

Original Article

Validation of an Analytical Method for Determination of Benzo[a]pyrene Bread using QuEChERS Method by GC-MS

Samira Eslamizad^a, Hassan Yazdanpanah^{a,b}, Katayon Javidnia^b, Ramezan Sadeghi^{d,b}, Mitra Bayat^c, Sara Shahabipour^c, Najmeh Khalighian^c and Farzad Kobarfard^{e,f,*}

^aFood Safety Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^bDepartment of Toxicology and Pharmacology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^cMedicinal and Natural Products Chemistry Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran. ^dDepartment of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran. ^ePhytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^fDepartment of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

A fast and simple modified QuEChERS (quick, easy, cheap, rugged and safe) extraction method based on spiked calibration curves and direct sample introduction was developed for determination of Benzo[a]pyrene (BaP) in bread by gas chromatography-mass spectrometry single quadrupole selected ion monitoring (GC/MS-SQ-SIM). Sample preparation includes: extraction of BaP into acetone followed by cleanup with dispersive solid phase extraction. The use of spiked samples for constructing the calibration curve substantially reduced adverse matrix-related effects. The average recovery of BaP at 6 concentration levels was in range of 95-120%. The method was proved to be reproducible with relative standard deviation less than 14.5% for all of the concentration levels. The limit of detection and limit of quantification were 0.3 ng/g and 0.5 ng/g, respectively. Correlation coefficient of 0.997 was obtained for spiked calibration standards over the concentration range of 0.5-20 ng/g. To the best of our knowledge, this is the first time that a QuEChERS method is used for the analysis of BaP in breads. The developed method was used for determination of BaP in 29 traditional (Sangak) and industrial (Senan) bread samples collected from Tehran in 2014. These results showed that two Sangak samples were contaminated with BaP. Therefore, a comprehensive survey for monitoring of BaP in Sangak bread samples seems to be needed. This is the first report concerning contamination of bread samples with BaP in Iran.

Keywords: Polycyclic aromatic hydrocarbons; Benzo[a]pyrene; bread samples; QuEChERS; Gas chromatography-Mass Spectrometry; Iran.

Introduction

Benzo[a]pyrene (BaP) is a member of

Polycyclic Aromatic Hydrocarbons (PAHs) that their molecular structures include two or more combined aromatic rings with adjacent rings sharing two or more carbon atoms (1). PAHs are produced during the incomplete burning of coal, oil, gas, wood, waste, or other organic materials,

* Corresponding author:

E-mail: farzadkf@yahoo.com

such as tobacco and food components and spread in the environment (2, 3). More than 100 PAHs have been recognized in nature, 16 of them were classified as “priority pollutants” according to the U.S. Environmental Protection Agency (EPA). It has been reported that BaP is a proper marker of the occurrence of the carcinogenic PAHs in food and their health outcomes (4-6).

BaP is the only compound in PAHs group with sufficient toxicological evidence to authorize the setting of a guideline (7). Toxicological studies on individual PAHs in animals, principally on BaP, have revealed a variety of toxicological impacts, such as reproductive and developmental toxicity, hematological effects and immunotoxicity (8). International Agency for Research on Cancer (IARC) reported that BaP is carcinogenic to humans (Group 1) (9). Food can be polluted by PAHs that are present in the soil, air or water, as well as those that are formed during thermal food processing or certain home cooking practices such as baking (10, 11).

Based on the studies reported about PAHs exposure, in the non tobacco-smoking and non-occupationally exposed population, food is the major source of human exposure to PAHs, and cereals constitute one of the main causal sources (10-13).

There are some reports (11, 13-16) concerning contamination of BaP in different types of bread.

According to the food basket survey in Iran, bread is the most consumed food and the average per capita consumption of bread in a day is 450 g (17). Bread's contamination by PAHs can be dependent on both the contamination of bakery raw resources, mostly flour, and the baking process. An important subject is also the temperature of this thermal process and its influence on bread's contamination level (11).

According to the existing regulations set by European Commission, the content of BaP in some foodstuffs and baby foods should not go beyond 1 ng/g (5).

In Iran, a few research have been conducted on determination of BaP in different matrices such as water (drinking water, sea water, surface water and urban runoff) (18-21), soil (22, 23), type of sediments (24-26), atmosphere (27) and plants (28). However there is no information

regarding contamination of bread with BaP in Iran. Therefore the aim of this study was to develop a QuEChERS method for the analysis of BaP in bread samples and application of the developed method for analysis of BaP in bread samples.

There is no national procedure for regular monitoring of BaP in foods and there are no regulations laid down for BaP in bread in Iran.

The quantification of trace analytes in complex solid matrices is a critical challenge for analytical chemists (29). Analytical methods have been developed for determination of PAH loads in numerous matrices with the characteristic factor being the mode of sample preparation. Common preparation methods comprise soxhlet extraction, solid-liquid extraction, sonication assisted extraction or accelerated solvent extraction followed by a sample cleanup step using solid-phase extraction or gel permeation chromatography. Nevertheless, these methods are often toil, solvent and time demanding, need advanced analytical expertise and lab apparatus and depend on the utilization of chlorinated extraction solvents. To overcome these challenges, QuEChERS (quick, easy, cheap, effective, rugged and safe) based sample preparation procedures have been used for analysis of PAHs in tea (30, 31), rice (32), meat of animal origin (33), sea urchin (34) and seafood such as mussel, finfish, shrimp, oysters and scallops (35-39). To the best of our knowledge there is no report about the analysis of BaP in bread and cereal products by using QuEChERS method.

The QuEChERS method is based on work done and published by Anastassiades *et al* (40). QuEChERS was developed using an extraction procedure for pesticide residues in fruits and vegetables. This method uses a single-step buffered acetonitrile (MeCN) extraction and salting out liquid-liquid partitioning from water in the sample with MgSO₄. Dispersive-solid-phase extraction (dispersive-SPE) cleanup is used to remove polar matrix elements, organic acids, excess water, and other components with a mixture of primary secondary amine (PSA) sorbent and MgSO₄; afterward the extracts are analyzed by means of mass spectrometry (MS) techniques following a chromatographic analytical separation (35, 40,

41). The stream-lined nature of the QuEChERS procedure has resulted in its application in the analysis of veterinary pharmaceuticals, mycotoxins, phthalates and oil dispersion surfactants (35). To the best of our knowledge analysis of BaP by QuEChERS method has not been reported in bread in the scientific literature presented till the date.

The main objective of this study was to develop a method using QuEChERS method for determination of BaP in bread samples by GC-MS.

Experimental

Sample collection

Industrial bread samples (Senan) and Sangak breads (a traditional bread in Iran) were collected from 26 supermarkets and 3 bakeries in Tehran, respectively. All of the samples were prepared from wheat flour. All samples were covered with aluminum foil in order to prevent photo degradation and then transported to the lab. Each sample was separately cut into small pieces and then blended. After mixing, the samples were stored in amber glass bottles with Teflon-lined caps at -20°C until extraction process.

Chemicals

Standard concentrations of BaP and Anthracene-d10 (ISTD) were prepared from Sigma Aldrich (St. Louis, Mo., U.S.A.). All solvents including, acetone, acetonitrile, ethyl acetate, toluene, methanol and isooctane were purchased in HPLC grade from Merck (Darmstadt, Germany). Anhydrous MgSO₄ and sodium chloride were obtained from Chem Lab NV, Belgium and Primary Secondary Amine (PSA) SPE Bulk Sorbent was purchased from Varian, Italy. Ultrapure water was obtained using a water purification system (Econolab, Oklahoma, U.S.A.).

Preparation of standards

BaP and Anthracene-d10 stock solutions were prepared by weighing exactly 5 mg of each standard, dissolved separately in 5 mL of toluene. Stock solutions of the BaP and Anthracene-d10 were made at 1000 µg/mL in toluene. Intermediate standard solutions of

BaP (in acetone) included concentrations of 10000, 1000 and 100 ng/mL and intermediate standard solutions for Anthracene-d10 (in acetone) included concentrations of 10000 and 1000 ng/mL. Bread samples were spiked with calibration standards in acetone at the following concentrations: 500, 250, 125, 50, 25, 12.5, and 5 ng/mL. For this purpose, 200 µL of each standard stock solution was added to 5 g of blank bread samples. An aliquot of 50 µL of Anthracene-d10 solution in acetone (1000 ng/mL) was added to the spiked bread sample as internal standard.

All standard solutions were prepared in amber color volumetric flask (to avoid light exposure) and stored at 4°C whenever not in use. The samples so obtained were treated as described in sample preparation section.

QuEChERS Sample preparation

The QuEChERS method developed for analysis of pesticide by Anastassiades *et al* [40], was used with some modifications for the analysis of BaP in bread. The extraction procedure was as follows: (1) To 5 g sample weighed into a 50 mL "PAH free" centrifuge tube was added (2), 50 µL of the ISTD solution (1000 ng/ml), followed by (3), addition of 5 mL deionized water, and then (4), 10 mL acetone and the mixture was shaken with a vortex for 60 s (mini vortexer, Heidolph Germany). Also (5) 6 g MgSO₄ and 1.5 g NaCl were added and immediately hand-shaken and vortex-shaken for 30 s. (6) The tube was centrifuged at 4000 RPM for 5 min (Hettich, Universal 320 R Centrifuge); (7) the whole acetone extract was transferred to a dSPE cleanup tube containing 400 mg PSA and 1200 mg MgSO₄ and vortex-shaken for 30 sec. (8) The dSPE tube was centrifuged at 4000 RPM for 5 min, then (9), 6 mL of the extract was transferred into a 10 mL amber vial and dried under gentle nitrogen flow at ambient temperature. (10) The dried extract was re-dissolved in 100 µL acetone and vortex-shaken for 30 sec and sonicated for 60 sec (Euronda S.P.A, Italy) and finally, (11) the extract was transferred to an amber GC vial.

Gas Chromatography-Mass Spectrometry Conditions

BaP analysis was conducted using an Agilent

Table 1. Quantifier ions and qualifier ions used in the selected ion-monitoring of BaP and Anthracene-d10 by gas chromatography-mass spectrometry.

Compound	Quantifier ion	Qualifier ion
BaP	252	253, 250, 126
Anthracene-d10	188	189, 187, 160

7000-Triple-Quad mass spectrometer coupled with 7890 A gas chromatography. Separation of PAHs was performed using a 5% phenyl-methyl siloxane (HB-5MS) bonded-phase fused-silica capillary column (Hewlett-Packard, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The carrier gas was helium (purity 99.9995 %) which was further purified by passage through a helium gas purifier Agilent model RMSH-2. The injection port was run in splitless mode and the injection volume was 2 μL. The oven temperature program was 80°C for 1.5 min, raised to 290°C at a rate of 50°C/min and maintained at this temperature for 10 min and the total run time was 15.2 min. The MS transfer line and ion source temperatures were adjusted at 290°C and 230°C, respectively. GC-MS was performed in EI mode. The mass spectra were collected by electronic impact at 70 eV. Detection of BaP and ISTD was carried out using SIM mode. The following ions were monitored: m/z 252 (for BaP) and m/z 188 (for ISTD). Determination was done based on the ratio between the peak areas of BaP to that of the ISTD. The Quantifier ions and qualifier ions masses (m/z) are shown in Table 1.

Method validation

For method validation, the parameters assessed were linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision.

For construction of spiked calibration curve, spiked bread samples at concentration levels of 0.5, 2, 5, 8, 10 and 20 ng/g were prepared in triplicates and then treated according to the procedure described previously. The recoveries were calculated for spiked samples at three levels (1, 3 and 16 ng/g) using the spiked calibration curves.

Results

The development of QuEChERS extraction procedure required the evaluation of the influence of different parameters on BaP extraction efficiency such as extraction solvent, shaking time and influence of the elapsed time after addition of the salts.

Method optimization

Extraction Solvent

Several experiments were performed in order to select the best extraction solvent. Acetonitrile, ethyl acetate and acetone were tried. The results obtained are shown in Table 2. Acetone was found to be the most suitable extraction solvent for BaP.

Extraction time

The effect of shaking time on the BaP extraction efficiency was studied. Initial vortex shaking for 1 min followed by 25 min of vibration shaking was used to achieve the maximum extraction yield. The results revealed that no significant improvement could be achieved after 25 min of agitation with vibrating motion. Therefore one min of manual shaking was selected as optimum extraction time.

Influence of the elapsed time after adding the salts

Increasing the elapsed time after the addition of 6 g MgSO₄ and 1.5 g NaCl, up to one hour did not change the efficiency of BaP extraction. Therefore, the samples were treated immediately after adding the salts.

Table 2. Effect of different extraction solvents on the average recoveries percentage (n = 3) and repeatability (RSD %) of BaP analyzed in bread samples using QuEChERS method and GC-MS

Extraction solvent	Acetonitrile	Ethylacetate	Acetone
Recovery ± RSD (%)	54.96 ± 27.92	49.15 ± 3.18	111.18 ± 24.08

Table 3. Retention time and calibration data for BaP

Spiked calibration range (ng/g; n = 18)	Retention Time (min)	Regression equation (n = 6)	R ²	LOD (ng/g)*	LOQ (ng/g)*
0.5-20	11.2	y = 0.070x - 0.003	0.997	0.3	0.5

* According to wet weight

Method validation

Linearity. Spiked calibration standards at levels of 0.5, 2, 5, 8, 10 and 20 ng/g were prepared by the addition of 200 µL of 12.5, 50, 125, 200, 250 and 500 ng/mL standard stock solutions to 5 g of blank bread samples in each case.

Quantification of the BaP in bread samples was performed by using an internal standard method. Calibration curves showed a linear relationship between the concentration and peak area ratios, with a correlation coefficient of 0.997. Table 3 shows the values of the Validation parameters for BaP quantification.

LOD and LOQ. The value of estimated LOD was 0.3 ng/g. Recovery and precision in spiked samples at levels of 0.5 and 1 ng/g were 119.9 ± 0.90 and 119.2 ± 3.68 , respectively. Therefore, the value of LOQ was determined as 0.5 ng/g.

Recovery. The extraction recoveries were determined by applying the full procedure to triplicate samples in three days at three spiking levels including 1, 3 and 16 ng/g. The extraction recovery was expressed as recovery percentage. Proper recoveries (110.5-119.85%) of BaP from spiked samples were obtained, which were in the acceptable range of European Commissions regulation (42). The values of recovery percentage are presented in Table 4.

Precision. The values obtained for RSD% were less than 11.68 %. Table 4 shows the calculated RSD % for each spiking level which were below the RSD_R (22 %) determined by European Commission.

The HorRAT value, *i.e.*, $HoR = RSD_R$ (measured)/predicted RSD_R (Horwitz), gives a comparison of the actual precision measured

with the precision predicted by the Horwitz equation. A HorRAT value of 1 usually indicates satisfactory interlaboratory precision, whereas a value > 2 usually indicates unsatisfactory performance of the method (43).

In this study, the obtained HorRAT values, ranging from 0.4 to 0.5, confirmed satisfactory interlaboratory precision.

Application of the optimized method-analysis of unknown bread samples

To establish the capability and the suitability of the developed method, a variety of bread samples were analyzed for the presence of BaP. BaP was not detected in 26 industrial bread samples (Senan bread), but 2 out of 3 Sangak bread samples were contaminated at the levels of 2.73 and 3.19 (ng/g of wet weight) which is higher than the permissible limit of European Commission regulatory control value for BaP (1 µg/ kg of wet weight) in processed cereal-based foods and baby foods for infants and young children (44). The results are presented in Table 5. Figures 1 and 2 show the chromatograms obtained for industrial and traditional bread samples, respectively.

Three Sangak samples were analyzed to evaluate the practicality of the method. Considering the few Sangak samples analyzed in this study, a comprehensive survey for monitoring of BaP in Sangak bread samples seems to be needed.

Discussion

QuEChERS method was first introduced by Anastassiades and coworkers for extraction

Table 4. BaP recoveries \pm RSD (%) for homogenized bread samples using the optimized QuEChERS method at three spiking levels (n = 3).

Spiking levels (ng/g)	1	3	16
Recovery \pm RSD (%)	119.22 ± 0.77	119.85 ± 9.3	110.5 ± 11.68

Table 5. Incidence, mean, median and maximum levels of BaP in bread samples.

Bread type	Incidence (%)	Mean	Median	Maximum
Traditional bread (Sangak)	66.66	1.97	2.73	3.19
Industrial bread (Senan)	ND*	-	-	-

* Not detected

and cleanup of pesticide residues in fruits and vegetables. This method uses a single-step buffered acetonitrile (MeCN) extraction and salting out liquid-liquid partitioning from the water in the sample with MgSO₄. Dispersive-solid-phase extraction (dispersive-SPE) cleanup is used to remove polar matrix elements, organic acids, excess water, and other components with a mixture of primary secondary amine (PSA) sorbent and MgSO₄; afterward the extracts are analyzed by means of mass spectrometry (MS) techniques following a chromatographic analytical separation (35, 40 and 41).

As per the scientific literature available till the date, analysis of BaP by QuEChERS has not been reported in bread.

Different extraction methods such as Soxhlet apparatus and solid-phase extraction have been used for analysis of BaP in bread (13-16). Comparison of the proposed QuEChERS method with other methods for extraction and determination of PAHs in bread is shown in Table 6.

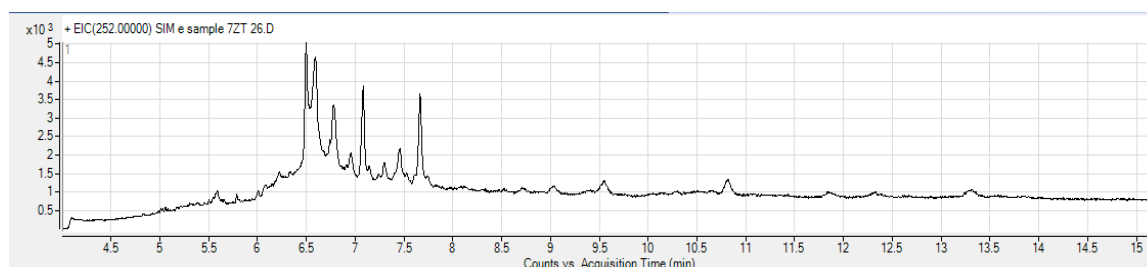
Compared to the other methods shown in Table 6, the extraction time of our method is shorter than most of the other methods. The extraction solvent which is used in the method presented in this study is less toxic compared to the solvents used in the other methods. The proposed method needed less extraction solvent.

Some of the reported methods (Soxhlet, SPE and SLE) need special equipment for extraction and are more expensive compared to the QuEChERS method presented in this study. In the methods which Soxhlet, SPE and SLE are used the extraction time is longer than our method. Recovery of the proposed method is better than Soxhlet method (15, 16) and is comparable with that of the other methods.

In our proposed method, acetone is used as extraction solvent, which is more volatile than other extraction solvents used in the other methods (11, 13-16), therefore, less time is needed in this method for drying the samples.

Major advantages of the developed method are the simplicity of operation, the rapidity to achieve a very high sample throughput and low cost. Thus, the proposed method represents simple and low-cost alternative to the conventional methods and is suitable for screening and quality control programs in food studies.

To the best of our knowledge, there are only few reports for determination of PAH pollution in bread. Tawfic *et al.* (16) reported that BaP concentration in mazot, solar, solid waste and electricity operated bakeries had an average of 20.6, 14.7, 45.1 and 4.4 µg kg⁻¹, respectively. Results show that the samples collected from mazot, solar and solid waste operated

**Figure 1.** GC-MS chromatogram of BaP in industrial bread sample.

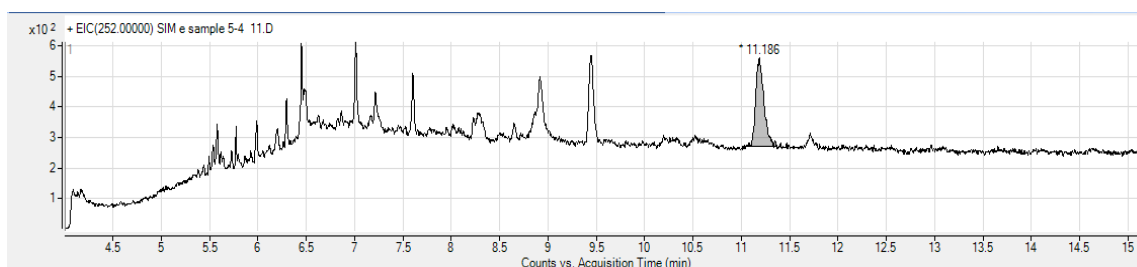


Figure 2. GC-MS chromatogram of BaP in Sangak bread sample.

bakeries have had a wide spectrum of PAHs, in comparison to the bread samples collected from electricity operated bakeries. Estimated daily intakes of PAHs via bread consumption were 48.2, 28.5, 80.1, and 4.8 mg per person per day for bread produced in bakeries using mazot, solar, solid waste and electricity, respectively. Meanwhile, the estimated daily intakes of BaP were 3.69, 2.65, 8.1, and 0.81 mg per person per day for bread sample baked with mazot, solar, solid waste and electricity, respectively.

Rey-Salgueiro *et al.* (14) reported the BaP concentration in 3 of 24 selected samples

(Commercial toasted bread samples) in a range of 0.13-0.23 $\mu\text{g/kg}$.

In Kuwait, Al-Rashdan *et al.* (15) reported no detectable amount of BaP in most of samples of bread baked from white wheat flour. The concentration of BaP in Five samples exceeded the legal permissible limits (1 $\mu\text{g/kg}$) proposed by the European Union for processed cereal-based foods (44). In their investigation, the original white wheat flour was analyzed in parallel to the toasted white bread and BaP was not detected in white flour, supplied by the Kuwait Flour Mills and Bakeries Company.

Table 6. Comparison of the proposed QuEChERS method with other methods for extraction and determination of BaP in bread.

Authors	Extraction method	Detection system	LOD (ng/ml)	LOQ (ng/ml)	DLR b (ng/ml)	ISTD	r ²	Recovery (%)	R.S.D. %	V _L ^a (mL)	Extraction solvent	Ref.
Ahmed <i>et al</i>	Soxhlet apparatus	GC-FID	-	-	-	-	-	82	6.7	> 400	n-hexane	(16)
Rey-Salgueiro <i>et al</i>	solid-liquid extraction (SLE)	(LC-FD)	0.02	0.07	0.02-40	-	≥ 0.999	103	5	>40	n-hexane	(14)
Al-Rashdan <i>et al</i>	Soxhlet apparatus	GC-MS	0.1	0.3	100-1000	d-PAHs	>0.995	82.39	4.99	>175	hexane: dichloro methane (1 : 1)	(15)
Orecchio and Papuzza	Soxhlet	GC-MS	0.015	0.05	100-2000 mg/l)	d-PAHs		95	7-25	>80	pentane	(13)
Ciecierska and Obiedziński	GPC	HPLC-FLD/ DAD and Confirmation by GC/MS	0.12	0.24	1-50	-	0.999	106	5.1	>150	hexane /acetone, 60:40, v/v	(11)
Eslamizad <i>et al</i>	The proposed method	GC-MS	0.3	0.5	0.5-20	d-PAHs	0.997	114.7	<14.5	10	Acetone	This study

^a Liquid-phase volume

^b Dynamic linear ranges

BaP in bread samples baked from brown wheat flour was detected in one sample and found to be equal to 11.13 µg/kg. The level of BaP in Sandwich breads baked from white wheat flour was in the range of 2.83-9.04 µg/kg in 2 of the 3 samples (15).

Dennis *et al.* (45) reported less than 0.1 µg/kg of BaP in white flour, although they reported 2.2 µg/kg in cereal-derived products enriched with high levels of edible oils.

Orecchio and Papuzza (13) showed carcinogenic PAHs, expressed as BaP equivalent ranged from 1.1 to 26 µg/kg dry weight.

In samples of bread baked using wood as fuel, concentrations of BaP ranged from 0.13 to 9.4 µg/kg d.w (dry weight). Five out of 15 samples exceeded the legal limits (1 µg/kg) proposed by the European Commission for processed cereal-based foods (44).

Conclusions

To the best of our knowledge, this is the first time that a QuEChERS method is used for the analysis of BaP in bread samples. The developed QuEChERS method offers an efficient, cost effective, and easy sample preparation procedure for the determination of BaP in bread. Comparing with the other extraction methods used for analysis of BaP in bread, this extraction method is rather simple and cheap. The method is advantageous in terms of total solvent utilization and also the extraction solvent is less toxic. The use of spiked calibration curves for constructing the calibration curve substantially reduced adverse matrix-related effects. This method is cheaper than the conventional method practiced for extraction of BaP from food matrices.

Correlation coefficient, recovery percentage, and precision were within the standard EU No 836/2011 (42) in this study, which indicate the reproducibility and accuracy of the extraction method.

Moreover, the results of this study revealed that PAHs may often be contained in breads, representing the need to develop and introduce efficient procedures to prevent more severe PAHs contamination in bread in the future.

The preliminary results showed that the

Sangak bread samples could be contaminated with BaP and therefore routine monitoring of BaP in traditional bread samples seems to be necessary.

Acknowledgments

The authors are very grateful to the Medicinal and Natural Products Chemistry Research Centre, Shiraz University of Medical Sciences and Central Research Laboratories of Shahid Beheshti University of Medical Sciences for their supports.

References

- (1) Terzi G, Celik T, and Nisbet C. Determination of Benzo[a]pyrene in Turkish doner kebab samples cooked with charcoal or gas fire. *Irish Journal of Agricultural & Food Research*. (2008) 47: 187-193.
- (2) Kumari R, Chaturvedi P, Ansari N, Murthy R and Patel D. Optimization and validation of an extraction method for the analysis of polycyclic aromatic hydrocarbons in chocolate candies. *Journal of food science*. (2012) 77: T34-T40.
- (3) Rey-Salgueiro L, Martinez-Carballo E, Garcia-Falcon M, Gonzalez-Barreiro C, Simal-Gándara J. Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. *J. Food chemistry*. (2009) 115: 814-819.
- (4) Kasim N, Osman R, and Saim L. I. Determination of Benzo[a]pyrene in Malaysian commercialized coffee powder using solid phase extraction and gas chromatography. *Malaysian Journal of Analytical Sciences*. (2012). 16: 39-42.
- (5) Bogusz M. J, Hajj El, Abu Ehaideb S, Hassan Z, Al-Tufail H. Rapid determination of Benzo[a]pyrene in olive oil samples with solid-phase extraction and low-pressure, wide-bore gas chromatography-mass spectrometry and fast liquid chromatography with fluorescence detection. *J. of chromatography A* (2004) 1026: 1-7.
- (6) Food Survey Information Sheet. Polycyclic aromatic hydrocarbons in cereals, cereal products, vegetables, vegetables products and traditional smoked foods. NUMBER 01/12 April 2012. Available from: URL: <http://multimedia.food.gov.uk/multimedia/pdfs/poly-aromatic-hydrocarbons.pdf>.
- (7) Muyela B, Shitandi A, and Ngunjiri R, Determination of Benzo[a]pyrene levels in smoked and oil fried Lates niloticus. *International Food Research Journal* (2012) 19: 1595-1600.
- (8) Food Safety Authority of Ireland. Polycyclic Aromatic Hydrocarbons (PAHs) in Food. Toxicology factsheet series. May 2009. Available from: URL: http://www.fsai.ie/resources_and_publications/factsheets.html International Agency for Research on Cancer. Agents

- (9) classified by the IARC monographs, volumes 1-109. *World Health Organization. Lyon, France* [Online]. (2011). Available from: URL: <http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf>. Janoszka B. HPLC-fluorescence analysis of polycyclic aromatic hydrocarbons (PAHs) in pork meat and its gravy fried without additives and in the presence of onion and garlic. *J. Food chemistry*. (2011) 126: 1344-1353.
- (10) Ciecierska M and Obiedzinski M. Polycyclic aromatic hydrocarbons in the bakery chain. *J. Food chemistry*. (2013) 141: 1-9.
- (11) Gomez-Ruiz J.A and Wenzl T. Evaluation of gas chromatography columns for the analysis of the 15 + 1 EU-priority polycyclic aromatic hydrocarbons (PAHs). *J. Analytical and bioanalytical chemistry*. (2009) 393: 1697-1707.
- (12) Orecchio S and Papuzza V. Levels, fingerprint and daily intake of polycyclic aromatic hydrocarbons (PAHs) in bread baked using wood as fuel. *Journal of hazardous materials*. (2009) 164: 876-883.
- (13) Rey-Salgueiro L, Garcia-Falcon M, Mercedes Sonia Martinez-Carballo, Simal-Gandara E, Simal-Gandara J. Effects of toasting procedures on the levels of polycyclic aromatic hydrocarbons in toasted bread. *J. Food Chemistry*. (2008) 108: 607-615.
- (14) Al-Rashdan A, Helaleh MI, Nisar A, Ibtisam A and Al-Ballam Z. Determination of the levels of polycyclic aromatic hydrocarbons in toasted bread using Gas Chromatography Mass Spectrometry. *International journal of analytical chemistry* (2010) 2010: 1-8.
- (15) Ahmed MT, Abdel Hadi ES, Samahy SEI and Youssof K. The influence of baking fuel on residues of polycyclic aromatic hydrocarbons and heavy metals in bread. *Journal of hazardous materials* (2000) 80: 1-8.
- (16) National Nutrition and Food Technology Research Institute. Comprehensive Study of Food Basket Pattern, and Nutrition Status in Iran during 2000-2002. Shahid Beheshti University of Medical Sciences, Tehran. (2004) 20.
- (17) Karyab H, Yunesian M, Nasserli S, Mahvi AH, Ahmadkhaniha R, Rastkari N and Nabizadeh N. Polycyclic Aromatic Hydrocarbons in drinking water of Tehran, Iran. *Journal of Environmental Health Science and Engineering* (2013) 11: 1-7.
- (18) Mahvi A and Mardani G. Determination of phenanthrene in urban runoff of Tehran, capital of Iran. *Iranian Journal of Environmental Health Science & Engineering*. (2005) 2: 5-11.
- (19) Hosseini M, Peery S, Karami O, Bastami AA and Monikh FA. Distribution of Polycyclic Aromatic Hydrocarbons in Water (Persian Gulf) (Iran). *American-Eurasian Journal of Toxicological Sciences*. (2012) 4: 94-97.
- (20) Taheri Azad L, Esmaili Sari A and Rezai Tavabe K. Determination of rest-oil pollution (polycyclic aromatic hydrocarbons) in surface water of three international wetlands of Iran. *Journal of Environmental Research and Development*. (2009) 4: 310-320.
- (21) Vossoughi M, Yaghmaei S, Alemzadeh I and Safekordi A. Some investigation on bioremediation of PAHs contaminated soil in Iran tar refinery. *J International journal of engineering transactions*. (2002) 15: 1-10.
- (22) Bostani A and Charkhabi AH. Concentration distribution of polycyclic aromatic hydrocarbons (PAHs) resulted from petroleum products in soils of the south Iran. *International Journal of Agronomy and Plant Production*. (2013) 4: 1819-1825.
- (23) Nikpour Y and Ghanemi K. Distribution and Sources of Polycyclic Aromatic Hydrocarbons in the Sediment of Bushehr Coastal Zone-Iran. *J Iranica Journal of Energy & Environment*. (2012) 3: 173-179.
- (24) Mirvakili H and Hadjizadeh Zaker N. Evaluation of PAHs in Hydrocarbons Pollution, Biodegradation and Weathering Extent in Surface Sediments of the Kharg Island in the Persian Gulf. *International Journal of Environmental Research*. (2014) 8: 387-394.
- (25) Mohammadizadeh C and Javanmard A. Some Organophosphorous Pesticide (OPPs) and Polycyclic Aromatic Hydrocarbons (PAHs) along the Eastern Caspian Sea Coast, Mazandaran State, Iran. *E-Journal of Science & Technology*. (2011) 6: 85-92.
- (26) Halek F, Bidhendi GRN, Hashtroudi M and Kavousi A. Distribution of Polycyclic Aromatic Hydrocarbons in Gas phase in urban atmosphere. *International Journal of Environmental Research*. (2008) 2: T01A013.
- (27) Zare MH. Polycyclic Aromatic Hydrocarbons (PAHs) in plants of Shadegan wetland: Halocnemum strobilaceum and Suaeda maritima. *J Journal of the persian gulf (marine science)*. (2011) 2: 37-41.
- (28) German-Hernandez M, Pino V, Anderson JL and Afonso AM. A novel in situ preconcentration method with ionic liquid-based surfactants resulting in enhanced sensitivity for the extraction of polycyclic aromatic hydrocarbons from toasted cereals. *J. Journal of Chromatography A*. (2012) 1227: 29-37.
- (29) Sadowska-Rociek A, Surma M and Cieslik E. Comparison of different modifications on QuEChERS sample preparation method for PAHs determination in black, green, red and white tea. *J. Environmental Science and Pollution Research*. (2014) 21: 1326-1338.
- (30) Pincemaille J, Schummer C, Heinen E and Moris G. Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas and tea infusions. *J. Food chemistry*. (2014) 145: 807-813.
- (31) Escarrone A, Caldas S, Furlong E, Meneghetti V, Fagundes C, Arias J and Primel E. Polycyclic aromatic hydrocarbons in rice grain dried by different processes: Evaluation of a quick, easy, cheap, effective, rugged and safe extraction method. *J. Food chemistry*. (2014) 146: 597-602.
- (32) Surma M, Sadowska-Rociek A and Cieslik E. The application of d-SPE in the QuEChERS method for the determination of PAHs in food of animal origin with GC-MS detection. *J. European Food Research and Technology*. (2014) 238: 1029-1036.

- Angioni A, Porcu L, Secci M and Addis P. QuEChERS method for the determination of PAH compounds in Sardinia Sea Urchin (*Paracentrotus lividus*) Roe, using gas chromatography ITMS-MS analysis. *J. Food Analytical Methods*. (2012) 5: 1131-1136.
- Forsberg ND, Wilson GR and Anderson KA. (35) Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPE and GC-MS. *Journal of agricultural and food chemistry*. (2011) 59: 8108-8116.
- Sapozhnikova Y and Lehotay SJ. Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography-tandem mass spectrometry. *J. Analytica chimica acta*. (2013) 758: 80-92.
- Johnson YS. Determination of Polycyclic Aromatic Hydrocarbons in Edible Seafood by QuEChERS-Based Extraction and Gas Chromatography-Tandem Mass Spectrometry. *Journal of food science*. (2012) 77: T131-T137.
- Joao Ramalhosa M, Paiga P, Morais S, Delerue-Matos C and Prior Pinto Oliveira MB. Analysis of polycyclic aromatic hydrocarbons in fish: evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method. *Journal of separation science*. (2009) 32: 3529-3538.
- Cai ShS, Stevens J and Syage JA. Ultra high performance liquid chromatography-atmospheric pressure photoionization-mass spectrometry for high-sensitivity analysis of US Environmental Protection Agency sixteen priority pollutant polynuclear aromatic hydrocarbons in oysters. *Journal of Chromatography A*. (2012) 1227: 138-144.
- (40) Anastassiades M, Lehotay SJ, Stajnbaher D and Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal of AOAC international*. (2003) 86: 412-431.
- (41) Lehotay S. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. *Journal of AOAC International*. (2007) 90: 485-520.
- (42) European Commission. Commission regulation 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and Benzo[a]pyrene in foodstuffs. *Eur. Union*. (2011) L215: 9-16.
- (43) Buchgraber M and Wasik A. Validation of an analytical method for the simultaneous determination of nine intense sweeteners by HPLC-ELSD. (2007). Available from: URL: <http://www.irmm.jrc.be>, <http://www.jrc.ec.europa.eu>
- (44) European Commission. EC Commission No, Regulation 2006 of 19 December 2006, Setting Maximum Levels for certain Contaminants in Foodstuffs. *Official Journal of the European Union*. (2006) L 364/22.
- (45) John Dennis M, Massey RC, McWeeny DJ, Larsson B, Eriksson A and Sahlberg G. Comparison of a capillary gas chromatographic and a high-performance liquid chromatographic method of analysis for polycyclic aromatic hydrocarbons in food. *Journal of Chromatography A*. (1984) 285: 127-133.

This article is available online at <http://www.ijpr.ir>