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Review

Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods

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Abstract

This review deals with analytical methods for polycyclic aromatic hydrocarbon (PAH) determination in oils and fats. The data reported in the introduction deal with PAH dietary intake from this group of food and contamination levels recently found by some authors in different vegetable oils, stressing the importance of establishing a method suitable for routine analyses. Traditional sample preparation relies on tedious, time-consuming procedures. They generally consist of an extraction step (liquid–liquid partition, caffeine complexation, saponification) followed by one or more purification procedures (column chromatography, thin-layer chromatography, solid-phase extraction). The analytical determination is usually carried out by HPLC and spectrofluorometric detection, or through high-resolution capillary GC coupled to flame ionisation detection or mass spectrometry. LC is a valid alternative to the traditional sample preparation, and off-line LC–LC allows performing an accurate PAH analysis in less than 2 h. Also supercritical fluid extraction, allowing performing both extraction and clean-up in one combined step, is a promising technique. Hyphenated techniques such as LC–GC and LC–LC–GC seem to be very promising. A completely on-line method for alkylated PAH determination in oils or lipidic extracts contaminated with mineral oil involves a two-dimensional LC-step with intermediate eluent evaporation and GC transfer through a vaporiser/overflow interface.

Keywords: Reviews; Oils; Fats; Sample preparation; Polynuclear aromatic hydrocarbons

Contents

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of well known carcinogenic compounds originating from incomplete combustion of organic compounds and geochemical processes. At high temperatures organic compounds are partially cracked to smaller unstable fragments (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs (pyrosynthesis) [1,2]. Aromatization may also occur at lower temperatures (100–150°C) but requires much more time and originates large amounts of alkylated PAHs. This is the case of natural fossil fuel formation, the result of organic material degradation over a period of thousands of years [3].

Due to the wide distribution of PAHs in the environment and their lipophilic nature, edible oils and fats can be heavily contaminated with these xenobiotic substances.

1.1. Sources

Different routes of PAH contamination in vegetable oils have been suggested.

The seed drying processes using direct firing for production of hot air can be responsible for major PAH contamination of some vegetable oils such as coconut and grapeseed oils [4–9]. Fortunately, refining can drastically reduce the amount of these contaminants. The deodorization process seems to have little effect on high molecular PAHs and removes mainly ''light'' PAHs (up to four aromatic rings), while higher condensed ''heavy'' PAHs are mainly removed by charcoal treatment [4,10].

Air pollution with dust and particulate containing large quantities of pyrolytically generated PAHs may contaminate the plants or the raw material via atmospheric fallout during the growing period and most of this superficial contamination can be transferred to the final product [1,2]. High PAH contents were found in Italian virgin olive oils deriving from plants exposed to industrial emission of pitch condensate {benz[*a*]pyrene (BaP) and phenanthrene, up to ca. 60 and 3800 ppb, respectively} and, to a lesser extent, in virgin oils coming from plants exposed to vehicular exhausts [11]. Also the practice to fire the field after harvesting may be a suspect operation for what concerns PAH contamination [12]. Analyses of rapeseed samples, purified in the laboratory by rinsing seeds with organic solvent in an ultrasonic bath, revealed that solid particles, which contaminate rapeseeds during harvesting, transport and storage, contributed to PAH contamination to the extent of 36% (light PAH) to 64% (heavy PAH) on average [13].

Simko et al. [14] demonstrated the possibility of vegetable oil contamination with PAHs by rediffusion from recycled polyethylene film used for oil packaging.

There are controversial results about the possibility of some vegetables to translocate PAHs from contaminated soil and water, and about biochemical synthesis [15,16].

A different form of contamination may derive by contact with mineral oil residues, rich in naturally occurring alkylated PAHs that have been generated by geochemical processes [1]. For example the practice to store and transport oil seeds in jute bags treated with mineral oils (before the spinning of the jute fibres) can cause migration of PAHs to the food [17,18]. Traces of the lubricating oils used for maintenance of extraction plants can be occasionally found in vegetable oils.

1.2. Legal limits

Although the carcinogenic properties of PAHs have been demonstrated [1,19], due to the difficulty of extrapolating toxicity data from animals to humans, it has not yet been possible to fix PAH levels which constitute a health risk [19,3].

Some European countries such as Germany, Austria and Poland adopted a legal limit of 1 ppb for BaP content in smoked foodstuff [1], but there are no legal limits for PAH content in oils and fats. Considering the appreciable BaP (and other PAH) findings for some vegetable oils and considering that smoked foodstuffs represent only a small proportion of the average diet, while oils and fats are far more important, the lack of a legal limit for the latter appears as a contradiction. In spite of this situation the German Society for Fat Science have fixed the following limits: 25 ppb for total PAHs and 5 ppb for the heavy fraction [6,9,24].

1.3. Occurrence

As reported by several authors [20–23], fats and oils represent one of the major sources of PAHs in the diet because of their lipophilic nature.

Dennis et al. [5,20] revealed that 80% of the total dietary intake of BaP in the UK, often used as an indicator of overall PAH contamination, came from two food groups: cereals and oils/fats. High BaP contents in retail vegetable oils (on average 1.29 ppb) and lower amounts in retail fish and animal-derived oils and fats, such as butter (on average 0.06 ppb of BaP), were found. Margarine resulted to be the major dietary source of PAH in the oil and fat total diet group, accounting for 70% of BaP intake from these commodities. High concentrations of BaP, up to 2.2 ppb, were detected in cereal-derived products containing high levels of edible oils, such as pudding-based desserts, biscuits and cakes, and in infant formulae.

Larsson et al. [4] found in crude coconut oils amounts of BaP and total PAHs ranging from 20 to 34 ppb and from 2600 to 3700 ppb, respectively. However, the corresponding refined product showed considerably lower PAH contents (0.1–0.3 for BaP and 2–59 ppb for total PAHs).

Gertz and Kogelheide [6] found, in products coming from the Italian and French markets, mean content for light and heavy PAHs of 114.7 and 94.7 ppb, respectively. According to Balenovic et al. [7] grapeseed oils displayed heavy PAH levels (on average 108 ppb) 15 times higher on average than other vegetable oils analysed. Light PAH contents were, on average, only twice as high, compared to the average value of the other oils.

Balenovic et al. [7] tested a large number of vegetable-origin oil samples for PAHs. Almost all the oils analysed showed total PAHs above 25 ppb, due to the large amount of light PAHs making them unfit for human consumption. Also among the 11 different kinds of vegetable oil analysed by Gertz and Kogelheide [6], most of the unrefined ones contained more than 25 ppb of total PAHs. With the exception of grapeseed samples, containing high levels of PAHs, other refined products only occasionally exceeded the 25-ppb limit. As reported by Speer et al. [24], virgin olive oil displayed total PAH levels always exceeding the 25-ppb limit (54.4–110.8 ppb). In comparison to other oils, which showed similar contamination by ''heavy'' PAHs, the high amount of ''light'' was remarkable (53–105.6 ppb) due to the fact that extra virgin olive oil is unrefined. Also Moret et al. [25] found total PAH concentrations exceeding 25 ppb for most of the 51 virgin olive oils analysed. Only one sample contained more than 5 ppb for the sum of heavy PAHs and more than 1 ppb for BaP. Among the five olive oils analysed, four had a heavy PAH content higher than 5 ppb. As remarked by some authors [7] the limit of 25 ppb for total PAH seems unrealistic, especially if dealing with unrefined products (extra virgin olive oil), while a limit of 5 ppb for heavy PAHs and 1 ppb for BaP is recommended.

2. Traditional methods for polycyclic aromatic hydrocarbon analysis

2.1. Extraction

This preparative step aims at extracting PAHs from the rest of lipids, which are dissolved by the same solvent as PAHs, as selectively as possible.

The three procedures mostly used for this purpose are: liquid–liquid partition following the scheme reported by Grimmer and Böhnke, caffeine complexation and saponification.

With the partition method the oil sample is dissolved in an organic solvent such as cyclohexane (CH) and PAHs are extracted with a solution of dimethylformamide (DMF)–water (9:1) [22,24,26–28] or dimethyl sulfoxide (DMSO) [29,30], while most of the lipidic matter (mainly constituted of triglycerides) remains in the organic phase. Isolation is performed by dilution with water, in order to change the coefficients of partition of the PAHs between the two phases, and back-partition into CH, which may be more easily evaporated (at lower temperature). This procedure allows reducing the mass of the residue to a 10% of the initial value.

Some authors [12,31,32] described a relatively rapid method for determining PAHs in fats and oils, utilising the phenomenon of caffeine–PAH complex formation. The sample is dissolved in CH and the PAHs are selectively extracted by means of vigorous shaking in a caffeine–formic acid solution. After

decomposing the complex, with an aqueous sodium chloride solution (2%), the PAHs are extracted with CH.

The saponification method allows to reach a considerable concentration of the PAH fraction (from 10 g of oil we can obtain 100–200 mg of unsaponifiable matter). The oil is saponified under reflux during at least 40 min with alcoholic KOH and unsaponifiable matter is extracted with CH [6,7,33,34]. Some authors have suggested that the harsh alkaline digestive treatment could have an effect on the more labile PAHs in the sample [35,36].

Moret et al. [37] compared the three extraction procedures above described (with minor modifications) on an olive oil sample. Sample purification was performed by solid-phase extraction (SPE) clean-up and analytical determination by HPLC and spectrofluorometric detection. The three methods (saponification, caffeine complexation and liquid–liquid partition with CH and DMF–water) resulted to be not equivalent for the degree of purification obtained and the capacity to get a squalene free fraction. Squalene is contained in olive oils at about 50% of unsaponifiable matter and represents a problem because it co-elutes with PAHs from the cartridge, and is able to trap them, especially the heavier ones. The saponification method gave appreciable squalene residue, meaning that an additional purification step is needed, while the liquid–liquid partition method showed a higher purification power, as no appreciable amount of squalene remained in the samples. Liquid–liquid partition followed by SPE clean-up resulted to be the best method. Recoveries ranged between 42.5 and 92.2%, depending on molecular masses while RSDs varied from 2.5 to 12% except for naphthalene (Na) (31.9%) probably due to its volatility.

2.2. Purification

The extract obtained with one of the methods above described inevitably contains substantial amounts of material other than PAH which may interfere with the following analytical determination. In relation to the degree of purification needed, depending also on the selectivity of the final determination, different clean-up procedures, such as thin-layer chromatography (TLC) and column chromatography on different adsorbent materials, are traditionally applied [2].

Most of these procedures rely on tedious steps on columns packed with different material such as silica gel, alumina, Florisil and Sephadex LH-20. Among these, silica gel columns (deactivated with 15% of water) used alone or in combination with other materials, mainly Sephadex LH-20, are widely used. After loading the sample, the column is eluted with CH; a first fraction containing the more apolar compounds (alkanes) is discharged while the PAH fraction is collected leaving the coloured substances and more polar compounds in the column [38]. Preparation and use of these columns is very time-consuming, requires large volumes of solvents and give not always reproducible results.

Some authors [37] evaluated successfully the possibility of using SPE silica cartridges instead of the packed silica columns in the purification step. Data obtained applying the two purification procedures on the same fortified oil sample produced similar results, although, in many cases, recoveries and RSDs were a little better for SPE purification. Moreover, SPE cartridges require lower amounts of solvent and shorter analysis time.

Swetman et al. [9] employed silica cartridges for sample preparation of coconut oil: 30 mg of the oil dissolved in hexane were loaded on a 500-mg silica SPE cartridge and PAHs eluted with a mixture of dichloromethane–hexane (30:70).

2.3. Analytical determination

Early semi-quantitative procedures based on paper chromatography (PC) and TLC, followed by ultraviolet (UV) detection or fluorescence spectroscopy, have been almost completely replaced by modern high resolution (HR) GC and high-performance liquid chromatography (HPLC) techniques.

Capillary GC is widely used for separating PAHs. The high resolving power of capillary columns and the possibility to use mass spectrometry (MS) in the single ion monitoring (SIM) mode allows simultaneous detection of known and unknown PAHs at extremely low concentrations. Weak polar stationary phases such as OV-1701 provided better separation than the traditionally recommended SE-52 and SE-54. Among injection techniques, the ones most often

utilised are splitless and cold on-column injection, the latter being the most precise for PAH quantification [39].

Capillary GC is the best technique to use with samples contaminated with mineral oil [40]. PAH fractions derived from mineral oil are much more complex than those obtained from combustion sources. They are very rich in alkylated species and, even with the high resolving power of capillary GC, a detailed compositional analysis is far from being possible. The approach is towards group-type analysis [2]. GC–MS cannot be used for identifying the thousands of unresolved components, and flame ionization detection (FID) represents, in this case, the only way of quantitating hydrocarbons of unknown identity [41].

When parent non-substitute PAHs predominate, HPLC is the preferable technique. HPLC is somewhat faster than HRGC and, even though it offers lower resolution efficiency in separating low-molecular mass PAHs, reversed-phase (RP) columns can readily separate a number of PAHs isomers that are difficult to separate by GC [27,42]. The most widely used columns are the RP ones (C_{18}) employing acetonitrile–water or methanol–water mobile phase and gradient elution.

As a lot of PAHs exhibit strong fluorescence, HPLC, coupled to a spectrofluorometric detector, represents the most powerful technique for what concerns sensitivity and selectivity (comparable only to that obtained with MS-SIM detection). HPLC technique has also the advantage to allow detection of high-molecular mass PAHs which cannot be detected by GC methods because of the thermal decomposition occurring at high temperatures [39].

A schematic description of the traditional methods used by some authors for PAH determination in oils and fat samples is reported in Table 1.

3. Alternative methods for sample preparation

Stijve and Diserens [43] used an alternative method for the isolation of PAHs from large test portions of fats and oils. A 30 g sample of lipid material was adsorbed on synthetic calcium silicate (Calflo E) and

Table 1
Traditional methods for PAH analysis in edible fats and oils[a]

Sample extraction	Sample purification	Analytical determination	Ref.
Liquid–liquid partition with DMF–water and CE	Silica gel column + Sephadex LH-20 column	GC–MS	[26]
Liquid–liquid partition with DMF–water and CE	Silica gel column + GPC on S-X3 column	GC–MS	[24]
Liquid–liquid partition with DMSO and CE	Silica gel column + TLC on silica gel	GC–FID	[15,16]
Liquid–liquid partition with DMF–water and CE	Silica gel column	GC–MS (SIM) HPLC–FL	[4,22] [28]
Liquid–liquid partition with DMF–water and CE	SPE on silica gel cartridge (500 mg)	HPLC–SF	[25]
Caffeine complexation and extraction with CE	Silica gel column + TLC on Al_2O_3–Al sheet	UV-spectroscopy fluorescence measurem.	[31,12]
Caffeine complexation and extraction with CE	Silica gel column+HPTLC	GC–FID	[32]
Saponification and extraction with CE	Liquid–liquid partition + silica gel column	HPLC–SF HPLC–FL	[6] [34]
Saponification and extraction with CE	Silica gel column	HPLC–SF TLC, HPLC–FL, GC–MS	[33] [7]<

[a] CE=cyclohexane; DMF=dimethylformamide; DMSO=dimethylsulfoxide; FL=fluorometric detection; SF=spectrofluorometric detection.

extracted with a mixture of acetonitrile–acetone (9:1). The evaporated extract contained only small amounts of co-extracted fat and can be used for PAH analysis with current methods. PAH recoveries ranged from 72 to 87%.

A new approach to quantitative isolation of organic chemicals, such as PAHs, polychlorinated biphenyls (PCBs) and organochlorine pesticides, from lipid samples using a semi-permeable membrane was recently reported [44]. The method is non-destructive and more than 20 g of lipid can be dialysed in a single membrane. The lipid content can be reduced by 90–99%, depending on species and amount of lipid. Neither triglycerides nor phospholipids were obtained in the dialysate fraction.

The potential of supercritical fluid extraction (SFE) as an alternative to the classic solvent-based extraction and clean-up methods is slowly being recognised. SFE is often favoured for its ability to perform selective extractions and therefore renders any further sample preparation superfluous. As known, the ability to perform selective extraction and clean-up with SFE strongly depends on the range of the polarity of the analytes. Supercritical CO_2 solubilizes not only contaminants but also lipids. Recently, some authors [45] developed a selective SFE extraction method that allows GC–MS quantification of PAHs in the presence of a substantial lipid background. The method is based on the addition of C_{18} absorbent beads to the initial sample slurry, which is placed in the SFE chamber. During SFE, lipids are preferentially retained on the C_{18} beads. Using this approach on model lipid spiked with PAHs, recoveries ranged between 94 and 100% while only 9–17% of the lipid material present was co-extracted from the same test sample.

4. Liquid chromatography for off-line sample preparation

Perrin et al. [46] realised sample preparation by donor acceptor complex chromatography (DACC) with a tetrachlorophtalimidopropyl (TPCI) modified silica.

Molecular complexes are formed by weak interactions (dipole–dipole, dipole–induced dipole and dispersion forces) between the electron transfers from the donor components to the acceptor. The straight of the complexes formed between PAHs (electron-donors) and the acceptor phase increases with the number of aromatic rings. Triglycerides and tocopherols together with light PAHs were eluted with a mixture of hexane and methyl *tert*.-butyl ether (MTBE), while heavy PAHs were eluted with a stronger eluent (methylene chloride). The fraction so obtained was evaporated and analysed by RP-HPLC in combination with spectrofluorometric detection. PAH recoveries were determined on a rapeseed oil spiked with different amounts of PAHs. At a level of 10-ppb, recoveries ranged between 106 and 121% (RSDs 4–27%), while at the 1-ppb level they varied from 78 to 100% (RSDs 13–30%).

Untreated silica columns can also be advantageously used for LC pre-separation of triglycerides from the hydrocarburic fraction. The capacity of a silica column to retain fat (for columns of the same particle size) depends on column size, mobile phase composition, as well as type and by-products (free acids and polymerised material) of the fat injected [41,47].

Moret et al. [48,49] described a fast off-line LC–LC method, employing a large silica column, for PAH isolation from edible oils and fats. Using an optimised mobile phase (pentane with 5% of dichloromethane), this large silica column (250×4.6 mm I.D., 5 μm particle size) was able to retain up to 100 mg of fat and other interferents, allowing the polyaromatic fraction (from Na to IP) to elute in a few millilitres. After PAH elution the column needed to be backflushed with 10 ml of dichloromethane (filled from a pressurised reservoir). The entire sample preparation step can be automated by using an additional backflush valve and a programmable switching valve box. By switching the backflush valve, the mobile phase from the pumps pushes the dichloromethane into the rear end of the LC column thereby causing triglycerides and all remaining oil sample constituents to elute via a waste outlet. Of course it is also possible to wash the column by simply turning it upside-down. Once collected and concentrated, the PAH fraction was injected into a RP column for PAH quantitation by spectrofluorometric detection.

Fig. 1 shows the spectrofluorometric trace obtained applying the entire procedure to extra virgin

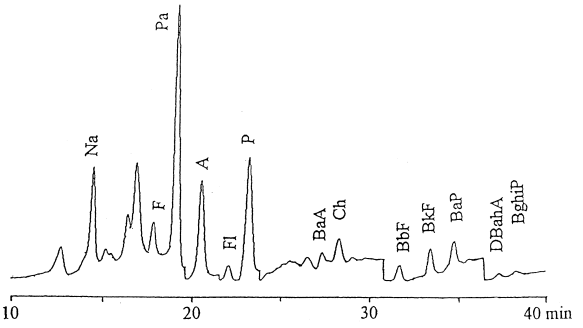

Fig. 1. Spectrofluorometric trace of an extra virgin olive oil analysed with the off-line LC–LC method (from Ref [49]).

olive oil. The HPLC chromatogram appears clean and sensitivity is adequate to detect PAHs at the level at which they are generally present in oil samples. Also in the case of fat extracted from smoked fish samples, the degree of purification achieved with LC sample preparation is comparable to that obtained with traditional procedures such as liquid–liquid partition followed by SPE clean-up. The repeatability of the whole method was tested on extra virgin olive oil. RSDs ranged from 2.3 to 10.8%, with the exception of Na (21.5%), presenting a higher value related to its volatility. Recoveries, calculated on a peanut oil sample spiked with amounts of PAHs ranging from 4 to 40 ppb for each compound, varied from 37.6 to 102.1% depending on molecular masses, but were quantitative practically starting from Pa. The lower recoveries registered for the light PAHs are due to the evaporation step.

The possibility to perform this method by on-line LC–LC, by using an on-line solvent evaporator (SE) able to couple a silica column with a reversed-phase one (C_{18}), is now under investigation. As reported later, a similar SE has already been used to couple two normal-phase LC columns [50].

5. Hyphenated techniques

An on-line LC–GC–MS technique was developed by Vreuls et al. [51] for PAH determination in vegetable oils. A 100×2 mm silica column was used for sample pre-treatment. Up to 2 mg of oil were injected in the LC system. After the fraction of interest had been eluted, triglycerides were removed by backflushing the column with 1 ml of MTBE contained in a loop mounted on the backflush valve and filled from a pressurised reservoir. By using pentane with 2% of MTBE as mobile phase, the PAH fraction was separated from the alkane fraction and eluted in less than 300 μl. The appropriate LC fraction containing PAHs was transferred to the GC column (22 m×0.32 mm I.D. coated with 0.17 μm methylsilicone) using a loop-type interface. This method allows for direct analysis of oil samples after simple dilution with pentane to prevent overloading of the LC column with triglycerides. According to the authors, detection limits for various PAHs were about 20 ppb in the full scan mode and about 0.5 ppb with selective ion monitoring.

Moret et al. realised a completely on-line LC–LC–GC method for the analysis of alkylated PAHs in edible oil and fat extract contaminated with mineral oil residues [42,43]. This method involved a first LC pre-separation on a large silica column (250×4.6 mm I.D.) able to retain large amounts of fat, on-line evaporation of a 6 ml fraction (containing PAHs from naphthalene to perilene), PAH fractioning on an amino column and GC transfer through a vaporizer/overflow interface.

The SE (prototype from Fison/CE Instrument) consisted of two thermostatted (40°C) aluminum blocks containing a notch into which a steel capillary tubing (1/16 in. O.D. and 1 mm I.D; 1 in.=2.54 cm.) packed with silica gel (35–70 mesh) and deactivated by mild silylation was fitted (vaporising chamber). The SE worked on the principles of concurrent evaporation and overflow: the solvent being evaporated during its introduction and vapours being discharged by their expansion and vapour pressure. Vacuum was applied to the SE outlet in order to keep the evaporation temperature increasing the retentive power of the vaporising chamber low [50].

When the PAH fraction starts to be eluted, the solvent is fed into the vaporising chamber where the analytes are retained. At the end of the transfer, the silica column is backflushed to remove any residual fat and the analytes are transferred by a second LC pump into the amino column for analysis of aromatics according to the number of rings (group type analysis). Fig. 2 shows an application of the method to non-refined linseed oil contaminated with about 300 mg/kg of mineral oil material. The composition

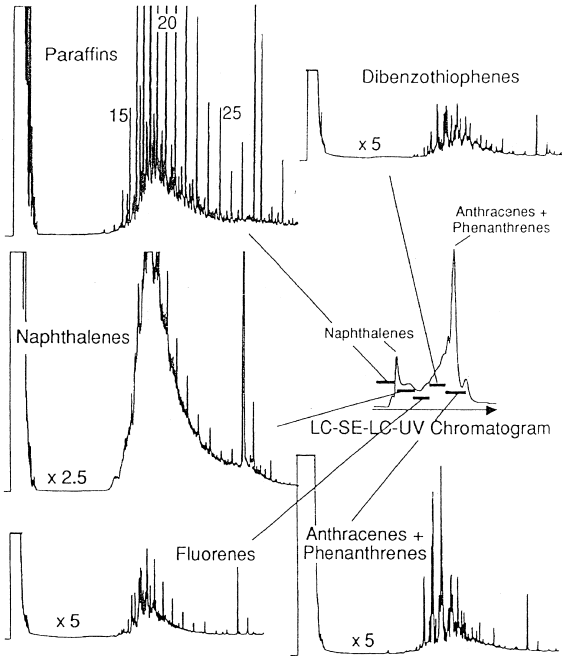

Fig. 2. LC–SE–LC–UV and –GC–FID traces of alkylated PAHs (derived from mineral oil contamination) in non-refined linseed oil (from Ref. [42]).

of the aromatics corresponds to a fraction of crude mineral oil similar to the batching oil used for jute fibres: the linseeds could have been transported and stored in jute bags [42].

6. Conclusions

Sample preparation for PAH analysis, if carried out with traditional procedures, is inadequate for rapid determinations of PAH content in edible fats and oils. Extraction generally involves liquid–liquid extraction, often the major cause of poor profile reproducibility, especially with emulsion-forming samples. Moreover purification steps are time-consuming procedures. Large volumes of solvent are also involved in traditional sample preparation, so that, in order to achieve a reasonably low detection limit, it is necessary to concentrate the final extract to a very small volume. Of course, this operation results in a concentration of potentially interfering impurities.

These disadvantages can be overcome by performing sample preparation with a simple LC step, aiming at separating PAHs from triglycerides and other interferents prior to their analytical determination.

The recent development of the so-called hyphenated techniques (LC–GC, LC–LC–GC), has introduced new possibilities for the PAH analysis. Hyphenated techniques drastically reduce analysis time, volume of solvents consumed and sample manipulation, and are also suitable for routine analyses.

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