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DETERMINATION OF BENZO[a]PYRENE IN MALAYSIAN COMMERCIALIZED COFFEE POWDER USING SOLID PHASE EXTRACTION AND GAS CHROMATOGRAPHY

(Penentuan Benzo[a]pirena di dalam Serbuk Kopi di Pasaran Menggunakan Ekstraksi Fasa Pepejal dan Kromatografi Gas)

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Abstract

Roasting is a critical process in coffee production as it enables the development of flavor and aroma. Benzo[a]pyrene (BaP) is a nondesirable product of incomplete combustion at temperatures between 300 and 600 °C and may be produced during roasting step. In this study, selected samples of roasted coffee powder were analysed for BaP. Extraction of BaP was achieved using C_{18} solid phase extraction (SPE) prior to analysis by gas chromatography. Calibration curve prepared with concentrations ranged between 3 – 50 ppm showed good linearity with r = 0.999. The limit of detection (LOD) was 0.25 ppm and the limit of quantification (LOQ) was 0.85 ppm. Recovery of BaP obtained from spiked sample (3 ppm) was 88.7 % with RSD (n=3) of 5.4 %. Benzo[a]pyrene was detected in all samples, at level ranging from 0.14 to 0.62 ppb .

Keywords: Benzo[a]pyrene, solid phase extraction, roasting, coffee

Abstrak

Memanggang adalah proses yang kritikal di dalam penghasilan kopi kerana ia membolehkan pembentukan rasa dan aroma. Benzo[a]pirena (BaP) adalah bahan sampingan yang terhasil daripada pembakaran tidak lengkap pada suhu antara 300 dan 600^{o}C semasa proses pemanggangan. Dalam kajian ini, BaP dalam serbuk kopi terpilih telah dianalisa. Pengekstrakan dilakukan menggunakan ekstraksi fasa pepejal C_{18} dianalisis menggunakan gas kromatografi. Graf kalibrasi yang telah disediakan menggunakan kepekatan antara 3-50 ppm menghasilkan hubungan linear dengan r = 0.999. Had pengesanan (LOD) BaP adalah 0.25 ppm dan had pengesanan kuantiti (LOQ) adalah 0.85 ppm. Perolehan semula BaP daripada sampel yang telah ditambahkan dengan BaP (3 ppm) adalah 88.7 % dengan RSD (n=3) 5.4 %. Benzo[a]pyrene telah dikesan di dalam semua sampel, pada tahap diantara 0.14 sehingga 0.62 ppb .

Kata kunci: Benzo[a]pirena, ekstraksi fasa pepejal, pemanggangan, kopi

Introduction

Roasting is a critical process in coffee production as it enables the development of flavor and aroma. At the same time, roasting may lead to the formation of nondesirable compounds, such as polycyclic aromatic hydrocarbons (PAHs) [1]. Contamination of PAHs by intense thermal processing occur due to generation by direct pyrolysis of food nutrients and, also due to direct deposition of PAHs from smoke of different thermal agents [2].

Toxicological investigation showed different carcinogenic potency for various PAHs mixtures [3,4]. Very mutagenic and carcinogenic is benzo[a]pyrene and it has been accepted as a marker of carcinogenic PAHs in food and environmental samples [5]. According to existing regulations by European Commision [6], the content of BaP in some foods and baby foods should not exceed 1 ppb.

In this paper, an analytical method for the BaP determination in coffee using solid phase extraction and gas chromatography was evaluated and the amount of BaP in various coffee samples was determined.

Experimental

Chemicals

Benzo[a]pyrene standard from Sigma-Aldrich (Steinheim, Germany) had a minimum of 97% purity. A stock solution of BaP 1000 ppm was prepared by dissolving BaP in deionized water from Milli-Q. The standard solutions were prepared by conveniently diluting the stock solution with deionized water from Milli-Q. Methanol and n-hexane were purchased from Merck (Darmstadt, Germany).

Sample Preparation

Seven local coffee brands were obtained from local stores. Each coffee sample was weighed (3g to 5g) depending on the particle size. The weighed coffee was dissolved with Milli-Q water in 100 mL volumetric flask. The solution may be filtered using a glass fibre filter if neccesary.

Calibration Curve

Calibration curve was in the range of 3-50 ppm from the stock solution.

Solid Phase Extraction

Solid Phase Extraction was performed using Isolute C18 (EC), 1g cartridge (Biotage Europe). The SPE cartridges were conditioned successively using 6 mL methanol and 6 mL Milli-Q water. After sample loading, cartridge was vacuum dried for 1 hour. Then, 10 mL Milli-Q water was used to wash the cartridge and vacuum dried for 30 minutes. After elution using 6 mL hexane, the collected extract was evaporated and concentrated to 1mL under a gentle stream of nitrogen.

Chromatographic procedure

All chromatographic measurements were done on an HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Flame Ionization Detector (FID). GC procedure was carried out using (a) column coated with HP-5MS 5% Phenyl methyl siloxane, capillary size 30.0m x 250µm x 0.25µm nominal, from Bios Analytique (France), (b) carrier gas N_2 with 15.34 psi and 23.2 mL min^{-1}, (c) 2 µL of sample using splitless injection, (d) temperature programming started at 90°C, then increased to 250°C (18°C min^{-1}) and ended with 310°C (15° C min^{-1}) held for 7 minutes, and (e) time retention registered approximately 13.57 min (Figure 1).

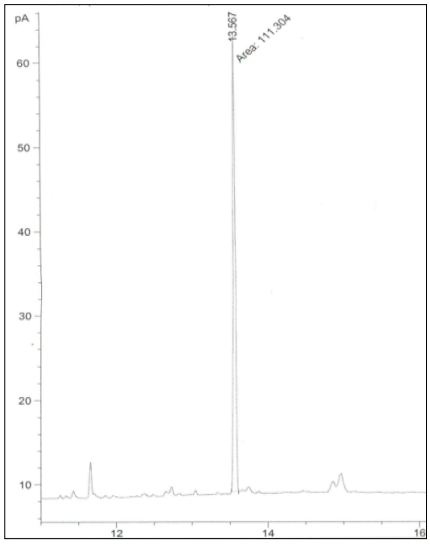

Figure 1: Chromatogram for BaP standard (20 ppm)

Results and Discussion

Method validation

For method validation, the parameters evaluated were linearity, limit of detection (LOD), limit of quantification (LOQ), % recovery and % relative standard deviation (RSD).

Linearity

The linearity of relative response (area versus concentration of standard) was evaluated. The calibration curves was evaluated in the range 3.0 - 50.0 ppm by injecting five standards in concentration range. The obtained linear regression equation for BaP standard was as follows: regression equation for the concentration of benzo[a]pyrene standard was y = 6.2 x- 1.7 for concentration at 3, 5, 10, 20and 50 ppm. Good linearity with correlation coefficient of r = 0.9995 was obtained.

Limit of detection (LOD)

In this study, the LOD was estimated as the concentration that produced a peak height of three times the background noise (ratio of signal to noise = 3). The value of LOD estimated was 0.25 ppm.

Limit of quantification (LOQ)

The limit of quantification (LOQ) was calculated based on the sample concentration with a peak height of ten times the background noise (ratio of signal to noise = 10). The value for LOQ was 0.85 ppm.

Percent recovery and relative standard deviation in %

Good recovery (88.7 %) of benzo[a]pyrene from spike samples was obtained with relative standard deviation (RSD) of 5.4% using spiked samples of 3 ppm.

Analysis of coffee samples.

Figure 2 shows a chromatogram of a selected coffee extract. The amount of benzo[a]pyrene detected in coffee samples were in range of 0.14 to 0.62 ppb (Table 1). BaP detected in all samples are lower than the permissible limit (1 ppb) set by European Commission.

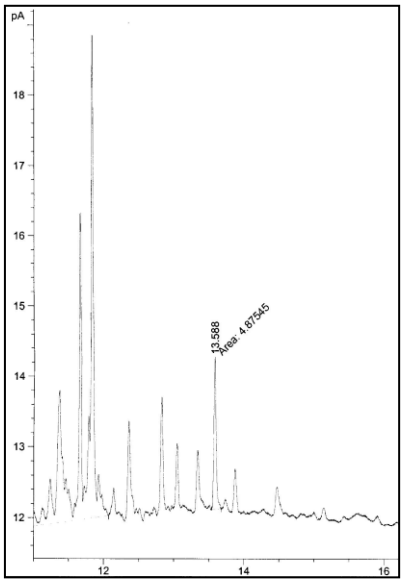

Figure 2. Chromatogram of a coffee extract

Table 1: Amount of BaP in coffee samples

Sample	Concentration of benzo[a]pyrene (ug/kg)
A	0.52 ± 0.07
B	0.29 ± 0.03
C	0.22 ± 0.01
D	0.62 ± 0.03
F	0.45 ± 0.06
G	0.14 ± 0.02
H	0.50 ± 0.06

Conclusion

The amount of benzo[a]pyrene in all of the coffee samples analyzed is below the permissible limit set by the EU for foods and baby foods. However, since benzo[a]pyrene is considered a carcinogenic compound, monitoring the amount of this compound in a popular drink such as coffee is crucial. Thus, a simple and reliable method discussed in this study may provide a significant contribution in assessing the dietary exposure of consumers to carcinogens.

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