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Acetic acid liquid-liquid extraction and UHPLC-DAD detection of polycyclic aromatic hydrocarbons in toasted and fried foods

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings. They have very low water solubility and are highly lipophilic. Acetic acid is a polar protic solvent that is often used in reactions involving carbocation intermediates. In this study, acetic acid, coupled with other organic solvents, was optimized and used for the extraction of eleven PAHs in two types of foods: toasted and fried. The UHPLC-DAD method, coupled with a C18 column, was validated and applied to analyze eleven PAHs in the food samples. The LOD and LOQ values obtained ranged from 0.0049 to 0.373 μg L^{-1}. The recoveries of the PAHs ranged from 47.3% to 119.7%. The analysis results show that light PAHs were commonly found in both types of food. Some fried foods are highly carcinogenic due to the presence of BaP and Group 2B PAHs. Generally, toasted foods are safe to consume.

Keywords: Malaysian food; organic solvent; matrix effect; validation; HPLC.

INTRODUCTION

Prolonged thermal food processing, such as grilling, roasting, and frying, may induce the production of polycyclic aromatic hydrocarbons (PAHs). Many studies have shown that some PAHs are highly carcinogenic, and long-term exposure to them may increase the risk of human cancers.[1] Animal studies have indicated that certain PAHs may affect the hematopoietic and immune systems and can cause reproductive, neurological, and developmental effects.[2] Due to the carcinogenic effects of PAHs, the International Agency for Research on Cancer (IARC) classifies benzo[a]pyrene (BaP) as carcinogenic to humans (Group 1), while compounds such as benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), and chrysene (Chr) are classified as possibly

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carcinogenic to humans (Group 2B).[3] Accordingly, the maximum limits for BaP and the sum of Group 2B PAHs in foodstuffs were set at 2.0 μg kg^{-1} and 12.0 μg kg^{-1}, respectively.[4] Due to BaP's carcinogenicity, it is often used as a marker for the presence and carcinogenic effects of PAHs in food. However, the committee also emphasized that the analysis of multiple PAHs is still necessary to gather more information on contamination levels and possible changes in the PAHs formation profile in foods.[5]

Various extraction methods have been used to recover PAHs from foods, including conventional techniques like liquid-liquid extraction (LLE), solid-phase extraction (SPE), and supercritical fluid extraction (SFE), as well as more advanced methods such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), liquid-liquid microextraction (LLME), and the QuEChERS method (quick, easy, cheap, effective, rugged, and safe).[1] The main disadvantages of conventional techniques are the large amounts of organic solvents consumed, lengthy extraction times, and high energy use.[6] The introduction of LLME has addressed many of these drawbacks, as it uses microliter volumes of solvent. LLME is a miniaturized version of LLE, where an extraction solvent is mixed with a dispersive solvent and rapidly injected into the aqueous sample. The resulting cloudy solution is then centrifuged to separate it into a two-phase system, allowing for easy recovery of the extraction solvent for analysis. MAE uses electromagnetic radiation to break the cellular matrix, releasing intracellular compounds into the extraction solvents.[7] UAE enhances mass transfer rates and solvent penetration, leading to higher extraction yields and shorter extraction times.[6] PLE employs liquid solvents at temperatures above their atmospheric boiling points but below their critical points, improving solubility and mass transfer properties.[7] MAE, UAE, and PLE are considered the most practical methods for industrial-scale use due to the availability of equipment, short extraction times, and low solvent consumption rates.[8] The QuEChERS technique is a type of dispersive solid-phase extraction (dSPE) used for sample preparation. It effectively uses adsorbent fillers to interact with impurities in the matrix, achieving high impurity removal.[9]

The PAHs profiles of various foods, especially meat variants such as grilled and smoked meats, have been widely studied.[1,10] However, limited research has been conducted on the PAHs profiles of Satay (grilling), Roasted Chicken (roasting), and Youtiao or Chinese doughnut sticks (frying). Acetic acid, one of the most widely used carboxylic acids, is often employed in reactions like the synthesis of acetic esters.11 It is also used as a solvent, for example, in the production of cellulose acetate.12 In this study, acetic acid, coupled with selected organic solvents, was applied for the first time in the extraction of PAHs from selected foods. The effects of acetic acid composition and the types of organic solvents, used for the extraction, were optimized. The PAHs content and profile in

the foods were determined using the UHPLC-DAD method. Separation was performed using a traditional C18 column rather than a more selective and expensive PAH column. The UHPLC-DAD method was developed and validated before being applied to determine eleven PAHs in the selected foods. The focus was on indigenous toasted and fried foods, commonly prepared from or mixed with flour, which are popular as appetizers or snacks in Southern and Southeast Asia. These foods are typically served for breakfast or afternoon tea. They can be categorized into toasted (Roti Canai, Chapati, Thosai, Kuih Tayap, and Apam Balik) and fried (Youtiao, Keropok Lekor, Pisang Goreng, Cekodok, and Fried Chicken) foods. The appearance of these foods is shown in Fig. S1. The determination of PAHs in these items has not been previously reported. The PAHs analyzed (ANT, FLR, FLT, PHE, PYR, CHR, BaA, BbF, BkF, BaP, Ghi) are among the most prevalent PAHs found in smoked, grilled, and fried foods.

EXPERIMENTAL

Preparation of standard solutions: 0.05 g of each PAH (ANT, FLR, FLT, PHE, PYR, CHR, BaA, BbF, BkF, BaP, Ghi) (Table S1) was measured to prepare a stock solution (100 mg L^{-1}) containing the eleven PAHs in a 500 mL volumetric flask. Acetonitrile was used to dissolve the PAHs, and the solution was made up to 500 mL. The flask was left overnight at room temperature to ensure complete dissolution of the PAHs stock solution. This stock solution was further diluted 100-fold to prepare a working solution of 1 mg L^{-1}. From this, a series of standard solutions (25 mL each) with concentrations of 10, 50, 200, 800, and 1000 µg L^{-1} were prepared, which were used to obtain calibration curves of peak areas as a function of PAH standard concentrations. These standard solutions were covered with aluminum foil and stored at 4°C until analysis.

Optimization of acetic acid extraction: Different acetic acid compositions (0%, 30%, 50%, and 70%) combined with an organic solvent (i.e., acetonitrile) were prepared for the optimization study. Two selected food samples (3 g each), Chapati and Keropok Lekor (representing the toasted and fried categories, respectively), were placed into separate beakers and spiked with eleven PAHs at a concentration of 5000 µg L^{-1} (50 mL). The samples were stirred overnight at 300 rpm using a mechanical stirrer. The spiked food samples were filtered and subsequently extracted with 10 mL of a mixed solvent containing different acetic acid compositions, stirred at 300 rpm for 30 minutes. After stirring, the food solids were removed by vacuum filtration. One milliliter of the filtrate was diluted to 5 mL with acetonitrile and then injected into the HPLC-DAD system for PAH analysis. Once the optimum acetic acid composition was determined, the most suitable solvent for extraction was evaluated by replacing acetonitrile with four other solvents: toluene, dichloromethane, ethyl acetate, and ethanol. A 10 mL extraction solvent consisting of acetic acid and one of the selected solvents was added separately to each type of food sample. The samples were stirred at 300 rpm for 30 minutes, filtered, and then diluted fivefold before analysis.

Liquid-liquid extraction (LLE): Two selected food samples, Chapati and Keropok Lekor, were spiked with the eleven PAHs at concentrations of 5000 µg L^{-1}. The spiked food samples were extracted with a 10 mL mixed solvent of acetonitrile and acetic acid (70:30% v/v) using a magnetic stirrer for 30 minutes. After stirring, the food solids were removed by vacuum filtration. One mL of the filtrate was diluted to 5 mL with acetonitrile and then injected into the

HPLC-DAD system for PAH analysis. This procedure is referred to as acetic acid extraction without SPE.

Solid phase extraction (SPE): In a separate experiment, after LLE, the two selected food samples (Chapati and Keropok) underwent SPE clean-up. The SPE method followed Hamidi et al.[10] with modifications and it was conducted using a Supelco C18 SPE cartridge (ENVI-18, 6 mL / 0.5 g). The PAH clean-up was evaluated based on the percentage recovery when passed through the SPE cartridge (VacElut, 16 x 150 mm, Agilent, Palo Alto, CA, USA). The cartridge was conditioned with 10 mL of the respective mixed solvents (acetic acid and acetonitrile in varying ratios) used for extraction prior to the clean-up procedure. Elution was performed using 3 mL of toluene, and the process was repeated three times. Toluene was chosen due to its potential for π-π interactions with better aromatic selectivity. Toluene was evaporated using a rotary evaporator, and the residue was diluted with 4 mL of HPLC-grade acetonitrile. The solution was filtered through a membrane before injection into the HPLC system. This procedure is referred to as acid extraction with SPE.

Sample extraction: A total of 10 different foodstuffs were purchased from various food stalls in the Klang Valley region, Malaysia. These included 5 types of toasted foods (Roti Canai, Chapati, Thosai, Kuih Tayap, and Apam Balik) and 5 types of fried foods (Youtiao, Keropok Lekor, Pisang Goreng, Cekodok, and Fried Chicken). All samples were carefully packed in polyethylene bags and stored at 4°C until analysis. For the extraction, 3 g of each food sample was cut into equal sizes of 1 cm x 1 cm. A 10 mL solvent mixture of acetic acid and acetonitrile (30:70) was added to the food sample, which was then sonicated for 5 minutes, followed by mechanical stirring for 30 minutes at 300 rpm at room temperature. The food sample was separated from the filtrate by vacuum filtration using a Buchner funnel. The filtrate was diluted fivefold prior to analysis. Details of the food descriptions are summarized in Table 1.

Instrumentation and chromatographic conditions: The separation and quantification of PAHs were performed using an HPLC system (Thermo Technologies, California, US) consisting of a Thermo UltiMate 3000 Quaternary Pump, an Agilent 1260 Infinity Diode-Array Detector, a Thermo UltiMate 3000 standard autosampler injector with a 10 µL loop, and a reversed-phase Ascentic 5 µm C18 column (250 mm x 4.6 mm). A gradient elution using ultrapure water and HPLC-grade acetonitrile was varied from 0% to 40% acetonitrile over 30 minutes. The flow rate, wavelength, and column temperature were set at 1.0 mL/min, 259 nm, and 30°C, respectively.

TABLE 1: Description of food samples analyzed.

Food Type	Name of Food	Description
Toasted Food	Roti Canai	Indian flatbread dish served by the Indian-Muslim community popularly as breakfast or for tea time. The dough is made out of fat, flour and water and is flattened and oiled before toasting.
	Chapati	A type of bread served by the Indian community for breakfast. It is made out of wheat flour, also known as 'atta' flour mixed into a dough.
	Thosai	It is a type of "pancake" that is originated from India which is made from mainly rice and black gram. This food resembles a crepe in appearance and is normally served as breakfast.
	Kuih Tayap	Also known as pandan pancake rolls. It is a Nyonya sweet desert made from crepe batter
	Apam Balik	A type of pancake served as a sweet desert, originating in the Chinese cuisine. The batter is made from flour, eggs, sugar, baking soda, coconut milk and water.
Fried Food	Youtiao	It is a "fried bread stick" which is a popular breakfast food among the Chinese society. It is normally eaten as a side dish and always consume together with porridge.
	Keropok Lekor	Also known as fish crackers. It is a traditional Malay snack and is made by grinding fish, mixing with sago and deep frying it.
	Pisang Goreng	Also known as goreng pisang. It is a traditional Malay snack made by coating sliced bananas in flour and deep frying them. Sometimes served with soy sauce.
	Cekodok	Also known as fried banana balls served by the Malay community as snacks. The batter is made by mashing bananas together with plain flour and rice flour and subsequently deep fried.
	Fried Chicken	The chicken pieces are floured and deep-fried. Commonly consumed as a side dish together with other types of foods.

RESULTS AND DISCUSSION

Development and optimization of liquid-liquid extraction

Effect of acid composition

The primary goal of this section was to develop an effective extraction method for PAH analysis. Various acid compositions and solvent types were tested for extracting PAHs from two selected food samples, Chapati and Keropok Lekor. The effect of solid-phase extraction (SPE) was examined. The optimized conditions were applied to determine the PAH profiles in selected toasted and fried foods.

The tested acid compositions were 0%, 30%, 50%, and 70% v/v of acetic acid. The solvents tried included toluene, dichloromethane, ethyl acetate, and ethanol, all of which are miscible with acetic acid. Two sets of extraction experiments were conducted for each acetic acid composition: one without SPE (direct acetic acid

extraction), and one with SPE following acetic acid extraction. In the primary trials, acetonitrile with different acetic acid compositions was used as the extraction solvent, as acetonitrile is the most polar solvent (polarity index, 5.8) compared to ethyl acetate (4.4), toluene (2.6), dichloromethane (3.1), and ethanol (5.2).

The extraction method's effectiveness was evaluated by the percentage recovery after spiking a mixture of eleven PAHs into two food samples (Chapati and Keropok Lekor). Results showed that acetonitrile with 30% acetic acid, without SPE, yielded higher percentage recoveries (mean 70.9% and 109.5% for Chapati and Keropok Lekor, respectively) for the eleven PAHs (Figures 1a and 1b). Other acetic acid compositions (0%, 50%, and 70%) resulted in lower recoveries (mean 55.8%, 44.0%, and 38.2% for Chapati; 101.0%, 56.2%, and 94.1% for Keropok Lekor, respectively). Similar trends were observed for Chapati with SPE (Fig. 1a). However, for Keropok Lekor, the recovery for 30% acetic acid with SPE (50.5%) was slightly lower than for other compositions (58.3%, 55.3%, and 69.2% for 0%, 50%, and 70% acetic acid, respectively) (Fig. 1b). Details on the effects of acetic acid composition on recoveries are provided as supplementary data (Table S2).

Overall, recoveries without SPE were higher than those with SPE, likely due to the semi-adsorption of PAHs onto the SPE adsorbent. The higher recoveries without SPE may be attributed to acetic acid enhancing PAH solubility in organic solvents, with maximum solubility occurring at 30% acetic acid. In contrast, higher acetic acid concentrations (50% and 70%) resulted in lower recoveries, possibly due to increased solvent polarity reducing PAH solubility. In the presence of water, acetic acid dissociates into acetate and H^+ ions, but without water, it acts as a polar covalent compound, binding to other compounds via covalent bonding.[13] The protic nature of acetic acid (dielectric constant 6.2) may also facilitate the dissolution of both polar and non-polar compounds like PAHs.[14] This explains why 30% acetic acid yielded optimal solubility for PAHs, and this condition will be used to select the best organic solvent for PAH extraction in the next section.

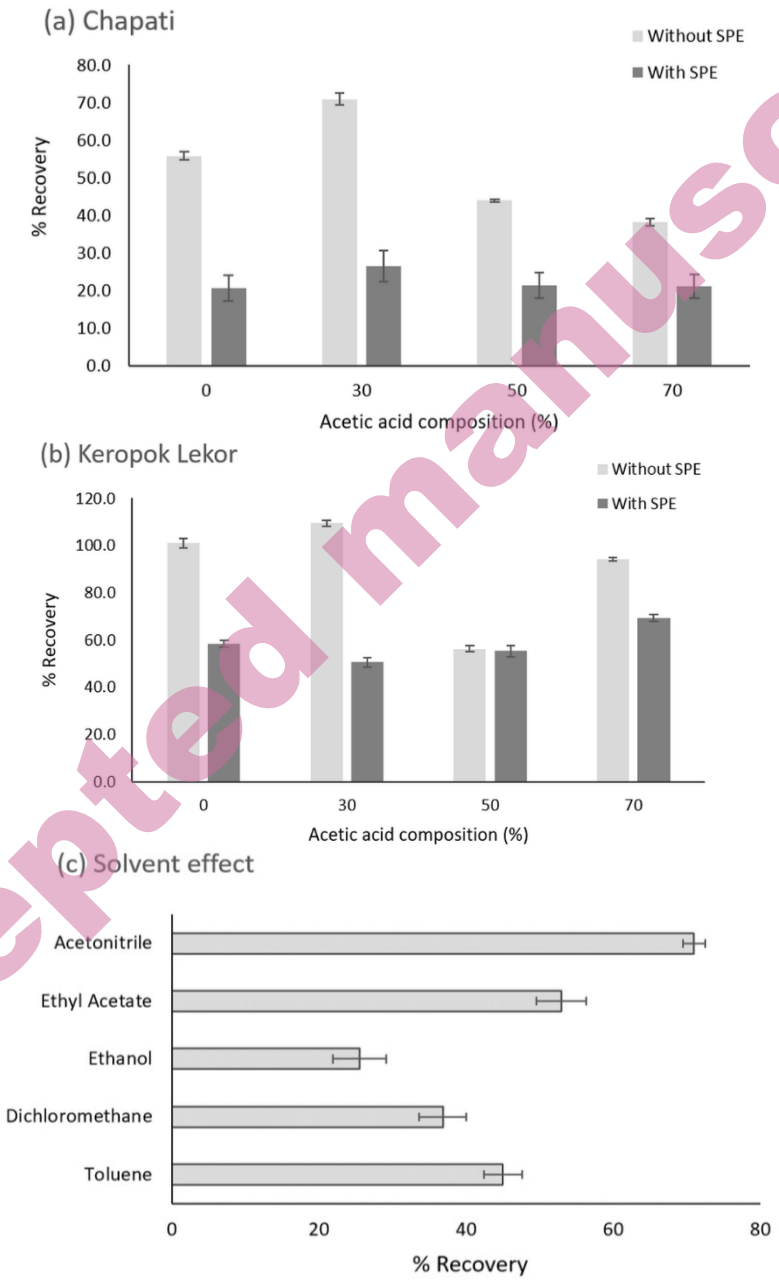

Fig. 1. Effects of different acetic acid compositions on the percentage recoveries of eleven PAHs with and without SPE for Chapati (a) and Keropok Lekor (b); (c) the effects of different organic solvents on the percentage recoveries of eleven PAHs after spiking to a selected food sample (acetic acid composition is 30%).

Effect of organic solvent

In this study, different organic solvents were used to extract spiked PAHs from the selected food samples (Chapati and Keropok Lekor), using the optimum 30% acetic acid composition without SPE. The percentage recoveries of the solvents in descending order were: acetonitrile (70.9%), ethyl acetate (52.9%), toluene (45.0%), dichloromethane (36.8%), and ethanol (25.5%) (Fig. 1c). Acetonitrile gave the highest percentage recovery. Detailed recovery data for the solvents are presented in Table S3 (supplementary data).

When correlating solvent extraction ability to their polarity indices, the expected order (from highest to lowest) would be acetonitrile (P = 5.8), ethanol (P = 5.2), ethyl acetate (P = 4.4), dichloromethane (P = 3.1), and toluene (P = 2.6) (Zarrinmehr et al., 2022).[15] However, toluene showed higher recovery than expected, and ethanol showed lower recovery. The higher extraction power of toluene compared to ethanol may result from π-π interactions between toluene and PAHs, while ethanol's poor performance could be due to the unfavorable interaction between its highly polar hydroxyl group (-OH) and the non-polar aromatic rings of PAHs.[15]

Acetonitrile's high extraction power can be explained by the presence of the relatively less polar cyano group (-CN) compared to ethanol's hydroxyl group (-OH). The smaller difference in electronegativity between carbon and nitrogen (2.5 and 3.0) in -CN, compared to oxygen and hydrogen (3.5 and 2.1) in -OH, combined with acetonitrile's higher polarity index and shorter carbon chain, may account for its superior performance.[16] Thus, acetonitrile with 30% acetic acid provided the highest extraction power for PAHs.

HPLC method development and optimization

Before analyzing PAHs using a gradient reversed-phase HPLC-DAD method, an isocratic elution with the following solvent compositions was tested: solvent A (40–80% acetonitrile, ACN) and solvent B (60–20% H_2O) on an Ascentic ODS column (250 mm × 4.6 mm, 5 µm, USA). However, this method was unsatisfactory due to the co-elution of CHR and BaA, as well as BbF and BkF. To address this issue, gradient elution was employed, achieving complete separation with good resolution and sensitivity using the following solvent program: 0 - 5 min 40% ACN, 5 - 10 min 30% ACN, 10 - 15 min 20% ACN, 15 - 16.5 min 30% ACN, 16.5 - 18 min 20 % ACN, 18 -25 min 30% ACN, 25 - 30 min 0% ACN, 30 - 35 min 40% ACN. To our knowledge, the complete separation of CHR and BaA (both with four aromatic rings) and BbF and BkF (with similar 4.5-ring structures) on a C18 column with DAD detection has not been reported in the literature, except when using selective columns for PAHs or C18 columns with fluorescence detection.[1, 10] The optimized conditions for the best compromise between resolution and sensitivity were: a flow rate of 1.0 mL/min, a column temperature of 30°C, and a detection wavelength of 259 nm. Fig. 2a shows the chromatogram

for the eleven PAHs standards using the optimized gradient method, while representative chromatograms for food samples are shown in Fig. 2b-c.

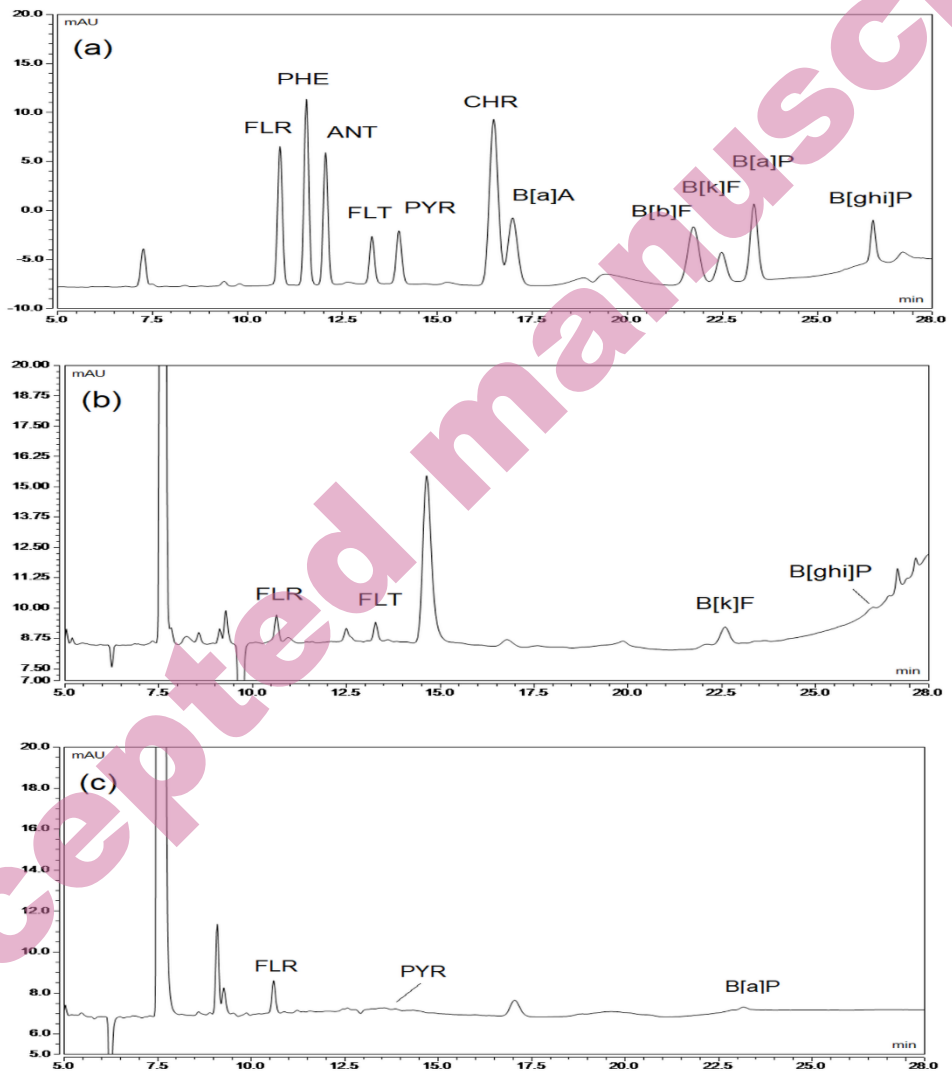

Fig. 2. Chromatogram of PAHs obtained using the HPLC-DAD method under the optimum condition: (a) Standard PAHs (5000 µg/L), (b) Thosai, sample 3 and (c) Youtiao, sample 3.

Validation and comparison with previously reported method

The presence of PAHs in the food samples was identified by comparing the peak retention times with those of the corresponding standards. The chromatographic method's reliability was confirmed by validating parameters such

as linear range, repeatability, reproducibility, limits of detection (LOD) and quantitation (LOQ), and recovery. These validation results are summarized in Tables S4 and S5 of the supplementary data. Excellent linearity was observed, with a range of 10-1000 µg L^{-1} for all eleven PAHs, and correlation coefficients exceeding 0.99 (R^2: 0.9984-0.9997), showing a strong relationship between peak area and analyte concentration.

The LOD and LOQ were calculated using the formulae 3.3 s/S and 10 s/S, where "s" is the standard deviation of the y-intercept, and "S" is the slope of the regression analysis.[8] The LOD values for the PAHs ranged from 0.0049 to 0.1232 µg L^{-1}, and the LOQ values ranged from 0.0149 to 0.373 µg L^{-1}, aligning with those reported by others, such as Mahmoudpour et al.[17], who analyzed smoked rice using HPLC-UV with C18 columns, and Hamidi et al.[10], who studied grilled meat using HPLC-FL with PAH-specific columns. Mahmoudpour et al.[17] found LOD and LOQ values of 0.05-0.12 µg L^{-1} and 0.14-0.38 µg L^{-1}, respectively, and Hamidi et al.[12] reported values of 0.03-3.00 µg L^{-1} and 0.08-9.00 µg L^{-1}, respectively.

Repeatability and reproducibility were assessed using relative standard deviation (RSD) values from three consecutive injections of seven standard PAH solutions (10-1000 µg L^{-1}). For intraday repeatability (same-day analysis), RSD ranged from 0.03% to 0.76% for retention time and 1.10% to 3.78% for peak area. Interday repeatability (over five days) showed similarly satisfactory results. Recovery tests were done by spiking the food samples with PAH standards at three concentrations (50, 200, and 400 µg/L). Recovery values ranged from 47.3% to 119.7% in Chapati and from 77.3% to 119.6% in Keropok Lekor (Table S5). Comparable results with low recoveries for some PAHs were also observed by Zachara et al.[2] in tea samples and by Hamidi et al.[12] in charcoal-grilled beef and chicken. These low recoveries may be attributed to differences in food type and composition, as suggested by Navarro et al.[18], who noted that whole-wheat flour may adsorb PAHs, leading to incomplete desorption in Chapati samples. All eleven PAHs were successfully resolved and eluted in under 28 minutes using a C18 column, a satisfactory result in line with other studies.[1, 10] The elution time was faster compared to those reported in similar studies.[1, 2]

In general, PAHs extraction from food samples involves liquid-liquid extraction (LLE) followed by solid-phase extraction (SPE).[19] Commonly used solvents like n-hexane and toluene benefit from π-π interactions with PAHs, and SPE cartridges are often used to purify the extracted solvent, reducing solvent use and improving recoveries. However, SPE can be time-consuming and require multiple steps.[6] Newer microextraction techniques like solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), and liquid-phase microextraction (LPME) address some limitations of LLE and SPE, such as high solvent consumption and extended extraction times. SPME, for instance, uses

minimal solvent but has issues with limited fiber life and sample carryover. SBSE is selective toward non-polar solutes and requires a long equilibrium time. LPME, particularly dispersive liquid-liquid microextraction (DLLME), is a simpler and more cost-effective approach but uses halogenated solvents that can pose environmental and health risks.[6]

The method developed in this study improves upon LLE without requiring SPE or SPME, achieving higher PAH recoveries (mean recoveries of 70.9% for Chapati and 109.5% for Keropok Lekor) using acetonitrile with 30% acetic acid. This is notably better than when SPE was employed (recoveries of 26.4% and 50.5%, respectively), likely due to the partial adsorption of PAHs onto the SPE adsorbent. This method is simpler and faster as it avoids the additional time and steps associated with SPE or SPME. Its analytical performance in terms of linearity, LOD, LOQ, repeatability, reproducibility, and recovery are comparable to other reported methods.[17,19] Moreover, the exclusion of macromolecules such as proteins and fats were made easier by their unfavorable interaction with the protic acidic solvent.[20]

Matrix effect

The matrix effect (ME) was evaluated by comparing the slopes of the linear equations for the eleven PAHs in both the solvent and the matrix.[8] This effect, calculated using the following formula:

$$ME = \frac{slope\ with\ matrix - slope\ without\ matrix}{slope\ without\ matrix} x\ 100\% \quad (1)$$

was investigated for two food categories: Chapati and Keropok Lekor. The results, provided in Table S6, indicated a more significant matrix effect in Chapati than in Keropok Lekor. For Chapati, several PAHs, including FLR (-58.39%), PHE (-54.92%), ANT (-49.85%), CHR (42.62%), B[b]F (29.58%), and B[a]P (29.93%), showed substantial ME, exceeding the cut-off value of ±20%.[8] In contrast, only PHE (33.40%) and CHR (24.70%) exhibited significant ME in Keropok Lekor.

Statistical analysis using the t-test revealed significant differences ($p < 0.05$) in the slopes of PAHs spiked to Chapati but not to Keropok Lekor (Table S6), likely due to flour adsorption in Chapati.[18] Food products like Roti Canai, Thosai, and Youtiao, which contain high amounts of dehydrated flour, are expected to experience similar flour adsorption.[21] Conversely, food items such as Kuih Tayap, Apam Balik, and Pisang Goreng, which have lower flour content and higher levels of other ingredients (e.g., coconut, banana), are less prone to this effect.[22] Based on these findings, food samples suspected of flour adsorption were quantitated using the linear plot for Chapati, while samples with less flour adsorption were quantitated using the Keropok Lekor model. The LOD and LOQ values obtained in the food matrices (Chapati: mean LOD 0.3544 mg L^{-1}, mean LOQ 1.0742 mg L^{-1}; Keropok Lekor: mean LOD 0.3447 mg L^{-1}, mean LOQ 1.0445 mg L^{-1}) were

higher than those in the solvent (mean LOD 0.2847 mg L^{-1}, mean LOQ 0.8630 mg L^{-1}). Thus, the matrix-derived LOD and LOQ values were used for all analyses.

Analysis of food samples

The developed method was applied to analyze PAHs in 10 food samples, categorized as toasted (Roti Canai, Chapati, Thosai, Kuih Tayap, Apam Balik) and fried (Youtiao, Keropok Lekor, Pisang Goreng, Cekodok, Fried Chicken). Each sample was collected from different food stalls and analyzed in triplicate. Quantification was performed using the external standard method, with calibration plots fitted by least squares linear regression. The analysis results are summarized in Table S7.

As shown in Table S7, light PAHs (compounds with two to four fused benzene rings, including FLR, PHE, ANT, FLT, PYR, CHR, and BaA) were detected in both toasted and fried foods. Heavy PAHs (compounds with four or more aromatic rings, such as BbF, BkF, BaP, and Ghi) were found in fried foods. Light PAHs accounted for 82.3% of the total PAHs (mean total: 3525.2 μg kg^{-1}), while heavy PAHs contributed 17.7% (mean total: 757.5 μg kg^{-1}) to the total PAHs (mean total: 4282.7 μg kg^{-1}).

Fried foods generally contained more PAHs than toasted foods. About 64.4% of light PAHs were detected in fried foods, compared to 35.6% in toasted foods. Similarly, 86.5% of heavy PAHs were found in fried foods, whereas only 13.5% were detected in toasted foods. This suggests that fried foods contain higher amounts of both light and heavy PAHs.

Light PAHs are generally less toxic, as the International Agency for Research on Cancer (IARC) classifies them as Group 3 (not carcinogenic).[3] These include FLR, PHE, ANT, FLT, PYR, and B[ghi]P. In contrast, B[a]P, a Group 1 carcinogen, and other heavy PAHs like BaA, BbF, BkF, and CHR, classified as Group 2B (possible carcinogens), pose higher toxicity concerns. Fried foods were found to have higher levels of Group 2B PAHs (mean total: 487.7 μg kg^{-1}) compared to toasted foods (mean total: 193.2 μg kg^{-1}). Additionally, the total PAH content in fried foods (mean total: 2925.3 μg kg^{-1}) was significantly higher than in toasted foods (mean total: 1357.4 μg kg^{-1}). This could be attributed to the higher temperatures involved in frying, which promotes PAH formation.

CONCLUSION

This study introduced a novel PAH extraction method using acetic acid coupled with organic solvents for analyzing toasted and fried foods. The method optimized acid compositions and solvent types, with 30% acetic acid coupled with acetonitrile showing improved extraction efficiency and eliminating the need for SPE. The analysis of 10 food samples revealed that light PAHs are commonly found in both toasted and fried foods, while heavy PAHs are more prevalent in fried foods. Fried foods were found to contain higher levels of both light and heavy

PAHs, with some fried items posing higher carcinogenic risks due to the presence of BaP and Group 2B PAHs. Overall, this extraction method provides a simple and effective alternative for PAH analysis in food samples.

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И З В О Д

ТЕЧНО-ТЕЧНА ЕКСТРАКЦИЈА СИРЋЕТНОМ КИСЕЛИНОМ И UHPLC-DAD ОДРЕЂИВАЊЕ ПОЛИЦИКЛИЧНИХ АРОМАТИЧНИХ УГЉОВОДОНИКА У ТОСТИРАНОЈ И ПРЖЕНОЈ ХРАНИ

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Полициклични ароматични угљоводоници су класа органских једињења која садрже два или више спојених ароматичних прстенова. Имају веома ниску растворљивост у води и веома су липофилни. Сирћетна киселина је поларни протонски растварач који се често користи у реакцијама које укључују карбокатјон интермедијере. У овом раду смеша сирћетне киселине и других органских растварача, је оптимизована и коришћена за екстракцију једанаест полицикличних ароматичних угљоводоника у тостираној и прженој храни. UHPLC-DAD метода са C18 колоном, је валидирана и примењена за анализу једанаест полицикличних ароматичних угљоводоника у узорцима хране. Добијене вредности LOD и LOQ кретале су се од 0.0049 to 0.373 μg L^{-1}, а рикавери од 47,3% до 119,7%. Резултати анализе показују да се лаки полициклични ароматични угљоводоници обично налазе у обе врсте хране. Нека пржена храна је веома канцерогена због присуства BaP и полицикличних ароматичних угљоводоника групе 2Б. Тостирана храна је безбедна за конзумирање.

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