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Green detection of polycyclic aromatic hydrocarbons in heat-processed meat products by HPLC-MS/MS using deep eutectic solvent-assisted DLLME combined with magnetic nanomaterials

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ABSTRACT: This study presents a green and efficient method for the rapid detection of polycyclic aromatic hydrocarbons (PAHs) in heat-processed meats. The method combines deep eutectic solvents (DES) with magnetic nanomaterials (Fe_3O_4@C_{18}) to perform dispersive liquid-liquid microextraction (DLLME), followed by HPLC-MS/MS determination of 16 priority PAHs. Under optimized conditions, the method showed excellent linearity ($R^2 \geqslant 0.99$) over the range of 0.1–50 ng/ml, with limits of detection between 0.01 and 0.06 ng/ml and recoveries of 80.9–102.4% (RSD < 8%). The developed method exhibited high sensitivity and accuracy, and was successfully applied to commercial meat products, where elevated levels of PAHs were detected in grilled lamb skewers and cured pork. The approach offers environmental sustainability, operational simplicity, and strong applicability for monitoring and risk assessment of PAHs in complex food matrices.

KEYWORDS: polycyclic aromatic hydrocarbons, deep eutectic solvents, magnetic nanomaterials, DLLME, food safety

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic, persistent organic pollutants ubiquitously distributed in the environment. Composed of two or more fused aromatic rings, PAHs are mainly generated through the incomplete combustion of organic matter and thermal degradation of lipids during food processing [1–3]. Owing to their carcinogenic, mutagenic, and teratogenic potential, PAHs are classified as priority contaminants by the U.S. Environmental Protection Agency (USEPA) and the European Food Safety Authority (EFSA) [4–6]. High-toxicity compounds, such as benzo[a]pyrene (BaP), are strictly regulated in thermally processed meats, fried foods, and infant formula products [7, 8].

Accurate detection of trace-level PAHs in complex food matrices remains a major challenge in food safety and analytical chemistry. Conventional sample preparation techniques, such as liquid-liquid extraction (LLE) [9], solid-phase extraction (SPE) [10], and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [11], are widely employed. However, these methods present notable limitations, including tedious procedures, high solvent consumption, and limited sensitivity and selectivity. Dispersive liquid-liquid microextraction (DLLME) [12, 13] has emerged as a promising alternative method. This technique offers several advantages, including rapid operation, ease of use, and high efficiency in analyte enrichment. However, traditional DLLME systems often rely on halogenated solvents and centrifugation, limiting their environmental sustainability and hindering automation.

Deep Eutectic Solvents (DESs) are a class of green solvents that have attracted considerable interest in analytical chemistry due to their favorable properties, such as low toxicity, biodegradability, and tunable solvation capacity [14, 15]. DESs are typically formed by mixing a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), yielding eutectic mixtures that remain liquid at relatively low temperatures. These solvents have demonstrated strong solubilization ability for a wide range of organic compounds, including PAHs, making them ideal for sample preparation and extraction in environmental and food safety analyses [16, 17]. For instance, a choline chloride-proline-based natural deep eutectic solvent (NADES) has been successfully applied for the green extraction of bioactive compounds from coffee husk waste, highlighting its strong solubilization ability and environmental compatibility [18]. Recent studies have highlighted the ability of DESs to replace conventional solvents in various applications, offering a more sustainable and environmentally friendly alternative [19–21].

In parallel, the development of magnetic nanomaterials, such as Fe_3O_4-based nanoparticles, has created new opportunities for simplifying sample preparation. In particular, Fe_3O_4@C_{18} nanoparticles exhibit strong hydrophobic interactions with PAHs and can be rapidly separated using an external magnetic field, eliminating the need for centrifugation and enhancing compatibility with automated workflows [22]. While previous studies have explored the application of DESs or mag-

netic nanomaterials in water and plant matrices, few systematic reports have examined their combined use in lipid-rich food products such as processed meats, where the complex fat content presents significant analytical challenges.

Taken together, these considerations highlight a critical research gap: although DESs and magnetic nanomaterials have been separately reported for PAH analysis, their integration into a single method for high-fat food matrices remains limited. This study presents a novel method that integrates DES-assisted DLLME with Fe_3O_4@C_{18} magnetic nanoparticles and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for determining 16 priority PAHs in thermally processed meats. The method combines environmental safety, simplicity, and high enrichment efficiency. Following systematic optimization and validation, the method was successfully applied to commercial samples of cured pork, sausage, and grilled meats. The results not only demonstrate the method's practical value but also highlight its novelty in extending DES-magnetic nanomaterial-based microextraction strategies to complex, high-fat food matrices.

MATERIALS AND METHODS

Instruments and reagents

Quantitative analysis of PAHs was performed using a HPLC system coupled with a triple quadrupole mass spectrometer (HPLC-MS/MS; 1290/6470B, Agilent Technologies, USA). Phase separation during sample pretreatment was carried out with a centrifuge (ThermoFisher Scientific, USA). Sample and standard solutions were stored in a low-temperature freezer (Haier, China). Ultrasonic-assisted extraction was conducted using an ultrasonic cleaner (Shanghai Ultrasonic Instrument Co., China). Nitrogen evaporation was performed using a nitrogen blow concentrator (Pierce, USA).

Organic solvents including methanol, acetonitrile, n-hexane, ethyl acetate, and acetone (all ⩾ 99.9% purity, chromatography grade) were purchased from Merck (Germany). Ultrapure water used throughout the experiments was obtained from a Milli-Q system (Millipore, USA). The green extraction solvent—DES—was prepared by heating and stirring a 1:2 molar mixture of choline chloride (⩾ 98%, Sigma-Aldrich, USA) and lactic acid (⩾ 90%, Sinopharm Chemical Reagent Co., China) at 80 °C for 30 min until a clear, homogeneous liquid was formed. The resulting DES was cooled and stored at room temperature. Magnetic nanoparticles Fe_3O_4@C_{18} (average particle size ∼50 nm) were obtained from Odyssey Chemical Technology Co. (Beijing, China). These particles were surface-modified with C_{18} alkyl chains to enhance hydrophobicity and magnetic responsiveness. They were employed to facilitate rapid magnetic separation and enrichment of PAHs following extraction. Fe_3O_4@C_{18} was chosen over commonly used Fe_3O_4 or Fe_3O_4@SiO_2 because the C_{18} modification provides stronger hydrophobic interactions with PAHs, resulting in higher extraction efficiency in complex food matrices.

A total of 16 PAHs, regulated by the European Union (EU), were selected as target analytes: benzo[c]fluorene (BcL), benzo[j]fluoranthene (BjF), benzo[ghi]perylene (BgP), benzo[a]anthracene (BaA), cyclopenta[cd]pyrene (CPP), indeno[1,2,3-cd]pyrene (IcP), chrysene (Chr), benzo[a]pyrene (BaP), 5-methylchrysene (5MC), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), dibenz[a,h]anthracene (DhA), dibenz[a,i]pyrene (DiP), dibenz[a,e]pyrene (DeP), dibenz[a,l]pyrene (DIP), and dibenz[a,h]pyrene (DhP). All standards were purchased from Dr. Ehrenstorfer GmbH (Germany) at a concentration of 500 mg/l and stored at −20 °C in the dark after dilution with methanol.

Sample collection and preparation

A total of 40 batches of commercially available meat products were collected, comprising 10 batches each of cured pork, sausage, grilled chicken wings, and grilled lamb skewers. All samples were stored at −18 °C prior to analysis. Before processing, samples were thawed at room temperature and cut into pieces with a maximum particle size of 0.5 mm. For subsequent analysis, 0.5000 g (±0.0001 g) of each homogenized sample was accurately weighed.

Preparation of standard solutions

Sixteen PAHs were used as standard compounds. Each stock solution (1.00 ml) was diluted with methanol to 10 mg/l, and 500 µl of each diluted solution was combined to prepare a mixed stock solution, which was stored in amber volumetric flasks at −20 °C and under light-protected conditions. Working standard solutions, ranging from 0.1 to 50 ng/ml, were freshly prepared by serial dilution of the stock solution with methanol. These were used to construct calibration curves and perform quantitative analysis of samples.

To evaluate the accuracy and recovery efficiency of the developed method using DES and magnetic nanoparticles (Fe_3O_4@C_{18}), blank meat samples—confirmed by preliminary screening to contain no detectable PAHs—were spiked at low, medium, and high concentration levels (1, 10, and 50 ng/g). These spiked samples underwent the full sample preparation procedure. Recovery experiments were conducted to assess the method's accuracy, precision, and matrix applicability.

Preparation of DES

DES was prepared by mixing choline chloride (13.95 g, 0.10 mol) with lactic acid (18.02 g, 0.20 mol), mixed at a molar ratio of 1:2. The mixture was stirred at 80 °C for 30 min using a thermostatic magnetic stirrer

until a clear and homogeneous liquid was obtained. The resulting solvent was cooled and stored at room temperature until use.

Sample treatment procedure

Approximately 5.0 g of homogenized meat sample (accurate to 0.0001 g) was transferred into a 50 ml polypropylene centrifuge tube. A volume of 200 µl DES (choline chloride:lactic acid = 1:2) was added as the green extraction solvent, followed by 400 µl methanol as the dispersant. The mixture was vortexed and then subjected to ultrasonic extraction at 60 °C for 30 min.

Subsequently, 30 mg of surface-modified Fe_3O_4@C_{18} magnetic nanoparticles were added, and the solution was shaken for 5 min to promote analyte enrichment. An external neodymium magnet (diameter 40 mm, magnetic strength ⩾ 4000 Gs) was then applied to attract the nanoparticles to the tube wall, enabling rapid magnetic separation. The supernatant was collected, filtered through a 0.22 µm microporous membrane, and analyzed using HPLC-MS/MS. A schematic workflow of the sample preparation procedure is presented in Fig. 1.

Liquid chromatography conditions

Chromatographic separation was achieved on a PAH-specific Eclipse column (2.1 mm × 100 mm, 1.8 µm; Agilent Technologies). The column temperature was maintained at 40 °C. Mobile phase A consisted of 0.05% (v/v) aqueous acetic acid, and mobile phase B was acetonitrile containing 5% methanol. The flow rate was set to 0.3 ml/min, with an injection volume of 2 µl. A gradient elution program was applied as follows: 0–5 min: 60% A and 40% B; 5–8 min: A decreased linearly from 60% to 45%, while B increased from 40% to 55%; 8–11 min: A and B isocratic at 45% and 55%, respectively; 11–15 min: A decreased from 45% to 40%, and B increased from 55% to 60%; A representative chromatogram is presented in Fig. 2.

Mass spectrometry conditions

Mass spectrometric analysis was performed using an atmospheric pressure chemical ionization (APCI) source operated in positive ion mode. Quantification was conducted under multiple reaction monitoring (MRM) mode. The following instrument settings were applied: Drying gas (N_2) temperature: 300 °C; Drying gas flow rate: 5 l/min; Curtain gas (N_2) pressure: 20–30 psi; Nebulizer pressure: 50 psi; Capillary voltage: 2,500 V; Compound-specific MRM transitions and optimized parameters for the 16 target PAHs are summarized in Table 1.

Table 1 Mass spectrometry parameters of the 16 EU priority-controlled PAHs.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Retention time (min)
BcL	179.3	152.3	30	12.3
BaA	229.5	202.5	35	18.2
CPP	229.4	201.6	35	19.1
Chr	179.8	152.4	30	13.4
5MC	179.6	152.1	30	14.0
BbF	253.8	226.2	40	20.2
BkF	253.1	226.1	40	21.5
BjF	253.0	226.7	40	22.3
BaP	253.5	226.6	45	25.4
IcP	277.8	250.0	45	26.6
DhA	279.3	252.5	50	28.0
BgP	277.4	250.3	45	27.5
DiP	303.1	276.5	50	29.6
DeP	303.5	276.7	50	30.4
DIP	303.8	276.9	50	31.5
DhP	303.1	276.1	50	32.1

RESULTS AND DISCUSSION

Optimization of sample pretreatment conditions

DESs have demonstrated broad potential for pretreating trace pollutants due to their low toxicity, biodegradability, and environmentally friendly synthesis. Both the molar ratio and volume of DESs significantly affect their extraction performance. In this study, choline chloride (ChCl) and lactic acid were chosen as the hydrogen bond acceptor and donor, respectively. Three DES systems were prepared at molar ratios of 1:1, 1:2, and 1:3. The extraction efficiency for 16 EU priority PAHs was evaluated for each system at different volumes (Fig. 3a). The DES with a ChCl:lactic acid ratio of 1:2 showed the best extraction performance, achieving the highest mean recovery (93.5%) at a volume of 200 µl. At the same volume, the 1:1 system exhibited a significantly lower recovery (74.2%). This may be attributed to insufficient hydrogen bonding, which restricted solute dissolution and mass transfer [23]. In contrast, although the 1:3 system contained more lactic acid, its increased viscosity may have impeded analyte diffusion, leading to a slightly lower recovery (88.6%) than the 1:2 system. When the volume of the 1:2 DES exceeded 200 µl (e.g., 300 or 400 µl), the average recovery slightly decreased. This suggests that an excessive solvent volume may cause uneven dispersion or disrupt phase volume balance [24]. Overall, a moderate molar ratio (1:2) with a volume of 200 µl provided a stable and efficient microenvironment. This condition facilitated effective PAH release and enrichment, providing a theoretical and practical foundation for designing a green, magnetically-assisted extraction system.

In DLLME, the dispersant is critical for forming a stable emulsion of the extractant, which significantly improves the mass transfer efficiency of target analytes. Considering the specific physicochemical properties of DESs, this study evaluated the effects of three commonly used dispersants—methanol, acetonitrile, and acetone—at specific volumes of 200, 300, 400, and 500 µl on the average recovery of 16 PAHs. The aim was to optimize extraction performance and enhance

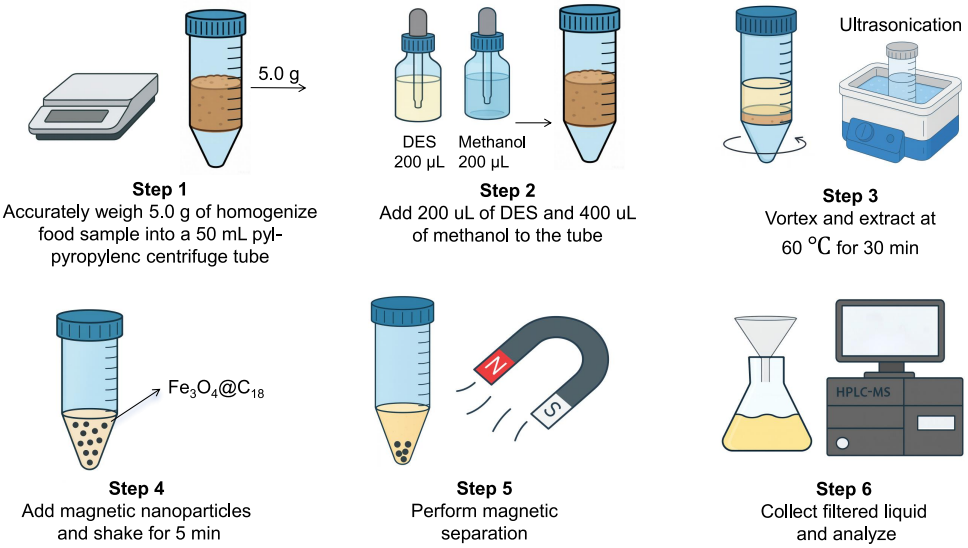

Fig. 1 Workflow of the sample pretreatment process.

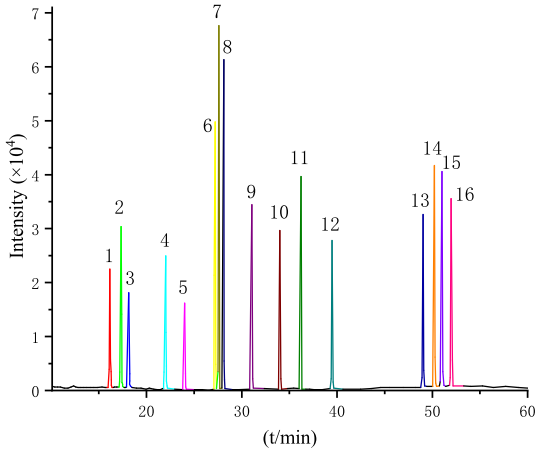

Fig. 2 Ion chromatograms of 16 EU priority PAHs: 1. BcL; 2. BaA; 3. CPP; 4. CHR; 5. 5MC; 6. BbF; 7. BkF; 8. BjF; 9. BaP; 10. IcP; 11. DhA; 12. BgP; 13. DiP; 14. DeP; 15. DIP; 16. DhP.

system stability. As shown in Fig. 3b, the type of dispersant significantly affected extraction efficiency. Across all tested volumes, methanol consistently outperformed both acetonitrile and acetone. The highest mean recovery (87.3%) was obtained with 400 µl of methanol, indicating its strong emulsification compatibility with the DES phase. While acetonitrile and acetone are traditionally used as dispersants in DLLME due to their polarity, their performance was suboptimal in the high-viscosity DES system. The emulsions they formed showed poor dispersion, limiting analyte migration and resulting in recoveries typically below 75%. When methanol was used, recovery increased from 200 to 400 µl, reflecting enhanced emulsification and improved mass transfer. However, a slight decrease in extraction efficiency was observed at 500 µl, likely due to dilution of the system. A similar trend was observed with the other two dispersants, although their overall performance was significantly lower. In summary, methanol exhibited superior dispersibility and system stability in the DES-DLLME platform. A volume of 400 µl was identified as the optimal addition level, yielding the highest overall extraction efficiency under the conditions of this study.

In DLLME systems assisted by DESs, the high viscosity of the extraction phase poses challenges for phase separation. Traditional centrifugation methods are time-consuming and not well-suited for automation. To address this, three types of magnetic nanomaterials—Fe_3O_4, Fe_3O_4@SiO_2, and Fe_3O_4@C_{18}—were introduced to replace centrifugation with external magnetic separation. Their magnetic responsiveness was compared, and the optimal dosage of the best-performing material was further optimized to enhance both separation efficiency and extraction performance. As shown in Fig. 3c, Fe_3O_4@C_{18} demonstrated the fastest separation time, averaging just 30 s, significantly shorter than Fe_3O_4 (80 s) and Fe_3O_4@SiO_2 (55 s). The superior performance is attributed to the hydrophobic C_{18} chains, which enhance the affinity between the nanoparticles and the hydrophobic DES phase, improving magnetic aggregation within the emulsion system [25]. In contrast, unmodified Fe_3O_4 particles, with their higher surface polarity, exhibited poor dispersion and a slower magnetic response. As a result, Fe_3O_4@C_{18} demonstrated clear advantages in ease of use and separation speed. After identifying Fe_3O_4@C_{18} as the most effective material, its dosage was further optimized. As shown in Fig. 3d, increasing the dosage from 10 mg to 30 mg resulted in a steady increase in the average recovery

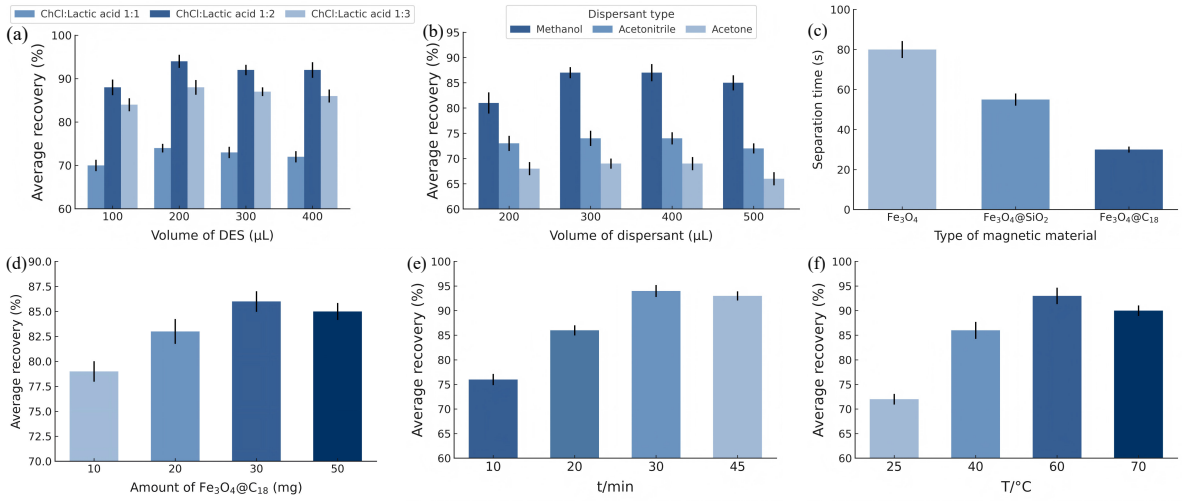

Fig. 3 Optimization of DES-assisted DLLME conditions for PAH extraction. (a) Effect of DES volume and molar ratio on mean recovery of 16 EU priority PAHs. (b) Effect of dispersant type and volume on mean PAH recovery. (c) Phase separation time comparison of three magnetic materials in DES-DLLME system. (d) Effect of $Fe_3O_4@C_{18}$ dosage on PAH recovery. (e) Effect of ultrasonic extraction time on PAH recovery. (f) Effect of extraction temperature on PAH recovery. Data points represent mean values from triplicate experiments ($n = 3$); error bars indicate standard deviation (SD).

of the 16 PAHs, from 79.5% to 86.1%. However, increasing the dosage to 50 mg did not yield a significant improvement in recovery. This suggests that a dosage of 30 mg provides an optimal balance between extraction efficiency and material utilization. Excessive dosage may not only lead to resource wastage but also cause nonspecific adsorption, which could reduce analyte recovery. In conclusion, $Fe_3O_4@C_{18}$, with its hydrophobic surface modification, significantly enhanced the magnetic responsiveness and separation efficiency of the DES-based extraction phase. A dosage of 30 mg was found to be optimal, enabling rapid separation and efficient enrichment of PAHs.

Extraction time and temperature are crucial parameters in microextraction systems, as they directly influencing mass transfer rates, solute solubility, and solvent-matrix interaction efficiency. In this study, the DES composition was fixed at a 1:2 molar ratio of ChCl to lactic acid, with a volume of 200 μl. Under these conditions, the effects of ultrasonic extraction time and temperature on PAH recovery from meat matrices were systematically investigated. As shown in Fig. 3e, increasing ultrasonic extraction time from 10 to 30 min significantly improved recovery, increasing from 76.8% to 93.5%. However, extending the extraction time to 45 min did not result in further improvement and even caused a slight decrease in recovery. This decline may be attributed to localized heating during ultrasonication, which could degrade thermally sensitive analytes or alter the viscosity of the DES system. Therefore, an extraction time of 30 min was chosen as optimal, balancing efficiency and practicality. Temperature optimization results (Fig. 3f) showed continuous improvement in recovery as the temperature increased from 25 °C to 60 °C. This trend suggests that higher temperatures reduce the viscosity of the DES and enhance analyte diffusion rates. However, when the temperature was increased further to 70 °C, the average recovery slightly decreased from 93.5% to 90.2%. This reduction is likely due to thermal degradation of PAHs or compromised stability of the DES system at higher temperatures. Based on these findings, 60 °C was selected as the optimal extraction temperature, providing a balance between improved extraction performance and system stability.

Determination of linearity and detection limits

Standard solutions of 16 PAHs were prepared at concentrations ranging from 0.1 to 100 ng/ml to assess the method's linearity. Calibration curves and correlation coefficients (R^2) were determined for each analyte. As shown in Table 2, all analytes exhibited excellent linearity, with R^2 values $\geqslant 0.99$. The method detection limits (LODs), defined as the signal-to-noise ratio (S/N) of 3, ranged from 0.01 to 0.06 ng/ml. The limits of quantification (LOQs), calculated at an S/N of 10, ranged from 0.03 to 0.21 ng/ml.

Evaluation of method accuracy and precision

The accuracy and precision of the developed method were systematically evaluated based on the matrix spiking protocol outlined in Materials and Methods. A low-background cured pork sample, pre-screened to ensure the absence of target analytes, was used as the blank matrix. The sample was spiked at three concentration levels (1, 10, and 50 ng/g) and processed

Table 2 Linear equations, R^2, LOD and LOQs of the 16 PAHs.

Analyte	Linear range (ng/ml)	Regression equation	R^2	LOD (ng/ml)	LOQ (ng/ml)
BaP	0.1–50	$y = 630.1x + 9.3$	0.9987	0.051	0.168
BbF	0.1–50	$y = 2397.8x - 63.0$	0.9998	0.067	0.221
BkF	0.1–50	$y = 2431.3x + 93.9$	0.9994	0.06	0.198
BaA	0.1–50	$y = 2116.8x + 55.0$	0.9991	0.05	0.165
Chr	0.1–50	$y = 1109.2x + 87.9$	0.9983	0.073	0.241
5MC	0.1–50	$y = 695.3x + 79.0$	0.9983	0.04	0.132
BjF	0.1–50	$y = 1868.5x + 19.6$	0.9981	0.05	0.165
CPP	0.1–50	$y = 1380.3x + 84.4$	0.9996	0.056	0.185
BcL	0.1–50	$y = 744.1x - 82.3$	0.9991	0.062	0.205
IcP	0.1–50	$y = 1490.4x - 60.8$	0.9993	0.085	0.28
BgP	0.1–50	$y = 568.8x - 91.0$	0.9982	0.044	0.145
DhA	0.1–50	$y = 2318.6x - 34.9$	0.9998	0.066	0.218
DiP	0.1–50	$y = 1017.6x - 22.3$	0.9996	0.071	0.234
DeP	0.1–50	$y = 1825.0x - 45.7$	0.9984	0.033	0.109
DlP	0.1–50	$y = 1123.4x + 65.7$	0.9983	0.073	0.241
DhP	0.1–50	$y = 1540.1x - 28.6$	0.9983	0.042	0.139

Table 3 Recoveries and precisions of 16 EU priority PAHs at three spiking levels in cured pork matrix ($n = 6$).

Analyte	Low concentration (1 ng/g)		Medium concentration (10 ng/g)		High concentration (50 ng/g)	
	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
BaP	95.3	4.1	98.7	3.5	102.4	3.8
BbF	89.2	5.6	93.5	4.8	97.8	4.2
BkF	86.4	6.3	90.1	5.5	94.6	4.9
BaA	92.7	4.8	96.3	3.9	101.1	3.5
Chr	88.5	5.9	92.8	5.1	96.2	4.7
5MC	85.7	6.7	89.4	6.0	93.1	5.3
BjF	84.2	7.1	88.6	6.5	92.5	5.8
CPP	91.6	4.5	95.9	4.0	100.3	3.6
BcL	82.5	7.8	86.3	7.2	90.8	6.4
IcP	87.3	6.0	91.7	5.4	95.4	4.8
BgP	83.9	7.5	87.5	6.9	91.2	6.1
DhA	84.6	7.3	88.1	6.7	92.7	5.9
DiP	88.6	6.2	92.1	5.7	96.5	4.9
DeP	81.7	7.8	85.4	7.5	89.9	6.7
DlP	83.2	7.6	86.9	7.0	90.5	6.3
DhP	80.9	8.0	84.7	7.8	88.3	7.1

according to the standard workflow. Each spiking level was analyzed in six replicates ($n = 6$) to determine recovery rates and relative standard deviations (RSDs). The results showed that the average recoveries for the 16 PAHs ranged from 80.9% to 102.4%, with RSDs between 3.5% and 8.0% (Table 3). These values meet the technical criteria recommended by the Association of Official Analytical Chemists (AOAC) for trace analysis (RSD < 10%), demonstrating the method's good accuracy and repeatability in complex meat matrices. Furthermore, the method exhibited excellent sensitivity and stability, even at the lowest spiking level (1 ng/g). This highlights the strong enrichment capacity of the DES-Fe_3O_4@C_{18} system for hydrophobic contaminants.

Detection of PAHs in commercial food products

To further validate the practical application of the microextraction-mass spectrometry method based on DES and magnetic nanomaterials, this study systematically detected 16 PAHs regulated by the European Union in four types of commercially available heat-processed meat products (cured meat, sausage, roasted chicken wings, and roasted lamb skewers, each with 10 batches). The results showed significant differences in both the detection rates and concentration levels of PAHs across the different food samples. Roasted lamb skewers exhibited the highest contamination levels, with most of the 16 PAHs detected in over 70% of the samples. The estimated median concentrations of highly toxic PAHs, such as BaP, BbF, and BgP, were 3.85, 3.2, and 3.45 ng/g, respectively. Cured meat samples also exhibited relatively high detection rates and concentration levels for various PAHs (e.g., BaP with a median concentration of 2.95 ng/g), likely due to the long-term smoking process. In contrast, sausage samples had lower detection rates for most PAHs, with some compounds undetected, and the estimated median concentrations were generally below 1.0 ng/g, indicating relatively low PAH formation during pro-

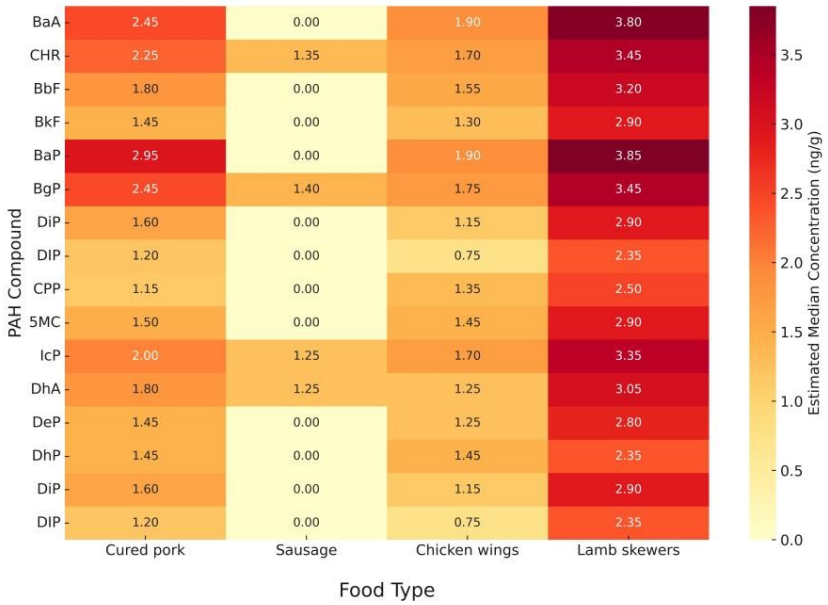

Fig. 4 Heatmap of estimated median concentrations (ng/g) of 16 EU priority polycyclic aromatic hydrocarbons (PAHs) detected in four types of thermally processed meat products ($n = 10$).

cessing. The contamination level in roasted chicken wings was intermediate, between sausages and cured meat, with greater variability. To provide a clearer overview of the PAH contamination differences across food samples, Fig. 4 presents a heatmap showing the estimated median concentrations (ng/g) of 16 PAHs in the four sample types. The heatmap indicates that pollutants such as BaP, BaA, CHR, and DiP appeared in darker regions for roasted lamb skewers and cured meat, indicating a higher potential health risk. Overall, PAHs were detected in the majority of the tested samples. Specifically, 35 out of 40 samples (87.5%) contained detectable levels of at least one PAH, while 5 samples (12.5%), mainly from sausages, were below the detection limit.

Comparison with other methods in the literature

To evaluate the practical application and overall performance of the sample preparation method developed in this study, a comparative analysis was conducted with five widely used PAH sample treatment techniques. Table 4 summarizes the performance of each method across these parameters. The comparison reveals that although traditional methods such as LLE and Magnetic Solid-Phase Extraction (MSPE) show good recovery rates and applicability in specific matrices, they are often associated with complex procedures, large solvent usage, and additional purification steps. In contrast, green analytical techniques such as Solid-Phase Microextraction (SPME) and QuEChERS offer advantages in terms of simplified procedures and environmental friendliness. For example, limits of detection (LODs) of 0.05–0.1 ng/g with recoveries of 94–122% and RSDs below 5% has been reported in QuEChERS–GC-MS/MS [27], whereas HS-SPME–GC-MS showed higher LODs of 1–4 ng/g with recoveries of 88–105% and RSDs in the range of 4–11% [28]. However, these methods often face challenges such as matrix interference or insufficient extraction efficiency for high molecular weight PAHs, especially in high-fat and complex matrices. By comparison, the improved DLLME method developed in this study, based on DES and $Fe_3O_4@C_{18}$ magnetic nanomaterials, achieved lower LODs (0.01–0.06 ng/ml), high recoveries (80.9–102.4%), and reproducibility with RSDs below 8.0%, while also avoiding halogenated solvents and simplifying phase separation. This method is particularly well-suited for detecting trace contaminants in high-fat, heat-processed foods.

Compared with commonly reported magnetic nanomaterials such as Fe3O4 and $Fe_3O_4@SiO_2$, the $Fe_3O_4@C_{18}$ used in this study exhibits stronger hydrophobic interactions with PAHs due to the long-chain alkyl modification. This results in superior extraction efficiency, higher enrichment factors, and lower detection limits, particularly in high-fat and complex food matrices. Although its synthesis cost is relatively higher, the improved selectivity and analytical performance justify its use, highlighting its advantage over cheaper alternatives in applications requiring high sensitivity and accuracy.

Despite these advantages, some limitations should be acknowledged. First, the relatively high cost of synthesizing and modifying magnetic nanomaterials may limit their large-scale application in routine food testing laboratories. Second, the long-term stability of

Table 4 Comparative performance of different sample preparation methods for PAHs detection in food.

Method	Extractant type	Centrifugation/Filtration required	Extraction time	Recovery (%)	LOD	Applicable matrices	Environmental Sustainability
MSPE + GC-MS [26]	MWCNTs-Fe_3O_4 nanomaterials	Magnetic separation	∼30 min + purification	93.4–101.6	0.06–1.12 µg/kg	Infant foods	Medium (recyclable nanomaterials)
LLE + UHPLC-DAD [27]	Acetate + acetonitrile	Filtration as a substitute for centrifugation	∼30 min	47.3–119.7	0.0049–0.373 µg/l	Fried/baked foods	Medium (organic solvent used)
QuEChERS + GC-MS [28]	Acetonitrile + water + Z-Sep + Florisil	Two-step centrifugation	20–25 min	94–122	0.17–1.1 µg/kg	Olive fruit	High (green purification agents)
HS-SPME + GC-MS [29]	PDMS solid-phase fiber (solvent-free)	None (auto-sampling)	90 min	88–105	1–4 ng/g	Plant buds	High (solvent-free)
MSE-DLLME + GC-MS [30]	Fe_3O_4@TEOS + DES + n-hexane	Magnetic separation	Ultrasound + microwave, ∼60 min	73–92	29–82 ng/kg	Grilled meat samples	Medium (contains n-hexane)
Method used in this work	DES + magnetic nanomaterials	Magnetic separation, no centrifugation	∼30 min	80.9–102.4	0.01–0.06 ng/ml	Heat-processed meat products	High (green solvent, rapid phase separation)

GC-MS, gas chromatography-mass spectrometry; UHPLC-DAD, ultra-high-performance liquid chromatography with diode-array detection; HS-SPME, headspace solid-phase microextraction; MSE-DLLME, Matrix Solid-Phase Extraction Coupled with Dispersive Liquid-Liquid Microextraction.

these nanomaterials remains uncertain, as potential issues such as aggregation or surface degradation could affect reproducibility during prolonged use. Finally, although the method has been validated for thermally processed meats, its broader applicability to other complex food matrices (e.g., dairy products, cereals, and edible oils) requires further evaluation. Future research should therefore focus on reducing costs, enhancing stability, and validating the method across diverse food systems to facilitate the wider adoption of this green extraction approach.

CONCLUSION

This study established a green and efficient method for detecting PAHs in heat-processed meat products using DES-assisted DLLME coupled with magnetic nanomaterials (Fe_3O_4@C_{18}) and HPLC-MS/MS. The optimized method exhibited excellent linearity, low detection limits, and satisfactory recoveries, confirming its reliability for trace analysis in complex food matrices. The integration of DESs with magnetic nanomaterials improved extraction efficiency, eliminated the need for toxic solvents and centrifugation, and simplified sample handling. Application to commercial meat products demonstrated its practicality for PAH monitoring and food safety assessment, which is particularly relevant given the widespread environmental occurrence of PAHs, including severe contamination reported in estuarine surface soils [31]. Despite its advantages, the relatively high cost and uncertain long-term stability of magnetic materials may limit large-scale implementation; therefore, future research should focus on improving reusability and expanding its application to other high-fat or complex food systems.

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