the fraction or the use of specific detectors.

Isolation techniques similar to those described for analysis of volatile components (Section 4) have been used to obtain the fractions containing the aromatic hydrocarbons.

A dynamic headspace technique was used for the qualitative analysis of VAHs. A nitrogen stream was passed through an olive oil sample (60 g) and the volatiles trapped on activated charcoal [46]. The trapped compounds were desorbed with carbon disulfide and fractioned by a silica gel column. The non-polar fraction was analyzed by GC–MS [40].

A static headspace was used for quantitative determination. After equilibrium with the air contained in the headspace, a gas volume was sucked through a fused-silica capillary column (5 cm×0.32 mm I.D.) packed with activated charcoal. The column was then connected to the GC analytical column (30 m×0.25 mm I.D.) coated with OV-1701 (14% cyanopropylphenylmethylpolysiloxane) and thermally desorbed and determined by GC–MS using 4-chlorotoluene as internal standard [53]. Dynamic headspace and GC–MS analysis has been used to quantitatively analyze VAHs in soybean oil [42].

In other procedures, the oil headspace air was purged with a nitrogen stream and the volatiles adsorbed on a Tenax TA porous polymer. The volatile compounds were then thermally desorbed and cryofocused in a short fused-silica capillary column cooled at −120°C with liquid nitrogen. The cryogenic trap was heated up to 200°C and the volatiles analyzed by GC–MS using a capillary column coated with free fatty acid phase (FFAP) (polyethylene glycol for acidic compounds). The

quantification was done by means of a selected-ion monitoring GC–MS of the ion appearing at m/z 78, 91, 92 and 106 using external standard calibration curves [54].

6. Steroidal hydrocarbons

Most edible oils, depending on type and designation, undergo considerable processing prior to retail sale. Generally, the crude oil is obtained by pressing and/or solvent extraction, followed by degumming, neutralization, bleaching, hydrogenation, dewaxing and deodorization to yield a refined product [55].

Significant amounts of hydrocarbons with steroidal skeleton (sterenes) are formed in vegetable oils as dehydration products of the Δ^5-sterols, because of bleaching with acidic earths and deodorization at high temperatures during the refining process. Each Δ^5-sterol give rise to three sterene isomers with the two double bonds at the 3,5-, 2,4- and 2,5-positions, the first one being the most abundant. Among these hydrocarbons, the stigmastadienes are the most abundant in all refined vegetable oils since they derive from β-sitosterol by dehydration [56]. The composition of the steroidal hydrocarbon fraction enables one to identify the oil origin, since their composition reflects that of the sterol [57]. This fact allows the detection of desterolized seed oils (oils treated strongly with bleaching earth) in refined olive oils by the analysis of the steroidal hydrocarbons composition [58].

The isolation of steroidal hydrocarbons in crude vegetable oils is usually performed by means of low-pressure column chromatography of the unsaponifiable matter of 20 g of oil, using light petroleum as eluent. For quantitative determination of sterenes in crude vegetable oils, the first fraction eluted from the column chromatography is discarded and the second one analyzed by GC on a fused-silica capillary column coated with 5% phenylmethylpolysiloxane. The method has been standardized by IUPAC [59] and adopted by the European Union [60], the International Standard Office [61] and the American Oil Chemists Society [62]. Fig. 3 shows a typical GC profile of the steroidal hydrocarbon fraction.

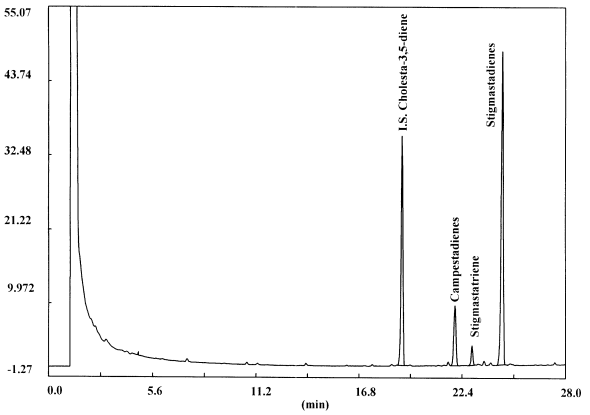

Fig. 3. Typical GC profile of the steroidal hydrocarbon fraction of refined oil. The fraction was injected on a low polar GC column (5% diphenyl–95% methylpolysiloxane) (25 m×0.25 mm I.D., 0.1 μm film thickness), with a temperature program of 235°C for 6 min and then to 285°C at 2°C/min and a hydrogen flow-rate of 1 ml/min.

In refined oils, steroidal hydrocarbons concentrations are higher than in crude oils (1–50 mg/kg) and the saponification step can be avoided. The oil dissolved in hexane is directly fractioned on a silica gel column and the corresponding fraction analyzed as described earlier [56]. Nevertheless, strongly refined olive oils contain isoprenoid alkenes deriving from squalene isomerization, and these compounds interfere with the gas chromatographic analysis of sterenes. Therefore, an improved isolation was developed using silica gel impregnated with silver nitrate allowing the isolation of sterenes from the oil, free of squalene derivatives [63].

Among many methods of isolation, HPLC of the hydrocarbon fraction on a silica gel column and subsequent analysis by off-line GC has been proposed for a quick screening of a large number of samples with the aim of verifying whether the stigmastadiene content is within the legal limit [64].

On-line HPLC–GC using a silica gel column has also been used to detect refined oils by the determination of sterene hydrocarbons [65], and its comparison with the official method was also carried out [66].

An alternative to GC analysis is reversed-phase HPLC using UV detection. The oil crude or refined is directly fractioned on a silica gel column and the

hydrocarbon fraction analyzed by HPLC on an RP-18 column using UV–Vis detection at 235 nm [67].

The 3,5-steradienes are the main dehydration products of Δ^5-sterols, but other isomers are also formed together with degradation products of Δ^7-sterols, methylsterols and triterpenic alcohols. In order to identify these minor compounds, new isolation procedures have been used. Mennie et al. [68] isolated the hydrocarbon fraction (except squalene) from several refined vegetable oils using a silica gel column, and then eliminated the aromatic ones by HPLC on a silica gel column. Subsequent analysis by GC–MS suggested the presence of the 3,5-, 2,5-, and 4,6-steradienes. On the other hand, Grob et al. [69] using on-line HPLC–HPLC–GC–MS on silica gel columns identified the 3,5-, 2,4-, 2,5-steradienes, the 3,5-cyclo-6-enes and 2,4,6-trienes. The first column separates the sterenes (except squalene) from the triacylglycerols and other polar compounds, and the second one separates different fractions identified with UV detection; stereradienes at 235 nm and steratrienes at 309 nm. Several fractions isolated by the LC–LC system were transferred to the GC–MS system provided with methylsilicone- or Carbowax-type columns.

Using low-pressure column chromatography on silica gel impregnated with silver nitrate, fractions containing 3,5-, 2,4- and 2,5-sterenes were isolated by Cert and Moreda [63] in a different order to that obtained using HPLC on silica gel [69]. Fig. 4 shows the different profiles obtained from the analysis of the corresponding the 3,5-, 2,4- and 2,5-sterene fractions obtained using silver ion column chromatography from a mixture of refined olive oil and desterolised sunflower oil.

Mariani and Grob [70] identified by GC–MS two epimeric sterenes at the C_{24} carbon atom, one deriving from campesterol (24α-methylcholesterol) and the other from 22,23-dihydrobrassicasterol (24β-methylcholesterol).

The GC–MS analysis of the sterene fraction isolated from refined oils revealed other compounds which mass spectra had a molecular ion two mass units less than those sterenes derived from dehydration of sterols. The mass spectra were in agreement with the presence of three double bonds in the ring system [68,69,71]. The formation of these products can be rationalized by the loss of two molecules of water from the hydroxy derivatives of sterols [72].

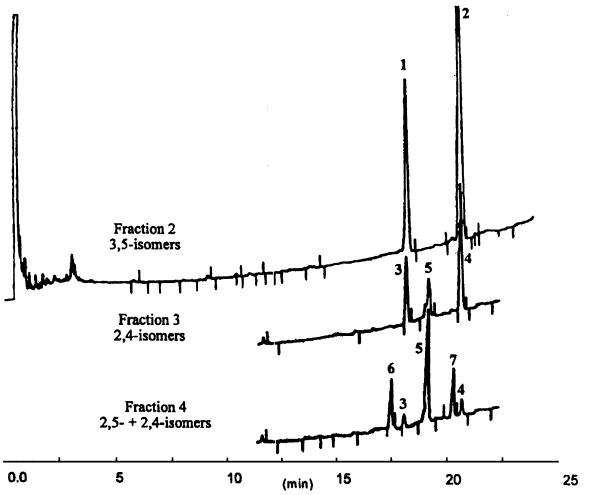

Fig. 4. Different GC profiles obtained from the analysis of the corresponding the 3,5-, 2,4- and 2,5-sterene fractions obtained using silver ion column chromatography from a mixture of refined olive oil and desterolised sunflower oil. (1) 3,5-Campestadiene, (2) 3,5-stigmastadiene, (3) 2,4-campestadiene, (4) 2,4-stigmastadiene, (5) stigmastatriene, (6) 2,5-campestadiene, (7) 2,5-stigmastadiene. The chromatographic conditions were similar to those stated in Fig. 2.

7. Carotenes

Carotenoids are divided into two groups; carotenes and xantophylls. While carotenes are purely hydrocarbons, xantophylls are oxygenated at the ends groups and hence polar. In olive oils, the main carotene is β-carotene, which is regarded as a singlet oxygen quencher that inhibits the chlorophyll-induced photooxidation [73]. The amount of carotenes in olive oils depends fundamentally on the cultivars and state of ripeness [74].

The carotenes were determined after cold saponification of the oil [75], by means of reversed-phase HPLC with UV detection at 458 nm. The carotenes were not significantly affected by saponification [76]. Two reversed-phase columns differing in the degree of carbon loading, functional groups in the stationary phase endcapping and pore size were used to aid in the identification of the *cis*-/*trans*-isomers in carotene concentrates of crude palm oil. The less lipophilic ones were separated on the column with less percentage of carbon and higher pore size and the more lipophilic with the other one. The α-carotene isomers could not be separated [77].

A chromatographic method was developed using

direct injection of an oil solution in hexane–2-propanol onto a HPLC system provided with a silica gel column and UV detection at 452 nm [78,79].

In order to eliminate interferences, several procedures of isolation and clean up have been described. In olive oil, liquid–liquid partition with *N*,*N*-dimethylformamide (DMF)–hexane yields a hexane fraction containing lipids and carotenes. Moreover, a quick method to isolate carotenes by solid-phase extraction on a C_{18} cartridge has been described. In both methods, quantification of β-carotene was performed using the coefficient extinction in hexane $E^{1\%}_{660}=613$ [80].

8. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) represent a very important group of chemical carcinogens or co-carcinogens [81,82]. PAHs are compounds consisting of two or more fused carbocyclic aromatic rings, which may or may not have substituent groups attached to one or more rings. These compounds are generally formed by incomplete combustion and high-temperature pyrolysis reactions, such as the burning of coal, oil and other forms of organic matters.

Due to the wide distribution of PAHs in the environment and their lipophilic nature, edible vegetable oils can be contaminated with these substances. Different routes of PAH contamination in vegetable oils have been suggested. The oilseed drying processes using direct combustion can be responsible for major PAH contamination of some edible oil such as sunflower, soybean, palm [83], grape seed [84] and coconut [85] oils. Other sources of contamination include air pollution with dust containing particles of pyrosynthesized PAHs, that may contaminate the plants or the raw material and hence the final product [86]. Contamination by rediffusion from recycled polyethylene bottles used for oil packaging [87], from mineral oils employed for softening fibers used in storage of oil seed [88,89], and finally from lubricating oils used for maintenance of extraction plants are also possible [16]. Nevertheless, the endogenous origin of some PAHs has not been ruled out.

PAHs are present in vegetable oils at concentrations under 100 μg/kg, the ''light'' PAHs being more abundant than the ''heavy'' ones [83,86]. Table 3 shows the concentrations of the major PAHs in several edible vegetable oils. Refining reduces the amount of PAHs depending on the refining conditions adopted, the loss of ''light'' PAHs is greater than the ''heavy'' ones [90].

Actually, there are no official regulations that include limits to the presence of PAHs in edible vegetable oils, although, several countries have adopted limits for such compounds, particularly for benzo[*a*]pyrene (BaP). There are great concerns within the European Union about this subject and official regulations may be adopted in the near future.

As always with trace analysis, the determination

Table 3
Polycyclic aromatic hydrocarbons concentration in several vegetable oils[a]

PAH	Oil (μg/kg)						
	Sunflower	Linseed	Wheat germ	Olive	Safflower	Maize germ	Sesame
Phenanthrene	3.7	41.4	69.4	39.3	0.5	0.9	18.2
Anthracene	0.3	4.0	4.4	2.2	n.d.	0.1	0.2
Fluoranthene	3.1	9.9	18.2	8.5	0.6	2.4	1.2
Pyrene	2.2	6.3	13.2	12.9	0.4	2.5	1.2
Benzo[*a*]anthracene	0.8	2.2	2.4	9.0	0.2	1.3	<0.1
Chrysene	1.5	5.9	5.6	4.6	0.7	2.5	0.6
Benzo[*b*]fluoranthene	1.6	2.5	6.4	2.4	1.1	2.5	0.1
Benzo[*k*]fluoranthene							
Benzo[*a*]pyrene	0.7	0.9	1.3	0.7	0.2	1.3	n.d.
Dibenzo[*a*,*h*]anthracene	<0.1	<0.1	n.d.	n.d.	n.d.	0.1	n.d.
Benzo[*ghi*]perylene	0.5	0.8	0.4	0.2	0.2	0.7	n.d.
Indeno[1,2,3-*cd*]pyrene	0.5	0.7	0.3	0.3	0.3	0.7	n.d.

[a] Ref. [86].

of PAHs in lipids is beset with many difficulties, resulting from the low level of individual PAHs (μg/kg), the complexity of extraction and clean-up procedures, and the need for good chromatographic resolution and very sensitive detection for final quantification.

Several methods of PAH extraction and clean up have been described. The isolation of PAHs is usually carried out by liquid–liquid partition of the oil using cyclohexane and dimethylformamide–water (90:10) [91]. Hopia et al. [92] performed an extraction of the solution of the oil in cyclohexane with methanol–water (4:1) prior to the liquid–liquid partition. Clean up of the extracts is needed to separate PAHs from other organic compounds, and it is performed using column chromatography on silica gel [92,93]. Furthermore, Speer et al. [86] purified the PAH extracts using silica gel column chromatography followed by size-exclusion chromatography. Several other procedures have been used to clean-up the extracts, among them are column chromatography on Florisil [94] and XAD-2 resin [95]. Moret et al. [96] simplified greatly the purification procedure using silica gel SPE cartridges instead of packed columns.

Other authors [97] performed the liquid–liquid partition of the oil dissolved in pentane using dimethyl sulfoxide (DMSO) followed by TLC fractionation on silica gel plates.

Moreover, the saponification of the oil has been

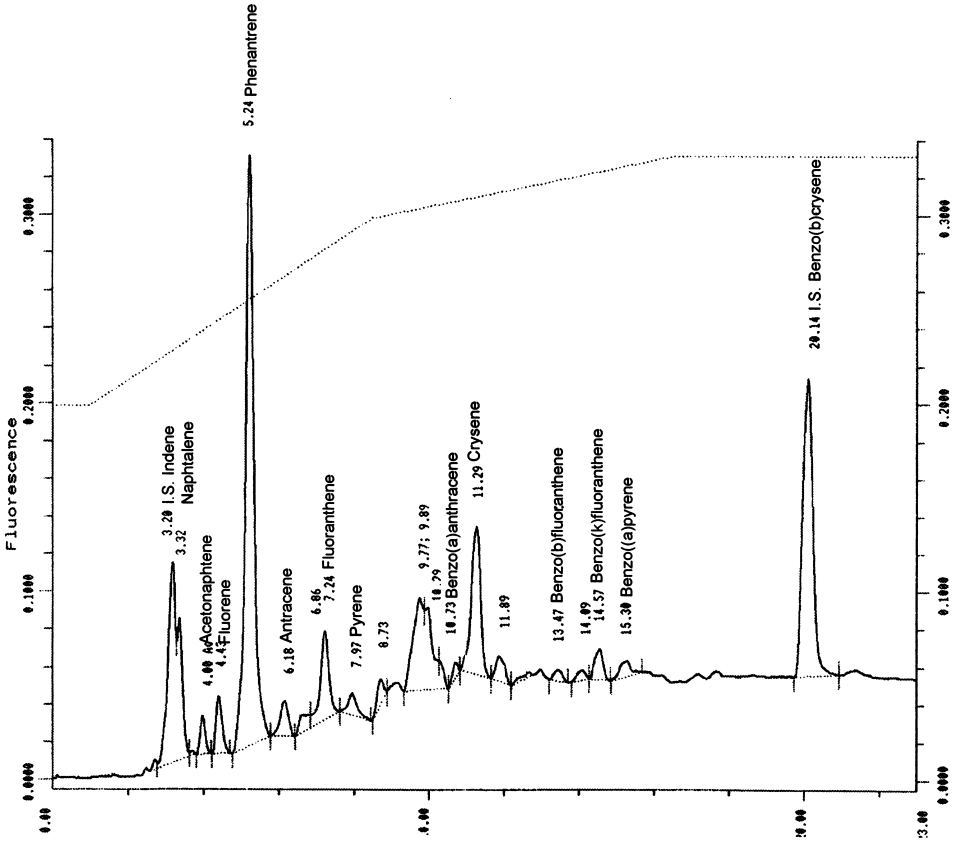

Fig. 5. HPLC profile of PAHs from a virgin olive oil sample with programmed fluorescence detection. The sample was analyzed using a special reversed-phase HPLC column (Vydac TP201, 25 cm×2.1 mm I.D.) at a flow-rate of 1 ml/min using gradient elution of acetonitrile–water (60:40) for 3 min and then to 100% acetonitrile. The detection was done by programmable fluorescence detector (initial λ_{ex} 280 nm, λ_{em} 330 nm; t=4.60 min, λ_{ex} 246 nm, λ_{em} 370 nm; t=6.50 min, λ_{ex} 280 nm, λ_{em} 450 nm; t=7.50 min, λ_{ex} 275 nm, λ_{em} 390 nm; t=12.70 min, λ_{ex} 290 nm, λ_{em} 403 nm; t=18.65 min, λ_{ex} 305 nm, λ_{em} 500 nm; t=19.5 min, λ_{ex} 230 nm, λ_{em} 400 nm).

also used to extract the PAHs from the triacylglycerols matrix followed by the clean-up of the unsaponifiable matter using several methods, such as, liquid–liquid partition using cyclohexane–DMF–water [83], silica gel column chromatography, and silica gel SPE cartridges [96].

The PAHs can be also isolated from the oil by complexation with caffeine–formic acid. The PAHs are back extracted with cyclohexane after destroying the complexes with aqueous sodium chloride. The clean-up was carried out by silica gel column chromatography [98] or by silica gel SPE cartridges [95].

The extraction method using liquid–liquid partition of the oil and subsequent clean-up by column chromatography packed with XAD-2 resin or by silica gel SPE cartridges showed better reproducibility and recovery percentage than the complexation with caffeine–formic acid [95,96].

The isolation of the PAH fraction by HPLC was performed either using electron acceptor stationary phases (tetrachlorophthalimidopropyl-modified silica), allowing the elimination of neutral lipids and tocopherols which can interfere with PAHs [99], or by a silica gel column, the recovery of the ''light'' PAHs being low [100].

Other methods of isolation comprises adsorption of the lipid material on synthetic calcium silicate (Caflo E) and extraction with acetonitrile–water (90:10) [101] and quantitative isolation using semipermeable membranes, in which neither triacylglycerols nor phospholipids were encountered in the dialyzed fraction [102].

Supercritical fluid extraction (SFE) has also been extensively used as an alternative to solvent extraction and clean-up. Recently the technique has been used to isolate PAHs from lipid matrices [103].

The quantification of PAHs is usually performed by reversed-phase HPLC in combination with fluorescence detection [83,90,93]. Fig. 5 shows a reversed-phase HPLC profile of PAHs from a virgin olive oil sample with programmable fluorescence detection of the PAH fraction isolated using liquid–liquid partition. Other authors used reversed-phase HPLC equipped with UV detection at 254 nm [99]. The availability of special reversed stationary phases increases resolution [99,104], and in conjunction with selective wavelength-programmed fluorescence detection provide a rapid and effective method for PAH determination [95].

The analysis has also been performed by GC on a capillary column (100% dimethylpolysiloxane) with FID [97]. Because of its lack of selectivity, special precautions must be taken with the chemicals used in the clean-up step in order to avoid contamination. Therefore, the identification and assessment of PAHs in vegetable oils have been carried out by GC–MS which provides the necessary selectivity [86,92].

The on-line HPLC–GC–MS system allows, in one step, the analysis of PAHs in vegetable oils with high selectivity and sensitivity [105].

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