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Polycyclic aromatic hydrocarbons in fruit juices

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are compounds widespread in the environment, many of them showing carcinogenic effects. These compounds can reach the food chain by different ways and, therefore, the analysis of PAHs in food is a matter of concern. The purpose of this paper is to determine the polycyclic aromatic hydrocarbons from fruit juices using high performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD). The higher concentration value (2.92µg/L) was obtained for benzo[k]fluoranthene in grapefruit juice while in orange juice almost the all studied samples were under detection limit.

Keywords: polycyclic aromatic hydrocarbons, HPLC-FLD, fruit juices

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds characterized by a structure made up of carbon and hydrogen atoms forming two or more fused aromatic rings without any heteroatom or substituent.

The compounds containing five or more aromatic rings are known as "heavy" PAHs, whereas those containing less than five rings are named "light" PAHs. Both kinds of PAHs are non-polar compounds showing high lipophilic nature, therefore heavy PAHs are more stable and toxic than the other group.

PAHs are ubiquitous environmental contaminants which are widespread in the air bonded to particulate matter. In spite of PAHs show hydrophobic properties (especially heavy PAHs), they are also found in water. These compounds are produced during a variety of combustion and pyrolysis processes from anthropogenic and natural sources. A high amount of PAHs are emitted from processing coal, during incomplete combustion of organic matter (e.g. wood and fossil fuels), from motor vehicle exhaust and cigarettes. Forest fires, volcanoes or hydrothermal processes are natural emission sources of PAHs [1].

The sources of PAH in food are mainly environmental pollution, food processing (drying, smoking) and cooking (roasting, grilling and frying). These compounds occur as contaminants in different kinds of foodstuffs including dairy products, vegetables, fruits, oils, cereals, and smoked meats [2-5].

The 64th Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed all relevant information related to the toxicology, epidemiology, intake assessment, analytical methodology, formation, fate, and occurrence of PAHs in food. Overall, the Committee concluded that PAHs are clearly genotoxic as shown by in vitro and in vivo assays, and include benz [a] anthracene, benzo [a] pyrene, benzo [b] fluoranthene, benzo [ghi] perylene, benzo [k] fluoranthene, chrysene, dibenz [a,h] anthracene, and indeno [1,2,3-cd] pyrene are of interest [6].

Trace analysis of PAHs can be performed mainly by GC/MS or HPLC/fluorescence. The last technique has higher selectivity and lower quantification limits than GC/MS, and has been widely used for analyses of environmental factors, food, and biological samples [7-10].

The purpose of this paper was to determine 15 polycyclic aromatic hydrocarbons from fresh juice fruits (lemon, grapefruit, orange kiwi and tangerine) using high performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD).

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2. Experimental

2.1. Reagents

Standards of PAHs: Acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), naphthalene (Np), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benzo[α]anthracene (B[α]An), benzo[k]fluoranthene (B[k]Fl), chrysene (Chry), pyrene (Py), benzo[ghi]perylene (B[ghi]Pe), benzo[α]pyrene (B[α]Py), dibenzo[α,h]anthracene dB[α,h]An, indeno[1,2,3–cd]pyrene (I[1,2,3–cd]Py) were supplied by International Atomic Energy Agency, Monaco laboratory.

Silica gel was assayed for preconcentration step and it was obtained from Merck, Darmstadt, Germany. As eluents were assayed two organic solvents: n-hexane, supplied by Merck, Darmstadt, Germany and dichlormethane supplied by J.T. Baker. Anhydrous sodium sulphate (granulated for residue analysis) was activated at 200^0C for 2h before use. All glassware were washed with detergent, rinsed with deionised water and acetone before use.

2.2. Sampling

The studies were performed on fresh fruit juices (lemon, grapefruit, orange, kiwi and tangerine). Lemon was imported from Greece, grapefruit from Turkey, orange and tangerine from Italy and kiwi from Chile. Fruit juice was obtained by squeezing the fruit purchased from local market, avoiding any contamination of juice with peel's fruit.

2.3. Sample preparation

Each sample of fruit juice (10 mL) was treated with 2.5 mL hexane and was easily stirred by magnetic stirrer for one hour. Samples were placed into the separating funnel and the phases were separated for at least 5 minutes. The extract was passed through anhydrous sodium sulfate and concentrated to 2 mL using a rotary evaporator with a bath temperature of 30^0 C and a slight decrease in pressure at 200 hPa. The extracts were not evaporated to dryness because may occur the loss of compounds with two or three ring. For extract purification columns containing silica were used. The columns were washed by rinsing the silica with a five higher volume than the volume of silica layer using a mixture of dichloromethane / hexane (1:1). The extract was transferred on the silica column and the polycyclic aromatic hydrocarbons from the column were eluted with a mixture of dichloromethane / hexane (1:1). After that, in elute were added 2.5 ml NN- dimethylformamide, and was mixed by stirring, concentrated using a rotary evaporator and the concentrated aliquot was blown down with nitrogen. The extract was diluted at 2 mL with the same solvent that was used to prepare reference solutions (acetonitrile). The extract was kept in a cool and dark place until chromatographic analysis was performed.

2.4. Instrumental analysis

The chromatographic conditions include a Varian HPLC apparatus equipped with a 230 Controller pump, ProStar autosampler, and a 360 Fluorescence detector (FL) (Varian Inc., Palo Alto, CA, USA). The wavelength program was: for naphthalene, acenaphthene, and fluorene the excitation wavelength 220 nm and emission wavelength 322 nm (0 – 9.6 min), for phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo(e)pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz(a,h)anthracene, and benzo[g,h,i]perylene 240 nm and 398 nm (9.7–29.3 min) and for indeno[1,2,3-cd]pyrene 300 nm and 498 nm (29.4–35.0 min). A Supelcosil LC-PAH column (250 mm _ 4.6 mm _ 5 mm) obtained from Supelco was used at 22^0C. Gradient ACN: water elution began with 60% acetonitrile (5 min) and increased to 100% ACN in 20 min, remaining for 15 min in this last condition. The flow rate used was 1.5 mL/min. The injection volume was 50 mL. The peaks were identified by comparison with the retention time for authentic PAH standards.

3. Results and Discussions

Accuracy and precision were evaluated using spiked juice fruits samples containing three concentrations of each PAH. Control samples (no spiked juice fruits) were also analyzed in order to

evaluate the selectivity of the method. The samples were analyzed in triplicate. Recovery experiments were carried out by spiking studied samples with three different concentrations of PAHs standard solution. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples and were between 80-100%. In **figure 1** the chromatogram of a standard mixture of PAHs is presented. Linearity was observed in the range of 0.01–100 µg/L, with the correlation coefficients (r) ranging from 0.9995 to 0.9998. The LODs, based on signal-to-noise ratio (S/N) of 3, ranged from 0.001 to 0.01 µg L^{-1}.

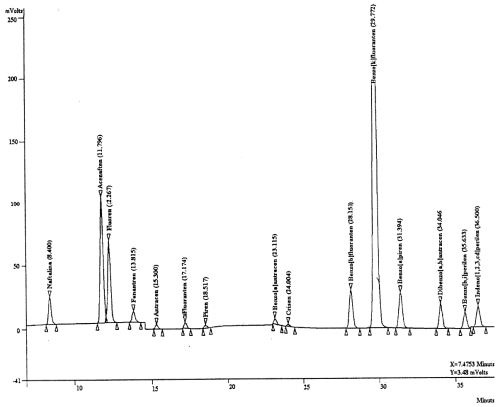

Fig. 1. The chromatogram of the standard mixture of polycyclic aromatic hydrocarbons

In **tables 1** and **2** are presented the average of PAH concentrations for fruit juices.

Table 1. PAH concentrations for lemon, orange and grapefruit juices

PAH	Concentrations (µg/L)		
	Lemon juice	Orange juice	Grape fruit juice
I[1,2,3-cd]Py	< LOD	0.28	1.08
Np	0.777	0.32	0.27
Acy	1.574	0.81	0.46
Ace	1.767	1.09	1.18
F	0.185	1.02	0.40
Ph	0.111	0.179	0.16
An	2.32	< LOD	0.80
Fl	0.158	< LOD	0.10
Py	0.145	< LOD	1.26
B[α]An	0.197	< LOD	0.19
Chry	< LOD	< LOD	1.10
B[k]Fl	0.144	< LOD	2.92
B[α]Py	0.151	< LOD	2.28
B[ghi]Pe	< LOD	< LOD	2.43
dB[α,h]An	0.247	< LOD	1.72

LOD: limit of detection;
Np, naphthalene; Acy, acenaphthylene; Ace, acenaphthene; F, fluorine; Ph, phenanthrene; An, anthracene; Fl, fluoranthene; Py, pyrene; B[a]An, benzo[a]anthracene; Chry, chrysene; B[k]Fl, benzo[k]fluoranthene; B[α]Py, benzo[α]pyrene; B[ghi]P, benzo[ghi]perylene; dB[α,h]An, dibenzo[α,h]anthracene; I[1,2,3-cd]Py, indeno[1,2,3-cd]pyrene.

Table 2. PAH concentrations for kiwi and tangerine juices

PAH	Concentrations (µg/L)	
	Kiwi juice	Tangerine juice
I[1,2,3-cd]Py	0.96	0.12
Np	0.37	0.33
Acy	1.64	1.06
Ace	1.37	1.20
F	0.61	0.28
Ph	0.44	0.43
An	1.90	2.19
Fl	0.86	0.13
Py	0.83	0.16
B[α]An	1.66	0.13
Chry	1.04	0.13
B[k]Fl	0.68	0.14
B[α]Py	0.67	0.12
B[ghi]P	1.27	< LOD
dB[α,h]An	1.95	0.10

LOD: limit of detection;
Np, naphthalene; Acy, acenaphthylene; Ace, acenaphthene; F, fluorine; Ph, phenanthrene; An, anthracene; Fl, fluoranthene; Py, pyrene; B[a]An, benzo[a]anthracene; Chry, chrysene; B[k]Fl, benzo[k]fluoranthene; B[α]Py, benzo[α]pyrene; B[ghi]P, benzo[ghi]perylene; dB[α,h]An, dibenzo[α,h]anthracene; I[1,2,3-cd]Py, indeno[1,2,3-cd]pyrene.

A very wide range of PAH concentrations is observed for all studied samples. The higher value (2.92 µg/L) was obtained for benzo[k]fluoranthene in grapefruit juice while in orange juice almost the all studied samples were under detection limit.

The higher concentration founded in lemon juice was for anthracene (2.32 mg/L), while indeno[1,2,3–cd]pyrene, chrysene and benzo[ghi]perylene were under the detection limit. All 15 aromatic polycyclic hydrocarbons were present in kiwi and grapefruit juices studied.

In assessing consumer exposure to PAHs in food, JECFA estimated a representative mean intake of 4 ng benzo(a)pyrene/kg bw per day and a high-level intake of 10 ng benzo(a)pyrene/kg bw per day [11].

Following the findings of this survey, and taking the JECFA assessment into account, it can be concluded that the levels of PAHs from fruit juices are not of concern for human health.

4. Conclusions

The higher concentration value (2.92 µg/L) was obtained for benzo[k]fluoranthene in grapefruit juice while in orange juice almost the all studied samples were under detection limit.

According with JECFA assessment the levels of PAHs from studied fruit juices are not of concern for human health.

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