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Gas chromatographic determination of fatty acids contained in different lipid classes after their separation by solid-phase extraction

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Abstract

A quick and successful procedure is presented for the separation of polar lipids, monoacylglycerols (MAGs), diacylglycerols and triacylglycerols (TAGs) and for fatty acid determination in the above-mentioned lipid fractions by gas chromatographic analysis, which was acceptable for physiological and nutrition studies. In the analysis of edible oils and biological tissue samples, lipid classes were separated and purified by solid-phase extraction (SPE) using an aminopropylsilica column. Fatty acids in the *sn*-2 position in edible oil TAGs were determined after previous 1,3-specific lipase hydrolysis and separation of 2-MAGs by SPE using an aminopropylsilica column. A preliminary study of the solid-phase extraction separation of lipid classes with stock standard solutions using styrene–divinylbenzene–methacrylate copolymer (Nexus), octadecylsilica (C_{18}) and aminopropylsilica (NH_2) was carried out and it was shown that NH_2 was the best sorbent for the above-mentioned purpose.

Keywords: Oils; Fatty acids; Lipids; Acylglycerols; Glycerols

1. Introduction

Major ongoing research is directed towards better understanding of the role diet plays in obesity, cardiovascular disease, and cancer [1–3]. Determination of the fatty acid composition of different lipid classes is very important for lipid class characterisation and to explain physical differences in lipid fractions. The physical properties of membranes are the basis upon which the fatty acid composition of phospholipids in biomembranes is determined, which is directly related to lipid consumption in humans and animals [4]. Dietary fats can modulate the composition of the fatty acid moieties of membrane phospholipids in tissues [5]. However, when studying the effects of diet, previous intake needs to be characterized. The separation of different lipid classes and determination of their fatty acid composition are parts of an analytical procedure central to a wide range of studies in human and animal biology, and food characterisation [6].

During chemical analysis of biological samples it is necessary to first carry out some operations to isolate an analyte free of interfering compounds so as to ensure its quantitative determination. The difficulties inherent in the separation of complex biological samples, such as 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 with this method. Solid-

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phase extraction (SPE) is considered one of the most powerful techniques currently available for rapid and selective sample preparation. It is effective for lipid separation because it enriches minor components of the sample, which is especially important in biological samples, where trace amounts of those compounds are being dealt with. It has elicited growing interest because it is rapid and reliable, requires less solvent, and can be easily automated [7].

A wide variety of the chromatography supports are utilised in lipid separation processes [6,8]. Numerous quantitative techniques (HPLC, GC, UV) have benefited from a separation method such as SPE and used it as a first step in analysis. The strategy chosen for the analysis of specific lipids will depend on the nature of the information required.

Many papers where SPE was used in the analyses of different lipid classes have been published [7,9–18]. Using the SPE method Kaluzny et al. were able to isolate up to ten different lipid classes with a reasonable degree of purity by sequential elution with solvents of increasing polarity [17].

SPE cartridges packed with silica gel have been used by lipid analysts for the fractionation of total lipid extracts into simpler classes [18]. Similar types of separation have been accomplished on SPE columns with bonded aminopropyl groups. The SPE procedure on aminopropyl cartridges was used for the separation of lipid mixture from fish gills and human melanoma tumor tissues, into free ceramides, neutral glycosphingolipids, neutral phospholipids (sphingomyelin) and a fraction containing acidic phospholipids and phosphorylated sphingoid bases, phosphoceramides and sulfatides [7]. The procedure using aminopropyl solid-phase extraction columns has also been used to separate polyhydroxyalkanoates (PHAs), phospholipids, sterols, triacylglycerols (TAGs), diacylglycerols (DAGs), and monoacylglycerols (MAGs) and steryl esters from microbial lipid mixture [9]. Burdge et al. have developed a method for rapid separation of phosphatidylcholine (PC), non-esterified fatty acids (NEFAs), cholesterol ester and TAGs from total plasma lipid extracts by SPE using aminopropylsilica columns [10].

Powell used chemically bonded octadecylsilyl columns for the extraction and isolation of prostaglandins from urine, plasma and tissue homogenates [14]. A similar procedure was introduced for the isolation of water-soluble lipids such as gangliosides from aqueous extracts [13].

In this paper, we propose a procedure for the determination of fatty acids in polar lipids, TAGs, DAGs and MAGs from edible oils and biological tissue extracts in order to determine their relationships. The procedure involves the SPE separation, methylation and gas chromatographic method. The same separation procedure was used for *sn*-2 monoacylglycerol resolution from lipolysis mixture obtained after 1,3-specific lipolysis. We used the proposed method to study the effects of dietary fatty acids (from corn and olive oil) on the fatty acid composition of polar lipids in tissue samples (spleen, lung and submandibular gland tissues).

2. Experimental

2.1. Chemicals and SPE columns

Chloroform, acetone, methanol, *n*-hexane, methylene chloride, ethyl acetate, diethyl ether, light petroleum, acetic acid and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO, USA). Reference substances were used in resolution and recovery in a preliminary phase, when individual lipid classes were extracted on different sorbents. 1-Monoolein, 2-monoolein, mixed isomers of 1,3-diolein (85%) and 1,2-diolein (15%) and triolein were obtained from Sigma. All fatty acid methyl ester (FAME) standards were obtained from Sigma. Polar lipids were prepared as the reference standard for their recovery test; they were extracted according to Pinkart et al. from commercial soybean lecithin samples (Sojara, Zadar, Croatia) [9].

Pancreatic lipase (EC 3.1.1.3, type 2, crude from porcine pancreas, activity 220 U/mg protein with olive oil at pH 7.7), sodium cholate and calcium chloride were purchased from Sigma.

The solid-phase extraction cartridges, aminopropylsilica (NH_2) and octadecylsilica (C_{18}) (Bond Elut, 3-ml volume, 500-mg sorbents) used for the separation of lipid classes and for the resolution of lipolysis mixture were purchased from Varian (Harbor City, CA, USA). Abselut (styrene–divinylbenzene–methacrylate copolymer (Nexus) bonded phase, sorbent mass 30 mg) was donated for the test

separation of lipid classes by Varian. TLC silica gel 60 plates were purchased from Merck (Darmstadt, Germany).

2.2. Samples

The used olive oil sample was taken from an olive oil product made from a mixture from various olive cultivars during the 1997–1998 harvest and processed in an industrial oil mill in Punat, Island of Krk, Croatia, by using a continuous three phase system with centrifugation. The olive oil sample (acidity<1%) was collected from an individual producer and stored at 4 °C in the dark until analysis. The refined corn oil sample was obtained from Oleificio Zucchi, Cremona, Italy.

Tissues for analysis (average amounts were 90 mg of spleen, 126 mg of lung and 159 mg of submandibular gland) were obtained from 2–3-month-old male Balb/C mice. Animals were divided into three groups of six animals each. The control group was fed a standard diet (Faculty of Biotechnology, Domžale, Slovenia), the second group (fed olive oil, FOO) was fed a standard diet supplemented with 5% (w/w) olive oil and third group (fed corn oil, FCO) was fed a standard diet supplemented with 5% (w/w) corn oil. The animals were sacrificed by spinal dislocation, under ether anesthesia. The tissues were removed with plastic instruments, washed with saline solution to remove blood, weighed and stored at −70 °C until analysis.

2.3. Sample preparation

Stationary phases and elution solvents for SPE were selected in a preliminary study. The quantities and composition of all elution systems were determined by experimentation according to modified methods of Pinkart et al. [9] and Neff et al. [19]. The separation recovery of individual lipid classes was obtained gravimetrically with stock standard solutions of polar lipids (2, 5, 10 mg ml^{-1}), 1-monoolein (0.2, 0.5, 0.85 mg ml^{-1}), 2-monoolein (0.2, 0.5, 0.8 mg ml^{-1}), mixed isomers of 1,2- and 1,3-diolein (1, 2, 5 mg ml^{-1}) and triolein (1, 2, 4 mg ml^{-1}) on the three solid sorbents: Nexus, C_{18} and NH_2. The purity of the separated lipid fractions was confirmed by TLC [20] after previous SPE separation of standard lipid mixture, prepared from the lowest previously mentioned concentrations of individual stock standard solutions. Application of the modified procedure on NH_2 column according to Pinkart et al. (Fig. 1) gave higher yields, so this method was used for further characterisation of edible oils and biological tissue samples [9].

Total lipids were extracted from tissue samples with a chloroform–methanol (2:1, v/v) mixture according to Folch et al. [21]. During the extraction procedure, lipids were protected against oxidation by adding 0.05 mg ml^{-1} BHT to the solvents. The average amount of total lipids (in chloroform) from spleen was 0.76 mg ml^{-1}, lung 1.29 mg ml^{-1} and submandibular glands 2.45 mg ml^{-1}. Polar lipids were separated on the SPE column from those samples by the previously proposed method [9]. FAMEs contained in polar lipids were obtained after methylation according to European Union regulations [22].

Samples of edible oils (olive and corn oil) were dissolved in chloroform (10 mg ml^{-1}), the solution was loaded on the NH_2 column and the lipids were separated into classes by the proposed solvent system. FAMEs contained in polar lipids, TAGs, DAGs and MAGs were obtained after previous methylation according to European Union regulations [22].

2-MAGs were obtained after lipolysis of the TAG fraction of edible oils (olive and corn oil) by using lipase directed cleavage of fatty acids at the glycerol 1,3 carbons according to EEC Directive 2568 [22]. The lipid mixture obtained after lipolysis, average amount 1.1 mg ml^{-1}, was applied on the aminopropylsilica column to separate 2-MAGs. FAMEs contained in 2-MAGs were obtained after previous methylation according to European Union regulations [22].

2.4. Chromatographic conditions

GC analyses were carried out using an Autosystem XL (Perkin-Elmer, Norwalk, CT, USA) with flame-ionisation detection (FID). Chromatography software from Perkin-Elmer Nelson (Turbochrom 4, rev. 4.1.) was used for data acquisition from the FID. Hydrogen was obtained with a Claind hydrogen generator.

Test portions, in form of the FAMEs, were

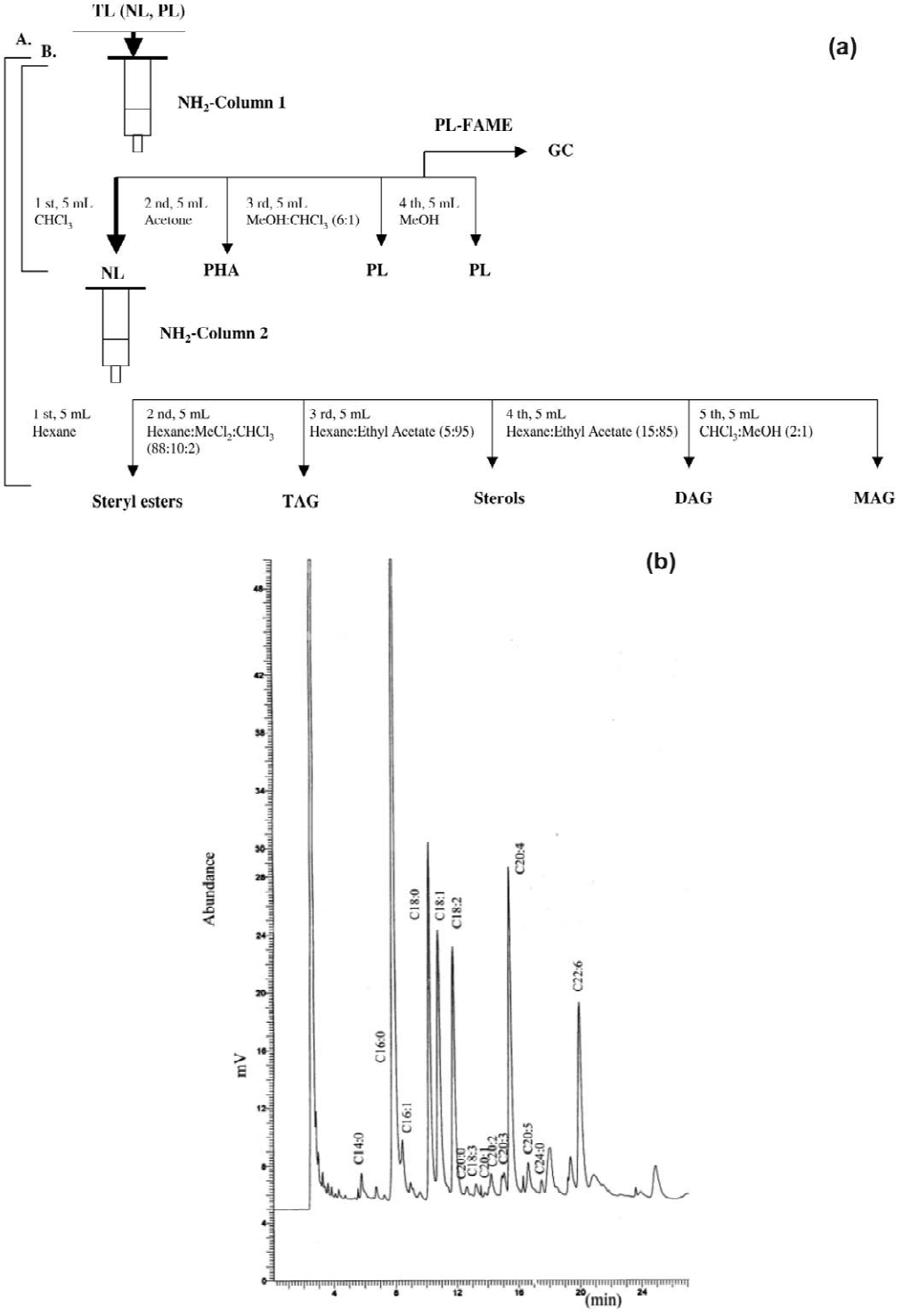

Fig. 1. (a) Scheme followed for the isolation of polar lipids in spleen tissue, using the aminopropyl column. (A) Elution regimes used for the separation of lipid classes of the edible oil samples. TL, total lipids; NL, neutral lipids; PL, polar lipids. (B) Elution regimes used for the separation of polar lipid of the tissue samples (spleen). (b) Typical gas chromatogram of the FAMEs in polar lipids isolated from tissue samples, on an SP-2330 capillary column, 30 m×0.32 mm I.D., 0.2 μm film thickness. The GC oven was maintained at 140 °C, programmed at 5 °C min^{-1} to 220 °C and then held isothermal for 25 min. Splitting ratio, 100:1. Compound names are shown on the peaks.

examined in triplicate and 1 μl of each solution in chloroform was injected into the GC system. An SP-2330 capillary column (Supelco, Bellefonte, PA, USA), 30 m×0.32 mm I.D., 0.2 μm film thickness, was used. Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in programmed temperature mode from 140 to 220 °C, at 5 °C min^{-1} and then isothermal for 25 min. The detector temperature was 350 °C and injector temperature was 300 °C. The results were expressed as a percentage of individual fatty acids in lipid fractions.

After comparison of several analytical variants (different SPE sorbents, solvent regimes and standard concentrations) and subsequent SPE–GC development of an improved draft standard for quantitation of examined lipid classes, this draft was checked for accuracy. Partial SPE–GC validation was carried out for the analysis of the polar lipid FAMEs isolated from the examined tissues and FAMEs from TAGs, DAGs, MAGs and 2-MAGs. Validation was proven only in the examined tissue samples, because of the unlimited lipids in the edible oils. Acceptable accuracy was obtained. Repeatability and recovery trials were carried out (the relative standard error of the former was ~10%), and the quantification limit was 10 μg ml^{-1} of polar lipids from tissue samples. From the GC chromatogram shown in Fig. 1b it is obvious that good selectivity of FAMEs from tissue samples was accomplished.

3. Results and discussion

Analytical methodology is important for a large number of studies dealing with lipid compound determination in biological samples and edible oils. The knowledge obtained from lipid analysis and the determination of their chemical composition will allow us to find out more about the influence that the lipids have on human and animal nutrition [23].

During the biochemical study of the mechanism of dietary lipid absorption, a quick and reliable analytical procedure is required. As the amount of the tissue samples from the mice used was limited, isolation techniques of the lipid compounds of interest from these samples required the precision of the separation procedure. In our experiments, the SPE technique was shown to be more applicable than TLC for the semi-preparative isolation of lipid classes prior to FAME gas chromatographic analysis. Although the use of silicon, NH_2, quaternary amino, C_{18} and diol-phase columns for the separation of lipid classes has been reported [24,25], separately or together, we optimised conditions for the SPE procedure on polar NH_2 and hydrophobic C_{18} columns. We used two elution regimes with stock standard solutions to obtain maximum recovery and purity of each lipid fraction [9,19]. By using a novel sorbent material, Nexus, SPE cartridge with hydrophilic and lipophilic properties, with the same elution procedures, obtained recoveries of stock standard solutions were lower than for the two above-mentioned sorbents. When comparing the recoveries of these two sorbents, the aminopropyl column was shown to be more applicable for this purpose. Due to a series of different interactions, the aminopropyl-bonded column initially separates non-polar and polar lipids. The extraction of total lipids by the modified procedure was complete and comparable with a reference procedure [9]. Recoveries of polar lipids, TAGs, DAGs, MAGs and 2-MAGs from stock standard solutions using the SPE aminopropyl column were 66±7.5, 78±6.8, 94±2.5, 95±3.5 and 95±3.5, respectively.

Fig. 1 shows the scheme followed for the isolation of lipid classes from the examined samples. An example of the isolation (Fig. 1a) and quantification of FAMEs in polar lipids isolated from spleen tissue samples (Fig. 1b), with representative chromatogram can also be seen. Spleen tissue sample was chosen as tissue with the smallest mass; the average amount of polar lipids was 0.76 mg ml^{-1}. As show in Fig. 1a and b, the proposed methodology was employed successfully for microanalyses of the studied samples.

This paper focuses on the limited lipid quantities from the tissue samples obtained in the SPE, and determination of their fatty acid composition. We were dealing especially with effects of dietary lipids on the polar lipid fraction content in tissue samples of spleen, lung, and submandibular glands.

In the first step of our study, we characterised edible oils without previous extraction; they were examined as monounsaturated fatty acid diet (MUFA; olive oil) and polyunsaturated fatty acid diet (PUFA; corn oil). These characterisations were important because of the nutritional characteristics of

Table 1
Fatty acid composition of the polar and non-polar lipid classes from olive oil sample after previous separation using the amino-propyl SPE column and GC analysis of FAMEs in separated lipid fractions

Fatty acid	Fatty acid composition (mol.%)				
	Polar lipids	TAGs	DAGs	MAGs	2-MAGs
C14:0	Trace	Trace	0.2	Trace	Trace
C16:0	21.4	12.7	40.9	26.0	10.7
C16:1	1.5	1.5	0.8	2.5	1.6
C17:0	Trace	Trace	Trace	Trace	Trace
C18:0	9.8	3.7	25.4	33.2	1.8
C18:1	56.2	70.7	27.5	29.7	77.4
C18:2	9.0	9.7	4.1	6.9	7.1
C20:0	Trace	Trace	Trace	Trace	0.3
C18:3	1.7	0.4	0.2	0.4	0.4
C20:1	0.4	0.6	0.6	0.7	0.5
C22:0	Trace	0.3	0.3	Trace	Trace
C22:1	Trace	0.4	Trace	0.6	Trace
C24:0	Trace	Trace	Trace	Trace	0.1

Trace is <0.05%.

the studied fatty acids such as degree of unsaturation, PUFA:MUFA ratio, oxidizability, etc. Tables 1 and 2 present the fatty acid composition of non-polar and polar lipid fractions from olive and corn oil samples after previous separation of these fractions using two NH_2 columns by the solvent elution regime previously described. The SPE aminopropyl column shows a diacylglycerol isomerization tendency [6]. This is important in the analysis of diacylglycerols, which were not considered in our study. Carelli et al. [26] developed an SPE–HPLC procedure using a diol phase column to quantify phospholipids present in sunflower oil at different stages of processing, which were found in a wide range of concentrations (0.1–1.2%). In our work, by using one NH_2 column for lipid separation, the phospholipid content in corn oil was 0.9%, and 0.6% in olive oil. At these polar lipid concentrations, the fatty acid composition in edible oils was successfully determined by GC analysis, as well as in other examined lipid classes. The analytical data provide useful information about differences in fatty acid composition affected by the type and genetic modification of seed oils [27]. In our work, oleic acid was predominant in the olive oil sample in the polar lipid and TAG fractions, palmitic acid in the DAG fraction, stearic acid in the MAG fraction and oleic acid in the 2-MAG fraction. Linoleic acid was predominant in the corn oil sample in the polar lipid and TAG fractions, palmitic acid in the MAG fraction, oleic acid in the DAG fraction and linoleic acid in the 2-MAG fraction. The nutritional quality of oils is determined by their fatty acid composition as well as by the distribution of these fatty acids in TAGs. The standard regio-specific analysis procedure [22] for edible oils was modified using SPE–GC instead of TLC–GC using the NH_2 column for 2-MAG separation from the lipid mixture after 1,3-specific lipase hydrolysis [28]. Major fatty acids found in the *sn*-2 position were oleic acid for the olive oil sample and linoleic acid for the corn oil sample and we expected them to have the biggest effect on the polar lipid fatty acid composition.

Table 2
Fatty acid composition of the polar and non-polar lipid classes from corn oil sample after previous separation using aminopropyl SPE column and GC analysis of FAMEs in separated lipid fractions

Fatty acid	Fatty acid composition (mol.%)				
	Polar lipids	TAGs	DAGs	MAGs	2-MAGs
C14:0	0.1	Trace	0.1	Trace	Trace
C16:0	21.0	13.1	12.8	27.2	10.4
C16:1	Trace	Trace	0.1	1.4	0.3
C17:0	Trace	0.5	0.1	Trace	0.1
C18:0	11.7	5.9	10.5	19.7	2.9
C18:1	27.2	27.0	33.2	23.6	32.8
C18:2	38.1	51.4	31.1	24.4	51.0
C20:0	Trace	0.5	Trace	Trace	0.4
C18:3	1.5	0.9	4.1	0.3	1.3
C20:1	0.4	0.7	8.0	3.4	0.4
C22:0	Trace	Trace	Trace	0.1	Trace
C22:1	Trace	Trace	Trace	Trace	0.1
C24:0	Trace	Trace	Trace	Trace	0.2

Trace is <0.05%.

NH_2 columns were used also to isolate neutral and polar lipids from different sources, such as microbial cultures and tissues [7,9]. Application of this method for different tissues allowed the authors to assert that the efficiency of the separation depends on the type of tissue. In order to simplify the separation, we proposed a small modification of the procedure described by Pinkart et al. [9], as shown in Fig. 1a.

Relative percentages of FAMEs in the polar lipid fraction isolated from the examined tissue samples

Table 3
Fatty acid composition (mol.%) of the total phospholipids in the mice spleen tissue samples in control and after feeding diets supplemented with 5% olive oil (FOO) and 5% corn (FCO) after previous separation using aminopropyl SPE column and GC analysis

Fatty acid	Control	FOO group	FCO group
C14:0	0.49±0.08	0.45±0.09	0.47±0.08
C14:1	0.00	0.00	0.00
C16:0	33.86±2.26	35.94±3.08	32.39±3.46
C16:1	1.63±0.46	1.45±0.50	1.20±0.30
C18:0	14.03±1.70	12.24±1.55	13.35±0.81
C18:1	12.00±0.49	17.30±2.17	12.73±0.97
C18:2	8.90±1.38	8.03±0.10	12.10±2.40
C20:0	0.54±0.26	0.45±0.05	0.30±0.08
C18:3	0.73±0.10	1.06±0.27	0.61±0.12
C20:1	0.00	0.00	0.09±0.08
C22:0	0.00	0.00	0.00
C20:2	0.81±0.09	0.95±0.22	1.25±0.17
C20:3	0.86±0.09	0.81±0.28	0.56±0.04
C20:4	13.74±1.35	11.46±1.50	13.91±1.08
C20:5	0.83±0.13	0.77±0.13	0.68±0.14
C24:0	1.12±0.47	0.46±0.21	0.47±0.18
C22:6	10.46±1.43	8.65±1.41	9.89±1.50

Data are means±SD (n=6 per group).

Table 4
Fatty acid composition (mol.%) of the total phospholipids in the mice tissue samples in control and after feeding diets supplemented with 5% olive oil (FOO) and 5% corn oil (FCO) after previous separation using aminopropyl SPE column and GC analysis

Fatty acid	Control	FOO group	FCO Group
C14:0	1.00±0.09	0.88±0.24	1.21±0.35
C14:1	0.00	0.00	0.00
C16:0	48.86±1.21	41.91±3.49	46.77±1.13
C16:1	4.09±0.05	3.32±0.61	3.06±0.18
C18:0	8.93±0.34	10.66±0.87	9.72±0.37
C18:1	10.85±0.67	13.50±0.18	9.75±0.96
C18:2	6.80±0.36	7.40±0.81	9.25±0.35
C20:0	0.23±0.09	0.22±0.14	0.19±0.10
C18:3	0.37±0.21	0.52±0.16	0.34±0.11
C20:1	0.00	0.00	0.00
C22:0	0.00	0.00	0.00
C20:2	0.44±0.05	0.40±0.11	0.61±0.10
C20:3	0.58±0.10	0.78±0.20	0.76±0.08
C20:4	7.10±0.35	8.34:±1.67	7.33±0.41
C20:5	1.27±0.29	0.58±0.02	0.49±0.06
C24:0	0.33±0.15	0.45±0.08	1.24±1.15
C22:6	9.15±0.41	11.04±1.11	9.28±1.02

Data are means±SD (n=6 per group).

are shown in Tables 3–5. As shown in Table 3, palmitic acid predominated in the fatty acid composition of polar lipid species in spleen tissue samples. The differences depended on dietary fat intake. Table 4 presents the phospholipid fatty acid composition in control and supplemented diets for lung tissue samples. Palmitic acid was again the predominant fatty acid in the lung. Table 5 shows the fatty acid composition of the polar lipid fraction in submandibular gland tissue samples, where palmitic acid was the major fatty acid present. Considering the fatty acid composition of lipid fractions from the fed oils, it was evident that the major fatty acid in olive oil present in the polar lipid, TAG and 2-MAG fractions (oleic acid) affected oleic acid concentration in all examined tissues. The major fatty acid present in polar lipids, TAGs, MAGs and 2-MAGs in corn oil (linoleic acid) affected linoleic acid concentration in all examined tissues. We used this methodology extensively to analyse the phospholipid fatty acid composition from more than 150 samples over a 1-year period. These results showed that the used method is very sensitive for analysing phospholipid fatty acid composition. Total lipids in

Table 5
Fatty acid composition (mol.%) of the total phospholipids in the mice submandibular gland tissue samples in control and after feeding diets supplemented with 5% olive oil (FOO) and 5% (FCO) after previous separation using aminopropyl SPE column and GC analysis

	Control	FOO group	FCO group
C14:0	0.27±0.04	0.26±0.04	0.32±0.18
C14:1	0.00	0.00	0.00
C16:0	28.75±1.48	27.90±1.70	29.18±5.07
C16:1	1.44±0.15	1.16±0.14	0.89±0.36
C18:0	11.24±0.53	12.79±0.72	14.60±1.38
C18:1	13.63±0.33	17.40±2.29	13.01±1.89
C18:2	20.69±0.97	19.29±0.98	21.84±0.50
C20:0	0.33±0.06	0.32±0.13	0.32±0.17
C18:3	0.25±0.01	0.73±0.53	2.08±0.91
C20:1	0.67±0.06	0.36±0.36	0.01±0.01
C22.0	1.15±0.29	0.00	0.00
C20:2	0.70±0.09	0.44±0.43	0.95±0.10
C20:3	0.85±0.54	0.78±0.23	0.79±0.48
C20:4	9.55±0.54	9.63±2.18	8.24±2.60
C20:5	1.74±0.12	0.75±0.66	0.69±0.13
C24:0	0.43±0.18	0.40±0.20	0.55±0.10
C22:6	8.30±0.73	7.79±2.53	6.52±2.77

Data are means±SD (n=6 per per group).

spleen tissues were in the concentration range 0.20–0.79 mg ml^{-1}, in the lung 0.80–1.29 mg ml^{-1} and in the submandibular glands 1.80–2.45 mg ml^{-1}.

4. Conclusions

On the basis of the obtained results we suggest that the SPE method with an aminopropylsilica column with appropriate solvent regime is an effective tool for the separation of lipid classes in microquantities of sample for analysis. The presented data confirm that the proposed methodology, SPE–GC, is appropriate even for as limited amounts of samples as used in this investigation.

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