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Screening of edible oils for polycyclic aromatic hydrocarbons using microwave-assisted liquid–liquid and solid phase extraction coupled to one- to three-way fluorescence spectroscopy analysis

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ABSTRACT

The potential of microwave-assisted liquid–liquid and solid phase extraction coupled with fluorescence spectroscopy and employing one- to three-way spectral data was assessed in terms of their capacity for the rapid detection of heavy polycyclic aromatic hydrocarbons (PAHs) in olive and sunflower oils. Tocopherols and pigments groups (chlorophyll and pheophytin) present in oil matrices were the main interference compounds in the detection of PAHs using fluorescence spectroscopy. Partial spectral overlap and inner-filter effects were observed in the emission range of the analytes. The effectiveness of removing these interferences using solid phase extraction (silica, C18 and graphitized carbon black) was examined. Solid phase extraction with silica was the most effective method for the removal of pigments and tocopherol and allowed for the detection of PAHs in edible oils using fluorescence spectroscopy. The limit of detection was observed to depend on the use of one-, two- or three-way fluorescence spectral data in the range of 0.8 to 7.0 $\mu g\,kg^{-1}$. The individual recoveries of PAHs following the microwave-assisted L–L extraction and SPE with silica were assessed using HPLC–FD with satisfactory results.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds that have been recognized as carcinogenic and mutagenic, and whose regulation is of interest in the environmental and food fields. PAHs with high molecular weight (5–6 fused aromatic rings) have lipophilic character. Human beings are exposed to PAH contamination principally by the direct inhalation of polluted air or tobacco smoke, direct skin contact with polluted soils, soot or tars and the intake of contaminated water or foods, mainly fatty foods (animal or vegetable) [1]. Accordingly, several heavy PAH have been detected in edible oils, including olive and sunflower oils [2]. In 2001, different European countries produced legislation limiting the concentration of eight heavy PAHs in olive pomace oils: benzo[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]-fluoranthene (BkF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), indeno [1,2,3–c,d]-pyrene (IP), dibenz[*a,h*]anthracene (DBahA), and benzo[*g,h,i*]perylene (BghiP). The maximum value of 2 $\mu g\,kg^{-1}$ for each PAH and 5 $\mu g\,kg^{-1}$ for the sum of eight heavy PAHs was established [3,4].

The determination of PAHs in these complex matrixes requires extraction and purification for the partial or complete removal of lipidic components co-extracted with the target compound. The analysis of extracts is performed mainly using high performance liquid chromatography coupled to fluorescence detection (HPLC–FD) [5–8] or using gas chromatography (GC) coupled to tandem mass spectrometry (GC–MS/MS) [9–12]. The sample preparation and the subsequent chromatographic analyses are often time and solvent consuming and hinder sample throughput of the analytical process. This fact can be a major disadvantage if a large number of samples must be analyzed; therefore, it would be desirable to have a screening method to sift out the positive samples, which could be then be confirmed by a chromatographic method. Ideally, the screening method must detect the presence of a specific class of analytes at the concentration of interest, provide a low rate of false compliant samples, and exhibit high throughput, short analysis time, good selectivity, low cost and semi-quantitative or quantitative results.

PAHs detection by using fluorescence spectroscopy is a faster alternative to chromatography for the determination of these compounds in different types of samples. Several methods based on multivariate analysis of fluorimetric data have been reported for detecting PAHs in water samples [13–16]. Some of the advantages of these methods include minimum sample treatment and direct

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determination of analytes without performing an additional separation. As a preliminary approach, these methods provide qualitative information about the presence of PAHs in water samples. Subsequently, by using different chemometric techniques to resolve the partially overlapping fluorescence spectra of the analytes, analytical selectivity can be achieved.

Using a similar method, fluorescence spectroscopy could be employed to detect the presence of PAHs in edible oil samples, and can be performed as an initial screening prior to the chromatographic analysis. However, interferences could occur during the analysis of this kind of samples. For example, the inner filter effect can be observed due to interactions with the molecular environment of the fluorophore. This effect occurs when one component absorbs the emission corresponding to another component in the sample. When the first component does not fluoresce at the analyte emission wavelength, the standard addition method is an alternative for overcoming this problem. Alternatively, a pre-treatment intended to eliminate or diminish the inner filter effect and/or overlap with other fluorophores is required. Moreover, in the case of edible oils, the residual level of PAHs requires a pre-concentration step. However, this sample pre-treatment must be as simple as possible, and one that avoids excessive consumption of time and reagents.

Traditional sample pre-treatment methods applied for the detection of PAHs in oils and fats involve extraction and purification steps. The more commonly applied approach for PAHs extraction from edible vegetable oils is liquid partitioning with organic solvents by mechanical or manual shaking, followed by a clean-up with solid-phase extraction (SPE) using different sorbents or gel permeation chromatography [1,17]. The principal drawback of these methodologies is the low throughput of samples and poor reproducibility. Other techniques involve extraction and clean-up with a single SPE [6,7,18] or tandem-SPE [5] after sample dilution. As an alternative, a saponification step prior to the liquid partitioning is also utilized to reduce the lipidic content (e.g., triacylglicerols) by using mixtures of KOH or NaOH solutions containing ethanol or methanol [17].

In this study, the suitability of liquid–liquid extraction assisted by microwave energy and coupled to different clean-up alternatives for the rapid detection by fluorescence spectroscopy was investigated for seven heavy PAHs in edible oils that are currently included in the legislation. The ability to remove the principal sources of interference on the fluorescence of the analytes was evaluated by using solid phase extraction with silica, C18 and graphitized carbon black. The capability of using one- to three-way spectral fluorescence data for the detection of PAH in oily matrices was evaluated. Finally, the individual recoveries of PAHs with the proposed extraction were assessed by HPLC–FD and compared with a traditional liquid–liquid extraction method. The results confirm the possibility of connecting the proposed sample treatment to fluorescence spectroscopy and multivariate calibration for the quantitative determination of PAHs in edible oils.

2. Experimental

2.1. Instruments and apparatus

A Milestone (Sorisole, Bergamo, Italy) MLS 1200MEGA microwave oven equipped with a high performance microwave digestion unit model mls-1200Mega, an exhaust module model EM-45/A, a terminal Mega-240 and a 10-position rotor was used for the liquid–liquid extraction of oil samples.

Fluorescence measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer (Mulgrave, Victoria, Australia) equipped with two Czerny-Turner monochromators and a xenon flash lamp, and connected to a PC via an IEEE 488 (GPIB) serial interface.

The HPLC–FD system used for the chromatographic analysis consisted of a Waters (Milford, MA, USA) chromatograph equipped with a 600 quaternary gradient pump, a 717 Plus autosampler and a 2475 multi λ fluorescence detector. System control, data acquisition and process were performed using Empower software. The separation column was an Inertsil ODS column (5 μm average particle size, 250 × 4.6 mm i.d.) connected to a Symmetry™ C18 120 Å, 10 mm (5 μm average particle size, 3.9 × 20 mm) guard column.

2.2. Chemicals and reagents

Acetonitrile, dichloromethane and isopropanol of liquid chromatography grade as well as acetone and *n*-hexane of pesticide residue analysis grade were obtained from Merck (Darmstadt, Germany).

Stock solutions (100 mg L^{-1}) were prepared in acetonitrile from the corresponding solid compounds obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) BaP, BaA and DBahA, Accustandard, Inc. (New Haven, CT, USA) for BkF, BbF and IP; and BghiP from Supelco (Bellefonte, PA, USA). Using these stock solutions, dilute multi-component standards containing 0.1 mg L^{-1} of each compound and 0.02 mg L^{-1} for BkF were prepared in hexane and used for the fortification of oil samples.

The chlorophyll *a* standard was supplied by Sigma-Aldrich (St. Louis, MO, USA), pheophytin *a* was obtained via acidification with 1 M hydrochloric acid of the respective chlorophyll *a* solution according to a previously described procedure [19].

Discovery® DSC-Si silica (12 mL, 2 g), ENVI-Carb® (6 mL, 0.5 g), ENVI-Carb/LC-NH_2 (6 mL, 1 g) cartridges from Supelco and Super-SPE® C18 (6 mL, 0.5 g) cartridge from Luknova (Mansfield, MA, USA) were used for the solid phase extraction clean-up.

2.3. Oil samples

Recovery and optimization studies were performed using a commercial organic extra virgin olive oil and common sunflower oils purchased at a local supermarket. Prior to the extraction and clean-up procedures, these samples were spiked with a solution of PAHs dissolved in hexane.

2.4. Conventional and microwave-assisted liquid–liquid extraction

The procedure proposed by Martínez López et al. [6], with some modifications, was used as for the conventional L–L extraction method. Samples of 1.00 ± 0.01 g of oil were placed in centrifuge tubes and treated with 10 mL of acetonitrile. The mixture was shaken vigorously for 3 min in a vortex shaker and sonicated for 5 min. The resulting suspensions were centrifuged at 2500 rpm for 5 min and the upper layer was separated using a Pasteur pipet. The extraction was repeated by adding 10 mL of acetonitrile to the remaining oil. After three extraction steps, the acetonitrile mixture of extracts was evaporated to dryness using a vacuum rotary evaporator equipped with a 65 °C water bath. The residue was weighed to determine the oil residue and re-dissolved in 2 mL of hexane for the direct measurement of the emission spectrum or in 1 mL of hexane for the subsequent solid phase extraction step.

A glass system previously designed in our laboratory was used for the microwave assisted extraction (MAE) [20]. An aliquot of 1.00 ± 0.01 g of olive oil was accurately weighed and placed in a 50 mL Erlenmeyer flask equipped with a ground–glass joint. Subsequently, 30 mL of acetonitrile were added and the air-cooled condenser was attached to the flask using the ground–glass joint. The glass system was placed in a microwave oven and heated for 19 min at 150 W. A maximum of eight and minimum of six samples were extracted each time. After cooling

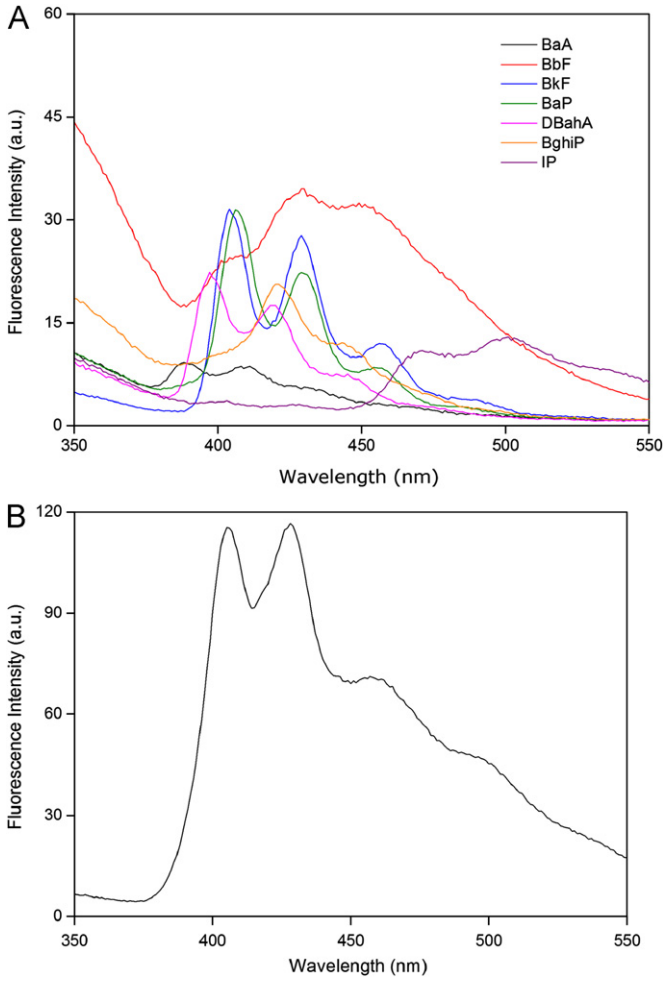

Fig. 1. Emission spectra of seven pure PAHs obtained at 2 μg L^{-1} in hexane (0.5 μg L^{-1} for BkF and 4 μg L^{-1} for BghiP and IP) using the excitation wavelength of 290 nm (A). Emission spectrum of a mixture of seven PAHs (B).

to room temperature, the inner wall of the condenser was rinsed with 2 mL of acetonitrile and removed from the flask. The upper layer was separated, evaporated to dryness and the residue was weighed and re-dissolved as described above.

2.5. Solid phase extraction

The final extract was obtained in the L–L extraction (1 mL) and loaded onto a 2-g silica SPE cartridge (Discovery™ Supelco), which was pre-washed with 5 mL of dichloromethane and pre-conditioned with 5 mL of *n*-hexane. PAHs were eluted with a mixture of *n*-hexane and dichloromethane 80:20 (*v*/*v*). The eluate containing the PAH fraction (the first 15 mL) was collected into a conical vial. The flow rate was adjusted to approximately 1 drop per second. The collected fraction was evaporated to dryness under a nitrogen stream and the residue was dissolved in 2 mL of hexane for spectroscopic analysis or 2 mL of isopropanol for HPLC–FD analysis. Central composite design was used to optimize the volume of the elution solvent and its volumetric ratio (hexane–dichloromethane). This design was composed of a 2^2 full factorial design with four added axial points ($\pm\alpha = \pm 1.41$) and two central points. Statistical software (Statgraphics Centurion XV for Windows, Rockville, MD, USA) was used to build the experimental design and analyze the experimental data.

2.6. Fluorescence analysis

The fluorescence spectra used for the zero- and first-order calibrations were collected in the range of 300 to 700 nm every 2 nm at a scanning rate of 600 nm min^{-1} and at an excitation wavelength of 290 nm. The excitation–emission spectra used for the second-order calibration were obtained using the following settings: λ_{exc}=250–400 nm each 5 nm and λ_{em}=370–550 nm each 2 nm, at a scanning rate of 600 nm min^{-1}. The excitation and emission slit width was 10 nm. For the first- and second-order calibration, the spectra were saved in ASCII format prior to analysis. A routine was employed to perform the partial least square (PLS) and unfolded PLS (U-PLS) analysis in MATLAB 7.6 using a graphical interface with the MVC1 and MVC2 toolbox, which is available on the internet (〈www.chemometry.com〉).

A calibration curve was prepared to determine the analytical figures of merit for the detection of PAHs in edible oils using MAE–SPE and fluorescence spectroscopy. To this end, extracts of a blank olive oil, obtained using microwave assisted L–L extraction, were evaporated and re-dissolved in 1 mL of hexane, fortified with increasing quantities of PAHs detailed in Table 1(3.0 to 88 μg kg^{-1} total concentration), cleaned using SPE on silica, evaporated, re-dissolved in 2 mL of hexane and analyzed by fluorescence spectroscopy under the conditions described above.

2.7. Chromatographic analysis

The temperature of the column in the HPLC–FD system was kept constant at 30 °C to obtain reproducible retention times for the PAHs. The detection of PAHs was performed at four channels available using the fluorescence detector: channel A (excitation wavelength: 220 nm; emission wavelength: 330 nm); channel B (292/410 nm); channel C (292/426 nm); channel D (300/500 nm). The mobile phase consisted of acetonitrile (A) and water (B) at a flow rate of 1.4 mL min^{-1}. The optimized elution program was: 0–10 min, linear gradient from 70 to 90% A; held for 5 min; 15–20 min, linear gradient from 90 to 100% A; held for 2 min and returned to the initial conditions within 3 min. Isocratic conditioning was performed for 10 min to afford column re-equilibration. The identification of PAHs was performed by comparison of the observed retention times (tr/min) with those obtained using analytical standards under the same conditions. Peak identity was also confirmed using the co-injection method wherein the extracts were spiked with their corresponding standards.

3. Results and discussion

3.1. Absorption and emission spectra of analytes and matrices

The emission spectra of the analytes as well as the absorption and emission spectra of the matrices were obtained in hexane. Fig. 1A provides the emission spectra of seven pure PAHs examined at 2 μg L^{-1} in hexane (0.5 μg L^{-1} for BkF and 4 μg L^{-1} for BghiP and IP) at the excitation wavelength of 290 nm. Due to the difference in the quantum efficiency, it is advisable to use a higher dilution for BkF and a lower dilution for BghiP and IP. Although each PAH has its own specific emission, overlap clearly occurs. Fig. 1B provides the emission spectrum of a mixture of seven PAHs with the same concentration. As a result, four wavelengths corresponding to the maximum emission of the PAHs mixture are observed (403, 426, 455 and 500 nm). Fig. 2 provides the absorption and emission spectra (obtained at the excitation wavelength of 290 nm) of extra virgin olive oil and sunflower oil diluted in hexane. The band in both edible oils, with

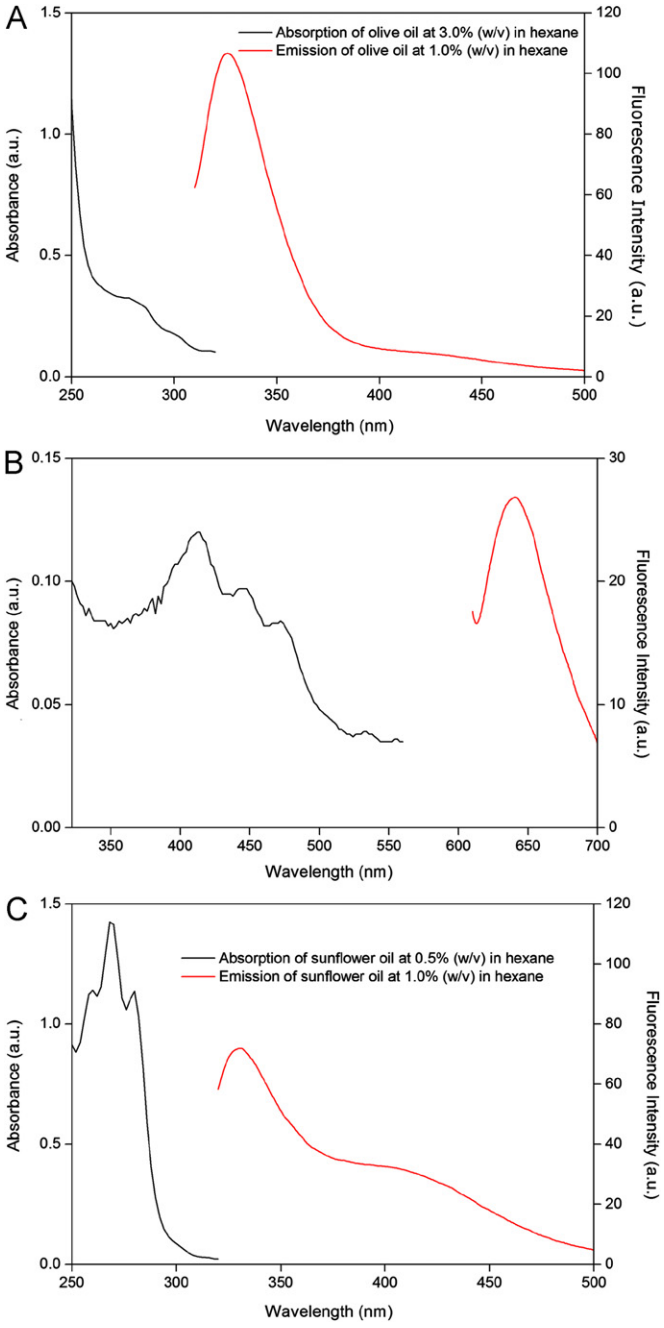

Fig. 2. Absorption and emission (λ excitation=290 nm) spectra of virgin olive oil (A) and (B) and sunflower oil (C) diluted in hexane.

absorption in the range of 250–320 nm and emission in the range of 310–370 nm (Fig. 2A and C), has been ascribed to tochopherols [21]. In the case of olive oil, recent studies demonstrate that phenolic compounds based on tyrosol and hydroxytyrosol and derived from phenolic glycosides present in olives contribute to the observed absorption–emission [22,23]. A second long-wavelength band with absorption between 360 and 510 nm and emission between 610 and 700 nm is observed specifically in olive oil (Fig. 2B) and is ascribed to different pigments [22–24]. The absorption band at 360–450 nm is attributed to the chlorophyll group, including chlorophyll *a* and *b* as well as pheophytins *a* and *b* [19], which overlap with the band in the range of 450–520 nm ascribed to carotenoid pigments. In contrast, a less intense fluorescence band at 410 nm is found in sunflower refined oil (Fig. 1C) and has been ascribed to the oxidation products [21]. In agreement with these observations, the detection of the emission band of PAH at 380–550 nm is possible in the presence of olive or sunflower oil matrices.

3.2. Microwave assisted L–L extraction of PAHs from oil

In a previous study, we proposed a microwave-assisted liquid–liquid extraction at atmospheric pressure (APMAE) to assist with the extraction of organophosphorus pesticides from olive and avocado oil using a home-made, inexpensive and simple glass system [20]. In this work, this system was applied for the extraction of PAHs from olive and sunflower oil, providing an alternative to the conventional L–L extraction for fluorimetric detection. First, the fluorescence of PAHs was measured in the presence of co-extracted compounds from oil. To this end, microwave assisted L–L extraction was applied on a blank olive oil sample under the conditions previously described, fortified with increasing quantities of PAHs (see Table 1) and analyzed using fluorescence spectroscopy.

The mean value of residues co-extracted from oil in these samples was 91 $\pm$ 7 mg. As can be observed in Fig. 3A, the emission of PAHs appears into the low fluorescence wavelength of the edible oils (380–550 nm). However, the co-extracted tocopherol (emission at 310–370 nm) exhibits partial spectral overlap with the analytes. Moreover, the low fluorescence of the blank oil extract, attributed to the chlorophyll group, was observed at 380–500 nm and also overlaps with the PAHs spectra. An increase in the emission intensity of PAHs with increasing concentration was not observed in the presence of the olive oil matrix. This fact is attributed to the inner filter effect produced principally by the chlorophyll–pheophytin group, which is co-extracted in the microwave assisted L–L extraction and exhibits an absorption band at 360–450 nm that significantly overlaps with the emission band of the PAHs. Among the vegetable oils, olive oil contains one of the highest amounts of the chlorophyll pigments. The total pigment content in olive oil was in the range of 10–30 mg/kg with a typical distribution composed of pheophytin 55%, lutein 28%, β-carotene 8% and chlorophyll 5% [25,26].

In the case of sunflower oil, the inner filter effect due to the pigments was not observed. In contrast, the principal interference was the partial spectral overlap of the co-extracted tocopherol with the PAHs emission. Chlorophyll is nearly absent in refined sunflower oil samples. Further, sunflower oil has a higher concentration of tocopherol than olive oil (671 and 158 mg/kg, respectively, according to Sikorska et al. [21]). Thus, the elimination of these interferences with a clean-up step is mandatory for the selective detection of PAHs in virgin olive oil and sunflower oil by fluorescence spectroscopy.

3.3. Clean-up method

Solid phase extraction was evaluated to eliminate or diminish the effect of co-extracted natural compounds on the emission of PAHs.

Table 1
Individual and total concentrations of PAHs added to the olive oil extract obtained using microwave assisted extraction.

Added ($\mu g\ kg^{-1}$)								
Sample	BaA	BbF	BkF	BaP	DBahA	BghiP	IP	Total
E1	0.4	0.4	0.1	0.4	0.4	0.4	0.8	3.0
E2	1.0	1.0	0.25	1.0	1.0	1.0	2.0	8.0
E3	2.0	2.0	0.50	2.0	2.0	2.0	4.0	14
E4	3.0	3.0	0.75	3.0	3.0	3.0	6.0	22
E5	5.0	5.0	1.2	5.0	5.0	5.0	10	36
E6	8.0	8.0	2.0	8.0	8.0	8.0	16	58
E7	12.0	12.0	3.0	12.0	12.0	12.0	24	88

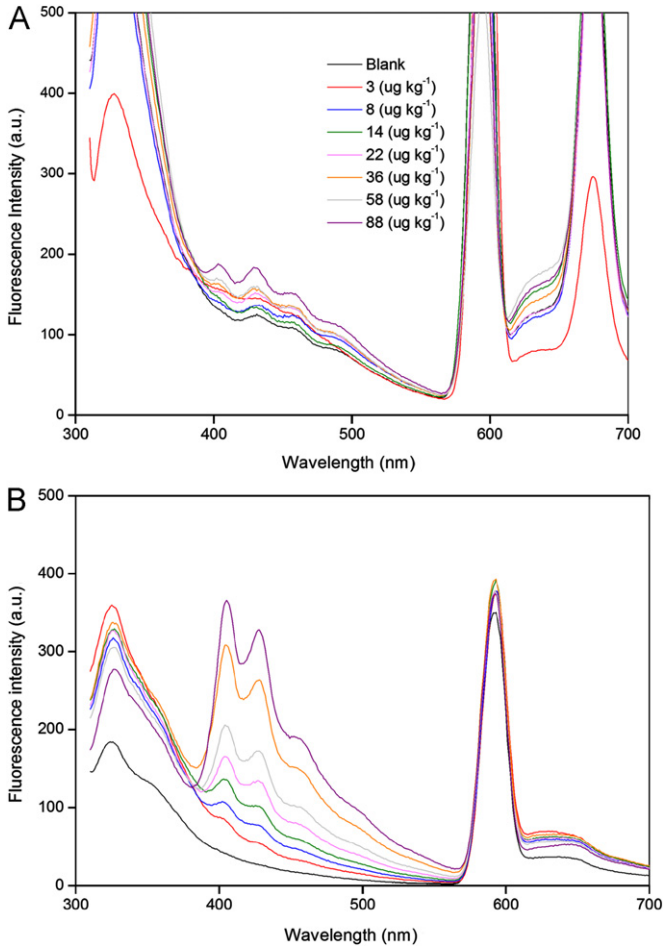

Fig. 3. Emission spectra of a mixture of PAHs (0, 3, 8, 14, 22, 36, 58 and 88 μg kg^{-1} total concentration) in the presence of an olive oil extract obtained using microwave assisted extraction (A) and SPE with silica (B).

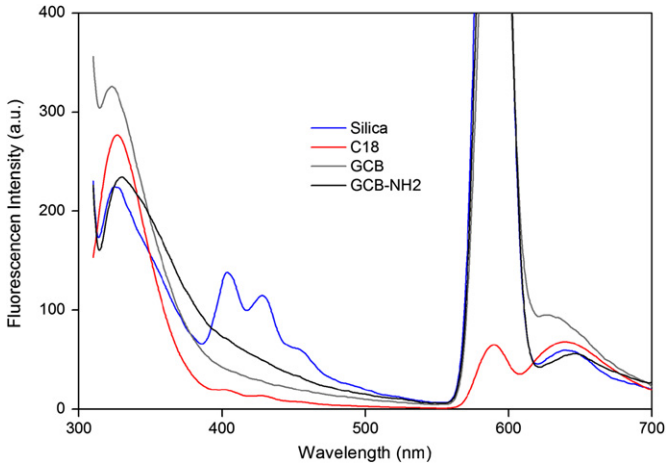

Fig. 4. Emission spectra of a mixture of PAHs (42 μg kg^{-1} total concentration) in the presence of an olive oil extract obtained after SPE with silica, C18, graphitized carbon black (GCB) and graphitized carbon black-aminopropyl silica (GCB–NH_2).

Four different phases were evaluated in terms of efficiency in removing interferences from oil: Silica (1 g), C18 (0.5 g), graphitized carbon black (0.5 g GCB) and dual phase GCB-aminopropyl silica (0.5 g GCB+0.5 g NH_2). For this purpose, 0.2 g of olive oil was spiked with 30 μg kg^{-1} of each PAH, diluted with 1 mL of hexane, and added to the different cartridges. Based on previous studies [7,18], the elution was performed with 7 mL of hexane–dichloromethane 70:30 for silica, 7 mL of acetonitrile for C18 and 10 mL of acetonitrile for both GBC cartridges. The eluates were evaporated under nitrogen and re-dissolved in 2 mL of hexane.

The emission bands ascribed to PAHs were only observed for extracts obtained from silica and C18 (Fig. 4), with the latter exhibiting a significant decrease in sensitivity. As discussed earlier, this finding is attributed to the inner filter effect produced by the pigments. The results demonstrate that only silica was efficient enough to eliminate this interference, producing more intense emission spectra and decreasing the emission of tocopherol at 340 nm. In contrast, extracts from graphitized carbon exhibited a complete absence of emission bands for PAHs, even when using 20 mL of acetonitrile as the eluent solvent. Although carbon has a high affinity for planar pigments, it also exhibits a significant affinity for PAHs, which were irreversibly adsorbed onto the carbon.

The effect of pheophytin-*a* and chlorophyll-*a* on the PAH emission was evaluated by comparing the fluorescence spectrum of the analyte mixture in the presence of pure pigments at their expected concentrations in olive oil (20 and 1 mg/kg, respectively) before and after the clean-up with silica (Fig. 5). According to the absorption and emission spectra, an inner-filter effect was produced by both pigments. These compounds clearly interfere by absorbing either part of the excitation energy (primary inner filter effect) or the emission energy (secondary inner filter effect) of the PAHs, leading to emission profiles that are different in both intensity and shape. As a result, the emission of PAHs is completely masked or confounded by the signals from the pigments. However, the emission profile of the analytes was recovered in a satisfactory manner after the solid phase extraction treatment wherein the pigments were adsorbed onto the silica. The efficient adsorption of pheophytin, chlorophyll and the oxygenated carotenoid lutein on silica in the presence of hexane was previously reported [27].

Thus, a further optimization of the clean-up process was performed with 2 g of silica and the microwave assisted liquid–liquid extraction method using the central composite design. The volume of the elution solvent (15 to 26 mL) and its volumetric ratio (hexane–dichloromethane, 90:10 to 50:50) were optimized. Only this mixture of solvents was evaluated because previous experience and other works [7,18] have demonstrated that this is the most appropriate mixture for the elution of PAHs from silica. The co-extracted oil residue obtained using the microwave assisted extraction was dissolved in 1 mL of hexane, spiked with 12 μg kg^{-1} of each PAH (excepting BkF with 3 μg kg^{-1}) and loaded into a cartridge (to maintain 50 mg of oil residue per gram of silica). The total recovery of PAHs and the emission of tocopherol at 340 nm were simultaneously optimized using the Derringer desirability function [28]. In this analysis, each individual response "*i*" was associated with its own partial desirability (d_i), which varies from 0 to 1 according to the closeness of the response to its target value. The two individual desirability values were combined as geometric means to obtain the overall desirability at all points in the experimental domain ($D=(d_1 \times d_2)^{1/2}$), whose values were utilized in the optimization. The partial desirability of PAHs recovery was obtained by "target is best" fixed at 95% (bilateral; upper limit 105%; weight factors, $s=t=1$; impact factor, $I=1$), and the partial desirability of the emission at 340 nm was obtained by its minimization (unilateral; weight factor, $s=3$; impact factor, $I=3$). The model provided an adequate representation of the data because the lack of fit was not significant (p-value=0.073) and the coefficient of determination was 0.8450. Higher values of D were observed over the entire volume range of dichloromethane between 20 and 30% *v*/*v*. Thus, the optimal volume for the elution of PAHs that provides minimum co-elution of the interferences was 15 mL of 20% *v*/*v* DCM in hexane (monitored through the elution profile from the silica

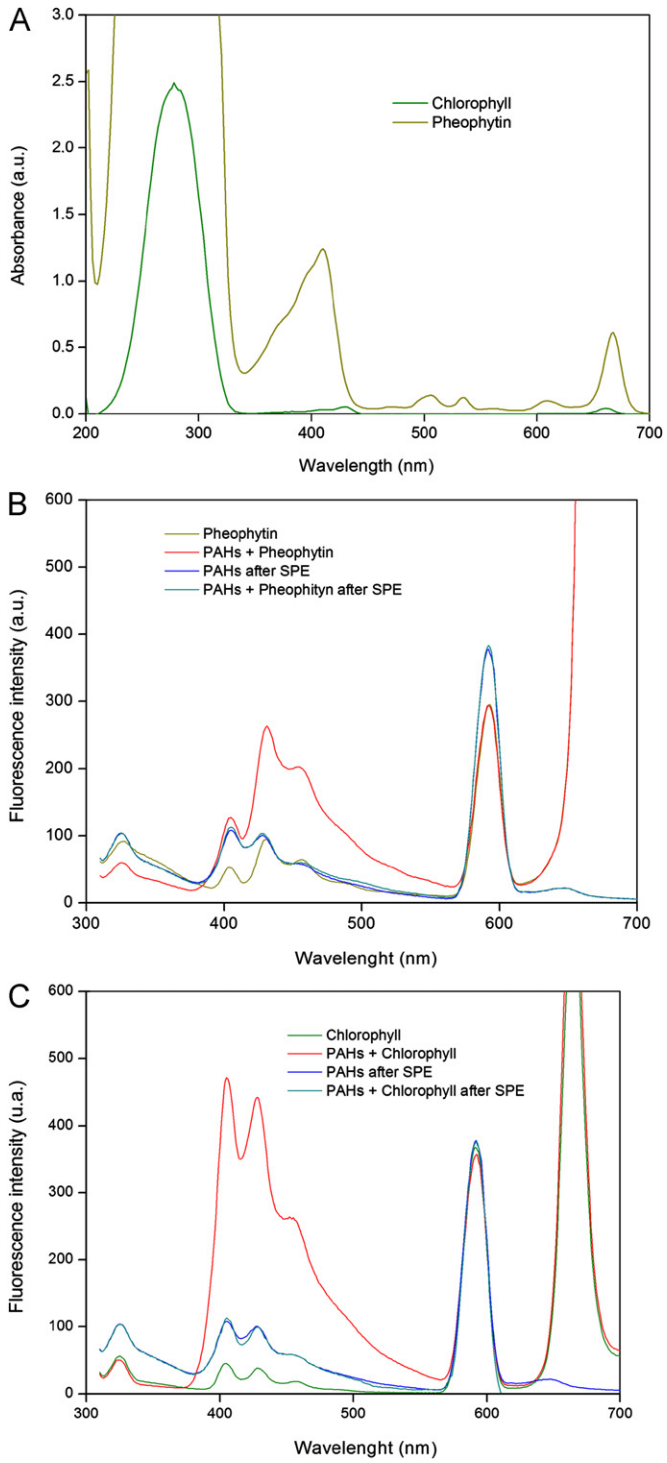

Fig. 5. Absorption spectra of pheophytin and chlorophyll (A). Emission spectra of PAHs in the presence of pheophytin (B) and chlorophyll (C) before and after SPE with silica.

cartridge). Under these conditions, the total recovery of PAHs was higher than or equal to 94% for olive and sunflower oils. This recovery value was obtained by comparing the fluorescence of the final extract with that of a blank extract after the addition of a standard solution at the wavelength corresponding to the maximum emission of the PAHs mixture. Similarly, the extracts obtained using the microwave-assisted L–L extraction and fortified with increasing quantities of PAHs (Fig. 3A) were also cleaned with silica using the optimal conditions. As a result, the characteristic emission spectra of the PAHs mixture were obtained (Fig. 3B), with a proportional increase in the emission intensity with increasing concentration.

3.4. Analytical figure of merit for PAHs in edible oil using MAE–SPE fluorescence

To establish the capability of MAE–SPE and fluorescence spectroscopy as a screening method for the determination of the total concentration of PAHs in edible oils, analytical figures of merit were obtained from univariate and multivariate calibration using a curve in the range of 3.0 to 88 μg kg^{-1}. For univariate calibration, the maximum emission at four wavelengths of the PAHs mixture and its mean value were considered. In contrast, the multivariate calibration was performed on the two- and three-way spectral data using the partial least square (PLS) and unfolded PLS (U-PLS) analyses, respectively. For the PLS, the wavelength range was 380–590 nm (excitation at 290 nm), whereas for the U-PLS, the wavelength ranges were 265–350 nm for the excitation and 392–480 for the emission. The raw excitation emission matrices (for U-PLS) or the mean centred fluorescence spectra (for PLS) were correlated with the total concentration of PAHs in the extract. The mean centering preprocessing option was used to correct some baseline problems present in the two-way spectral data. The analytical figures of merit for the multivariate calibration were obtained according to Olivieri et al. [29] and the results are summarized in Table 2.

For the univariate calibration, a linear response was observed for emission at four single wavelengths and its mean value in the range of 8.0–58 μg kg^{-1}, with r values of 0.9953–0.9977. Moreover, the relative standard deviation of the slope (S_b/b; where S_b is the standard deviation of the slope and b is the slope), which gives a better representation of the linearity of the analytical data, was equal or less than 5.6%. The analytical sensitivity ($S_{y/x}/b$; where S_y/x is the standard deviation of the regression), which indicates the minimal difference in the concentration detected by the method, was between 1.6 and 2.2 μg kg^{-1}. The limit of detection (LOD) was obtained from the regression model using 3.3($S_{y/x}/b$) and was in the range of 5.2–7.0 μg kg^{-1}. Additionally, the limit of quantification from the regression model (LOQ) was also obtained using 10($S_{y/x}/b$), and ranged 16 to 22 μg kg^{-1}.

For the multivariate calibration, the results were obtained from the regression models after a full cross-validation. As shown in Table 2, the multivariate calibration exhibited lower LODs that the univariate ones. The best analytical sensitivity (0.2 μg kg^{-1}) and lowest limit of detection (0.8 μg kg^{-1}) were obtained using the three-way spectral data with the U-PLS calibration. The parameters that account for the minimum detectable net concentration are most likely the most relevant to the screening method. Although the MAE–SPE does not have a pre-concentration factor, a clean extract was obtained and high sensitivity was achieved using fluorescence data from two- or three-way spectral data, allowing for the detection of PAHs in oil samples at sub μg kg^{-1} levels.

Another set of olive and sunflower oil blanks as well as low level spiked olive oil samples were analyzed with the method. The results are summarized in Table 3. The prediction of PAHs concentration in all blank samples was zero, equal or below the LOD: no false positives were detected. In contrast, false negative samples were not observed using the U-PLS algorithm, even if the sample contained a unique PAH with low quantum efficiency (BghiP in OO3). The comparison of the proposed method with the performance of the only method reported by Zougagh et al. [30] for the screening of PAHs in vegetable oils is fairly satisfactory. The reported LODs (12 to 16 μg kg^{-1}) are 5 to 20 higher than the values reported here. Due to the fluorimetric detection at one wavelength (400 nm), the monitored PAHs are limited to those that have similar fluorescence properties, which is not required for the multi-way spectral data approach presented here. Moreover, the

Table 2
Analytical figure of merit for the total PAHs concentration in edible oil matrix using the MAE–SPE fluorescence spectroscopy method obtained at four different wavelengths and using a multivariate calibration.

Univariate calibration						
Wavelength (nm)	Linear range (μg kg^{-1})	R	S_b/b (%)	$S_{y/x}/b$ (μg kg^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
403	8.0–58	0.9953	5.6	2.2	7.0	22
426	8.0–58	0.9967	4.6	1.9	6.1	19
455	8.0–58	0.9977	3.9	1.6	5.2	16
500	8.0–58	0.9972	4.6	1.7	5.7	17
Mean emission	8.0–58	0.9970	4.4	1.8	5.9	18

Multivariate calibration Wavelength range (nm)	Linear range (μg L^{-1})	R	Number of PC	Analytical sensitivity (μg kg^{-1})[a]	LOD (μg kg^{-1})[b]	LOQ (μg kg^{-1})[c]
PLS 380–590	3.0–58	0.9940	1	0.8	2.6	7.6
U-PLS 265–350 excitation 392–480 emission	3.0–58	0.9946	2	0.2	0.8	2.4

[a] Obtained from sX/sensitivity, where sX=spectral noise level.
[b] Obtained from 3.3 (sX/sensitivity).
[c] Obtained from 10 (sX/sensitivity).

Table 3
Prediction of total PAH concentrations in blank olive (BOO), blank sunflower oil (BSO) and low level spiked olive oil (OO) using MAE–SPE fluorescence spectroscopy and univariate (mean emission) or multivariate calibrations.

Added (μg kg^{-1})									Predicted (μg kg^{-1})		
Sample	BaA	BbF	BkF	BaP	DBahA	BghiP	IP	Total	Univariate (mean emission)	PLS	U-PLS
BOO1	–	–	–	–	–	–	–	–	0	0	0.8
BOO2	–	–	–	–	–	–	–	–	0	0	0.6
BOO3	–	–	–	–	–	–	–	–	0	2.6	0
BSO1	–	–	–	–	–	–	–	–	0	0	0
BSO2	–	–	–	–	–	–	–	–	0	0	0
BSO3	–	–	–	–	–	–	–	–	0	0	0.4
BSO4	–	–	–	–	–	–	–	–	0	0	0
OO1	4	0.25	1.0	0.25	0.25	0.25	5	11	6.1	3.6	11.6
OO2	0.25	0.25	0.16	0.25	0.25	0.25	1.0	2.4	0	0	3.0
OO3	0	0	0	0	0	4	0	4	0	0	2.4
OO4	0.4	0.40	0.1	0.4	0.4	0.40	0.8	3.0	1.6	0.8	3.0
LOD									**5.9**	**2.6**	**0.8**

Table 4
Individual and total recoveries (%) of seven PAHs from a spiked olive oil sample obtained using microwave assisted liquid–liquid extraction or classical liquid–liquid extraction.

		BaA	BbF	BkF	BaP	DBahA	BghiP	IP	Total
Added (μg kg^{-1})		4	4	1.6	4	4	4	10	31.6
HPLC–FLD	MAE–SPE	84 (7)[a]	78 (2)	83 (2)	67 (3)	72 (2)	62 (2)	72 (2)	74 (8)[b]
	LLE–SPE	88 (2)	81 (2)	78 (2)	77 (2)	75 (2)	67 (2)	77 (2)	78 (9)[b]
PLS	MAE–SPE	–	–	–	–	–	–	–	70 (2)
	LLE–SPE	–	–	–	–	–	–	–	90 (3)
U-PLS	MAE–SPE	–	–	–	–	–	–	–	88 (2)
	LLE–SPE	–	–	–	–	–	–	–	79 (9)

[a] Standard deviation (n=3).
[b] Mean obtained from de individual recovery of PAHs (n=7).

occurrence of samples that were incorrectly classified (false positive or negative) was not discussed in the aforementioned study.

Consequently, the proposed method can detect the presence of PAHs at the levels required by the European and international regulations [4] and also provide semi-quantitative results. In light of these results, a future study will be aimed at developing a quantitative multivariate calibration method for the determination of PAHs in edible oils.

3.5. Extraction efficiency of MAE–SPE

With the intention of evaluating the extraction capacity of the MAE–SPE method, oil samples were fortified with seven PAHs, extracted and analyzed using HPLC–FD. The calibration curve was prepared in isopropanol. The recoveries ranged from 62–84% with an SD equal or lower than 3%, with the exception of BaA (Table 4). No significant differences were observed between these values and those obtained using the conventional L–L extraction method (paired t-test; p-value=0.27 at 95% confidence). Furthermore, these values are in the range of acceptable recovery requirements according to the concentrations of the analytes (60 to 120% for 1–10 μg kg^{-1}) and the complexity of the matrix [31]. Moreover, the MAE process does not involve the formation of emulsions; it is possible to extract as many as eight samples simultaneously with less intervention in the process and similar solvent consumption as with the classic L–L extraction. Therefore, MAE coupled to SPE

with silica is adequate for the detection of PAHs in edible oils using fluorescence spectroscopy. The total recoveries were also predicted by PLS and U-PLS with only one significant difference observed between the value predicted by U-PLS for MAE (88%) and the one found by HPLC–FDL (74%) (t-test for means; $n_1=3$, $n_2=7$, p-value=0.02 at 95% of confidence); equivalent to a relative error of 19% (Table 4).

4. Conclusions

Microwave-assisted liquid–liquid and solid phase extraction on silica coupled to fluorescence spectroscopy and univariate or multivariate data analysis can be used for the rapid and facile detection of heavy polycyclic aromatic hydrocarbons (PAHs) in olive and sunflower oils. This screening method can detect the presence of analytes at the level set by the European and international regulations, providing semi-quantitative results. The combination of L–L extraction and SPE on silica with fluorescence spectroscopy makes it possible to extract the analytes, eliminate the main interferences for the detection of PAHs (tocopherol and chlorophyll–pheopytin) and achieve good analytical sensitivity. The limit of detection obtained depends on the use of one-, two- or three-way fluorescence spectral data in the range of 0.8 to 7.0 $\mu g\,kg^{-1}$. The lowest rate of false compliant samples was obtained using the three-way spectral data. The individual recoveries of PAHs assessed by HPLC–FD were in the range of 62–84%. This study provides a glimpse into the possibility of connecting the proposed method to multivariate calibration for the quantitative determination of PAHs in edible oils. Thus, future studies will develop a quantitative multivariate calibration method that employs three-way spectral data for the detection of PAHs in edible oils.

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