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Polycyclic Aromatic Hydrocarbon Determination by Reversed-Phase High-Performance Liquid Chromatography in Olive Oils on the Iranian Market

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Abstract. Despite the carcinogenic properties of some PAHs, and although edible oils are particularly prone to PAH contamination, no international legal limits for PAH in edible oils have been yet established however, a number of methods for such analysis have been published most of which are time consuming and unsuitable for routine analysis, as they do not permit analysis of a large number of sample per day. In this paper, HPLC with spectro fluorimetric detection was applied to the determination of Polycyclic aromatic hydrocarbons (PAHs) in 7 Iranian olive oil and 2 turkeys imported olive oils.The analysis of some blends of refined and virgin oils shows that the distributions of light and heavy PAHs are different with the content of the former being lower in refined samples.

Keywords: Polycyclic Aromatic Hydrocarbons (PAHs), HPLC, Olive Oils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of well known carcinogenic compound originating from incomplete combustion of organic compounds and geochemical processes. Through atmospheric fallout these apolar substances can contaminate crops and are easily transferred to the final products, especially when the matrix has a lipidic nature (e.g.vegetable oil)[1].The German Society for Fat Science has introduced a limiting value of 5 ppb for the sum of both light and heavy PAH (2-6 benzene rings) [2,3].

More recently, Spanish law fixed a limit of 5ppb for the sum of heavy PAHs and a limit of 2 ppb for each heavy PAH [4]. The goal of this work was to determine the level of <light> and <Heavy> PAH´s in olive oils available on Iranian market.

2. Materials and Methods

Iranian olive oils (samples 1-7) and turkey imported olive oil were collected randomly from different local shops. The specification of all samples was showed in table 1.

Table 1: Characteristics of all olive oils sample

Samples	Labels specification
M1, M2, M3, M5	Virgin Olive Oil
M_4	Pure Olive Oil 100%
M_6	Extra Virgin Olive Oil
M_7	Deodorize Olive oil
M_8	Olive Oil
M_9	Refined Olive Oil

Samples were stored in the darkness in near-full bottles, at temperatures never exceeding 20˚C.hexan and dichloromethane. HPLC grade (J.T,Baker). Standard PAH mixture, 610M, in 1ml of

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methanol/dichloromethane(Supelco, Bellefonte, PA, USA) Standard mixture. Standard PAHs mixture 610M,Supelco (Bellofonte, Pa, USA).

2.1. Apparatus

Sample preparation was performed with SPE cartridges packed with 5g of silica phase (Mega Bond Elut, 20ml, Palo Alto, Ca, USA).The analytical determination of PAHs was carried out with Varian mod. 9010 HPLC Gradient pump equipped with a Rheodyne 7161 injector with a 30µl loop.

The column was a C18 reversed phase, 250 ×3mm ID × 5µm partial size (Supelcosil LC-PAH, SUPELCO), thermostatted at 38°c with a column heater.

Mobile phase: A: Acetonitrile B: Water. At flow rate of 1ml/min.

Excitation and emission spectra of each PAH present in the standard mixture were recorded and nine changes of excitation and emission wavelength were then applied to obtain the maximum sensivity possible for each compound (or group of them).

2.2. Sample Preparation

2/5 ± 0/001 of oil were exactly weighted into a 10ml volumetric flask and diluted to volume with n-hexane, then 1ml of the sample solution was loaded onto a 5g silica SPE cartridge previously washed with 20ml of dichloromethane dried completely by means of vacuum, and conditioned with 20ml of n-hexane. PAHs were eluted with a mixture of n-hexane and dichloromethane 70:30 (V/V). The collected fraction was concentrated under a nitrogen stream.

Allowing the residual solvent evaporate spontaneously at room temperature, in order to minimize volatile PAH losses. The residue was dissolved in 100µl of Acetonitrile and injected into the HPLC apparatus.

3. Results and Discussion

Olive oils imported from Turkey (samples 8,9) also showed variable levels of contamination by B(a)P. Table (2)

Table 2: Polycyclic aromatic hydrocarbon (PAHs) in all olive oil samples

Abr.	Name	M1	M2	M3	M4	M5	M6	M7	M8	M9
Na	Naphthalene	-4.39	6.86	-1.27	3.82	-5.05	-3.46	-5.34	-8/0	-9.71
Ac	Acenaphthylene	-1.29	-0.96	-0.24	-1.08	-1.26	-1.23	-1.33	-1.28	-1.34
F	Fluorene	-1.08	-0.04	-1.5	-1.04	-1.16	0.51	-1.99	-1.98	-1.59
Pa	Phenantheren	10.3	8.1	0.1	0.43	12.3	11.3	0.35	0.4	-8.3
A	Anthracene	0.81	0.67	0.15	0.1	1.37	0.67	0.11	0.07	0.7
FI	Fluoranthene	-0.63	2.69	1.5	-0.65	5.4	3.41	1.73	0.39	9.74
P	Pyrene	5.87	3.61	0.7	1.33	7.31	4.68	2.47	0.01	12.8
BaA	Benz[a]anthracene	0.56	0.16	0.4	0.31	0.49	0.08	0.13	0.08	1.09
Ch	Chrysene	0.12	0.07	0.1	0.23	0.39	0.26	0.41	0.01	1.97
BeP	Benzo[e]pyrene	0	0	0	0	0	0	0	0	0
BbF	Benzo[b]fluoranthene	0.78	0.21	0.54	0.41	0.3	0.04	0.06	0.15	1.23
BkF	Benzo[k]fluoranthene	0.2	0.13	0.2	0.14	0.19	0.03	0.04	0.09	0.27
BaP	Benzo[a]pyrene	0.25	0.11	0.17	0.18	0.12	-0.03	0.01	0.1	1.02
DBahA	Dibenz[a,h]anthracene	0.01	0.19	0.04	0.01	0.03	-0.01	0	0.13	0.12
BghiP	Benzo[ghi]perylene	0.27	0.25	0.15	0.15	0.22	0	0.01	0.14	0.44
IP	Indeno[1,2,3-cd] Pyrene	0.07	0.03	0.07	0.22	0.08	0.02	0	0.14	0.46
Sum of Heavy PAHs		2.14	1.07	1.57	1.42	1.42	0.12	0.26	0.82	4.63

4. Conclusion

It was found that in all samples, the amount of total PAH's is lower than 25 ppb and less than 5 ppb for heavy PAH's. Also one of the Turkish samples (M9) analyzed in this study contained more than 1 ppb BaP. It was known that refining reduce the amount of PAH's, depending on the refining conditions adopted. for

example, olive oil samples, that is a mixture of virgin and refined oils, contain lower amount of PAH's. The presence of 'Light' PAH's can be explained by the fact that this kind of oil is not refined, and therefore the steps of deodorization that usually eliminate these compounds do not exist.

Based on the data obtained and in face of the present dietary habit of Iranians olive oils do not seem to be an important source of Polycyclic aromatic hydrocarbons in the diet.

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