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Research paper



The Effects of Parameters on the Efficiency of DLLME in Extracting of PAHs from Vegetable Samples

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Abstract

An effective analytical method based on microwave-assisted extraction (MAE) and dispersive liquid-liquid microextraction (DLLME) followed by gas chromatography-flame ionization detector (GC-FID) was developed for the determination of polycyclic aromatic hydrocarbons (PAHs) in vegetable samples. In most cases, the details of the parameters influencing the efficiency of DLLME in extraction are not well studied. Understanding the reactions of solvents in extraction is the important task on selecting of an appropriate solvent in the process. The effects of parameters affecting the extraction efficiency of DLLME, including extraction solvent and dispersive solvent, extraction time and MAE, such as solvent, microwave power and irradiation time, were studied and explained. The impacts of physiochemical properties of the selected extraction solvents on the extraction efficiency were also investigated. The results indicated that extraction solvents with low viscosity and low polarity have better extraction efficiency in extraction of PAHs from vegetable sample. No significant difference was observed for the effects of selected dispersive solvents and extraction time on extraction efficiency. In MAE, the types of solvent, microwave power and irradiation time implied some critical effects on the extraction efficiency of DLLME.

Keywords: *Microwave-assisted extraction; dispersive liquid-liquid microextraction; vegetable; polycyclic aromatic hydrocarbon; gas chromatography-flame ionization detector*

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are groups of hydrocarbon compounds consisted of two or more fused benzene rings [1]. These PAHs are released from incomplete combustion of organic matters. Joint FAO/WHO Expert Committee on Food Additives (JECFA) [2] and International Agency for Research on Cancer (IARC) [3] have classified some PAHs as carcinogens including chrysene, benzo[a]pyrene, benz[a]anthracene and etc. The animal tests reported some PAHs were the significant factor that causing non-genotoxic diseases [4, 5]. The exposure of PAHs to human can be through various pathways and the three main routes are inhalation, direct dermal contact, and ingestion [6]. The ingestion of food and water are considered as the major route for PAHs intake [7]. The occurrences of PAHs in the raw vegetables and fruits which may due to air pollution were reported [8, 9].

The determination of PAHs from food sample is very crucial because of its perilous properties. Direct determination of PAHs from vegetable samples usually is impossible because they contain complex constituents such as phytoconstituents [10], plant lipid [11] and phenolic compounds [12]. Owing to this fact, some conventional methods such as solid-phase extraction (SPE) [13] and liquid-liquid extraction (LLE) [14] have been developed for the sample pre-treatment and clean-up before the instrumental analysis. However, these methods experienced several disadvantages such as labor-intensive, time consuming and expensive.

To overcome the disadvantages of conventional methods, many researches put the efforts toward the development of sample preparation techniques with low solvent consumption, economic and rapid. In 2006, Rezaee and his co-workers [15] introduced a new miniaturized sample preparation technique which named as dispersive liquid-liquid microextraction (DLLME). DLLME has obtained increasing attentions from the analytical chemists and successfully utilized for extraction of the analytes of different structures. DLLME has several advantages include miniaturized volume of solvent consumption, simplicity, short extraction time, cost effective, high enrichment factor, and high recovery [16]. It used sample solution, dispersive solvent and extraction solvent as ternary component solvent system for extraction [15]. Chlorinated solvents are generally selected as the extraction solvents in DLLME as their extraction efficiency is relatively high. However, most of these chlorinated solvents are highly toxic [17]. Despite of that, DLLME is not well suited for extraction of analytes from the complex food samples. The previous studies reported that dilution of vegetable samples was required to minimize the effect of matrix interferences on the extraction efficiency of DLLME [18]. However, the matrix components still existed and impeded the accumulation of extraction solvent. This will cause the issue for obtaining the clean chromatogram as the extraction phase was not clean enough. To overcome this problem, high volume of extraction solvent was needed.

In recent years, microwave-assisted extraction (MAE) has been developed as alternative approach for sample preparation. It exhibits many excellent characteristics such as requires minimize volume of organic solvents, short extraction time and increases the extraction recovery [19, 20]. Best in our knowledges, there is no study use the MAE-DLLME technique for the determination of PAHs in vegetable. No one has explained the details of the parameters affecting the efficiency of DLLME in extraction.

In the present study, MAE was combined with DLLME (MAE-DLLME) for extraction of PAHs from vegetables followed by

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analysis using gas-chromatography-flame ionization detector (GC-FID). To overcome the disadvantage of consuming high-toxicity chlorinated solvents as extraction solvents in conventional DLLME, low toxic alcohol and brominated solvents were chosen as extraction solvents. It is important to understand the details of the parameters influencing the efficiency of DLLME in extraction for the development of DLLME applications in the future. Hence, the impacts of physiochemical properties of the chosen alcohol and brominated solvents on extraction efficiency were also evaluated and discussed.

2. Experimental

2.1. Reagents and Materials

The EPA 525 PAH Mix B with concentration of 500 µg/mL in acetone (acenaphthylene, phenanthrene, anthracene, pyrene, fluorene, benzo[a]anthracene, benzo[b]fluoranthene, chrysene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[ghi]perylene dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) and biphenyl solution (internal standard) with concentration of 2000 µg/mL in methanol were purchased from Sigma-Aldirch (USA). Working solution (10 mg/L) of 13 PAHs and biphenyl was prepared in acetone and methanol respectively. Stock solutions and working solutions were stored at 4 °C in a refrigerator prior to use.
Acetonitrile, acetone, methanol and hexane were obtained by purchasing from Fisher Scientific (Loughborough, UK). The solvents of 1-bromo-3-methylbutane, 1-bromobutane, bromobenzene, 2-bromo-2-methylbutane, 2-heptanol, 2-octanol and 2-ethyl-1-hexanol were bought from Merck (Darmstadt, Germany). All the chemicals used were of the analytical reagent grade and highest purity.

2.2. Instrumentations

The sample was analyzed using a gas chromatograph (GC, Agilent 7890A) equipped with a flame ionization detector (FID). The PAHs were separated using SLB-5ms capillary column (30 m×0.25 mm ID, 0.25 µm film thickness). Helium gas (Air products, UK) with the purity of 99.9995% was used as a carrier gas at a constant flow rate of 1.67 mL min^{-1} and it was filtered through Agilent Big Universal Trap-Helium purge (model RMSN-2) to discard hydrocarbons, water and oxygen. Injection was carried out in the splitless mode using Agilent 7683B automatic liquid sampler at 290 °C. The oven temperature was initially programmed from 70 °C for 1 min, ramped to 120 °C at 20 °C min^{-1} and holding for 1 min; Then ramped to 258 °C at 10 °C min^{-1} with holding for 1 min, up to 262 °C at 1 °C min^{-1} with holding for 2 min. Finally, the temperature was ramped to 280 °C at 5 °C min^{-1} and holding for 5.1 min. The temperature of FID was kept at 300 °C. The instrumental controller and data analysis was performed using Agilent Chemstation (B.04.02 version).

2.3. Sample Preparation

The PAHs-free cabbage that bought from the local hypermarket was used for the method optimization. The mass of 25 g vegetable was weighted, mixed with 300 mL of distilled water and homogenized using food processor (Philips, HR2001/70). After that, the homogenized sample was sieved and filtered. After centrifugation using Spinplus Centrifuge (Topscien) at the 4000 rpm for 5 minutes, 0.22 µm pore size nylon membrane filter (Jinlong) was used to filter the supernatant and 10 mL filtered sample was transferred into glass vessels. The sample was spiked with appropriate concentration of biphenyl and PAHs. Lastly, the sample was ready for DLLME or MAE-DLLME process.

2.4. Extraction Procedure

2.4.1. Microwave-Assisted

The volume of 4 mL acetone was added to the 10 mL spiked sample in glass bottle. Then, it was closed with cap and irradiated in the microwave oven at certain power for certain time. After cooled to room temperature by immersing in the water bath, the spiked sample was transferred into centrifuge tube and centrifuged at 4000 rpm for 5 min. The volume of 5 mL aqueous phase was separated and used for DLLME.

2.4.2. DLLME

The mixture of extraction solvent and dispersive solvent was quickly injected into the spiked sample using microvolume syringe. After gently shaken, the mixture was centrifuged at 4000 rpm for 3 min. Organic phase was separated from aqueous phase with syringe and analyzed using GC-FID.

3. Results and Discussions

In this study, all the parameters affecting the efficiency of DLLME and MAE parameters were studied using one-at-a-time method. The DLLME parameters were studied without combined with MAE. However, MAE parameters were studied with the combination of DLLME. One of the methods to determine the extraction efficiency is comparing the analytical extraction efficiency of extracted samples with the corresponding extracts of blanks that spiked with analytes [21]. The relative extraction recovery (ER) and relative enrichment factor (EF) were used to express the extraction efficiency.

3.1. The Effects of DLLME Parameters

3.1.1. Extraction Solvent

The selection of the appropriate extraction solvent is very crucial in DLLME to obtain the good extraction efficiency. In conventional DLLME, the extraction solvent was selected based on the following requirements which is more denser than water, low solubility in water and miscible with the dispersive solvent. It also must capable extracting analytes of interest and have suitable chromatographic behavior [22]. In this study, the high-density and the low-density extraction solvents were studied. Seven types of extraction solvent (1-bromo-3-methylbutane, 1-bromobutane, bromobenzene, 2-bromo-2-methylpropane, 2-heptanol, 2-octanol and 2-ethyl-1-hexnaol) were selected to study due to their low toxicity. Their properties are showed in Table 1.
The results in Fig. 1 show that 2-bromo-2-methylpropane was not able to extract 13 PAHs. Although cloudy solution was produced after the injection of 2-bromo-2-methylpropane with the dispersive solvent into the spiked sample, no organic phase was found after centrifugation. It might because 2-bromo-2-methylpropane carried out the S_N1 reaction (substitution, nucleophile, unimolecular reaction) with water [23]. The solvent of 2-bromo-2-methylpropane is insoluble in the water but it could dissolve in the acetone and reacted with water. Besides, it could dissociate spontaneously to tert-butyl carbocation and bromide ion [22]. The compound of tert-butyl carbocation is stable and able to react with water in fast rate. The final products formed are 2-methyl-2-propanol and hydrogen bromide. These two final products are miscible with water and acetone therefore no organic phase was produced after centrifugation. In addition, the organic phase color was changed from colorless to yellowish as some dissociated bromide ions reacted with other bromide ions and formed the yellowish bromine.

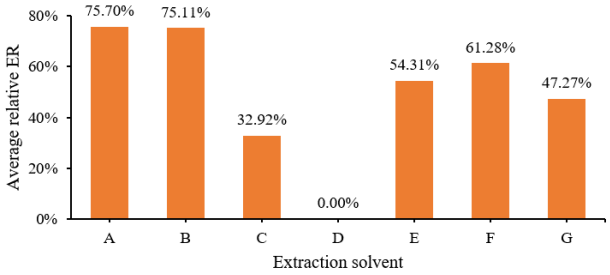
Table 1: Physiochemical properties of extraction solvents used in this study [24-26]

Extraction solvent	LD_{50} (mg/kg, oral) on rat	Density (g/mL)	Solubility in water (g/L)	Molecular Weight	Boiling point (°C)	Dielectric constant	Surface tension (dyn/cm)	Viscosity (mPa. s)
1-bromo-3-methylbutane	6150	1.261 at 25°C	0.196 at 16.5°C	151.047	121	6.33 at 18 °C	Not available	Not available
1-bromobutane	2761	1.276 at 25°C	0.869 at 25°C	137.018	101.4	7.315 at 10°C	26.28	0.606 at 25 °C
Bromobenzene	2383	1.492 at 25°C	0.41 at 25°C	157.008	156	5.45 at 20°C	35.97	1.074 at 25°C
2-bromo-2-methylpropane	4400	1.428 at 20°C	0.6 at 25°C	137.018	74	10.98 at 20°C	Not available	Not available
2-ethyl-1-hexanol	3730	0.933 at 25°C	0.88 at 25°C	130.228	186.2	7.58 at 25°C	Not available	6.27 at 25 °C
2-octanol	>3200	0.819 at 25 °C	1.120 at 25°C	130.228	179	8.13 at 20 °C	Not available	Not available
2-heptanol	2580	0.82 at 20°C	3.27 at 25 °C	116.20	159-161	9.72 at 21°C	Not available	3.95 at 25 °C

The density of three alcohol solvents was lower than water thus the organic phase formed was floated on the aqueous phase after centrifugation. The floated organic phase was difficult to collect if low volume of alcohol solvents was used. Thus, the high volume of 300 µL alcohol solvents was used. The result indicated that three selected alcohol solvents were able to extract all the 13 PAHs. The alcohol solvents of 2-ethyl-1-hexanol and 2-octanol showed the higher average relative ER (54.31% and 61.28% respectively) compared to 2-heptanol (47.27%). Previous studies showed that high viscosity would slow down the mass transfer of PAHs into the organic phase thus reduce the extraction efficiency [27, 28]. This might due to the type of bonding that forming viscosity. In some studies, the high viscosity was due to the excess addition of salts [27]. The salt formed ionic bonding and decrease the diffusion coefficients of analytes [29]. The ionic bonding was strong and not easily to be broken. In this studies, no ionic bond formed between molecules and the high viscosity of solvents was due to intermolecular forces between molecules. The intermolecular forces of 2-ethyl-1-hexanol and 2-octanol were nonpolar Van der Waal forces which due to the dominance of a long hydrocarbon chain over polar hydroxyl group [30]. Energy of Van Der Waals force is weak and easily to be broken. Furthermore, the previous studies stated that the presence of the alkyl groups and a long carbon chain [31, 32] reduced the polarity of the molecules. Fatemi et al. [33] reported that 2-ethyl-1-hexanol was able to extract PAHs from the water sample with high extraction efficiency. Furthermore, the viscosity could be reduced by adding solvents such as acetone and methanol [34]. However, the extraction efficiency of three selected alcohol solvents was lower than acceptable range (70-110%) which may due to the presence of matrix interference in cabbage samples.

Although the solvents of 1-bromobutane and 2-ethyl-1-hexanol have similar value of solubility in water, 1-bromobutane produced higher average relative ER. Therefore it could be stated that the solubility of extraction solvent in water is not a main factor which have the direct effect on extraction efficiency. The three selected alcohol solvents are more viscous than 1-bromobutane. The interaction between PAHs and 1-bromobutane may be more desirable since no energy is needed for broken down the intermolecular forces between 1-bromobutane molecules. For the alcohol solvents, energy is required to break down the intermolecular forces between alcohol molecules before interact with other molecules.

Based on Fig. 1, the extraction solvents that show the highest and second highest average relative ER were 1-bromo-3-methylbutane and 1-bromobutane. The properties of these two solvents were similar with bromobenzene. They can be categorized as nonpolar solvents since no polar interaction was formed between these solvent and water molecule. Moreover, they do not dissolve in water. The bromine atom is present in these three extraction solvents thus they can form halogen bond with PAHs. However, the results indicated that bromobenzene showed the second lowest relative extraction efficiency (32.93%). This might due to the interaction between molecules as bromobenzene could interact with aromatic compounds through halogen bonding, π-stacking interaction and CH/π interaction. Interestingly, bromobenzene also could bind to another bromobezene molecules due to presence of aromatic ring. The binding energy between bromobenzenes was lower than the interaction between bromobenzene and benzene. Furthermore, the interaction between bromobenzene and benzenes molecules was less than the interaction between bromobenzene molecules [35]. Therefore bromobenzene has low chance interacted with PAHs and has low extraction efficiency. Apart from that, bromine atoms in 1-bromobutane and 1-bromo-3-methylbutane bond to the carbon atoms that only have sigma bonds while bromine atoms in bromobenzene bond to the aromatic carbons that have sigma bonds and pi bonds. The properties of halogen bond in different structures are different [36]. Instead of different structure, the viscosity of bromobenzene is higher than 1-bromobutane.



A: 1-bromo-3-methybutane B: 1-bromobutane C: Bromobenzene
D: 2-bromo-2-methylpropane E: 2-ethyl-1-hexanol F: 2-octanol
G: 2-heptanol

Fig. 1: Effect of types of extraction solvent on average relative ER in DLLME. Extraction conditions: sample, 5 mL; Extraction solvent volume, 50 µL 1-bromobutane, bromobenzene, 2-bromo-2-methylpropane, 1-bromo-3-methylbutane, 300 µL 2-ethyl-1-hexanol, 2-octanol and 2-heptanol; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1 min; Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L.

The solvents of 1-bromo-3-methylbutane and 1-bromobutane have similar high average relative ER. However, 1-bromo-3-methylbutane has no clear viscosity value, its structure is almost similar with 1-bromobutane which have extra one methyl group in a carbon chain. The addition of one methyl group would not have a large effect on viscosity [37]. Hence their viscosity value might be similar. The toxicity of 1-bromobutane was higher than 1-

bromo-3-methylbutane, therefore 1-bromo-3-methylbutane was used as extraction solvent in the further study.

The solvent of 1-bromo-3-methylbutane as an extraction solvent was studied at four different volumes (25, 50, 75 and 100 µL) to evaluate the effect of volumes of extraction solvent on the extraction efficiency in DLLME. The results was illustrated in Fig. 2. The volumes of 50 µL, 75 µL and 100 µL were able to yield high average relative ER except 25 µL. In the volume of 25 µL, no organic phase was existed after centrifugation. It might be due to 1-bromo-3-methylbutane was fail to sediment in organic phase as the presence of matrix interference. The volumes of 50 µL, 75 µL and 100 µL have almost similar average relative ER but with increasing volume of extraction solvent from 50 µL, the average relative EF tends to decreased slightly, apparently because of the dilution effect. Hence, the volume of 50 µL was selected for the further study.

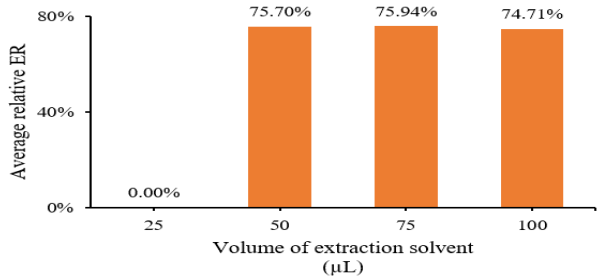


Fig. 2: Effect of volume of extraction solvent on average relative ER in DLLME. Extraction conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane) volume, 25, 50, 75 and 100 µL; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1 min; Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L.

3.1.2. Dispersive Solvent

The main criteria for the selection of dispersive solvent is it must miscible in extraction solvent and sample [38]. Three solvents namely acetone, acetonitrile and methanol were tested in this study. The results (Fig. 3) indicate that the average relative ER using acetone, acetonitrile and methanol were 75.90%, 73.01% and 73.42% respectively. No remarkable variation was obtained using three selected dispersive solvents. Three selected dispersive solvents induced stable emulsion successfully. The stable emulsion is important as extractant droplets which do not easily break up due to the dispersive solvent increases the interfacial tension [39] between the aqueous sample and the extraction solvent. During emulsion, extraction solvents can easily interact with PAHs that in the aqueous sample because both of them are nonpolar molecules. The interaction between dispersive solvent and extractant droplets was formed by weak Van der Waal force, thus their interaction was easily broken by centrifugation and formed the organic phase. Most of the dispersive solvent molecule was dissolved in the aqueous phase as they can form strong hydrogen bond with water molecules. Acetone was less toxic and low cost, therefore it was chosen for the further study.

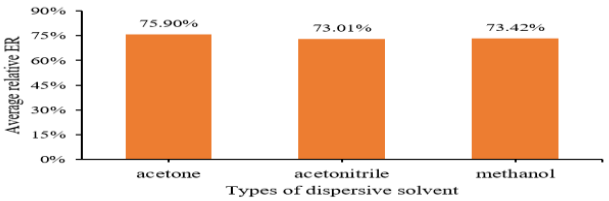


Fig. 3: Effect of types of dispersive solvent on average relative ER in DLLME. Extraction conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane) volume, 50 µL; Dispersive solvent (acetone, acetonitrile, methanol) volume, 800 µL; Room temperature; Extraction time, 1 min; Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L.

The volumes of acetone ranging 200, 400, 600, 800 and 1000 µL were investigated. The results (Fig. 4) show that the average relative ER was increased with the increasing of dispersive solvent volume up to 800 µL. The extraction solvent droplets might not stable in low volume of the dispersive solvent and the extraction solvent might dissolve in the aqueous phase if volume of dispersive solvent is too high. At 800 and 1000 µL of acetone, the average relative ER showed no remarkable difference (75.87% and 76.42% respectively). Thus, the volume of 800 µL of acetone was selected as an optimum volume.

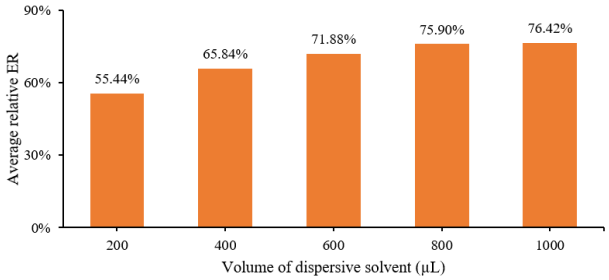


Fig. 4: Effect of volumes of dispersive solvent on average relative ER in DLLME. Extraction conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane) volume, 50 µL; Dispersive solvent volume, 200, 400, 600, 800 and 1000 µL; Room temperature; Extraction time, 1 min; Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L.

3.1.5. Extraction Time

Extraction time is the time spent between after the injection of the mixture of dispersive solvent with extraction solvent into the sample and before centrifugation. The effect of time was investigated in the range of 1 - 3 minutes. It was observed that no significant was found on the average relative ER (Fig. 5). It is because the extraction solvent droplet was formed quickly in the cloudy sample [15]. The large surface area of droplet favored fast mass transfer of 13 PAHs into the organic phase [40]. The time of 1 minute was chosen for the further experiments.

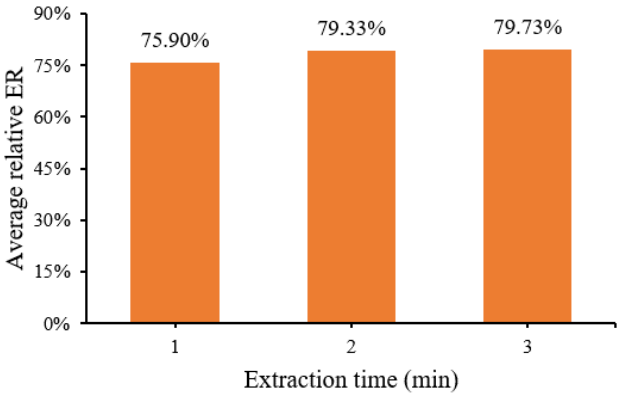


Fig. 5: Effect of extraction time on average relative ER in DLLME. Extraction conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane) volume, 50 µL; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1, 2 and 3 min; Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L.

3.2. Effects of MAE Parameters

The preliminary test of MAE-DLLME revealed that there is a possibly of volume of 1-bromo-3-methylbutane can be reduced to 30 µL from 50 µL after microwave due to the reduction of matrix interference. In order to reduce the exposure risk to the operator

and the environment, the volume of 30 µL of 1-bromo-3-methylbutane was used in MAE-DLLME.

3.2.1. Types of Solvent

It is crucial to evaluate the effect of types of solvent in MAE to improve the efficiency of the extraction process. In this study, the solvent was functioned as a medium to absorb the microwave-irradiation energy and transfer the energy into the subsequent heat so that the matrix component would be thermal degraded. Four types of solvent which were acetone, distilled water acetone/hexane (1:1, v/v) and hexane were selected to evaluate the efficiency of solvent. It was observed that hexane and acetone/hexane have successfully degraded the matrix component (Fig. 6). Nevertheless, no PAHs was detected. It might due to the 1-bromo-3-methylbutane (extraction solvent) was dissolved in the hexane. Thus, no organic phase was formed. The solvent of acetone was capable recovering 13 PAHs with high extraction efficiency (Fig. 6). The average relative ER of distilled water was very low (30.85%) compared to the acetone (103.69%). Although dielectric constant of water is high, its dissipation factor was considered remarkably lower than other selected solvent in this study [41]. Hence, the water disperses the heat with lower speed than the speed of water absorbs the microwave energy. The longer irradiation time or higher microwave power might be required for distilled water to absorb and dissipate heat to the sample.
Acetone which have the higher dissipation factor and dielectric constants strongly absorbed the microwave energy and rapidly transferred it into thermal energy. Kormin et al. [42] suggested that with the same experimental conditions, acetone which have low dipole moment will rotate easily when exposed to the electric field of microwave energy. This oscillation produced collisions with the surrounding molecules then the energy was transferred with the subsequent heating. The matrix interference was then thermally degraded and hence increased the extraction efficiency. Acetone was chosen as solvent in the further MAE process.

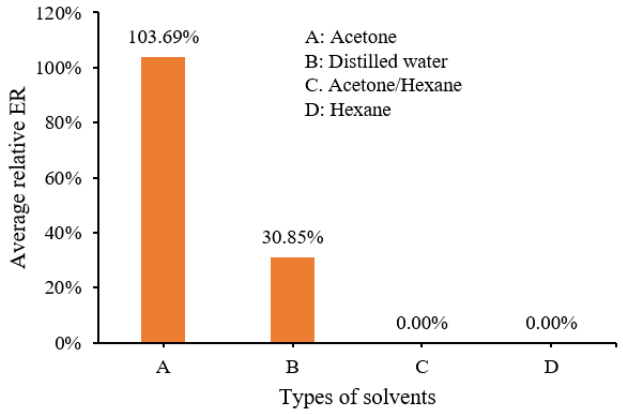


Fig. 6: Effect of types of solvents on average relative ER in MAE-DLLME. Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L. Microwave conditions: sample, 10 mL; Solvent (acetone, distilled water, acetone/hexane and hexane) volume, 4 mL; Microwave power, 200 W; Irradiation time, 1.5 min; DLLME conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane) volume, 30 µL; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1 min.

3.2.2. Microwave Power

The excessive or insufficient thermal energy will reduce the extraction efficiency. Therefore, microwave power should be studied closely. The effect of microwave power on extraction efficiency was evaluated in 100, 200, 300 and 400 W under uniform experimental conditions. The results (Fig. 7) show that under microwave power of 100 W, matrix interference was not degraded efficiently and the average relative ER was low. Under microwave power of 200 W and 300 W, the average relative ER was improved. In 400 W, the sample was burn. The pressure and temperature in 400 W were too high for solvent and vegetable samples. Since lower energy was consumed in 200 W than 300 W, thus 200 W was chosen for the further study.

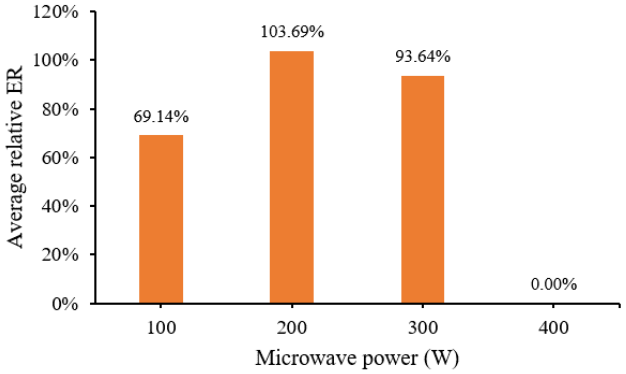


Fig. 7: Effect of microwave power on average relative ER in MAE-DLLME. Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L. Microwave conditions: sample, 10 mL; Solvent (acetone) volume, 4 mL; Microwave power, 100, 200, 300, 400 W; Irradiation time, 1.5 min; DLLME conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane), 30 µL; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1 min.

3.2.3. Irradiation Time.

To study the effect of microwave irradiation time on the extraction efficiency, four different irradiation times (0.5, 1, 1.5 and 2 min) were selected under fixed experimental condition. The results show that from 0.5 min to 1.5 min, the average ER was increased then decreased at 2 min (Fig. 8). The matrix interference was not degraded in the short irradiation time. Degradation of PAHs might be occurred if irradiation time is too long. The time of 1.5 min exposure at a microwave irradiation power of 200 W is the best among others irradiation time.

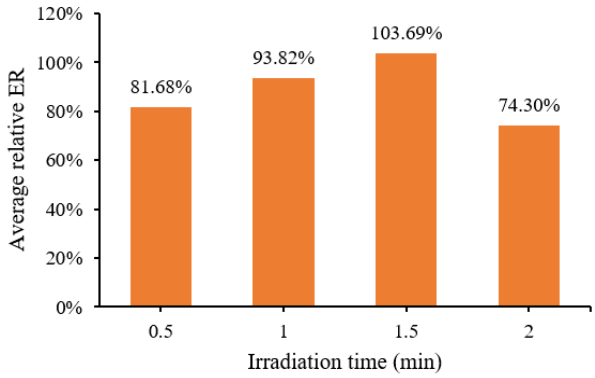


Fig. 8: Effect of irradiation time on average relative ER in MAE-DLLME. Concentration of PAHs, 80 µg/L; Concentration of internal standard (biphenyl), 40 µg/L. Microwave conditions: sample, 10 mL; Solvent (acetone) volume, 4 mL; Microwave power, 200 W; Irradiation time, 0.5, 1, 1.5, 2.0 min; DLLME conditions: sample, 5 mL; Extraction solvent (1-bromo-3-methylbutane), 30 µL; Dispersive solvent (acetone) volume, 800 µL; Room temperature; Extraction time, 1 min.

3. Conclusions

The effects of parameters on the efficiency of DLLME in extracting of 13 PAHs from vegetable samples have been studied in details. The low toxic brominated solvent, 1-bromo-3-methylbutane was able to extract all the 13 PAHs with high extraction efficiency due to the low viscosity and non-polarity. No significant differ-

ence was observed for the effect of the selected dispersive solvents and extraction time. In MAE, the types of solvent, irradiation time and microwave power showed significant effect on the extraction efficiency. The method of MAE combined with DLLME follow by GC-FID has been proposed for the determination of PAHs in vegetable samples.

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