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Review

Chromatographic analysis of minor constituents in vegetable oils

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Abstract

The main group of minor constituents belonging to vegetable oils are reviewed. Their importance in the characterization, origin and detection of oil mixtures are considered. Also, the determination of these minor components is of great value in establishing the oil quality and their genuineness. The most commonly used procedures (including the Official methodologies) normally applying chromatographic techniques are reviewed. The interference of each component within the determination of the other minor constituents are discussed. Furthermore, novel procedures for determining those compounds are also presented. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Vegetable oils are mainly constituted by triacylglycerols (95–98%) and complex mixtures of minor compounds (2–5%) of a wide range of chemical nature. These minor constituents show a broad qualitative and quantitative composition, depending on the vegetal species from they were obtained. Moreover, in the same species, content and composition of these components can vary due to the agronomic and climatic conditions, fruit or seed quality, oil extraction system and refining procedures. Finally, during storage of the oil, the hydrolysis, esterification and oxidation also originate changes in the minor constituents. Accordingly, the determination of the minor constituents is essential for the analytical assessment of the quality, origin, extraction method, refining procedure and possible adulteration of the vegetable oils.

The main groups of minor constituents present in vegetable oil are: fatty alcohols, wax esters, hydrocarbons, tocopherols and tocotrienols, phenolic compounds, volatiles, pigments, minor glyceridic compounds, phospholipids and triterpenic acids. The ability of an analytical method to characterize a vegetable oil is based on the identification and quantification of those compounds that are expected to be in connection with their origin and quality attributes. This is, however, a difficult task because these groups contain numerous species with a wide range of polarities, concentrations and chemical structures. Therefore, the methods require usually the isolation and analysis of minor constituents by means of several procedures of separation, identification and quantitation. An enrichment of the components of interest is usually necessary, but also a high separation efficiency and selectivity. Those characteristics are normally achievable by chromatographic techniques. To analyze the minor constituents of vegetable oils, a preliminary qualitative and quantitative isolation step from the triacylglycerol matrix is required. Three basic procedures are normally used: saponification, liquid–liquid partition and chromatographic techniques. The saponification (heating with alcoholic solution of potassium hydroxide) transforms the glyceridic compounds in polar soaps allowing the extraction of the unsaponifiable matter with hexane or diethyl ether. Nevertheless, this procedure is not appropriate for wax esters, sterol esters, phenols, pigments, minor glyceridic compounds and phospholipids, since they are altered during the saponification. Liquid–liquid partition with polar solvents (methanol, methanol–water, hexane–acetonitrile, etc.) is suitable for the isolation of phenols, polycyclic aromatic hydrocarbons and chlorophylls. Recently, supercritical fluid extraction has gained importance in the separation techniques, due to the possibility of modifying product solubilities through alteration of pressure and/or temperature, or adding modifiers, substituting a wide variety of liquid solvents. Column chromatography is widely used to separate fractions having constituents of similar polarities. Actually, the latter its being substituted by solid-phase extraction (SPE), as it is a quicker technique and saves solvent volumes. Finally, the isolation of volatile compounds is achieved by gas stripping or distillation. The isolated fractions are still complex and require further fractioning, usually by means of thin-layer chromatography (TLC) or preparative high-performance liquid chromatography (HPLC).

The qualitative and quantitative determination of the constituents is often done by capillary gas chromatography (GC) of the compounds or their derivatives. GC in general assumes that the compounds injected are volatile at the temperature of analysis and that they do not decompose at either the temperature of injection or analysis. In standardized analytical methods, flame ionization detection (FID) is the most widely used. Mass spectrometry (MS) allows obtaining molecular mass data, structural information and identification of compounds.

HPLC is used normally for separating non-volatile, high-molecular-mass constituents employing either adsorption or partition chromatography. Adsorption chromatography, namely normal phase, is widely used to separate classes of constituents according to the nature and number of polar functional groups. In normal-phase HPLC the adsorbent is silica gel and the eluent is a non-polar solvent. Reversed-phase HPLC, which is based on partition chromatography, is used to separate individual components that belongs to one constituent class. In this case, the stationary phase usually consist of a non-polar octadecylsilane (C_{18}) bonded phase, while the mobile phase is a polar solvent. Several detection

methods can be used in conjunction with HPLC, the ultraviolet–visible (UV–Vis) being the most commonly used. Other detection methods, such as refractive index (RI), FID, MS, evaporative light scattering (ELSD), fluorescence (FD) and electrochemical detection are also used.

A new technique have been developed and widely used in recent years, that combines HPLC and capillary GC achieving in one process the isolation of the fraction, its transfer, through an interface to the gas chromatograph and the GC analysis, allowing high separation efficiency and high sensitivity. On-line coupling provides a very interesting approach to integrate sample preparation into chromatographic procedure and, consequently, offers a new and practical alternative to traditional methods of sample preparation. Normally, the on-line HPLC–GC methods mainly refer to the use of normal phase in the LC separation and the transferring of the fraction by loop type interface using concurrent eluent evaporation. Furthermore, latterly the uses of reversed-phase HPLC separation enlarge the field of HPLC–GC applications. However, it must be taken into account that transfer to GC of polar solvents requires a new type of interface. The liquid fraction is transferred to the GC via a programmed thermal vaporized (PTV) injector packed with a porous polymer. Coupled chromatographic techniques (HPLC–GC) are being increasingly used, since they allow the isolation and determination of the compounds avoiding the sample preparation and clean up.

Supercritical fluid chromatography (SFC) is a relatively new separation technique that has features from both GC and HPLC, using supercritical CO_2 (SC-CO_2), as the mobile phase and both HPLC and GC detectors. Most applications are carried out near or slightly above room temperature, thus making SFC an ideal technique for separating thermally labile compounds.

2. Alcoholic compounds

Long-chain aliphatic and triterpenic alcohols, methylsterols, sterols and triterpenic dialcohols are important for the characterization of vegetable oils. They vary more widely between different oils than the fatty acid composition, and their content and composition in the oil are a rich source of information about the oil origin, providing information on the detection of mixtures. These compounds are present as free alcohols and fatty acid esters.

The saponification allows a detailed separation of these constituents, but does not provide information about their original structure, whether or not the component was esterified in the oil. The compositions of the free and esterified sterols, alcohols and triterpenic alcohols are not identical, and different extraction procedures or refining methods have different effects on free and esterified constituents. In addition, the ratio of free/esterified alcohols is related to the oil quality [1].

Official method for isolation of the total sterols and alcohols from olive oils implies saponification of the oil, extraction of the unsaponifiable matter with diethyl ether and washing of the extract with water. The extract is fractionated by TLC on silica gel plates yielding four separated bands containing: aliphatic alcohols together with triterpenic alcohols, methylsterols together with oleanolic aldehyde, sterols and triterpenic dialcohols [2]. Kawanishi et al. [3] used TLC on reversed-phase plates to separate alcohols from other interfering material in seed oils. A further development in the isolation of the sterol, and alcohol fraction is to pass the unsaponifiable matter through an aluminum oxide column [4], to avoid the extraction and washing steps. The method has been standardized by the International Standard Office (ISO) [5]. An alternative to the saponification involves alkali-catalyzed transesterification with KOH–methanol. The method was compared with saponification on sterol analysis, giving similar results [6].

The TLC fractioning of the unsaponifiable exhibits a problem in delimitating the corresponding band to be scrapped and extracted with solvents. Therefore, a HPLC method has been used to fractionate the unsaponifiable using a reversed-phase column, studying the effect of the mobile phase and the temperature over the separation efficiency, achieving good separations in several vegetable oils [7]. The HPLC separation was also carried out using a silica gel column and both UV–Vis [8] and RI detection [9]. In the latter, the precision of the determination of sterols and triterpenic dialcohols was noticeably better than the official method by TLC.

The isolation of sterols as free alcohols has been proposed by Horstmann and Montag [10] passing a solution of the oil through a silica gel solid-phase cartridges, circumventing the saponification. The triacylglycerols, wax esters and hydrocarbons were eliminated passing hexane, and the sterols recovered by passing hexane–diethyl ether [11]. However, the sterols co-eluted with the other alcohols and diacylglycerols, requiring a methylation with methanolic potash and subsequent fractionation by TLC [12].

Better results were obtained by Worthington and Hitchcock [13] using semi-preparative HPLC to separate free and esterified sterols from seed oils. The collected fractions were purified by TLC.

Ballesteros et al. [14] used an on-line isolation–preconcentration–extraction module to isolate free sterols from triacylglycerols. The method consists in a continuous-flow system with a silica gel column that retains the sterols and preconcentrates them prior to analysis. The sterols co-eluted with a reduced proportion of triacylglycerols, which were transesterified with acetyl chloride/methanol to fatty acid methyl esters.

Plank and Lorbeer [15] determine the free and esterified sterols by capillary GC after methanolysis under different conditions, being able to determine the concentration of the free sterols as well as their qualitative and quantitative composition and the total concentration of the sterol esters.

Conventional method for quantifying sterols, aliphatic and triterpenic alcohols involves capillary GC analysis, with low-polar stationary phase (5% phenylmethylsilicone) and FID, of the fraction isolated by TLC or semi-preparative HPLC as trimethylsilyl derivatives [2,5,9]. More polar phases (50% phenylmethylsilicone) has been used to detect peaks usually unresolved with non-polar columns [16,17].

For the identification of sterols, the analysis by electron impact ionization GC–MS of their acetyl esters [18] and their butyryl ester derivatives [19] has been used. The fragmentations of the derivatives are related to the fragmentation of free sterols.

Reversed-phase HPLC with UV–Vis detection has been used by Holen [20] to separate and identify free sterols, studying the effect of the column temperature and mobile phase on the efficiency of the separation.

Van Boven et al. [21] isolated and identified the methylsterols, fatty alcohols and triterpenic alcohols in jojoba oil by means of GC–MS of the free alcohols. On a further step Mariani et al. [22] analyzed the sterol fraction of several vegetable oils by GC–MS on highly polar columns (cyanopropylpolysiloxane) observing that the peak usually appearing at the time of campesterol was split in two: campesterol (24α-methylcholesterol) and 22,23-dihydrobrassicasterol (24β-methylcholesterol), both epimers at C_{24} carbon atom.

On-line coupled HPLC–GC was developed to resolve several problems at once. It allows the determination of free and esterified compounds in the same analysis and eliminates practically all-manual work. Some pivalic anhydride is added to the oil in order to esterify the free alcohols [23]. HPLC on silica gel columns isolates a fraction containing the free monoalcohols (as esters of pivalic acid) together with the fatty acid esters of those alcohols (wax esters). The fraction is then transferred on-line to GC obtaining a gas chromatographic profile showing the pivalic esters of the alcohols just before the wax esters [1,23]. Transferring a slightly later HPLC fraction to GC, free erythrodiol and uvaol, two triterpenic dialcohols, can be analyzed [24]. Afterwards, the on-line HPLC–GC technique was applied to samples of silylated oils obtaining similar results [25,26]. The main advantages of the method are the elimination of the saponification step and the good reproducibility as well as distinguish between esterified and free sterols giving additional information about the oil.

The determination of the sum of free and esterified alcohols involves the transesterification of an oil solution in methyl *tert*-butyl ether with sodium methylate in methanol, addition of water and subsequent extraction with hexane. The extract was then analyzed by on-line HPLC–GC using silica gel column, yielding a chromatographic profile showing the triterpenic and aliphatic alcohols and sterols [27].

The latter method offers an evident advantage, but the fact that the normal phase used may be deactivated by triacylglycerols, a backflush is needed after each injection. To avoid it, Señorans et al. [28] used on-line reversed-phase HPLC–GC to analyze free sterols in olive oil [29] and sunflower oil [30] using a PTV injector, packed with Tenax TA. The method

has been applied also to determine free erythrodiol and uvaol [31].

The determination of Δ8(14)- and Δ14-sterols has been proposed for detecting desterolized sunflower oil in olive oil, since these sterols arise from the isomerization of Δ7-stigmastenol. The determination of such compounds using the usual GC conditions is rather difficult due to they have similar retention times than β-sitosterol. Therefore, an elimination of the majority of β-sitosterol by means of TLC on silica gel plates and subsequent GC analysis over a polar column (70% phenylmethylsilicone) was necessary [32]. Furthermore, Biederman et al. [33], [34] used semipreparative HPLC and also on-line HPLC–GC for the determination of the Δ8(14)- and Δ14-sterols.

3. Wax esters

The wax esters, formed by the reaction of alcohols (aliphatic, triterpenic, methylsterols and sterols) and free fatty acids are present in seed and fruits. During the oil extraction process, a fraction of these esters is transferred into the oil, depending on the oil extraction system. So solvent extracted oils contain higher concentration of wax esters compared with cold-pressed and centrifuged ones. C_{40}, C_{42}, C_{44} and C_{46} waxes, deriving from straight chain alcohols are very abundant in olive pomace oils while low concentrations are found in olive oils. In addition, great concentration of waxes yields turbidness during refining or storage. Consequently, the determination of waxes is important to evaluate the quality and genuineness of some vegetable oils. Methods to isolate them have been described using TLC on silica gel plates [35] or on silica gel plates impregnated with silver nitrate [36], [37]. However, the usual procedure to separate wax esters is column chromatography [38], being used to detect olive pomace oil in olive oil [39] and to assess the effect of the extraction procedures in the quality of sunflower oil [40]. This method has been adopted by the European Union as an official method for olive oils [41]. The separation is affected by the composition of the mobile phase (hexane–diethyl ether, 99:1), the temperature and the activation of the silica gel. The wax esters eluted just before the triacylglycerols, thereby, the addition of a colorant (Sudan I) with a R_F similar to that of the triacylglycerols allows to visualize when the elution of wax esters ends [42]. Henon [36] and Kiosseoglu and Boskou [37] also suggested the separation of wax esters from vegetable oils using column chromatography on silica gel impregnated with silver nitrate.

Amelio et al. [43,44] used HPLC to isolate the wax ester fraction from vegetable oils using a silica gel column. In a recent work, Nota et al. [45] isolated and quantified wax esters from olive oils using a silica gel SPE cartridges. The methodology improves that proposed by EU Regulation 183/93 [41]. The authors demonstrated that samples with high acidity values (up to 10%) did not deactivate silica gel, neither produce matrix effect on the separations, and nor bring irreversible adsorption.

The isolation of wax esters from vegetable oils using supercritical fluid extraction (supercritical carbon dioxide as mobile phase) has been reported by García et al. [46].

The fraction isolated by above procedures, contains also other minor components such as the hydrocarbons, and methyl and ethyl fatty esters but they do not interfere in the quantitation of wax by GC analysis. After isolation of the wax ester fraction, the quantitation is normally carried out by a gas chromatographic system equipped with on-column injector, FID and using a short capillary fused-silica column (10 m) coated with 5% phenylmethylsilicone. The straight long chain aliphatic waxes are separated according to the carbon number but the steryl and triterpenic esters are not well resolved [38,40,41,43]. On normal length capillary columns (25 m) coated with polar phases (50% or 65% phenylmethylsilicone) separations among aliphatic waxes with the same carbon atom number were achieved [45]. Operating on these polar stationary phases the separation of the steryl esters by the fatty acid and sterol moieties has been described [47].

On the other hand, Kawanishi et al. [3] optimized the quantification of wax esters using the combination of TLC with FID by the system commercialized as Iatroscan.

Finally, the direct determination of wax esters from the oil can be performed using the coupled on-line HPLC–GC system. The HPLC, equipped with a silica gel column transfers the apolar fraction

to the GC via a loop type interface. However, the described methods realize a derivatization of the oil previous to the fractioning, in order to obtain in the same chromatographic profile the free alcohols and wax ester, as described in Section 2 [1,23,25,26].

4. Hydrocarbons

4.1. Aliphatic, sesquiterpenic and steroidal hydrocarbons

In virgin olive oil, the major hydrocarbon is the squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,20-tetracosahexaene) a terpenoid hydrocarbon occurring in high concentrations (800–12 000 mg/kg). It is accompanied by *n*-alkanes in the range of C_8–C_{35}, being the more abundant the comprehended between C_{21} and C_{35}, in which alkanes with an odd number of carbon atoms predominated over those of even numbers [48]. Besides, also low amounts of unsaturated aliphatic, sesquiterpenic (α-farnesene), low-molecular-mass aromatic (from benzene to tetramethylbenzene, including styrene) [49], and polycyclic aromatic (mainly low-molecular-mass ones) hydrocarbons [50] have been detected. In other crude vegetable oils the hydrocarbons series is similar to those encountered in olive oil, although the concentration of squalene is significantly lower (excepting pumpkin oil) [51]. The origin of the volatile aromatic hydrocarbons in virgin olive oils has been attributed to the metabolism inside the fruits and the equilibrium of olives and oils with the air environmental pollution [52,53].

During the refining process, the squalene isomerize [54] yielding a number of components with molecular mass 410. In the same way, isoprenoid alkenes of molecular mass 408 have been characterized [48,51] attributed to dehydration products of oxidized squalene [55].

Significant amounts of hydrocarbons with steroidal skeleton are formed in vegetable oils because of thermal treatments during the refining process, as dehydration products of the Δ5-sterols. Among these hydrocarbons, the stigmasta-3,5-diene is the most abundant in all refined vegetable oils since it derives from the β-sitosterol by dehydration [56]. The composition of steroidal hydrocarbon fraction enables to identify the oil origin, since their composition reflects that of the sterols [33]. 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 [57].

In crude vegetable oils, the isolation of the hydrocarbon fraction is performed usually by means of saponification of 20 g of sample, since hydrocarbon concentrations are quite low. The unsaponifiable is fractioned by means of a silica gel low-pressure column using light petroleum as eluent. The first fraction contains saturated and unsaturated aliphatic hydrocarbons, and cyclic sesquiterpenes. The second one contains α-farnesene and allofarnesenes, the third contains steroidal hydrocarbons and the fourth squalene. The identification of the compounds was carried out by mass spectra of the compounds, and those of their hydrogenated and dimethylsulfide derivatives [48].

For quantitative determination of stigmastadienes in crude vegetable oils, the first and second fractions are discarded and the third is analyzed by GC on a fused-silica capillary column coated with 5% phenylmethylsilicone. The method has been standardized by IUPAC [58] and adopted by the European Union [59] and the International Standard Office [60].

In refined oils, steroidal hydrocarbons concentrations are higher than in crude oils (1–50 mg/kg) and the saponification step can be avoided. A solution of the oil in hexane is directly separated on a silica gel column and the corresponding fraction is analyzed as described above [56]. Nevertheless, many refined olive oils contain isoprenoid olefins deriving from squalene isomerization. These compounds interfere the gas chromatographic analysis of sterenes and an improved isolation of these compounds are required. Cert and Moreda [61] developed and optimized a new silver-ion stationary phase for column chromatography allowing the isolation of sterenes from the oil, free of squalene derivatives.

The isolation of the hydrocarbon fraction by an HPLC on a silica gel column and subsequent analysis by GC is proposed for a quick screening of large number of samples with the aim of verifying whether the stigmastadiene content is lower or higher the legal limit [44].

The on-line HPLC–GC using a silica gel column has been used to detect refined oils by the determination of sterene hydrocarbons and polyolefins [62,63].

An alternative to GC analysis is the reversed-phase HPLC using UV detection at 235 nm. The oil is directly fractioned on a silica gel column and the hydrocarbon fraction analyzed by HPLC on a RP-18 column using UV–Vis at 235 nm [64].

The 3,5-steradienes are the main dehydration products of Δ5-sterols, but other isomers are also formed together with degradation products of Δ7-sterols, methylsterols and triterpenic alcohols. In order to identify these minor compounds, new isolation procedures has been used. Menie et al. [65] 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. [66] 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 and identified with an UV detector the different fractions; stereradienes at 235 nm and steratrienes at 309 nm. Several fractions isolated by the LC–LC system were transferred to the GC–MS provided with a methylsilicone or carbowax-type column.

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 [61] in a different order to that obtained using HPLC on silica gel [66].

Mariani and Grob [67] identified by GC–MS two epimeric sterenes at C_{24} carbon atom, one deriving from campesterol (24α-methylcholesterol) and the other from 22,23-dihydrobrassicasterol (24β-methylcholesterol).

4.2. Squalene

In crude vegetable oils with high concentration of squalene (olive and pumpkin seed oils), the interferences from other hydrocarbons in the GC analysis are negligible. Therefore, the European Union official method for wax determination [41], using a short capillary column coated with a low-polarity phase, allows the simultaneous determination of squalene and waxes if squalane is used as internal standard.

In vegetable oils with low squalene concentrations, the use of a normal length (25 m) capillary column is advisable in order to prevent possible overlapping with saturated hydrocarbons. In refined vegetable oils containing significant amounts of isoprenoid polyolefins, the use of a more polar stationary phase in the GC analysis is necessary to avoid the overlap [51].

4.3. Low-molecular-mass aromatic hydrocarbons

For the qualitative analysis of these volatile aromatic hydrocarbons (VAHs), a dynamic headspace technique was used, passing a nitrogen stream through an olive oil sample (60 g) and trapping the volatiles on an activated charcoal [68]. The trapped compounds were desorbed with carbon disulfide and fractioned by a silica gel column. The apolar fraction was analyzed by GC–MS [49].

For a quantitative determination, a static headspace was used. 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 and thermally desorbed and determined by GC–MS using 4-chlorotoluene as internal standard [52]. Morchio et al. [53] analyzed the VAHs in edible oils, with particular reference to virgin olive oils, using a trap–purge–desorption system (Tekmar LSC 2000) to isolate and transfer the volatiles onto the gas chromatograph. The oil headspace air was purged with a nitrogen stream and the volatiles adsorbed on a Tenax TA porous polymer [69]. The volatile compounds were then thermally desorbed and cryofocused in a short fused 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). 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.

4.4. Polycyclic aromatic hydrocarbons

Separate mention has to be made about polycyclic aromatic hydrocarbons (PAHs). These are present in vegetable oils in concentrations under 100 μg/kg, the ''light'' PAHs being more abundant than the ''heavy'' ones [50,70]. Refining reduces the amount of PAHs depending on the refining conditions adopted, the loss of ''light'' PAHs being greater that of the ''heavy'' ones [71]. Although, their origin is not clear, they may arise from different sources, including atmospheric pollution, uptake by the plants from PAH-contaminated soils or contamination through the extraction solvent. The high concentrations found in many grape seed oils (200 μg/kg) has been attributed to the direct drying of pressed grapes with fuel gases [50].

As always with trace analysis, the determination 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 needs for good chromatographic resolution and very sensitive detector for final quantification.

Several methods of PAH extraction and clean up have been described. The isolation of PAHs usually is carried out by liquid–liquid partition of the oil using cyclohexane and dimethylformamide–water (90:10) [72]. Hopia et al. [73] perform 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 [73,74]. Furthermore, Speer and Montag [70] purified the PAH extracts using silica gel column chromatography followed by a size exclusion chromatography. Several other procedures have been used to clean-up the extracts, among them are column chromatography on Florisil [75] and XAD-2 resin [76]. Moret et al. [77] simplified greatly the purification procedure using silica gel SPE cartridges instead of packed columns.

Besides, Menichini et al. [78] carry out the liquid–liquid partition of the oil dissolved in pentane using dimetyl sulfoxide and followed by TLC fractionation on silica gel plates.

Moreover, the saponification of the oil has been also used to extract the PAHs from the triacylglycerol matrix followed of the clean-up of the unsaponifiable matter using several methods, such as liquid–liquid partition using cyclohexane–dimethylformamide–water [50], silica gel column chromatography, and silica gel SPE cartridges [77].

The PAHs can also be 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 [79] or by silica gel SPE cartridges [76]. 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 than the complexation with caffeine–formic acid [76,77].

The isolation of the PAH fraction by HPLC was performed using either electron acceptor stationary phases, allowing the elimination of neutral lipids and tocopherols which can interfere with PAHs [80], or by silica gel column, although the recovery of the ''light'' PAHs was low [81].

The quantitation of PAHs is usually performed by reversed-phase HPLC in combination with fluorescence detection [50,71,74]. Other authors used reversed-phase HPLC with UV detection set at 254 nm [81]. The availability of special reversed stationary phases increases resolution [80,82], and in conjunction with selective wavelength-programmed fluorescence detection provide a rapid and effective method for PAH determination [76].

The analysis has been performed also by capillary GC–FID [78]. 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 providing the necessary selectivity [70,73].

The on-line HPLC–GC–MS system allows, in one step, the analysis of PAHs in vegetable oils with high selectivity and sensitivity [83].

5. Tocopherols and tocotrienols

There are four natural tocopherols (α, β, γ, δ) and

four tocotrienols (αT3, βT3, γT3, δT3), all R-configuration, which form the vitamin E group. The two classes differ by the presence of three double bonds in the side-chain of the tocotrienol series. The vitamin E group is lipid-soluble and the members are abundant in most vegetable oils in varying amounts (70–1900 mg/kg) [84,85].

Tocopherols in crude vegetable oils are partially destroyed by refining treatments. A further decomposition during storage is possible; an additional effect of light was of greater influence (higher decrease of the content) than the effect of the oxygen. Coors and Montag [86] studied the influence of these parameters on total tocopherol content and tocopherol distribution in several refined and crude vegetable oils during storage.

The most commonly used method for the analysis of tocopherols and tocotrienols is the direct HPLC analysis of the oil sample using normal phase and fluorescence detection [86,87]. The method with either fluorescence or UV detection has been standardized by the IUPAC [88] and the AOCS [89]. Besides, purification of oils before the analysis has been carried out by TLC in order to eliminate sterols [90] or by gel permeation chromatography to separate tocopherols from triacylglycerols [91].

The HPLC techniques have been also used with UV detection and reversed-phase columns [92,93]. The separation with reversed-phase columns presents the advantage of short equilibrium and analysis time and, high reproducibility of retention time but has the disadvantage of not resolve between β and γ isomers of both tocopherols and tocotrienols. On the other hand, normal-phase HPLC allows a good separation of all isomers but the analysis time is longer and the retention times are more variable. Likewise, the complete separation of all isomers is achieved using HPLC with a diol column [94]. Amperometric detection [95] and ELSD [91] have been compared with fluorescence, being both less sensitive than fluorescence and ELSD needing a very clean sample.

The capillary GC analysis of tocopherols and tocotrienols normally implies warm saponification, TLC separation, and formation of the trimethylsilyl derivatives prior to the chromatographic injection [96,97]. Mariani et al. [98] analyzed the trimethylsilyl derivatives, isolated by column chromatography, by means of GC–FID obtaining in the same chromatographic profile free alcohols, hydrocarbons, wax esters and tocopherols. Besides, Mariani and Bellan [99] using GC–MS achieve the identification even of tocodienols and dehydrotocopherols in some vegetable oils (palm, safflower and sunflower oil). Furthermore, Lechner et al. [100] isolated the silyl derivatives of the tocopherol by SPE and quantified them by on-column GC–FID. Recently, an automatic removal of the triacylglycerols by a continuous on-line transesterification pre-concentration module was used prior to GC–FID determination of α-tocopherol and α-tocopheryl acetate [14].

Using on-line normal-phase HPLC–GC, the laborious sample preparation needed for the GC analysis is circumvented and the determination of the silyl derivatives of the tocopherols together with the squalene, free alcohols, wax esters and hydrocarbons was carried out in the same run [26]. Some variability on retention times can be observed if triacylglycerol concentrations vary from one sample to another, because triacylglycerols deactivated the silica gel column affecting performance. Therefore, the use of reversed-phase HPLC–GC equipped with a PTV injector allows direct analysis of the oil and the use of more polar solvents and large volumes of eluent in the simultaneous analysis of free alcohols, tocopherols and squalene [101].

Recently, some authors demonstrated that tocopherol can be isolated and quantified using SFC–FID detector, allowing the separation of the tocopherols from complex lipidic mixtures. Furthermore, the SFC was coupled to a MS to permit identification of the tocopherol peaks [102].

6. Phenolic compounds

Phenolic compounds are a group of polar components, which contain one or more aromatic hydroxylated rings. Some of them show antioxidant activity and are responsible of the bitter taste of the vegetable oils. They are present in significant amounts (<350 ppm) in virgin olive oils and crude grape seed oils, but they are destroyed in the various refining stages. In olive oil, the main phenols are secoiridoid compounds originated by degradation of the glucosides oleuropein and ligstroside. The sub-

sequent hydrolysis of these compounds leads to the formation of simple phenols such as tyrosol and hydroxytirosol [103]. Minor amounts of phenolic acids as caffeic, *p*-coumaric and syringic, and flavonoids as luteolin and apigenin are also detected.

The isolation of the phenolic fraction from olive oils is the key point of the differences in the quantitative results described in the literature. Traditionally isolation has been performed by liquid–liquid partition of the oil solution in hexane with several portions of water–methanol, followed by solvent evaporation of the aqueous extract, and clean-up of the residue by solvent partition [104]. Extraction with tetrahydrofurane–water and posterior centrifugation has been also assayed [105]. These extraction procedures are very laborious, and alteration of the phenolic compounds could occur. Consequently, attempts to isolate the phenolic fraction by reversed-phase SPE using C_{18} [106,107] and C_8 [108] cartridges have been done, but incomplete extraction of phenolic fraction [105] and partial oil separation [108] have been reported. Other methods has been used to isolate and clean-up the phenolic fraction, among this stand the semipreparative reversed-phase HPLC and clean-up by silica gel TLC [108], and SPE using diol cartridges [109].

The usual method to analyze the phenolic fraction is the reversed-phase HPLC using isocratic elution with aqueous solution of sulfuric acid–acetonitrile [108] or with methanol–aqueous acetic acid [110]. The technique has been also applied using gradient elution with methanol–aqueous acetic acid [111] or acetonitrile–aqueous acetic acid [93]. In both procedures the system was equipped with a UV–Vis detector operated at 225, 240 or 280 nm. Because some phenolic compounds show several absorption maxima, the use of simultaneous multiple UV (photodiode array) detection is recommended for identification purposes [104,108,112].

Coulometric electrode array detection coupled to HPLC was also used for qualitative and quantitative determination of phenolic compounds in vegetable oils. The advantage of this method, besides of the easy sample preparation, was the possibility of separating and quantifying co-eluted substances with different oxidation potentials. The detector measures the different potentials, which the phenolic compounds are oxidized [113]. In addition, amperometric detector was also used to quantify phenolic compounds in olive oils, showing great sensitivity and stability, although the contamination of the electrode with the oxidation products is an important disadvantage [114].

Solinas [115] developed a GC method for the quantitative evaluation of phenolic compounds in virgin olive oils. The methodology implies liquid–liquid extraction of the phenolic compounds, clean-up of the methanolic extract followed by an azeotropic distillation to remove the solvents, low-pressure column chromatography to clean-up the extracts, and finally capillary GC analysis of the trimethylsilyl derivatives. Although, the method permits to characterize the simplest compounds, other linked phenols occurring in large quantities were not identified. Furthermore, Angerosa et al. [116] used a modified extraction procedure followed by capillary GC–MS to identify either the simple and the linked phenols present in virgin olive oils. Other authors used HPLC–MS to identify the structure of the phenolic compounds present in fractions previously separated by other procedures [105].

7. Volatile compounds

Flavor and aroma of the oils are generated by a number of volatile constituents that are present at extremely low concentrations. The volatile fraction of virgin olive oils has been profusely studied, and includes saturated, unsaturated, aromatic and terpenic hydrocarbons, as well as, alcohols, aldehydes, esters and ethers [49]. The influence of the olive cultivar, maturity, cultivation practices and extraction procedures on the volatile profile of olive oils has been reviewed by Kiritsakis [117]. The effect of olive alterations has been also studied [118,119]. In other crude vegetable oils, some compounds has been identified, such as α-pirene, calarene and kaurene in sunflower oil [120] and the enantiomers of the (E)-5-methyl-2-hepten-4-one (filbertone) in hazelnut oil [121]. In addition, during oxidation of crude and refined vegetable oils, various compounds responsible of undesirable flavor are formed. The analysis of pentane and hexanal has been proposed to evaluate the oxidation rate [122]. Likewise, the 3-methylnonane-2,4-diene is considered a significant

contributor to the reversion odor of soy bean oil [123].

The term volatile fraction is confusing and there is not an agreement about its exactly definition, mainly due to the experimental conditions applied by each research group for their obtention [124]. Thus, depending on the temperature and time used in the obtention of the volatile fraction, different results can be found. This is evident in the different collaborative study carried out by the AOCS [125].

Several isolation methods has been reported, all using GC–FID analysis, namely direct injection, static headspace, dynamic headspace, high vacuum distillation and on-line LC–GC.

In 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 precolumn. The injector is maintained at 200°C and the components of low and intermediate boiling points are volatilized and transferred to the column. Using this method, fat degradation products interfere [120], and the low amounts of sample (1 μl) allows the detection of major components only.

Due to the poor sensitivity, the static headspace is used only to quantify hexane residues in vegetable oils obtained by solvent extraction. However, using GC with electronic-capture detection (ECD), the method is applied to the quantitation of halogenated solvent residues in olive oils [2]. Snyder and Mounts [126] studied the volatiles produced from oxidized vegetable oils using a multiple headspace extraction (MHE) technique. The headspace over the oil heated at 90°C was sampled three times, and each sample injected consecutively onto the gas chromatography column maintained at −20°C. The MHE provided a more reproducible measurement than the obtained by single headspace. Furthermore, Yang and Peppard [127] applied the solid-phase microextraction (SPME) technique to absorb the volatiles present in the equilibrated headspace. A fused-silica fiber coated with polydimethylsiloxane was inserted during 2 min in the headspace of the oil under stirring.

The 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. [68] optimized the trapping of the volatile compounds on activated charcoal and desorbing with carbon disulphide. Moreover, the fractioning of the carbon disulphide extract was performed by Olías et al. [49] using silica gel columns. Other authors desorbed the volatiles trapped on charcoal with dithyl ether or acetonitrile [115]. The dithyl ether extract was analyzed by static headspace GC yielding a total volatile profile. On the other hand, the acetonitrile extract was analyzed, by reversed-phase HPLC with UV detector, either directly or after derivatization with 2,4-dinitrophenylhydrazine. The direct analysis gave a profile of the compounds having conjugated double bonds, whereas the analysis of the derivatized extract yields a profile of the carbonilic compounds.

The most used method consists in 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 in the analytical column by ballistic heating and quantified by GC–MS. The method has been used in the analysis of the volatile fraction in olive oil [53,128] and of oxidized products in oxidized soybean oil [122].

Supercritical fluid extraction (SFE) was applied to the extraction of the volatile fraction of the oil impregnating a silica gel support. The volatile were concentrated on Tenax TA trap placed in the outlet of the carrier fluid. The analysis was carried out similarly as the above-mentioned method. This extraction procedure yields higher concentrations of semi-volatile compounds than the dynamic headspace. Changing the experimental parameters of SFE, different GC profiles were obtained.

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 [120]. The on-line HPLC–GC on reversed-phase columns using a PTV as interface has been used to detect filbertone in hazelnut oil [129]. The effect of the packing material used for trapping the transferred solutes, desorption temperature and eluent composition of the mobile phase were investigated in terms of their influence on the sensitivity.

To identify minor components of the volatile fraction, large oil samples (400–1000 g) were subjected 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 [123]. The distillated was condensed and the solvent evaporated. The residue was fractionated on a silica gel column [130] or by HPLC [131]. When quantitation was needed, stable-isotope dilution technique was applied [132]. To detect trace amounts of thiols in the volatile extracts from olive oil obtained by means of SDSE, reversible covalent chromatography using Affi-gel 501 was used to separate fractions [133]. The thiol fraction was analyzed by multidimensional gas chromatography using moving capillary stream switching system (Fisons), being the column outlet connected to a GC–MS system [134].

For the volatile analysis in hazelnut oil, Blanch et al. [121] compared SDSE and SFE procedures in conjunction with GC equipped with a PTV injector. On-line reversed-phase HPLC–GC was also used. Better precision was achieved in the determination of filbertone by using both SDSE and on-line reversed-phase HPLC–GC analysis in comparison with SFE, although, the latter showed higher sensitivity.

In the analysis of oxidation products, the oils were derivatized with pentafluorophenyl hydrazine at room temperature. The compounds containing a carbonyl moiety yields the corresponding hydrazones, isolated by means of reversed-phase SPE (C_{18}) and analyzed by GC with either ECD, nitrogen–phosphorus detection, FID or selected-ion monitoring MS. ECD showed the better selectivity [135].

Finally, the sensorial analysis of the aroma can be achieved by splitting the eluent of the GC column to the FID system and to a sniffing port [136]. An olfatometric GC method to determining the odor threshold values has been also described [137].

8. Pigments

Chlorophylls and carotenoids are the main pigments in vegetable oils, being pheophytin the principal component of the chlorophyll group. 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 carotenoids are β-carotene and lutein. The type and amount of pigment in vegetable oils depend fundamentally on the species, cultivars, state of ripeness, and agronomic conditions, etc., and, in general, undergo a considerable variation during storage and preparation as edible oils. Both chlorophylls and carotenoids are considered to have an important role in keeping the quality of edible oils, mainly due to their action as photo-sensitizers or singlet oxygen quenchers respectively [106,138].

The carotenoids were determined after cold saponification of the oil [139], by means of reversed-phase HPLC [140]. The complexity of the carotenoid mixtures, as well as the variation in their polarity difficult the separation of carotenoids in a single run. Consequently, the combination of two coupled reversed-phase columns of different content on C_{18} and different porous size was used to aid in the identification of the *cis-/trans*-isomers. The less lipophilic ones were separated on the column with less percentage of C_{18} and higher porous size and the more lipophilic by the other one [141].

For the determination of chlorophylls, a spectrophotometric method has been standardized by the IUPAC [142]. Nevertheless, to quantify the different components of the pigment fraction, a chromatographic method was developed using direct injection of the oil onto an HPLC provided with a reversed-phase column and UV detection at 408, 430 and 450 nm [143]. The method allowed the detection of β-carotene, chlorophylls *a* and *b*, and pheophytines *a* and *b* simultaneously [144]. An improvement of the method was achieved using silica gel columns and UV–Vis diode-array detection [145].

In order to eliminate interferences, several procedures of isolation and clean up of the pigment fraction has been described. In olive oil, liquid–liquid partition with acetone–hexane and N,N-dimethylformamide(DMF)–hexane has been reported. The hexane elutes the carotenes and the DMF retains the chlorophylls, chlorophyll derivatives and the rest of the carotenoids [146]. The pigments contained in the DMF phase were separated by TLC and identified by white and UV light and by the UV–Vis spectra of the extracts obtained from the scrapped silica bands [147].

Mínguez-Mosquera et al. [146] described a quick method to isolate both the chlorophyll and carotenoid pigments by solid-phase extraction on a C_{18} station-

ary phase column and subsequent separation by ion-pair reversed-phase HPLC using detection at 410 and 430 nm.

9. Minor glyceridic polar compounds

Water, temperature and oxygen are considered as the main factors affecting oil degradation. Thus, water present in food is the cause of the hydrolysis which produce free fatty acids, partial glycerides and glycerol capable of generate from dehydration acrolein [148,149]. In the presence of oxygen, glyceride autoxidation leads to formation of hydroperoxide derivatives. The primary oxidation product produces other oxidized triacylglycerols monomers (alcohols, epoxi, ketones) and polymers. In addition, the high temperatures used in the deodorization step during the oil refining and during frying or cooking uses, lead to the cycling of the unsaturated fatty acids and polymerization of triacylglycerols (TGs). The above-mentioned factors alter in more or less extension all the oil constituents depending on the unsaturation and on their initial quantity in the original oil. Consequently, altered products coming from TGs will be more important quantitatively than the minor constituents, as TGs are the major constituents. They are called generically minor glyceridic polar compounds and have been more extensively studied than those coming from non-glyceridic constituents.

The intensity of the changes is narrowly related to the duration of the processes and to the oil composition. Besides, degradations are not independent, and the compounds interact once and toward to a more advance-altered stage, increasing the complexity of the process and the compounds obtained.

Many chemical and physicochemical parameters has been proposed for measuring the oil alteration, even, countless compounds have been isolated and identified. Actually, it is well accepted that analytical indexes widely used in the past, supply only partial information about oil degradation, being advisable the evaluation of polar glyceridic compounds. Over the past 10 years, there has been a considerable advance in the quantitation of altered components due to the advances in the analytical techniques.

9.1. Total polar glyceridic compounds

Total polar glyceridic compounds are defined as the sum of glyceridic materials that are not triacylglycerols, and their determination stands out as the most objective quantitative method to evaluate total degradation in fats and oils [150,151]. The methodology, to determine them, was standardized by the IUPAC [152] and adopted by the AOAC in 1984 [153], and consists in the separation, on a silica gel column, of the oil in two fractions. The first, containing non-altered triacylglycerols, elutes using an admixture of hexane–diethyl ether (87:13), the second, eluting with diethyl ether, contains the more polar compounds, namely dimers (DTGs), polymers (PTGs), oxidized triacylglycerols monomers (oxTGMs), diacylglycerols (DGs), monoacylglycerols (MGs), free fatty acids (FFAs), and other polar minor constituents. Both fractions are quantified by gravimetry. To simplify the procedure, different authors proposed the use of small columns in place of the classical, diminishing the oil sample quantity, as well as, the volume of solvent used for the elution. Thus, Sebedio et al. [154] proved that silica gel SPE columns yield the same results than classical silica gel columns. Furthermore, other authors have generalized the use of the technique using silica gel and also amino phases as support [155–160].

Later, Sebedio et al. [161] proposed the use of the TLC–FID technique (Iatroscan) to determine quantitatively total polar compounds, using Chromarod S II as support and hexane–diethyl ether–acetic acid (97:3:1) as eluent.

9.2. Polar compounds composition

The determination of the composition of the polar fraction is the most commonly used methodology to evaluate the alteration in heated oils. It requires the use of high-performance size-exclusion chromatography (HPSEC) to separate the components according to their molecular size.

A direct method for the evaluation of PTGs in heated oils was standardized by the IUPAC [162]. A liquid chromatograph equipped with a column of styrene–divinylbenzene of 100 Å and 5 μm and refractive index as detector are used. Nevertheless,

oil samples containing less than 3% (w/w) of PTGs are not suitable for evaluation.

A considerable improvement was achieved when HPSEC was applied to the polar fraction obtained from a previous separation of the polar and non-polar compounds by column chromatography on silica gel, since interferences by hydrocarbons, waxes and TGs are eliminated. The use of two SEC columns connected in series (100 and 500 Å, styrene–divinylbenzene) allowed the determination of triacylglycerols polymers, triacylglycerols dimers, oxidized triacylglycerol monomers, diacylglycerols and monoacylglycerols and thus to evaluate the thermal, oxidative and hydrolytic degradations [163,164]. This methodology was applied to oils coming from frying processes [165–167], as well as to crude and refined ones [168,169]. Undoubted advantages were achieved when SPE on silica gel cartridges was used instead of column chromatography using monoacylglycerols as internal standard [158]. In this case, the method goes without the mass of the fraction injected in the liquid chromatograph and total alteration is calculated using the area and mass of the standard [156,157].

9.3. Diacylglycerols

Diacylglycerols are the major components of the polar fraction in fresh olive oils. They are present owing to an incomplete synthesis of TGs, as they are intermediates in the formation of TGs, and are generated by hydrolysis of TGs during the oil obtention. Processes such as refining or frying do not produce noticeable increase in their quantities [170].

For the quantification of total diacylglycerols, the polar fraction is isolated from the oil by SPE on amino phase [155] or silica gel cartridges [158], and the polar fraction analyzed by HPSEC with either IR detection or ELSD. Direct analysis of the oil by reversed-phase HPLC allows the separation of the diacylglycerols according their equivalent carbon number (ECN) [171], but the overlapping between some DGs with different ECNs and isomeric structures, and interferences from sterols were observed [172]. The use of HPLC on a silica gel column and ELSD gave chromatograms showing only two peaks, corresponding to the 1,2- and 1,3-diacylglycerols [173].

The direct injection of silylated oils into a gas chromatograph equipped with a low-polarity capillary column has been standardized by the IUPAC [174]. This procedure allows separation according to carbon number and isomeric structure [175]. The introduction of fused-silica capillary columns coated with polar phases of high thermal stability (65% phenylmethylsilicone) permitted separations on the basis of carbon number, 1,2- and 1,3-isomeric structures and number of double bonds in the molecule [176,172]. Nevertheless, the direct injection of silylated oils implies analysis of the triacylglycerols, the majority components of the sample. Therefore, the isolation of diacylglycerols before the GC analysis is advisable. For the previous isolation of the diacylglycerols, TLC has been proposed [177], but SPE using diol phase cartridges is recommended since acidic or basic phases produce isomerization of the diacylglycerols [172].

10. Phospholipids

Phospholipids (PLs) are important constituents of crude oilseeds. The measurement of PLs is important in determining the stability and quality of vegetable oils. Phospholipids are undesirable in oil since they are responsible for oil discoloration during deodorization and steam distillation and losses of neutral lipids during neutralization. They affect the stability of the oil by chelating metals and increasing the amount of metal ions. The removal of PLs results in elimination of iron and copper, which increases the oxidative stability and facilitates the refining process.

Spectrometric methods have been used to determine the concentration of PLs and phosphorus in vegetable oils, however, these method are tedious and inaccurate.

In concentrated with high concentration of PLs, labeled lecithin, the separation and identification of the substances can be made by an standardized method using two-dimensional TLC [178]. The quantitative analysis of PL classes was also standardized by the IUPAC using HPLC with a silica gel column, UV detection at 206 nm and isocratic elution [179]. Nevertheless, the clean up of the enriched samples before the HPLC analysis is recommended. The purification by SPE was investi-

gated in different stationary phases (silica gel, aminopropyl, C_{18} and C_8) resulting in the C_8 phase giving the best qualitative and quantitative recovery [180].

In routine work, PL-rich layers obtained from degumming of soybean oil were purified on a silica gel column. The extract was analyzed by one-dimensional TLC, the spots developed with iodine and then quantified by imaging densiometry [181].

The SPE on aminopropyl cartridge allowed a rapid and sequential separation of the individual PL classes using different solvent mixtures for elution [182].

The low concentration of PL in vegetable oils requires some pre-concentration step, achieved by column chromatography [183–185] or SPE [182]. The silica gel SPE cartridges has been used to isolate the PL from crude soybean oils [186,187], while diol-phase ones were applied to the isolation of PL in sunflower oil [188]. In crude peanut oil, PL was enriched by HPLC using a 2 cm silica gel precolumn and hexane as mobile phase. Repeated injections were made, neutral lipids were eluted with the hexane, and PL retained on the pre-column. Then, the pre-column was connected to the analytical column and eluted with a polar solvent [189]. This system was improved and automatized by Singleton and Stikeleather [190].

The quantitative analysis of the PL fraction is usually performed by HPLC using silica gel column and UV detector with either isocratic [179,188] and programmed gradient [186,187,189]. ELSD has been used in conjunction with preparative HPLC to isolate PL classes from soybean oil. In each PL class, the separation of molecular species differing in the fatty acid composition was achieved using reversed-phase HPLC [184]. Several reversed-phases were investigated and the best separation of the PL species was obtained with octadecanoyl poly(vinyl alcohol) phase [185].

11. Triterpenic acids

Virgin olive oils contains minor amounts of oleanolic and maslinic acids, but significant amounts (0.1% and 0.4% respectively) were isolated from crude olive pomace oils [191]. These compounds seem to be responsible for the gelatinous precipitates in crude olive-pomace oil [192] and the turbidity observed in some physically refined olive oils [193].

The more accurate method for determining triterpenic acids in vegetable oils consists in the isolation of the triterpenic acids by means of SPE cartridges packed with aminopropyl phase and subsequent analysis by GC. The solution of the oil in hexane is passed through the cartridge and eluted with several solvent admixtures in order to elute hydrocarbons, waxes, triacylglycerols, alcohols and diacylglycerols. The elution with diethyl ether–acetic acid (98:2) desorbs the triterpenic acids and the free fatty acids. The silyl derivatives are analyzed by GC using a capillary column coated with 5% phenylmethyl silicone [194].

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