RESIDUES OF POLYCYCLIC AROMATIC HYDROCARBONS IN DIFFERENT TYPES OF COFFEE♦

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Abstract: The aim of this work is the development of a simple, fast and quantitative method for polycyclic aromatic hydrocarbons (PAHs) potentially generated by roasting coffee beans. The roasting process is the most important in the coffee industry for the development of the characteristic flavor of the bean mix. The method involves Soxhlet extraction with hexane, clean-up based on silica adsorption chromatography, concentration to dryness and injection of the acetonitrile solution of the residue for HPLC analysis with fluorescence detection. The method allowed confirming or not the presence of the 12 selected PAHs in green coffee and roasted coffee bean samples.

Keywords: *coffee, PAHs, HPLC, fluorescence detection*

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INTRODUCTION

Coffee is one of the most popular beverages in the world; seventy five per cent of soft drinks consumed regularly are coffee.

Coffee belongs to *Rubiaceae* family along with more than 70 other species, but two of them are of significant economic importance, *Arabica Coffee* and *Robusta Coffee.* At present, coffees consisting of 100% Arabica beans are preferred by coffee drinkers since they are considered of better quality than Robusta coffees.

Coffee's chemical composition is determined by a complex interaction of agricultural factors, roasting, blending, and brewing.

In order of abundance, typical values for the water soluble constituents are: phenolic polymers (pulp) 8%, polysaccharides 6%, chlorogenic acids 4%, minerals 3%, water 2%, caffeine 1%, organic acids 0.5%, sugars 0.3%, lipids 0.2%, and aroma 0.1%. Green coffee beans contain up to 10% of chlorogenic acids, i.e., various isomers of hydroxy-cinnamoyl esters of quinic acid (a common plant constituent). Common to most plants and fruits, green coffee beans can contain as much as 10% of dry weight of chlorogenic acids. These are mixtures of mono- and di-esters of 3-substituted 4-hydroxycinnamic acid and quinic acid, a sugar-like molecule [1].

In the roasting process, approximately half of the chlorogenic acids lose a molecule of water, thereby forming an internal ester bond that results in a mixture of non-acidic quinolactones (quinides) as a result of generation of compounds from pyrolysis.

Coffee contains several beneficial antioxidants and is one of the richest sources of chlorogenic acid. Chlorogenic acids are a family of esters formed between cinamic acids and quinic acid and they are a major contributor of antioxidant activity to coffee [2].

Nowadays there is an increasing concern over the potential hazardous effects that polycyclic aromatic hydrocarbons (PAHs) may have on human health. PAHs are a group of compound who's mutagenic and/or carcinogenic effects are well known [3].

PAHs are important environmental pollutants originating from a wide variety of natural and anthropogenic sources and they are generally formed during the incomplete combustion or pyrolysis of organic matter. Because combustion of organic materials is involved in countless natural processes or human activities, PAHs are omnipresent and abundant pollutants in air, soil, water and food.

A few research papers have been published on PAHs in food samples, such as vegetable oils, fruits, seafood, tea, coffee, rice, potato, toasted bread, tomato, pepper, honey and propolis [4-8]. For the determination of these compounds in food samples were used different techniques, such as gas chromatography with mass spectrometric detection [9] or HPLC [10].

In order to minimize health risk as well as for enforcement activities, monitoring of PAHs residues is increasingly important and essential. This paper reports original studies concerning PAHs determination in different types of coffee available at supermarkets by high pressure liquid chromatography with fluorescence detector (HPLC–FLD) and this study is useful in quality control of coffee samples.

EXPERIMENTAL

Chemicals and reagents

There were used the following PAHs as analytical standards: naphthalene (Np), acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benzo[a]anthracene (B[a]An), benzo[k]fluoranthene (B[k]Fl), chrysene (Chry), pyrene (Py), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (B[a]Py), dibenzo[a,h]anthracene (dB[a,h]An), indeno[1,2,3–cd]pyrene (I[1,2,3–cd]Py) from Supelco.
As sorbent materials: silica gel and aluminum oxide were assayed for preconcentration step. Silica gel 60 (0.2 – 0.5 mm) and aluminum oxide 90 (0.063 – 0.200 mm) were obtained from Merck, Darmstadt, Germany and was activated at 420 ^{o}C for 4 h before use. Anhydrous sodium sulfate (granulated for residue analysis – Merck) was activated at 200 ^{o}C for 2 h before use.
As eluents were assayed four organic solvents: n-hexane and acetonitrile, supplied by Merck, Darmstadt, Germany, petroleum ether and ethylic ether supplied by J.T. Baker.
From the PAH mix solution (PAH Calibration Mix – Supelco), a new PAH solution at levels from 10 $\mu g.mL^{-1}$ to 100 $\mu g.mL^{-1}$ was prepared into acetonitrile and this solution was diluted to construct calibration lines for the PAHs.

Sampling

Samples of *Arabica* and *Robusta* coffee (green coffee bean and roasted coffee bean) available at supermarkets were analyzed. All these samples were registered to the same producer and no indication as regards the country of origin was given on the label.

PAHs extraction and analysis

Approximately, 10 g of each coffee sample was weighed and mixed with 2 g anhydrous sodium sulfate. The samples were Soxhlet extracted for 4 h with hexane as solvent. The solvent was removed under reduced pressure at 40 ^{o}C. The extracts were then evaporated under vacuum using a rotary evaporator.
The extract was then cleaned using silica adsorption chromatography prepared with 5 g of activated aluminum oxide, 5 g of activated silica-gel and capped with anhydrous sodium sulfate. The PAHs extract was applied to the top of the column and eluted with petroleum ether : ethylic ether (94 : 6) mixture. Afterward, the eluted fraction was finally concentrated to about 1 mL and dried under a flow of nitrogen. Finally, the residue was dissolved in 2 mL acetonitrile.
The final concentrated extracts were analyzed using a high pressure liquid chromatograph (HPLC, Varian) equipped with ProStar 240 Quaternary pump, a ProStar autosampler and ProStar 363 fluorescence detector. The optimized instrumental parameters for the chromatographic analysis of PAHs were as follows:

- *injection loop*: 20 μL;
- *ChromSep HPLC Column* SS 100 x 4.6 mm;

- *elution conditions*: 32 min linear gradient elution from 70:30 acetonitrile/water to 93:07 acetonitrile/water, followed by 3 min linear gradient to 100% acetonitrile and held for 5 min; Flow rate was 1 $mL.min^{-1}$ throughout. *Elution temperature:* 30 °C;
- *fluorescence detection:* 16 min $\lambda_{ex.}$ at 274 nm and λ_{em} at 414 nm, followed by 7 min λ_{ex} at 296 nm and λ_{em} at 406 nm followed by 9 min at λ_{ex} at 300 nm and $\lambda_{em\ at}$ 470 nm.

RESULTS AND DISCUSSION

The detection limits (LOD) were calculated on the basis of the noise obtained with the analysis of blank coffee samples. LODs were defined as the concentration of the analyte that produced a signal-noise ratio of three. The noise values were determined by measuring the amplitude signal by fluorescence analysis of a blank reagent (n = 7). In order to verify the accuracy and precision of the analytical procedure, recovery experiments were carried out by spiking coffee samples (n = 7) with a mixture of standard PAH of 2 $\mu g.kg^{-1}$. The values are given in Table 1.
In general, recoveries obtained were satisfactory for determinations at the $\mu g.kg^{-1}$ level.
It was found that use of an internal standard was not necessary as the method performance characteristics were already satisfactory. Fluorescence detection was selected to maximize the analytic response, while reducing interferences from the matrix to a minimum.

Table 1. *Statistical parameters*

PAHs	Instrument linearity range [$\mu g.L^{-1}$]	LOD[a] [$ng.kg^{-1}$]	Absolute[a] recovery [%]
naphthalene	0.2-10	0.05	99
acenaphthylene	0.2-10	0.04	85
acenaphthene	0.2-10	0.04	96
fluorene	0.2-10	0.02	102
phenanthrene	0.2-10	0.01	88
anthracene	0.2-10	0.01	84
fluoranthene	0.2-10	0.01	102
pyrene	0.2-10	0.01	97
benzo[a]anthracene	0.2-10	0.04	83
chrysene	0.2-10	0.04	91
benzo[k]fluoranthene	0.2-10	0.02	89
benzo[a]pyrene	0.2-10	0.02	105
dibenzo[a,h]anthracene	0.2-10	0.04	87
benzo[ghi]perylene	0.2-10	0.04	95
indeno[1,2,3–cd]pyrene	0.2-10	0.04	103

a - 7 determinations

Table 2. *PAHs concentrations from coffee samples*

PAHs	Concentrations [µg.kg^{-1}]			
	Arabica coffee		Robusta coffee	
	Green coffee bean	Roasted coffee bean	Green coffee bean	Roasted coffee bean
naphthalene	0.578	1.005	0.458	0.903
acenaphthylene	ND	ND	0.076	0.135
acenaphthene	0.168	0.178	0.028	ND
fluorene	0.235	0.255	0.067	0.184
phenanthrene	0.097	0.389	0.087	0.497
anthracene	0.007	0.024	ND	0.009
fluoranthene	0.004	0.078	0.042	0.073
pyrene	0.015	0.017	0.017	0.014
benzo[a]anthracene	0.007	0.015	ND	ND
chrysene	ND	ND	ND	ND
benzo[k]fluoranthene	ND	0.006	ND	0.008
benzo[a]pyrene	ND	ND	ND	ND
benzo[ghi]perylene	0.039	0.044	0.026	0.149
dibenzo[a,h]anthracene	0.005	0.018	0.007	ND
indeno[1,2,3–cd]pyrene	0.051	0.129	0.150	0.323

ND - not detected;

Table 2 shows the concentrations of PAHs found in coffee samples. Naphtalene, fluorene, anthracene, fluoranthene, pyrene, benzoperylene and indenopyrene occurred in all samples analysed. The highest concentration of analyzed PAHs was found in roasted coffee bean (1.005 µg.kg^{-1} for naphtalene). Chrysene and benzopyrene were not detected in any sample.

Among the carcinogenic PAHs (Table 3), indenopyrene was the most representative, being found in all samples with the highest concentration in coffee (0.323 µg.kg^{-1}).

Table 3. *PAHs, their carcinogenicity*

Compound	Carcinogenicity
Benzo(a)anthracene	+
Chrysene	±
Benzo(k)fluoranthene	+
Benzo(a)pyrene	+
Dibenzo(ah)anthracene	+
Indeno[1,2,3–cd]pyrene	+

Sufficient evidence (+); limited evidence (±); [11]

The results of this limited study indicated a contamination of coffee samples by PAHs. Higher concentrations of PAHs were detected in all roasted coffee samples than green coffee samples. That confirms that their levels are dependent on the location of the growing sites of plant (maybe on the exposed surface of the vegetable to the air pollution) and to the roasting process.

The versatility of HPLC as analytical tool makes it an ideal technique for analytical quality control and research and development laboratories in the food industry. The determination of PAHs in coffee samples at the ppb level was performed in a short time. The analysis procedures proved not to affect their stability and are quantitative as indicated by the method recoveries (better than 87%). Detection and quantification limits for the PAHs in coffee samples were found to be satisfactory and much lower than the restrictions given in proposals of EU Directives for B[a]P (1 $\mu g.kg^{-1}$).

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