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Journal of Chromatography A, 885 (2000) 321–341

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Update on solid-phase extraction for the analysis of lipid classes and related compounds

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Abstract

This article provides information on the different procedures and methodologies developed when solid-phase extraction (SPE) is used for lipid component separation. The analytical systematics, established by different authors and designed to separate groups of compounds and also specific components by using a combination of chromatographic supports and solvents are presented. The review has been divided into three parts, which we consider well defined: edible fats and oils, fatty foods and biological samples. Separations of non-polar and polar lipids is the most extensive systematic, although many other published methods have been established to isolate specific components or a reduced number of components from edible fats and oils, fatty foods or biological samples susceptible to further analysis by other quantitative techniques.
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Keywords: Reviews; Solid-phase extraction; Lipids

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PII: S0021-9673(00)00181-3

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1. Introduction

The aim of any technique, be it column chromatography (CC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and mainly the coupled techniques (HPLC-GC) as well as thin-layer chromatography-flame ionization detection (TLC-FID) for the quantitative analysis of different kinds of lipids, would be to supply as much information as possible about each compound with minimal interference or overlaps with other similar components. Nevertheless, it is necessary to carry out a minimum number of previous operations and steps in order to isolate a sample free of interfering compounds before the quantitative determination can be done. Thus, liquid–liquid or liquid–solid extractions, as well as TLC, are classical techniques for separating the different classes of lipids found in edible fats and oils, biological fluids and plasma. In liquid–liquid extractions, chloroform, diethyl ether or ethyl acetate is used in variable proportions to isolate the various lipidic components [1,2]. This methodology is a lengthy procedure, since multi-step extractions and in some cases large volumes of organic solvents may be needed to accomplish an efficient extraction [3]. Furthermore, the formation of an emulsion is very common, which diminishes the efficiency for extracting some compounds. Thus, the more complex glycolipids and especially the gangliosides, acyl CoA esters and the more polar prostaglandins are partially or completely lost in the aqueous layer during the extraction of the solvent [4].

Regarding liquid–solid extractions, the adsorption of compounds from the liquid phase takes place in a solid adsorbent. The recoveries, and consequently the effectiveness, of the procedure will depend on the type of solvent used. Fractionation of lipids by liquid–solid chromatography has been widely used and reviewed by Mangold [5] and previously by other authors [6–9].

TLC is also one of the more common techniques used for the qualitative separation of the different lipid classes because it is the faster and more efficient one and has been used for a many years. However, the difficulties inherent in the separation of complex biological samples, the oxidative deterioration of polyunsaturated lipids during the TLC process and the problems that arise when the limit of the sample is exceeded are well-known inconveniences of this technique.

Solid-phase extraction columns were invented in the mid-1970s [10], and in 1978 Waters promoted the Sep-Pak cartridges, a convenient, dry packed silica-based disposable columns [11]. Two years later, Williams and McCluer and also Powell published the first papers on the application of SPE in the field of lipids. The first one deals with the isolation of gangliosides from animal tissue extracts [12], while the second one is on the fractionation of arachidonic metabolites [13]. Later, the principles of the SPE, the methods, isolation, and fractionation of lipids in biological and food matrices were extensively reviewed by Wachob [14], Christie [15], Ebeler and Shibamoto [16] and Ebeler and Ebeler [17].

1.1. Principles

SPE is just a CC, which is employed as a liquid–liquid extraction substitute and therefore follows almost the same basic principles of the HPLC. It is considered one of the most powerful techniques currently available for rapid and selective sample preparation [18].

In a SPE process, the lipidic components of interest are retained in the adsorbent placed in these disposable extraction mini-columns. Actually, a wide

variety of the chromatography supports are utilized in lipid separation processes.

The versatility of the SPE allows it to be used for a large number of purposes, for example as an effective method of sample purification, or for compound isolation and removal of reagent excess [14]. Thus, methyl ester samples intended for GC analysis can be purified by passing them through an adsorption cartridge, which may remove the peroxides and hydroxides [19]. It is effective because it enriches the minor components of a sample and is a quick procedure for separation. A great number of quantitative techniques (HPLC, GC, UV) have adopted the benefit of the SPE and used it as a first step. The common strategy chosen for the analysis of specific lipids will depend on the nature of the information needed, and the need to do so can be frequently solved by adopting some established analytical routine.

As SPE is based on the principle of liquid chromatography, strong but reversible interactions between the analyte and the stationary phase occur in these columns. Typical interactions are, for example, non-polar interactions between the C–H links of the analyte and the C–H links of the adsorbent (Van der Waals forces). The most common support for non-polar interactions are C_{18} cartridges, although C_8 and C_2 are also used for lipid separations. Regarding polar interactions, they include the links created by hydrogen bonding, dipole–dipole forces, etc. These interactions are typical of all the cyano (CN), amino (NH_2), diol or silica (Si) supports. In third place, we have the ion-exchange interactions occurring in a phase such as the one with quaternary amines, benzenesulfonic acids or propylsulfonic acids. These interactions take place when the analytes have negative or positive ionic charges (Table 1) [11].

One of the major problems that often occurs when we read a paper that mentions the pretreatment of these samples is that an incomplete description of the

Table 1
Properties of the supports

	Separation mode	Phase		Properties
Normal silica phase	Normal phase	Silica	$-Si-OH$	The support is polar and the matrix non-polar; thus, non-polar compounds pass through the cartridge depending on the eluent used
Modified silica phases (bonded phases)	Normal or reverse phase	NH_2 (aminopropyl) CN (cyanopropyl) Diol (2,3-dihydroxypropoxypropyl)	$-Si-(CH_2)_3NH_2$ $-Si-(CH_3)(CH_2)_3CN$ $-Si-(CH_2)_3OCH_2CH(OH)CH_2OH$	Moderately polar, they are used as an alternative to silica
	Reverse phase	C_{18} (octadecyl)	$-Si-C_{18}H_{37}$	It has strong hydrophobicity and is used to adsorb analytes from aqueous solutions
		C_8 (octyl)	$-Si-C_8H_{17}$	It has moderate hydrophobicity and retains less than C_{18}
		C_2 (ethyl) Phenyl	$-Si-C_2H_5$ $-Si-Ph$	They have low hydrophobic character
	Ion exchange	Quaternary amine	$-Si-(CH_2)_3N^+(CH_3)_3$	Strong anion exchanger used to extract compounds capable of carrying a negative charge from both aqueous an non-aqueous solutions
		Propylbenzenesulfonate Propylsulfonate	$-Si-(CH_2)_3C_6H_4-SO_3^-$ $-Si-(CH_2)_3SO_3^-$	Strong cation exchanger used to extract positively charged basic compounds

steps followed is given. The procedure of the SPE, as applied to lipid separation, is often poorly described, including the work that went into the development of the method. Although many published methods are shown and described as being thorough and reproducible, they are in practice difficult to repeat successfully if the authors do not even give the minimum details. Reasons concerning the chosen SPE cartridge, the conditioning of the phase, the volume and quantity of the sample loaded, the flow-rates as well as the suppliers are usually not commented upon. If the method described is really that good, it should contain a discussion of the logic behind it. Each one of the details commented above has its own role to play and by putting all together we achieve the process.

2. Compounds from edible fats and oils

Edible fats and oils are a complex mixture of TG (90–95%), DG (1–2%), FFA (≈0.5%), PL and other minor components (≈2.5% in the case of oils, or 10% in the case of animal fats (Table 2). In the majority of the cases, the main valuable components are standardized by official analytical methods.

Any one of the components contributes to the texture and flavor of natural fats and oils and most of them play a role in human nutrition. Therefore, they are important for both the food and pharmaceutical industries [20,21]. Good information about their analysis and chemical composition will allow us to find out more about the influence they have on the diet.

Table 2
Constituents of edible fats and oils

Glyceridic compounds	Non-glyceridic compounds
Triglycerides	Hydrocarbons
Diglycerides	Free fatty acids
Monoglycerides	Waxes
Phospholipids	Fatty acid ethyl and methyl esters
	Sterols, methyl sterols
	Alcohols, triterpenic dialcohols
	Vitamins; tocopherols
	Pigments
	Terpenic acids
	Other constituents and contaminants (phenols, metals, etc.)

The presence of oxygen, temperature, time and storage conditions can alter the compounds mentioned above. Thus, even a group of altered compounds such as ox-S, CM, TG-D, TG-P and ox-TGM originating from the original components can be found [22].

While the use of SPE for separating different classes of lipids from biological matter has been widely applied for a long time, its use is not very common in the analysis of edible fats and oils. Thus, in spite of the need of the oil industry to save time and reagents, most of the official methods they apply do not take into account or include this type of separation [23].

The most common fractionations carried out in edible fats and oils are separations into a non-polar (TG) and a relatively polar (oxidation products) fraction. Other, more complex fractionations of components with an increased polarity or the isolation of specific substances are also successfully described using SPE.

2.1. Non-polar and polar fractions

The most widely cited solid-phase extraction packing for a simple separation of non-polar and polar fraction from edible oils is silica gel. Hexane–diethyl ether (92:8) and methanol, hexane–diethyl ether (90:10) and diethyl ether or hexane–diethyl ether (90:10) and chloroform–methanol (2:1) were used to fractionate non-polar and polar lipids (those that are more polar than triglycerides) in heated and frying fats and oils [24–28]. All these analytical methods are based on the well-standardized IUPAC method [29], where 1 g of oil sample is separated using 20 g of silica gel and 150 cc of petroleum ether–diethyl ether (87:13) and subsequently 150 cc of diethyl ether. This procedure is time-consuming, slow and uses a lot of solvent. The use of solid-phase extraction makes the method easy and quick, apart from saving solvent. Samples and reagents can be reduced at least by a factor of 10. Nevertheless, it seems to be more difficult to standardize, since Si-cartridges (as was demonstrated in the case of CN-bonded phases [30]) differ from one supplier to another and even among batches coming from the same source [26]. The reason seems to be the water content, which greatly influences cartridge efficiency,

it becoming necessary to dry the cartridge before the analysis or to use solvents with controlled humidity [31]. The use of an appropriate internal standard and subsequent quantification of the polar fraction by HPLC makes this methodology more advantageous than the standardized one.

When Hopia et al. isolated the polar compounds of edible oils, they preferred amino over silica columns, because amino cartridges are slightly less polar than those with silica and the moisture has less effect on the capacity for lipid adsorption [24].

Silica cartridges are also used to purify the non-polar fraction (triglycerides) of virgin olive oils from other polar compounds in order get interference-free TG, which can then be analyzed by RP-HPLC. The methodology is similar to the process described before: 100 mg of oil sample and 13 ml of hexane–diethyl ether (87:13) elute the TG. In this case, the triglyceride fraction is of interest and any other solvent is used to recover the more polar components. The methodology proposed serves to obtain a group of chromatographic peaks of TG corresponding to the equivalent carbon number (ECN) 42, which allows elucidation if virgin olive oils have been diluted with other seed oils [32].

In addition, purification of TG can be accomplished by using Florisil columns and eluting with hexane–diethyl ether (80:20) [33]. Subsequently, stereospecific studies of triacyl-*sn*-glycerols from different vegetable oils such as safflower, sunflower, olive and palm, have been carried out isolating and obtaining the diacyl-*sn*-glycerol urethane derivatives by C_{18}-SPE. The authors advise that the methodology described is not suitable for application to more complex TG, such as those from milk fat or fish oils.

Analysis of TG having different degrees of unsaturation through the use of fragmentation by argentation chromatography (TLC or CC) has been extensively used in the past. Silica gel was the most widely used adsorbent for TG separations [34–39]. An optimum percentage of impregnation of Ag^+ in classical CC seems to be between 5 and 10%; other percentages can have a considerable effect on adsorption activity. As this adsorbent seems to be very inconvenient, other stationary phases using cation-exchange support consisting of macroreticular sulfonic acid resins or silica gel with chemically bonded methyl–, benzene– or *p*-propylbenzene–sulfonic acid groups have been also used for this purpose. They achieve good silver ion retention levels, avoiding the difficulties inherent in the silica gel and loading less Ag^+ for the impregnation of the column. In this way, Christie [40] as well as Ulberth and Achs [41] were the first to use benzenesulfonic acid SPE columns loaded with silver ions to separate FAME. On these columns, further TG were also successfully separated in different fractions [42]. This methodology was also used by Kemppinen and Kalo [43], who described a separation of TG in four fractions from lipase-modified butter oil according to the degree of unsaturation. The method, fractionation on a *p*-propylbenzene–sulfonic acid SPE column loaded with silver ions, is an adaptation of Christie's experiment [42] on FAME fractionation, and provides a useful and rapid alternative to the $AgNO_3$-TLC methodology [44]. Saturated TG elute with 6 ml of penthane–dichloromethane (50:50), monoenes with 5 ml of acetone–dichlorometane (1:99), dienes with acetone–dichloromethane (5:95) and trienes with 5 ml of acetone. Afterwards, these fractions were studied by GC and, based on FA composition, the degree of overlapping of each fraction was determined.

2.2. Isolation of the minor components of edible oils

The isolation of minor components of edible oils (wax esters (WS), diglycerides (DG), sterols (S), monoglycerides (MG), free fatty acids (FFA), hydroxypentacyclic triterpene acids (HPTA), phospholipids (PL)) and of specifically altered or oxidized compounds have been described in different papers using Si, NH_2, NH_4^+, C_{18} as well as diol-phase columns. The precision required for any isolation by SPE depends on the analytical technique used afterwards for the quantitative determination of the compounds that are of interest. Thus, sometimes the latter attend others, which do not disturb their quantitation. For instance, the DG fraction is isolated together with free sterols, alcohols, or HPTA. However, these compounds do not interfere in their determination by GC. A similar situation occurs when determining wax esters by GC when they were previously isolated using Si cartridges.

2.2.1. Diglycerides

After TG, DG are the major compounds present in edible fats and oils. They arise as intermediate products in TG biosynthesis and/or are present owing to the acidic or enzymatic hydrolysis of TG. A large number of analytical methods for evaluation purposes are described in the literature [45–49]. The determinations have been performed using two different methodologies. The first consists in the isolation and subsequent analysis, while the second is carried out by direct analysis using different techniques. The most widely accepted method is the one standardized by the IUPAC Commission on Oils, Fats and Derivatives, which consists in the direct injection of the silylated oils into a gas chromatograph equipped with a low-polarity capillary column [50].

The authors of this paper [51] described an analytical systematic that combined the use of a diol bonded phase column and GC for the quantitative determination of DG isomers in vegetable oils. Different chromatographic supports, among them NH_2 and Si, produced isomerization (Table 3). Thus, it shows the rate of isomerization as a percentage of dipalmitin isomers through SPE-bonded phases. Only the diol cartridge served as support for isolating DG without isomerization. The fraction of interest was isolated with chloroform–methanol (2:1), discarding a first fraction with hexane–methylene chloride containing the majority of TG, the main interfering compounds. Then, it is silylated and injected into a gas chromatograph equipped with a polar phase capillary column. As commented before, other oil components such as free sterols and alcohol are present in this fraction, but none of them interfere in the process. Fig. 1 shows the procedure followed and includes two representative chromatograms with the main isomers found when the diol and amino cartridges are used. As can be observed, strong isomerization occurs when the sample passes through the amino phase. Other phases have also been shown to have the same inconvenient, only the diol phase cartridges do not produce isomerization. The proposed methodology has also been employed successfully by other authors [52,53].

These results do not invalidate the works on the use of amino columns for total DG quantitation and other applications. Moreover, the great value of these columns for the isolation of acidic compounds has been demonstrated [47,54–56]. Nevertheless, it demonstrates that a lot of care must be taken before choosing a support until more explanation and importance is given to the adsorbent employed.

An example of the clear advantage of amino columns can be seen in the procedure shown in Fig. 2. The properties that these columns have for retaining mostly acidic compounds have been used to isolate terpenic acid present mainly in olive oils [57]. Hydroxypentacyclic triterpene acids (HPTA), mainly oleanolic (3α-hydroxyolean-12-en-28-oic) and maslinic (2α,3β-dihydroxyolean-12-ene-28-oic), inappropriately called waxes by many, are present in the external part of the olive fruit (cuticle). During the oil extraction process, they pass to the oil, yielding quantities of approximately 200 ppm, which increase when the fruits deteriorate or are damaged. This determination could be another and more objective, quality parameter that should be taken into account for evaluating virgin olive oils. As can be observed in the procedure, FFA and HPTA are first retained in the NH_2 phase and, afterwards, are isolated by elution with diethyl ether–acetic acid and, finally, after silylating are quantified by GC on a non-polar phase.

Table 3
Isomerization rate of 1,2-PP and 1,3-PP through SPE cartridges[a]

Phase	Isomerization (%)	
	1,3→1,2	1,2→1,3
SPE diol	N[b]	N
SPE Silica	3.7	4.3
SPE amino	35.0	63.5

[a] Adapted from Ref. [51].
[b] N, negligible.

2.2.2. Wax esters

Wax esters are a group of compounds formed by the esterification of alcohols with fatty acids, are present in variable quantities in edible fats and oils. For example, in extra virgin olive oils these compounds reach concentrations lower than 250 ppm, as dictated by EEC Regulation 183/93 [58].

They are very important in the case of some fish oils where their high concentration interferes strong-

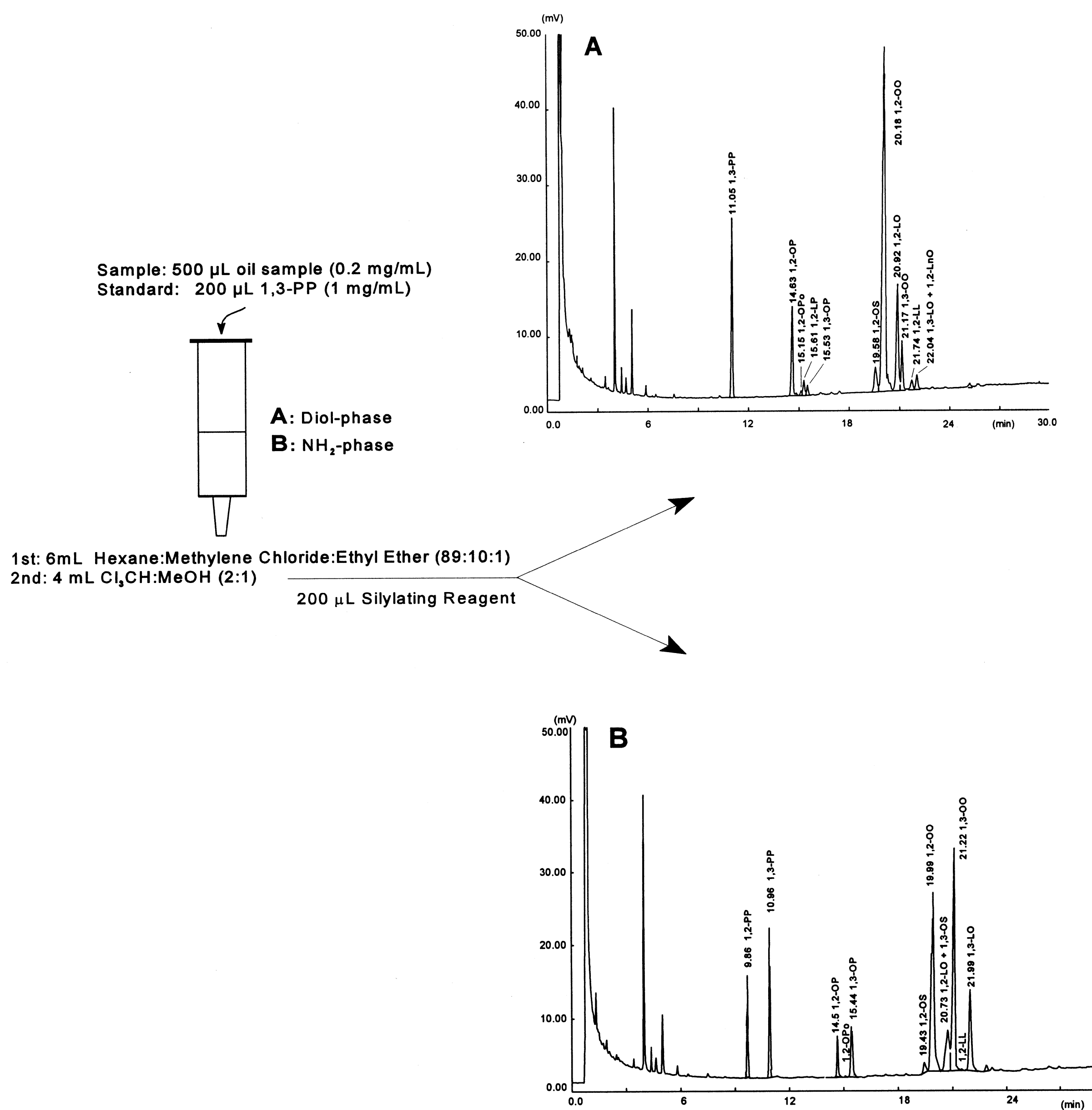

Fig. 1. Combination of SPE and GC for the quantitative determination of diglycerides. (A) Using diol phase, (B) using NH_2 phase.

ly with other analytical determinations, as is the case with the lipids from *Ruvettus pretiosus* [59].

Their high molecular weight makes them highly insoluble in water. If the alcoholic group has a linear chain of carbons the result is an aliphatic wax, which has between 36 and 46 carbons. In the case where the union of triterpenic alcohols, sterols or methyl sterols produces esterification with fatty acids, the result is a triterpenic wax. Different attempts to reduce time and simplify their analysis have been made [60–62]. One of them, published in a recent work by Nota et al., isolated and quantified wax esters from olive oils by SPE using a silica cartridge [61]. The methodology improved on the official

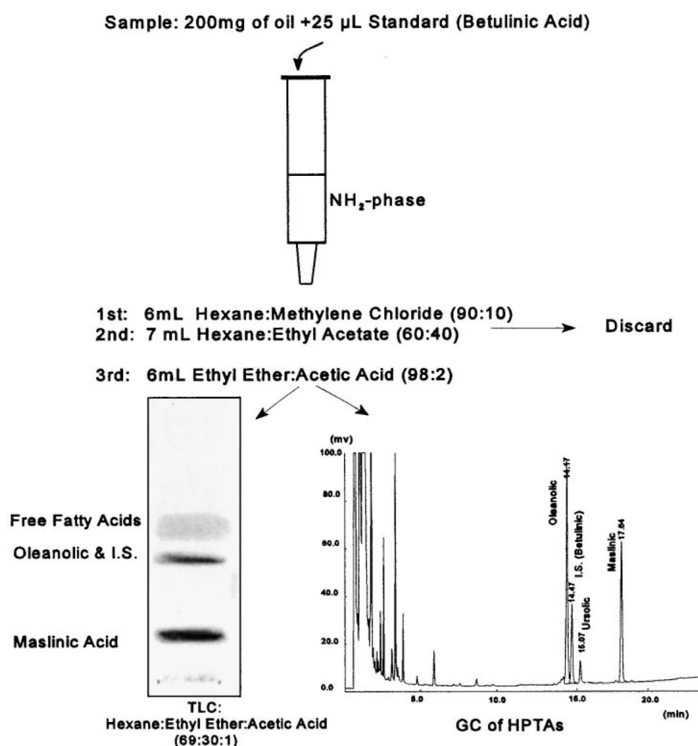

Fig. 2. Scheme followed for the isolation and quantification of HPTAs, using NH_2 columns.

method established by EEC Regulation 183/93 [58]. One gram of Si-phase column is employed to separate wax esters from 20 mg of oil, and a solution of lauryl arachidonate is used as an internal standard, as done in the official methodology. The eluent used is carbon tetrachloride, which elutes the waxes, retaining the rest of the olive oil components in the cartridge. The authors demonstrate that in their methodology samples with high acidity values (up to 10%) do not deactivate the silica gel, do not cause a matrix effect on the separations and do not bring irreversible adsorption. Although data deviations are higher than with the official methods, they do not exceed 5.7%. When they analyzed extra virgin olive oils, which are characterized by a low wax content, the deviation for other categories does not exceed 3.6%. Their study leads to the conclusion that this methodology is an advantageous alternative to the EEC procedure. The fraction isolated by the authors also contained other minor components present in olive oils, such as the hydrocarbon fraction and free esters as well. Neither one of them interferes significantly in the quantification of waxes.

2.2.3. Sterols

Sterols are a group of non-glyceridic minor components present in edible fats and oils. The most frequent one is β-sitosterol (≈90%) in vegetable oils [63,64]. Other minor sterols also present in the oils are α-cholestanol, campesterol, campestanol, stigmasterol, Δ7-campesterol, clerosterol, sitostanol, Δ5–24 stigmasterol, Δ7-stigmasterol, Δ7-avensterol and cholesterol, which is the most important sterol found in animal fats. As the β-sitosterol structure resembles cholesterol, its study and formation of oxidation products is important due to their similitude. Thus, as with cholesterol products, they have been demonstrated to play an important role in atherosclerosis [65,66]. The presence of β-sitosterol oxidation products could also induce desirable biological effects [67]. Their content is also very important, especially for investigating the quality of

olive oil. As with lineal or triterpenic alcohols, they can be present in free form or esterified with fatty acids [68,69]. The present EEC Regulation No. 2568/91 requires analyses for this type of research and fixes the physical and chemical characteristics of the products [70]. The technique requires substantial manual operations, which makes the methodology tedious. Thus, several papers have described alternative methods for replacing the official methodology [69]. In particular, SPE has been proposed for the determination of both free and combined sterols, and for total sterol content in unsaponifiable matter [71] as well as for the isolation of its oxides. Thus, NH_4^+ phase cartridges were proposed by Amelio et al. in 1992 for isolating sterols, erythrodiol, uvaol and alkanols in olive oils. The chosen phase has the property of removing all the remaining soap once the saponification process is completed, making it possible to obtain a fraction susceptible for analysis by HPLC or GC.

Nourooz-Zadeh and Appelqvist [67] analyzed sterols and sterol oxides from edible oils. Their proposed methodology includes a separation of the lipids on a Lipidex-500 column to isolate free sterols, and a subsequent separation on a NH_2 cartridge to enrich the sterol oxide fraction. The efficiency of the combined Lipidex-5000 and NH_2 cartridge system was studied, resulting in a recovery of 85–88% depending on the compounds. The analytical procedure allowed the authors to study crude and refined soybean oils and virgin olive oils after 1 year of having obtained them.

Si-cartridges were chosen by Chung and Lucy [72], who described a method for separating sterols, particularly for determining the amount of esterified and free cholesterol in lard. A sample solution (100 mg of lard in 1 ml hexane) is applied to a Si-cartridge that has been previously conditioned with 4 ml of hexane. The cartridge is eluted with 8 ml of hexane–ethyl acetate (92:8). This is the fraction that contains the cholesterol esters. Two more fractions are collected by passing 4 ml of hexane–ethyl acetate (90:10) and 8 ml of hexane–ethyl acetate (80:20). These two fractions combined contain free cholesterol. The fraction containing the esterified cholesterol is saponified and both the free and esterified fractions are quantified by GC. The recoveries exceeded 90%, so the authors concluded that cholesterol esters in lard represent about 32% of the total cholesterol.

2.2.4. Phospholipids

The development of methodologies for the quantitative analysis of PL becomes important mainly for evaluating the efficiency of the degumming process during the refining of edible oils [21,73–76]. It also serves to monitor the quality of non-refined oils and the effect that environmental conditions, such as humidity, storage abuse or a lengthy storage, have on the oilseeds and on oil quality. The analysis done on vegetable oils is rather recent. The low PL concentrations present in vegetable oils require a method for concentrating them before the HPLC analysis can take place. This can be achieved by traditional column chromatography or by SPE. The advantages of the SPE as opposed to the isolation of phospholipids are reported by Mounts and Nash [22] and by Nash and Frankel [75], who analyzed phospholipids in crude soybean oils using silica cartridges as well as by Singleton, who studied phospholipids in crude peanut oils [21,76]. More recently, Carelli et al. [77] developed an SPE-HPLC procedure using a diol phase cartridge to successfully recover, separate and quantify the classes of phospholipids present in sunflower oil, which were found in a wide range of concentrations (0.1–1.2%), and to apply the method to study the effect of processing on phospholipids composition. Their methodology begins by conditioning the cartridge with methanol, chloroform, and hexane, in that order. After having loaded the sample, a first fraction, which elutes with chloroform, is rejected. Non-phospholipids elute in this fraction. Afterwards, phospholipids are collected, eluting with methanol containing a solution of ammonium.

2.2.5. Minor altered products

Hydroxy and hydroperoxy fatty acids, sterol oxides, cyclic monomers, oxidized triglycerides monomers, dimers and polymers are slightly altered compounds formed in edible fats and oils by different processes such as temperature, storage and, in general, oil manipulation. They play a very important role in animal and human health.

Wilson et al. [78] developed an accurate and sensitive capillary GC–MS method for the quantita-

tive analysis of specific oxidation products of fatty acids by using SPE as a previous step for concentrating the oxidation products. Lipid samples containing hydroperoxy and hydroxy acids produced by peroxidation of linoleic, arachidonic, eicosapentaenoic and docosahexaenoic fatty acids are first hydrogenated to produce stable saturated hydroxy lipids and then saponified and methylated. The methoxy FAME are purified and concentrated by Si cartridges previously conditioned with 5 ml of the admixture hexane–ethyl acetate (98:2). The fraction containing monohydroxy FAME was separated from non-oxygen-containing FAME, using at first 5 ml of hexane–ethyl acetate (80:20), then discarding it. The fraction containing MH-FAME is obtained increasing the polarity of the eluent.

Other minor altered compounds present in heated vegetable oils are cyclic fatty acid monomers. As they are very well absorbed, they have been found to be potentially toxic. Many studies on their structure and toxic effects have been carried out [79].

Rojo and Perkins [80] described the application of a combined SPE procedure for isolating and purifying a fraction containing all the monomeric cyclic fatty acid methyl esters as nonurea adducting (NUAF). Extraction of the nonpolar lipid components from nonurea adducting (NUA) filtrates is performed on a reversed-phase C_{18}-bonded silica cartridge previously conditioned with methylene chloride, methanol and a blank solution of urea. Next, the sample is passed through a silica gel cartridge. The stepwise elution of the SPE-retained materials through silica gel are: firstly, 4 ml of hexane–methylene chloride (3:1); secondly, the hydrogenated cyclic fatty acid methyl esters (HCFAME) are collected with 3 ml of methylene; and, finally, use of 2 ml of methanol collects more polar compounds present in the NUA filtrate. The use of phenanthrene as internal standard allows the quantitative determination, although authors recommend the use of naturally occurring or synthetic cyclic fatty acids. The recoveries were determined, exceeding 97% in all cases.

In 1991, Sebedio et al. [81] modified the procedure previously studied by Rojo and Perkins [80] and described an analytical evaluation of CFAM with C_{18} reversed-phase columns for studying fried oils.

An analysis of oxidation products from unsaturated triglycerides by a combination of SPE-HPSEC was used to study the autoxidation of TG mixtures by Hopia et al. [24] and also to study heated [82,83] or frying oils [25,26]. Polar compounds formed mainly by TG alterations during the heating process are separated by NH_2 cartridges or Si cartridges and are further analyzed by HPSEC.

Other minor compounds formed by the alteration of PL during beef cooking were investigated by Caboni et al. [84]. The authors purified PL by SPE on a NH_2 phase column. After PL isolation, they were quantified by HPLC equipped with a silica column and a light-scattering evaporative detector. The authors demonstrated that cooking treatments did not affect the PL composition or the fatty acids content of single PL in ground beef.

Johansson et al. [85] investigated the presence of heterocyclic amines (HA) during the frying of beef hamburgers. Fried beef hamburgers and their corresponding residues in the pan were purified using SPE and analyzed by HPLC with a photodiode UV array and fluorescence detection.

2.3. Other compounds present in edible fats and oils

2.3.1. Phenols

These are an important group of naturally occurring compounds found in raw oils and mainly in virgin olive oil, in contrast to both seed and refined olive oils. Recent interest in the activity of the antioxidants of higher plants is due, at least in part, to their potential health benefits. Thus, studies done on virgin olive oils demonstrated that there is a narrow relationship between the total quantity and quality of certain phenolic compounds and the quality of the oil. On the other hand, in olive oils, it has been demonstrated that most phenols are responsible for the bitter taste [86,87], a quality appreciated in certain countries. Furthermore, they improve the resistance of oil to oxidation [88], being even more effective than some recognized antioxidants such as BHT. The nutritional and therapeutic effect of the phenolic components present in olive oils [89,90] is yet another characteristic recently studied [91–93]. Specific methodologies, using HPLC with UV detection (280 and 225 nm) after SPE have been developed during the present decade for determining the presence of phenolic compounds. Thus, Gutiérrez

and co-workers in 1989 [86] and in 1992 published methodologies using C_{18} columns [87]. The authors used hexane to remove the non-polar fraction and a mixture of water–methanol (30:70) to elute the phenolic compounds retained in the support, which were further studied by HPLC. The group of isolated phenols corresponded directly to the sensory evaluation carried out by the panel evaluating the bitter taste.

The methodology is identical to that followed by Mannio et al. [94] and Papadopoulus and Tsimidou [95] except for modifying the elution of the polyphenols. In these cases, the fraction of interest eluted only with methanol.

A more complex fractionation of the phenolic compounds was achieved by Litridou et al. [96], who used a previous method of liquid–liquid extraction, as described by Vázquez-Roncero et al. [97], to obtain a polar fraction, which is subsequently separated using C_{18} cartridges. The authors obtained eight fractions eluting with different proportions of methanol–water (20:80, 25:75, 30:70, 45:55, 60:40, 80:20), pure methanol, and chloroform in methanol (30:70). Each fraction consisted of five 1-ml subfractions. The 40 subfractions collected were examined under UV at 280 nm and RP-HPLC.

In addition, a C_8-bonded phase has been used to isolate phenols in a work published by Pirisi et al. [98]. In their methodology, a mixture of hexane–ciclohexane (50:50) is used to remove the non-polar fraction. Subsequently, they employed CH_3CH to elute the phenols. The recoveries ranged from 70 to 105% (SD=±4–10%).

New carboxylic acid-derivatized materials have also been tested as adsorbents by Eder et al. [99] for phenol extractions. In their work, the efficiency of other commercially available columns (Si and C_{18}) was compared. The authors emphasized that all COOH resins could be recycled at least 30 times without seeing any loss of performance. Nevertheless, this adsorbent does not confer any other significant advantages compared to the others previously used.

3. Analysis of lipids from fatty foods

Lipids from foodstuffs (frequently processed with animal fat) are a complex mixture of TG (80–90%), with lesser amounts of phospholipids, mono- and diglycerides, FFA and C. As a consequence of the temperature or the atmosphere present during storage or processing, the fat components also undergo changes with the result that altered compounds appear.

In contrast to edible oil studies, components of food lipid analysis need a previous extraction from the food matrix in order to obtain a fraction that is free of non-lipidic components which can then be susceptible to further separation of lipid classes.

The procedure chosen will depend on the complexity of the matrix itself. Thus, the extraction of lipids from fried or baked foods such as dough will not present the same level of difficulty as that from animal or plant tissue. In the latter, lipids can be strongly associated to other lipids and also to protein [100]. On the other hand, the extraction procedure will depend on the sort of information that is needed. For this reason, there are non-destructive methods that allow the further study of the different lipid classes while others are based on methylation or acid or alkaline digestion that allow the determination of total lipid quantities, but only partial studies of the fraction obtained.

Different classical extraction procedures have been described in the literature and applied for years to obtain lipid fractions that are free of non-lipidic components.

They are based on the use of organic solvents of hydrophobic character that allow the selective solving of the lipids so they can be separated from the remainder hydrophobic and non-lipidic components. The most exhaustive and widely cited study is the one published by Folch et al., in which the almost total extraction of lipids and pigments is described [101]. This procedure uses a chloroform–methanol (2:1) mixture and is employed when samples have extraction problems due to their humidity and presence of polyphenol. Subsequently, Bligh and Dyer [102], Mancha et al. [103] and Hara and Radin [104] introduced several modifications to the Folch procedure in order to minimize the problems inherent in that methodology by using different solvents having the main advantages of being non-toxic and allowing to obtain a lipidic fraction in the upper layer. More recently, other methodologies have tried to improve those proposed previously [105–111].

3.1. Different lipid classes

A single Si phase column and 17 solvent mixtures of increasing polarity allowed Prieto et al. [112] to fractionate lipids from wheat flour into eight classes: SE, TG, FA, 1,3- and 1,2-DG, MG, PC and LPC, as well as MGG and DGG of high purity and homogeneity. The authors also used a combination of NH_2 and Si phases to separate PL (PC, LPC, NAPE and NALPE), by elution with a mixture of seven solvents.

Additionally, the separation of different classes of lipids was carried out by García Regueiro et al. [113], who studied the lipid composition of cured ham. The authors used a combination of SPE (NH_2 and Si columns) and GC and LC for their study. Once the lipid sample was extracted using a chloroform–methanol (2:1) mixture and the neutral lipids and FA were separated on a NH_2 column, a first fraction was collected with chloroform–2-propanol (neutral lipids) and a second one with diethyl ether–acetic acid (98:2) (FA). The first fraction was applied to a second NH_2 column by obtaining a first fraction with 5 ml of hexane–dichloromethane–Et_2O (89:10:1) in order to get TG and CE, and a second fraction was collected with Cl_3CH/MeOH (2:1) to obtain MG, DG and C. The first fraction was once again subjected to a separation with a Si column for separating the CE (with hexane–diethyl ether (98:2)) and TG with hexane–diethyl ether (90:10).

Simoneau and German [114] investigated the effect of adding cocoa butter to milkfat fractions of short- and medium-chain triglycerides. The fractionation method [115] implied the use of C_{18} phase and the solvents EtOH–hexane–ACN in different proportions, plus $CHCl_3$–ACN and MTBE–ACN. The procedure allowed the separation of TG by fatty acid chain length without resorting to unsaturation. The TG rich in short-chain fatty acids had a lower affinity with the C_{18} phase and were the first to be washed off the column.

3.2. Cholesterol and its oxidation products

Cholesterol is the major sterol in human and animal tissue and also the most relevant in foodstuffs.

When cholesterol is exposed to air at room temperature it oxidizes to 7-hydroperoxide [116]. The rate of oxidation increases as the temperature rises, as is the case during the processing of fatty food when frying or baking. The hydroperoxides decompose rapidly to over 30 oxidation products, which the literature generally labels cholesterol oxidation products (COPs) [117]. The importance of these compounds lies in the fact that they seem to be implicated in the development of human diseases.

Several methods have been developed for analyzing the COPs from TLC or CC to HPLC, GC or even with nuclear magnetic resonance techniques [118,119]. Packed silicic acid columns and silica gel columns have been widely utilized to fractionate it from triacylglycerols and other lipids. However, these columns cannot avoid the interference between C and some COPs, e.g., 7α-hydroxycholesterol. Furthermore, the column chromatography procedure involves cumbersome and tedious preparatory steps [120–126].

Recently, SPE has been used intensively for the cleanup of COPs to avoid the formation of artifacts and/or breakdown of COPs during saponification. They have been used effectively to enrich COPs from cholesterol and other lipids [127–131].

The content of COPs in lipids from egg powder range from 0 to 100 μg/g. For this reason, it is necessary to isolate the COPs from other major lipids in order to increase their relative concentration prior to GC analysis. Some parameters that affect the efficiency of SPE and the accuracy of quantification such as solvent flow-rate and conditions of derivatization were also investigated.

Schmarr et al. [132] studied COPs in lipids from foodstuffs, with a new method involving transesterification, SPE and gas chromatography analysis. A 500-mg bonded amino phase cartridge previously conditioned with hexane was loaded with the transesterified lipids. The recoveries depended on the class of derivative compounds, and were >85%. The analysis of the results allowed the authors to conclude that COPS are lower than 0.5–3 ppm with the exception of liverwurst, which had up to five times higher concentrations of oxysterols.

Regarding the presence of COPs in milk powders, Dionisi et al. [133] compared four of the most frequently used methods for analyzing them with the purpose of identifying the best, motivated by the

huge number of contradictory results previously reported in the literature. A method involving direct saponification of the sample was compared with another three classical methods involving preliminary fat extraction. After saponification, all of the samples were enriched by SPE on amino cartridges and analyzed by GC–MS. The four methods were applied to the analysis of powdered milk containing different levels of COPs. The results demonstrated that direct saponification and subsequent separation by NH_2 phase and GC–MS is the best compromise for quantification, thus avoiding the preliminary fat extraction.

Furthermore, Johnson and co-workers [134,135] proposed the quantification of the COPs using NH_2 columns to separate them from TG and normal C. Once the sample is loaded onto the column, three fractions are collected. The first one by using 4 ml of the mixture of hexane–dichloromethane (90:10), the second with 6 ml of hexane–dichloromethane (50:50) and the third one having acetone; a fraction is thus eluted containing the COPs. NH_2 columns were also used by Sallin et al. [136] to extract COPs in milk and dairy products prior to their analysis by RP-HPLC.

García Regueiro and Maraschiello [137] used Si and Florisil columns to separate eight cholesterol oxides from lipids of poultry meat. A first fraction was obtained with a silica column using chloroform–methanol (90:10). This fraction was subjected to a second separation applying it to a Florisil column. The TG were obtained with hexane–diethyl ether (90:10) and diethyl ether–methanol (80:20), isolating the COPs which were further quantified by GC.

Ulberth and Roessler [138] compared the efficiencies of several SPE for cleanup of COPs. A combination of a Si followed by a NH_2 cartridge was found to be the optimum solution for removing the matrix components. COPs added at the concentration of 150 ppb to the milk fat were recovered (90%) by the method proposed, except for cholestanetriol, for which the recovery rate was only 52%. The quantification of the COPs was done by GC–MS.

3.3. Normal and altered fatty acids

Hydroxy fatty acids, geometric isomers with conjugated double bonds and, generally speaking, products from fatty acid oxidation, are believed to be implicated in some degenerative processes such as coronary artery disease [139,140]. They arise from processing conditions and aging.

Wilson et al. [141] proposed a simple and reliable method for preparing the concentrates of methyl or ethyl esters of *n*-3 polyunsaturated fatty acids by solid-phase extraction using NH_2 columns. After preparation of FAEE and FAME from fish oil and subsequent extraction, the samples were purified using 500 mg NH_2-bonded column preconditioned with dichloromethane and hexane. They carried out two elutions, the first with hexane (4 ml) and the second with dichloromethane (4 ml). The authors observed that their recoveries depended on the sample loaded, and were also related to the degree of FAME unsaturation. That is because the NH_2 phase is an adsorbent that exhibits strong hydrogen bonding properties and also functions as a weak anion exchanger [142,143]. As PUFA has strong dipoles at methylene-interrupted carbon atoms, strong interactions between the hydrogen atoms of these carbons and the NH_2 phase take place. These interactions do not equal those with SFA and MUFA, which exhibit weaker dipoles than PUFA [144]. Besides, SFA may be eluted using only hexane because it is a non-polar solvent and has an elutropic strength of zero [143], which does not help the dipole–dipole interactions.

The application of the methodology for the quantitative determination of oxidized lipids in other foods was later described by the authors [145]. Indeed, it was possible to distinguish the general type of oil used in the preparation of these foods because the ratio between the 9- and 13-C_{18} isomers was higher in oleic acid-rich food (prepared from olive oil) than that in foods prepared from sunflower oil (rich in linoleic acid). The identification of specific isomers using this method has many potential applications, especially when considering absorption of oxidized fatty acids from the diet.

De Jong et al. [146], using NH_2 columns, described a procedure for estimating the amount of free fatty acids in fermented dairy products. Large concentrations (7–15 g/l) of lactic acid can interfere with the estimation of FFA. Using a mixture of heptane–diethyl ether (50:50), approximately 3% of lactic acid elute and the rest is retained. Neverthe-

less, the procedure was not intended for estimating the lactic acid content of dairy products.

4. Lipid classes in biological samples: fluids and tissues

The importance of the huge number of studies done on the lipid constituents found in biological samples and especially in plasma cannot be underestimated due to their incidence in cardiovascular diseases. Thus, an increase in the cholesterol level and consequently of low density lipoproteins (LDL) is directly related to cardiovascular disease. This is a classical hypothesis, which predicts the adverse effect that diets rich in saturated FA can have and on the other hand recognizes the benefit of diets rich in mono- and polyunsaturated FA. Nevertheless, other authors including us consider that plasmatic cholesterol alone is not enough to cause a cardiovascular risk. Thus, we think that diet, or more exactly some components in it, have a significant influence on cellular parameters. Thus, it has been demonstrated that some components of olive oil, such as the polyphenol oleouropein, have an inhibiting effect on LDL oxidation, drastically reducing the production of eicosanoids [147].

4.1. Neutral and polar lipid separations

Juaneda and Rocquelin [148] published one of the most emblematic works on the use of SPE for NL and PL separations in biological samples. The procedure consists in the use of a Si cartridge and 20 ml of chloroform for NL elution. Afterwards, PL are collected by eluting with 30 ml of methanol. The authors use about 30 mg of lipids, and advise that for quantities greater than 100 mg there is a risk of cartridge saturation, so separation is not guaranteed. They compare the procedure with the traditional separation on CC and conclude that there are no noticeable differences in the final results seen between them. Furthermore, SPE is easier and faster, so the authors advise to reuse them five or six times. Their separation procedure has been followed successfully for many years by a considerable number of authors [149–184].

Amino-bonded phase columns were used more recently by Bateman and Jenkins [185] also to isolate NL and PL from microbial cultures. They applied their system to stomach samples from cows. The total lipids were extracted in heptane–2-propanol (1:4). The columns were preconditioned by passing hexane, then loading them with the lipids: NL eluted with 4 ml chloroform–2-propanol and PL with methanol. An intermediate fraction containing FFA was collected with 8 ml diethyl ether–acetic acid (98:2). They did not believe in the need of a fourth step for recovering the acidic PL, as recommended by Kim and Salem [186]. They verified that there was no cross-contamination even if columns were overloaded intentionally. As mentioned before, after Kim and Salem completed the three steps described above, they used a mixture of hexane–2-propanol–ethanol and 0.1 *M* ammonium acetate and formic acid (420:350:100:50:0.5) in water containing 5% phosphoric acid to elute the acidic phospholipids which do not elute with the neutral phospholipids using methanol. The application of this method for different tissues allowed the authors to assert that the efficiency of the separation depended for the most part on the own tissue. Thus, they found fractions containing pure lipids and other containing mixture of lipid classes of similar polarity. These fractions may then be more easily separated by other chromatographic techniques for a more complete analysis of lipid classes.

4.2. Separations of different lipid classes

Also in 1985, Kaluzny et al. [47] published the other very emblematic work on the separation of different classes of lipids. Although their methodology is more complex, as can be seen in the diagram in Fig. 3, it has been carefully followed by a great number of authors. The authors used, as can be appreciated in the diagram, three NH_2 columns and eight different eluents to separate C, CE, TG, DG, MG, FA and PL [187–206].

In order to simplify the fractionations, and also to avoid some methodological disadvantages, different modifications based on the method of Kaluzny et al. have been published [207–230].

4.3. Separations of specific compounds

4.3.1. Fatty acids and their oxidation products

The major C_{20}-polyunsaturated FA in most mam-

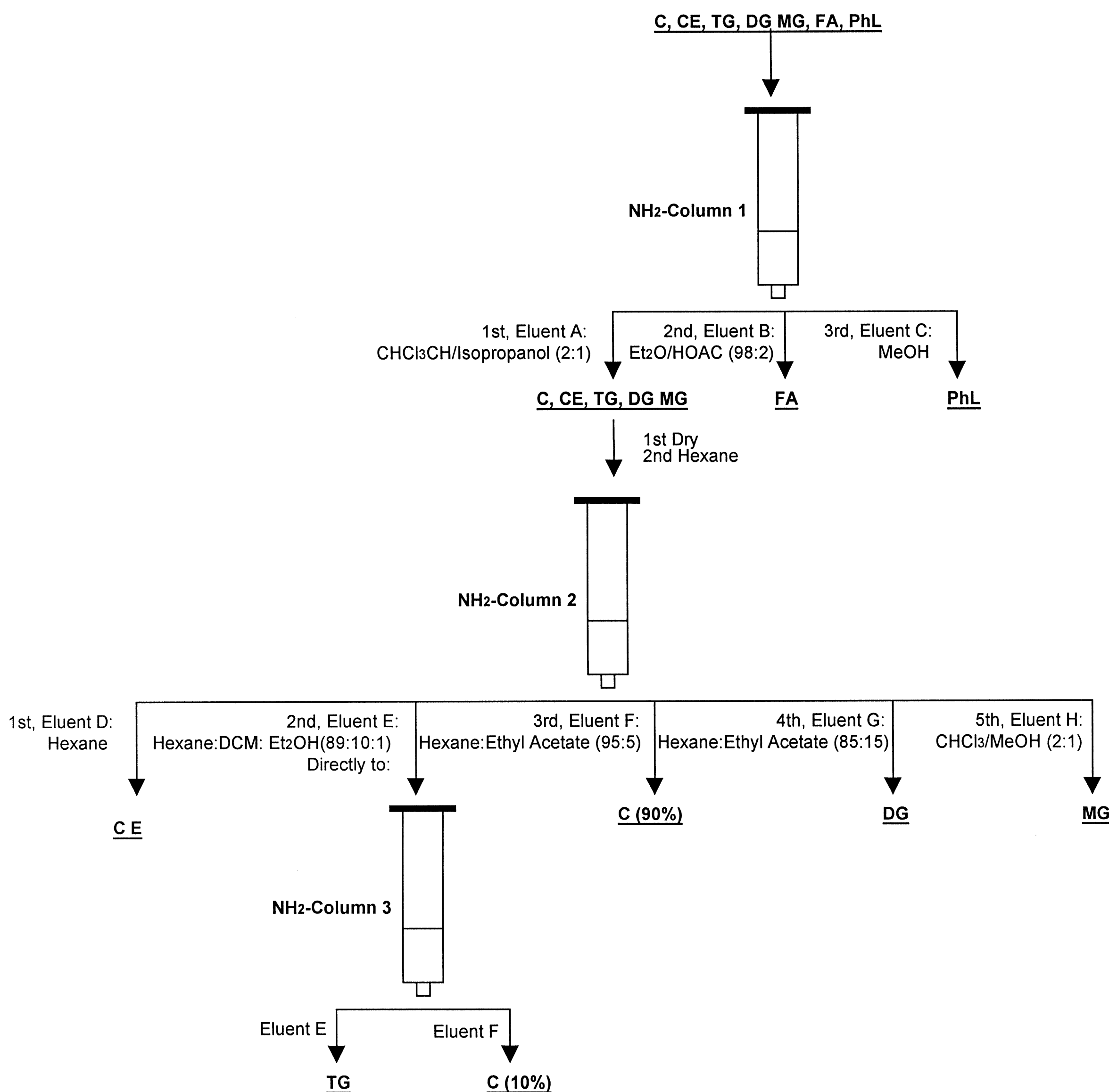

Fig. 3. Fractionation of lipid classes using NH_2 columns (scheme modified from Ref. [47]).

malian systems is arachidonic acid, $C_{20:4\omega6}$ (5,8,11,14-*cis*-eicosatetraenoic acid). $C_{20:4\omega6}$ is metabolized to a large family of oxygenated C_{20}-FA collectively termed eicosanoids [231]. They comprise PGs, Txs, 5-HETE and LT. All are formed in every tissue of the body, so their biological activities are of great physiological and pathophysiological importance. For example, they are implicated in the control of vascular tone, renal function, platelet aggregation, hypersensitivity and inflammation.

Following Kaluzny's procedure, Prasad et al. [56] analyzed arachidonic acid from tissue lipids and compared the recovery of the acid isolated from NH_2 column and TLC concluding that the bonded-phase column method yielded a higher recovery of arachidonic acid as compared to that of TLC.

Peroxidized arachidonic acid and other polyunsaturated fatty acids by scission yield a three-carbon compound: malondialdehyde (MDA) which is one of the main products of lipid peroxidation [232]. It has

been widely used as an index of lipoperoxidation in biological and medical sciences as well as in the food industry. Suttnar et al. [233] developed a SPE procedure in which the affinity of the MDA by different C_{18} and Si cartridges was checked and MDA was measured in blood plasma, platelet concentrates and erythrocytes. The authors obtained the best results using a 200-mg LiChrolut C_{18} column as a concentration pretreatment for quantifying MDA by HPLC with a fluorescence detector (Ex., 514 nm; Em., 556 nm). They concluded that the utilization of SPE in the sample preparation of MDA derivatives for HPLC analysis eliminates the use of *n*-butanol in the extraction step. Only 800 μl of pure methanol must be evaporated using LiChrolut C_{18} to obtain MDA for further analysis.

Octadecyl and also silica phases have successfully been utilized to extract unsaturated lipids, particularly the arachidonic acid metabolites, from biological fluids [234–237].

4.3.2. Fatty acid ethyl and methyl esters

A two-column SPE method was developed by Bernhardt et al. [238] to purify FAEE with a recovery of 70±3% using ethyl oleate as a recovery marker. The interest of FAEE determination lies in the fact that they have shown to inhibit protein synthesis and decrease the rate of cell proliferation. They are present predominantly in organs like the pancreas and liver, which are often damaged by ethanol abuse and are synthesized via FAEE synthase.

The first step in their procedure involves the use of a NH_2 column, modifying the procedure described by Kaluzny. FAEE and cholesteryl esters elute by passing hexane, after which GC–MS analysis of FAEE may be performed. The method can be used to isolate small quantities of FAEE from lipid extracts of human plasma. The extraction of the lipids from sera from patients with detectable blood ethanol was done adding 2 ml of acetone to 1 ml of serum followed by 5 ml of hexane. The recovery of the FAEE in the hexane–acetone layer was determined to be 96.3±1.2%. Individual FAEE species can subsequently be isolated and analyzed by HPLC without CE interference. If necessary, FAEE and CE can be separated in a second SPE step which involves the use of an ODS column with isopropanol–water (5:1). They demonstrated that NH_2 phase columns could be used at least four times without cross-contamination from one sample to another. Although the data about the recovery of saturated or monounsaturated FAEE are good, the authors observed that *n*-3 ethyl esters of eicosapentaenoate (20:5) and docohexaenoate (22:6) eluted from the NH_2 column only if the column was preconditioned with dichloromethane and hexane and therefore was not as good for the others.

The same conclusions were reported by Wilson et al. when they studied the enrichment of *n*-3 PUFA using NH_2 columns [141].

When Si-phase columns are used, a more polar fluent than hexane is necessary to obtain the fraction of esters. The finality of the SPE procedure described by Wilson et al. [78] was to separate normal FAME from monohydroxy fatty acid methyl esters. The individual positional isomers of MHFA derived from linoleic, arachidonic, eicosapentaenoic or docosahexaenoic acids were determined subsequently by capillary gas chromatography–mass spectrometry and the methodology was applied to plasma samples, adipose tissue, oils, and foods. The Si column was previously conditioned using hexane–ethyl acetate (98:2). The non-altered FAME eluted using hexane–ethyl acetate (95:5), and the admixture hexane–ethyl acetate (80:20) is necessary to elute the monohydroxy. Nevertheless, the authors suggested always checking the exact proportions of hexane–ethyl acetate (between 80:20 and 70:30) for the most suitable elution of monohydroxy because, as has been confirmed by other authors, various batches of silica cartridge columns differ in their activity. They routinely discarded the columns after their use, but suggested their reuse after eluting them with 100% ethyl acetate and removing the solvent under an inert atmosphere. The authors have used the methodology extensively to analyze hydroxy fatty acids from more than 1000 samples over a 2-year period. After having completed their study, they concluded that the method is very sensitive and only 0.5 ml of plasma, 10 mg of adipose tissue, 10 mg of oil and 1 mg of lipid from food extracts are sufficient for measuring the levels of hydroxy C_{18} and hydroxy C_{20} isomers.

4.3.3. Phospholipids and their oxidation products

The determination of the content of phospholipids

in animals has become an important research topic because they are, with proteins, the major components of biological membranes. On the other hand their study is a way of determining the physiological state of the cell membrane in all tissues.

Salari [239] published a study about PL from plasma where the effectiveness of the C_{18} and Si phase was compared for PL recovery. The study showed that neither C_{18} or Si supports adsorbed significantly more phospholipids from plasma than when water and isopropanol–acetonitrile (1:1) were used as eluents; only amberlite polymeric resins showed effectiveness with a recovery exceeding 85%. Thereby, the author discarded both phases for not being selective enough for the adsorption of phospholipids.

When PL are peroxidized, a huge number of compounds are produced [240,241]. Some of these products can be identified as short-chain PL with hydroxyl, aldehyde or carboxyl groups in the T-position [242,243]. These species are formed by radical-mediated degradation of unsaturated acyl groups, which are usually located in the *sn*-2 position of mammalian PL. Due to the heterogeneity of the oxidation products, their presence in biological tissue has not been firmly established. Schlame et al. [244] developed a technique for the quantitative analysis of PL using an oxidized acyl chain. The methodology included: (1) lipid extraction; (2) chromatographic enrichment of the phospholipids; (3) derivatization; (4) purification by Si-SPE of the derivatives; and (5) RP-HPLC-fluorescence detector analysis. The method was effective for monitoring the generation of oxPh from human plasma. As an internal standard, the authors used 1-palmitoyl-2-suberoyl-PC. Their methodology did not produce oxidation artifacts, so they concluded that human blood contains oxidatively fragmented PC in submicromolar concentrations.

5. Nomenclature

C	Cholesterol
CC	Column chromatography
CE	Cholesteryl ester
CN	Cyanopropyl
COPs	Cholesterol oxidation products
C_{18}	Octadecyl
C_8	Octyl
C_2	Ethyl
DG	Diglyceride
FA	Fatty acids
FFA	Free fatty acids
GC	Gas chromatography
5-HETE	5-hydroxyeicosatetraenoic acid
HPLC	High-performance liquid chromatography
HPSEC	High-performance size-exclusion chromatography
HPTA	Hydroxypentacyclic triterpene acids
LT	Leukotriene
MS	Mass spectrometry
MG	Monoglyceride
MUFA	Monounsaturated fatty acid
NH_2	Aminopropyl
NH_4^+	Quaternary amino
OxTG	Oxidized triglycerides
PGs	Prostaglandins
PL	Phospholipids
PUFA	Polyunsaturated fatty acid
S	Sterols
SFA	Saturated fatty acid
Si	Silica
SPE	Solid phase extraction
TG	Triglyceride
TG-D	Triglyceride dimers
TG-P	Triglyceride polymers
TLC	Thin-layer chromatography
TLC-FID	Thin-layer chromatography-flame ionization detection
Txs	Thromboxanes
WS	Waxes

References

[1] R.D. McDowall, J. Chromatogr. 492 (1989) 3.

[2] M. Zief, in: Sample Preparation Technology: Solid-Phase Extraction for Sample Preparation, Zymark Corporation, Hopkinton, MA, 1982.

[3] J.J. Myher, A. Kuksis, J. Chromatogr. 671 (1995) 3.

[4] N.A. Shaikh, Anal. Biochem. 216 (1994) 313.

[5] H.D. Mangold, CRC Handbook of Chromatography: Lipids, Vol. 1, CRC Press, Boca Raton, FL, 1984, pp. 387.

[6] R.A. Stein, V. Lawson, Prog. Chem. Fats Lipids 8 (1966) 373.

[7] C. Litchfield, in: Analysis of Triglycerides, Academic Press, New York, 1972.

[8] A. Kuksis, J. Chromatogr. 143 (1977) 3.
[9] W.W. Christie, in: Lipid Analysis: Isolation, Separation, Identification, and Structural Analysis of Lipids, 2nd Edition, Pergamon, New York, 1982.
[10] E.M. Thurman, M.S. Mills, in: Solid-Phase Extraction. Principles and Practices, Wiley, New York, 1998, p. 344.
[11] P.D. McDonald, E.S.P. Bouvier (Eds.), Solid Phase Extraction, Applications Guide and Bibliography, 6th Edition, Waters Corporation, Milford, MA 1995, 645 pp.
[12] M.A. Williams, R.H. McCluer, J. Neurochem. 35 (1980) 266.
[13] W.S. Powell, Prostaglandins 20 (1980) 947.
[14] G.D. Wachob, in: E.G. Perkins (Ed.), Analyses of Fats, Oils and Lipoproteins, American Oil Chemists' Society, Champaign, IL, 1991, pp. 122–137.
[15] W.W. Christie, Lipid Tech. 3 (1991) 31.
[16] S.E. Ebeler, T. Shibamoto, in: T. Shibamoto (Ed.), Lipid Chromatographic Analysis, Marcel Dekker, New York, 1994, pp. 1–49.
[17] S.E. Ebeler, J.D. Ebeler, Inform 7 (1996) 1094.
[18] B. Tippins, Am. Lab. 19 (1987) 107.
[19] A. Kuksis, in: E. Heftman (Ed.), Chromatography, Elsevier, Amsterdam, 1992, p. 171.
[20] R.L. Kimmey, E.G. Perkins, J. Am. Oil Chem. Soc. 69 (1992) 772.
[21] J.A. Singleton, J. Am. Oil Chem. Soc. 70 (1993) 637.
[22] T.L. Mounts, A.M. Nash, J. Am. Oil Chem. Soc. 67 (1990) 757.
[23] M. Amelio, R. Rizzo, F. Varazini, J. Chromatogr. 606 (1999) 179.
[24] A.I. Hopia, V.I. Piironen, P.E. Koivistoinen, L.T. Hyvoenen, J. Am. Oil Chem. Soc. 69 (1992) 772.
[25] G. Márquez-Ruiz, M. Martín-Polvillo, M.C. Dobarganes, 21st ISF, 1995, p. 365.
[26] G. Márquez-Ruiz, N. Jorge, M. Martín-Polvillo, M.C. Dobarganes, J. Chromatogr. 749 (1996) 55.
[27] J.L. Sebedio, Ch. Septier, A. Grandgirard, J. Am. Oil Chem. Soc. 63 (1986) 1541.
[28] G. Márquez-Ruiz, M.C. Dobarganes, in: R.E. McDonald, M.M. Mossoba (Eds.), New Techniques and Applications in Lipid Analysis, AOCS Press, Washington, DC, 1997, pp. 216–233.
[29] IUPAC Standard Method 2.507, Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th Edition, International Union of Pure and Applied Chemistry, Blackwell, Oxford, 1987.
[30] M.R.S. Shreedhara, L.J. Crane, J. Chromatogr. 542 (1991) 205.
[31] H.C. Blunk, H. Steinhart, Z. Lebensm. Unters. Forsch. 190 (1990) 123.
[32] A. Cert, W. Moreda, Grasas Aceites 51(3) (2000) in press.
[33] W.W. Christie, Damyanova, B. Nikolova, P. Laakso, B. Herslof, J. Am. Oil Chem. Soc. 68 (1991) 695.
[34] E.W. Hammond, J. Chromatrogr. 203 (1981) 397.
[35] V. Ruíz-Gutiérrez, L.J.R. Barron, J. Chromatogr. B 671 (1995) 133.
[36] B. Nikolova-Damyanova, in: W.W. Christie (Ed.), Advances in Lipid Methodology I, The Oily Press, Dundee, UK, 1992, p. 181.
[37] E.C. Smith, A.D. Jones, E.W. Hammond, J. Chromatogr. 188 (1980) 205.
[38] B.S.J. Jeffrey, J. Am. Oil Chem. Soc. 68 (1991) 289.
[39] H. Kallio, T. Vauhkonen, R.R. Linko, J. Agric. Food Chem. 39 (1991) 1537.
[40] W.W. Christie, J. Lipid Res. 30 (1989) 1471.
[41] F. Ulberth, E. Achs, J. Chromatogr. 504 (1990) 202.
[42] W.W. Christie, J. Sci. Food Agric. 52 (1990) 573.
[43] A. Kemppinen, P. Kalo, J. Am. Oil Chem. Soc. 70 (1993) 1203.
[44] A. Kuksis, W.C. Breckenridge, in: Dairy Lipids and Lipid Metabolism, AVI Publishing, Westport, 1968, p. 28.
[45] N. Cortesi, P. Rovelli, E. Fedeli, Riv. Ital. Sostanze Grasse 69 (1992) 305.
[46] N. Frega, F. Bocci, G. Lercker, Riv. Ital. Sostanze Grasse 70 (1993) 153.
[47] M.A. Kaluzny, L.A. Duncan, M.V. Merritt, D.E. Epps, J. Lipid Res. 26 (1985) 135.
[48] G. Amelotti, A. Daghetta, Ferrario, Riv. Ital. Sostanze Grasse 66 (1989) 681.
[49] A.M. Leone, M. Santoro, V.A. Liuzzi, E.L. Notte, G. Gambacorta, Riv. Ital. Sostanze Grasse 65 (1988) 613.
[50] H. Brüschweiler, A. Dieffenbacher, Pure Appl. Chem. 63 (1991) 1153.
[51] M.C. Pérez-Camino, W. Moreda, A. Cert, J. Chromatrogr. A 721 (1996) 305.
[52] Y.M. Pacheco, M.C. Pérez-Camino, A. Cert, E. Montero, V. Ruiz-Gutiérrez, J. Chromatogr. B 714 (1998) 127.
[53] L.S. Conte, O. Koprivnjak, Fiorasi, L. Pizzale, Riv. Ital. Sostanze Grasse 74 (1997) 411.
[54] C. de Jong, H.T. Badings, J. High Resolut. Chromatogr. 13 (1990) 94.
[55] J. Egberts, R. Buiskool, Clin. Biochem. 34 (1988) 163.
[56] M.R. Prasad, R.M. Jones, H.S. Young, L.B. Kaplinsky, D.K. Das, J. Chromatogr. 428 (1988) 221.
[57] M.C. Pérez-Camino, A. Cert, J. Agric. Food Chem. 47 (1999) 1558.
[58] EEC.Regulation 183/93 of Commission of 29 January 1993 modifying EEC Regulation 2568/91 regarding the characteristics of olive oils, Com. Eur. 30/01/93 (1993).
[59] V. Ruíz-Gutiérrez, F.J. García Muriana, G. Pérez-Zarza, L. Bravo, J. Fish. Biol. 50 (1999) 1353.
[60] K. Grob, M. Lanfranchi, C. Mariani, J. Agric. Food Chem. 47 (1989) 202.
[61] G. Nota, D. Naviglio, R. Romano, R. Romano, V. Sabia, S.S. Musso, C. Improta, J. Agric. Food Chem. 47 (1999) 202.
[62] K. Grob, M. Lanfranchi, C. Mariani, J. Am. Oil Chem. Soc. 67 (1990) 626.
[63] S.P. Kochhar, Prog. Lipid. Rev. 22 (1983) 161.
[64] W.R. Morrison, Cereal Chem. 55 (1978) 548.
[65] H. Imai, N.T. Werthessen, V. Subramanyan, P.W. LeQuesne, A.H. Soloway, M. Kanisawa, Science 207 (1980) 651.
[66] S.K. Peng, C.B. Taylor, E.H. Mosbasch, W.Y. Huang, B. Mikkelson, Atherosclerosis 41 (1982) 395.
[67] J. Nourooz-Zadeh, L.A. Appelqvist, J. Am. Oil Chem. Soc. 69 (1992) 288.
[68] C. Mariani, E. Fedeli, K. Grob, Riv. Ital. Sostanze Grasse 68 (1991) 233.

[69] R.E. Worthington, H.L. Hitvhcock, J. Am. Oil Chem. Soc. 61 (1984) 1085.
[70] EEC.Regulation 2568/91, Official Journal of the European Communities, Commission Regulation 2568/91 (1991).
[71] P. Horstmann, A. Montag, Fette Seifen Anstrichm. 88 (1986) 262.
[72] W.Ch. Chung, S.H. Lucy, Food Sci. 23 (1996) 367.
[73] D. Firestone (Ed.), Official Methods and Recommended Practices, 5th Edition, AOCS, Champaign, IL, 1997.
[74] IUPAC, Standard Methods for the Analysis of Oils, Fats and Derivatives, 1st Supplement to 7th Edition, 1992.
[75] A.M. Nash, E.N. Frankel, J. Am. Oil Chem. Soc. 63 (1986) 244.
[76] J.A. Singleton, L.F. Stikeleather, J. Am. Oil Chem. Soc. 72 (1995) 481.
[77] A.A. Carelli, MI.V. Brevedan, G.H. Crapiste, J. Am. Oil Chem. Soc. 74 (1997) 511.
[78] R. Wilson, R. Smith, P. Wilson, M.J. Shepherd, R.A. Riemersma, Anal. Biochem. 248 (1997) 76.
[79] J.L. Sebedio, A. Grandgirard, Prog. Lipid Res. 28 (1989) 303.
[80] J.A. Rojo, E.G. Perkins, J. Am. Oil Chem. Soc. 66 (1989) 1593.
[81] J.L. Sebedio, J. Kaitaranta, A. Grandgirard, Y. Malkki, J. Am. Oil Chem. Soc. 68 (1991) 299.
[82] T. Albi, A. Lanzón, A. Guinda, M. León, M.C. Pérez-Camino, in: D. Barrera-Arellano, M.A.B. regitano dÀrce, L.Ap.G. Gonçalves (Eds.), Proceedings of the 6th Latin American Congress and Exibit on fats and Oils processing, Campinas, Brasil, 1995, 167–171, 1995, p. 167.
[83] T. Albi, A. Lanzón, A. Guinda, M. León, M.C. Pérez-Camino, J. Agric. Food Chem. 45 (1997) 3795.
[84] M.F. Caboni, S. Menotta, G. Lercker, J. Chromatogr. 683 (1994) 59.
[85] M.A.E. Johansson, L. Fredholm, I. Bjerne, M. Jaegerstad, Food Chem. Toxicol. 33 (1995) 993.
[86] F. Gutiérrez, M.A. Albi, R. Palma, J.J. Rios, J.M. Olías, J. Food Sci. 54 (1989) 68.
[87] F. Gutiérrez, S. Perdiguero, R. Gutiérrez, J.M. Olías, J. Am. Oil Chem. Soc. 69 (1992) 394.
[88] R. Gutiérrez, C. Janer del Valle, F. Gutiérrez, A. Vázquez, Grasas Aceites 28 (1977) 101.
[89] B. Berra, D. Caruso, N. Cortesi, F. Fedeli, M.F. Raseti, G. Galli, Riv. Ital. Sostanze Grasse 72 (1995) 285.
[90] R. Maestro-Durán, R. León-Cabello, V. Ruíz-Gutiérrez, P. Fiesta, A. Vázquez, Grasas Aceites 45 (1994) 332.
[91] R. Maestro-Durán, R. León-Cabello, V. Ruíz-Gutiérrez, Grasas Aceites 45 (1994) 265.
[92] C. Manna, P. Galletti, V. Cucciolla, O. Moltedo, A. Leone, V. Zappia, J. Nutr. 127 (1997) 286.
[93] F. Visioli, C. Galli, J. Agric. Food Chem. 46 (1998) 4292.
[94] S. Mannino, M.S. Cosio, M. Bertuccioli, Ital. J. Food Sci. 4 (1993) 363.
[95] G. Papadopoulos, M. Tsimidou, Proceedings of XV International Conference of Group Polyphenols, II (1992) 192.
[96] M. Litridou, J. Linssen, H. Schols, M. Bergmans, M. Posthumus, M. Tsimidou, D. Boskou, J. Agric. Food Chem. 74 (1997) 169.
[97] A. Vázquez Roncero, C. Janer del Valle, M.L. Janer del Valle, Grasas Aceites 27 (1976) 185.
[98] F. Pirisi, A. Angioni, P. Cabras, V.L. Garau, M.T. Sanjust di Teulada, M.K. dos Santos, G. Bandino, J. Chromatogr. 768 (1997) 207.
[99] K. Eder, M.R. Buchmeiser, G.K. Bonn, J. Chromatrogr. 810 (1998) 43.
[100] F. Shahidi, J.P.D. Wanasundara, in: C.C. Akoh, D.B. Min (Eds.), Food Lipids, Chemistry, Nutrition and Biotechnology, Marcel Dekker, Inc., New York, NY, 1998, pp. 115–136.
[101] J. Folch, M. Less, G.H. Sloane Stanley, J. Biol. Chem. 226 (1957) 497.
[102] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911.
[103] M. Mancha, G.B. Stokes, P.K. Stumpf, Anal. Biochem. 68 (1975) 600.
[104] A. Hara, N.S. Raden, Anal. Biochem. 90 (1978) 420.
[105] S.L. Melton, R.E. Moyers, G.G. Playford, J. Am. Oil Chem. Soc. 56 (1979) 489.
[106] W.N. Marmer, R.J. Maxwell, Lipids 16 (1981) 365.
[107] K. Elmer-Frohlich, P.A. Lachance, J. Am. Oil Chem. Soc. 69 (1992) 243.
[108] M.C. Erickson, J. Sci. Food Agric. 59 (1992) 529.
[109] M.C. Erickson, Comp. Biochem. Physiol. 101A (1992) 171.
[110] M.C. Erickson, J. Food Sci. 58 (1993) 84.
[111] C.M. Lee, B. Trevino, M. Chaiyawat, J. Am. Oil Chem. Soc. 79 (1996) 487.
[112] J.A. Prieto, A. Ebri, C. Collar, J. Am. Oil Chem. Soc. 69 (1992) 387.
[113] J.A. García Regueiro, J. Gilbert, I. Díaz, J. Chromatogr. 667 (1994) 225.
[114] C. Simoneau, J.B. German, J. Am. Oil Chem. Soc. 73 (1996) 955.
[115] C. Simoneau, P. Fairley, J.M. Krochta, J.B. German, J. Am. Oil Chem. Soc. 71 (1994) 795.
[116] L.L. Smith, in: Cholesterol Oxidation, Plenum, New York, 1981.
[117] S. McCluskey, R. Devery, Trends Food Sci. Technol. 4 (1993) 175.
[118] A. Fontana, F. Antoniazzi, G. Cimino, G. Mazza, B.E. Trivellone, B. Zanone, J. Biol. Chem. 57 (1992) 869.
[119] A. Fontana, F. Antoniazzi, M.L. Ciavatta, E. Trivellone, G. Cimino, J. Food Sci. 58 (1993) 1286.
[120] S.R. Missler, B.A. Wasilchuk, C. Merrit, J. Am. Oil Chem. Soc. 50 (1985) 595.
[121] F.J. Monahan, J.I. Gray, A.M. Booren, E.R. Miller, D.J. Buckley, J. Agric. Food Chem. 40 (1992) 1310.
[122] J.N. Morgan, D.J. Armstrong, J. Food Sci. 52 (1987) 1224.
[123] S.W. Park, P.B. Addis, J. Food Sci. 50 (1985) 1437.
[124] B.D. Sander, P.B. Addis, S.W. Park, D. Smith, J. Food Prot. 52 (1989) 109.
[125] K. Sugino, Terao, H. Urakami, S. Matsushita, J. Agric. Food Chem. 34 (1986) 36.
[126] L.S. Tsai, C.A. Hudson, J. Am. Oil Chem. Soc. 58 (1981) 931.
[127] J.N. Morgan, D.J. Armstrong, J. Food Sci. 57 (1992) 43.
[128] J. Nourooz-Zadeh, J. Agric. Food Chem. 38 (1990) 1667.

[129] S.-M. Lai, J.I. Gray, M.E. Zabik, J. Agric. Food Chem. 43 (1995) 1122.
[130] E. Boselli, M.F. Caboni, G. Lercker, Food Res. Technol. 205 (1997) 356.
[131] W.R. Stenzel, H.P. Wunderlich, Arch. Lebensmittelhyg. 46 (1995) 103.
[132] H.G. Schmarr, H.B. Gross, T. Shibamoto, J. Agric. Food Chem. 44 (1996) 512.
[133] F. Dionisi, P.A. Golay, J.M. Aeschlimann, L.B. Fay, J. Agric. Food Chem. 46 (1998) 2227.
[134] J.H. Johnson, P. McIntyre, J. Zdunek, J. Chromatogr. A 718 (1995) 371.
[135] C.B. Johnson, J. Chromatogr. A 736 (1996) 205.
[136] C. Sallin, E. Baumann, U. Buetikofer, R. Sieber, J.O. Bosset, Mitt. Geb. Lebensmitteluntersuch. Hyg. 84 (1993) 141.
[137] J.A. García Regueiro, C. Maraschiello, J. Chromatogr. A 764 (1997) 279.
[138] F. Ulberth, D. Roessler, J. Agric. Food Chem. 46 (1998) 2634.
[139] J.M.C. Gutteridge, Free Radic. Res. Commun. 19 (1993) 145.
[140] K. Ytrehus, A.C. Hegstad, Acta Physiol. Scand. 142 (1991) 81.
[141] R. Wilson, R.J. Henderson, I.C. Burkow, J.R. Sargent, Lipids 28 (1993) 51.
[142] K.C. Van Horne, in: Sorbent Extraction Technology, Analytichem International, Harbor City, 1985.
[143] M. Zief, R. Kiser, in: Solid Phase Extraction for Sample Preparation, J.T. Baker, Deventer, The Netherlands, 1988.
[144] V.I. Minkin, O.A. Osipov, Y.A. Zhdanov, in: Dipole Moments in Organic Chemistry, Plenum, New York, 1970.
[145] R. Wilson, R. Smith, P. Wilson, M.J. Shepherd, R.A. Riemersma, Proc. Nutr. Soc. 55 (1996) 246A.
[146] C. de Jong, K. Palma, R. Neeter, Netherlands Milk Dairy J. 48 (1994) 151.
[147] R. Puerta de la, V. Ruíz-Gutiérrez, J.R.S. Hoult, Biochem. Pharmacol. 15 (1999) 4459.
[148] P. Juaneda, G. Rocquelin, Lipids 20 (1985) 40.
[149] J.M. Alessandri, R. Christon, T.S. Arfi, A. Riazi, C.L. Leger, J. Chromatogr. 432 (1988) 75.
[150] J.M. Alessandri, Ph. Guesnet, T.S. Arfi, G. Durand, Biochim. Biophys. Acta 1986 (1991) 340.
[151] P.O. Astorg, J. Chevalier, Lipids 22 (1987) 1025.
[152] P.O. Astorg, A. Grynberg, J. Chevalier, M. Degois, Nutr. Rep. Int. 40 (1989) 949.
[153] P.O. Astorg, J. Chevalier, Nutr. Res. 11 (1991) 71.
[154] U.O. Barcelli, D.C. Beach, V.E. Pollak, Lipids 23 (1988) 309.
[155] U.O. Barcelli, D.C. Beach, B. Thompson, M. Weiss, V.E. Pollak, Lipids 23 (1988) 1059.
[156] G. Bruckner, P. Webb, L. Greenwell, C. Chow, D. Richardson, Atherosclerosis 66 (1987) 237.
[157] F. Cheillan, H. lafont, E. Termine, Y. Hamann, G. Lesgards, Lipids 24 (1989) 224.
[158] Z.Y. Chen, W.W. Nawar, J. Food Sci. 56 (1991) 398.
[159] A. Chevalier, L. Demaison, A. Grynberg, P. Athias, J. Mol. Cell. Cardiol. 22 (1990) 1177.
[160] G.L. Crozier, M. Traitler, H. Fleith, P.A. Finot, Lipids 24 (1989) 460.
[161] C. Fressinaud, M. Rigaud, J.M. Vallat, J. Neurochem. 46 (1986) 1549.
[162] C. Fressinaud, J.M. Vallat, J. Durand, F. Archambeaud-Mouveroux, M. Rigaud, J. Neurochem. 49 (1987) 797.
[163] C. Fressinaud, J.M. Vallat, M. Rigaud, C. Cassagne, G. Labourdette, L.L. Sarliève, Neurochem. Intern. 16 (1990) 27.
[164] A. Grynberg, M. Degois, L. Guenot, P. Athias, Biol. Cell. 57 (1986) 89.
[165] A. Grynberg, E. Fantini, P. Athias, M. Degois, L. Guenot, M. Courtois, S. Khatami, J. Mol. Cell. Cardiol. 20 (1988) 863.
[166] F.A. Hoekstra, E.W. Van der Val, J. Plant Physiol. 133 (1988) 257.
[167] F.A. Hoekstra, T. Van Roekel, Plant Physiol. 88 (1988) 626.
[168] F.A. Hoekstra, J.H. Crowe, L.M. Crowe, Plant Physiol. 97 (1991) 1073.
[169] M. Ishinaga, K.K. Carroll, Biochem. Cell Biol. 66 (1988) 1163.
[170] M. Ishinaga, M. Tanimoto, Y. Taniguchi, K. Mukai, K. Yokoro, Agric. Biol. Chem. 54 (1990) 1091.
[171] M. Ishinaga, M. Tanimoto, S. Sugiyama, R. Kumamoto, K. Yokoro, Biochem. Cell Biol. 69 (1991) 655.
[172] D.R. Janero, B. Burghardt, Lipids 23 (1988) 452.
[173] D.R. Janero, B. Burghardt, Lipids 24 (1989) 125.
[174] D.R. Janero, B. Burghardt, J. Mol. Cell. Cardiol. 21 (1989) 1111.
[175] S.H. Kim, C.V. Morr, A. Seo, J.G. Surak, J. Food Sci. 54 (1989) 25.
[176] J. Leclerc, B. Chanussot, M.L. Miller, J.P. Poisson, J. Belleville, Reprod. Nutr. Dev. 29 (1989) 269.
[177] A. Leseigneur-Meynier, G. Gandemer, Meat Sci. 29 (1991) 229.
[178] G. Nalbone, A. Grynberg, A. Chevalier, J. Leonardi, E. Termine, H. Lafont, Lipids 25 (1990) 301.
[179] C. Nicolas, Y. Demarne, M.J. Lecourtier, C. Lhuillery, Comp. Biochem. Physiol. 96B (1990) 195.
[180] C. Nicolas, Y. Demarne, M.J. Lecourtier, C. Lhuillery, Int. J. Obesity 14 (1990) 537.
[181] E. Ribot, A. Grandgirard, J.L. Sébédio, A. Grynberg, P. Athias, Lipids 27 (1992) 79.
[182] T.W. Robinson, D.P. Duncan, H.J. Forman, Prostaglandins 36 (1988) 443.
[183] T.W. Robinson, H.J. Forman, Prostaglandins 40 (1990) 13.
[184] Sallin, C. Rose, R. Sieber, J.O. Bosset, R. Tabacchi, Mitt. Geb. Lebensmitteluntersuch. Hyg. 87 (1996) 137.
[185] I.I. Bateman, T.C. Jenkins, J. Agric. Food Chem. 45 (1997) 132.
[186] H.-Y. Kim, N. Salem Jr., J. Lipid Res. 31 (1990) 2285.
[187] R. Asmis, A. Jörg, Eur. J. Biochem. 187 (1990) 475.
[188] M.H. Conte, J.K.B. Bishop, Lipids 23 (1988) 493.
[189] R. Diefenbach, H. Keweloh, H.J. Rehm, Appl. Microbiol. Biotechnol. 36 (1992) 530.
[190] W. Druml, R.A. Kelly, R.C. May, W.E. Mitch, J. Clin. Invest. 81 (1988) 1197.

[191] T. Hamazaki, M. Urakaze, M. Makuta, A. Ozawa, Y. Soda, H. Tatsumi, S. Yano, A. Kumagai, Lipids 22 (1987) 994.
[192] R. Huopalahti, P. Laakso, J. Saaristo, R. Linko, H. Kallio, J. High Resolut. Chromatogr. 11 (1988) 899.
[193] H. Kallio, P. Laakso, R. Huopalahti, R. Linko, P. Oksman, Anal. Chem. 61 (1989) 698.
[194] P. Kalo, P. Elo, M. Antila, Milchwissen Schaft 43 (1988) 421.
[195] R.A. Kelly, D.S. O'Hara, W.E. Mitch, T.W. Smith, J. Biol. Chem. 261 (1986) 11704.
[196] R.A. Kelly, D.S. O'Hara, V. Kelley, J. Chromatogr. 416 (1987) 247.
[197] R.A. Kelly, J.M. Pfeffer, W.E. Mithch, T.W. Smith, Hypertension 10 (1987) 198.
[198] R.A. Kelly, M.L. Canessa, T.I. Steinman, W.E. Mitch, Kidney Int. 35 (1989) 595.
[199] M.D. Maloney, L.H. Semprevivo, Parasitol. Res. 77 (1991) 294.
[200] R. Natarajan, W.D. Dunn, N. Stern, J. Nadler, Mol. Cell. Endocrinol. 72 (1990) 73.
[201] K.V.V. Nurmela, L.T. Satama, J. Chromatogr. 435 (1988) 139.
[202] V.L. Puchinger, H. Stachelberger, Fat Sci. Technol. 90 (1988) 540.
[203] T. Takata, T. Mimoura, H. Takada, M. Sakaguchi, M. Yamamura, K. Hioki, M. Yamamoto, Carcinogenesis 11 (1990) 2015.
[204] D.M. Tal, M.D. Yanuck, G. Van Hall, S.J.D. Karlish, Biochim. Biophys. Acta 985 (1989) 55.
[205] V.G. Weissmann, H. Kubel, W. Lange, Holzforschung 48 (1989) 75.
[206] V.G. Weissmann, W. Lange, Holzforschung 45 (1991) 285.
[207] J. Aufenanger, R. Kattermann, J. Clin. Chem. Clin. Biochem. 27 (1989) 605.
[208] T.A. Berkhout, L.M. Havekes, N.J. Pearce, P.H.E. Groot, Biochem. J. 272 (1990) 181.
[209] M. Gronn, E. Christensen, T.A. Hagve, Biochim. Biophys. Acta 1081 (1991) 85.
[210] R.A. Coleman, E.B. Haynes, J. Lipid Res. 28 (1987) 1335.
[211] R.A. Coleman, E.B. Haynes, T.M. Sand, R.A. Davis, J. Lipid Res. 29 (1988) 33.
[212] M.D. Englen, S.M. Taylor, W.W. Laegreid, H.D. Liggitt, R.M. Silflow, R.G. Breeze, R.W. Leid, Exp. Lung Res. 15 (1989) 511.
[213] M. Enslen, H. Milon, A. Malnoë, Lipids 26 (1991) 203.
[214] D.L. Hachey, M.R. Thomas, E.A. Emken, C. Garza, L. Brown-Booth, R.O. Adlof, P.D. Klein, J. Lipid Res. 22 (1987) 994.
[215] E.B. Hoving, G. Jansen, M. Volmer, J.J. Doormaal, F.A.J. Muskiet, J. Chromatogr. 434 (1988) 395.
[216] J.L. Kervin, N.D. Duddles, J. Bacteriol. 17 (1989) 3831.
[217] J.L. Kervin, N.D. Duddles, R.K. Washino, J. Invert. Pathol. 58 (1991) 408.
[218] H.F. Krug, H. Culig, D.M. Taylor, Anal. Chem. 330 (1988) 436.
[219] H.F. Krug, H. Culig, Mol. Pharmacol. 39 (1991) 511.
[220] M. Rimpler, A. Gerull, M.L. Döwald, W. Zaremba, E. Degen, Anal. Chem. 327 (1987) 37.
[221] M.D. Saltarelli, K. Yamada, J.T. Coyle, J. Neurosci. 10 (1990) 62.
[222] W. Sattler, G.M. Kostener, G. Waeg, H. Esterbauer, Biochim. Biophys. Acta 1081 (1991) 65.
[223] O. Schmitz, G. Danneberg, B. Hundeshagen, A. Klinger, H. Bothe, J. Plant Physiol. 139 (1991) 106.
[224] K. Shimada, J. Yanagisawa, F. Nakayama, Hepatology 13 (1991) 438.
[225] J.P. Slotte, L. Grönberg, J. Lipid Res. 31 (1990) 2235.
[226] Y. Takagi, C.A. Dyer, L.K. Curtiss, J. Lipid Res. 29 (1988) 859.
[227] H.J. Verkade, E.B. Hoving, F.A. Muskiet, J. Clin. Nutr. 53 (1991) 643.
[228] H.H. Winkler, R.M. Daugherty, Infect. Immunol. 57 (1989) 36.
[229] I. Zamir, E. Grushka, G. Cividalli, J. Chromatogr. 565 (1991) 424.
[230] I. Zamir, E. Grushka, J. Chromatogr. 567 (1991) 319.
[231] D. Tsikas, J. Chromatogr B 717 (1998) 201.
[232] S.K. Jain, Biochim. Biophys. Acta 937 (1988) 205.
[233] J. Suttnar, J. Cermak, J.E. Dyr, Anal. Biochem. 249 (1997) 20.
[234] S.A. Metz, M.E. Hall, T.W. Harper, R.C. Murphy, J. Chromatogr. 233 (1982) 193.
[235] F.J. Zijlstra, J.E. Vincent, J. Chromatogr. 311 (1984) 39.
[236] J.R. Luderer, D.L. Riley, L.M. Demers, J. Chromatogr. 273 (1983) 402.
[237] W.C. Pickett, M.B. Douglas, Prostaglandins 29 (1985) 83.
[238] T.G. Bernhardt, P.A. Cannistraro, D.A. Bird, K.M. Doyle, M. Laposata, J. Chromatogr B. 675 (1996) 189.
[239] H. Salari, J. Chromatogr. 419 (1987) 103.
[240] K.E. Stremler, D.M. Stafforini, S.M. Prescott, T.M. McIntyre, J. Biol. Chem. 266 (1991) 11095.
[241] A. Tokumura, Prog. Lipid Res. 34 (1995) 151.
[242] T. Tanaka, H. Minamino, S. Unezaki, A. Tsukatani, A. Tokumura, Biochim. Biophys. Acta 1166 (1993) 264.
[243] T. Tanaka, A. Tokumura, H. Tsukatani, Biosci. Biotech. Biochem. 59 (1995) 1389.
[244] M. Schlame, R. Haupt, I. Wiswedel, W.J. Kox, B. Rustow, J. Lipid Res. 37 (1996) 2608.