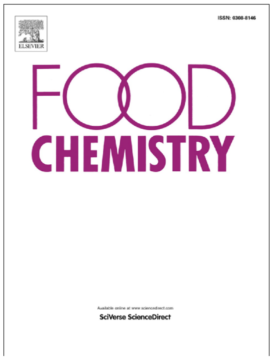

Extraction and analytical approaches for the determination of post-food processing major carcinogens: A comprehensive review towards healthier processed food

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Extraction and analytical approaches for the determination of post-food processing major carcinogens: a comprehensive review towards healthier processed food

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Abstract

Different food processing methods, e.g. fermentation, grilling, frying, etc., to improve food sensory attributes or shelf-stability are typically employed in different cuisines worldwide. These methods may illicit *in-situ* health-hazardous chemicals *via* thermal or enzymatic-mediated processes or chemical interactions with food preservatives. This review provides a comparative overview of the occurrence, extraction, and determination of the major food carcinogens such as nitrosamines (NAs), biogenic amines (BAs), heterocyclic aromatic amines (HAAs), polycyclic aromatic hydrocarbons (PAHs), ethyl carbamate (EC), and malondialdehyde (MDA). Their carcinogenicity levels vary from group 1 (carcinogenic to humans) e.g. benzo[a]pyrene, group 2A (probably carcinogenic to humans) e.g. N-nitrosodiethylamine, group 2B (possibly carcinogenic to humans) e.g. chrysene or group 3 (non-classifiable as carcinogenic to humans) e.g. MDA. Chromatography-based methods are the most predominant techniques used for their analysis. LC-MS is widely used for both volatile/non-volatile NAs, HAAs, BAs, and EC, whereas GC-MS is applied more for volatile NAs, PAHs and MDA.

Keywords:

Food carcinogens; nitrosamines; biogenic amines; heterocyclic aromatic amines; polycyclic aromatic hydrocarbons; ethyl carbamate; malondialdehyde

1. Introduction

Various processing methods are applied to produce tasty food for consumption or for preservation purposes. However, some of these methods generate pro-carcinogens and carcinogens. For example, a strong correlation existed between processed meat, either being salted, cured, fermented, or smoked, and the increased risk of colorectal cancer (Farvid et al., 2021; Santarelli, Pierre, & Corpet, 2008). Salt-preserved food, such as pickled vegetables or dried/salted fish, was found to increase stomach cancer. In addition, an association between fermented food known as Cantonese-style salted fish and nasopharynx cancer was also reported. The International Agency for Research on Cancer (IARC) also stated that 50 g processed meat/day increase the incidence of pancreatic, colon, breast, and prostate cancer by 19%, 18%, 9%, and 4%, respectively (Stewart & Wild, 2014). The IARC classified compounds, based on their carcinogenicity level, into group 1 (carcinogenic to humans), group 2A (probably carcinogenic to humans), group 2B (possibly carcinogenic to humans), and group 3 (not classifiable as carcinogenic to humans). For example, polycyclic aromatic hydrocarbons (PAHs) contain compounds belonging to all IARC groups: group 1 as benzo[a]pyrene, group 2A as dibenzo[a,h]anthracene, group 2B as chrysene, and group 3 as pyrene (IARC, 2010b). While nitrosamines (NAs) and heterocyclic aromatic amines (HAAs) contain compounds classified as group 2A and group 2B (Smoke & Smoking, 2004).

Several carcinogenic compounds are formed in processed meat and cooked red meat. Among these toxic chemicals, NAs are produced due to the addition of nitrites and nitrates used as preservatives in processed meat (Cantwell & Elliott, 2017). PAHs and HAAs are mainly formed due to extensive thermal cooking as in grilling (Farhadian, Jinap, Faridah, & Zaidul, 2012), barbecuing (Pan et al., 2020), roasting and frying (Xu et al., 2021). PAHs are commonly found in smoked fish and meat, and HAAs are found in well done meat and poultry (Turesky, 2018). As polar HAAs are primarily produced at typical cooking temperatures ranging from 100 to 300°C, while non-polar HAAs are typically generated at temperatures exceeding 300°C. However, lower amounts of HAAs are found in stewed meat dishes (M. Khan, Bertus, Busquets, & Puignou, 2009; M. R. Khan, Naushad, Alothman, Alsohaimi, & Algamdi, 2015). Furthermore, biogenic amines (BAs) and ethyl carbamate (EC) are produced more upon storage and fermentation of fish and alcoholic beverages (Cao, Xu, Xue, Feng, & Zhang, 2019; Kobets, Smith, & Williams, 2022; Mo, He, Xu, Huang, & Ren, 2014; Rabie, Peres, & Malcata, 2014). It should be noted that BAs are also considered as precursors of NAs (EFSA, 2007; Hazards, 2011) and add more to their health risks. Moreover, malondialdehyde

(MDA) is a reactive lipid peroxidation aldehyde which is present in practically all lipid-containing diets, (0.1 to 10 mg/kg) (Demir et al., 2024).

The toxicity of these compounds is correlated to their metabolic activation, which leads to the formation of reactive intermediates that could attack DNA and vital proteins. For example, the mutagenic effect of NAs is attributed to the formation of reactive species such as alkyl diazonium and carbonyl compounds *via* cytochrome P450 (Paulinus & Tinuade, 2013; Xie et al., 2023). Similarly, the carcinogenic effect of EC is mediated *via* cytochrome P450 and esterase enzymes, resulting in vinyl carbamate epoxides, which form DNA and RNA adducts (Jiao, Dong, & Chen, 2014), (Sakano, Oikawa, Hiraku, & Kawanishi, 2002; Weber & Sharypov, 2009). PAHs are metabolised *via* cytochrome P450, epoxide hydrolase, and aldo-keto reductase increasing cellular oxidative stress and generating diol-epoxides, radical cations, and o-quinones, which can form adducts with DNA, and cellular proteins (Bulanda & Janoszka, 2022; Moorthy, Chu, & Carlin, 2015). HAAs are also metabolised *via* cytochrome P450 yielding *N*-hydroxylamines which are further metabolised by acetyltransferase and sulfotransferase into highly reactive compounds attacking DNA and proteins. Another HAAs' metabolic interaction in the presence of nitric oxide generates reactive diazonium ion compounds, which also induce DNA damage (Bulanda & Janoszka, 2022; X. Chen, Jia, Zhu, Mao, & Zhang, 2020).

Accordingly, several health hazards are associated with the ingestion of these chemicals warranting the development of methods that can allow for their rapid detection at sensitive levels for routine screening in order to meet food industry needs. The analytical workflow for the detection of these hazards typically proceeds with food matrix homogenisation, analytes extraction, and concentration, and finally detection and quantification. Analytes extraction is undertaken using classical methods either liquid-liquid extraction (LLE) or solid phase extraction (SPE) for enrichment purposes. However, these methods have limitations, including the use of large amounts of toxic solvents and lengthy processing time. On the contrary, eco-friendly micro-extraction techniques including dispersive liquid-liquid micro-extraction (DLLME) and solid phase micro-extraction (SPME) are simple, not labor-intensive approaches. Moreover, these microextraction techniques minimize heavily toxic solvents, while effectively enriching targeted analytes prior to detection (Jagirani & Soylak, 2022). For example, DLLME could successfully reduce solvents consumption at the microliter range.

This review presents an updated overview of analytical methods used for the detection of food carcinogens i.e., NAs, BAs, EC, PAHs, HAAs, and MDA. In the following subsections, the details for each family of compounds regarding needed optimisation in extraction and detection are presented to aid researchers or food analysts in identifying the best methods in different food matrices. Each subsection delves into the occurrence, extraction and analytical strategies for each food contaminant, highlighting the major chemical forms found in food, the best methods for their assessment together with advantages and any limitations of the method.

2. Nitrosamines (NAs) occurrence in different food sources, extraction, and analysis

NAs are chemical compounds of the structure $R_1(R_2)N\text{-}N{=}O$, where R_1 and R_2 are aliphatic, cyclic (aromatic, or heterocyclic) groups. NAs are produced due to a reaction of nitrites (NO_2^-) with naturally occurring amines in food as secondary and tertiary amines (EFSA et al., 2023). For example, nitrites and nitrates are added as preservative to processed meat, i.e., bacon, sausages, and ham during their processing (Cantwell & Elliott, 2017; Honikel, 2008). Nitrite is added as pickling salt to beer and some dairy products, leading to NAs formation during manufacturing (Crews, 2010). Different cooking methods also result in NAs generation, such as, frying dry-cured sausages which increases NAs content by about 30% whereas microwaving or boiling cooking methods did not affect the NAs levels (Škaljac et al., 2014).

NAs are typically classified as volatile (VNAs) and nonvolatile (NVNAs). Extensive carcinogenicity and genotoxicity studies reported by The European Food Safety Agency (EFSA), on NAs, indicated the carcinogenic effect of VNAs over NVNAs. Examples of the major NAs in dietary sources include nitrosodimethylamine (NDMA), *N*-Nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), N-nitrosomethylaniline, nitrososarcosine, *N*-nitrosomorpholine (NMOR), *N*-nitrosopiperidine (NPIP), and *N*-nitrosopyrrolidine (NPYR) (EFSA et al., 2023). Previously, the IARC classified NDEA and NDMA as group 2A, and NMEA, NPYR, NPIP as group 2B carcinogens (Smoke & Smoking, 2004). Despite the fact that NVNAs are typically detected at higher levels than VNAs, yet NVNAs do not exhibit carcinogenic activity like VNAs. However, NVNAs are still of concern, as they may get converted during food processing to their corresponding decarboxylated VNAs, viz. nitrososarcosine and *N*-nitrosoproline (NPRO) to NDMA and NPYR, respectively (Herrmann, Duedahl-Olesen, & Granby, 2015). Several extraction methods and analytical techniques are used to determine NAs in different foods and beverages as presented in **Table 1.**

2.1. NAs Extraction methods

To remove interferences and achieve acceptable recoveries, it is crucial to implement a reliable protocol for the clean-up and efficient extraction of the targeted analytes. Several extraction methods including the conventional methods and micro-extraction-based ones were used for NAs. However, applying LLE mostly necessitates SPE for analytes' enrichment and purification. Each method has its merits and demerits regarding simplicity, time-consumption, cost, etc. as explained in detail for each carcinogen below. Examples for the major techniques are presented in the next subsections for conventional and modern ones.

2.1.1. NAs liquid-liquid extraction (LLE)

Solvents of different polarities can greatly influence the extraction efficiency of targeted NAs in complex matrices. A study was conducted *via* GC-MS/MS on dry-cured mackerel samples, collected from the Chinese market,to detect 9 NAs compounds. Using solvents of different polarities such as acetonitrile, acetone, n-hexane, and ethyl acetate, acetonitrile was selected as the best solvent with recovery yields of 77.10%-112.9%. On the contrary, acetone and n-hexane resulted in a low recovery of <70%, whereas ethyl acetate failed to detect low MW NDMA (Dong, Li, et al., 2020). In another study, acidified acetonitrile with 1% formic acid suppresses the dissociation of the acidic NAs analogues such as *N*-nitrosohydroxyproline and *N*-nitrososarcosine. This led to improved extraction efficiency using organic solvents (Herrmann, Duedahl-Olesen, & Granby, 2014).

After LLE, the clean-up step is necessary to remove any interferences remaining in the organic extract, which could be achieved *via* saponification using sodium hydroxide (NaOH). However, a study on meat samples applying 0.1 M phosphate buffer (pH 7.0) as a cleaning solution resulted in higher extraction efficiency of VNAs, except for NDPH, compared to an alkaline 0.1 N NaOH solution. This was attributed to the weak acidic nature of these VNAs; hence, their solubilisation in the organic phase was enhanced at neutral pH, in addition to the effect of phosphate buffer in solubilising polar co-extracts that are potentially present in the organic phase after LLE step (Scheeren, Sabik, Gariepy, Terra, & Arul, 2015).

Volatile NAs that are nonpolar and have low molecular weight are separated *via* distillation from foods. Steam distillation is one of the conventional techniques for extracting NAs from food matrices followed by LLE using a low boiling point organic solvent such as dichloromethane (DCM) which is subsequently removed under vacuum (S. Wang et al., 2023; Q. Zhu, Wang, Liu, & Zhang, 2015).

2.1.2. NAs solid phase extraction (SPE)

SPE is widely used for sample enrichment and purification. However, a sample pretreatment step was achieved *via* steam distillation. The aqueous distillate was subjected to SPE using activated charcoal (Sanches Filho, Rios, Valcárcel, Zanin, & Caramão, 2003). The extraction efficiency was enhanced, with improvement in sensitivity after enrichment *via* SPE as reflected by lower LOD at *ca*. 10-fold decrease (Sanches Filho et al., 2003). A fast and simple purification method based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was realized for the extraction of 7 VNAs in Chinese Sichuan salted vegetables. Salt-assisted extraction *via* dispersive SPE (d-SPE) yielded higher recovery (91.5-106%) (Q. Zhang et al., 2019). QuEChERS was applied widely in different food matrices, such as cooked bacon (Lehotay, Sapozhnikova, Han, & Johnston, 2015) and soy sauce (Zeng, Bai, Xian, Dong, & Luo, 2016) resulting in acceptable recoveries as listed in **Table 1.**

2.1.3. NAs Micro-extraction methods

2.1.3.1. NAs dispersive LLME (DLLME)

DLLME is an excellent alternative to LLE as it limits the use of large amounts of organic solvents and increases method sensitivity by concentrating analytes in small portions. DLLME is based on a ternary solvent system (two immiscible solvents and a dispersive miscible solvent). The DLLME efficiency may be improved *via* either ultrasonic-assisted extraction as in the extraction of certain NAs which yields a satisfactory recovery ranging from 92.80 – 102.10% (Lu et al., 2017). Microwave-assisted extraction (MAE) displayed good NAs recovery from sausage and salami ranging from 83.9 – 109.4% (Ramezani, Hosseini, Kamankesh, Ghasemzadeh-Mohammadi, & Mohammadi, 2015).

2.1.3.2. NAs solid phase micro-extraction (SPME)

SPME is a sensitive and robust technique for NAs extraction from dietary sources due to its high extraction efficiency, mostly suited for high volatile and low molecular weight NAs, and in complex matrices that comprise lipids and proteins. Four main parameters should be optimised to obtain high extraction efficiency by SPME including temperature, extraction time to allow sufficient equilibrium for partitioning the analytes between the extracting phase and the fibers' surface, as well as SPME fibers and the ionic strength of the solution (Lona-Ramirez, Gonzalez-Alatorre, Rico-Ramírez, Perez-Perez, & Castrejón-González, 2016).

Fiber selectivity is the most detrimental factor in SPME technique, which depends on the physiochemical characteristics of the targeted analytes. For example, polydimethylsiloxane/divinylbenzene (PDMS-DVB- 65 μm) coated fiber showed higher recovery than polyacrylate-coated fiber (PA, 85 μm) in extracting NDMA and NDEA compounds as PDMS-DVB-coated fiber has an intermediate polarity compared to the highly polar PA-coated fiber. However, PA-coated fiber was found better in the extraction of the more polar compounds NPIP and NPYR (Andrade, Reyes, & Rath, 2005). In another study, head-space SPME fitted with carboxen (CAR)/DVB/PDMS fiber was selected to extract 9 VNAs from meat samples. Excellent recovery levels were detected at 92–113%, with an LOD and LOQ of 3.6 µg/kg and 12 µg/kg, respectively. However, unsatisfactory results with LOD of 56 µg/kg and LOQ 188 µg/kg was reported for NDMA, likely attributed to the conditioned temperature for extraction, as the highest recovery for NDMA was detected at 45℃, whereas set temperature in this method was at 65℃ (Roasa, Liu, & Shao, 2019).

Despite conventional LLE's widespread application, intensive labour requirement, and prolonged extraction time are considered a major challenge especially at large scale as it requires extract clean up to remove any interferences that could disturb analytes' detection. Also, in many studies, it is essential to apply SPE for NAs enrichment for better sensitivity level. In contrast, microextraction techniques provide eco-friendly alternatives that solved some of these issues regarding reducing the amounts of organic solvents used and relying on microwave-assisted extraction (MAE) and ultrasonic-assisted extraction, which efficiently assist in rupturing food matrices and liberate NAs in a shorter time. Moreover, microwave heating offers several advantages over conventional heating as the mixture directly absorbs energy, leading to a rapid temperature gradient that improves extraction efficiency. These advantages have established microwave-assisted approaches as a significant aspect of green chemistry due to their eco-friendliness compared to conventional methods. The best extraction and highest recovery were achieved *via* \`fiber\` based SPME. A comparison between conventional extraction and micro-extraction methods for NAs is presented in **(Figure 1).**

2.2. NAs analytical techniques

Accurate analysis of NAs requires a combination of efficient separation techniques as chromatographic or capillary electrophoresis coupled to sensitive detectors such as MS, fluorescence detector (FLD), or thermal energy analyzer (Andrade et al., 2005). Capillary electrophoresis coupled with UV-Vis detector was also applied for the analysis of NAs in preserved sausages (Sanches Filho et al., 2003). Examples represented with their validation are listed in **Table 1.**

2.2.1. NAs Liquid chromatography- ultraviolet/ fluorescence detector (LC-UV/FLD)

NAs are not fluorescent on their own, warranting a derivatisation reaction using 2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate. For example, 5 NAs (NPYR, NDMA, NDEA, NDPA, NDBA) were analysed using HPLC-FLD which afforded a high sensitivity with satisfactory detection and quantification limits within 0.01–0.07 µg/kg and 0.03 to 0.21 µg/kg, respectively, as well as high recovery values within 92.80–102.10% range (Lu et al., 2017), as shown in **Table 1**. Another 5 VNAs (NDMA, NDEA, NPYR, NPIP, NMOR) were extracted from edible marine food and analysed using HPLC-UV method which exhibited a robust, rapid method with low detection limit at 0.17 - 0.4 µg/kg (Bhangare, Sahu, & Pandit, 2013).

2.2.2. NAs liquid-chromatography-mass spectrometry (LC-MS)

LC-MS is considered a potential technique for the analysis of VNAs and NVNAs (Herrmann et al., 2014). Although both VNAs & NVNAs can be ionized by either atmospheric pressure chemical ionization (APCI) or electrospray ionisation (ESI), APCI showed higher sensitivity than ESI attributed to the reduction in matrix interferences and an increase in the signal to noise ratio, especially for VNAs (LOD 0.2 - 1 µg/kg). The same procedure was employed to determine NAs in Danish meat, with LOD at 2.3 - 4.2 µg/kg for NVNAs versus 0.1–0.5 µg/kg for VNAs (Niklas, Herrmann, Pedersen, Jakobsen, & Duedahl-Olesen, 2022).

2.2.3. NAs gas chromatography- mass spectrometry (GC-MS)

VNAs exhibit physiochemical characteristics as they are nonpolar and low molecular weight compounds, which makes them ideally suited for GC-MS analysis (Yuan Yuan et al., 2015). For example, analysis of 7 VNAs using GC-MS/MS in different food products firstly extracted by SPE sorbents: Extrelut NT and Florisil, and ionisation was done by positive chemical ionisation (CI) using ammonia as reagent gas to enhance their ionisation alongside Multiple reaction monitoring (MRM) for quantification. The high sensitivity and accuracy were presented by 0.10–0.25 µg/kg and 0.30–0.74 µg/kg for LOD and LOQ, respectively (Seo et al., 2022). In another study, 8 VNAs *viz*. NDMA, NMEA, NDEA, NPYR, NDPA, NPIP, NDBA, and *N*-nitrosodiphenylamine, were detected in 28 selected Chinese meat products with an LOD and LOQ of 0.057–0.495 µg/kg and 0.189–1.649 µg/kg, respectively using GC-EI/MS(Yuan Yuan et al., 2015).

Among all reviewed analytical techniques, poor sensitivity was obtained through applying micellar electrokinetic chromatography (MEKC)-PDA and GC coupled to thermal energy analyser (TEA), whereas GC-MS showed excellent performance with good sensitivity as shown in **Table 1**. The key drawback for GC-MS is that it is limited to VNAs, unlike LC-MS, which could be used for both VNAs and NVNAs. UV-Vis detector and FLD were also used for NAs detection with a satisfactory sensitivity result. FL based detectors are affordable and easier to operate than MS, aside from enhanced sensitivity upon derivatisation. However, since NAs are not fluorescent on their own, derivatisation is indispensable in this approach, which potentially increases the analysis time. Nevertheless, the best sensitivity was attained *via* LC-FLD.

3. Biogenic amines (BAs) occurrence in different food sources, extraction, and analysis

BAs are a type of nitrogen-containing aliphatic, aromatic or heterocyclic compounds generated mainly *via* thermal or enzymatic-mediated decarboxylation from their corresponding amino acids. According to the number of amino groups in the molecule, BAs are classified to mono-, di-, and poly-amines. The most common BAs found in foods are histamine, tyramine, cadaverine, phenylethylamine, spermine, spermidine, putrescine, and tryptamine. These BAs are derived from histidine, tyrosine, lysine, phenylalanine, arginine, ornithine, glutamic, and tryptophan, respectively (Naila, Flint, Fletcher, Bremer, & Meerdink, 2010). Fermentation is one of the typical food processing methods that results in BAs generation (**Figure 2**). It was reported that the maximum allowed limits of BAs in foods for human consumption such as phenylethylamine (Phe), histamine (His), tyramine (Tyr), and total BAs were at *ca*. 30, 50–100, 100–800, & 200–1000 mg/kg, respectively (Ec, 2003; Kandasamy et al., 2021; El-Sayed, Ibrahim, & Farag, 2022).

For BAs determination in food, different preparation methods were reported that mostly depend on food matrices to eliminate any interferences. For instance, the addition of acids i.e., trichloroacetic acid, perchloric acid, and hydrochloric acid for protein precipitation occurs especially in food with high protein content (**Figure 2**) (Erim, 2013). Multiple extraction methods are employed to extract BAs, such as LLE, SPE, or micro-extraction-based approaches as DLLME and SPME. The ‘Dilute and shoot’ strategy is also a convenient technique to extract BAs from low-protein content samples such as wine and beer, in which samples are diluted, filtered, and subjected to analytes concentration prior to detection (Manetta et al., 2016). A list of the major methods for BAs` extraction alongside their analysis is listed in **Table 2.**

3.1. BAs extraction methods

3.1.1. BAs liquid-liquid extraction (LLE)

LLE was used to extract 8 BAs including tryptamine, phenylethylamine, putrescine, cadaverine, His., Tyr., spermidine, and spermine from Turkish dairy products. Diethyl ether was used for extraction from acidified samples and derivatisation by dansyl chloride (DNS-Cl). The method resulted in excellent recovery at 95 – 100.7% (Pekcici, Guler, & Topkafa, 2021). A more polar solvent acetonitrile was used to extract the same 8 BAs, from acidified wine. This method proved high sensitivity with LOD and LOQ at 0.001 – 0.050 mg/L and 0.005 – 0.167 mg/L, respectively. The recoveries of the targeted analytes were up to 111.6%

(Y. Liu, Han, Liu, & Wang, 2020). Furthermore, salting-out-assisted liquid-liquid extraction enhanced the extraction efficiency of BAs from cheese by providing phase separation, which increased analyte recovery. Among different salts, NaCl was the most favoured as Cl^- anion did not disturb the basic pH of the derivatisation matrix at which BAs are deprotonated, which is ideal for derivatisation. High sensitivity with LOD, LOQ, and good recovery at 0.015 – 1.77 mg/L, 0.050 – 5.91 mg/L, and 94 – 110%, respectively were detected (Ramos, Brandão, & Rodrigues, 2020).

3.1.2. BAs solid phase extraction (SPE)

Compounds present in wine samples are susceptible to derivatisation alongside targeted BAs, which adversely affected the method reliability. Therefore, adsorbents such as poly(vinylpolypyrrolidone) PVPP, as well as primary, secondary amine (PSA), and octadecylsilane (C18) are used to successfully remove polyphenolics, polar and non-polar impurities, respectively (Daniel, Dos Santos, Vidal, & do Lago, 2015) and (Xian et al., 2016).

PVPP can adsorb BAs *via* π- π interactions, hydrogen bonds, and Van der Waals forces. PVPP was used in the assay of 4 BAs in whey protein *viz*. His., Tyr., spermine, and dopamine. Low LODs (0.22–0.47 µg/L) and LOQs (0.74–1.6 µg/L) were detected with moderate recovery at 64–87% (Mirzaei, Mogaddam, & Khandaghi, 2022). Also, cation exchange d-SPE sorbent (cation exchange polymeric Dowex® 50W X8) was used for determination of BAs in wine samples for clean-up and analytes concentration as prior to derivatisation using benzoyl chloride (Bnz-Cl). This leads to remove interferences concurrent with satisfactory recovery (72 - 99%) (Milheiro, Ferreira, Filipe-Ribeiro, Cosme, & Nunes, 2019). These results indicated that applying d-SPE resulted in 5-folds enrichment of BAs levels compared to poor sensitivity (LOD: 0.2-2.5 mg/L) results obtained from direct analysis using same derivatization, albeit without the d-SPE step (Özdestan & Üren, 2009).

3.1.3. BAs micro-extraction methods

3.1.3.1. BAs dispersive liquid-liquid micro-extraction (DLLME)

One of DLLME merits in BAs extraction lies in providing *in situ* derivatisation in which extraction and derivatisation occur simultaneously. Applications include extraction of BAs in different liquors (Cunha, Lopes, & Fernandes, 2017), and beers (Almeida, Fernandes, & Cunha, 2012). Toluene and acetonitrile were used as extractors and dispersive solvents, respectively, and isobutyl chloroformate (IBCF) for derivatisation. The reported recovery values in the two studies were at 72 - 113%, and 79–108%, respectively.

DLLME can also be assisted with eco-friendly techniques such as microwave or ultrasonication (**Figure 2**), which are effective in reducing the time needed for sample preparation particularly within complex food matrices. For example, extraction of BAs from cheese samples employed microwave-assisted extraction (MAE) for 3 min at 500 MHz combined with DLLME. MAE is a process that employs microwave energy to heat solvents in the vicinity of a sample, in order to release the analytes from the sample matrix into the solvent. Results showed that this combination exhibited lower LODs and LOQs in the ranges of 5.9 - 14.0 µg/kg and 19.7 - 46.2 µg/kg, respectively, as well as high recovery values of 97 - 103% (Mohammadi, Kamankesh, Hadian, Mortazavian, & Mohammadi, 2017).

7 BAs i.e., PUT, CAD, His, TRP, Tyr, SPD, and SPM were determined in different food and beverage matrices by DLLME with ionic liquid instead of organic solvent. 1-Butyl-3-methylimidazolium hexafluorophosphate and acetonitrile were used as extractor and dispersive solvents, respectively, followed by derivatisation using DNS-Cl. This method proved cost-effective, fast with a low LOD and LOQ of 0.06 - 0.3 mg/L and 0.19 - 0.99 mg/L, respectively with similar high recovery (70.7 - 118.4%) (Bani, Saaid, & Saad, 2020).

3.1.3.2. BAs solid phase micro-extraction (SPME)

Direct immersion-SPME was successfully employed to derivatise and extract BAs simultaneously. For example, 4 non-volatile BAs (PUT, CAD, His, and Tyr) were determined in fish samples. In brief, the fiber polydimethylsiloxane/divinylbenzene (PDMS/DVB) was dipped in a mixture of isooctane as an extraction solvent and isobutyl chloroformate (IBCF) as a derivatisation reagent. This method provided high sensitivity with LOD and LOQ of 2.98 – 45 µg/kg and 9.83 – 149 µg/kg, respectively alongside recovery of 78.9 – 110% (J. Huang et al., 2016). Similarly, extraction of 14 BAs by direct immersion of polyacrylate-SPME fiber for 40 min in wine sample containing an IBCF for derivatisation showed LODs and LOQs at 0.009 – 0.859 µg/L and 0.028 – 1.634 µg/L, respectively, and recoveries values from 65% - 107% (Papageorgiou, Lambropoulou, Morrison, Namieśnik, & Płotka-Wasylka, 2018).

The performance of fabricated SPME was better than commercial SPME fiber as manifested in case of functionalised mobile composition of matter (MCM) mesoporous silica coat (MCM-T-H) on the SPME fiber, which enhanced the fiber hydrophobicity and acidity, found effective in extracting 6 nonpolar derivatised BAs from pork and fish samples. Results showed a higher extraction efficiency than commercial carboxen (CAR)/DVB/PDMS-SPME with good robustness and stability up to 50 extraction cycles, LOD and LOQ of 1.1 – 27 µg/L and 3.5 – 89 µg/L, respectively. Improved detection was attributed to the high affinity of primary BAs towards the acidic SPME MCM-T-H (M. Chen, Lan, Pan, & Zhang, 2023).

Since extraction of BAs is a detrimental step in their analysis, micro-extraction techniques such as DLLME and SPME provided both higher accuracy and sensitivity. They shortened analysis time as they offer *in situ*-extraction and derivatisation as well as low solvent consumption, unlike conventional LLE which requires high solvent consumption or SPE that resulted in lower accuracy.

3.2. BAs analytical methods

Various chromatographic methods were implemented to measure BAs such as ion-exchange chromatography with colorimetric detection, GC-MS, HPLC-MS/MS and HPLC-PDA (Kandasamy et al., 2021; Loizzo et al., 2013; Rabie, Elsaidy, El-Badawy, Siliha, & Malcata, 2011). Examples are listed in **Table 2.** Although LC-based methods are the most reported techniques used for BAs analysis.

3.2.1. BAs, liquid chromatography- ultraviolet/fluorescence detector (LC-UV-/FLD)

Next to extraction, pre-column derivatisation is typically needed to improve BAs separation on the reversed phase column and further detection using either UV or FLD detectors as they are polar low molecular weight compounds, without either chromophores or fluorophores (Valente, Santos, Gonçalves, Rodrigues, & Barros, 2012) and (Zotou & Notou, 2013). Furthermore, post-column derivatisation is applied to limit interferences, and reduces the time in sample preparation compared to pre-column derivatszation (Triki, Jiménez-Colmenero, Herrero, & Ruiz-Capillas, 2012; Zhao et al., 2007).

Several derivatisation agents were reported for BAs analysis including dansyl chloride (DNS-Cl) owing to its ability to derivatise primary and secondary amines rapidly yielding stable derivatives (Y. Liu et al., 2020), in addition to benzoyl chloride (Bnz-Cl) which also produces stable derivatised amines within 20 minutes (Yañez, Saavedra, Martínez, Córdova, & Ganga, 2012). In contrast, *o*-phthalaldehyde used for BAs derivatisation can only derivatise primary amines and results in unstable derivatives, despite its quick reaction time (Y.-j. Zhang et al., 2019).

The amounts of DNS-Cl and ammonia used for the derivatisation reaction should be monitored carefully as they can directly affect extraction efficiency. Excess levels can increase the susceptibility to react with already-formed derivatized BAs and substantially disrupt the method's accuracy (Y. Liu et al., 2020). BA's successful derivatization process necessitates pH adjustment in the alkaline range to allow the deprotonated form of BAs to become more prevalent for derivatisation and extraction (Ramos et al., 2020) and (Wang, Liu, & Qu, 2021).

In a study targeting 6 BAs in sausage and cheese samples, four derivatising agents were used: DNS-Cl, dabsyl chloride, 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl), and Bnz-Cl followed by detection using UV-Vis detector. The study suggested that dansyl and benzoyl derivatives were more stable and suitable for HPLC than FMOC and dansyl analogues. LOD values were within 0.03 to 0.36 mg/kg and 0.09 to 0.38 mg/kg for dansyl and benzoyl derivatives, respectively (S.-J. Liu, Xu, Ma, & Guo, 2018). In another study, BAs were extracted using dispersive-SPE composed of cation exchange resin followed by derivatisation using Bnz-Cl. This resulted however in a low sensitivity level of LOD within 0.133-0.509 mg/L range (Milheiro et al., 2019). However, an enhancement in sensitivity was detected during the analysis of 6 derivatised BAs in wine and fish samples using DNS-Cl with LOD/LOQ values within 1.3–3.9/4.1–9.9 µg/L, and 1.2–3.8/3.9–9.6 µg/kg, respectively (Cao et al., 2019). It is noteworthy to mention that derivatisation plays a major role in enhancing BAs separation and detection due to their polar and non-volatile nature. However, another option to improve BAs detection without derivatisation process is ion pairing reagent that can be added to the mobile phase used in LC for determining 14 BAs in different beverages, such as beer, dairy beverage, herbal tea, and vinegar. The ion pair reagent promoted the retention of polar compounds as BAs on the stationary phase (Sun, Guo, Semin, & Cheetham, 2011).

3.2.2. BAs liquid chromatography-mass spectrometry (LC-MS)

Compared with FLD, MS operated in product ion mode was found more sensitive, accurate, less time-consuming, demonstrating it more appropriate for analysis in foodstuff, with no need for a derivatisation step, unlike FLD. Improved LOD was detected in (+)ESI/MS/MS at 0.02-0.2 mg/ kg versus 1-4 mg/kg in the case of FLD for the 6 underivatised BAs and their dansyl derivatives by MS/MS and FLD, respectively (Sagratini et al., 2012). In another study, UPLC-MS/MS operated in MRM mode was employed for the detection of 7 BAs extracted from soy sauce using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). UPLC column provided a rapid analysis time of 4 min alongside a sensitive detection method of BAs with LOD and LOQ values of 4–8 µg/kg and 15–30 µg/kg, in addition to recoveries of 84% and 115% (Dong & Xiao, 2017) making it suitable for large screening of samples in the food industry.

3.2.3. BAs gas chromatography-mass spectrometry (GC-MS)

LC columns are more suited for the analysis of BAs compared with GC columns as BAs are polar compounds with low volatility, that is why GC-MS is not commonly used, and derivatisation is a necessity (M. Chen et al., 2023).

Despite this fact, DLLME- GC-MS resulted in high sensitivity with LOD range within 0.3 to 2.9 µg /L for determination of BAs in beer samples. Isobutyl chloroformate (IBCF) was used for derivatisation (Almeida et al., 2012). Same protocol was tested in wine samples, but with propyl chloroformate as a derivatising agent resulting in comparable sensitivity to LC-based techniques by 1.1 to 4.1 µg/L and 3.3 to 11.7 µg/L for LOD and LOQ, respectively (Płotka-Wasylka, Simeonov, & Namieśnik, 2016).

According to this literature survey, chromatography-based methods are the most predominant techniques used for BAs analysis. Other hyphenated techniques such as capillary electrophoresis (CE) was also reported for BAs analysis. For example, CE coupled with contactless conductivity detection revealed good sensitivity (LOD 41 - 98 µg/L) in fermented dairy products without any pre-concentration or derivatisation steps (Adımcılar, Öztekin, & Erim, 2018). Whereas, coupling of CE with MS/MS led to sensitivity enhancement (LOD of 1 - 2 µg/L) and separation within 10 min during the analysis of 9 BAs in beer and wine (Daniel et al., 2015). Among all the reported hyphenated techniques, GC-MS exhibited the highest sensitivity in the picogram range (300 picogram/Kg) (Almeida et al., 2012).

One of the main limitations for the analysis of BAs are the need of separation techniques including HPLC, GC, or CE, which require centralised laboratories, trained personnel and are considerably more expensive. Prolonged chromatographic separations are usually required to exclude the interference of amino acids present in food matrices. The use of a low level of derivatising reagent and minimizing the analytical time are the current challenges facing the analysis of BAs.

4. Ethyl carbamate (EC) occurrence in different food sources, extraction, and analysis

EC, or urethane is an ester of carbamic with the formula $CH_3CH_2OCNH_2$. It is a low molecular weight compound classified by the International Agency for Research on Cancer (IARC) as (Group 2A,; a possible carcinogen to humans (IARC, 2010a). EC is naturally produced in fermented food, i.e., yogurt and cheese, and in alcoholic beverages (Gowd, Su, Karlovsky, & Chen, 2018; G. Li, Zhong, Wang, & Gao, 2017). Precursors used in EC products include hydrocyanic acid, urea, and ethanol during the fermentation process. The lowest levels were found in beer at 0-5 µg/L versus highest in fruit brandy at 260 µg/L (EFSA, 2007).

4.1. EC extraction methods

The Association of Official Agricultural Chemists (AOAC) developed an official method to extract EC from soy sauce and alcoholic beverages, which was realised *via* LLE using dichloromethane (DCM) followed by SPE using Chem Elut in order to eliminate interferences (Canas, Joe Jr, Diachenko, & Burns, 1994; Stadler & Lineback, 2009). In order to apply the AOAC method with solid fermented food, a clean-up step is mandatory using SPE such as

Florisil column prior to GC-MS (Chung, Kwong, & Chen, 2010). DCM is widely used in extraction process from different fermented food, i.e., yogurt, cheese, bread, fermented fish, soybean, liquors and wine (Chung et al., 2010; Jagerdeo, Dugar, Foster, & Schenck, 2002; Lim & Lee, 2011) or a less toxic solvent such as diethyl ether (Nóbrega et al., 2015). For a summary of extraction methods used for EC in different food types, see **Table 3**.

4.1.1. EC, liquid-liquid extraction (LLE)

Extraction of EC from Korean traditional rice wines, *takju* and *yakju*, was done by conventional LLE using chloroform. A poor recovery (16%) was obtained which slightly increased (24%) upon pre-saturation with NaCl before LLE step. Whereas, an additional pH adjustment step resulted in an obvious increase in recovery (81%) salt saturation and pH adjustment to 9, resulted in uncharged EC, minimising its solubility in the aqueous phase and hence, its solubilisation in the organic phase was enhanced in the *takju* sample. On the contrary to the noticeable pH effect on increasing EC recovery in the *takju* sample, a slight decrease (≈ 5%) in EC recovery was observed in the *yakju* samples suggestive of a matrix effect (Woo et al., 2001).

Due to the presence of EC at trace levels, it is warranted to eliminate interferences that adversely affect EC detection by GC-MS. This is a labourious procedure as AOAC official methods are time consuming when adding an extra cleaning up stage with a suitable sorbent and requiring eco-unfriendly organic solvents. To avoid these drawbacks, many solutions are proposed to limit the use of organic solvents. For example, assisted salt-based aqueous two-phases systems (ATPS) extraction techniques exhibited an alternative approach to limit organic solvents consumption. ATPS exhibited satisfactory EC extraction from Maesil wine being partitioned between 2 phases, such as water and ethanol, with the addition of phosphate-concreted salt to improve EC concentration in the ethanolic phase at a recovery of 75.6% (Magollah et al., 2022). Although ATPS showed comparable results to LLE without using an organic solvent, estimated extraction time was longer at 2.8 h, which is considered a drawback.

4.1.2. EC solid phase extraction (SPE)

SPE reduces matrices interferences and enhances sensitivity and accuracy as reported by (Ryu et al., 2015), with chem Elut (diatomaceous earth) as the most often used sorbent for EC extraction. Different extraction sorbents as Chem Elut, Florisil, and primary, secondary amine (PSA), were tested for extracting EC from Chinese liquor. The procedure involves loading liquor samples onto the column followed by EC elution using DCM followed by a concentration step. The results showed that Chem Elut had the best recovery values with 85.29% - 100.82%, with LOD and LOQ of 1.10 and 3.65 µg/L, respectively, versus lowest recoveries in case of PSA and Florisil (Xia et al., 2014). However, this was opposite to results obtained using diatomaceous earth and Florisil for pressurized liquid extraction of EC from complex fermented solid food. Higher EC recovery was obtained using Florisil due to the higher efficiency of Florisil in removing fats and pigments (Liao & Luo, 2014). In another example, ethyl acetate was used to elute EC from alcoholic beverages adsorbed on ENV+ column (a hyper cross-linked styrene−divinylbenzene copolymer) for SPE (Mirzoian & Mabud, 2006). It should be noted that ethanol has to be removed first as it hindered EC retention on ENV+ column, a prerequisite step for the detection of EC in alcoholic beverages.

4.1.3. EC micro-extraction methods

A myriad of SPME based setups were reported in the literature as alternatives to conventional methods. For example, carbowax/divinylbenzene (CW/DVB) fiber was found to be an efficient and reproducible fiber for EC extraction in headspace analysis (Lachenmeier, Nerlich, & Kuballa, 2006; Whiton & Zoecklein, 2002). CW-DVB fiber was used for EC extraction from alcoholic beverages, with high sensitivity (LOD 3 μg/L) and recovery (92.8–97.5%) (Y. Zhang & Zhang, 2008).

A polyethylene glycol/hydroxy-terminated silicone oil fiber is another fabricated fiber used for EC extraction from wine samples. The extraction was assisted with anhydrous Na_2SO_4 within 10 min at 35°C revealing an efficient extraction of EC at LOD of 34 μg/L (Ye et al., 2011). In another example, a headspace single drop microextraction was employed to extract EC from wine samples using a butyl acetate droplet from fortified wine samples. In this study, matrix modifiers such as anhydrous sodium carbonate and sodium sulfate (3:2, w/w) were added to reduce water interference and improve EC extraction under slightly alkaline conditions. This method provided high recovery within 95-99% and LOD of 1.5 μg/L (Z. Ma et al., 2022).

Some of the well-known EC extraction methods are LLE, aqueous two-phases systems, SPE, and SPME and each one has its own merits and demerits. For example, LLE has a relative short extraction time with good reproducibility, though with large consumption of eco-unfriendly organic solvent. Concerning SPE, using a Chem-Elut column showed high reproducibility, but it suffered long extraction time and was costly. Whereas, SPME represents an ecofriendly approach, it has disadvantages of its low reproducibility along with low durability and the high cost of the fiber used.

4.2. EC analytical techniques

Various analytical approaches were developed for EC analysis with hyphenated techniques as the most common ones. GC-MS is the official method stated by the AOAC in alcoholic beverages and soy sauce, which was further modified to reduce any co-extracts by including SPE for a clean-up step prior to GC-MS (Canas et al., 1994; Mo et al., 2014). In addition, LC-based techniques-coupled with affordable and low-cost detectors such as fluorescence detector (FLD), and highly sensitive MS detector were reported in the literature.

4.2.1. EC liquid chromatography- fluorescence detector (LC- FLD)

Although GC-MS is a certified analytical based technique, it requires lengthy preparation steps. Therefore, LC-based approaches are found to be alternative solutions in which fewer steps for the sample treatment are employed. For example, the low-cost FLD requires derivatisation by 9-xanthydrol as a reagent (Herbert, Santos, Bastos, Barros, & Alves, 2002). Moreover, analysis was realised on different wine samples without previous sample extraction or concentration. LC-FLD displayed high recovery (96%) with good sensitivity (LOD 4.2 μg/L) (Herbert et al., 2002).

4.2.2. EC liquid chromatography- mass spectrometry (LC- MS)

Since EC is poorly ionized by electrospray ESI, APCI was found to be an excellent ionisation method in LC-MS-based approaches. A study was conducted to determine EC in Korean rice wine by UPLC-APCI-MS/MS within a 5 minute run time, which leads to high accuracy, and precision with recovery of 96.3%–100.4%, and LOD & LOQ values at 0.17 and 0.51 μg/L, respectively (G.-h. Lee et al., 2017).

4.2.3. EC gas chromatography- mass spectrometry (GC-MS)

The presence of EC at trace levels in complex matrices requires an extra clean-up step to eliminate any interferences that may reduce GC-MS sensitivity. Triple quadrupole mass spectrometry (GC–MS/MS) is known for its high sensitivity preferably used in complex food matrices. GC–EI/MS/MS was applied on MRM mode for EC confirmation and determination in different food matrices such as soy sauce, alcohol, curry products, tomato sauce, salad dressing, and flavouring of instant noodles. In this method, purification by Extrelut™ NT and PSA was found essential to eliminate interferences. Satisfactory results were reported with LOD and LOQ at 1.8 and 4.7 μg /kg, respectively, as well as high recovery values within 88.6–112% (Mo et al., 2014). In cases of high EC level, sample pre-treatment is unnecessary. For example, rapid analysis within 2 min and without sample pre-treatment for sugar cane spirits was reported for EC analysis using direct injection mass spectrometry at an acceptable LOQ and LOD of 75.0 and 7.5 μg/L, respectively (Ribeiro et al., 2018).

From the above data, it can be concluded that hyphenated techniques such as GC-MS/MS and LC-MS/MS are frequently used for EC determination in different food matrices with comparable sensitivity in the picogram range. UPLC-MS/MS displayed fast analysis with slightly higher sensitivity (G.-h. Lee et al., 2017) than that in GC-MS/MS (Liao & Luo, 2014). However, the later requires extensive sample pretreatment to get rid of interferences for proper quantification. HPLC-FLD also provides higher sensitivity, in the low microgram range, though with a derivatisation step to impart fluorophore in EC, which in turn has a negative impact on analysis time.

5. Polycyclic aromatic hydrocarbons (PAHs) occurrence in different food sources, extraction and analysis

PAHs are produced naturally or as end-product of an anthropogenic heat-based process, such as incomplete combustion of organic matter. Food contamination with PAHs is commonly derived from the packaging process, environmental contamination, and food processing (Kim et al., 2022). Moreover, direct and indirect contact between food and heat sources (smoke, emissions, and fumes) can also cause PAHs accumulation on its surface. It was observed that smoke and flames produced by charcoal after it is ignited promoted PAHs and adhered to the surface of beef. The different types of charcoal used as a heat source also impact the formation of PAHs in barbecuing (Z. Zhu et al., 2022).

PAHs are a group of organic compounds made up of carbon and hydrogen atoms that encompass two or more fused aromatic rings which are present in multiple configurations. PAHs are classified based on molecular weights into light PAHs (up to four rings) and heavy PAHs (more than four rings). Light PAHs are known for their volatility, and less toxicity compared to heavy PAHs, which are more lipophilic (Onopiuk et al., 2021). The physical and chemical properties of PAHs are highly related to their ring number and ring linkage pattern (Wu, Gong, Yan, Sun, & Zhang, 2020). According to the standing committee on foodstuffs, high levels of PAHs were found in smoked fish and meat at 130 - 200 µg/kg (Directorate & Opinions, 2002). EFSA reported that the 4 major PAHs (PAH4) (benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (Chry) should be used as a marker of genotoxicity and carcinogenic effects of PAHs in food. Their levels are used as an indicator for PAHs occurrence in food (X. Li, Gao, Deng, Ren, & Teng, 2023).

Among these 4 PAHs (EFSA, 2008), International Agency for Research on Cancer (IARC) classified BaP as carcinogenic to human (group 1), whereas the other 3 PAHs were classified as (Group 2B) possibly carcinogenic (IARC, 2010b). The European Commission issued a maximum limit of PAH4 at 10 μg/kg versus 2 μg/kg for BaP (Sampaio et al., 2021). In 2008, EFSA decided that monitoring total PAH4 or PAH8 (PAH4, benzo[k]fluoranthene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene) concentration is an appropriate indicator for PAHs contamination in food. Generation of PAHs resulted from the pyrolysis of organic components (carbohydrates, fats, proteins) during high-temperature food processing as in grilling, smoking, frying, roasting, toasting, etc. (Farhadian, Jinap, Hanifah, & Zaidul, 2011; Rey-Salgueiro, García-Falcón, Martínez-Carballo, & Simal-Gándara, 2008). Furthermore, chemical modification due to the heating source itself, such as fuel and oil used while cooking, upon exposure to high temperatures, also generates PAHs (Hamidi, Hajeb, Selamat, & Razis, 2016; Singh, Agarwal, & Simal-Gandara, 2020).

5.1. PAHs extraction methods

As PAHs are non-polar, hydrophobic in nature with very low aqueous solubility, though the extraction of PAHs is a critical step for proper extraction, especially in complex food matrices containing fats and proteins. Therefore, an alkaline hydrolysis (saponification) is carried out to hydrolyse sample fats and proteins allowing for PAHs to be retained in these hydrophobic matrices and to be readily released into the extraction solvents (**Figure 3**). Usually, the saponification step is achieved using alcoholic (methanolic or ethanolic) KOH but ethanol is preferable. As methanol increases the susceptibility of transesterification resulting in methyl fatty acids esters which may be extracted alongside PAHs during the LLE step (Kamankesh, Mohammadi, Hosseini, & Tehrani, 2015). The LLE step was carried out using different solvents such as n-hexane (Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Wongmaneepratip & Vangnai, 2017) or cyclohexane (Zachara, Gałkowska, & Juszczak, 2017), then clean up by SPE (Samiee et al., 2020) or gel permeation chromatography (Malesa-Ciećwierz, Szulecka, & Adamczyk, 2019; Slámová, Fraňková, Hubáčková, & Banout, 2017). A summary of PAHs extraction and analysis methods is listed in **Table 4.**

5.1.1 PAHs solid phase extraction (SPE)

The international standard (15753) established two methods to determine 16 PAHs in animal and vegetable fats and oils: a general method, and a specific method for coconut oil and vegetable oils with short-chain fatty acids (**Figure 3**). Briefly, it involves LLE using acetone/acetonitrile (40:60, v/v) mixture followed by SPE purification on C18 and Florisil cartridges, then analysis by HPLC-FLD. However, ISO 15753 has some limitations particularly for naphthalene, acenaphthene, and fluorene compounds quantification owing to their high volatility. In addition, palm and olive pomace are not possible as food matrices due to interferences which hinder PAHs accurate analysis (Palade, Negoiță, Adascălului, & Mihai, 2023).

However, alternative approaches were addressed to overcome these shortcomings. For example, freezing the sample at −18 to −25 °C for 24 h after homogenisation with a mixture of acetonitrile and acetone aided in fatty substances precipitation, and the remaining organic fraction was subjected to further purification *via* alumina-N SPE cartridges and NH_2-cartridge consecutively. This method yielded high recovery at 75 – 110%, as well as 0.09 – 1.97 µg/kg and 0.29 – 5.99 µg/kg for LOD and LOQ, respectively. As shown in **Figure 3**, a typical ISO method aided by ultrasonication during LLE lead to a recovery of 81.5 to 113.8% with low LOD (0.5 – 2.93 µg/kg).

Another example, in which 22 PAHs were analysed in pork samples using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), followed by GC–MS/MS. The extraction was achieved *via* salt-assisted LLE followed by a clean-up step using sorbents such as primary, secondary amine (PSA) and C18 end-capped. This method showed satisfactory recovery (80% -101%), with improved sensitivity (LOD and LOQ; 0.03 – 0.5 µg/kg and 0.1 – 1.5 µg/kg, respectively) (Lai, Lee, Cao, Zhang, & Chen, 2023).

5.1.2. PAHs micro-extraction methods

5.1.2.1. PAHs dispersive liquid-liquid micro-extraction (DLLME)

Microwave-assisted (MA) DLLME enhanced the extraction efficiency (**Figure 3**) of 13 PAHs from vegetables by *ca*. 10% *via* MA-DLLME instead of DLLME alone, and highlighting the potential of a combined approach in PAHs recovery from complex matrices (Kin, Hwang, & Shing, 2020). In another study of combinatorial extraction in smoked rice to extract 4 PAHs (BaP, BaA, BbF, and CRY) showed high recovery (87 – 98%) alongside good sensitivity (LOD 0.05 – 0.12 µg/L). A sample pretreatment step was realised *via* microwave-assisted alkaline hydrolysis followed by pH adjustment to 5, prior to extraction, using DLLME (Mahmoudpour, Mohtadinia, Mousavi, Ansarin, & Nemati, 2017). Likewise, 16 PAHs in grilled meat were extracted using MA-DLLME at 500 MHz for 1.5 min using ethylene tetrachloride and acetone as extraction and disperser solvents, respectively. The efficiency of the extraction was reflected by a recovery (85 – 104%) with high sensitivity (LOD 0.15 – 0.3 µg/kg) (Kamankesh et al., 2015).

Although DLLME fulfills some of the green extraction requirements by minimising organic solvents, many attempts have emerged toward eliminating eco-unfriendly organic solvents. For example, applying ionic liquids in DLLME rather than organic solvents for extraction of 6 PAHs from tea exemplified by ethyl-dimethyl-(2-methoxyethyl) ammonium tris(pentafluoroethyl) trifluorophosphate and acetonitrile (1:2, v/v). Although the method showed moderate recoveries between 56 – 94% (RSD 1.9 – 4.4%), it exhibited high sensitivity with a LOD of 2.0 and 30.8 ng/L (Rivera-Vera et al., 2019).

5.1.2.2. PAHs solid phase micro-extraction (SPME)

Several commercial fibers were used for PAHs extraction, such as polydimethylsiloxane (PDMS), carboxen/PDMS (CAR/PDMS), and divinylbenzene(DVB)/CAR/PDMS (Jalili, Barkhordari, & Ghiasvand, 2020). Moreover, a novel fabricated SPME, with a distinctive design, including a large surface area and surface modification by diverse functionalizs d coats enhanced its selectivity towards PAHs. For example, direct immersion SPME fiber, fabricated of a covalent organic framework grafted on a stainless-steel wire, provided high extraction efficiency for 16 PAHs from grilled meat and durability up to 200 cycles with recoveries from 85.1 – 102.8%. The high performance of fabricated SPME allowed an efficient solvent-free extraction technique within 50 minutes at 40 °C (T.-T. Ma et al., 2019). UiO-66/MoS2 coating is another example of head-space SPME used to extract PAHs from fish samples with 2.1 – 4.5 folds higher adsorption capacity than commercial DVB/PDMS/CAR fiber coating. Recovery values ranged from 80.2–101%, lower LOD (0.11 –1.40 ng/kg), compared to DVB/PDMS (LOD 20 – 60 ng/kg) (Al-Alam, Fajloun, Chbani, & Millet, 2017; Yongli Yuan et al., 2019).

PAHs extraction from complex food matrices requires saponification to eliminate any fatty substances that adversely affect extraction process. ISO 15753 is a lengthy method that is not suitable for all PAHs or fatty matrices. Instead, application of QuEChERS after LLE resulted in excellent accuracy and sensitivity – as well as in DLLME and SPME. Unlike commercial fibers, newly fabricated ones were designed to adsorb PAHs being durable, and to provide high recovery and sensitivity levels.

5.2. PAHs analytical methods

HPLC-UV or PDA detectors, as well as GC- FID, were reported for PAH analysis. However, according to their limited selectivity and sensitivity, other detectors as FLD and MS offered higher sensitivity and were predominantly used, enabling accurate detection and quantification within the low typical nanogram range found in food (EFSA, 2008). A summary of PAHs extraction and analysis methods is presented in **Table 4.**

5.2.1. PAHs liquid chromatography- ultraviolet/ fluorescence detector (LC-UV/FLD)

HPLC coupled with FLD or UV-Vis detector was used widely in PAHs determination, being sensitive for most PAHs, regardless of their M.wt. or volatility. Although FLD is more sensitive than UV detector, the latter is still used, especially for non-fluorescent compounds such as cyclopenta[c,d]pyrene (Ciecierska, 2020). HPLC-FLD was widely used for PAHs analysis in different foodstuffs such as smoked cheese, coffee beans, cocoa beans, meat, etc., (Fatma Aygün & Kabadayi, 2005; K. Lee & Shin, 2010; Suchanová, Hajšlová, Tomaniová, Kocourek, & Babička, 2008). Seven PAHs Compounds were determined using HPLC-FLD in roasted coffee at high sensitivity level of 0.016 – 0.497 µg/kg, and 0.054 – 1.656 µg/kg for LOD and LOQ, respectively (K. Lee & Shin, 2010). Higher sensitivity levels were observed in other dietary sources *via* HPLC-FLD in roasted sesame seeds (LOD 0.08 µg/kg) (Cheng, Liu, Wang, Liu, & Liu, 2015) and grilled beef meat (LOD: 0.01 - 0.03 µg/kg) (Farhadian et al., 2011)

5.2.2. PAHs liquid-chromatography-mass spectrometry (LC-MS)

Mass spectrometry is advantageous compared with FLD for non-fluorescent PAHs with higher selectivity for structurally related PAHs based on its strong elucidation power, especially in high resolution MS/MS mode (alkylated PAHs) (Ciecierska, 2020; Šimko, 2002). A rapid analysis of 4 PAHs compounds using HPLC-(+)ESI/HRMS in smoked bacon samples exhibited high sensitivity in the nanogram range (LOD of 100 – 250 ng/kg), alongside a satisfactory recovery of 73.9 – 99.8% (Merlo et al., 2021).

5.2.3. PAHs gas chromatography- mass spectrometry (GC-MS)

16 PAHs were detected in smoked meat samples *via* GC-EI/MS in SIM mode within 23 min analysis time. The method displayed good recovery (74 – 117%) with high sensitivity (LOD 0.24 – 7.60 µg/kg) (Al-Thaiban, Al-Tamimi, & Helaleh, 2018). In another example, application of GC-EI/MS for determining 16 PAHs in meat and fish samples revealed high sensitivity in the low nanogram range (LOD 3 – 70 ng/kg) with high recovery 85 – 105% (Rascón et al., 2019a). Furthermore, excellent sensitivity results were reached using triple quadrupole tandem mass spectrometer (QqQ) in MRM mode for the assay of 23 PAHs from thinly sliced roasted pork. Low LOD and LOQ results were in the range of 0.03 – 0.3 µg/L and 0.1 – 0.9 µg/L (Hung, Lee, Inbaraj, Sridhar, & Chen, 2021). Another example, GC-EI/MS/MS on MRM mode was used for detecting 4 PAHs in different foods of plant and animal origin at comparable LOD and LOQ (Urban & Lesueur, 2017).

Considering PAHs volatile nature, GC-MS is the most frequently reported technique considering its simplicity compared to LC-MS, and high sensitivity to be applied for different food matrices as listed in **Table 4**.

6. Heterocyclic aromatic amines (HAAs) occurrence in different food sources, extraction, and analysis

HAAs are a group of more than 30 compounds which are structurally composed of one or more nitrogen-based heterocyclic rings. These compounds are produced upon exposure of protein-rich food such as meat, poultry, and fish to high temperatures during cooking with 3 main contributors in their formation: amino acids, creati(ni)ne, and reducing sugars (Nadeem et al., 2021). HAAs are further subclassified into two groups that are IQ- and non IQ-type, which are namely amino-imidazoazaarenes, and amino-carbolines, receptively. The HAAs-IQ type group are identified as thermic/ polar HAAs that are formed predominantly within temperature range 100 – 300 °C including 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). In contrast, above 300°C HAAs-non IQ are produced, hence called pyrolytic/non-polar HAAs exemplified by 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC), and 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) (Oz et al., 2023).

According to toxicological studies, HAAs is a multisite carcinogneic agent including breast, colon, gastric, pancreatic, prostate, and lung (Guo et al., 2018). In contrast, the International Agency for Research on Cancer (IARC) has classified IQ in (group 2A), a probable human carcinogen, and other 8 HAAs compounds as (group 2B), a possible human carcinogenic, to include 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), MeIQx, PhIP, AαC, MeAαC, Trp-P-1, 3-amino-1-methyl-5H-pyrido[4,3-b]indol (Trp-P-2), and 2-amino-6-methyldipyrido[1,2-a:3`,2`-d]imidazole (Glu-P-1) (Smoke & Smoking, 2004).

6.1. HAAs extraction methods

6.1.1. *HAAs conventional extraction method*

Firstly, food samples are homogenised with organic solvents, alcoholic mixtures, or aqueous acidic/basic solutions to liberate HAAs compounds from their matrix components. Therefore, a saponification step was carried out followed by a filtration or centrifugation step, and finally extraction using LLE and SPE (Alaejos, Ayala, González, & Afonso, 2008; Toribio, Galceran, & Puignou, 2000).

Considering HAAs structural diversity, application of tandem sorbents with different properties to extract all HAAs seems warranted. For example, Gross and Grüter method was widely applied to extract polar and non-polar HAAs from different food. In brief, After LLE, extract is subjected to two different SPE sorbents, this results in two different fractions according to the polarity of HAAs (Gross & Grüter, 1992). However, this technique was simplified to elute both polar/nonpolar HAAs in one fraction (Santos et al., 2004), though with poor recovery (54 – 65%) of 5 HAAs; IQ, MeIQ, MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), and PhIP in cooked camel meat burgers (Rizwan Khan, Naushad, & Abdullah Alothman, 2017). Low recovery levels for 15 HAAs likewise detected from pan-fried beefsteak within 35 – 70% (J. Lee, Dong, Jung, & Shin, 2011). Therefore, extensive clean-up steps appeared to adversely affect the extraction process, with the two-step SPE approach being reduced to a single step by applying specific sorbents that could allow recovery of both polar and non-polar HAAs in one fraction from complex food matrices, **Figure 4.**

One of these sorbents is the mixed-mode Oasis mixed-mode cation exchange (MCX) SPE cartridge that is a cation exchange sorbent and could form hydrophobic interactions, and subsequently allowing for adsorption of polar and less polar HAAs (Haskaraca, Demirok Soncu, Kolsarıcı, Öz, & Juneja, 2017; Messner & Murkovic, 2004; Turesky, Taylor, Schnackenberg, Freeman, & Holland, 2005).

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), was reported for HAAs extraction displaying moderate to good recovery (59.4-104%). The Oasis MCX cartridge was included as a part of QuEChERS instead of primary, secondary amine (PSA) and C18 sorbent and resulted in comparable recoveries of HAAs (54 – 122%) (Chevolleau et al., 2020). Furthermore, enhancement in HAAs recovery was reported upon applying the Oasis MCX cartridge after classical QuEChERS extraction method for detection of 8 HAAs in braised beef. Briefly, extraction was done using QuEChERS method, with acetonitrile as the extracting solvent, and $MgSO_4/CH_3COONa$ as salt mixture. In addition to d-SPE, such as C18 and PSA, an extra purification step was applied using a mixed-mode polymeric sorbent (Oasis MCX cartridge). This SPE sorbent showed good retention of all tested HAAs, either polar or non-polar with subsequent recovery improvement reaching 110.47%, higher than 38 – 99% previously reported (Batool et al., 2023).

A high recovery of 14 HAAs (82.9 – 115.4%) was achieved using SPE from Chinese traditional bacon and sausage attributed to ionic interaction between benzenesulfonic acid groups grafted onto the cation-exchange SPE stationary phase and the positively charged nitrogen embedded in HAAs compounds (Dong, Xian, Li, Wu, et al., 2020). Another novel magnetic-SPE was formulated using Fe_3O_4@COP nanocomposites to extract 6 HAAs from thermally processed meat. Samples containing the magnetic sorbent were mixed using a vortex, and further eluted with methanol showing high recovery (82.0 – 109.5%) (Feng et al., 2022).

6.1.2. HAAs Micro-extraction techniques.

Microwave energy has a great effect in releasing of analytes from complex tissues in a short time at high yields. For example, extraction of 3 HAAs, from hamburger patties using microwave-assisted extraction (MAE)-DLLME within 1.5 minute at 500 MHz. Then, DLLME with 1-octanol and methanol were used as extracting and dispersive solvents, respectively yielding a high recovery of polar HAAs (90 – 105.0%), alongside good sensitivity (LODs 0.06 – 0.21 µg/kg) (Aeenehvand et al., 2016). A fast and green MAE/DLLME was developed for the extraction of 5 non-polar HAAs using an ionic liquid solution at high sensitivity (LOD 0.35 – 2.4 µg/L) and good recovery (69 – 100%) (Feng et al., 2022).

Extraction of polar/nonpolar HAAs in one fraction utilising one single SPE (Oasis MCX cartridge) step enhanced extraction efficiency with higher recovery compared with the official two-step SPE. Furthermore, a simple and quick QuEChERS method showed improved recovery *via* a mixed-mode polymeric sorbent (Oasis MCX cartridge) as a part of QuEChERS sorbents or for cleaning up the extract. High extraction efficiency levels for HAAS after applying MAE-DLLME, as an eco-friendly approach was also observed, and comparable to the well-established protocol LLE-SPE as listed in **Table 5.**

6.2. HAAs analytical techniques

Separation techniques, particularly LC were widely applied for polar and non-polar HAAs analysis after their extraction from complex food matrices. LC-based methods provide several advantages; being suited to a broader range of HAAs regardless of their polarity, alongside compatibility with detectors as UV -Vis and FLD. Additionally, higher accuracy and precision are obtained through MS utilising different ionisation modes such as APPI, APCI, and ESI (Nadeem et al., 2021). Unlike GC-MS, despite its sensitivity and simplicity, it is primarily suited to non-polar and volatile HAAs (Dong, Xian, Li, Bai, & Zeng, 2020).

6.2.1. HAAs, liquid chromatography- ultraviolet/ fluorescence detector (LC- UV-Vis/FLD)

Most HAAs can be analysed using LC coupled to UV-Vis or FLD (H. Wang et al., 2023). For example, the determination of 5 HAAs in meatballs was done using two types of detectors, PDA and FLD, with PDA used for the detection of MeIQx, 4,8-DiMeIQx and IQ, whereas FLD was used for 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) due to its strong fluorescence properties. The assay LOD and LOQ for examined HAAs were at 0.04 – 1.40 µg/kg, and 0.13 – 4.40 µg/kg, respectively with a recovery of 68.9 – 87.8% (Özdestan, Kaçar, Keşkekoğlu, & Üren, 2014). Another study for the separation of three polar HAAs from hamburger patties *viz.* IQ, MeIQ, and MeIQx using HPLC-UV showed comparable LOD and LOQ values to the previous study (Özdestan et al., 2014) at 0.06 – 0.21 and 0.15 – 0.70 µg/kg, respectively, with slightly better recovery ranging from 90–105%. In comparison with other methods, this assay was found simple, rapid, of high accuracy and precision, with a linear dynamic range from 1 – 200 µg/kg (Aeenehvand et al., 2016) posing it as a solution for industrial food applications.

6.2.2. HAAs, liquid chromatography- mass spectrometry (LC-MS)

LC- MS using APCI for ionisation and operated in an MRM mode was employed to analyse 16 HAAs in cooked beef. Separation was done within less than 4 minutes using a C8 column (Chevolleau et al., 2020). Ionisation using ESI mode coupled with UPLC in positive ion mode was used for the analysis of 11 HAAs from roasted pork. A result was achieved within 17 min. LOD and LOQ for spiked meat matrices ranged from 0.026 to 0.659 and 0.023 -0.275 µg/kg, respectively, with good recovery levels between 50-110% (Yan et al., 2014).

6.2.3. HAAs gas chromatography-mass spectrometry (GC-MS)

Despite GC advantages, including high selectivity and sensitivity, due to the necessity for a derivatisation step, GC employment for HAAs detection is rare, being limited to non-polar HAAs. For example, *N*-methyl-*N*-*tert.*-butyldimethylsilyltrifluoroacetamide is commonly used as a derivatising agent for HAAs prior to GC-MS analysis to take place at 90 °C for 90 min adding to sample preparation time with LOD of (0.05 – 0.3 µg/kg) in food samples (Casal, Mendes, Fernandes, Oliveira, & Ferreira, 2004). Another study using GC–EI-MS/MS operated in an MRM mode was used to detect HAAs in meat samples. The derivatization reaction was within only 8 min using *N*, *N*-dimethylformamide di-*tert*-butylacetal (F. Zhang et al., 2008).

Comparison of the commonly reported protocols for HAAs, HPLC-coupled with UV-Vis or FLD depending on the HAAs structure offers excellent sensitivity results with low LOD. Further, UPLC-ESI/MS/MS showed exceptional sensitivity by LOD ranging from 0.005 to 0.05 µg/kg (Lai et al., 2023), **Table 5.** Compared to LC-MS, GC-MS appears to be more suited for volatile non-polar HAAs post a derivatization step that adds to sample preparation time.

7. Malondialdehyde (MDA) occurrence in different food sources, extraction, and analysis

Aldehydic substances, such as malondialdehyde and hydroxylated α, β-unsaturated aldehydes such as 4-hydroxy-2-nonenal (HNE), and 4-hydroxy-2-hexenal (HHE), are typically generated due to lipid peroxidation, especially in fat-rich food such as oil. MDA is a three carbon dialdehyde where HNE and HHE are unsaturated mono-aldehyde and produced mainly from the decomposition of ω-3 and ω-6 polyunsaturated fatty acids. Their potential toxicity was attributed to their high reactivity with proteins and DNA (Papastergiadis et al., 2014). Significant attention was given to these products due to their toxic hazards (Ayala, Muñoz, & Argüelles, 2014). Among all aldehydes, MDA is the most commonly reported (Barriuso, Astiasarán, & Ansorena, 2013). EFSA established a threshold of toxicological concern values for MDA, HNE, and HHE at 30, 1.5, and 1.5 µg/kg/day, respectively (L. Ma et al., 2023).

Owing to MDA high reactivity, its major part might be bound to different species in different food matrices. Hence, it is crucial to determine total MDA level, and in turn choose suitable sample pretreatment and extraction conditions. particularly hydrolysis. Extraction of MDA from food involves alkaline hydrolysis for MDA release, followed by an acid-based protein precipitation and then derivatization. The derivatized MDA was subjected to LLE extraction using organic solvents prior to the main analytical step (Jung, Nam, & Jo, 2016; Mendes, Cardoso, & Pestana, 2009).

Different extraction steps were reported for MDA, HHE, and HNE extraction from French fries and beef samples, in which solid samples were first homogenized and extracted using petroleum ether. Then, ethereal layer was evaporated till dryness and reconstituted in aq. ethanol. The ethanolic solution was derivatized by using 2,4-dinitrophenylhydrazine (DNPH) in an acidic medium, with derivatized MDA subjected to LLE extraction using dichloromethane (DCM), the organic layer was subjected for further analysis by UHPLC-QqQ-MS/MS. Although, this method presented excellent recovery result between 95.56 – 104.22% with high sensitivity (LOD 0.2 – 5 µg/kg), it required a lengthy sample preparation protocol alongside large solvents consumption (L. Ma et al., 2023).

SPE was incorporated for sample purification to extract MDA from French fries frying oils collected post LLE in DCM. The DCM fraction was further introduced to SPE column packed with anhydrous sodium sulfate further derivatized using DNPH. Separation was performed using HPLC coupled to PDA for detection with LOD and LOQ values at 0.012 mg/L and 0.020 mg/L, respectively (L. Ma et al., 2021).

Ultrasonic DLLME presents a fast and straightforward method to detect MDA, and HNE in different beverages including fruit juices, black tea, beer and coffee. In this study, extraction and derivatization were performed simultaneously with acetonitrile/chloroform as extraction and dispersion solvents, and DNPH for derivatization. The mixture was ultrasonicated for 5 min at 60 °C, then centrifuged to separate hydrazine derivatives for subsequent GC-MS analysis. This method resulted in high accuracy and excellent reproducibility by 94.0 – 102.4% for recovery and RSD lower than 4.5% (Custodio-Mendoza et al., 2022).

The MDA reported analytical strategies can be classified as derivatization-based methodologies. For example, 2-thiobarbituric acid (TBA)-spectroscopic assay method based on a condensation reaction between TBA and MDA resulted in a colored product to be measured spectrophotometrically. Moreover, it requires a high temperature (90 °C) during the derivatization step leading to increased aldehyde content. However, it exhibited a lack of selectivity and sensitivity as it detected total aldehydic content. Although TBA method is the most widely used for MDA detection, it is also considered a standard marker for lipids peroxidation because of the tendency of a derivatizing agent TBA to react with other reactive compounds, not only MDA leading to inaccuracy of estimated MDA content (Reitznerová et al., 2017). Though, determination of MDA by chromatographic techniques is more reliable and accurate than the direct TBA-spectrophotometric methods as in HPLC-PDA and GC-MS (Custodio-Mendoza et al., 2022; L. Ma et al., 2021). Although chromatographic method’s detection and quantitative limits are suitable in the low microgram range, they are still relatively time-consuming and require dedicated laboratory equipment (L. Ma et al., 2023). Therefore, a more selective, sensitive, and fast applicable method is needed.

8. Conclusions and future directions

Many carcinogens are produced during different food processing methods i.e., fermentation, grilling, frying, broiling, etc. The carcinogens produced depends on the predisposing factors such as the availability of their precursors, additives, and processing conditions. Nowadays, it is advisable to limit the application of the hazardous cooking methods by replacing them with safer options like microwave or boiling to minimise the carcinogens released (Škaljac et al., 2014). Where this is not possible, it is necessary to develop and implement assessment methods for their detection and quantification. Therefore, application of an efficient extraction method followed by highly sensitive analytical technique is a major goal for human and food safety.

According to the food matrix type and the targeted analyte, a sample pretreatment step is required prior to the main extraction process (LLE, SPE, etc.). For example, an alkaline hydrolysis or a saponification step is carried out as in case of certain hydrophobic carcinogens,e.g. PAHs and HAAs. Whereas, an acid aided-deproteinisation step is employed in case of high-protein foods. The homogenised 'pretreated' samples are subjected to either conventional or microextraction techniques. Different approaches were presented to afford a fast, eco-friendly and efficient extraction method. For example, direct extraction techniques *via* head-space were adopted for volatile analytes *versus* selective immersion sorbents for non-volatile ones. Therefore, rapid sample pretreatment and efficient extraction and enrichment techniques should be developed.

Each carcinogen has its unique structure with a subsequent difference in their physiochemical properties, which controls their polarity and volatility. The hyphenated techniques are the most reported in the literature presenting the main focus of this study, and their choice depends on the analytes and their matrices. LC-MS is widely used for both VNAs /NVNAs, polar/non-polar HAAs, BAs, and EC, whereas, GC-MS is more suited for volatile compounds, such as MDA, VNAs and PAHs. However, highly sensitive hyphenated techniques still face the disadvantages of being time-consuming and costly. It is necessary to establish fast and cost-effective methods, while ensuring sensitivity especially with the increasing developed of bench top spectroscopic techniques.

Fast, reproducible and "simultaneous determination" or "multi-class detection" methods are still scarce in the literature regarding food carcinogens assay, without any extraction protocol reported to simultaneously extract more than one carcinogen category. Although, a saponification step followed by an organic-solvent LLE should concurrently extract different analytes such as PAHs, HAAs and MDA. Albeit, an additional SPE or a derivatisation step is needed to efficiently extract these targeted analytes to be suitable for proper detection *via* GC-

MS or LC-FLD. On the contrary, UPLC coupled with MS presents an effective approach for multi-class detection, though with high operational costs aside from the use of a labelled standard for MS quantification.

Conventional extraction methods are laborious suffering from prolonged extraction times, large consumption of organic solvents with a high environmental burden, and low recovery of trace-level carcinogens. Whereas, microextraction presents a rapid and efficient extraction approach with minimum organic solvents consumption withrelatively easy operational protocols compared to the conventional procedures. It can be used for an extensive range of structures and polarities of compounds. Moreover, it can be hyphenated with different chromatographic techniques such as (HPLC, UPLC or GC). Therefore, in order to achieve automation, high throughput and real-time analysis, which may be challenging, a one-step (on-line) extraction and detection system seems essential. Further development in microextraction techniques with new materials is needed to cover the full targeted analytes, alongside, the advancement of new analytical strategies to guarantee the highest sensitivity levels of the targeted food carcinogens.

Conflicts of Interest

None to declare.

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Table 1. List of the different extraction and analytical methods for NAs in different food types

Analyte	**Food type**	**Sample preparation**	**Analytical technique**	**Validation parameters**	**References**
NDMA, NMEA, NDEA, NDPA, NDBA, NPIP, NPYR, NMOR, NMPhA	Dry-cured mackerel	Salting out-LLE	GC-EI/MS/MS	LOD: 0.08 – 0.15 µg/kg LOQ: 0.25 – 0.5 µg/kg Recovery: 77.1 – 112.9%	(Dong, Li, et al., 2020)
NDMA, NPYR, NDEA, NDBA, NDPA	Different fermented/ salted Foodstuff	LLE labelling with fluorescent probe followed by DLLME	RP-HPLC-FLD (Quantitative) RP-HPLC-(+)APCI/MS/MS (Qualitative)	LOD: 0.01 – 0.07 µg/kg LOQ: 0.03 – 0.21 µg/kg Recovery: 92.8 – 102.1%	(Lu et al., 2017)
NDMA NMEA, NDEA, NDPA, NDBA, NPIP, NPYR	Chinese Sichuan salted vegetables	QuEChERS	GC-EI/MS/MS	LOD: 0.02 – 0.15 µg/kg LOQ: 0.07 – 0.50 µg/kg Recovery: 91.5 – 106.1%	(Q. Zhang et al., 2019)
NDMA, NDEA, NPYR, NPIP, NDPA	Cooked Bacon	QuEChERS	GC-EI/MS/MS	Recovery: 70 – 120%	(Lehotay et al., 2015)
NDMA, NDEA, NDPA, NDBA, NPIP, NPYR	Soy sauce	QuEChERS	GC-EI/MS	LOD|: 0.4 – 0.9 µg/kg LOQ: 1.2 – 3.0 µg/kg Recovery: 80.2 – 112%	(Zeng et al., 2016)
NDMA, NDEA, NPYR, NPIP, NDPA, NSC336	Chinese dried aquatic products	Steam distillation- liquid -liquid extraction (LLE)	RP-UPLC-(+)APCI/MS/MS	LOD:0.3 – 0.5 µg/kg LOQ: 1.0 – 1.5 µg/kg Recovery: 88.3 – 108.7%	(S. Huang et al., 2023)
NDMA, NDEA, NPYR, NPIP, NDPA, NDBA	Cooked meat	Ultrasonic or autoclave-assisted LLE	GC-EI/MS	LOD: 0.05 – 0.3 µg/kg LOQ: 0.85 – 1.5 µg/kg Recovery: 85% to 89%	(Abdullah et al., 2022)
NDMA, NDEA, NDBA, NPIP, NPIP	294 various samples of fish	Two-step SPE	GC-(+)CI/MS	LOD: 0.10 µg/kg LOQ: 0.35 µg/kg Recovery: 79 – 88%	(Yurchenko & Mölder, 2006)

NDMA, NDEA NPIP, NPYR	Sausages	Head space SPME	GC-TEA	LOD: 3 µg/Kg LOQ: 10 µg/Kg Recovery: 105 – 110%	(Andrade et al., 2005)
DMN, NMOR, NPYR, DEN, NPIP	Canned sausages	Steam distillation- SPE	MEKC-PDA	LOD: 160 – 240 µg/kg LOQ: 520 – 820 µg/kg Recovery: 68.8 – 105.0%	(Sanches Filho et al., 2003)

Electron impact ionization (EI), fluorescence detector (FLD), chemical ionization (CI), atmospheric pressure chemical ionization (APCI), Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), micellar electrokinetic chromatography (MEKC), photodiode array detector (PDA), N-nitroso-N-methylaniline (NMPhA), N-nitrosodiisopropylamine (NSC336), dimethylnitrosamine (DMN), diethylnitrosamine (DEN), N-nitrosomorpholine (NMOR), Thermal energy analyzer (TEA).

Table 2. List of the different extraction and analysis methods for BAs in different food types

Analytes	Food type	Sample preparation	Analytical technique	Validation parameters	References
Methylamine, PUT, Tyr, TRP, Phe, cysteamine, SPD	Fruit juices alcoholic beverage	Salting-out-assisted LLE Followed by pre-column derivatization using 1-naphthylisothiocyanate	RP-HPLC - UV (254 nm) Mobile phase: acetonitrile-water, 60:40 (v/v). Isocratic elution: 1 ml/min	LOD:1.1 μg/L LOQ: 3.2 μg/L Recovery: 92.8 – 109.5%	(Jain, Gupta, & Verma, 2015)
Serine, SPD, PUT, His, CAD, Phe, Tyr, URO	Beer, Wine	SPE: poly(vinylpolypyrrolidone) PVPP	CE – ESI/MS/MS PVA-coated capillary column Running buffer: 0.5 M acetic acid (pH 2.5)	LOD: 1 – 2 μg/ L LOQ: 3 – 8 μg/L Recovery: up to 113%	(Daniel et al., 2015)
SPD, PUT, CAD, Tyr, Phe, His, ethylamine, propylamine, butylamine, amylamine, hexylamine, spermine	wines	d-SPE Followed by pre-column derivatization using Bnz-Cl	RP-HPLC-PDA Mobile phase: 5 mM sodium tetraborate (pH 8) & methanol Gradient elution: 1 ml/min	LOD: 0.133 – 0.509 mg/L LOQ: 0.331 – 1.54 mg/L Recovery: 72 –99%	(Milheiro et al., 2019)
Methylamine, Phe, PUT, CAD, His, Tyr. dimethylamine, ethylamine, isopropylamine, diethylamine, isobutylamine, 2-methylbutylamine, pyrrolidine, isoamylamine,	Beer	In situ derivatization -DLLME using isobutyl chloroformate (IBCF) for derivatization	GC- MS: SIM mode Carrier gas: Helium Total run time: 25 min	LODs: 0.3 – 2.9 μg/L LOQs: 1.0 – 9.5 μg/L Recovery: 72 – 113%	(Almeida et al., 2012)

morfoline, piperidine, amylamine, 1,3-diaminopropane					
TRP, Phe, His, Tyr, SPD, spermine	Wine, fish samples	In situ derivatization -A magnetic ionic liquid -DLLME using DNS-Cl for derivatization	RP-HPLC-UV. Gradient elution: 1 ml/min Mobile phase: water & acetonitrile	LOD: 1.2 – 3.9 µg/kg LOQ: 3.9 – 9.9 µg/kg Recovery: 93.2 –103.1%	(Cao, Xu, Xue, Feng, & Zhang, 2019)

Putrescine (PUT), Tryptamine (TRP), Phenylethylamine (Phe), Spermidine (SPD), Cadaverine (CAD), Tryptamine (Tyr), Urocanic acid (URO).

Table 3. List of extraction and analytical methods for EC in different food types

Food type	**Sample preparation**	**Analytical techniques**	**Validation parameters**	**References**
Chinese Liquor	N.d	GC – EI/MS	LOD: 0.56 µg/L LOQ: 1.87 µg/L Recovery (RSD): 97 – 104% (< 1%)	(W. Zhang et al., 2017)
Fermented solid food	Pressurized liquid extraction - SPE with Florisil.	GC- EI/MS/MS	LOD: 0.3 µg/kg LOQ: 1.0 µg/kg Recovery: 98 – 107%	(Liao & Luo, 2014)
Fermented food	Matrix solid-phase dispersive extraction (MSPDE)	GC – EI/MS/MS	LOD: 1.8 µg/kg LOQ: 4.7 µg/kg Recovery (RSD): 88.6–112% (< 10%)	(Mo et al., 2014)
Wine	HS-SPME: Carbowax/ divinylbenzene	GC-EI/MS	LOD: 9.6 µg/L Recovery (RSD): 85%- 121% (< 15%)	(Whiton & Zoecklein, 2002)
Stone-fruit spirits	HS-SPME Carbowax/ divinylbenzene	GC- EI/MS/MS	LOD: 30 µg/L LOQ: 110 µg/L	(Lachenmeier et al., 2006)
90 Samples of Chinese spirits	SPE, followed by pre-column derivatization	HPLC- FLD: λ_{ex} 233 nm, λ_{em} 600 nm Mobile phase: sodium acetate / acetonitrile Isocratic elution	LOD: 1.82 µg/L LOQ: 5.34 µg/L Recovery (RSD): 96.67% (6.59%)	(G. Li et al., 2015)
42 Wine samples	Simple dilution in water or ethanol followed by pre-column derivatization	HPLC- FLD: λ_{ex} 233 nm, λ_{em} 600 nm Mobile phase:sodium acetate / acetonitrile Isocratic elution; 0.45 ml/min.	LOD: 4.2 µg/L Recovery: 96%	(Herbert et al., 2002)
Chinese yellow rice wine	Analyte concentration by vacuum evaporation followed by pre-column derivatization	HPLC- FLD: λ_{ex} 233 nm, λ_{em} 600 nm Isocratic elution Mobile phase: methanol–water	LOD: 73.2 µg/L LOQ: 243.9 µg/L	(Fu et al., 2010)

			Recovery (RSD): 98.30 – 101.30% (< 5%)	

Table 4. Examples of extraction and analysis methods for PAHs in different food types

Analytes	**Food type**	**Sample preparation**	**Analytical techniques**	**Validation parameters**	**References**
4 PAH[a]	Smoked meat; beef stripe, pork, & chicken fillet.	Saponification followed by two step extraction LLE and SPE	GC- FID	LOD: 0.1 – 0.3 μg/kg LOQ: 0.3 – 0.9 μg/kg Recovery (RSD): 94% (< 16%)	(Olatunji, Fatoki, Opeolu, & Ximba, 2014)
16 PAHs[b]	Meat & fish samples	Saponification followed by two step extraction ultrasonic assisted-LLE & RP-SPE	GC-EI/MS	LOD: 0.003-0.070 μg/kg Recovery (RSD): 85 – 105% (< 8%)	(Rascón et al., 2019a)
13 PAH[c]	Dry fermented sausage	QuEChERS	GC-EI/MS	LOD: 0.4 – 0.6 μg/g	(Škaljac et al., 2014)
16 PAHs[b]	Beer, wine, cider, soft drinks, fruit juice, tea, & coffee	RP-SPE	GC-EI/MS	LOD: 0.02–0.6 ng/L Recovery (RSD): 90 – 103% (< 7%)	(Rascón, Azzouz, & Ballesteros, 2019b)
16 PAHs[b]	Barbecue smoke	Magnetic SPE using magnetic nanoparticles (TCLN@Fe3O4 MNPs)	GC-EI/MS	LOD: 2 – 5.4 ng/L LOQ: 3.7 – 16.4 ng/L Recovery (RSD): 73 – 91% (< 11%)	(Pan et al., 2020)
1 PAH: benzo (a) pyrene	Rousted sesame seeds	LLE	RP-HPLC-FLD λ_{ex} 384 nm, λ_{em} 406 nm Isocratic elution.	LOD: 0.08 μg/kg LOQ: 12.26 μg/kg Recovery (RSD): 102% (3%)	(Cheng et al., 2015)
3 PAHs[d]	Grilled beef meat	Saponification-SPE	RP-HPLC-FLD Isocratic elution	LOD: 0.01 – 0.03 μg/kg LOQ: 0.04 – 0.10. μg/kg Recovery (RSD): 75 – 102% (< 9%)	(Farhadian et al., 2012)
4 PAHs[a]	Smoked bacon	Pressurized liquid extraction;	RP-HPLC- (+) ESI/MS Gradient elution	LOD: 0.1 – 0.25 μg/kg	(Merlo et al., 2021)

				LOQ: 0.50 µg/kg Recovery: 74 – 100%	
4 PAHs[a]	Dark chocolate	SLE Clean-up; gel permeation chromatography and SPE Silica cartridges	RP-HPLC-APPI/MS Gradient elution	LOD: 0.016 – 0.024 µg/kg LOQ: 0.054 – 0.081 µg/kg Recovery (RSD): 86 – 102% (< 11%)	(Rozentale, Zacs, & Bartkevics, 2019)

[a] 4 PAHs: benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene.

[b] 16 PAHs: the 4 PAHs + benzo[k]fluoranthene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene.

[c] 13 PAHs: the 16 PAHs, except naphthalene, acenaphthene, and fluorene.

[d] 3 PAHs: benzo[a]pyrene, benzo[b]fluoranthene, and fluorene.

Table 5. List of extraction and analysis methods for HAAs in different food types

Analytes (N° of HAAs)	**Food type**	**Extraction method**	**Analytical Techniques**	**Validation parameters**	**References**
8 HAAs[a]	Fast-food meat products in China	Saponification LLE / Two-steps SPE (PRS and C-18)	HPLC- UV-Vis / FLD Mobile phase: aq. ammonium acetate & acetonitrile	LOD: 0.06 - 0.65 µg/kg LOQ: 0.22 - 2.19 µg/kg Recovery: 61 – 84%.	(I. A. Khan et al., 2019)
21 HAAs[b]	Freeze-dried pork and pork jerky	QuEChERS: $MgSO_4$ and CH_3COONa salts d-SPE: PSA and C18	RP-UPLC-MS/MS Mobile phase: aq. ammonium acetate & acetonitrile	LOD: 0.005 -0.05 µg/kg LOQ: 0.01 - 0.1 µg/kg Recovery: 59.4 – 104%	(Lai et al., 2023)
4 HAAs[c]	Grilled Pork Samples	Ion-Pair-Based Surfactant-Assisted-DLLME)	HPLC-PDA Mobil phase 0.2% acetic acid and acetonitrile	LOD: 0.01µg/ kg LOQ: 0.03 µg/ kg Recovery: 90 – 106%	(Vichapong, Srijaranai, Santaladchaiyakit, Kanchanamayoon, & Burakham, 2016)
18 HAAs[d]	Roasted and pan-fried pork, and beef patties	LLE-single step SPE on Oasis MCX SPE cartridge	HPLC –ESI/MS Mobile phase: aq. ammonium acetate & acetonitrile	LOD: 0.02–0.6 µg/kg LOQ: 0.05–2.0 µg/kg Recovery (71.3 – 114.8%)	(Xu et al., 2021)

[a] 8 HAAs: 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), Harman, and Norharman.

[b] 21 HAAs: IQ, MeIQ, MeIQx, 4,8-DiMeIQx, AαC, PhIP, Harman, Norharman, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), isoIQ, 2-aminodipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-2), 2-amino-3-methyl-imidazo[4,5-f]quinoxaline (IQx), 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1), 2-amino-1-methylimidazo[4,5-*b*]quinoline (IQ[4,5-*b*]), 2-amino-1,6-dimethyl-furo[3,2-*e*]imidazo[4,5-*b*]pyridine (IFP), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indol (Trp-P-2), 2-amino-6-methyldipyrido[1,2-a:3`,2`-d]imidazole (Glu-P-1), 2-amino-5-phenylpyridine (Phe-P-1), and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC).

[c] 4 HAAs: MeIQ, 4,8-DiMeIQx, PhIP, and Harman.

[d] 18 HAAs: IQ, DMIP, MeIQ, IQx, MeIQx, IQ[4,5-b], IFP, 7,8-DiMeIQx, 4,8-DiMeIQx, Norharman, Harman, Phe-P-1, Trp-P-2, PhIP, Trp-P-1, AαC, MeAαC, and 2-amino-1,5,6-trimethylimidazo[4,5-b]pyridine.

Declaration of Interest

- the work described has not been published previously.
- the article is not under consideration for publication elsewhere.
- the article's publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.
- if accepted, the article will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

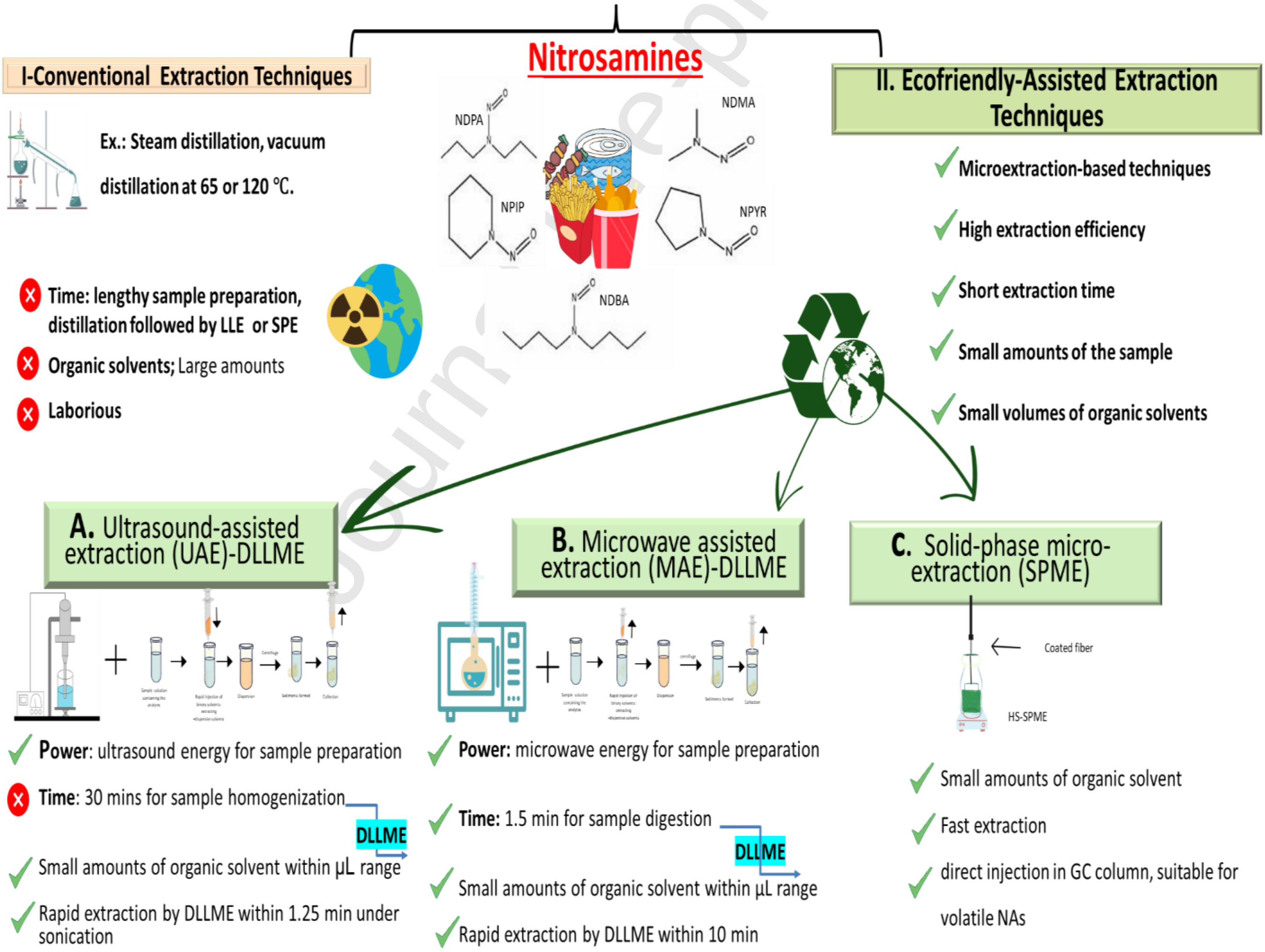


Figure 1. Sample preparation for Nitrosamines (NAs) extraction 1) Conventional extraction techniques 2) Micro-extraction techniques: a) UAE-DLLME, b) MAE-DLLME, c) SPME

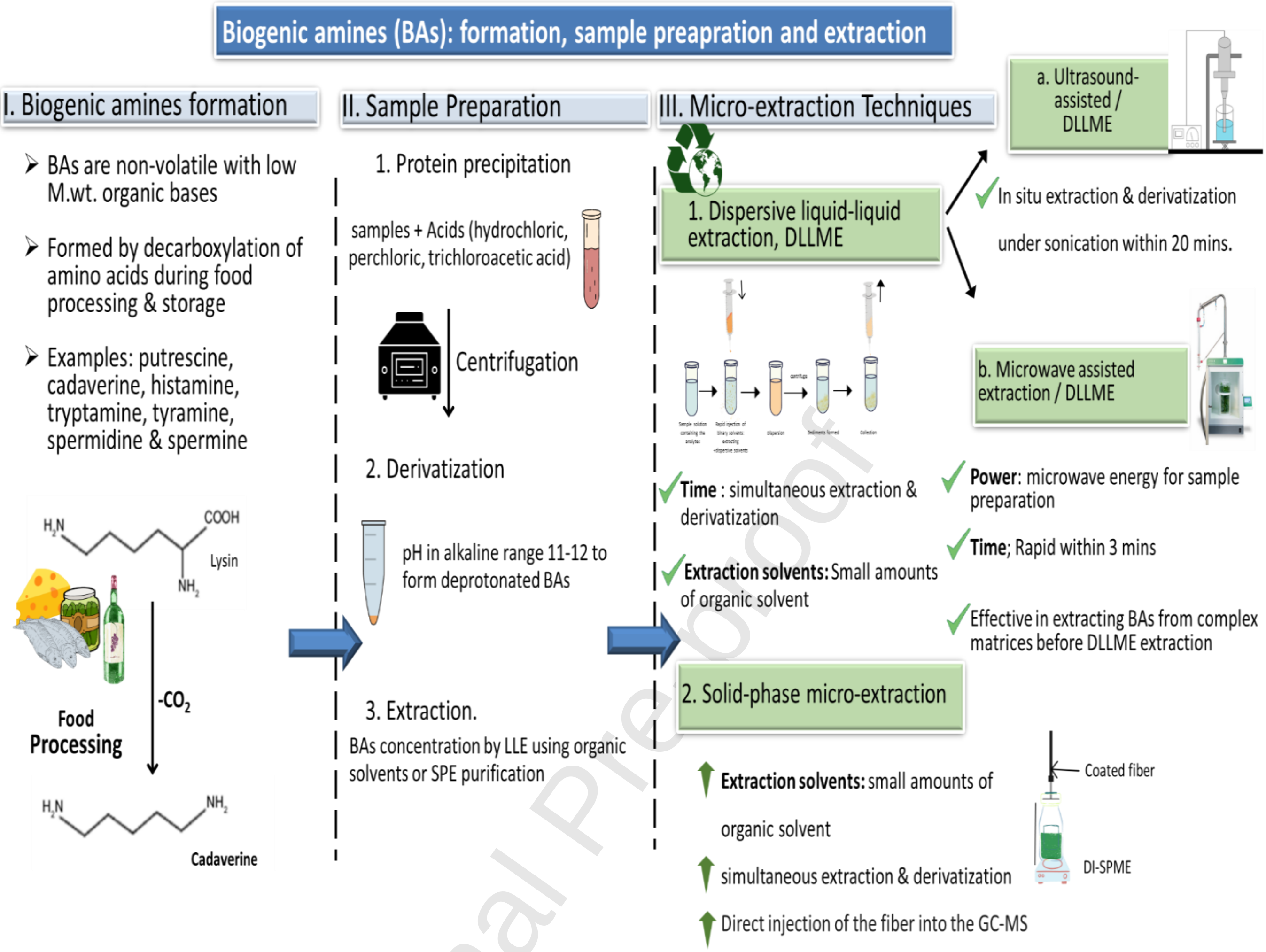


Figure 2. (A) Biogenic amines formation while food processing (B) Sample preparation to extract BAs from complex matrices as cheese (C) Examples of eco-friendly extraction techniques.

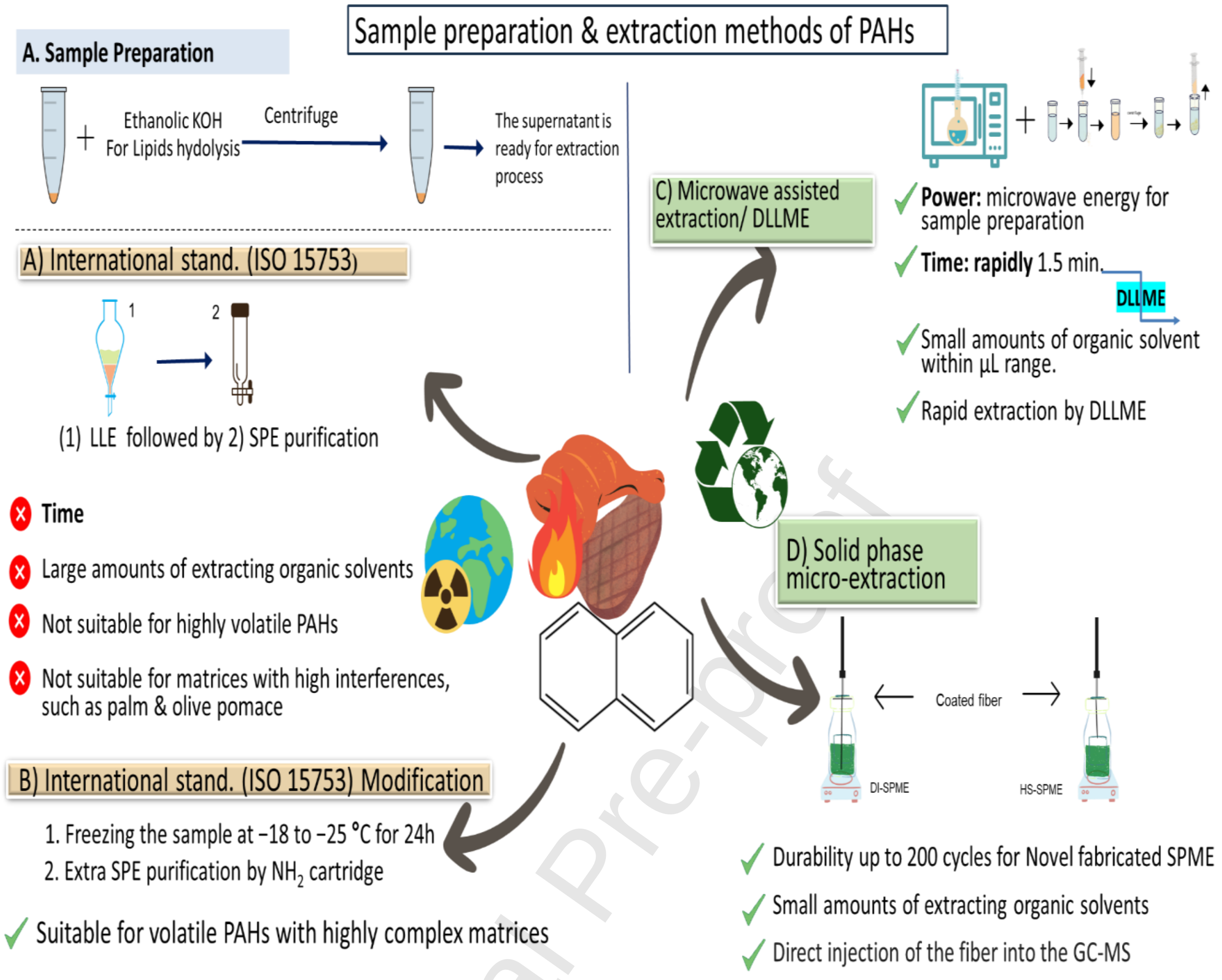


Figure 3. Sample preparation for PAHs extraction from complex matrices A) the standard ISO method (15753) to extract PAHs from animal and vegetable fats B) Modifications of the ISO method to avoid limitation C) Microwave-assisted DLLME D) Solid phase micro-extraction using novel fabricated fibers instead of commercial ones

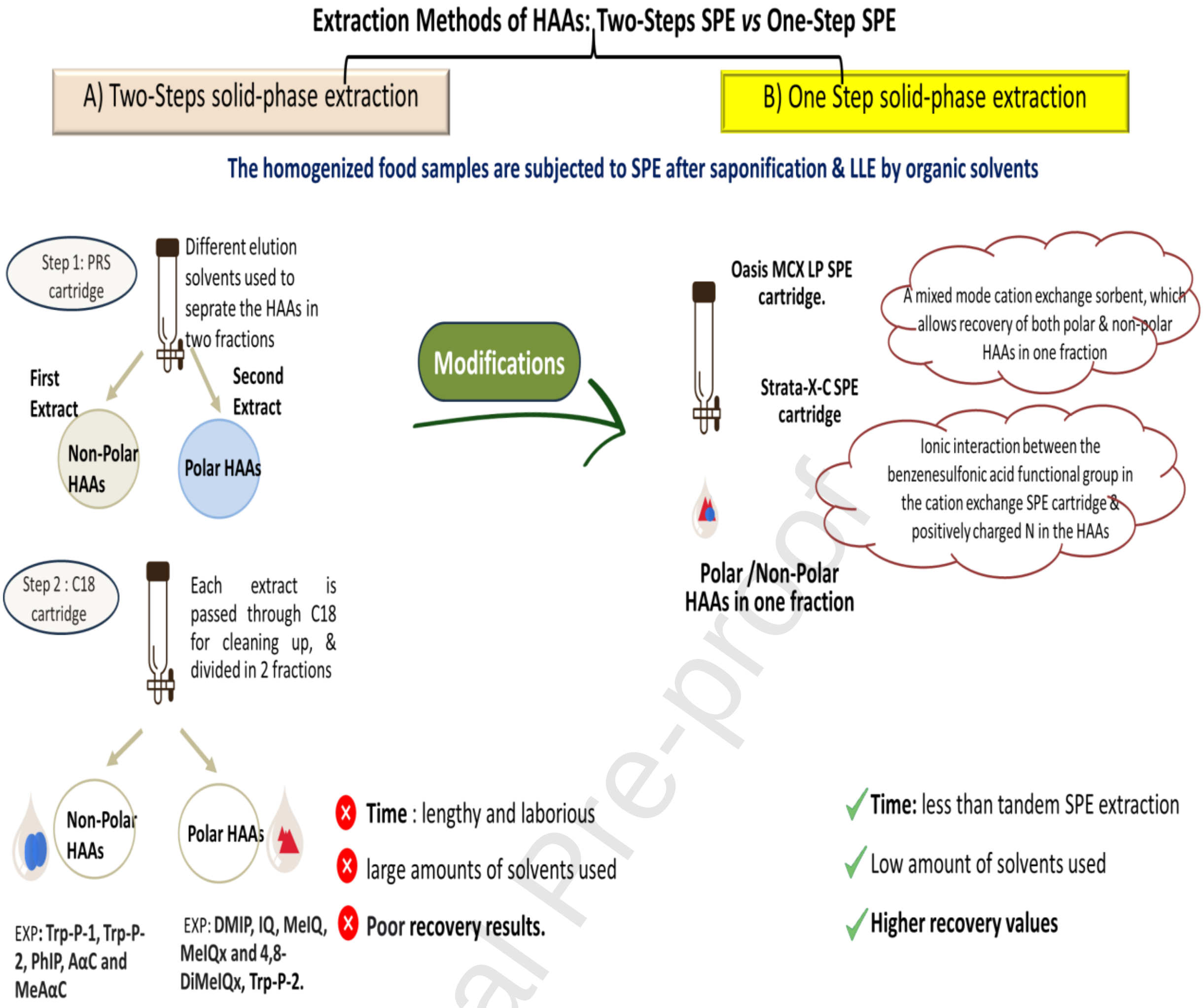
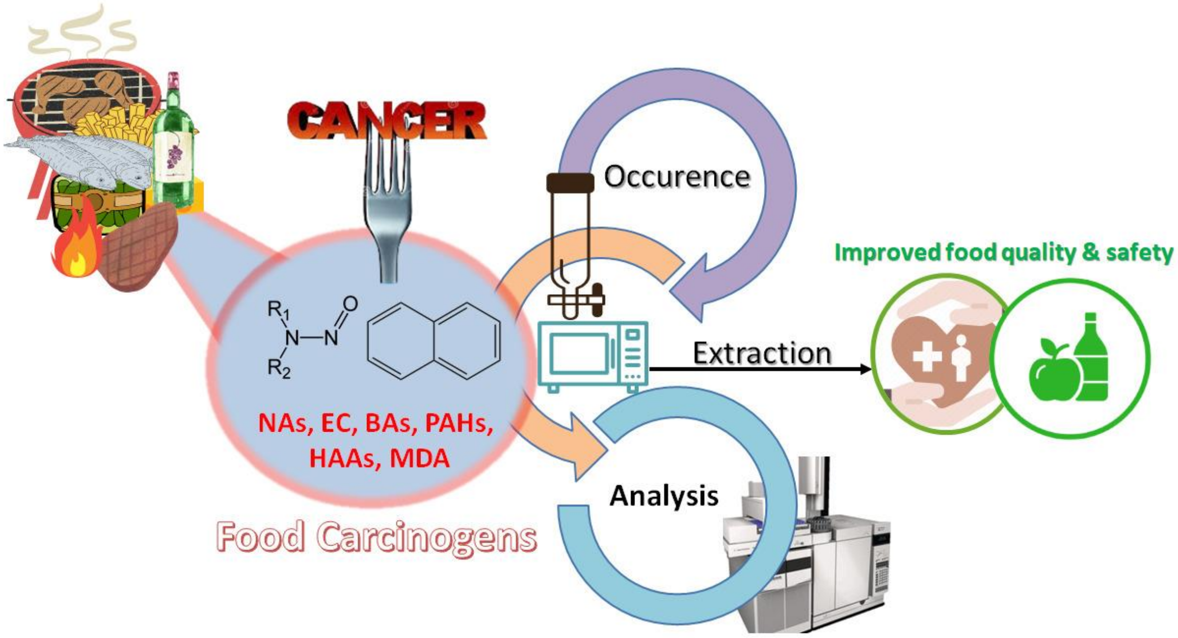


Figure 4. Extraction methods of HAAs A) Two-steps SPE, B) One-step SPE method

Graphical Abstract



Highlights

- Recapitulate 6 potential classes of post-processing food carcinogens and their health hazards.
- Insight on the occurrence of food carcinogens and their production action mechanism upon processing.
- A comprehensive overview on sample preparation strategies targeting eco-friendly multi-class extraction.
- Outline the advantages and limitations of the reported analytical approaches with emphasis on needed future development.
- New perspectives for tracing food carcinogens for healthier processed food are presented.