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Determination of polycyclic aromatic hydrocarbons in vegetable oils using solid-phase microextraction–comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry

Giorgia Purcaro [a,b,*], Paul Morrison [b], Sabrina Moret [a], Lanfranco S. Conte [a], Philip J. Marriott [b]

[a] *Department of Food Science, University of Udine, via Marangoni 97, 33100 Udine, Italy*
[b] *Australian Centre for Research on Separation Science, School of Applied Sciences, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia*

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Abstract

A simple and fast solid-phase microextraction method coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry was developed for analysis of polycyclic aromatic hydrocarbons in edible oil, performed directly in a hexane solution of the oil. Sampling conditions (solvent used, extraction time, extraction temperature and fiber rinsing time) were optimized by using a sample of oil fortified with a standard solution of polycyclic aromatic hydrocarbons. The method was validated by calculating linear range, correlation coefficient, accuracy, repeatability, detection limit and quantification limit. The method was applied to several oils collected from the market and directly from an olive pomace extraction plant.

Keywords: Polycyclic aromatic hydrocarbon (PAH); Edible oil; Solid-phase microextraction (SPME); GC × GC; Time-of-flight mass spectrometry (TOF-MS)

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds produced through incomplete combustion or pyrolysis of organic matter [1]. Some PAHs have been proved to be carcinogenic and genotoxic [2]. They can be assimilated by humans through ingestion, inhalation, or skin contact [3]. The occurrence of PAHs in food is due to environmental contamination (deposition of airborne particulates on crops or growth in contaminated soil), technological processing or home-cooking (i.e. grilling and smoking) and in low amounts from contaminated packaging material [4–7]. Contamination by technological processing of foods depends on a number of parameters: heating time and temperature, fuel used, distance from the heat source, impinging of smoke onto the product and drainage of fat. Due to the lipophilic nature of these compounds, fats and oils can be highly contaminated [8].

The US Environmental Protection Agency (EPA) highlighted 16 PAHs as priority pollutants [9]. Investigations on PAHs have generally focused on these PAHs (EPA PAHs) or on benzo[a]pyrene (BaP; considered the most hazardous).

In early 2005 the European Commission fixed a new limit for allowable amount of only one PAH, BaP in foods [10], and furthermore it recommended [11] that all the Member States investigate the levels of 15 PAHs pointed out by the Opinion of the Scientific Committee on Food (SCF) expressed in 2002 [12] and one PAH (benzo[c]fluorene) highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2005 [13] (16 EU PAHs). The European Union (EU) should have reviewed the maximum level for PAHs by 1 April 2007.

The most widespread techniques for PAH analysis are high-performance liquid chromatography (HPLC) coupled with a spectrofluorimetric detector and gas chromatography–mass spectrometry (GC–MS). Recently, methods using comprehensive two-dimensional chromatography have been reported (both LC × LC [14] and GC × GC [15,16]). The high separation

* Corresponding author at: Department of Food Science, University of Udine, via Marangoni 97, 33100 Udine, Italy. Tel.: +39 0432 590724; fax: +39 0432 590719.
E-mail address: giopurcaro@libero.it (G. Purcaro).

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power of GC × GC, allows separation of the target compounds from interferences. GC × GC also provides a chemically structured chromatogram that gives useful additional identification information. The narrow peaks obtained from the fast separation in the second dimension (^{2}D) require fast detectors. The time-of-flight mass spectrometry (TOF-MS) detector has been largely used to achieve this aim and to allow the identification of the numerous separated compounds.

In recent years many methods for PAH sample preparation have been proposed. Among them the most common is solid-phase extraction (SPE) [17]. Several methods using solid-phase microextraction (SPME) for the analysis of PAHs in head space or directly from water matrix have been reported [18–21]. Only one study [22] has reported the use of SPME for extraction of components (dioxins) directly from organic solvent.

The aim of this work was to develop an SPME method for analysis of PAHs in vegetable oils using a GC × GC–TOF-MS system. Considering the new European legislation, the study was focused on the EU PAHs.

2. Experimental

2.1. Reagents and standards

Two stock standard PAHs solutions were mixed in order to evaluate the affinity of the fiber to a large range of components.

The first stock standard (std-1a) in methylene chloride/benzene (1:1) (Cerilliant Corporation, Round Rock, TX, USA), contained 2000 μg/mL of each of the following components: acenaphtene (Ac), acenaphthylene (Ap), anthracene (A), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (Ch), dibenz[ah]anthracene (DBahA), fluoranthene (Fl), fluorene (F), indeno[1,2,3-cd]pyrene (IP), naphtalene (Na), phenanthrene (Pa), pyrene (P). This solution was diluted to give working solutions of 10 μg/mL (std-1b).

The second stock standard PAHs mixture (std-2) in cyclohexane (Dr. Ehrenstorfer GmbH, Augsburg, Germany), consisted of 10 mg/L of each of the following components: BaA, BbF, BjF, BkF, benzo[c]fluorene (BcF), BghiP, BaP, Ch, cyclopenta(cd)pyrene (CPP), DBahA, dibenz[ae]pyrene (DBaeP), dibenz[ah]pyrene (DBahP), dibenz[ai]pyrene (DBaiP), dibenz[al]pyrene (DBalP), IP, 5-methylchrysene (5-MeCh).

Hexane, cyclohexane, acetone and acetonitrile were all HPLC grade (Ajax Finechem, Seven Hills, Australia).

2.2. SPME conditions

The SPME fiber used was a 15 μm film thickness Carbopack Z/PDMS (Supelco, Bellefonte, PA, USA). The same fiber, directly dipped into an organic solution, was used by Maeoka et al. [22] for dioxin analysis. Therefore, it was presumed to be suitable for the purpose of the present study. The primary mode of extraction of this fiber is the π–π interaction between the carbon surface and the analytes. The flat co-planar compounds can have greater interaction with the carbon surface and are retained while other analytes will not be retained as efficiently. This becomes especially critical in a nonpolar solvent matrix. Under these conditions the effect of PDMS is greatly minimized, although sorption into the PDMS phase would play a much greater role in water based matrix.

To determine the optimum conditions, 100, 150, 200, 250 and 500 μL portions of a spiked oil sample (ranging between 10 and 40 μg/kg, depending on the individual PAH) was made up to 1.5 mL using different solvents (hexane, cyclohexane, isooctane and acetonitrile). Extraction time and temperature were also investigated. In order to remove triglyceride surface residues from the fiber before injection, the fiber was rinsed in hexane. Different rinse times were tested (30 s, 1, 2 and 5 min). All the solutions were stirred at a constant rate throughout the extraction period. The optimum conditions were found to be: 200 μL of oil made up to 1.5 mL using hexane, absorption time of 30 min at room temperature, followed by a 1 min rinse in hexane.

2.3. GC × GC–TOF-MS analysis

All analyses were performed using an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA, USA) fitted with a longitudinally modulating cryogenic system (Chromatography Concepts, Doncaster, Australia). The detection system used was a Pegasus III TOF-MS (LECO Corporation, St. Joseph, MI, USA) with data interpretation provided by LECO ChromaTOF software.

The column set comprised of a guard column (5 m × 0.32 mm I.D.) followed by a 5% phenyl polysilphenylene-siloxane (BPX5) first dimension capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness), a 50% phenyl polysilphenylene-siloxane (BPX50) second dimension capillary column (1 m × 0.1 mm I.D., 0.1 μm film thickness) and 0.5 m × 0.1 mm transfer line. All the columns were from SGE International (Ringwood, Australia).

Oven temperature program conditions were 40 °C hold for 2 min, then the temperature was increased to 210 °C at 30 °C/min, then to 360 °C at 5 °C/min (15 min hold time at 360 °C). A modulation period of 3 s was used in all analyses and cryogenic trap temperature control was set at 25 °C.

The MS transfer line and MS source temperatures were 300 °C and 250 °C, respectively. Data were collected at a nominal acquisition rate of 100 spectra/s. Electron ionisation mass spectra were recorded at 70 eV and the detector voltage was set at −1650 V.

Splitless injections were performed at 340 °C, with the fiber held in the injector, for 10 min.

2.4. Analytical performance

A working standard solution (std 3) was prepared by using one volume of std-1b and two volumes of std-2 to produce similar amounts of each PAH component of interest in the final solution.

Calibration curves were constructed through spiking of a PAH-free oil sample at six different concentrations (each analy-