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Assessment of the levels of benzo-a-pyrene in olive oil in the State of Kuwait

NISAR AHMED*, ALI S. AL-OMAIR, MAHA AL-SINAN, SULAIMAN AL-KANDARI AND ZANAIB AL-BALLAM

Central Analytical Laboratory, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, 13109, Kuwait Tel: 00965-4899062 Fax: 00965-4989059. E-mail: chromlab@kisr.edu.kw

ABSTRACT

Kuwait, like many of the Arabian Gulf countries, imports most of its olive oil requirements from a variety of countries. The present study was carried out to make a preliminary assessment of the status of olive oil in the Kuwaiti market using appropriate method. Spanish olive oil, which is highly consumed in Kuwaiti food, was found contaminated with benzo(a)pyrene. Pomace virgin and extra virgin oil were collected from supermarket shelves and analyzed. The analytical method proposed by de Vos and van Dokkum including saponification, clean-up by silica gel, alumina and florasil, prior to high-resolution gas chromatography/mass spectrometry was modified for benzo(a)pyrene analysis of a large number of samples in the shortest possible time. The method was employed to conduct a preliminary survey of the olive oil market in Kuwait. Because of the low detection level, the single ion monitoring mass spectra was used for quantitative determination. Quality control measures were incorporated into the analytical procedure to ensure good performance during sample preparation and analyses. Deuterated internal standard having four ions was spiked at the level of 1.0 microgram in each sample. The level of benzo(a)pyrene varied between 2 to 39 ppb in pomace olive oil. Extra virgin and virgin olive oil showed negative results for benzo (a) pyrene.

Keywords: benzo(a)pyrene; gas chromatography/mass spectrometry; olive oil.

INTRODUCTION

Polycyclic aromatic hydrocarbons (also called PAH, PAHs, PAH's, polyaromates, polyaromatic hydrocarbons) are regarded as potentially genotoxic and carcinogenic to humans. Some PAHs ("EPA list") were chosen by the US EPA to be analyzed in the environment (water, soil, air, food). This list includes acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo(b)fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo (ah) anthracene, benzo (ghi)

perylene, indeno (123-cd) pyrene. benzo-a-pyrene (Bap) is classified as the most toxic compound among this group. There is compelling evidence of Bap genotoxicity. Its metabolites bind to DNA, and it can act as an initiator of tumorigenesis.

Spanish pomace olive oil has ban found to be contaminated by Bap. Kuwait imports about 23 types of Spanish olive oil from variety of countries, which is used as both food and medicine. The Kuwaiti Government has presently banned the sale of Spanish olive oil on safety grounds based on our lab results, which showed this product contaminated with Bap.

In the pomace vegetables oils, Bap might be formed by heating during the elaboration process. The Council Regulation (2001), EU countries producing olive residual oil introduced into their National legislation, a maximum level of 2 μg/kg for each of the eight most carcinogenic PAHs including Bap, with a combined maximum level of 5 μg/kg and a maximum content of 25 μg/kg for all PAHs.

The Canadian Food Assessment Agency (2001) has determined on an interim basis that the presence of polycyclic aromatic hydrocarbons in olive pomace oil in excess of 3 parts per billion (ppb) constitutes an unacceptable risk to human health. The 3 ppb level is calculated on the basis of the toxic equivalence to Bap.

Major problems associated with the determination of Bap in complex matrices, such as olive oils, are the low analyte level (g/kg) and the diversity of potential interferences present. The Scientific Committee on Food (2002) examined the possibility of using benzo(a)pyrene that usually is included in any analysis, as a marker for PAH in foods. Menichini *et al.* (1991) analyzed six olive oils and seven virgin olive oils, mostly consumed in Italy, for 28 polycyclic aromatic hydrocarbons (PAHs) and found phenanthrene concentrations up to 40 ng/g. In the Netherlands, De Vos *et al.* (1990) conducted a market basket study and analyzed 221 different food items. The highest PAH concentration (36 ng/g) was measured for chrysene in sugar and sweets. Lenges *et al.* (1976) investigated fish samples from the Belgium market and found high levels of benzo(a)pyrene (11.1 ng/g). Al-Yakoob *et al.* (1993) reported anthracene concentrations of 78.4 and 57.9 ng/g in the edible tissue of two species of fish caught in the Arabian Gulf. Hussain and Naeemi (1997) reported 12 PAHs in 327 foodstuff samples originated from animals reared in Kuwait. However, carcinogenic PAHs concentrations were relatively low in most of the samples with chrysene having the highest concentration among the carcinogenic PAHs. In the above studies, the on-line coupling of liquid chromatography, gas chromatography and mass chromatography (LC-GC) was used for identification of PAHs in vegetable oils (Vreuls *et al.* 1991). In a recent study, Lage Yusty and Cortizo Daviña (2004) utilized HPLC with a fluorescence

detector after supercritical fluid extraction to analyze eight PAHs in olive oils. Detection of 0.5 μg/kg oil was achieved for most of the PAHs.

In order to monitor the quality of imported olive oil and to ensure the safety of the consumers, it is imperative that a monitoring system be put in place to address the concerns of consumer health. The aim of this study was to inspect olive oil and to adapt better analytical techniques for the optimal detection and quantitation of Bap. Secondly, a preliminary survey was conducted to assess the present status of the levels of Bap olive oils in the Kuwaiti local market. Saponification and silica gel column clean up, alumina and florasil were used prior to analysis by gas chromatography/ mass spectrometry in a shortest possible time. Better sensitivity, fewer interferences and results that are more accurate were obtained using this method with internal standards having 4 characteristic ions.

EXPERIMENTAL

Sampling

Twenty three commercial Spanish olive oils (Extra virgin, virgin and pomace) collected from supermarket shelves were stored in a dark cabinet after spiking with deuterated internal standard of PAHs (1.0 μg/g). Italian olive oil was used as blank and recovery was verified with benzo (a) pyrene (100 ppb) after labeling.

Analytical procedure

The PAHs standards and internal standards were purchased from Hewlett- Packard (HP). All solvents were distilled in glass and were of high purity grade. Standard solutions of PAHs were prepared in hexane having concentrations of 100, 500 and 1000 ppb along with internal standard (1000 ng/μl). Silica gel (silica Woelm, 63-200 Mesh, active) was obtained from Woelm Pharma and deactivated with 15% water before use as described by Grimmer & Boehnke (1975).

A 5 g olive oil sample after spiking with internal standard (1.0 μ/g) was boiled under reflux with 100 ml of 2M potassium hydroxide in ethanol-water (9:1 v/v) in a 300 ml conical flask. After completion of saponification (1.5 hr) De Vos, *et al.* (1990), 100 ml of cyclohexane was slowly added through the condenser. After 10 min, the mixture was cooled by adding 100 ml of cold water through the condenser.

The mixture was allowed to stand for one hour in the dark. A 70 ml aliquot of the clear cyclohexane layer, representing 70% of the original sample, was concentrated to near 2.0 ml. The concentrate was applied on a silica gel column as described by

Grimmer and Boehnke (1975). The elute was concentrated to near dryness on a rotary evaporator and the residue was dissolved in 1.0 ml of cyclohexane.

PAH, analysis was performed on a Hewlett-Packard (HP) 5890 Series 11 gas chromatograph and a HP 5972 mass spectrometer. Chromatographic resolution was achieved with a 30 m x 250 μm DB-5 capillary column with a 0.25-m film thickness (J&W Scientific, Folsom, CA) using helium carrier gas. PAHs were analyzed by GC-MS using selected ion monitoring (SIM) and quantitated using the method of internal standards. Bap and internal standards along with the quantification and confirmation ions are listed in Table I.

Table 1: Characteristic ions of benzo(a)pyrene & internal-standards

Compounds Intensity	Target ion	Confirmation ion	M/Z-Relative
Benzo (a) pyrene	252	250, 253	21.3, 22.6
D10-Acenaphthene	164	165	11.6
D10- Phenanthrene	188	189	14.4
D12-Chrysene	240	241, 236	17.3, 24.6
D12- Perylene	264	260, 265	24.3, 22.4

Prior sample set, analysis the GC-MS system performance and calibration were verified for all analytes. The mass spectrometer was tuned immediately before the running of a sample batch using the system's operating software programs (AUTOTUNE) with perfluorotributylamine (PFTBA) calibration gas. Specific detection of low-level analytes such as Bap requires the GC/MS be tuned to optimize sensitivity within a certain mass region. Mass spectrometer parameters were adjusted using tuning masses of 69, 219, and 414 because maximum sensitivity for Bap was desired in this analysis. Using SIM, the mass spectrometer was tuned to maximize response in the 50-450amu range, rather than optimizing for response at 502amu or across the entire 10-650 amu range of Mass Selective Detector. Hexane was injected prior to the running of a sample set to insure the system was free from contaminants or interfering peaks. The relative standard deviation (RSD) between a calibration standard and a performance standard should be within 20%. Other significant gas chromatographic parameters are Carrier gas: helium (99.999%), injector: split/splitless, Injection volume: 2 l, Transfer line: 300°C. The mass spectrometer was operated in the electron ionization (EI) mode with ion source and quadrapole temperatures of 250°C and 100°C, respectively. Gas chromatography (GC) oven temperature program for PAH analysis was:

Initial Temperature: 45°C Initial Time: 2.00min Rate: 10°C/min Final Temperature: 290°C Final Hold Time: 8min Total Run Time: 33.70 min.

The extracted samples, lab blanks and matrix spikes were stored in 4 mL amber vials at -20°C until analysis, contained approximately 1 mL of solvent (cyclohexane) and were previously spiked with 1 mL of the internal standard solution. Standards and samples are brought to room temperature before injection. After the hexane blank was injected, a calibration standard was injected followed by the samples. All injections were performed using an auto sampler. The mass spectrometer was turned on after a 4.0-minute solvent delay. Data was acquired in the selected ion mode. Data was collected and stored within the system's HP computer. Mass chromatograms were generated, and their peaks were integrated with the accompanying software programs.

A reagent blank was analyzed simultaneously with each series of samples. None of the blanks had benzo(a)pyrene levels exceeding the detection limits (0.5 μg/kg). A quantitation limit of 0.65 μg/kg was established by this method.

Quality control

In order to verify the precision and accuracy of the method a blank oil sample was spiked with 100 ppb Bap and internal standard. A reagent blank was analyzed along with each set. Sunflower oils, Italian and corn oils were also analyzed as control samples.

RESULT AND DISCUSSION

Among 23 Spanish olive oils, 11 were found contaminated with Bap. Other PAHs were found in trace levels. The Bap levels found in pomace olive oils ranged from 2 to 39 ng/g (Table 2). Extra virgin oils and gold olive oil had Bap below the detection limit. Some olive oil samples contained ions 253 and 260 as impurity ions. During the clean-up step, silica gel with 5g alumina and silica gel with 5g florasil were used to remove these impurities but this extra clean-up procedure was not very helpful. In single ion monitoring mode ions 253 and 260 were used as confirmation ion for benzo(a)pyrene and D12-perylene (internal-std). (Table1). Using the extracted ion option it was necessary to extract only ions 252 and 250 for benzo(a)pyrene and ion 264 for D12-perylene. Deuterated chrysene-D12 (ion 240) was used for quantification of benzo(a)pyrene and deuterated perylene-D12 for confirmation. In some olive oil samples, there was shift in retention time of benzo(a)pyrene because of sample matrix. Deuterated perylene-D12 was used for confirmation because it eluted just after benzo(a)pyrene. Chromatograms of standard with all internal-std ions and olive oil sample with internal-std ions are given in Figures 1 and 2. Elution order of all PAHs is given in Table 3.

Table 2: Concentrations of benzo(a)pyrene in olive oils

Samples	Benzo(a)pyrene (ng/g)
1 A	15.7
2 A	16
3 A	32
4 A	1.0
4 AR	1.0
5 A	32
6 A	5
7 A	< 0.5
8 A	19
9 A	< 0.5
10 A	< 0.5
11 A	3.5
12 A	< 0.5
6 B	< 0.5
7 B	18
8 B	39
9 B	1.0
10 B	7.6
11 B	1.0
12 B	< 0.5
13B	< 0.5
14 B	24
15 B	< 0.5

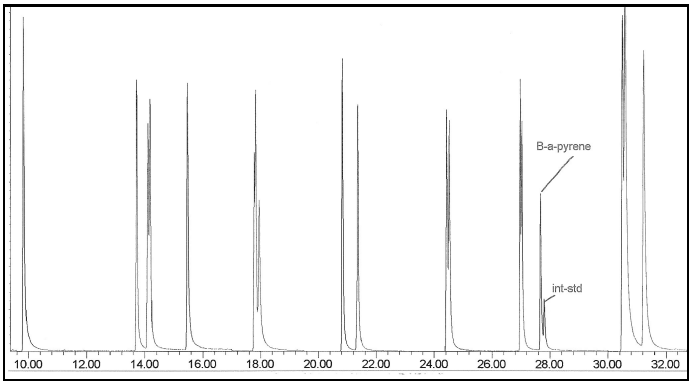

Figure 1: Chromatogram of standard PAHs along with all characteristic internal standard ions. GC/MS conditions: Column, DB-5 capillary column; GC oven temperature program, initial temperature 45^0C; initial time, 2.00 min: Rate 10^0C/min, final Temperature, 290^0C; Final Hold Time, 8 min, Total Run Time, 33.70 min.

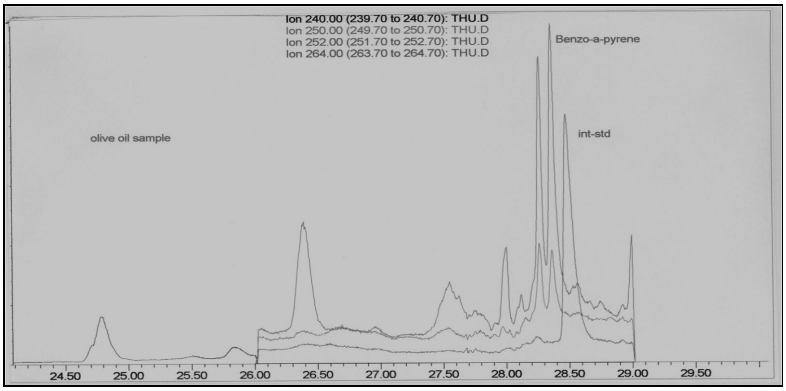

Figure 2: Chromatogram of olive oil sample along with all characteristic internal standard ions. GC/MS conditions are same as given in Fig 1.

Table 3: Calibration standards with elution order

Retention Order	Compound	Target ion and confirmation ion	Concentration (ng/g)
13.85	Acenaphthene-D10	164, 162	1000
9.58	Naphthalene	128, 127	100
13.47	Acenaphthylene	152, 153	100
13.94	Acenaphthene	153, 154	100
15.21	Fluorene	166, 165	100
17.55	Phenanthrene-D10	188, 189	1000
17.60	Phenanthrene	178,179	100
17.72	Anthracene	178, 179	100
20.61	Fluoranthene	202, 200	100
21.17	Pyrene	202, 200	100
24.27	Chrysene-D12	240, 236	1000
24.25	Benzo(a)anthracene	228,226	100
24.35	Chrycene	228, 226	100
26,.84	Benzo(b)Fluoranthene	252, 250	100
26. 89	Benzo(k)Fluoranthene	252, 250	100
27.55	Benzo(a)pyrene	252, 250, 253	100
27.73	Perylene-D12	264, 260	1000
30.95	Indeno(1,2,3,cd)pyrene	276, 277	100
31.07	Dibenzo(a,h)anthracene	278, 279	100
31.87	Benzo(g,h,I)perylene	276, 277	100

CONCLUSIONS

This study showed that high levels of PAHs were found in Pomace Spanish olive oil. This contamination is believed to result from the process used to produce olive oil from the pulp left over after the extraction of pure olive oil. This pulp is dried by heating it and after that, solvents are added to extract additional oil. Olive residue oil may contain high levels of PAHs of which Bap is the most harmful.

The present results demonstrated the ability to detect Bap at very low concentrations down to 0.5 ppb.

The results of our investigations have enabled the Kuwaiti Ministry of Health and the Municipality who control food safety to remove contaminated pomace oil from supermarket shelves and in the future to evaluate the degree of contamination. However, based on the results obtained, the high level of benzo(a)pyrene (Bap) in olive oil (Spanish olive pomace oil), the Government effectively demanded the removal of this oil from the market.

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تحديد مستوى البنزو (أ) بايرين في زيت الزيتون في دولة الكويت

نصار أحمد - علي العمير - مها السنان - سليمان الكندري - زينب البلام

المختبر التحليلي المركزي، معهد الكويت للأبحاث العلمية
ص.ب. 24885. الصفاة 13109 الكويت

خلاصة

تعتبر الكويت مثل أي دولة في الخليج العربي، تستورد الكثير من زيت الزيتون من مختلف الدول. وتم في هذه الدراسة تحديد مستوى مادة البنزو(أ)بايرين المسرطنة في زيت الزيتون وتوصلت الدراسة إلى أن يحتوي زيت الزيتون الأسباني على نسبة عالية من البنزو(أ)بايرين.

وقد تم التحليل عن طريق جهاز كروماتوغرافيا الغاز لكتلة الأطياف مستخدما معيار داخلي يحتوي على أربع أيونات تم إضافتها (1 ميكروجرام) في كل عينة. وقد كان مستوى البنزو (أ) بايرين في العينات المحللة في حدود (2-39 جزء من البليون). وقد كانت النتائج سلبية.