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Original Research Article

Application of QuEChERS-EMR-Lipid-DLLME method for the determination of polycyclic aromatic hydrocarbons in smoked food of animal origin

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ABSTRACT

The aim of this study was to develop an effective sample preparation procedure for the determination of polycyclic aromatic hydrocarbons (PAHs) in smoked fatty products of animal origin (fish, cheese and sausage). Two different approaches were tested: classical QuEChERS and procedure with the use of Enhanced Matrix Removal (EMR)-Lipid material. Two techniques of extract preconcentration: under nitrogen stream and with the use of dispersive liquid-liquid microextraction (DLLME), were also taken into consideration. All samples were analysed using gas chromatography-mass spectrometry. The results showed the optimised sample preparation procedure was composed of three steps: 1) QuEChERS extraction, 2) clean-up by EMR-Lipid material and 3) extract preconcentration by DLLME. The obtained recovery rates within the range of 50–120% were received for all compounds with relative standard deviation (RSD) values lower than 16.7%. The proposed method is fast and effective and can be successfully applied for PAHs determination in difficult matrices such as heat-treated food of animal origin with high fat content. The research also discovered the significance of the quality of the laboratory disposables. Contaminants present in plastic consumables can be transferred to the sample extract contributing to its contamination and can also lead to failure of analytical equipment.

1. Introduction

Smoking is one of the oldest food preserving technologies. It has been used by mankind for over 10,000 years. It is believed that man would hang his catch over the fire as a protection against canines and subsequently the preserving effect of smoke was probably discovered (Šimko, 2002, 2009). The first evidence of smoking as a technological process dates back 90,000 years to Poland where the oldest smoking house was discovered by archaeologists in a Stone Age colony located in Zwierzyniec, near Krakow (Möhler, 1978). Ever since, smoking started to be widely used not only for special organoleptic profiles of smoked products, but also for the inactivating effect of smoke (and heat) on enzymes and microorganisms (Essumang et al., 2010; Šimko, 2002). Smoking is usually used for preservation of fish and its products as well as meat and meat products. Apart from that, other foods can also be subjected to smoke treatment e.g. cheeses or even fruits (Suchanová et al., 2008; Fasano et al., 2016; Surma et al., 2018). According to Stołyhwo and Sikorski (2005) in Europe about 15 % of the total quantity of fish for human consumption is offered on the market in the form of either cold- or hot-smoked products. Currently, we suppose, the technology is mainly used to enrich the foods with its specific taste, odour, and appearance, as there is a high demand for it on the market (Šimko, 2005; Hui et al., 2001; Essumang et al., 2013). It is assumed that the technology is today applied in many forms to treat 40–60 % of meat products (Sikorski, 2004) and 15 % of fish (Stołyhwo and Sikorski, 2005).

The preservation effect is generally attributed to antioxidant and antimicrobial properties of phenolic compounds contained in smoke. The rate of deposition of different components depends on temperature, humidity, flow rate, and density of the smoke, water solubility and volatility of particular compounds, as well as shelf life, and wholesomeness of the product (Borgstrom, 2012; Stołyhwo and Sikorski, 2005). Generation of wood and charcoal smoke during curing is a typical example of incomplete combustion, and it is known that polycyclic aromatic hydrocarbons (PAHs) are generated and released, and, in consequence, due to the contact of food with smoke and high temperature of this process, PAHs are transferred to smoked food. PAHs are a large group of hydrophobic organic compounds, containing two or

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more aromatic rings. The compounds containing five or more aromatic rings are known as 'heavy' PAHs, whereas those containing less than five rings are named 'light' PAHs. Both kinds of PAHs are non-polar compounds, showing high lipophilic nature, although heavy PAHs are more stable and toxic than the other group (Raters and Matissek, 2014). PAHs originate mainly from environmental sources (natural and anthropogenic) and food processing (e.g. heating, drying, smoking, grilling, roasting and frying) (Singh et al., 2016). PAHs show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo and are classified by International Agency for Research on Cancer (IARC) in both groups 2A and 2B and benzo(a)pyrene in group 1 (carcinogenic to humans) (IARC, International Agency for Research on Cancer, 2014). In European Union, as PAHs indicator in food sum of four of them (Σ4 PAHs) including benzo(a)anthracene, chrysene, benzo (b)fluoranthene, and benzo(a)pyrene has been designated (European Commission, 2011a). So far, maximum levels (MLs) were established only for several groups of smoked food, including smoked fish and smoked fishery products as well as smoked meat and smoked meat products. However, for other smoked products such as traditional smoked cheeses from east and central Europe, no MLs have been set until this date.

One of the main challenges in the determination of PAHs in smoked food of animal origin is their high fat content (e.g. lipids, triglycerides and fatty acids) and the extraction of PAHs from these complex matrices is usually laborious and often not effective enough. Fat residues in analysed extracts can contribute to the deterioration of chromatographic system (especially GC) but also can suppress signal of analytes. Therefore, there is a constant need of search for effective techniques for fat removal from smoked fatty samples.

The most common approach for the determination of PAHs in fatty foods involves saponification of lipids by methanolic or ethanolic KOH or NaOH solution followed by the isolation of the PAHs by liquid-liquid extraction (LLE) with cyclohexane, hexane, dichloromethane or its mixtures. Obtained extracts are then cleaned up using gel-permeation chromatography (GPC), solid phase extraction (SPE) or adsorption chromatography with the use of silica or Florisil sorbents. For the detection and quantification of PAHs, gas chromatography with mass spectrometry (GC-MS) and high-performance liquid chromatography with fluorometric detection (HPLC-FLD) are usually used (Silva et al., 2017; Slámová et al., 2017; Urban and Lesueur, 2017; Zachara et al., 2017).

The QuEChERS (quick, easy, cheap, effective, rugged, safe) method is another concept that can be applied for the PAHs determination in fatty food samples. It is characterized by short extraction and purification times, as well as low solvent consumption. In clean-up step mainly PSA (primary secondary amine), C_{18} (octadecyl), and Z-Sep (zirconium dioxide-based) sorbents are used for the fat removal, but also an implementation of freezing out has been reported in the literature (Rejczak and Tuzimski, 2015; Sadowska-Rociek et al., 2016; Kim et al., 2019). However, in the case of food with higher fat content, even these modifications might be insufficient, to achieve adequate sample cleanup and in consequence matrix co-extractives can affect analyte signals and even destroy the elements of analytical equipment (Lucas and Zhao, 2015). Additionally, these sorbents can exhibit non-selective interactions with analytes providing the loss of analysed compounds (Lucas and Zhao, 2015; Rejczak and Tuzimski, 2015).

Recently, a new material "enhanced matrix removal" (EMR-Lipid) has been proposed for the fat removal from fat-rich food products. The structure of EMR-Lipid is a proprietary secret, and it does not function as a conventional sorbent, but it dissolves to saturation in sample extract solution, and its mechanism is said to involve both size exclusion and hydrophobic interactions. Long-chain hydrocarbons associated with lipids fit within the EMR-Lipid structure, where they are trapped. The EMR-Lipid complex is either precipitated out of solution or remains in the aqueous phase during the final salting-out step (Lucas and Zhao, 2015; Han et al., 2016). The manufacturer claims that EMR-Lipid selectively removes lipids from QuEChERS extracts without loss of analytes (Huang et al., 2019).

Depending on the final determination method, the low levels of PAHs sometimes require application of an extract preconcentration step, such as e.g. evaporation in a stream of nitrogen and dissolution of the residues in a small volume of solvent that will then be injected to chromatographic system. However, in case of lighter PAHs, the stream of gas can lead to the loss of analytes. An alternative to this operation is the direct transfer of analytes from the extract into a small volume of another non-miscible solvent. This approach is used in dispersive liquid-liquid microextraction (DLLME) method that is based on the system of three solvents: aqueous sample, dispersive solvent and extraction solvent. The mixture of an extraction solvent (e.g. chloroform) and a dispersive solvent (water-organic miscible solvent, e.g. acetonitrile) is rapidly injected into an aqueous sample, forming a cloudy solution. After centrifugation, the analytes are preconcentrated into the phase of extraction solvent (Viñas et al., 2014; Kamankesh et al., 2015). Until now, DLLME has demonstrated promising results in extract preconcentration without any loss of analytes, also in the case of the determination of PAHs in food samples (Sadowska-Rociek et al., 2015; Petrarca and Godoy, 2018).

Therefore, the aim of this study was to develop an effective sample preparation procedure for the determination of PAHs in smoked fatty products of animal origin. Two different approaches were employed: 1) classical QuEChERS with PSA and C_{18} sorbents 2) procedure with the use of EMR-Lipid according to the manufacturer. We have also compared two different methods of extract preconcentration: under nitrogen stream and with the use of DLLME method. All samples were analysed using gas chromatography-mass spectrometry. Finally, some findings resulting from the use of plastic laboratory consumables have been also discussed.

2. Materials and methods

2.1. Chemicals and reagents

Polycyclic aromatic hydrocarbons suitable for EPA Method 610, anthracene-d_{10} (Internal Standard 1; IS1), chrysene-d_{12} (Internal Standard 2; IS2), hexachlorobenzene (Syringe Standard; SS) were obtained from Sigma-Aldrich, Saint Louis, Missouri, USA. Magnesium sulphate anhydrous p.a. and sodium chloride p.a. were purchased from Krakchemia SA, Krakow, Poland. Acetonitrile, chloroform and hexane, were purchased from Merck KGaA, Darmstadt, Germany. PSA, C_{18}, SPE Bulk Sorbents and EMR-Lipid material derived from Agilent Technologies, Santa Clara, California, USA. Deionised water (18 MΩ) was produced by a Milli-Q system (Millipore, Burlington, Massachusetts, USA). Stock, intermediate and working standard solutions of PAHs, chrysene-d_{12}, and anthracene-d_{10} (all at the concentration of 1 μg mL^{-1}) were prepared in hexane. Calibration standards of PAHs at the concentrations ranged from 2 to 400 ng mL^{-1} were prepared by diluting the standard mixture solution to the corresponding hexane volume. All reagents were at least of analytical purity.

2.2. Instrumentation

Analyses were carried out on a Varian 4000 GC-MS (Agilent Technologies, Santa Clara, California, USA) system consisted of 3800 gas chromatograph with a DB-5MS column (30 m x0.25 mm x0.25 μm; Agilent Technologies, Santa Clara, California, USA) and 4000 Ion Trap MS detector (Agilent Technologies, Santa Clara, California, USA). The GC oven was operated with the following temperature program: initial temperature 50 °C (1 min) – 15 °C min^{-1} – 300 °C (6.0 min) for PAHs. Helium 5.0 (Linde Group, Munich, Germany) was used as the GC carrier gas at a flow rate of 1.0 mL min^{-1}. The auto sampling injector was CP-1177 Split/Splitless Capillary Injector, with a temperature of 270 °C and with the volume of 1.0 μL for all standards and samples. Each injection

Table 1
Parameters of GC-MS analysis of examined compounds.

R_t [min]	Compound	Quantification ion	Confirmation ions
7.726	NaP	128.3	128.2, 102.2, 127.5
8.816	MeNaP2	142.3	142.2, 141.4, 115.3
8.975	MeNaP1	141.4	141.2, 142.2, 115.2
10.255	Acp	152.1	151.1, 151.3, 153.1
10.537	Ace	153.3	153.2, 154.2, 152.4
11.378	Flu	166.1	164.1, 165.1, 165.3
12.216	HCB	282.8	248.9, 284.1; 286.0
12.926	Phen	178.1	166.1, 178.2, 179.1
12.978	Ant-d_{10} (IS1)	188.0	188.1, 177.9, 189.2
13.011	Ant	178.1	165.1, 178.2, 179.1
14.852	Fla	202.5	202.4, 200.6, 201.7
15.217	Pyr	202.1	200.1, 202.3, 203.1
17.156	B[a]a	228.1	226.1, 228.3, 229.1
17.169	Chr-d_{12} (IS2)	240.1	240.2, 239.2, 241.2
17.21	Chr	228.1	226.1, 228.3, 229.1
18.985	B[b]f	252.1	250.1, 253.1, 253.3
19.039	B[k]f	252.1	250.1, 250.4, 253.1
19.645	B[a]p	252.1	250.1, 250.3, 253.2
22.516	I[cd]p	276.1	274.1, 277.1, 277.5
22.631	D[ah]a	278.2	276.0, 276.5, 279.1
23.359	B[ghi]P	276.0	274.1, 276.4, 277.0

R_t – retention time; NaP – naphthalene; MeNaP2 –2-methylnaphthalene; MeNaP1 –1-methylnaphthalene; Ace – acenaphthene; Acp – acenaphthylene; Flu – fluorene; HCB – hexachlorobenzene (syringe standard); Phen – phenanthrene; Ant-d_{10} – anthracene-d_{10} (internal standard); Ant – anthracene; Fla – Fluoranthene; Pyr – pyrene; B[a]a – benzo[*a*]anthracene; Chr-d_{12} – chrysene-d_{12} (internal standard); Chr – chrysene; B[b]f – benzo[*b*]fluoranthene; B[k]f – benzo[*k*]fluoranthene; B[a]p – benzo[*a*]pyrene; I[cd]p – indeno[1,2,3-*c*,*d*] pyrene; D[ah]a – dibenzo[*a*,*h*]anthracene; B[ghi]P –.benzo[*g*,*h*,*i*]perylene.

was repeated three times. The ion trap mass spectrometer was operated on the internal ionisation mode, scan from *m/z* 45 to 500 in full scan mode, used for the evaluation of the quality of sample extracts. Quantitative analyses were conducted in the selected ion monitoring mode (SIM mode) and analysed compounds were identified according to their ions and retention times (Table 1). The trap and the transfer line temperatures were set at 200 and 270 °C, respectively. The emission current of the ionisation filament was set at 15 μA. Acquisition and processing data were performed using Varian Star Workstation software and NIST 2.0 library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

2.3. Extraction and clean-up the sample

In the experiment, the samples of smoked mackerel obtained from the local market were used for the preparation of blank and spiked samples. Recovery studies in each case involved the samples being spiked at the level of 100 μg kg^{-1} with the PAH standard and internal standards solutions (anthracene-d_{10} and chrysene-d_{12}, both also at the level of 100 μg kg^{-1}). At the preparation phase before using the different proposed schemes. Blank samples and reagent blanks were prepared similarly to the fortified samples.

The tested procedures were presented in the Fig. 1. The experiment was based on the comparison of two different concepts of analyte extraction from the samples and its clean up: 1) classical QuEChERS, using freezing out, clean-up step with PSA and C_{18} 2) the protocol involving an implementation of EMR-Lipid according to its manufacturer (Lucas and Zhao, 2015).

The second part of the research included the selection of the best method of the final extract preconcentration: 1) by evaporation to dryness under the stream of nitrogen and dissolution of the residues in a small volume of hexane or 2) the use of DLLME method; the choice of the solvents and its volumes were based on the previous results, according to the procedure developed and optimised recently (Sadowska-Rociek et al., 2015; Surma et al., 2018). To summarize, four different variants combining different analyte extractions and final extract preconcentration were prepared, analysed and subsequently evaluated based on analyte recoveries and the quality of obtained chromatograms to develop effective sample preparation procedure. In all tested variants, the recovery values were calculated after the final preconcentration step, involving all conducted sample preparation stages.

3. Results and discussion

3.1. Comparison of different methods of sample preparation

Fig. 2 shows the comparison of PAHs recoveries obtained for the tested variants ("QuEChERS" – 1, "EMR-Lipid" – 2, in the combination with evaporation to dryness using the nitrogen stream). Generally, the recovery values within the acceptable range (50–120%, according to EU recommendation) were obtained only for QuEChERS method, but with the exception for NaP and MeNaP1, for which the recovery was below 50%. For two other compounds, Ant and B[a]a, the results of the recovery were exceptionally high (120% and 1.9%, respectively), although they were still in the acceptable range. This supposed the impact of the sample preparation method. Indeed, when analysing the chromatograms shown in Fig. 3, it was found that the procedure with the application of freezing out and the use of conventional QuEChERS sorbents (Fig. 3A), such as PSA and C_{18} did not provide sufficient removal of matrix co-extractives from the sample. Additionally, these undesirable matrix residues influenced on the analytes, which can be seen in the Fig. 3D, leading in consequence to suppression or enhancement of recoveries, which was mentioned previously. On contrary, in the second tested variant, in which EMR Lipid was incorporated for clean-up step, obtained chromatogram (Fig. 3B) was free from any undesirable compounds; however, the received recoveries were below 50% for almost all compounds, and Ace and Flu were not detected at all.

Regarding the sample preparation procedure there are two significant steps influencing the yield of PAHs recovery: extraction and clean-up step. The PAHs extraction in classical QuEChERS method is performed with acetonitrile (MeCN), usually in the presence of water, which releases the matrix components and improves the transfer of analytes into the solvent (Rejczak and Tuzimski, 2015). In the second investigated procedure (with EMR-Lipid application), no water was used during extraction step, as has been suggested by the manufacturer. Therefore, in this case, the lack of water can be a possible explanation for low PAHs recoveries. However, loss of compounds can be as well as result of the use of impropriate materials in clean up step, which can retain the analytes. Until now, the use of EMR-Lipid material for the PAHs determination in food with high fat content has been reported in the studies conducted by Lucas and Zhao (2015); Han et al. (2016) and as well as by Urban and Lesueur (2017) who, however, did not noticed any PAHs loss when EMR-Lipid was applied, even when the extraction was performed without addition of water. Nevertheless, it should be emphasised that aforementioned experiments were carried out using an untreated raw food samples, which, although contained a high level of fat, had not been previously smoked and therefore contained higher level of water, which probably resulted in better recovery values. Hence, it was concluded that in the case of smoked food with a low water level the sample preparation should be based on acetonitrile-water extraction of PAHs followed by clean up step with the application of EMR-Lipid material (variant 3 – "combined QuEChERS + EMR-Lipid") in Fig. 1). Indeed, the results confirming this hypothesis and the successful removal of matrix co-extractives were provided, but the recovery values for certain compounds were still below the acceptable limits (Fig. 2).

Therefore, in order to investigate the potential cause of loss of PAHs and to improve the recovery rate of the compounds, we decided to include DLLME, as an alternative technique of extract preconcentration before the GC-MS analysis, instead of conventional evaporation to

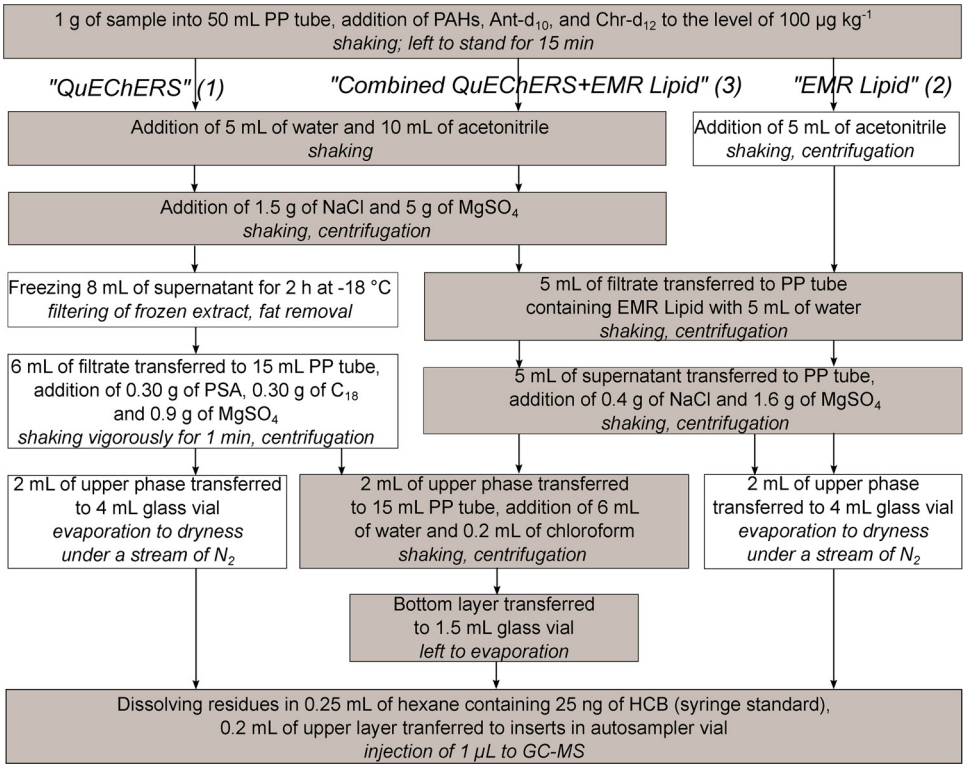

Fig. 1. Schema of the sample preparation process. PP – polypropylene, Ant-d_{10} – anthracene-d_{10}, Chr-d_{12} – chrysene-d_{12}.

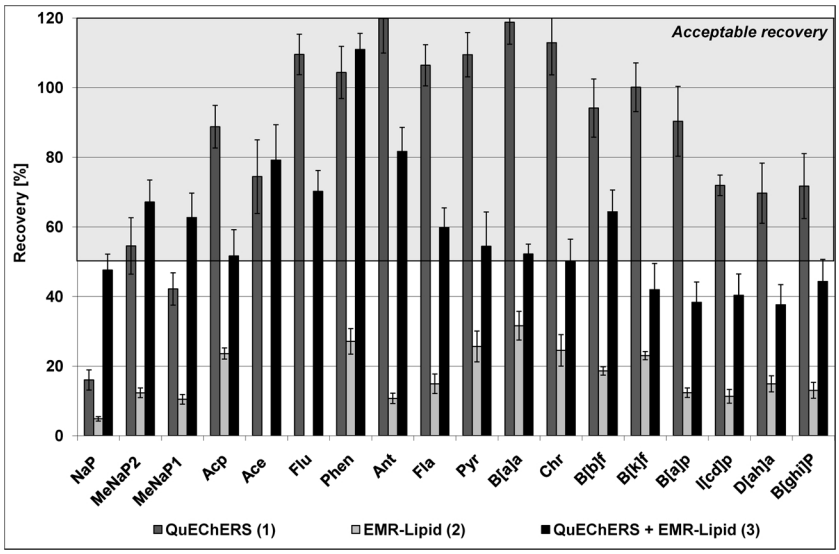

Fig. 2. Comparison of the PAH recoveries obtained in QuEChERS (1), EMR-Lipid (2) and combined method QuEChERS + EMR-Lipid (3) for smoked mackerel.

dryness by stream of nitrogen (see Fig. 1). As expected, the application of DLLME contributed to the increase in the recovery values, especially for two of the lightest compounds: Nap and MeNap1 (Fig. 4). This phenomenon can be explained by the implementation of chloroform, which can easily evaporate even without the incorporation of stream of nitrogen, comparing to acetonitrile that requires a longer time of evaporation process due to higher boiling point. Additionally, the use of stream of nitrogen in the latter case might lead to the partially loss of light PAHs. DLLME method was also used in QuEChERS and EMR-Lipid variant, but it did not improve the quality of the sample clean up (in the case of QuEChERS method), and, in the case of EMR-Lipid variant, the implementation of DLLME did not influence significantly on the PAHs recoveries, although the values were slightly higher when compared to the variant with the evaporation with nitrogen (data not shown in this study due to the lack of sufficient importance). This also suggests that the use of EMR-Lipid material without an effective extraction based on MeCN + H_2O, even if a DLLME preconcentration step is included in the procedure, does not contribute to an appropriate analyte recovery.

To summarise, the final version of optimised sample preparation procedure composed of three steps (Fig. 1, marked in grey colour): 1) QuEChERS extraction using water, acetonitrile followed by addition of NaCl and $MgSO_4$, 2) clean-up by EMR-Lipid material and 3) extract preconcentration by DLLME. This combination provided not only acceptable recovery data but also a satisfactory clean-up of the extract, which is shown in Fig. 3C.

This protocol was also tested for other smoked fatty matrices, such

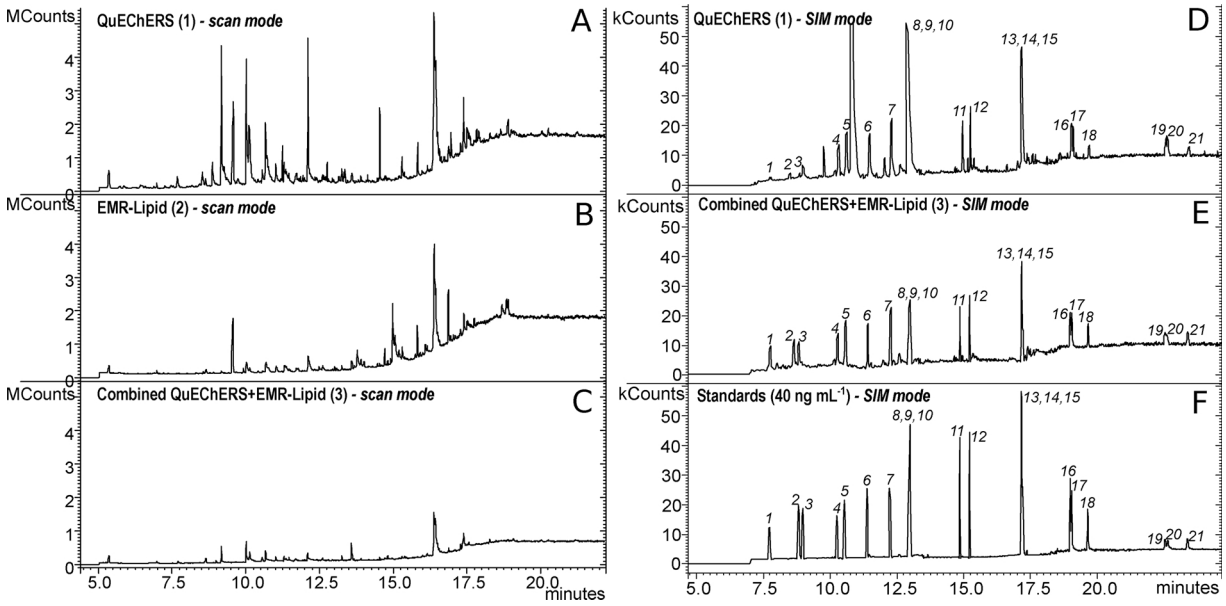

Fig. 3. Comparison of GC-MS chromatograms (smoked mackarel) for three tested analytical procedures. A – QuEChERS (1), full scan mode; B – EMR-Lipid (2), full scan mode; C – Combination QuEChERS + EMR-Lipid (3), full scan mode; D - QuEChERS (1), SIM mode: 1 – naphthalene; 2 –2-methylnaphthalene; 3 –1-methylnaphthalene; 4 – acenaphthene; 5 – acenaphthylene; 6 – fluorene; 7 – hexachlorobenzene (syringe standard); 8 – phenanthrene; 9 – anthracene-d_{10} (internal standard); 10 – anthracene; 11 – fluoranthene; 12 – pyrene; 13 – benzo[*a*]anthracene; 14 – chrysene-d_{12} (internal standard); 15 – chrysene; 16 – benzo[*b*]fluoranthene; 17 – benzo[*k*]fluoranthene; 18 – benzo[*a*]pyrene; 19 – indeno[1,2,3-*c*,*d*]pyrene; 20 – dibenzo[*a*,*h*]anthracene; 21 – benzo[*g*,*h*,*i*]perylene; E - Combination QuEChERS + EMR-Lipid (3), SIM mode; F – Standards, SIM mode.

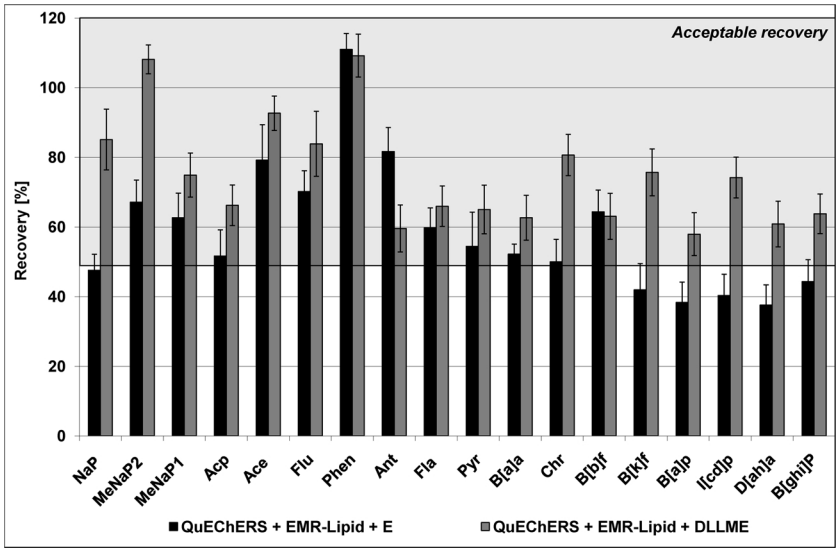

Fig. 4. Comparison of the PAH recoveries obtained by evaporation (E) and dispersive liquid-liquid microextraction (DLLME) in the combination QuEChERS + EMR-Lipid method (smoked mackarel).

as smoked cheese and smoked sausage. In each case the recovery rates within the range of 50–120% were obtained for all compounds (Fig. 5).

3.2. Method performance

The developed analytical procedure was subjected to in-house validation process that involved method linearity, limit of detection, limit of quantification, inter and intra-day precision, and accuracy according to the criteria established by the Commission Regulation 836/2011 (Commission Regulation 2011b). The calculations were conducted for the spiked samples of smoked fish, sausage and cheese, at the levels of 20 and 100 μg kg^{-1}. The results of the validation process are presented in Table 2 (to maintain the clarity of the table, the results are presented as the ranges of the values, obtained for all tested matrices) and Fig. 3E shows the chromatogram of the sample of smoked fish, spiked at the level of 100 μg kg^{-1}, analysed in SIM mode.

To sum up, linearity of the method was calculated based on the series of standard solutions in the range 2–400 ng mL^{-1}. The chromatogram of the PAH standards at the level of 40 μg mL^{-1} are presented in Fig. 3F. The received values of correlation coefficient (r) were higher than 0.99 for all compounds and matrices. Limit of detection (LOD) and limit of quantification (LOQ) were estimated on the basis of the signal of the background noise measured from the standard chromatograms at the lowest calibration level. The limit of detection was calculated as three times higher than the level of noise (S/N = 3), and the limit of quantification was equal to three times of the detection limit (LOQ = 3LOD). LOQs were lower than 0.9 μg kg^{-1} that is in accordance with the values established by EU (according to Commission Regulation 836/

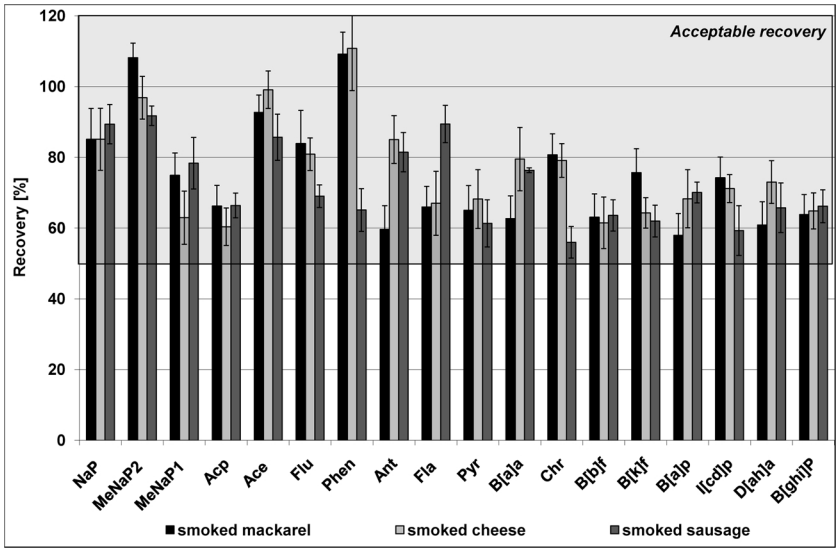

Fig. 5. Recovery values for all tested matrices (smoked mackerel, smoked cheese and smoked sausage) obtained at the level of 100 μg kg^{-1} by the final version of the procedure.

2011, LOQ should not exceed the level of 0.9 μg kg^{-1}).

The repeatability expressed as a relative standard deviation (RSD_r) was calculated from six spiking samples analysed on the same day whereas reproducibility (RSD_R) involved preparation and analysis from three different days. Received consistent deviations for all matrices were below 11.9% and 16.7%, respectively, with HORRAT values (calculated based on RSD_r and RSD_R, according to Horwitz equation; European Commission, 2011b) lower 2 for each of the compounds which was in good agreement with EU criteria.

The method accuracy was determined by the recovery using spiked samples, at two spiking levels. All results were found within acceptable limits and ranged from 55% to 103% for 20 μg/kg and 56–111% for 100 μg/kg (Figs. 5 and 6).

3.3. Contamination of extracts with residues from tubes

Regardless the fat and other co-extractives in smoked food samples, presence of certain contaminants that can be transferred from laboratory consumables into the sample is another issue that should be considered during sample preparation. Currently, most analytical procedures are based on the use of disposable plastic materials, such as polyethylene, polystyrene, polypropylene and others. Polypropylene tubes are also recommended for use in QuEChERS method, as well as in the protocol with the application of EMR-Lipid method. Additionally, manufacturers often sell ready-to-use sorbent or salt kits, already placed in plastic tubes.

In this method mentioned materials were also used. During the study, however, we observed huge peaks of oleonitrile and oleamide, which appeared on the chromatograms of almost all samples, including also blank reagent samples (Fig. 7) and they were not present only in the standard solutions used to prepare the calibration curve. The oleonitrile peak was not very high, but its retention time was close to the retention times of Pyr and Fla, which, in consequence could lead to the alteration of the analytes signal. In case of oleamide, although its

Table 2
Parameters of in-house validation study of target PAHs for all tested matrices.

Compound	Calibration slope	Correlation coefficient, r	Repeatability* (RSD_r, n = 6) [%]	Reproducibility* (RSD_R, n = 6) [%]	Recovery* (level 20 μg kg^{-1}) [%]	Recovery* (level 100 μg kg^{-1}) [%]	LOQ [μg kg^{-1}]
NaP	220	0.9938	0.62-4.61	5.56-8.77	85-91	85-89	0.42
MeNaP2	153	0.9916	1.40-6.33	2.76-16.7	89-98	92-108	0.39
MeNaP1	143	0.9903	2.38-7.01	5.30-11.5	61-93	63-78	0.39
Acp	232	0.9975	1.59-7.55	3.49-9.28	69-71	60-66	0.38
Ace	157	0.9963	2.01-11.9	6.50-12.3	78-99	86-93	0.55
Flu	169	0.9986	2.36-9.58	3.18-10.6	74-82	69-84	0.47
Phen	225	0.9956	3.67-9.58	6.04-11.9	75-103	65-111	0.37
Ant	254	0.9940	4.49-6.39	5.55-14.7	63-93	60-81	0.49
Fla	289	0.9944	2.80-5.67	5.26-13.6	69-99	66-89	0.61
Pyr	341	0.9917	4.42-9.85	6.68-13.3	59-81	61-68	0.70
B[a]a	209	0.9936	4.12-9.87	4.67-10.2	59-84	63-80	0.82
Chr	282	0.9988	4.54-6.46	4.45-12.7	64-72	56-81	0.85
B[b]f	321	0.9930	1.20-6.25	4.41-8.38	67-69	61-64	0.76
B[k]f	410	0.9972	1.17-7.53	4.48-8.31	53-67	62-76	0.68
B[a]p	317	0.9924	1.36-5.84	2.95-8.19	63-75	58-70	0.67
I[cd]p	313	0.9982	1.98-6.10	7.03-13.9	59-72	69-74	0.89
D[ah]a	307	0.9990	2.31-10.7	6.99-12.0	67-76	61-73	0.81
B[ghi]P	393	0.9983	2.28-9.32	4.65-15.8	59-84	64-66	0.89

NaP – naphthalene; MeNaP2 – 2-methylnaphthalene; MeNaP1 – 1-methylnaphthalene; Ace – acenaphthene; Acp – acenaphthylene; Flu – fluorene; Phen – phenanthrene; Ant – anthracene; Fla – fluoranthene; Pyr – pyrene; B[a]a – benzo[*a*]anthracene; Chr – chrysene; B[b]f – benzo[*b*]fluoranthene; B[k]f – benzo[*k*]fluoranthene; B[a]p – benzo[*a*]pyrene; I[cd]p – indeno[1,2,3-*c*,*d*]pyrene; D[ah]a – dibenzo[*a*,*h*]anthracene; B[ghi]P –.benzo[*g*,*h*,*i*]perylene.

* The results are presented as the ranges of the values, obtained for all tested matrices.

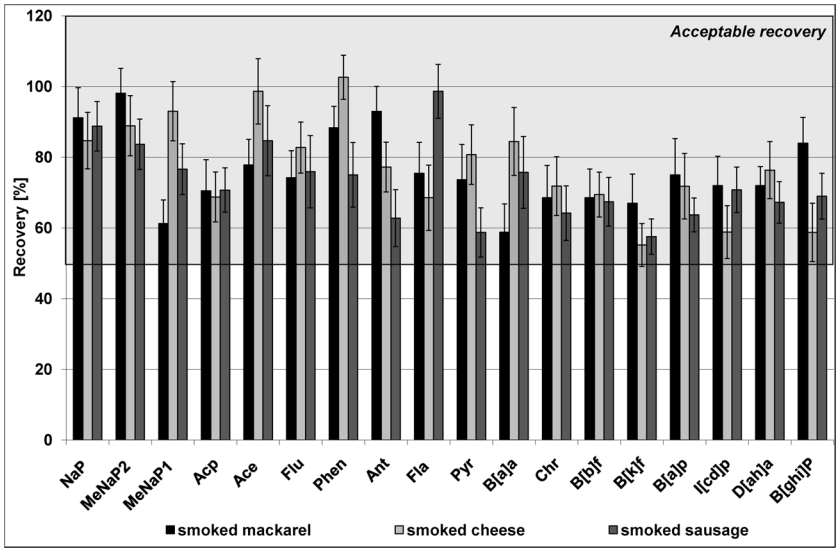

Fig. 6. Recovery values for all tested matrices (smoked mackerel, smoked cheese and smoked sausage) obtained at the level of 20 μg kg^{-1} by the final version of the procedure.

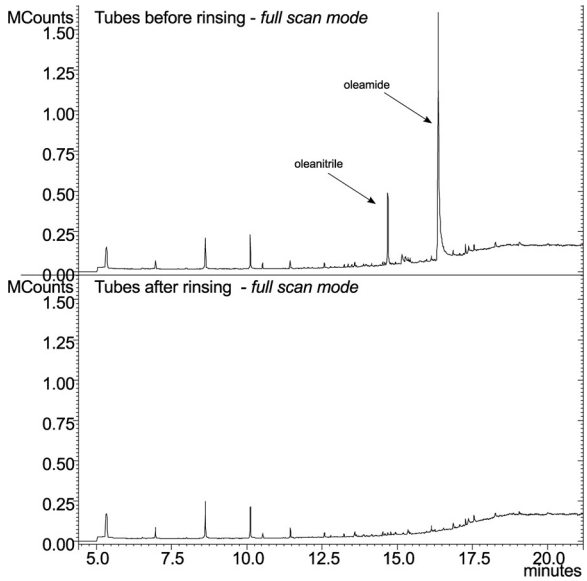

Fig. 7. Comparison of GC-MS chromatogram for originally new tubes and tubes after rinsing.

retention time and ions did not interfere with any of the PAHs, the signal was too high in view of the condition of ion trap used in this study. Additionally, we observed that oleamide had the tendency to retain on the chromatographic column after the analysis. Assuming that routine analyses of PAHs occurrence in food usually involve a lot of samples, the presence of such impurity in each analysed extract could significantly shorten the life of ion trap filaments as well as GC column.

Detailed investigation showed that the compounds did not come from the examined food samples but could be transferred from the reagents or disposable equipment used in the experiment. We discovered that they were washed out from polypropylene tubes (both 50 mL and 15 mL), even if we used a completely new products directly from manufacturer. Hence, it was necessary to implement an appropriate procedure of rinsing the tubes prior its use for sample preparation. Different solvents (acetone, acetonitrile, hexane, and ethanol) were tested to remove oleamide and oleanitrile. The tubes were filled with the solvent and placed in ultrasonic bath for 10 min. After that the solvent was removed, the tubes were dried and then used in the preparation of blank reagent samples. Consequently, it was decided to use hot ethanol as the most efficient solvent that washed out the contaminants. Fig. 6 demonstrates the chromatogram of blank reagent sample prepared in tubes rinsed previously in hot ethanol, which turned out to be the most effective solvent in removing oleamide and oleanitrile.

4. Conclusions

The experiment conducted in this study revealed that the combination of QuEChERS extraction method with clean-up step by EMR-Lipid and DLLME technique as an extract preconcentration resulted in successfully purified samples providing in the same time acceptable recoveries of PAHs in smoked fatty products. Although the proposed method is composed of several steps, it is fast and effective and can be successfully applied for PAHs determination in difficult matrices such as heat-treated food of animal origin with high fat content.

The research also discovered the significance of the quality of the laboratory disposables. Contaminants present in plastic consumables can be transferred to the sample extract contributing to its contamination and also can lead to failure of analytical equipment (chromatographs, spectrometers).

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Tereza Slámová: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Anna Sadowska-Rociek:** Methodology, Formal analysis, Validation, Investigation, Writing - original draft, Writing - review & editing. **Adéla Fraňková:** Conceptualization, Formal analysis, Investigation. **Magdalena Surma:** Formal analysis, Validation, Writing - review & editing. **Jan Banout:** Conceptualization, Supervision.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

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