Determination of Polycyclic Aromatic Hydrocarbon (PAH) on Foods using Numerous Extraction Methods: A Review

Premla Devi Chander*, Chai Mee Kin

College of Foundation and General Studies,
Universiti Tenaga Nasional, Jalan IKRAM-UNITEN,
43000 Kajang, Selangor.

**Corresponding email: premla@uniten.edu.my*

ABSTRACT

According to the European Union (EU) legislation, a strong attention has been focused on the presence of Polycyclic Aromatic Hydrocarbon (PAH) on the environment and also on foods. As known, the presence of PAH mainly on food leads to the activation of carcinogenic agent as the cause of the genotoxic and mutagenic production. Various analytical methods have been used to analyze the concentration of PAH on foods such as fruits and vegetables. The efficiency of PAH concentration on food samples depends on the types of extraction method implemented. The extraction methods were Accelerated Solvent Extraction (ASE), QuEChERS (acronymic name from quick, easy, cheap, effective, rugged and safe) extraction, Supramolecular solvent extraction (SUPRAS), Ultrasonication Extraction, Soxhlet extraction method and Dispersive Liquid-Liquid Microextraction (DLLME). Most of the mentioned extraction methods use the High-Resolution Gas Chromatography (HRGC), High Pressure Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS) to carry the analysis of PAH in fruits and vegetables. The percentage recoveries of each method have been discussed and it was known that SUPRAS showed the best result in percentage recovery and relative standard deviation. In the present review, all the implemented extraction of PAH methods on food were analyzed and discussed in terms of the advantages and the limitations on each extraction methods as well as the analytical performances.

Keywords: PAH; extraction methods; fruits; vegetables

1. INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) is the fusion of more than one type of aromatic rings and also from the class of hydrophobic organic molecules that causes various contaminations to the environment and also surroundings [1]. Those contamination on food causes harmful effects to the human beings as this compound is a carcinogenic to the human leads to cancer diseases. As known, PAH is the subset of Polycyclic Aromatic Compounds (PAC) which contains other elements (e.g. oxygen, nitrogen and sulphur) than carbon in one ring structure. There is two types of PAH can be classified such as light and heavy PAH. Light PAH is consist of four benzene rings and below meanwhile heavy PAH is consist of more than four fused benzene rings which are stable and more toxic compared to light PAH. In brief, some of the PAH mixtures may act as synergist therefore difficulties to inhibit the formation of carcinogenic agent [1,2].

There were many factors of the PAH formation on the environmental as well as on food production. One of the factors was the incomplete combustion of carbon materials, leads to the formation of PAH. Besides, there are various ways of PAH exposures on human. In such ways, when human tend to drink the contaminated water and also depends on how human practicing food consumption, packaging materials and also food production [2].

According to the year of 2005 legislation, mainly 15 types of PAH out of 33 were identified as carrying the traits of genotoxic and carcinogenic which was assessed by the Scientific Committee on Food (SCF). Such PAHs were, the benzo[a]anthracene, benzo [b] fluoranthene, benzo[j] fluoranthene, benzo[k] fluoranthene, benzo[ghi]perylene, benzo [a] pyrene, chrysene, cyclopental[cd] pyrene, dibenzo [a,h] anthracene, dibenzo[a,e] pyrene, , dibenzo[a,h] anthracene, dibenzo[a,i] pyrene, dibenzo[a,l] pyrene, Indeno[1,2,3,-cd] pyrene and 5-Methylchrysene. They used benzo [a]pyrene (BaP) as marker as it presents in the maximum of 20% compared to the total concentration of carcinogenic of PAH [2]. The Air Quality Standard uses the BaP as marker to monitors the exposure levels of PAH in the ambient air. Besides focused on one type of marker, the US Environmental Protection Agency (EPA) decided to increases the monitoring of harmful types of PAH in UK. Due to this, many analytical methods were developed and applied on various types of food as well as in environmental matrices [2].

Generally, PAH was extracted using the liquid-liquid extraction (LLE) method or the solid phase extraction (SPE) method [3]. However, both methods require high amount of organic solvent which is expensive and harmful. Besides, the extraction procedures are complex, tedious and time consuming. Recently, microextraction techniques play important role in the extraction of PAH from the various matrices. These microextraction techniques can be used in the food production, liquid samples as well as the complex matrices and fill the gaps of LLE and SPE methods [4].

Microextraction techniques comprise of high sensitivity, high extraction efficiency and enrichment factor, low cost, short extraction period as well as low amount of solvent usage leads to environmental friendly. There are two major categories of microextraction technniques. (a) Sorbent based microextraction such as Solid Phase Microextraction (SPME) and Stir-Bar Sorptive Extraction (SBSE). (b) Solvent based microextraction such as Single Drop-Microextraction (SDME), Hollow-Fiber Liquid Phase Microextraction (HF-LPME) and Dispersive Liquid-liquid Microextraction (DLLME).

In the present review, the implemented extraction methods of PAH on foods are well focused. Such extraction method that focused in this review are Accelerated Solvent Extraction (ASE), QuEChERS (acronymic name from quick, easy, cheap, effective, rugged and safe) extraction, Supramolecular solvent based extraction (SUPRAS), Ultrasonification extraction, Soxhlet extraction method and Dispersive Liquid-Liquid Microextraction (DLLME). The selection of extraction method plays an important role on obtaining the high percentage of recovery and efficiency of PAH concentration from food samples. The discussions are also based on the advantages and limitations of each approach as well as analytical performance in aspect of extraction efficiencies.

2. THE COMMON EXTRACTION METHODS IMPLEMENTED TO ANALYZE PAH IN FOODS

Based on the analytical analysis, there are few common extraction methods are used to extract the compounds of PAH from foods such as fruits and also vegetables. Most of the extraction methods use the High-Resolution Gas Chromatography (HRGC), High Pressure Liquid Chromatography (HPLC) and Gas Chromatography- Mass Spectrometry (GC-MS) to carry the analysis of PAH in fruits, dairy products and vegetables.

2.1 Accelerated Solvent Extraction (ASE)

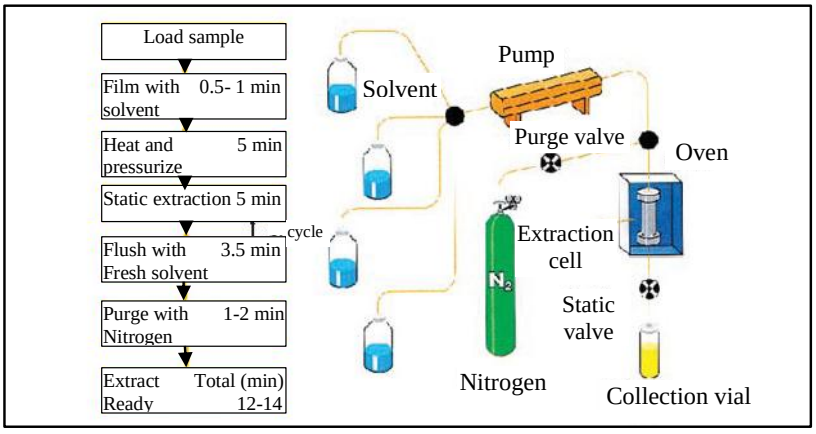


Figure 1: The Outline of ASE method [6]

This extraction method is also known as Pressurized Fluid Extraction (PFE). It acquires short period of time to extract the desired analytes from matrices, at combination of high temperature and pressure. Besides that, this method applies the requirement of U.S. EPA Method 3545. It is derived based on the theory of conventional liquid extraction[5]. As this extraction method able to transfer the existing solid extraction to accelerated mode. The implement of proper preparation techniques and operational parameters leads to attain the high efficiency of extraction.

Martorell, *et al*. (2010) used the ASE method, to extract the 16 types of PAHs [naphthalene, acenaphtylene, acenaphtene, benzo(a) anthracene, crysene, benzo(b) fluoranthene, fluorene, phenanthrene, anthracene, fluoranthene ,pyrene, benzo(a) pyrene, indeno(1,2,3,-c,d) pyrene, dibenzo (a,h) anthracene and benzo (g,h,i) perylene] in vegetables such as lettuce, tomato, cabbage, tubers and as well in fruits such as orange, apple, banana and strawberry [2].

The figure 1 shows the outline of ASE method. In which, the samples were kept into the extraction cell. Then, the solvents from one or more reservoirs were transferred via a pump into the extraction cell in which located in an oven. The process was further up with the heat up the samples by an oven for a short period of time, in the range of 10 to 20 minutes. Extractions were performed using the static mode, in which a nitrogen purge is used to transfer the extract to a collection vial [6].

In this ASE method, the samples were homogenized in which suitable isotope-labelled extraction standards were added. The purpose of adding the standard is to control the sample extraction. Hexane and acetone solution were mixed accordingly to its ratio, as it act as solvent extraction and further with chromatographic size exclusion as the cleaning process. The purpose of cleaning step in the method was to filter the analytes from contaminants and reduces the interference that eventually leads to damage to the instrument. Therefore, each sample undergoes chromatographic size exclusion and the process continued with combination of High-Resolution Mass Spectrometry (HRMS) with High-Resolution Gas Chromatography (HRGC) [2]. This extraction method is the modern version of conventional liquid extraction as it acquires short period of time and less solvent consuming in which it only requires two types of solvent majorly as extraction solvent such as hexane and acetone. As well as less cost taken as it only uses high temperature and force of pressure to extract the compound compared to other conventional methods [2]. The simplicity of extraction procedure is the result of modification made on the conventional liquid extraction. High temperature enable the fast transfer from surface of the particle to extraction solvent and also increases the diffusion of internal component of the polymer particles to the surface [7]

2.2 QuEChERs Extraction

Figure 2: The Procedure of QuEChERs Extraction (Steven, 2010)

This method known as an inexpensive method compared with other conventional extraction methods such as Liquid-Liquid extraction (LLE) and Solid Phase Extraction (SPE) methods. QuEChER meant by quick, easy, cheap, effective, rugged and also safe method compared to Liquid-Liquid extraction (LLE) and Solid Phase Extraction (SPE) methods. It has short procedures with less amount of time needed. This is due to the simplicity of this extraction method in which includes extraction solvent (e.g. ethyl acetate) further up with clean-up process before send the sample for further analysis. It has the capabilities of yielding high percentage of recoveries from 90 to 110% with RSD's <5% for wide range of GC amenable compounds compared to other methods. No hazardous extraction solvent such like chlorinated solvent is required. Besides, less amount of extraction solvent acquired and minor wastage is produce resulting of generating accurate extractions.

There were two steps acquired in the extraction method such was homogenized the samples and another step is acquires dispersive solid phase extraction (dSPE).

The figure 2 briefly explains the two steps in the extraction. The first 5 steps of the figure 2 are referring to the homogenization process. The sample were weighed, homogenized using the ceramic homogenizers which helps to reduce the shaking time and break down the salt agglomerates and increases the efficiency of the extraction. Meanwhile, the remaining steps refer to the second part of this extraction which was cleaning up process. In which the compilation centrifugation process, vortex and carry out dSPE step takes place and the samples were collected into the vial. The purpose of cleaning up step is to purify the analytes from the contaminants as well as to reduce the interference that disrupt analytical instrument and complicate the analyte identification.

Banerjee, et al. (2012) used the dSPE method as clean up step [8]. In which, the Primary Secondary Amide (PSA) used as common sorbent as it removes compounds like sugar compounds, lipids, organic, sterols, proteins and also the excessive water from the samples. The specialty of this method is the dSPE tube, also known as QuEChER tube contains magnesium sulphate and sodium chloride in which removes the water compounds from the samples and decreases the polar interferences. Both agents enhance the recovery of polar analytes. Moreover, this method has the potential of extracting large amount of samples in short time.

2.3 Supramolecular solvent (SUPRAS) Based Microextraction

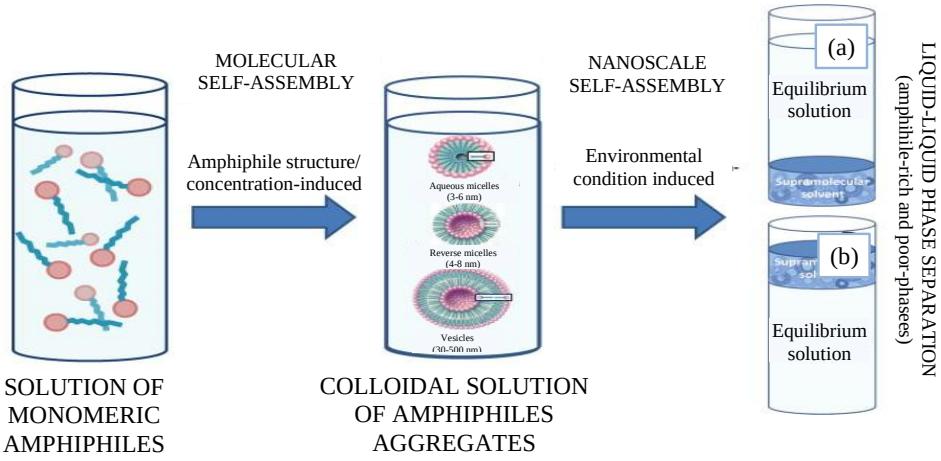


Figure 3: The formation of Supramolecular aggregation in self-assembly process [9].

This extraction is also known as SUPRAS and referred as coacervates and surfactant liquid-liquid phase separation. It is nanostructured liquids in which developed from the amphiphilic compounds [10,11].The generation of SUPPRAS occurs through the self-assembly process (Figure 3). This process enables the spontaneous separation of amphiphile from the large solution. The activation of self-assembly is dependable on the inducement of environmental condition. For instance, the pH modification and the presence of non-solvent for amphiphile lead to the activation of self- assembly. The benefit of using this extraction is SUPRAS has large interactions of analytes, as it has different regions of polarities and hydrophobic interactions. The higher concentration of amphiphiles leads to high number of binding sites of analytes in which increases the efficiency of this extraction is also compared to other methods as well as low amount of samples can be implement as this referring to microextraction [10,11].

Besides, this extraction is safety and harmless as it involve non-volatile and inflammable processes. This extraction is well known for the simultaneous extraction and for the cleaning up process as well. This method was used to extract the contaminants from the food and enhances the extraction efficiency. Besides that, it has compilation of

simple procedures, quantitative, cheap and also environmentally friendly compared to other methods [10,11].

The mixing of octanic acid with Bu4NOH and distilled water leads to the stimulation of self-assembly result the formation of SUPRAS, which was less dense than water. In addition the separation phase formed as the total density of conglomerates is lower than the solution. Therefore, the upper layer is isolated and store into closed glass vials for further usage. After the sample undergoes the homogenization, the samples were mixed with the SUPRAS in a safe lock 2ml microtube. The presence of glass pearls in the microtube enhances the sample dispersion as it undergoes the vortex. Finally, the SUPRAS with target PAH was transferred into a glass vial proceeds with chromatographic system. This extraction method has been used in Gomez & Sicilia (2010) and also in Jimenez, Gomez, & Rubio (2013) to extract the PAH compounds from foods [10,11]. Both methods used the similar mixing of extraction solvent to produce supramolecular formation.

2.4. Ultrasonication Extraction

This extraction method is unique compared to other extraction method as it acquires the ultrasonication to extract the PAH compounds from the samples. This method was implemented by Monica C et al, 2003 to extract 16 types of PAHs from foods[12]. After homogenization process, it undergoes the ultrasonication for short period using the sonicator. Then, remove the excessive of solvent from the sample by using the rotary evaporation. Ultrasonication stimulates the mechanical stress on the cells through the production of cavitation in the samples. Further, the samples are highly concentrated with rotary evaporation and preceded with the clean-up process using silica gel. Commonly, this extraction method does not required clean-up step after sonication as this is due to the energy production from the collapsing the cavitational bubbles leads to greater penetration of solvent into the cellular cell material. In which, it increases the efficiency of extraction by quick transfer of particles into extraction solvent. Besides that, it also homogenizes the samples with the extraction solvent well. Also, this extraction has short procedures with limited extraction solvent leads to short time consumption and save cost. This method requires the rotary evaporation which evaporates the solvents which have high boiling points. The exposure of solvents to the high temperature during the evaporation triggers the occurrence of side reactions in the sample leads to oxidation or breakage of analytes. Therefore, lowering of pressure and temperature enables to separation of analytes from the solvents without any inteferences [13].

2.4 Soxhlet Extraction

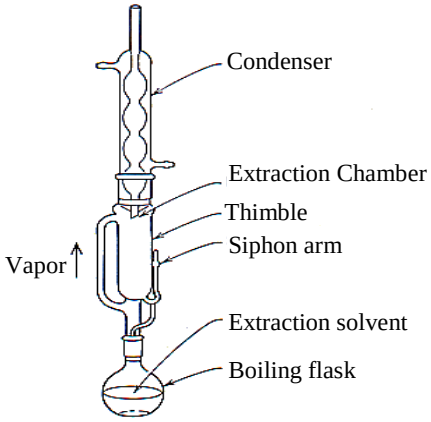
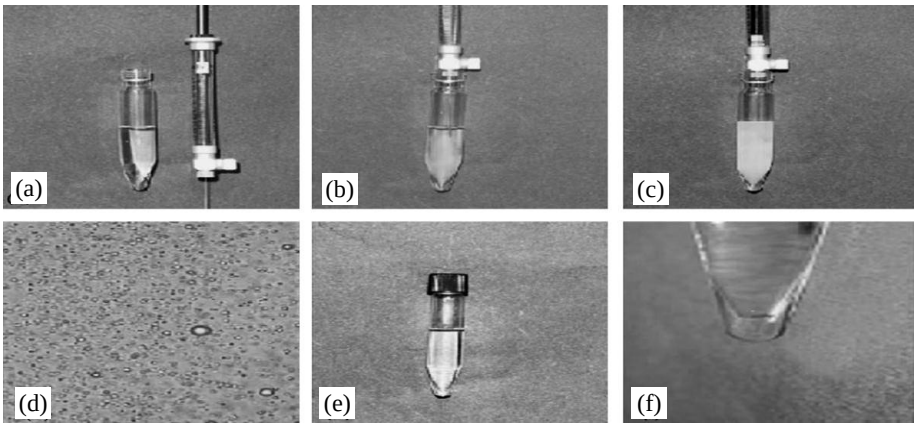


Figure 4: Soxhlet Extractor [14].

This method is known as semi continuous method in which involves the clean -up method using SPE cartridges. This extraction can use to extract the volatile and nonvolatile compounds from fatty matrix. This extraction able to remove the lipids from the samples and provides the strong interaction between matrix and solvent extraction [15].The potential benefit of using this as extraction method is the number of replica leads exposure with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium. The heat that applied to the distillation flask reaches the extraction cavity to some extent resulting the temperature of the system remains relatively high. There is not necessary of proceeding with clean-up step as well as inexpensive method. Common clean-up steps that implemented were Gel Permeation Chromatography (GPC) or saponification process. The purpose of this step is to increases the efficiency of the analyte extraction and also to purify the analyte from the contaminants [16]. Fast-track of extraction can be done by simultaneous extraction in parallel. It acquires little specialized abilities to operate the extraction and also is non-matrix dependent [15]. This extraction method can also implement if the desired analytes has the characteristic of low solubility in extraction solvent. As this technique, acquires the glassware in between a flask and a condenser involving the process of repeated refluxing solvent into flask which showed in Figure 4 [16].

2.6 Dispersive Liquid-liquid Nicroextraction (DLLME)



Fiqure 1: Overall procedure of DLLME technique to extract PAH from sample [17].

Dispersive liquid-liquid microextraction (DLLME) was discovered by Assadi and teammates in the year of 2006 [18]. Figure 1 shows the overall procedure of DLLME technique. First of all, this method acquires two types of solvent which is extraction solvent and dispersive solvent. Then, the mixtures of dispersive and extraction solvents is injected rapidly into an aqueous sample. A cloudy solution which consists of fine droplets of extraction solvents dispersing in the aqueous solution is formed. Two layers will be formed after the centrifugation process and the extraction solvent which contains the desired analyte is removed using the microsyringe and transfer into the vials and carry out for further analysis using chromatographic system. The advantages of using this method are simple and easy to carry out the extraction with short extraction procedure compare to LLE and SPE, low wastage of toxic solvents, low cost consumption, less time spent and also high extraction efficiency of analyte. This extraction method achieves high enrichment factor compared to other microextraction methods, in which the extraction solvent has the large surface area compared to the aqueous solution [18]. The enrichment factor and extraction recovery can be calculated as below:

$F= C_{sed}/C_o$

$R= (C_{sed}\ V_{sed}) / (C_o\ V_{aq})$

Where;

F=Enrichment factor,

C_{sed} = Concentration of sediment

C_o =initial concentration of analyte in aqueous sample

R=Extraction recovery

V_{sed} =Volume of sediment phase

V_{aq} =Volume of aqueous sample

The extraction efficiency of DLLME is affected by various parameters condition. There are selection of extraction solvent and dispersive solvent, effects of volume of

extraction solvent and dispersive solvent as well as the extraction time and also the effect of addition salt in the solution.

3. ASPECTS OF ANALYTICAL QUALITY

Table 1: The Summary of Extraction Methods and Clean-up step Implemented

NO	Types of Matrix	Extraction Method	Clean-up	Separation/ Detection	Recovery (%)	RSD (%)	Ref
1	Cheese	Soxhlet	GPC	LC-FLD	52-94	9-34	Suchanova *et al.*, 2008
2	Tea Leaves	Ultrasonification	Column (silica) Chromatography	LC-UV	>70	>20	[19]
3	Vegetables	ASE	Chromatographic size exclusion	HRGC	41-147.7	N.A	[2]
4	Vegetables	QuEChERS	DSPE	GC-EI-MS	>70	<20	[8]
5	Cereal based foods	SUPRAS	-	LC-FLD	>92	<5	[11]
6	Smoked Fish	DLLME	-	GC-MS	82.1-105.5	2.8-9	[20]

*GPC: gel permeation chromatography
LC-FLD: liquid chromatography coupled to fluorescence
LC-UV: LC couple with ultraviolet
ASE: Accelerated solvent extraction
HRGC: High-resolution gas chromatography (HRGC)
DSPE: dispersive solid phase extraction
GC-EI-MS: Gas chromatography triple quadrupole mass spectrometry
SUPRAS: supermolecular based extraction
N.A: Data not available.

3.1 Comparison of Analytical Performances

Based on Table 1, it was known that the percentage of recoveries for the six types of extraction methods were in the acceptable (70% to 120%). Therefore, it can be concluded that all the six types of extraction methods able to extract the PAH compounds from the samples. DLLME and SUPRAS show the best recoveries among the other extraction methods, due to the high solubility of the analytes into the extraction solvent. For instances, the formation of ordered aggregates and large concentration of amphiphiles leads to high solubilisation of analytes in SUPRAS extraction method as stated in Jimenez, Gomez, & Rubio, (2013); Gomez & Sicilia, (2010)[11]. The target of cleave the PAH compounds from the sample is high achieved using the respective method extraction. In SUPRAS extraction method, wide range of nonpolar compounds able to extracted as the ordered structure in SUPRAS consist of hydrophobic and hydrophilic induces the hydrophobic microenviroment in hydrocarbon region of the ordered structures. Besides, the polar properties of analytes able to target by polar groups in ordered aggregates as well. DLLME achieved the equilbrium state faster and also produced high extraction efficiency (82.1% – 105.5%) compared to ASE and Soxhlet. As known, DLLME method does not required the extraction solvent preparation like

SUPRAS. The rapid injection of extraction solvent and dispersive solvent leads to the formation of fine droplets that has the high solubility with the desired analyte enables the transfer of analyte from aqueous sample into the extraction solvent [17]. Meanwhile, the addition of clean up step also helps to increases the efficiency though its time consumption. Low recoveries obtain from ASE and Soxhlet extraction methods. This was due to the inappropriate extraction method leads to the contamination, the precision of extraction is low compared the other methods, some of the compounds loses during the extraction method or during the clean-up steps [14]. Also, the certain physiochemical properties of the compound are not eligible to use the respective extraction method. This is due to the concentration of analytes is lower than the limit of detection of the chromatographic system. In general, the good relative standard deviation is achieved (<20%) using SUPRAS and DLLME extraction method. The elevated relative standard deviation percentage might due to the time consumption especially the clean-up. There are other types of cleaning step can implemented to yields better recoveries such as saponification and gel permeation chromatography (based on size exclusion chromatography). This clean-up step can induce to remove the analytes from the lipids. Overall, the compounds that easily found on the food samples were Benzo[a] anthracene and Benzo[k] fluoranthene and the random compounds was chrysene. Besides, all sixth types of implemented extraction methods were obtain different result. This is due to different types of detection instruments were used. All of six methods able to detect the common types of PAHs from the food sample. Moreover, the efficiency of extract the respective PAH is highly dependable on various factors. Such factors are the selectivity of extraction method, cleaning-up process, selectivity of instrument and also the extraction solvent.

3.2 Common Limitations of Extraction Methods.

There is a space in each extraction method for better modification on the method to yield higher efficiency of extracting the desired compound. As known that, extraction method plays an important role to produce accurate results as well as the instrument used for the detection. In terms of extracting the different types of PAHs compounds from the various foods, there is some limitation on the five types of extraction method. Not all extraction method can be applied to all types of foods samples in order to produce precise results. Specifically, such like vegetables, dairy products, fruits, meat and many others. The common limitation based on the discussed extraction methods are large amount of solvent wastage is produce due to the repetition of samples extracting with the fresh solvents. Such like, the Soxhlet extraction method meanwhile large amount of solvent need to use in sonication process, resulting high solvent wastage [15]. Besides, most of the extraction methods has long complex procedure and also time consuming which leads to the low efficiency of extracting the PAH from the food which is not suitable for replication analysis. At times, the physiochemical properties of the foods lead difficulties to extract the PAH and produce many interference. For instance, soxhlet extraction is not suitable for the organic compound that is not stable. Besides, the solvent has to be evaporated to obtain concentrate analyte before undergoes for detection. In such ways,

there is potential of the degradable of labile compound due to high temperature. Ultrasonification extraction method is not suitable for the polymer adsorbents and also unstable substances [21].

3.3 Future Trends

There are many drawbacks from the present extraction methods such as acquiring complex extraction procedure, produce large amount of solvent wastage, expensive, tedious and also time consuming which initiates to potential extraction methods. Recently, microextraction techniques are one of potential extraction methods that solve the major drawbacks for conventional extraction methods. As known, microextraction techniques comprise of high sensitivity, high extraction efficiency and enrichment factor, low cost, short extraction period as well as low amount of solvent usage leads to environmental friendly [4]. Dispersive Liquid-Liquid Microextraction (DLLME) is a popular extraction method has been implemented to extract the PAH from liquid and solid matrices [17]. Though, this extraction method achieves high enrichment factor and extraction efficiency. However, the common implemented extraction solvent are chlorinated which is environmental unfriendly due to the high toxicity as well as mask the analyte peaks. Therefore, the current research should focus to replace the chlorinated extraction solvents with green extraction solvent that yields high extraction efficiency and enrichment factor [4]. The formulation of solvent extraction should include specific function and narrowed cleavage of desired analytes which eliminates the other interferences and yields high recoveries. Besides that, the extraction method should include less solvent consuming, capability of extracting large amount of samples with low cost and less time invested as well as the extracting solvent must be an environmental friendly and also includes simplify clean-up step leads to high recoveries and efficiencies. More research should focused on the implementing on the types of extraction solvents as same types of extraction solvent used in almost most of the extraction methods. Besides, more studies should look onto many types of PAHs instead of carcinogenic types of PAH. Also the more extraction methods should develop on the solid samples instead of focusing only on liquid samples. The data should analyses on the validation of developing the new extraction methods through carry out interlaboratory studies. All the efforts lead to improve the data of PAH concentrations in food and as well as the extraction methods.

3. CONCLUSION

The determination of PAH from food using numerous extraction method were discussed. The presence of polycyclic aromatic hydrocarbon in variety foods has been proven through many researches since early 1990s as well as the various extraction methods has been implanted to produce high efficiency of PAH detections. Each method has its own way in such the simplicity of the extraction procedure and the chemical properties of the extraction method to extract the PAH from the food. Most of the extraction method

requires the clean-up step as it purifies the analyte from the contaminations. The discussed extraction methods in this paper were ASE, Soxhlet, SUPRAS, QuEChERS ,ultrasonication extraction and DLLME . Among the extraction method, it was observed that SUPRAS showed the best result in terms of to obtain the concentration of PAH precisely with high percentage of recoveries such as (>90%) and lowest relative standard deviation which was (<5%).

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