

Polycyclic Aromatic Hydrocarbons in Spanish Olive Oils: Relationship between Benzo(a)pyrene and Total Polycyclic Aromatic Hydrocarbon Content

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Samples of Spanish virgin olive oils (VOOs) from different categories, origins, varieties, and commercial brands were analyzed by HPLC with a programmable fluorescence detector to determine the content of nine heavy polycyclic aromatic hydrocarbons (PAHs): benzo(a)anthracene, chrysene, benzo(e)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perilene, and indeno(1,2,3-c,d)pyrene. Samples of olive pomace and crude olive pomace oils were also investigated. Benzo(a)pyrene concentrations were below the allowed limit in the European Union (2 $\mu\text{g}/\text{kg}$) in 97% of the VOO samples. Only those samples coming from contaminated olive fruits or obtained in oil mills with highly polluted environments exceeded this value. High correlation coefficients (<0.99) were obtained between the contents of benzo(a)pyrene and the sum of the nine PAHs for all of the analyzed categories, suggesting that benzo(a)pyrene could be used as a marker of the content of these nine PAHs in olive oils.

KEYWORDS: PAHs; benzo(a)pyrene; virgin olive oil; olive oil; olive pomace oil; SPE; HPLC

INTRODUCTION

Because of the wide distribution of the polycyclic aromatic hydrocarbons (PAHs) in the environment and their lipophilic nature, olive oils can be easily contaminated with these substances. Different sources of contamination have been investigated, including the exposure of olives and olive oils to polluted environments and diesel exhaust (1, 2).

Although PAH levels in oils can be significantly reduced by refining (3, 4), this process cannot be applied to edible virgin olive oils (VOOs) because it is not allowed by the European Union and the International Olive Oil Council (IOC) in order to preserve its nutritional and organoleptic properties (5, 6). Therefore, once VOOs are contaminated, PAH levels cannot be reduced without losing the quality score of the oil.

Before 2005, there was no international legislation about PAHs in edible vegetable oils; there were only recommendations (7, 8) and local maximum limits (9–12). In 2001, the Spanish legislation established maximum limits for the following eight PAHs in refined olive pomace oil: benzo(a)pyrene (BaP); benzo(e)pyrene (BeP); benzo(a)anthracene (BaA); benzo(b)-fluoranthene (BbF); benzo(k)fluoranthene (BkF); dibenzo(a,h)-anthracene (DahA); benzo(g,h,i)perilene (BghiP); and indeno(1,2,3-c,d)pyrene (IP). The concentration of each PAH

individually could not exceed 2 $\mu\text{g}/\text{kg}$ of oil and their sum 5 $\mu\text{g}/\text{kg}$. Subsequently, the Scientific Committee on Food of the European Union concluded that BaP could be used as a marker for the occurrence and effect of some carcinogenic PAHs (13). In order to protect public health and to ensure market unity, the European Commission provisionally set a maximum level for BaP in some foods, establishing 2 $\mu\text{g}/\text{kg}$ of BaP as maximum limits for oils and fats (14). The European Commission also recommended the investigation of the levels of those PAHs highlighted to be carcinogenic by the Scientific Committee on

Table 1. PAH Levels ($\mu\text{g}/\text{kg}$) in Virgin Olive Oils Supplied by Different Andalusian Olive Oil Mills

PAHs	air pollution level					
	low ^a			high ^b		
	MV	SD	range	MV	SD	range
BaA	0.4	0.4	<LOQ–1.6	0.9	1.1	0.1–4.8
Chr	1.0	0.6	0.2–2.3	1.9	1.7	0.5–7.7
BeP	1.0	0.2	ND–1.1	2.8	1.7	ND–7.7
BbF	0.5	0.1	ND–0.7	1.7	1.6	<LOQ–6.7
BkF	0.1	0.0	<LOQ–0.2	0.5	0.5	<LOQ–2.1
BaP	0.2	0.1	<LOQ–0.5	0.7	0.8	<LOQ–3.6
DahA	<LOQ		ND–<LOQ	0.5	0.2	ND–0.9
BghiP	0.6	0.2	<LOQ–0.9	1.5	0.9	ND–3.8
IP	<LOQ		ND–<LOQ	1.5	0.5	ND–2.3
Σ PAHs	2.1	1.3	0.3–4.6	7.1	9.5	0.7–36.6

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^a $n = 31$. ^b $n = 33$; MV, mean value; SD, standard deviation; ND, not detected; <LOQ, below limit of quantitation.

Table 2. PAH Levels ($\mu\text{g}/\text{kg}$) in Extra Virgin Olive Oil Commercial Brands Purchased in Local Markets

EVOO PAHs	PDOs ^a			CB+WB ^b		
	MV	SD	range	MV	SD	range
BaA	0.2	0.1	<LOQ–0.5	0.2	0.1	<LOQ–0.2
Chr	0.6	0.3	0.3–1.4	0.6	0.2	0.4–0.9
BeP	<LOQ		ND–<LOQ	<LOQ		<LOQ–<LOQ
BbF	<LOQ		ND–<LOQ	0.5	0.03	<LOQ–0.5
BkF	0.2	0.03	<LOQ–0.2	0.1	0.03	<LOQ–0.2
BaP	0.1	0.003	<LOQ–0.1	0.2	0.1	<LOQ–0.3
DahA	<LOQ		ND–<LOQ	<LOQ		ND–<LOQ
BghiP	<LOQ		ND–<LOQ	<LOQ		ND–<LOQ
IP	<LOQ		ND–<LOQ	<LOQ		ND–<LOQ
Σ PAHs	0.8	0.5	0.3–2.2	1.0	0.5	0.4–1.9

^a PDO: protected designations of origin ($n = 10$). ^b CB, most consumed brands; WB, white-brands ($n = 11$); MV, mean value; SD, standard deviation; ND, not detected; <LOQ, below limit of quantitation.

Food as well as their relative proportions (15) for a future review of the suitability of maintaining BaP as a marker.

The studies on the content and distribution of PAHs in olive oils are scarce, and sometimes, their results are not comparable because of the differences in the PAHs determined and the analytical method used (4, 16–22).

Keeping in mind that Spain is the first VOO-producing country and that there is no comprehensive study on PAH levels in these oils, we propose in this article an investigation of the PAH content in the Spanish VOOs obtained following the usual elaboration practices as well as a confirmation of the relationship between BaP and total PAH concentrations. The analyzed PAHs have been those included in the Spanish legislation as well as chrysene since this PAH is in high concentrations in VOOs (2).

MATERIALS AND METHODS

Samples. To establish the PAH levels of the VOOs obtained in industrial olive mills, samples of different varieties (*Arbequina*, *Picual*, *Manzanilla*, *Hojiblanca*, and blends of unknown varieties) were supplied by 18 olive mills of Jaén, Sevilla, Córdoba, Huelva, and Málaga (Spain). The oils were obtained by continuous centrifugation in two-phase mode during the 2003/2004, 2004/2005, and 2005/2006 harvests. The samples were taken from oil tanks. VOOs coming from *Arbequina* olives contaminated by diesel exhaust were supplied by an oil mill located in Zaragoza (Spain).

To study the PAH levels in commercial oils, bottles of extra virgin olive oils (EVOOs) were purchased from local markets. The oils bought were from the most consumed brands (CB), white-brands (WB), and brands with protected designations of origin (PDO, high quality oils obtained following high quality standards in a strictly controlled environment). EVOOs with PDO came from the main Spanish producer regions: Andalusia (Sierra Mágina, Sierra de Segura, Baena, Priego de Córdoba and Estepa PDOs), Castilla-La Mancha (Montes de Toledo PDO), Aragón (Bajo Aragón PDO), Islas Baleares (Aceite de Mallorca PDO), and Catalonia (Les Garrigues and Siurana PDOs).

To study the olive and olive pomace oil categories in commercial oils, bottles of blends of VOO with refined olive oil or refined olive pomace oil were also purchased from local markets.

Crude olive pomace oils obtained by centrifugation and solvent extraction were supplied by several olive pomace extracting industries.

Materials. For chromatographic analysis, acetonitrile HPLC super purity solvent 190 (ROMIL, Cambridge, U.K.) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) were used. For the cleanup procedure, Si and NH_2 Bondesil adsorbents (Varian California, USA), *n*-hexane and toluene of Uvasol grade (Merck, Darmstadt, Germany), and an alkane mixture of boiling point 65–70 °C reagent grade (Scharlau, Barcelona, Spain) distilled using a Vigreux column were used.

Apparatus. The HPLC equipment was composed by a vacuum degasser for the mobile phase solvents, Gastorr 154 (Flom, Japan), an

autosampler System Gold 508, a binary pumping unit, System Gold 126, a Mistral peltier column thermostat unit (Beckman-Coulter, Fullerton, CA, USA), and a programmable fluorescence detector, LACHrom L-7485 (Hitachi-Merck, Japan). A reverse phase C-18 HPLC column (250 \times 4.6 mm i.d.) packed with Inertsil ODS-P (5 μm particle size) (GL Sciences Inc. Tokyo, Japan) was used together with a reverse phase C-18 high performance guard column (10 \times 2.1 mm i.d.) (5 μm particle size) packed with TP-201 (Vydac, CA, USA). The data were processed using 32 Karat Gold v. 5.0 acquisition software (Beckman-Coulter, Fullerton, CA, USA).

Analytical Procedure. All glassware were cleaned before use with *n*-hexane Uvasol several times. The purity of the solvents used was checked by HPLC-FLD.

The analytical method for PAHs determination in olive oils was described in detail in a previous paper (23). Briefly, it consists in the isolation of the hydrocarbon fraction and subsequent cleanup of PAHs by solid phase extraction using glass cartridges with Si and NH_2 adsorbents, respectively. The purified extract is dissolved in acetonitrile and the solution analyzed by RP-HPLC with a programmable fluorescence detector and using acetonitrile/water as the gradient mobile phase. The limits of quantification (LOQ) for each PAH were BaA, 0.1; Chr, 0.1; BeP, 0.8; BbF, 0.4; BkF, 0.1; BaP, 0.1; DahA, 0.2; BghiP, 0.4; and IP, 0.8 $\mu\text{g}/\text{kg}$.

This procedure fulfils the performance criteria of specificity, recovery, limit of detection (LOD), and limit of quantification (LOQ) included in the Commission Directive of 4 February 2005 laying down the methods of analysis for the official control of the levels of BaP in fats and oils (24) and in the Commission Regulation of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD, and BaP in foodstuffs (25).

RESULTS AND DISCUSSION

PAH Content in VOOs. Table 1 shows PAH levels of VOOs samples supplied by different Andalusian olive oil mills. In VOO samples, BaP, Chr, BeP, and BbF were the most abundant PAHs, and only DahA could be quantified in oils containing high levels of contamination. Total PAH contents (Table 1) were very variable (0.3–4.6 $\mu\text{g}/\text{kg}$), although the mean value (2.1 $\mu\text{g}/\text{kg}$) was low, and BaP never exceeded 0.5 $\mu\text{g}/\text{kg}$ (0.2 $\mu\text{g}/\text{kg}$ being the mean value).

However, VOO samples extracted in olive mills exposed to high levels of combustion fumes showed high PAH levels: in the particular case of an olive oil mill close to an olive pomace oil factory using solvent extraction, and in a second olive oil mill using olive stones as solid fuel yielded some VOO samples with a total PAH content up to 36.6 $\mu\text{g}/\text{kg}$. In the olive pomace oil factory close to the olive oil mill, the combustion fumes were used to dry the olive pomace before solvent extraction, whereas in the second olive oil mill, combustion was used only to produce vapor. In both cases, highly polluted environments were originated by the combustion of dried solid olive waste and olive stones, respectively. Moreover, the same effect (high PAH levels) was observed in VOO coming from olive fruits accidentally exposed to diesel exhaust. These results show the important effect of environmental pollution on the PAH content of virgin olive oils (2).

Table 2 shows the PAH levels of EVOO samples (PDO, WB, and CB samples). In all cases, PAH contents were very low (Table 2) since the total PAH and BaP mean values do not exceed 1.0 and 0.2 $\mu\text{g}/\text{kg}$, respectively. These results suggest that EVOOs, obtained following good manufacturing practices, contain low concentrations of PAHs.

In EVOOs and VOOs, the concentration of BaP was below its LOQ (0.1 $\mu\text{g}/\text{kg}$) and 1.0 $\mu\text{g}/\text{kg}$ in the 33.7% and 91.6% of samples, respectively (Figure 1). Only 3.1% of samples, which were obtained in highly polluted environments, had a BaP

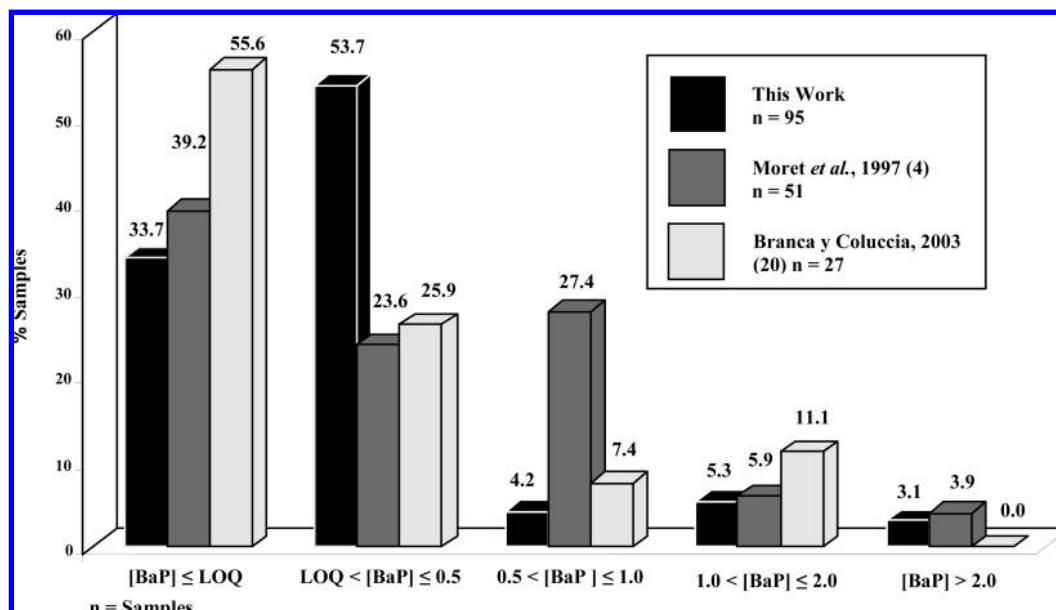


Figure 1. Distribution of virgin olive oil samples according to the BaP concentration ($\mu\text{g}/\text{kg}$).

Table 3. PAH Levels ($\mu\text{g}/\text{kg}$) in Lampante Virgin Olive Oils

PAHs	MV ^a	SD	range
BaA	0.7	0.5	0.2–1.6
Chr	1.6	0.6	0.9–2.2
BeP	<LOQ		<LOQ–<LOQ
BbF	<LOQ		<LOQ–<LOQ
BkF	0.1	0.05	<LOQ–0.1
BaP	0.2	0.1	0.1–0.2
DahA	<LOQ		<LOQ–<LOQ
BghiP	0.6	0.1	<LOQ–0.8
IP	<LOQ		ND–<LOQ
ΣPAHs	3.1	1.1	1.2–4.6

^a $n = 10$; MV, mean value; SD, standard deviation; ND, not detected; <LOQ, below limit of quantitation.

Table 4. PAH Levels ($\mu\text{g}/\text{kg}$) in Crude Olive Pomace Oils Extracted by a Two-Phase Centrifugation System

origin	olive mill ^a			olive pomace oil factory ^b		
	PAHs	MV	SD	range	MV	SD
BaA	0.6	0.4	<LOQ–1.4	2.7	0.5	1.9–3.0
Chr	1.1	1.0	0.2–2.8	3.4	0.4	2.7–3.7
BeP	<LOQ		ND–<LOQ	2.3	0.4	2.0–3.0
BbF	0.6	0.2	ND–0.7	1.5	0.5	0.9–2.0
BkF	0.2	0.1	<LOQ–0.3	0.7	0.2	0.5–0.9
BaP	0.2	0.1	<LOQ–0.4	1.3	0.3	0.9–1.6
DahA	<LOQ		ND–<LOQ	0.3	0.1	<LOQ–0.3
BghiP	0.5	0.2	ND–0.5	1.8	0.4	1.3–2.2
IP	<LOQ		ND–<LOQ	1.0	0.1	<LOQ–1.0
ΣPAHs	2.1	2.0	0.2–6.1	14.0	2.5	10.6–17.2

^a $n = 8$. ^b $n = 5$; MV, mean value; SD, standard deviation; ND, not detected; <LOQ, below limit of quantitation.

content higher than $2.0 \mu\text{g}/\text{kg}$. Therefore, almost all Spanish VOO-EVOO would be below the maximum limit established by the European Commission. Although the comparison between results obtained by different authors is difficult (the analytical methods have different LOQs, and the same PAHs are not always analyzed), these results are in agreement with those obtained in Italian oil samples, where nearly 90% of VOO samples had a BaP content lower than $1.0 \mu\text{g}/\text{kg}$ (4, 20).

Lampante VOO are usually obtained from bad quality olive fruits (overripe, dropped, damaged, and/or unhealthy fruits) and from olives piled in the oil mill for a long time. These oils are

Table 5. PAH Levels ($\mu\text{g}/\text{kg}$) in Blends of Virgin Olive Oil with Refined Olive or Refined Olive Pomace Oils Obtained before and after the Introduction of the Spanish Regulation on PAHs

data sampling	after 2001			before 2001		
	PAHs	MV ^a	SD	range	MV ^b	SD
BaA	0.2	0.1	0.1–0.6	11.9	5.7	3.9–16.9
Chr	0.9	0.5	0.2–2.1	37.9	22.0	18.9–60.5
BeP	2.2	0.9	<LOQ–3.3	40.3	17.0	20.1–57.6
BbF	0.8	0.5	<LOQ–1.6	33.7	17.6	16.7–51.9
BkF	0.2	0.1	<LOQ–0.5	7.9	3.5	4.5–11.5
BaP	0.4	0.2	ND–0.5	17.6	8.8	9.0–26.7
DahA	<LOQ		ND–<LOQ	3.1	2.0	0.7–4.4
BghiP	1.0	0.6	ND–1.8	35.4	26.2	9.5–61.5
IP	<LOQ		ND–<LOQ	16.5	11.9	5.8–28.5
ΣPAHs	3.9	2.3	0.4–8.5	204.1	112.5	99.8–320.1

^a $n = 10$. ^b $n = 4$; MV, mean value; SD, standard deviation; ND, not detected; <LOQ, below limit of quantitation.

not edible because they have bad organoleptic and physicochemical characteristics, and therefore, they must be subjected to a refining process. The analysis of these samples (Table 3) showed that PAH levels were low but slightly higher than those VOOs obtained in olive oil mills not exposed to high levels of combustion fumes.

Although some VOOs with BaP concentrations higher than $2 \mu\text{g}/\text{kg}$ were found, it is not a risk for human health because all of the VOOs with a BaP content exceeding the legal limit are classified in the Lampante category, and therefore, they must be refined using activated carbon to reduce the PAH level and then commercialized as refined olive oils.

PAH Levels in Olive and Olive Pomace Oil Categories. After VOO extraction, the olive paste contains a variable percentage of residual olive oil that can be obtained by a second centrifugation in two-phase mode or by solvent (hexane) extraction. The oil obtained, named crude olive pomace oil (COPO), must be refined in order to eliminate their undesirable organoleptic or physicochemical characteristics.

The extraction process by second continuous centrifugation can be carried out in the olive oil mill just after VOO extraction and from stored olive pomace paste or in olive pomace oil extraction plants. Table 4 shows that COPOs supplied by olive mills have low PAH content (0.2 – $6.1 \mu\text{g}/\text{kg}$, $2.1 \mu\text{g}/\text{kg}$ being

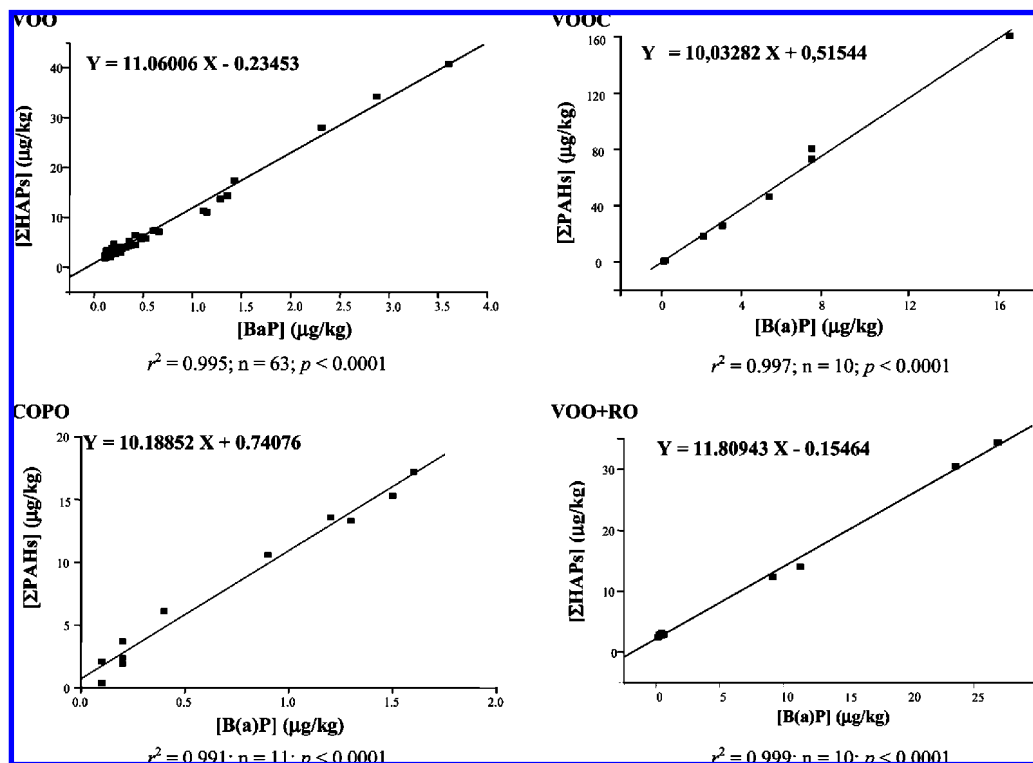


Figure 2. Relationship between concentrations ($\mu\text{g}/\text{kg}$) of BaP and the sum of the 9 PAHs quantified in olive oils.

the mean value) and similar to those of VOOs (0.3–4.6 $\mu\text{g}/\text{kg}$, 2.1 $\mu\text{g}/\text{kg}$ being the mean value). However, PAH concentrations in COPOs supplied by olive pomace oil extraction plants were much higher (up to 17.2 $\mu\text{g}/\text{kg}$). It may be due to the fact that in these factories, the olive pomace is stored outdoors and exposed to a contaminated environment for a long time. COPO can also be obtained by solvent extraction after the drying of the olive pomace. The drying process is usually carried out by the direct contact of olive pomace with combustion fumes, and consequently, an increase of the PAHs content occurs. In crude COPOs obtained from olive pomace dried with the combustion fumes of olive residues, BaP concentrations up to 274 $\mu\text{g}/\text{kg}$ were found. This fact shows the importance of the refining process: an adequate treatment of these oils with activated clays and activated carbon results in a reduction of PAH levels down to the maximum allowed limit (26) avoiding the risk for human health.

In commercial blends of VOO with refined olive oil (olive oil category) or refined olive pomace oil (olive pomace oil category), low PAH contents were found (Table 5), confirming the efficiency of the refining process to eliminate PAHs. Only in some olive pomace oils bottled before the introduction of the Spanish regulation on PAHs in olive pomace oils (9), high PAH concentrations were found, indicating that the oils were not treated with activated carbon.

Relationship between PAH and BaP Concentrations. To determine the relationship between BaP and total PAH content in olive oils, BaP concentrations were plotted versus the sum of concentrations of the nine analyzed PAHs (ΣPAHs). From all samples, those with BaP concentration equal to or higher than their LOQ (0.1 $\mu\text{g}/\text{kg}$) were chosen. VOOs obtained from the olive mills (including those located in polluted areas) and commercial brands showed a good correlation ($r^2 = 0.995$) (Figure 2), which was better than that ($r^2 = 0.778$) obtained by Legarda et al. (27) for Spanish VOO samples. This could

be because these authors did not analyze Chr, one of the most abundant PAHs in the samples.

Good correlations also were obtained for oils obtained from olives contaminated by diesel exhaust (2), crude olive pomace oils, and blends of virgin olive oil with refined olive oil or refined olive pomace oil (Figure 2). Relationships were similar for all the categories of olive and olive pomace oils suggesting that BaP is a good marker for the concentration of the nine heavy PAHs in olive oils.

ABBREVIATIONS USED

PAHs, polycyclic aromatic hydrocarbons; VOOs, virgin olive oils; EVOO, extra virgin olive oils; VOOC, virgin olive oils obtained from olives contaminated by diesel exhaust; COPO, crude olive pomace oil; RO, refined olive and olive pomace oils; IARC, international agency of research on cancer; SPE, solid phase extraction; HPLC, high performance liquid chromatography; FLD, fluorescence detector; BaP, benzo(a)pyrene; Chr, chrysene; BeP, benzo(e)pyrene; BaA, benzo(a)anthracene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; DahA, dibenzo(a,h)anthracene; BghiP, benzo(g,h,i)perilene; IP, indeno(1,2,3-c,d)pyrene; BbC, benzo(b)chrysene; ND, not detected; <LOQ, below of limit of quantification; r^2 , correlation coefficient.

ACKNOWLEDGMENT

We thank Rosario González Cordones and Manuel Rodríguez Aguilar for technical assistance.

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Received for review May 30, 2008. Revised manuscript received August 4, 2008. Accepted September 4, 2008. We are grateful to the Junta de Andalucía for its financial support (Project CAO01-005), and the Ministerio de Educación y Ciencia for the predoctoral fellowship (FPI, FP2000-6575).

JF8016699