

**Original Article****The potential of low temperature extraction method for analysis of polycyclic aromatic hydrocarbons in refined olive and refined pomace olive oils by HPLC/FLD**Zohreh Taghvaei¹, Zahra Piravivanak^{1*}, Keramatollah Rezaee², Mohammad Faraji¹, Sara Nanvazadeh¹

1- Faculty of Food Industry and Agriculture, Standard Research Institute of Iran (ISIRI), Karaj, Iran

2- Department of Food Science, Engineering and Technology, University of Tehran, Karaj, Iran

Received: April 2015

Accepted: June 2015

ABSTRACT

Background and Objectives: A method was developed and validated for determining 15 polycyclic aromatic hydrocarbons (PAHs) in refined olive and refined pomace olive oils using high performance liquid chromatography coupled with a fluorescence detector.

Materials and Methods: The sample preparation involved liquid-liquid extraction with organic solvent and low temperature clean-up. The low temperature allowed most of the lipids in the extract to be removed. Then alumina-N and NH₂ solid phase extraction cartridges were used for further clean-up.

Results: The results showed that the obtained chromatogram of extract was free of significant interferences. The PAH recoveries ranged from 81% to 114%. Limit of detection and limit of quantitation for individual PAHs were 0.09-1.97 µgkg⁻¹ and 0.29-5.99 µgkg⁻¹, respectively.

Conclusions: The performance of the present method was evaluated for determining PAHs in various types of real refined olive and refined pomace olive oil samples, and suitable results were obtained. The variable levels of PAHs were detected to range from 0.29 to 8.21 µgkg⁻¹ in real samples.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Refined olive oil, Refined pomace olive oil, Low temperature extraction, High performance liquid chromatography with fluorescence detection (HPLC/FLD)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent chemicals with two or more fused aromatic rings, and are highly stable contaminants that occur in soil, air, and food. They are a very important group of chemical carcinogens, 16 of which have been selected as priority pollutants by the United States Environmental Protection Agency (EPA) on the basis of their occurrence and carcinogenicity. The main sources for the human intake of PAHs are from the ambient air, drinking water, and food. Edible oils and fats are the largest contributory sources because of their lipophilic nature (1, 2). There are many organizations worldwide that set upper limit values for PAHs in fats and oils. For example, European

Union (No. 835/2011) has set a maximum level in fat and oils of 2 µgkg⁻¹ for benzo[a]pyrene and, and 10 µgkg⁻¹ for the sum of 4PAH, benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene (3).

The presence of polycyclic aromatic hydrocarbons (PAHs) in olive oils is caused by contamination on the skin of olives and contamination of oil during processing in the oil mill (4, 5). PAHs may also be formed directly in crude olive pomace oil as a result of drying heat process before the solvent extraction process obtains the residual oil, and consequently, higher PAH levels are usually detected in the crude oils obtained from pomace oil. This crude oil is not

*Address for correspondence: Zahra Piravivanak, Ph.D., Faculty of Food Industry and Agriculture, Standard Research Institute of Iran (ISIRI), Karaj, Iran. E-mail address: zpiravi@gmail.com

used directly for human consumption; rather it is refined to remove unwanted components and undesirable organoleptic properties. Olive oils contain high amounts of squalene ($800\text{--}8000\text{mgkg}^{-1}$). Squalene is isomerized in bleaching step, and decomposes the steroidal alcohols, yielding a great number of unsaturated hydrocarbons with cyclic moieties, which have polarities similar to those of PAHs (6-9).

Analysis of PAHs in vegetable oils is problematic owing to the complexity of the fat and oil matrix and the lipophilic nature of PAHs (10-13). Different procedures have been applied for sample preparation such as liquid-liquid extraction (14), solid-phase extraction (SPE) (15-16), solid-phase microextraction (17-19), supercritical-fluid extraction (11), and microwave-assisted extraction (20). Most of these techniques are complicated and time-consuming, and require large volumes of organic solvents. In contrast, the low-temperature clean-up method is one of the excellent techniques for eliminating fat interferences from the fat and oil samples (21-24). High performance liquid chromatography with fluorescence (25-27) or ultraviolet (UV) detection (28), gas chromatography-mass spectrometry (GC-MS) (29), and GC-MS-MS (30) are often used for the quantitative determination of PAHs in olive oils. HPLC-FLD methods are sensitive and most widely used assays (31). There are standardized methods based on the isolation of PAH fraction by column chromatography on SPE and analysis by HPLC using fluorescence detector (32-33); however, these methods are not applicable to refined olive pomace oil because of the interferences by different compounds in the HPLC analysis.

The main aim of this study was to develop and optimize a simple analytical method for quantifying PAHs in refined olive and refined pomace olive oils. The method consisted of the isolation of the hydrocarbon fraction, clean-up of PAH fraction using two SPE steps, and subsequent analysis by reverse-phase HPLC using a programmable wavelength fluorescent detector. The sample preparation was based on the extraction of PAHs from oil samples with acetonitrile/acetone solvent mixture and clean-up with alumina-N cartridge, the method described by Payanan et al. (2013). For specializing this method for

refined olive and refined pomace olive oils, the second clean-up step with NH_2 cartridge was applied (34). Then the method was validated to determine 15 PAHs that were priority pollutants by HPLC with fluorescence detection in refined olive and refined pomace olive oils. The PAHs recovery, limit of detection and limit of quantitation were also calculated. The method was applied to various brands of refined pomace olive and refined olive oil samples.

Materials and Methods

Experimental

Reagent and chemicals: The standard mixture of 16 EPA PAHs (PAH-mix 4S8743) was obtained from Sigma Aldrich (Bellefonte, PA). Hexane, acetone, acetonitrile, dichloromethane, and toluene solvents were HPLC-graded and purchased from Merck (Darmstadt, Germany). Deionized water was purified on a Milli-Q system (Millipore, Billerica, MA, USA). The stock and working standard solutions of PAHs were prepared in acetonitrile with the concentrations of $200\ \mu\text{gL}^{-1}$ and $50\ \mu\text{gL}^{-1}$, respectively. Eight standard solutions of PAHs in acetonitrile were prepared from the working standard solution for the calibration curve. They were stored at 4°C in darkness. The oil samples were bought from local supermarkets in Iran and stored at room temperature until analysis.

Instrument and analytical conditions: The HPLC equipment, a YL 9100 HPLC system, consisted of a YL 9101 vacuum degasser for the mobile phase solvents, a YL 9110 quaternary pump, a YL 9130 column compartment, and an FP-2020 plus programmable fluorescence detector co-operated with YL Clarity software program (Young Lin, Korea). A ZORBAX Eclipse ($150\ \text{mm} \times 4.6\ \text{mm i.d.}$, $5\ \mu\text{m}$ particle size; Agilent Technologies, USA) was used together with a C_{18} guard column ($10\ \text{mm} \times 2.1\ \text{mm i.d.}$) for chromatographic analyses. Injection volume was $20\ \mu\text{L}$. Separation was performed at 35°C using the gradient described in Table 1.

Table 1. Gradient elution program for HPLC separation

Time (min)	Solvent mixture A (%)	Solvent mixture B (%)
0	0	100
5	0	100
27	60	40
36	100	0
41	100	0
43	0	100
45	0	100

For the PAHs determination by fluorescence detector (FLD), the following programmed excitation and emission wavelengths (Ex/Em) were used: 270/324 nm (NPH, ACE, FL) at start, 248/375 nm (PHE, ANT) to 12.8 min, 280/462 nm (FT) to 16.8 min, 270/385 nm (PYR, BaA, CHR) to 18.1 min, 256/446 nm (BbF) to 28 min, 292/410 nm (BkF, BaP, DahA, BghiP) to 31.2 min, and 270/470 nm (IP) to 38 min. Alumina-N cartridges were Waters (Milford, MA, USA). The NH₂ cartridges 500 mg, 6 mL, LN 110924 was purchased from (Anpel, China). Additional equipment included an ultrasonic bath (Elma, Germany), an oven (Heraeus, Germany), a tabletop centrifuge (Dynamca, United Kingdom), a vortex mixer (Velp Scientifica, Italy), and a scale (Mettler Toledo, Switzerland).

Low-temperature extraction and alumina-N SPE clean-up: One gram of the oil sample was weighed into a 100 mL centrifuge tube, and 200 μ L of the working standard solution (50 μ g L⁻¹) and 8 mL of a 4:1 (v/v) solvent mixture of acetonitrile/acetone were added and shaken for 10 min. Then the solution was kept in the freezer at -18°C to -25°C for 24 h to allow the fat to precipitate. The extract was removed from the fat precipitate, and the residue was re-extracted with fresh solvent. The total extract was evaporated to dryness; the final residue was dissolved in 1 mL of 1:1 (v/v) of hexane/dichloromethane, purified by passage through alumina-N cartridge, and eluted with 10 mL of 1:1 (v/v) of hexane/dichloromethane. The resulting eluent was evaporated to the approximate volume of 0.50 mL.

NH₂ SPE cartridge clean-up: The NH₂ cartridge was conditioned by passing 30 mL of hexane under vacuum, and the concentrated solution obtained from the alumina-N cartridge was charged onto the cartridge. The column was then eluted with 25 mL of hexane/toluene (70:30), and the eluent evaporated in a rotary evaporator under vacuum just to dryness. The

residue was re-dissolved in 250 μ L of acetonitrile for HPLC-FLD analysis.

Statistical analysis: The experiments were designed by a completely randomized design. All results were the average of three separate experiments. Linear least-squares regression equations were used for calibration curves. Standard deviation of the PAH contents was calculated by the SPSS software (ver. 18.0; SPSS Inc., Chicago, IL, USA).

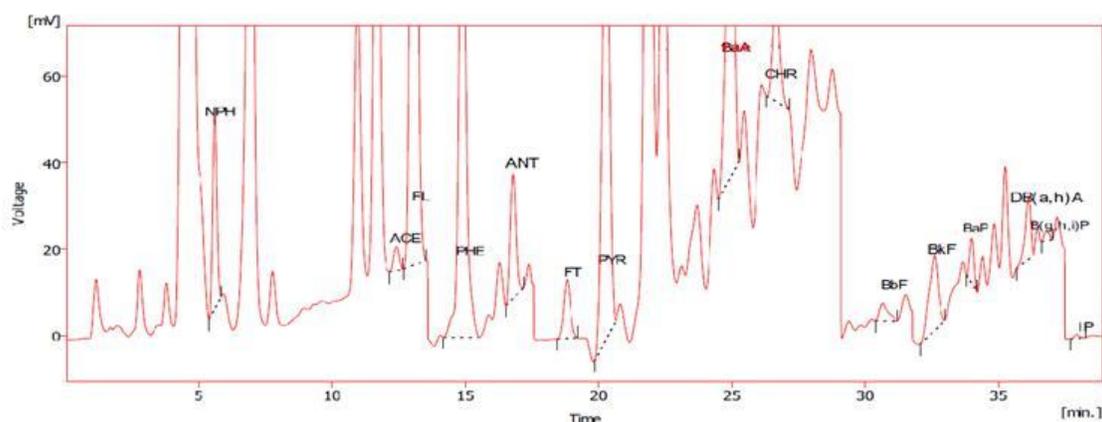
Results

Low-temperature extraction and SPE clean-up:

The extraction was typically achieved by adding an organic solvent for extraction, and subsequently, freezing the solution at -25°C. After freezing for 24 h the fat content was frozen out, and the extracted organic solvent was simply separated off. Then the oil residue in the extract was removed with the alumina-N SPE cartridge; however, huge interference peaks in the subsequent HPLC chromatograms for the detection of PAHs were obtained (Fig. 1a). Therefore, the use of NH₂ SPE cartridge was evaluated. The extract obtained was free of significant interferences (Fig. 1b), and the highest interference was removed with the NH₂ SPE cartridge.

Validation of the method: Limits of detection (LODs), limits of quantification (LOQs), linear ranges, and recoveries of PAHs were calculated under optimal conditions. The external standard calibration was chosen to quantify the analyte values for HPLC/FLD technique using eight multi-component calibration standards (0.01-90 μ g L⁻¹). The complete description of standard linearity supported by regression data is shown in Table 2. Analysis of the PAHs showed a linear relationship with high linear regression coefficients of determination for all of 15 the PAHs ($R^2 > 0.9929$). The PAH recoveries were in the range of 81–114%, and the LODs and LOQs were in the range of 0.2–3.2 μ g kg⁻¹ and 0.3–6 μ g kg⁻¹, respectively.

(a)



(b)

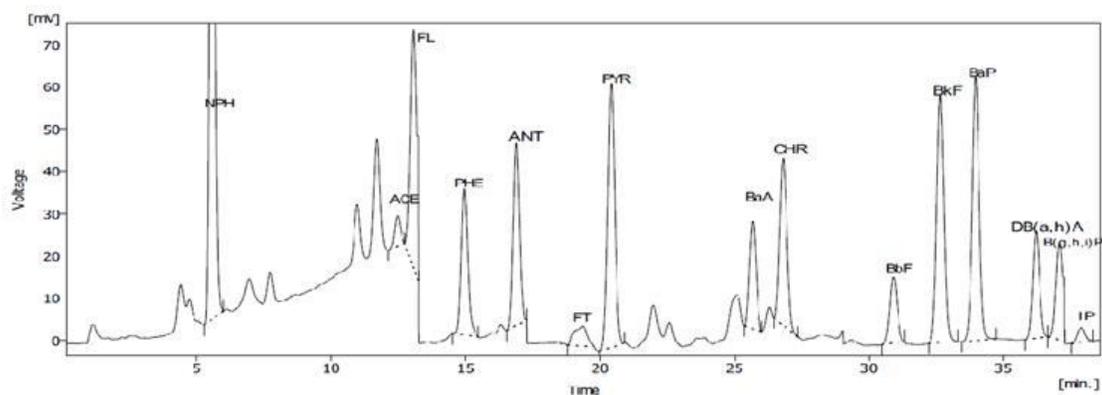


Fig 1. Chromatograms of HPLC-FLD analysis of PAHs after low temperature extraction method: (a) no NH_2 SPE cleanup and (b) followed by NH_2 SPE cleanup.

spiked PAHs solution concentration: $1 \mu\text{gL}^{-1}$ of NPH: Naphthalene; ACE: Acenaphthene; FL: Fluorine; PHE: Phenanthrene; ANT: Anthracene; FT: Fluoranthene; PYR: Pyrene; BaA: Benz[a]anthracene; CHR: Chrysene; BbF: Benzo[b]fluoranthene; BkF: Benzo[k]fluoranthene; BaP: Benzo[a]pyrene; DBaA: Dibenzo[a,h]anthracene; BghiP: Benzo[ghi]perylene and IP: Indeno[1,2,3-cd]pyrene.

Table 2. Performance criteria of method for determining PAHs; LOD, LOQ in μgkg^{-1} , instrument linearity in μgL^{-1} , and recoveries in %

PAH	Linearity		Recovery ^a ± RSD (%)	LOD (μgkg^{-1})	LOQ (μgkg^{-1})
	Range (μgL^{-1})	R ²			
NPH	0.01-30	0.9945	103±10	0.68	2.08
ACE	0.01-60	0.9997	114±6	0.29	0.88
FL	0.01-30	0.9985	113±4	0.12	0.35
PHE	0.01-60	0.9996	108±5	0.66	1.99
ANT	0.01-20	0.9995	105±3	0.11	0.35
FT	0.01-90	0.9995	85±5	1.97	5.99
PYR	0.01-30	0.9998	86±4	0.32	0.99
BaA	0.01-60	0.9988	101±7	0.16	0.48
CHR	0.01-30	0.9991	98±8	0.18	0.55
BbF	0.01-30	0.9979	84±3	0.28	0.85
BkF	0.01-12	0.9988	93±5	0.09	0.29
BaP	0.01-12	0.9964	92±4	0.09	0.29
DBaA	0.01-18	0.9989	81±6	0.15	0.46
BghiP	0.01-60	0.9989	100±7	0.59	1.80
IP	0.25-90	0.9929	86±10	0.87	2.65

^a NPH: Naphthalene; ACE: Acenaphthene; FL: Fluorine; PHE: Phenanthrene; ANT: Anthracene; FT: Fluoranthene; PYR: Pyrene; BaA: Benz[a]anthracene; CHR: Chrysene; BbF: Benzo[b]fluoranthene; BkF: Benzo[k]fluoranthene; BaP: Benzo[a]pyrene; DBaA: Dibenzo[a,h]anthracene; BghiP: Benzo[ghi]perylene and IP: Indeno[1,2,3-cd]pyrene.

^b Mean value for two levels of 5 and $10 \mu\text{gkg}^{-1}$ ± relative standard deviation (n = 3)

Table 3. Comparing the limit of detection and limit of quantitation for PAHs in vegetable oil for this work and in relation to the ones reported from other methods

Target analytes	Clean-up	Analysis method	LOQs (mg kg ⁻¹)	References
15 + 1 EU PAHs	Solid phase micro-extraction (SPME)	GC × GC TOFMS	0.4-3.7	15
16 EPA PAHs	Solid phase extraction (C18)	HPLC-FLD	0.3-6	10
16 EPA PAHs	SPE (silica gel)	GC-MS	0.3-3	42
16 EPA PAHs	Low temperature clean-up and SPE	HPLC-FLD	0.25–6.25	21
BaP	Solid phase clean-up (C18 and Florisil)	GC-MS	1	38
Heavy 8PAHs	Supercritical fluid extraction	HPLC-FLD	0.2-21	11

Determining PAHs in real samples: The proposed method was applied to the analysis of refined pomace olive and refined olive oil samples. All analyses were done at least in triplicate. The mean content of individual PAHs and the total sum of 15 PAHs content in the analyzed samples are presented in Table 4. NPH, ACE, FL and PHE were the compounds present to a greater extent. ANT, PYR, BaA, and CHR were also found at higher concentrations. None of the oil samples contained any important PAH content and exceeded the maximum limit for BaP and 4PAHs.

Table 4. PAHs contents (µg kg⁻¹) obtained in the analysis of refined olive and refined pomace olive oils by HPLC/FLD

	Mean concentrations of PAHs ± standard deviation	
	Refined pomace olive oil (n=5)	Refined olive oil (n=5)
NPH	8.21±3.23	7.25±3.00
ACE	7.53±4.05	6.10±3.05
FL	3.72±2.03	2.52±1.02
PHE	2.10±2.00	2.33±1.20
ANT	1.17±1.51	1.75±0.40
FT	<LOQ (5.99)	<LOQ (5.99)
PYR	1.74±2.50	<LOQ (0.99)
BaA	1.33±0.36	0.50±0.21
CHR	1.40±0.51	1.35±0.33
BbF	<LOQ (0.85)	<LOQ (0.85)
BkF	0.29±0.51	<LOQ (0.29)
BaP	0.30±0.35	<LOQ (0.29)
DBaA	0.58±1.10	0.70±0.30
BghiP	<LOQ (1.80)	<LOQ (1.80)
IP	<LOQ (2.65)	<LOQ (2.65)

^a NPH: Naphthalene; ACE: Acenaphthene; FL: Fluorine; PHE: Phenanthrene; ANT: Anthracene; FT: Fluoranthene; PYR: Pyrene; BaA: Benz[a]anthracene; CHR: Chrysene; BbF: Benzo[b]fluoranthene; BkF: Benzo[k]fluoranthene; BaP: Benzo[a]pyrene; DBaA: Dibenzo[a,h]anthracene; BghiP: Benzo[ghi]perylene and IP: Indeno[1,2,3-cd]pyrene. <LOQ: lower than quantification limit

Discussion

PAH extraction and clean-up: The low-temperature clean-up method is a potentially excellent technique for eliminating fat interferences from the fat and oil samples. This technique is simple, allows a high sample throughput, and utilizes a low amount of organic solvent (21-23). After this step, the highest interferences could be removed with the alumina-N SPE cartridge (4, 7, 35, 36); however, huge interference peaks still remained at the beginning (Fig. 1a). In addition, the heavy PAHs could not be reliably identified in Fig. 1a because of the presence of too many interfering peaks. These peaks were due to the interferences provided by the oil matrix and the existence of unsaturated hydrocarbons with cyclic moieties due to the squalene isomerization and steroidal alcohol decomposition in the refined pomace olive and refined olive oils (37-38). The use of NH₂ SPE cartridge allowed the non-aromatic hydrocarbons be eluted with hexane solvent but the heavy PAHs were left on the stationary phase because of the major interaction with the amino groups. Then the heavy PAHs were displaced with toluene aromatic solvent, and finally, the extract was free of significant interferences (Fig. 1b) because the highest interference was removed with the NH₂ SPE cartridge (34, 39-40).

Validation: Analysis of the PAHs showed linear relationship with high linear regression coefficients of determination for all of the 15PAHs ($R^2 > 0.9929$). The complete description of standard linearity supported by regression data is shown in Table 2. The results indicated that the developed extraction method provided reasonably good accuracy for the analysis of PAHs in the refined pomace olive and refined olive

oil samples in the tested range of concentrations (0.01-90 μgkg^{-1}).

In order to evaluate repeatability and recovery, blank samples of oils were spiked at two levels for all PAHs: 5 and 10 μgkg^{-1} . Repeatability was evaluated by performing three analyses the same day under the same conditions. The recoveries varied between 81 and 114% with 3-10 % of RSD, and were in the range set for BaP (50–120%) in Regulation 835/2011 (Table 2).

The LOD and LOQ defined as the concentration of the analyte producing the signal-to-noise ratio of 3 and 10, were obtained from the standard deviation of the blank samples ($n = 20$) and the slope of the calibration curve. The LODs and LOQs were in the range of 0.09-1.97 μgkg^{-1} and 0.29-5.99 μgkg^{-1} , respectively. The LOQs for 12 out of 15 PAHs were all below 2 μgkg^{-1} . For BaP, the LOQ (0.29 μgkg^{-1}) was lower than that of the required maximum level by the EU regulations (2 μgkg^{-1}). In addition, the LOQs of this method were broadly comparable with those reported by other researchers' techniques (Table 3).

Analysis of oil samples: The PAHs concentration in the refined pomace olive and refined olive oil samples were in the range of 0.29-8.21 μgkg^{-1} and 0.50-7.25 μgkg^{-1} , respectively, and surprisingly, the refined pomace olive oil contained high concentrations of all target PAHs. NPH, ACE, FL and PHE were the compounds present to a greater extent. ANT, PYR, BaA, and CHR were also found at higher concentrations. The rest of PAHs were detected at lower concentrations in all samples. FT, BbF, BghiP and IP in the refined pomace olive oil samples and FT, PYR, BbF, BkF, BaP, BghiP and IP in the refined olive oil samples were below the LOQ. The present and other authors have found similar results (21, 41). Mean content of the sum of 4PAHs (BaP, CHR, BaA, and BbF) in the refined pomace olive and refined olive oil samples was below 10 μgkg^{-1} . None of the oil samples contained any important PAH content and exceeded the maximum limit for BaP and 4PAHs, set by the EU regulations for oils and fats.

From the experimental results, it can be concluded that the method described by Paynan et al. (2013) modified from Moreda et al.'s (2004) work could be suitable for determining PAHs in refined olive and refined pomace olive oils. The highest interference in

the olive and refined pomace olive oil samples was removed with the NH_2 SPE cartridge. In this study, a method was developed and validated for determining 15 mutagenic and carcinogenic PAHs as well as two EPA indicators: PAHs in refined pomace olive and refined olive oils. The HPLC/FLD was selected as a powerful instrumental technique. The linearity, recoveries, LOD, LOQ, and RSD% of the developed procedure demonstrated its suitability for routine monitoring of PAHs in refined olive and refined pomace olive oils.

Financial disclosure

The authors declared no financial interest.

Funding/Support

This research was done with the financial support by the Standard Research Institute, Faculty of Food Industry and Agriculture (Project No. 91-05).

References

1. Danyi S, Bose F, Brasseur C, Schneider YJ, Larondelle Y, Pussemier L, et al. Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. *Anal Chem Acta* 2009; 633: 293–9.
2. Martorell I, Perelló G, Martí-Cid R, Castell V, Llobet JM, Domingo JL. Polycyclic aromatic hydrocarbons (PAH) in foods and estimated PAH intake by the population of Catalonia, Spain: Temporal trend. *Envir Inter* 2010; 36: 424–32.
3. European Commission EC (2011a) Commission Regulation (EU) No 835/2011 of 19 August 2011 amending regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official J Eur Union*, L215:4-8.
4. Moret S, Conte LS. Polycyclic aromatic hydrocarbons in edible fats and oils: Occurrence and analytical methods. *J Chromatogr A* 2000; 882:245-53.
5. Simko P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *J Chromatogr B* 2002; 770:2-18.
6. Lanzón A, Albi T, Cert A, Gracián J. The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *J Am Oil Chem Soc* 1994; 7: 285–91.
7. Moret S, Piani B, Bortolomeazzi R and Conte LS. HPLC determination of polycyclic aromatic hydrocarbons in

- olive oils. *Z Lebensm –Unters Forsch A* 1997; 205: 116–120.
8. Patterson HBN. Basic components and procedures, in *Bleaching and purifying fats and oils: Theory and practice*/Henry Basil Wilberforce Patterson. AOCS Press, Champaign, IL, 1992; 44–46.
 9. Le´on-Camacho M, Viera-Alcalde I, Ruiz-M´endez MV. Elimination of polycyclic aromatic hydrocarbons by bleaching of olive pomace oil. *Eur J Lipid Sci Tech* 2003; 105: 9–16.
 10. Barranco A, Alonso-Salces R M, Bakkali A, Berrueta L A, Gallo B, Vicente F, et al. Solid phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. *J Chromatogr A* 2003; 988: 33–40.
 11. Lage Yusty M A, Cortizo Daviña J L. Supercritical fluid extraction and high-performance liquid chromatography-fluorescence detection method for polycyclic aromatic hydrocarbons investigation in vegetable oil. *Food Cont* 2005; 16: 59–64
 12. Purcaro G, Moret S, Conte L S. Rapid SPE-HPLC determination of the 16 European priority polycyclic aromatic hydrocarbons in olive oils. *J Sep Sci* 2008; 31: 3936–44.
 13. Zougagha M, Redigolob H, R´ıosc A, Valc´arcea M. Screening and confirmation of PAHs in vegetable oil samples by use of supercritical fluid extraction in conjunction with liquid chromatography and fluorimetric detection. *Anal Chim Acta* 2004; 252: 265–71.
 14. Pandey M, Mishra K, Khanna S, Das M. Detection of polycyclic aromatic hydrocarbons in commonly consumed edible oils and their likely intake in the Indian population. *J Am Oil Chem Soc* 2004; 81(12): 1131–36.
 15. Purcaro G, Morrison P, Moret S, Conte L S, Marriott F J. Determination of polycyclic aromatic hydrocarbons in vegetable oils using solid-phase micro-extraction comprehensive two dimensional gas chromatography coupled with time of flight mass spectrometry. *J Chromatogr A* 2007; 1161: 284–91.
 16. Teixeira V H, Casal S, Oliveira M B. PAHs content in sunflower, soybean and virgin olive oils: Evaluation in commercial samples and during refining. *Food Chem* 2007; 104: 106–12.
 17. Purcaro G, Picardo M, Barp L, Moret S, Conte L. Direct-immersion solid-phase micro-extraction coupled to fast gas chromatography mass spectrometry as a purification step for polycyclic aromatic hydrocarbons determination in olive oil. *J Chromatogr A* 2013; 1307: 166–71.
 18. Ishizaki A, Saito K, Hanioka N, Narimatsu S, Kataoka H. Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high performance liquid chromatography fluorescence detection. *J Chromatogr A* 2010; 1217: 5555–63
 19. Plaza-Bolaños P, Frenich A G, Vidal J L M. Polycyclic aromatic hydrocarbons in food and beverages. Analytical methods and trends. *J Chromatogr A* 2010; 1217(41): 6303–26.
 20. Alarco´n F, Ba´ez M E, Bravo M, Richter P, Fuentes E. 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. *Talanta* 2012; 100: 439–46.
 21. Payanan T, Leepipatpiboon N, Varanusupakul P. Low-temperature cleanup with solid-phase extraction for the determination of polycyclic aromatic hydrocarbons in edible oils by reversed phase liquid chromatography with fluorescence detection. *Food Chem* 2013; 141: 2720–26.
 22. Chen S, Yu X, He X, Xie D, Fan Y, Peng J. Simplified pesticide multi-residues analysis in fish by low-temperature clean-up and solid-phase extraction coupled with gas chromatography/mass spectrometry. *Food Chem* 2009; 113(4): 1297–300.
 23. Goulart S M, de Queiroz M E L R, Neves A A., de Queiroz J H. Low temperature clean-up method for the determination of pyrethroids in milk using gas chromatography with electron capture detection. *Talanta* 2008; 75(5): 1320–23.
 24. Lentza-Rizos C, Avramides E J, Cherasco F. Low-temperature clean-up method for the determination of organophosphorus insecticides in olive oil. *J Chromatogr A* 2001; 912(1): 135–42.
 25. Rojo Camargo M C, Antonioli P R, Vicente E. Evaluation of polycyclic aromatic hydrocarbons content in different stages of soybean oils processing. *Food Chem* 2012; 135: 937–42.
 26. Chen Y H, Xia E Q, Xu X R, Li S, Ling W H, Wu S, et al. Evaluation of Benzo[a]pyrene in Food from China by High Performance Liquid Chromatography-Fluorescence Detection. *Int J Env Res. Public Health* 2012; 9: 4159–69
 27. Windal I, Boxus L, Hanot V. Validation of the analysis of the 15+1 European-priority polycyclic aromatic hydrocarbons by donor-acceptor complex chromatography and high-performance liquid chromatography ultraviolet/fluorescence detection. *J Chromatogr A* 2008; 1212(1–2): 16–22.

28. Dost K, Ideli C. Determination of polycyclic aromatic hydrocarbons in edible oils and barbecued food by HPLC/UV-Vis detection. *Food Chem* 2012; 133: 193-9.
29. Drabova L, Tomaniova M, Kalachova K, Kocourek V, Hajslova J, Pulkrabova J. Application of solid phase extraction and two-dimensional gas chromatography coupled with time-of-flight mass spectrometry for fast analysis of polycyclic aromatic hydrocarbons in vegetable oils. *Food Cont* 33; 2013: 489-97.
30. Veyrand B, Brosseau A, Sarcher L, Varlet V, Monteau F, Marchand P, et al. Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. *J Chromatogr A* 2007; 1149: 333-44.
31. Sekeroglu G, Fahrettin G, Sibel F. Determination of benzo(a)pyrene in vegetable oils by high performance liquid chromatography. *J Food Qual* 2007; 30: 300-308.
32. ISO, Method 15302 in international standard methods for animal and vegetable fats and oils. Determination of benzo(a)pyrene by reverse-phase high performance liquid chromatography. ISO 15302 (1987).
33. ISO, Method 15 753 in International Standard Methods for Animal and vegetable fats and oils. Determination of polycyclic aromatic hydrocarbons. ISO 15753 (2006).
34. Moreda W, Rodríguez-Acuña R, Pérez-Camino MC, Cert A. Determination of high molecular mass polycyclic aromatic hydrocarbons in refined olive pomace and other vegetable oils. *J Sci Food Agri* 2004; 84: 1759-64.
35. Barranco A, Alonso-Salces RM, Corta E, Berrueta LA, Gallo B, Vicente F, et al. Comparison of donor-acceptor and alumina columns for the clean-up of polycyclic aromatic hydrocarbons from edible oils. *Food Chem* 2004; 86(3):465-74.
36. Cert A, Moreda W, Pérez-Camino M C. Chromatographic analysis of minor constituents in vegetable oils. *J Chromatogr A* 2000; 881(1-2): 131-48.
37. Moret S, Conte L S. A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils. *J Sep Sci* 2002; 25: 96-100.
38. Bogusz M, El Hajj S A, Ehaideb Z, Hassan H, Al-Tufail M. Rapid determination of Benzo(a)pyrene in olive oil samples with solid-phase extraction and low-pressure, wide-bore gas chromatography with fluorescence detection. *J Chromatogr A* 2004; 1026: 1-7.
39. Rodríguez-Acuña R, Pérez-Camino MC, Moreda W, Cert A. Contamination source of virgin olive oils by polycyclic aromatic hydrocarbon. *Food Add Conta* 2007; 25(01):115-22.
40. Rodríguez-Acuña R, Pérez-Camino M C, Cert A, Moreda, W. Polycyclic aromatic hydrocarbons in Spanish Olive Oils: Relationship between benzo(a) pyrene and total polycyclic aromatic hydrocarbon content. *J Agri Food Chem* 2008; 56: 10428-32.
41. Wu S, Yu W. Liquid-liquid extraction of polycyclic aromatic hydrocarbons in four different edible oils from China. *Food Chemistry* 2012; 134: 597-601.
42. Fromberg A, Hojgard A, Duedahl-Olesen L. Analysis of polycyclic aromatic hydrocarbons in vegetable oils combining gel permeation chromatography with solid phase extraction cleanup. *Food Add Conta* 2007; 24: 758-67.