



SHORT COMMUNICATION

Solid-to-Liquid Extraction for Analysis of Polyaromatic Hydrocarbons in Tahdig of Potato by GC/MS: Comparison between Traditional Solvent and Sonication Methodologies

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KEYWORDS

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ABSTRACT: The main purpose of this study was to compare efficiency of two extraction methods for analysis of polyaromatic hydrocarbons (PAHs) in the tahdig of potato prepared with the solid edible oils. The PAHs separation was performed with conventional solvent extraction by Soxhlet and sonication methods. All samples were prepared under the same time (30 min) and temperature (180 °C) with solid edible oils. The extraction efficiencies were determined by subsequent optimized analysis by GC/MS. The amounts of extracted PAHs by Soxhlet and sonication methods were 74.2 ± 3.8 and 136.7 ± 2.1 $\mu\text{g/Kg}$, respectively. With the sonication, mean quantities of PAHs extracted from tahdig were 84% more than Soxhlet method and this improved limit of detection (LOD) and limit of quantification (LOQ) for some high molecular weight PAHs up to 0.01 and 0.03 $\mu\text{g/Kg}$, respectively. This study clearly demonstrated the sonication method was especially much efficient than the traditional Soxhlet method for detection of PAHs in the tahdig of potato with complicated matrix.

INTRODUCTION

Increasing levels of polyaromatic hydrocarbons (PAHs) in the environment and thus elevating human exposure, has led to increased cancer growth rates [1]. The presence of PAHs in food is a global concern because of their carcinogenic properties [2]. Food and feed are among the most important sources for human and animal exposure to PAHs [3]. Some food processing techniques such as smoking and cooking are the most responsible factors for the occurrence of PAHs in foodstuffs [4]. Various types of pot bottoms such as rice and potatoes are also another

popular source for exposure to some contaminants so that due to special cooking condition, tahdig can be significant source of PAHs in social diet. Although delicious, these foods are cooked at high temperatures and may contain harmful compounds [5]. The pot bottoms contain a lot of complicated compounds such as oil, oligosaccharides and polysaccharides, proteins and some of the minerals which makes difficult any control and analysis of contaminants, accurately. Recently, our research group evaluated acrylamide content of tahdig prepared by solid oils [5].

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However, the extraction of PAHs from food samples is faced with interfering compounds. Little is known about the best extraction method for determination of PAHs especially in tahdig as a complicated matrix. Obtaining validated results for determination of PAHs in tahdig provide rational diet suggestions and improve human health conditions [4]. Determination of PAHs in foodstuffs was reported previously by various techniques. Many Methods such as gas chromatography coupled with tandem mass spectrometry (GC/MS/MS) [6], liquid chromatography coupled with fluorescence detection (HPLC-FLD) [7], liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [8] and gas chromatography-mass spectrometry (GC-MS) [9] have been reported as efficient candidate methods for quantification phase. Although some of these methods have their valuable benefits, including high sensitivity and rapidity, they suffer from their low efficiency to extract and measure PAHs in tahdig as a complex matrix. This could be regarded as the main challenge to encourage researchers to design an effective sample preparation method for PAHs before instrumental analysis. In this research Soxhlet extraction was applied for determination of PAHs using n-hexane as extracting solvent. The sonication technique was also applied for extraction of PAHs from baked potato after it was prepared in cooking tahdig. The main aim of this study was comparison between traditional solvent and sonication methods for extraction and analysis of PAHs in tahdig of potato by GC/MS.

MATERIALS AND METHODS

Chemicals and Reagents

Pure PAHs standards were purchased from Sigma-Aldrich (Taufkirchen, Germany). All used solvents were of chromatography grade and obtained from Merck (Darmstadt, Germany) and used without any further treatment.

Sample Preparation

Potato samples were cut using a slicer in about 4 mm thickness. For executing the experiment and preparing required samples, rice was cooked in traditional way (dehydration method) at 180°C for 30 minutes and potatoes were used as the bottom of the pot. Edible rice and solid oil were purchased from retail local market and used them in cooking process. Before any further analysis, the bottom of the potato pots was completely separated from attached rice. The extraction step was performed by Soxhlet traditional extraction using a polar solvent and also sonication extraction using an appropriate sonicator (Model 55743-Fritsch, Germany).

Soxhlet Extraction method

The samples were dried in a vacuum oven at 30°C for 4h. Naphthalene was used as an internal standard. To extract fats, Soxhlet apparatus was used with methanol and potassium hydroxide and refluxed for 5 hours. The component of ethanol/NaOH caused enough saponification of the lipids. Then, the content of the flask was transferred to the separatory funnel containing immiscible n-hexane solvent. The final solution was concentrated to rotary vacuum at 40°C. The concentrated contents were passed through Sep-Pak silica gel and the final contents were transferred to a vial and dried under nitrogen flow. The residue was dissolved in 0.5 ml acetonitrile and toluene and then analyzed by GC/MS [9].

Sonication Extraction method

The second part of each sample was extracted by sonication method. In the ultrasonic bath, the samples were extracted with 150 ml of methanol for three times in 30 minutes as initial extraction phase and then by adding 20 ml of 0.7 M potassium hydroxide solution and 30 ml of distilled water for two times in 30 minutes were subjected to secondary phase extraction. The filtrates obtained from the later phase were transferred to the separator funnel containing n-hexane. The following steps were continued as in the previous method [10].

GC/MS analysis

Detection and analysis of PAHs were carried out by Agilent 5977A gas chromatography/mass spectrometer (GC/MS) apparatus. The dimension of HP5-MS capillary column was 30m×0.53 mm×0.4 μm. The injection temperature was set at 275°C. Helium was used as carrier gas and its velocity was set at 1.4 ml min⁻¹. The oven temperature was set at 80°C (held for 2 min) ramping at 50°C min⁻¹ to 230°C, then increased by 2°C min⁻¹ up to 260°C and then further increased by 8°C min⁻¹ up to 340°C (held for 5 min). The total run time included 35 min. The peak spectra for each compound were compared with the mass spectra of PAH standards. In the current study, 16 PAHs, including six low molecular weight PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene) and ten high molecular weight PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene) were assessed in tahdig of potato. The extracted amount of each PAH and total PAHs by Soxhlet and sonication techniques and recording limit of detection (LOD) and limit of quantification (LOQ) were majors factors that followed for evaluating superior method in extraction of PAHs from tahdig potato as a complicated matrix. All analyses were performed in triplicate, and in each case three similar samples were prepared for PAH determination.

Statistical analysis

Descriptive statistics were used in order to state the quantitative variables for PAHs by mean and standard deviation using SPSS version 21 software. Bar and column diagrams were drawn by prism. One-way analysis of variance (ANOVA) with post-hoc test was applied for depicting significance of difference between mean PAHs in two extraction groups. The results were considered as mean and standard deviation and differences of $p < 0.05$ were expressed as significant.

RESULTS AND DISCUSSION

After GC/MS analysis, only 10 PAHs were detected in tahdig of potato sample in both extraction methods. The identified PAHs were as: phenanthrene, anthracene, pyrene, fluoranthene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene and benzo[g,h,i]perylene. In tahdig samples extracted by both Soxhlet and sonication methods. Naphthalene, Acenaphthylene, Acenaphthene, Fluorene among low molecular weight and Chrysene and Indeno[1,2,3-cd]pyrene as high molecular weight PAHs were not detected. However, a significant difference between extracted amount of PAHs in Soxhlet and sonication methods was observed in nearly all extracted PAHs except for dibenz[a,h]anthracene ($p < 0.05$) (Table 1). Extraction of dibenz[a,h]anthracene from potato tahdig prepared with solid edible oil with $2.53 \pm 0.16 \mu\text{g Kg}^{-1}$ was improved in sonication method but in wasn't significant in comparison with Soxhlet method with $1.88 \pm 0.11 \mu\text{g Kg}^{-1}$ ($p = 0.1$). With the sonication, limit of detection (LOD) and limit of quantification (LOQ) improved for some high molecular weight PAHs from levels as high as 0.07 and 0.21 $\mu\text{g Kg}^{-1}$ to low levels as minimum as 0.01 and 0.03 $\mu\text{g/Kg}$, respectively (Table 1). The obtained LODs were compatible with European Commission Regulation No. 208/2005 (LOD $< 0.3 \mu\text{g Kg}^{-1}$) [11].

The advantage of sonication extraction was also obviously depicted in Figure 1 by superior extracted total PAHs. The total amounts of extracted PAHs by Soxhlet method was 74.2 ± 3.8 and was increased up to $136.7 \pm 2.1 \mu\text{g Kg}^{-1}$ in sonication procedure. With the sonication technique, mean quantities of PAHs extracted from tahdig were 84% more than Soxhlet method. There was a significant difference between total PAHs mean values in Soxhlet and sonication methods ($p < 0.05$). In our previous study, similar results were also observed and the advantages of sonication compared to the reflux method were approved for extraction of acrylamide from tahdig samples [5]. It is likely that due to the high temperature used in the Soxhlet system, some of the PAHs evaporated and this may be cause loss of some analytes during extraction process [5].

The comparison between the total amounts of PAHs in two extraction methods suggests the difference between them and introduces sonication as superior candidate (Figure 1).

Table 1. The level of total and selected PAHs (mean±SD $\mu\text{g Kg}^{-1}$) in tahdig samples extracted by Soxhlet and sonication methods.

PAHs Extraction	Benzo (a) pyrene			Fluoranthene			Benzo(b) fluoranthene		
	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean
Soxhlet	0.07	0.21	2.01±0.14 ^a	0.05	0.16	1.52±0.28 ^a	0.04	0.12	6.62±0.23 ^a
Sonication	0.01	0.05	7.92±0.28 ^b	0.01	0.03	9.61±0.56 ^b	0.02	0.07	11.61±0.49 ^b
PAHs Extraction	Anthracene			Phenanthrene			Pyrene		
	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean
Soxhlet	0.06	0.21	9.33±0.26 ^a	0.05	0.16	4.85±0.17 ^a	0.04	0.12	15.71±0.44 ^a
Sonication	0.02	0.09	19.11±0.24 ^b	0.02	0.06	17.91±0.51 ^b	0.02	0.06	29.44±0.21 ^b
PAHs Extraction	Benz[a]anthracene			Benzo[k]fluoranthene			Benzo[g,h,i]perylene		
	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean	LOD, $\mu\text{g Kg}^{-1}$	LOQ, $\mu\text{g Kg}^{-1}$	Mean
Soxhlet	0.07	0.21	13.18±0.27 ^a	0.05	0.16	8.12±0.14 ^a	0.04	0.12	1.93±0.35 ^a
Sonication	0.03	0.09	24.90±0.21 ^b	0.02	0.07	19.15±0.17 ^b	0.01	0.03	8.10±0.22 ^b

* Means with the different letters are significantly different from each other (P<0.05).

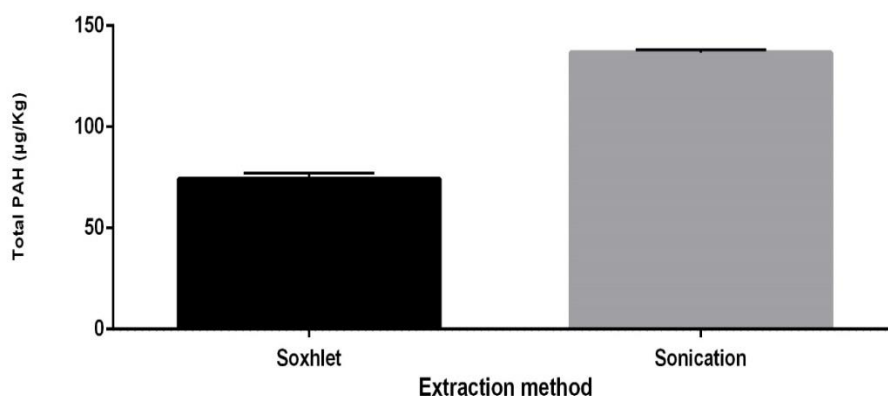


Figure 1. Comparison of total PAHs level ($\mu\text{g Kg}^{-1}$) in tahdig of potato extracted by Soxhlet and sonication methods (n=6)

Total ion chromatograms for PAHs extracted from tahdig of potato by Soxhlet and sonication methods were illustrated in Figure 2. The efficiency of extraction was

improved in sonication method by appearing PAHs with more abundance in Figure 2B in comparison with Figure 2A for Soxhlet method.

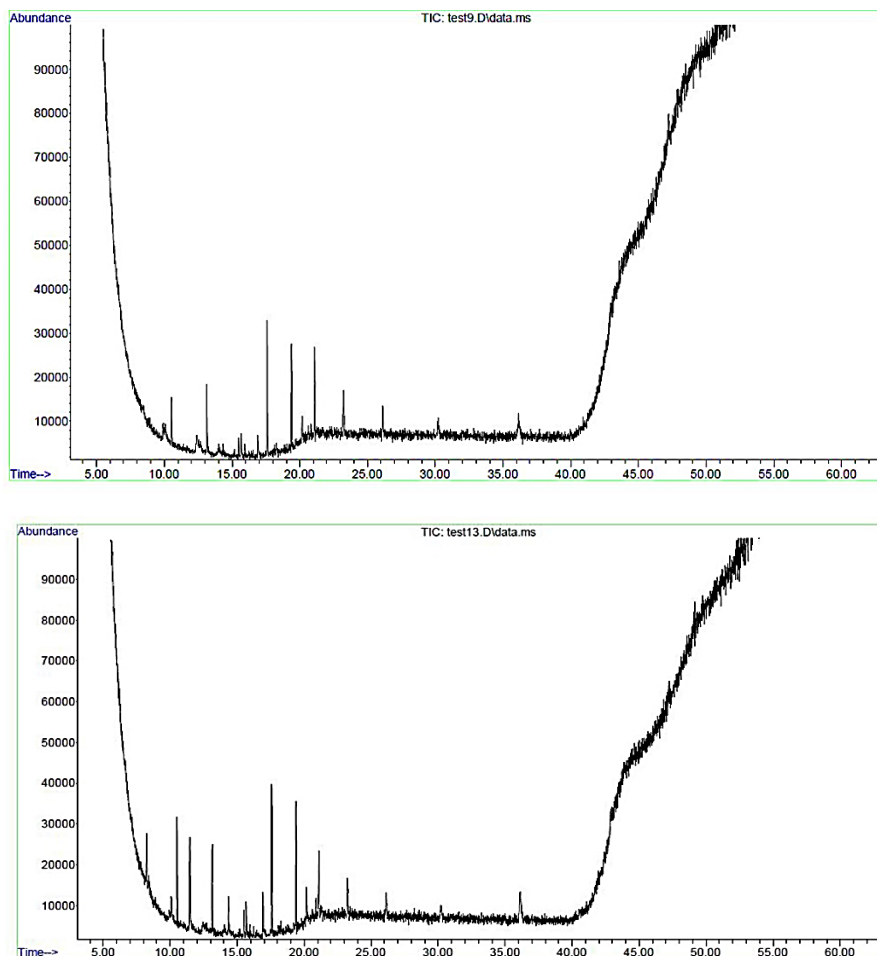


Figure 2. GC/MS analyses for tahdig of potato prepared with solid oil A: Soxhlet, B: Sonication extraction method.

CONCLUSIONS

The analytical results obviously confirmed that the PAHs contamination in tahdig of potato was engendered by the harsh processing i.e. frying in oil in elevated temperatures. Presence of complicated compounds in tahdig is the main cause for poor extraction and determination of concerning contaminants. Introducing efficient extraction method especially for high molecular weight PAHs is thoroughly essential due to their higher toxicity for human health. In this study, two kinds of extractions were applied for the

determination of PAHs in tahdig of potato cooked with solid oil. The levels of PAHs using sonication method met the criteria of limit of detection and recovery, as assigned by European Commission. A significant poor extraction in Soxhlet method was shown in comparison with sonication method. In conclusion the results demonstrated that sonication extraction method obtained higher total PAHs levels than Soxhlet extraction method and can be more appropriate extraction technique for detection of low levels of PAHs in tahdig as a complicated food matrix.

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Conflict of interest

The authors declare that they have no conflict of interest.

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