



HAL
open science

CONTAMINATION SOURCES OF VIRGIN OLIVE OILS BY POLYCYCLIC AROMATIC HYDROCARBONS

Wenceslao Moreda

► **To cite this version:**

Wenceslao Moreda. CONTAMINATION SOURCES OF VIRGIN OLIVE OILS BY POLYCYCLIC AROMATIC HYDROCARBONS. *Food Additives and Contaminants*, 2007, 25 (01), pp.115-122. 10.1080/02652030701459855 . hal-00577467

HAL Id: hal-00577467

<https://hal.science/hal-00577467>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**CONTAMINATION SOURCES OF VIRGIN OLIVE OILS BY
POLYCYCLIC AROMATIC HYDROCARBONS**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-102.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	08-May-2007
Complete List of Authors:	Moreda, Wenceslao; Instituto de la Grasa (CSIC), Food Quality and Characterization
Methods/Techniques:	Chromatography - GC/MS, Chromatography - HPLC, Quality assurance
Additives/Contaminants:	PAH, Process contaminants - PAH's
Food Types:	Oils and fats, Olive oil

SCHOLARONE™
Manuscripts

CONTAMINATION SOURCES OF VIRGIN OLIVE OILS BY POLYCYCLIC AROMATIC HYDROCARBONS

Rafael Rodríguez-Acuña, María del Carmen Pérez-Camino, Wenceslao Moreda* and Arturo Cert

Instituto de la Grasa (C.S.I.C.),

Avda. Padre García Tejero, 4,

E-41012, Seville, Spain

*E-mail: wmoreda@ig.csic.es

Abstract

The presence of polycyclic aromatic hydrocarbons (PAHs) in virgin olive oils is caused by contamination on the skin of olives, and also contamination of the oil during processing in the oil mill can occur. Contamination of olive fruits occurs on the olive skin, and it depends directly on the environmental pollution level and inversely on the fruit size. In the olive oil mill, the PAHs content can be increased by contamination of the oil during the extraction process if combustion fumes pollute the environment. Other factors during the extraction process, such as olive washing and talc addition, did not modify the PAHs levels of the oils. Very high concentrations of PAHs in oils were only found as a consequence of accidental exposure to contamination sources, such as the direct contact of olives with a diesel exhaust and oil extraction into a high polluted environment. Determination of 9 PAHs was carried out by isolation of the hydrocarbon fraction and subsequent clean-up by solid phase extraction, followed by RP-HPLC analysis using a programmable fluorescence detector.

Key words: *polycyclic aromatic hydrocarbons, virgin olive oil, olive fruit, contamination sources, PAHs.*

1
2 **Abbreviations:** VOO, Virgin Olive Oil; PAHs, Polycyclic Aromatic Hydrocarbons; IARC,
3 International Agency of Research on Cancer; SPE, Solid Phase Extraction; HPLC,
4 High Performance Liquid Chromatography; GC, Gas Chromatography; FLD,
5 Fluorescence Detector; MS, Mass Spectrometry; SIM, Single Ion Monitoring; BaP,
6 benzo(a)pyrene; Chr, chrysene; BeP, benzo(e)pyrene; BaA, benzo(a)anthracene;
7 BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; DahA
8 dibenzo(a,h)anthracene ; BghiPE, benzo(g,h,i)perilene; IP, indene(1,2,3-c,d)pyrene;
9 BbC, benzo(b)chrysene; ND, non detected; < LOQ, below of limit of quantification;
10 EC, European Commission; IOOC, International Olive Oil Council
11
12
13
14
15
16
17
18
19
20
21

22 INTRODUCTION

23
24 Polycyclic aromatic hydrocarbons (PAHs) are a group of contaminants that are widely present in the
25 environment, known to be cancer causing agents: several of them are classified by the International
26 Agency of Research on Cancer (IARC) in 2A and 2B groups [IARC, 1987]. They are generated by
27 incomplete combustion of organic matter arising, in part, from natural combustion (forest fires,
28 volcanic eruptions) and, for the most part, from human activities (engine exhaust, industrial
29 production, coal derived products, petroleum distillates, waste incineration, tobacco smoke) [Grova
30 et al. 2002].
31
32
33
34
35
36
37
38
39

40 Air pollution with dust and particle containing large quantities of PAHs may contaminate the plants
41 via atmospheric fallout during its growing period and most of this superficial contamination can be
42 transferred to the final product [Lee et al. 1981; Bories, 1988; Dennis et al. 1991; Bernd, 2002]. This
43 fact is much more important in industrial areas and highways than in rural areas, where
44 contamination of vegetables can be ten time higher. [Derache, 1990; Grova et al. 2002]. On the other
45 hand, PAHs may also be formed directly in food as a result of some heat processes (charcoal
46 grilling, roasting, smoke drying, and smoking) [Lijinsky y Shubik, 1965a,b; Guillén et al. 1997;
47 Moret and Conte, 2000; Šimko, 2002].
48
49
50
51
52
53
54
55

56 PAHs have been found in many different foods, including edible vegetable oils that, because of their
57 lipophilic nature, can be easily contaminated with these substances. Two main routes of PAH
58 contamination in vegetable oils have been suggested: the contact with polluted environment and the
59 drying process of the raw matter, using combustion fumes of organic matter [Gertz and Kogelheide,
60 1994; Moret and Conte, 2000; Bernd, 2002; EC, 2002]. However, in virgin olive oils (VOOs) only

1 the contact with the polluted air must be taken in account since the raw matter is not subjected to any
2 drying process.
3
4
5

6 Virgin olive oils (VOO) are usually obtained by a process involving several successive steps: olive
7 harvest by manual or mechanical procedures, transport of fruits from olive grove to olive oil mill,
8 piling of olives in the storage area, olive washing, crushing of olives in a hammer crusher, mixing of
9 the olive paste in a thermobearer and separation of the oil by centrifugation or pressing. During the
10 mixing step, oil drops combine, facilitating the release of the oil during centrifugation. However,
11 some olive varieties and, in general, fruits at the unripe stage cause emulsions. In this case, 1-3% of
12 micronized talc (hydrated magnesium silicate) is added to the olive paste in the thermobearer inlet
13 improving the centrifugation effectiveness with no loss of oil quality [Cert et al. 1996]. The talc is
14 eliminated during the centrifugation step together with the olive pomace.
15
16
17
18
19
20
21
22
23
24

25 Generally, the PAH content in crude vegetable oils can be reduced by refining using activated
26 carbon together with activated clays during the bleaching step [Patterson, 1992; Moret et al. 1997;
27 Texeira et al. 2007]. However, refining is not allowed for edible virgin olive oils [EC, 2001; IOOC,
28 2003] since organoleptic properties and chemical composition changes during this process. Due to
29 the PAHs levels in edible virgin olive oils cannot be reduced, the aim of this study was to detect and
30 evaluate the usual contamination sources by PAHs during the VOO obtaining process in order to
31 prevent the contamination hazard and obtain VOOs with the lowest PAHs levels, following the
32 European Commission recommendations [EC, 2005a,b].
33
34
35
36
37
38
39
40
41
42
43

44 MATERIALS AND METHODS

45 *Samples*

46 To determine PAHs contamination in olive fruits and oils, various sets of samples were collected:
47
48

49 1.- Olive fruits of *Koroneiki*, *Arbequina*, *Picual*, *Lechín*, *Hojiblanca*, and *Ascolano* varieties, all
50 being exposed to the same environmental pollution, and harvested at the same time from an olive
51 grove located in a medium polluted area of Seville (Spain). Moreover, olive fruits of *Picual* and
52 *Arbequina* varieties were harvested from the of the Instituto de la Grasa's olive garden (Seville,
53 Spain), located near the city centre.
54
55
56
57
58

59 2.- Olive fruits of *Picual* variety exposed to different environmental pollution were harvested at the
60 same time from the same wide orchard in Jimena (Jaén, Spain). The orchard includes trickle
irrigated and unirrigated groves growing in mountainous area and irrigated groves near a main road.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3.- Olives harvested by hand and by combine from an orchard located at Zaragoza (Spain) were used to establish the influence of diesel exhaust on the olive contamination and the effect of olive washing.

4.- The effect of talc addition and the scale of oil extraction process were studied in VOOs obtained from *Picual* and *Manzanilla* varieties growing in Córdoba (Spain), Seville (Spain) and Huelva (Spain).

5.- To study the effect of the environmental pollution during the oil obtaining process, VOOs were obtained by centrifugation in an olive oil mill close to an olive pomace oil extraction plant with high environmental pollution.

Materials

For oil extraction, tap water and micronized talc (Talcoliva[®], Luzenac, France) were used.

For sample clean-up procedure, Si and NH₂ Bondesil[®] adsorbents (Varian, California, USA), n-hexane and toluene Uvasol[®] grade (Merck, Darmstadt, Germany), and alkanes mixture of boiling point 65-70 °C reagent grade (Scharlau, Barcelona, Spain), distilled using a Vigreux column, were used.

For the HPLC 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 PAH identification, individual standard PAHs were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) at concentrations of 10 ng/μL BaA, Chr, BeP, BbF, BkF, BaP, DahA, BghiPE and IP in acetonitrile. A stock solution containing: BaA 0.50 μg/mL, Chr 0.50 μg/mL, BeP 1.0 μg/mL, BbF 0.50 μg/mL, BkF 0.125 μg/mL, BaP 0.25 μg/mL, DahA 0.25 μg/mL, BghiP 0.50 μg/mL and IP 1.75 μg/mL of the PAHs was prepared in acetonitrile and stored at 4 °C in darkness.

Apparatus

The HPLC equipment comprised a vacuum degasser for the mobile phase solvents Gastorr 154 (Flom, Japan), auto-sampler System Gold 508, binary pumping unit System Gold 126, 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 x 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 x 2.1 mm i.d.) packed with TP-201 (5 μm particle size) (Vydac, CA, USA). The data were processed using 32 Karat Gold v. 5.0 acquisition software (Beckman-Coulter, Fullerton, CA, USA).

1
2
3 The GC equipment comprised a Trace GC2000 gas chromatograph coupled to a GCQ/Polaris ion
4 trap mass spectrometer equipped with an AS2000 autosampler (ThermoFinnigan, Austin, TX, USA)
5 operating in single ion monitoring (SIM) mode for identification purposes. The column used was a
6 DB-5ms (J&W Scientific, CA, USA) fused silica capillary column (30 m long x 0.25 mm I.D. x 0.25
7 µm film thickness) coated with a non polar stationary phase (5% phenyl-methyl polysiloxane). The
8 data were processed using Xcalibur v. 1.4 acquisition software (ThermoFinnigan, Austin, Texas,
9 USA).

15 16 17 18 19 ***Analytical Procedures***

20
21 One of the key points in the determination of PAHs is the cleaning of the material used for its
22 determination, to avoid contamination, all glassware was cleaned several times with n-hexane
23 Uvasol[®] grade before use and the purity of the solvents was checked by HPLC-FLD analysis of the
24 concentrates.
25
26
27
28

29 30 ***PAH extraction from the olive skin***

31
32 PAHs were extracted from the olive skins by rinsing the fruits with n-hexane in an ultrasonic bath
33 as follows: The olives were weighed (500 g), placed into a 500 mL beaker (except *Ascolano* variety
34 that was placed into an 800 mL beaker), and then hexane was added until the fruits were covered.
35 The beaker was subjected to ultra sound for 5 min at maximum power at room temperature. The
36 hexane was then poured into a graduated cylinder and the olives washed again with hexane. The
37 volume of combined extracts of hexane were measured and transferred to amber bottles, which were
38 stored at -20 °C in darkness. Half the hexane volume was concentrated in a rotary evaporator at room
39 temperature under vacuum down to 2.5 mL volume, approximately. Then, the solution was analyzed
40 following the procedure set for the olive oil samples.
41
42
43
44
45
46
47
48

49 50 ***PAHs extraction from the talc***

51
52 Micronized talc (20 g) was placed into a 100 mL Erlenmeyer flask together with 50 mL of hexane
53 and the mixture was shaken vigorously. The flask containing the suspension was placed in an
54 ultrasonic bath for 5 min. at maximum power at room temperature. The suspension was allowed to
55 settle and the upper layer was collected with the aid of a pipette. The hexane volume was measured,
56 and stored in an amber bottle at -20 °C in darkness until analysis. The hexane was then concentrated
57 in a rotary evaporator at room temperature under vacuum down to 2.5 mL volume, approximately,
58 and analyzed following the procedure set for the olive oil sample.
59
60

Oil extraction method

The Abencor (MC2 Ingeniería y Sistemas, Seville, Spain) method was used to obtain the olive oils at laboratory scale, this system, which simulates the industrial process of olive oil production [Martinez et al. 1975]. The olive fruits (700 g) were milled in a hammer crusher and the olive paste was mixed in a thermob eater at 25 °C during 20 min, then 300 ml of hot water (40 °C) was added and the mixture mixed again for 10 min. The olive paste was centrifuged for 1 min and the liquid phase was poured onto a 500 mL graduated cylinder. The solid phase remaining in the centrifuge was centrifuged again after the addition of 100 mL of hot water. The combined liquid phases were left to settle and the upper layer was separated, filtered through filter paper, and stored in glass bottles at 5 °C.

Analysis of PAHs

Analysis of PAHs in oils and hexane extracts were carried out following the procedure previously described [Moreda et al. 2004]. The method involves isolation of the hydrocarbon fraction by solid phase extraction through silica gel phase (Si), subsequent clean up of PAHs using solid phase extraction through modified silica gel phase with NH₂ groups, and quantification of PAHs by RP-HPLC using a programmed fluorescence detector. The HPLC system is set up maintaining the column temperature at 20°C and using a gradient of acetonitrile/water as mobile phase at a flow rate of 1 mL/min. The analyzed PAHs were chrysene (Chr), one the most abundant PAH in olive oils, and those required by the Spanish legislation: benzo(a)anthracene, BaA; benzo(e)pyrene, BeP; benzo(b)fluoranthene, BbF; benzo(k)fluoranthene, BkF; benzo(a)pyrene, BaP; dibenzo(a,h)anthracene, DahA; benzo(g,h,i)perilene, BghiPE and indene(1,2,3-c,d)pyrene, IP [BOE, 2001].

Confirmation of PAHs identities

An extract obtained by rinsing olive fruits with hexane was purified by solid-phase extraction using silica gel and amino phases, as described in the analytical method [Moreda et al. 2004]. The residue was re-dissolved in 50 µL of heptane and aliquots were analyzed by GC-MS in SIM mode. The GC operating conditions were: helium as a carrier gas at 1 mL/min in constant flow mode. Injector temperature was 285 °C and “splitless” mode injection was used, being 1 minute the “splitless time”. The oven temperature was programmed as follow: the initial temperature was held for 3 min at 60 °C and then from 60 to 295 °C at 5 °C/min and maintained for 10 min.

The MS operation conditions were the following: ion source and transfer line temperatures 200 and 300 °C, respectively. The instrument was tuned in Electronic Impact (EI) positive mode using

perfluorotributylamina (FC-43) according to manufacturer's recommendations in order to achieve the maximum sensitivity. Parameters such as automatic gain control (AGC) and multiplier (1150 V, 10E5 gain) were set by automatic tuning. The electron energy was 70 eV and the emission current 250 μ A. The optimized parameter of buffer gas was set to 0.3 mL/min helium. For the single ion monitoring, the molecular ions of each PAHs were selected: m/z 228 for BaA and Chr; m/z 252 for BbF, BkF, BaP and BeP; m/z 276 for BghiP and IP; m/z 278 for DahA.

RESULTS AND DISCUSSION

The identity of each PAH in the hexane solution, obtained by rinsing the olive fruit surface and extraction of talc, was confirmed using both HPLC-FLD and GC-MS analysis by comparison of the retention times of each peak between samples and standard solution to avoid wrong identification of the chromatographic peaks. Once ensured the PAH identities, only HPLC-FLD analyses with quantitative purpose were made.

PAH Contamination of olives

Contamination of olives fruits during growth on the olive tree is a factor to be considered in the PAHs content in the oil, since the olive skin contacts with the oil during the crushing and mixing processes in the oil mill allowing the transfer of PAHs from the skin to the oil. Therefore, olive size, environmental pollution, and accidental contamination were taken into account in the present study.

Effect of environmental pollution during the olive growing

To establish the influence of the olive fruit size on the PAHs oil levels, olive fruits of several varieties were harvested at the same time from an olive orchard nearby to an airport. *Arbequina*, *Picual*, *Lechín*, and *Hojiblanca* varieties were selected because they are the most abundant in Andalusia, and *Koroneiki* and *Ascolano* varieties because their fruits were the smallest and biggest, respectively (Table 1). *Arbequina* and *Picual* olive fruits from trees growing in an urban area were also harvested. The PAHs present on the olive fruit surface were extracted with hexane in an ultrasonic bath and analyzed, being the PAH level per fruit proportional to its surface. Consequently, the PAHs content per Kg of olives is in inversely relation to the fruit size. Assuming an average oil content in fruits around 20% [IOOC, 1996], since samples were harvested at the same time in different ripeness stage, and the total transfer of PAHs from the surface to the oil, the theoretical PAH contamination of oils would be inversely proportional to the olive size (Table 1). This hypothesis was confirmed by the PAH contents in olive oils obtained from the olives using the Abencor system (Table 1). The comparison between the real PAH concentrations in oils and those calculated from the hexane extracts suggests that the contamination of the oils is mainly in the skin of the fruits, which is transferred to the oil during the extraction process. These results are in

1 agreement with those obtained by Cejpek et al. [1998] in rapeseed oil who found no significant
2 differences in PAH concentrations determined by means of a procedure including seed rinsing with
3 chloroform in an ultrasonic bath and that with chloroform extraction of the ground seed.
4
5
6
7

8 [Insert Table 1]
9
10
11
12

13 To establish the influence of environmental pollution on the olive grove, oils obtained by the
14 Abencor system from *Picual* olives harvested from different places of the same olive orchard,
15 exposed to different levels of pollution, were analyzed. The PAH concentrations were very low (the
16 sum of PAHs was below of 1 $\mu\text{g}/\text{kg}$) in oils coming from trees growing in mountainous area,
17 whereas those obtained from trees located near the roads were higher (Table 2). No differences in
18 PAHs concentration were found in oils coming from trickle irrigated and unirrigated olive trees from
19 the mountainous areas. These results indicated that the air pollution level on the tree is a significant
20 contamination source in olive oils.
21
22
23
24
25
26

27 [Insert Table 2]
28
29
30
31
32

33 ***Contamination during Olive Harvesting***

34 In the crop 2004/2005, several VOOs obtained in an olive oil mill showed high PAHs content (up to
35 30 $\mu\text{g}/\text{kg}$ of BaP). These concentrations were rather high and the possible contamination sources
36 were investigated. After checking the lack of pollution problems, the olive harvesting process was
37 then examined. The hexane extracts obtained by rinsing from various olive sets were analyzed:
38 olives harvested by hand, olives mechanically harvested, and olives harvested by hand after that the
39 combine had passed over the trees. Olives harvested by hand prior to passing the combine showed
40 very low PAH levels, the olives harvested after passing the combine showed a slightly increase in
41 the PAH level. The olives harvested mechanically by the combine reached very high PAH levels
42 (Table 3). When the exhaust pipe of the combine was enlarged, the PAH concentration diminished.
43 These results are in agreement with the fact that the combine circulate over the olive trees and the
44 harvested olives are carried to a hopper located close to the exhaust pipe outlet. When the exhaust
45 pipe was enlarged the concentration diminishes significantly. Therefore, the exposure to the diesel
46 exhaust fumes is the most important source of contamination. An appropriate design of the engine is
47 required to minimize the olive contamination.
48
49
50
51
52
53
54
55
56
57
58
59
60

[Insert Table 3]

1 Finally, to confirm that the PAHs are deposited mainly in the olive skin, olives contaminated by
2 the combine exhaust were rinsed with hexane in an ultrasonic bath and then, the oil extracted using
3 an Abencor system. The oil showed a low BaP content (1.8 µg/kg) in comparison with the oil
4 obtained from unwashed olives (27.0 µg/kg).
5
6

7
8 As expected, the PAHs composition in olive fruits depended on the contamination source (Table
9 4).
10

11 [Insert Table 4]
12
13

14 **VOO CONTAMINATION DURING THE EXTRACTION PROCESS**

15
16 The factors that could affect the PAHs concentration during the olive oil extraction process were
17 also studied. These factors include, olive washing, talc addition, scale of the extraction process and
18 environmental pollution in the olive oil mill.
19
20
21
22
23

24 **Olive washing**

25
26 Olive washing is a previous step in the process of olive oil extraction in order to remove earth
27 particles from the olive surface. The elimination of solid particles from the fruit surface might reduce
28 the contamination level. In order to check this hypothesis, two sets of highly contaminated olive
29 fruits were rinsed with hexane and tap water respectively. The latter was dried at 100°C and
30 extracted with hexane. The extracts were analyzed showing similar PAHs concentration. These facts
31 indicate that washings with water do not eliminate PAHs from the fruit skin.
32
33
34
35
36
37
38

39 **Talc Addition**

40
41 Nowadays, micronized talc is the only technical coadjutant allowed by EC legislation in the VOO
42 extraction process. In the hexane extract of talc, the PAHs concentrations were very low (Σ PAHs =
43 0.05 µg/kg). Assuming that the olive paste yield around 20% of oil and the talc is usually added in
44 proportion of 1-3%, the theoretical talc contribution to the final PAH level is negligible. To verify
45 the low effect of talc addition in the final PAH content, VOOs were obtained by an Abencor system
46 from *Arbequina* and *Picual* varieties both with and without addition of talc (2.8%). The total PAHs
47 concentrations were 1.0 µg/kg for *Arbequina* and 0.4 µg/kg for *Picual*, both with and without talc. In
48 conclusion, talc addition had no effects in VOO PAHs content.
49
50
51
52
53
54
55
56
57

58 **Scale of the Oil Extraction Process**

59
60 To study the influence of the industrial oil extraction process on PAHs concentration in VOOs,
olive fruits were processed in the experimental oil mill of the Instituto de la Grasa, located in a low

1 polluted area, using a Peralisi M-1 system (Peralisi España S.L., Zaragoza, Spain), tap water, 1% of
2 talc, and following the good manufacturing practices. The olive oil mill was composed by a hammer
3 crusher, a thermomixer at 31 °C, and horizontal and vertical centrifuges. In Table 5, the results
4 shows similar PAHs content in oils obtained at industry and laboratory scale whereas differences
5 were found according to olive origin confirming the influence of olive contamination.
6
7
8

9 [Insert Table 5]
10

11 **Environmental Pollution over the Olive Mill**

12

13
14
15 To evaluate this factor, an olive oil mill placed in the same area of an olive pomace oil extraction
16 plant was examined. In this industrial plant, exhausted olive pomace paste was burn as solid fuel for
17 drying the olive pomace and, consequently, the olive oil mill was surrounded by smoke and the
18 VOOs were obtained in a high polluted environment. In seasons before (2001/2002 and 2002/2003),
19 some samples contains very high PAHs levels, up to a 3.6 µg/kg of BaP and 39.6 µg/kg of total
20 PAHs. In this olive mill, the obtained olive oils were stored in outdoor tanks with ventilation shafts,
21 where the smoke could come into. In the season 2003/2004, every single tank was exhaustively
22 cleaned before starting the harvest season, and to avoid the incoming of the polluted air, a valve in
23 their ventilation shafts was installed. After the adoption of these measures, the PAHs concentrations
24 in VOOs were variable, but they did not reach those high levels which were observed in previous
25 crops (Table 6). The PAHs levels in the VOOs stored in the oil tanks remained constant even three
26 month after obtention. Then, the combustion fumes seem to be the main source of contamination of
27 the VOOs in the olive mills.
28
29
30
31
32
33
34
35
36
37
38
39

40 [Insert Table 6]
41
42
43
44

45 **CONCLUSIONS**

46

47
48 The PAHs content of olive fruits depends on the environmental pollution on the olive grove and
49 olive size. The PAHs are deposited on olive skin and they are transferred to the oil during the oil
50 extraction. The VOO extraction process does not increase the normal PAH background if it is carried
51 out in a clean environment. The high PAHs levels in the VOOs are due to high environmental
52 pollution by combustion fumes, both in the olive grove and the olive mill. Moreover, accidental and
53 significant contaminations may occur by exposure of olives to engine exhausts. Therefore, a correct
54 fruit harvest, and a suitable storage of the obtained VOOs are needed to avoid a virgin olive oil
55 contamination by PAH.
56
57
58
59
60

REFERENCES

- 1
2
3 Bernd R.T.S. 2002. Biomass burning: a review of organic tracers for smoke from incomplete
4 combustion. *Applied Geochemistry*. 17:129-162. ()
5
6
7
8 BOE. 2001. Order 14558 of 25th of July of 2001 in which is established the limits of certain
9 polycyclic aromatic hydrocarbons in olive pomace oil. *Spanish Official Bulletin* 178:23397-23398.
10
11
12 Bories G. 1988. Tossicologia e Sicurezza degli Alimenti. *Tecniche Nuove*, Milan.
13
14
15
16 Cejpek C., Hajšlová J., Kocourek V., Tomaniová, Cmolín J. 1998. Changes in PAH levels during
17 production of rapeseed oil. *Food Additives and Contaminants* 15:563-574.
18
19
20
21 Cert A., Alba J., León-Camacho M, Moreda W., Pérez-Camino M.C. 1996. Effects of talc addition
22 and operation mode on the quality and oxidative stability of virgin olive oils obtained by
23 centrifugation. *Journal of Agricultural and Food Chemistry* 44:3930-3934.
24
25
26
27 Dennis M.J., Massey R.C., Gripps G., Venn I., Howarthand N., Lee G. 1991. Factors affecting the
28 polycyclic aromatic hydrocarbon content of cereals, fats and other food products. *Food Additives*
29 *and Contaminants* 8:517-530.
30
31
32
33
34 Derache R. 1990. Toxicidad de los hidrocarburos aromáticos policíclicos y de los productos de
35 pirólisis en Toxicología y Seguridad de los Alimentos. *Ed. Omega*, Barcelona, Spain.
36
37
38
39 EC (European Commission) 2001. COUNCIL REGULATION (EC) No 1513/2001 of 23 July 2001,
40 Amending the regulation n° 136/66/EEC and (EC) n° 1638/98 as regarding the extension of the
41 period of validity of the aid scheme and the quality strategy for olive oil. *Official Journal of the*
42 *European Union*, L 201, 26/07/2001 P. 0004 – 0007.
43
44
45
46
47 EC (European Commission) 2002. Opinion of the Scientific Committee on Food on the risk to
48 human health of Polycyclic Aromatic Hydrocarbons in Food, Health and Consumer Protection
49 Directorate-General SCF/CS/CNTM/PAH/29 Final Report 1-65, Annex 1-194.
50
51
52
53
54 EC (European Commission) 2005a. COMMISSION REGULATION (EC) No 208/2005 of 4
55 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic
56 hydrocarbons. *Official Journal of the European Union*, L 34: 3- 4.
57
58
59
60 EC (European Commission) 2005b, COMMISSION RECOMMENDATION of 4 February 2005 on
the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods
(notified under document number C(2005) 256, *Official Journal of the European Union*, L34: 43-45.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Gertz C and Kogelheide H. 1994. Investigation and legal evaluation of polycyclic aromatic hydrocarbon in vegetable fats and oils. *Fat Science and Technology* 96:175-180.
- Grova N., feidt C., Crépineau C., Laurent C., Lafargue P.L., Hachimi A., Rychen G. 2002. Detection of polycyclic aromatic hydrocarbon levels in milk collected near potential contamination sources. *Journal of Agricultural and Food Chemistry* 50:4640-4642.
- Guillén M. D. , Sopelana P., Partearroyo M.A. 1997. Food as a source of polycyclic aromatic carcinogens. Review *Environmental Health* 12:133-146.
- IARC 1987. Polynuclear aromatic compounds, part 1: Chemical, environmental and experimental data in *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 32, Suppl. 7 International Agency for Research on Cancer, Lyon, France.
- IOOC (International Olive Oil Council) 1996. Enciclopedia mundial del olivo. *Plaza & Janés Editores*, Barcelona, Spain.
- IOOC (International Olive Oil Council) 2003. Trade standard applying to olive oils and olive-pomace oils COI/T.15/NC n°3 of 5 December 2003
- Lee M.L., Novotny M.V., Bartle K.D. 1981. Analytical Chemistry of Polycyclic Aromatic Compounds, *Academic Press*, London, UK. Lijinsky W., Shubik P. 1965. (a) Polynuclear hydrocarbon carcinogens in cooked meat and smoked foods. *Ind. Med. Surg.* 34:152-154.
- Lijinsky W., Shubik P. 1965. (b) Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science* 145:53-55.
- Martinez JM, Muñoz E, Alba J, Lanzón A. 1975. Report about the use of “Abencor” analyzer. *Grasas y Aceites* 26:379-385.
- Moreda W., Rodríguez-Acuña R., Pérez-Camino M.C., Cert A. 2004. Determination of high molecular mass polycyclic aromatic hydrocarbons in refined olive pomace and other vegetable oils. *Journal of Science of Food and Agriculture*, 84:1759-1764. Moret S., Piani B., Bortolomeazzi R., and Conte L.S. 1997. HPLC determination of polycyclic aromatic hydrocarbons in olive oils. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung A* 205:116-120.
- Moret S., Conte L.S. 2000. Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. *Journal of Chromatography A* 882:245-253.

1 Patterson H.B.N. 1992. Basic components and procedures, in Bleaching and purifying fats and oils:
2 Theory and practise / Henry Basil Wilberforce Patterson. *AOCS Press*, Champaign, IL, USA.
3

4
5 Šimko P. 2002. Determination of polycyclic aromatic hydrocarbons in smoked meat products and
6 smoke flavouring food additives. *Journal of Chromatography B* 770:2-18.
7

8
9
10 Teixeira V.H., Casal S., Oliveira B.P.P. 2007. PAHs content in sunflower, soybean and virgin olive
11 oils: Evaluation in commercial samples and during refining process. *Food Chemistry* 104:106–112.
12
13

14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Table 1: Morphological characteristics and PAHs concentrations ($\mu\text{g}/\text{kg}$) in olive fruits of different varieties exposed to the same pollution level.

Zone	Variety	Morphological characteristics			[ΣHAPs] ¹			
		Olives/100 g (approx.)	Medium weight (g/fruit)	Medium size (mm)	$\mu\text{g}/\text{Olive}$	in Olives ($\mu\text{g}/\text{kg}$)	in Oils* ($\mu\text{g}/\text{kg}$)	in Oils ($\mu\text{g}/\text{kg}$)
Airport surrounding	<i>Koroneiki</i>	126	0.795	15 x 11	0.00019	0.239	1.2	1.3
	<i>Arbequina</i>	40	2.486	18 x 17	0.00046	0.185	0.9	0.9
	<i>Picual</i>	25	4.018	28 x 19	0.00051	0.142	0.7	0.8
	<i>Lechin</i>	24	4.152	25 x 20	0.00057	0.137	0.7	0.8
	<i>Hojiblanca</i>	21	4.878	30 x 21	0.00079	0.162	0.8	0.8
	<i>Ascolana</i>	7	13.648	37 x 29	0.00140	0.103	0.5	0.5
Urban	<i>Arbequina</i>	107	0.93	13 x 12	0.00019	0.203	1.0	1.0
	<i>Picual</i>	26	3.78	22 x 17	0.00048	0.124	0.6	0.5

- Calculated assuming an oil yield of 20%; ¹ concentration as an average of two determinations

Table 2: PAHs content ($\mu\text{g}/\text{kg}$) in VOOs obtained from olive fruits of different areas in the same olive orchard.

Zone	Irrigation	Area	Variety	$[\Sigma\text{PAHs}]$
Rural	Trickle irrigation	Near Road	<i>Picual</i>	0.9
		Near Road	<i>Picual</i>	0.8
		Mountain	<i>Picual</i>	0.5
	Unirrigated	Mountain	<i>Picual</i>	0.5
		Mountain	<i>Picual</i>	0.5

For Peer Review Only

Table 3: PAHs levels ($\mu\text{g}/\text{kg}$) in oils coming from olives harvested by different methods,

[PAH]*	Sample			
	By Hand (prior to harvesting by combine)	Mechanically (by combine)	By Hand (After harvesting by combine)	Mechanically (After enlarge the exhaust pipe)
BaA	0.02	4.2	0.0	1.0
Chr	0.07	5.4	0.1	1.5
BeP	0.04	17.4	0.2	5.3
BbF	0.05	16.2	0.2	3.0
BkF	0.02	3.7	0.1	1.1
BaP	0.02	7.0	0.1	1.9
DahA	0.00	1.8	0.2	0.3
BghiPE	0.03	14.9	0.1	2.6
IP	0.04	10.1	0.1	1.8
ΣPAHs	0.30	80.6	1.1	18.5

*Calculated assuming an oil yield of 20%

Table 4: Ranges of PAHs compositions (%) in olives exposed to different environments

PAHs	Airport Surrounding	Urban Area	Harvested by Combine
BaA	11.5 - 13.2	7.5 - 8.3	4.8 - 6.1
Chr	57.3 - 46.5	20.9 - 24.0	6.3 - 9.2
BeP	7.6 - 10.3	14.8 - 15.5	18.5 - 27.0
BbF	13.5 - 16.9	17.5 - 18.6	16.1 - 20.0
BkF	5.3 - 6.2	7.0 - 7.3	4.5 - 7.3
BaP	2.5 - 3.0	5.5 - 6.4	9.6 - 13.7
DahA	ND	ND	1.6 - 2.4
BghiPE	2.1 - 3.3	14.8 - 16.5	13.7 - 18.8
IP	2.6 - 3.9	7.1 - 8.3	9.9 - 13.5

ND: non detected

Peer Review Only

Table 5: Comparison between the most abundant PAHs ($\mu\text{g}/\text{kg}$) in VOOs samples obtained at laboratory and industrial scales.

Variety Place Extraction Method	<i>Picual Cabra</i>		<i>Manzanilla, Villarrasa</i>		<i>Manzanilla, Dos Hermanas</i>	
	Laboratory	Industrial	Laboratory	Industrial	laboratory	Industrial
BaA	0.1	0.1	0.1	0.1	0.2	0,2
Chr	0.3	0.2	0.4	0.3	0.9	0,7
BeP	ND	ND	ND	ND	ND	ND
BbF	< LOQ	< LOQ	ND	ND	< LOQ	< LOQ
BkF	< LOQ	< LOQ	< LOQ	< LOQ	0.1	0.1
BaP	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
DahA	ND	ND	ND	ND	ND	ND
BghiPE	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
IP	ND	ND	ND	ND	< LOQ	< LOQ
Σ PAHs	0.4	0.3	0.5	0.4	1.2	1.0

ND: non detected
LOQ: limit of quantification

Table 6: PAH content ($\mu\text{g}/\text{kg}$) in VOOs samples from olive mill under high polluted environment.

Crop	2001/2002	2002/2003	2003/2004
BaA	0.5 - 1.9	0.3 - 4.8	0.2 - 0.7
Chr	1.5 - 2.6	0.9 - 7.7	0.5 - 1.5
BeP	0.9 - 3.2	< LOQ - 7.7	ND - 1.3
BbF	0.6 - 3.1	< LOQ - 6.7	< LOQ - 1.0
BkF	0.3 - 1.0	< LOQ - 2.1	< LOQ - 0.4
BaP	0.5 - 1.4	0.1 - 3.6	< LOQ - 0.6
DahA	ND - 0.4	< LOQ - 0.9	ND - < LOQ
BghiP	0.6 - 1.7	0.5 - 3.8	< LOQ - 0.7
IP	ND - 1.0	ND - 2.3	ND - < LOQ
ΣPAHs	4.9 - 16.1	1.8 - 39.6	0.7 - 6.2

ND: non detected

LOQ: limit of quantification

For Peer Review Only