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Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia

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Title: Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia

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Keywords: Olive oil contamination; polycyclic aromatic hydrocarbons (PAHs); mineral oil hydrocarbons (MOH); polyolefin oligomeric hydrocarbons (POSH); HPLC, on-line LC-GC.

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Abstract: The present paper investigated on the presence of some hydrocarbon contaminants, namely polycyclic aromatic hydrocarbons (PAHs), mineral oil hydrocarbons (MOH) comprising saturated (MOSH) and aromatic (MOAH) compounds, and polyolefin oligomeric saturated hydrocarbons (POSH) in olives and extra virgin olive oils from Tunisia. Olive fruits were collected in sites exposed to different environmental contamination, and the oil extracted both by physical mean (using an Abencor extractor) and with solvent (using microwave assisted extraction, MAE). Analytical determination was performed by SPE cleanup on silica cartridge followed by spectrofluorometric detection, for PAH, and on-line HPLC-GC-FID for MOH and POSH. Oils extracted from olives by physical mean, as well as extra virgin olive oils from the market, had PAH levels never exceeding the EU legal limits. All olive samples showed similar MOSH profiles, but not clear correlation between the variable contamination levels and considered sources of contamination, was evidenced. The average MOSH content in oil extracted from olives by solvent (11.1 mg/kg) was about four time higher than in oil extracted by physical mean (2.6 mg/kg). MOSH in extra virgin oil from the market ranged from 10.3 to 38 mg/kg, while MOAH were not detected. The higher MOSH levels found in oils from the market evidenced an important contribution due to oil processing and/or packaging. Two of the samples were clearly contaminated with polyolefin oligomeric hydrocarbons (POSH) migrated from the plastic cap.



Dipartimento di Scienze AgroAlimentari, Ambientali e Animali - Università degli Studi di Udine

Sabrina Moret, Associate Professor Department of Agri-Food, Environmental and Animal Sciences University of Udine, Via Sondrio 2A 33100 Udine Italy

> Editorial Office Food Control

Dear Editor,

here enclosed you can find our revised manuscript: "Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia" by Gharbi Ines, Sabrina Moret, Olfa Chaari, Manel Issaoui, Lanfranco S. Conte, Paolo Lucci and Mohamed Hammami.

The paper has been revised according with the referee suggestions.

We confirm that the paper is unpublished and has not been submitted for publication elsewhere. Also, the authors confirm that the institutions where they work agree to the submission of this paper to the journal.

Udine, October 21, 2016

Sincerely, Sabrina Moret

Replies to reviewer comments:

Reviewer #1: This is a very good written and valuable contribution to the topic of mineral oil in vegetable oils and should be published. There are some minor questions:

Abstract, line 34: Did you analyse also MOAH? When I got it right you did so, but didn't find any. As now clearly reported in the text we also analysed the MOAH. Since in all the samples MOAH resulted below the quantification limit, we did not reported MOAH data in the tables.

Table 1: Could you give an estimation what is high/medium or low vehicular traffic from your point of view? What is your rule of order?

As added in the text (paragraph 2.2), we made a rough estimation of the traffic intensity based on the road type (main road, secondary road), dimension of the urban sites in the surrounding, and population density. Based on available information we classified the roads into three groups (with low, medium and high traffic).

Figure 1: Why are samples OF3B, OF4 and OF8 missing?

We realized that there was a mistake in figure 1 (now we corrected it): we erroneously wrote OF3C instead of OF3B (sample OF3C doesn't exist). Samples OF4 and OF8 were only analysed for PAHs because unfortunately olive samples were no longer available.

Is there any indication that pesticides are still formulated with mineral oil?

Mineral oils are commonly used as fungistats and insecticides. Many pesticides exhibit limited water solubility, so mineral and vegetable oils often serve as the carriers to deliver the actives to plants or pests. A proof of the fact that pesticides formulated with mineral oils are still on the market can be easily found by a rapid search on the web, where many company publicize their products (see for example <u>http://www.break-thru.com/product/break-thru/Documents/Brochure-Oil-Enhancers-EN-web.pdf</u>)

A brief comment on the use of mineral oil based pesticides has been added in the text (paragraph 2.2).

Table 3 Why are samples OF4, OF3c and OF8 are missing?

As previously explained sample OF3C doesn't exist. Samples OF4 and OF8 were only analysed for PAHs because unfortunately olive samples were no longer available.

Page 17 line 370-372: I guess these peaks of endogenous n-alkanes had been subtracted fom the hump?

Of course they have been subtracted, as now better underlined in the tex.

Reviewer #2: In this work, the authors investigate the presence of polyciclic aromatic hydrocarbons, mineral oil hydrocarbons and polyolefin oligomeric saturated hydrocarbons in olives and extra virgin olive oils from Tunisia. In general, the work is clear and the results are relevant to food safety. However, some aspects must be corrected to consider this work for publication in this journal.

1) The introduction must be shortened. Some bibliographic aspects could be resumed. The introduction has been slightly shortened (only one of the three referees suggested this).

2) The analytical methodologies used in this research are based in publications of other authors. However, the correct use of these methods should be demonstrated using an adequate reference such as a certified method/material or conducting a recovery study. In the same sense, details of QA/QC procedures applied during analysis must be presented.

PAH analysis was performed by using a method previously validated by some of the authors (Moret and Conte, 2002), for this reason we did not report data concerning QA/QC. Concerning mineral oils a paragraph reporting the requested information has been added.

3) Only the mean concentrations of PAH, MOSH, MOAH and POSH are presented in result sections (Table 2,3 and 4). The estimation of measurement precision (repeteability or reproducibility) must be considered and showed the demonstrate the reliability of the results. *Repeatability data have been also added*

4) The Material and Methods section must be revised and corrected. Several abreviations (Ex.: say "mod." must to say "model") and chemical reagents (say "610 M" must say "610 Mix") are incorrectly presented. Several other errors were observed.

The abbreviations reported have been replaced as suggested and the paragraph has been revised and corrected when necessary

5) The english of the manuscript must be revised and edited by an expert to facilitate understanding.

The English has been revised with the help of an expert

Reviewer #3: Good paper on the determination of contaminants in olives and olive oils from Tunisia.

The main conclusions are interesting:

1) the PAH contamination of the olive oils comes directly from the contamination of the olives,

2) the MOSH contamination of the olives remains mainly in the olive pomace,

3) plastic cap used for the bottling of VOO can induce a POSH & MOSH migration to the bottled oil.

Some minor corrections are to be done :

- page 5 - line 120 : should it be "warning amounts" instead of "worning amounts" ? *We corrected the mistake*

- page 7 - line 154 : even if the analysed samples" cover about 50 % of commercial EVOO brands marketed in Tunisia", 5 samples cannot be represenative of the market, as we do not know if the oil in the bottle corresponds to the same blend from one lot to another. Please moderate your sentence.

We moderated the sentence as requested by the referee

- page 11 - line 244 : How did you manage to get rid of the squalene for the MOAH determination ? As you mentioned in page 17 that "MOAH data are not reported since they were all under the quantification limit (around 1 mg/kg)", you should described the way to quantify MOAH at a level of 1 mg/kg.

To eliminate the interference due to squalene, sample injection was preceeded by epoxidation according to the method developed by Biedermann et al. (2009). This is now specified in the text.

- page 11 - line 249 : Table 2 reports PAH concentrations in μ g/kg and not in mg/kg as mentioned in line 249.

We corrected the mistake.

- page 13 - line 291 : should it be "sources of contamination" instead of "sorces of contamination" ?

We corrected the mistake

- page 13 - line 311 : should it be "contained an average" instead of "contained on average" ? We checked the sentence, but we maintained it in its original form since it is correct.

- page 16 - line 352 : should it be "represents the background level" instead of "raprents the background level" ? We corrected the mistake

- page 17 - line 367 : replace "physica mean" by "physical mean" in the title of Figure 2. We corrected the mistake

- page 17 - lines 378-382: would you recommend to verify the presence of hopanes by GC/MS in order " to confirm if the contamination found in olive fruits is related to environmental conditions" ? Of course analysis of hopanes could help to confirm the environmental origin of the contamination but it's not enough. In my opinion a large scale investigation is needed, making also use of passive samplers to monitor air contamination.

- page 20 - line :440 : replace "differen sites" by "different sites". We corrected the mistake

Highlights

- Determination of PAH and mineral oil in olives and extra virgin olive oil
- Evaluation of background contamination in olive fruits
- Migration of polyolefin oligomeric hydrocarbons (POSH) from plastic caps

1	Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia
2	
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25 Abstract

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27 The present paper investigated on the presence of some hydrocarbon contaminants, namely 28 polycyclic aromatic hydrocarbons (PAHs), mineral oil hydrocarbons (MOH) comprising saturated (MOSH) and aromatic (MOAH) compounds, and polyolefin oligomeric saturated hydrocarbons 29 30 (POSH) in olives and extra virgin olive oils from Tunisia. Olive fruits were collected in sites 31 exposed to different environmental contamination, and the oil extracted both by physical mean 32 (using an Abencor extractor) and with solvent (using microwave assisted extraction, MAE). 33 Analytical determination was performed by SPE cleanup on silica cartridge followed by 34 spectrofluorometric detection, for PAH, and on-line HPLC-GC-FID for MOH and POSH. Oils 35 extracted from olives by physical mean, as well as extra virgin olive oils from the market, had PAH levels never exceeding the EU legal limits. All olive samples showed similar MOSH profiles, but 36 not clear correlation between the variable contamination levels and considered sources of 37 38 contamination, was evidenced. The average MOSH content in oil extracted from olives by solvent 39 (11.1 mg/kg) was about four time higher than in oil extracted by physical mean (2.6 mg/kg). MOSH 40 in extra virgin oil from the market ranged from 10.3 to 38 mg/kg, while MOAH were not detected. 41 The higher MOSH levels found in oils from the market evidenced an important contribution due to 42 oil processing and/or packaging. Two of the samples were clearly contaminated with polyolefin 43 oligomeric hydrocarbons (POSH) migrated from the plastic cap.

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- 45

Key Words: Olive oil contamination, polycyclic aromatic hydrocarbons (PAHs), mineral oil
hydrocarbons (MOH), polyolefin oligomeric hydrocarbons (POSH), HPLC, on-line LC-GC

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49 **1. Introduction**

50

Extra virgin olive oil is a staple food largely consumed in the Mediterranean Countries. Its benefits to health have prompted an increased demand worldwide (Luchetti, 2002). In Tunisia, olive oil production plays an important role in the agronomy and economy (Gharbi, 2015). Accounting for more than 4% of the world olive oil production, Tunisia is holding an important position in the olive oil market; it exports about 75% of its production and is ranked as the second largest exporter after the European Union with an average of 115 000 tons per year over the last five years (ONH, 2015).

Edible oils can be contaminated with polycyclic aromatic hydrocarbons (PAHs) and mineral oil hydrocarbons (MOH) which are both environmental and processing contaminants (EFSA, 2008, EFSA, 2012). Due to their similarity to MOH, some concerns also derive by possible migration of polyolefin oligomeric saturated hydrocarbons (POSH) (Biedermann-Brem, Kasprick, Simat & Grob, 2012) from polyolefin materials (PE and PP) in contact with oils.

63 PAHs are a class of organic compounds with 2-6 fused aromatic rings, produced at high temperature during incomplete combustion of organic matter, mainly combustion of fossil fuels, 64 motor vehicle exhausts and industrial emission. Forest fires, volcanoes or hydrothermal processes 65 66 are natural sources of PAHs (Poster, Schantz, Sander & Wise, 2006). Only heavy PAHs (4-6 benzene rings) are genotoxics and carcinogenic, while light PAHs (2-4 benzene rings) may act as 67 synergists (EFSA, 2008). The necessity for a legislation arose in 2001 after the finding of a highly 68 69 contaminated batch of olive pomace oil in the Czech Republic (Purcaro, Barp & Moret, 2016). 70 Regulation 1881/2006 (European Commission, 2006) harmonized the PAH legislation among EU 71 Member States and fixed a limit for the presence of benzo[a]pyrene (BaP) only, used as a marker of 72 the presence of genotoxic and carcinogenic PAHs. Later, EFSA (2008) recognized that BaP alone is not a suitable marker, and suggested to use PAH8, sum of benz[a]anthracene (BaA), chrysene (Ch), 73 74 benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, dibenz[a,h]anthracene (DBahA),

benzo[g,h,i]perylene (BghiP) and indeno [1, 2, 3-cd] pyrene (IP), or PAH4 (sum of BaA, Ch, BbF,
and BaP). Regulation 835/2011 (European Commission 2011) fixed a limit of 2 mg/kg for BaP and
10 mg/kg for PAH4 in vegetable oils and fats. No legal limit has been established for total PAHs,
even though the German Society of Fat Science considered a maximum acceptable level of 25
µg/kg for the sum of 16 PAHs (PAH8 plus 8 light PAHs) indicated as priority by the Environmental
Protection Agency (EPA).

81 MOH are complex mixtures of saturated (MOSH) and aromatic (MOAH) hydrocarbons generated 82 by geochemical processes, giving gas chromatographic (GC) traces characterized by "humps" of 83 unresolved peaks. MOSH consist of linear and branched alkanes (paraffins), and alkyl-substituted 84 cyclo-alkanes (naphthenes), whilst MOAH include 1-3-ring alkyl-substituted PAHs (parent PAHs represent less than 1-5% of total hydrocarbons). Neukom, Grob, Biedermann & Noti (2002), 85 86 observed that plant materials and edible oils are contaminated with mineral oil hydrocarbons from 87 the air (primarily from particulate matter), mostly originating from incomplete combustion of heating and diesel oils, engine lubricating oils, and road tar debris. 88

89 Depending on their composition and molecular range, mineral oil fractions may have different bioaccumulation potential and toxicity. MOSH from n-C16 to n-C35 accumulate in several tissues 90 91 (lymph nodes, spleen and liver) and cause microgranulomas in Fisher 344 rats. Exposure to MOAH 92 through food is considered of great concern due to the carcinogenic risk associated with this class of 93 hydrocarbons (Barp, Kornauth, Wuerger, Rudas, Biedermann, Reiner, Concin & Grob, 2014). No 94 legal limit are actually in force for mineral oil content in vegetable oils or other foodstuffs. 95 Nevertheless, a draft ordinance of the German Federal Ministry for Food and Agriculture (BMELV) on the presence of MOSH and MOAH in food, as consequence of migration from recycled 96 paperboard, established a limit of 2 mg/kg for MOSH n-C20-35 and 0.5 mg/kg for MOAH n-C10-97 98 35, and, recently, very restrictive limits have been requested in extra virgin olive oil by large scale 99 distribution in German.

Virgin olive oil is extracted from the olive fruit exclusively by mechanical processes. Environmental contamination generally proceeds via atmospheric deposition on growing crops. Thus, oil extracted from olive fruits is expected to contain a background contamination reflecting the contamination of the environment where the olive grows. High PAH concentrations were occasionally reported in oil obtained from olive collected in an olive-grove in a rural area with piles of old railways ties (Moret, Purcaro & Conte, 2007).

106 With the exception of some refining steps (decoloration and deodorization), which lead to a 107 decrease of the contamination (Cejpek, Hajslova, Kocourek, Tomaniová & Cmolík, 1998; Teixeira 108 Casal & Oliveira, 2007; Moret, Populin & Conte, 2010), edible oil processing generally contribute 109 to increase the contamination with both PAHs and MOH. Direct contact with combustion gases 110 during the drying process of grapeseeds (or the pomace) before oil extraction can lead to very high PAH load (Moret, Dudine & Conte, 2000). Storage of pomace under inadequate conditions 111 112 contributes to increase the MOH load (Moret, Populin, Conte, Grob, Neukom, 2003). Use of mineral oil based pesticide, mechanical harvesting, contact with lubricatings used for maintenance 113 114 of extraction plant, transport in jute bags, are some of the possible sources of contamination for 115 MOH (Moret & Conte, 2000; Brühl, 2016).

In conclusion, both MOH and PAHs can enter the oil product through different routes, along the production chain. Rapid alerts (RASSF) succeeded in the last years on the presence of warning amounts of these contaminants in vegetable oils, confirm the importance to mantain under control their presence.

The aim of the present work was to investigate, for the first time, the presence and origin of PAH and MOH in olives and virgin olive oil from Tunisia. To these purpose the background contamination already present in olive fruits differently exposed to some potential environmental contamination was evaluated and compared to the contamination found in bottled extra virgin olive oil from the market.

125

- 126 **2. Material and Methods**
- 127

128 2.1. Reagents and standards

All solvents used (Sigma-Aldrich, Milan, Italy) were of HPLC grade. Ultra pure water was obtained with a MilliQ filter system (Millipore, Bedford, MA, USA). To avoid contamination during sample preparation, all the glassware was carefully washed and rinsed with clean solvents (acetone and hexane) before use.

133 The 610 PAH mixture in 1 mL of methanol/dichloromethane (Supelco, Bellefonte, PA, USA)

134 consisted of: acenaphthene (Ac) (1000 µg/mL), fluoranthene (Fl) (200 µg/mL), naphthalene (Na)

135 (1000 μg/mL), BaA (100 μg/mL), BbF (200 μg/mL), BaP (100 μg/mL), BkF (100 μg/mL), Ch (100

- 136 µg/mL), acenaphthylene (Ap) (2000 µg/mL), anthracene (A) (100 µg/mL), BghiP (200 µg/mL),
- 137 fluorene (F) (200 μ g/mL), phenanthrene (Pa) (100 μ g/mL), DBahA (200 μ g/mL), IP (100 μ g/mL)
- 138 and pyrene (P) (100 $\mu g/mL).$

139 Internal standards for MOH analysis were purchased from Supelco (Milan, Italy) and the standard

140 solution was prepared by mixing 5-α-cholestane (Cho, 0.6 mg/mL), n-C11 (0.3 mg/mL), n-C13

141 (0.15 mg/mL), cyclohexylcyclohexane, (CyCy, 0.3 mg/mL), n-pentylbenzene (5B, 0.30 mg/mL), 1-

methylnaphthalene (1-MN, 0.30 mg/mL), 2-methylnaphthalene (2-MN, 0.30 mg/mL), tritertbutylbenzene (TBB, 0.3 mg/mL), and perylene (Per, 0.6 mg/mL) in toluene.

144 The C10-C40 *n*-alkane standard mixture (50 mg/L each) used to check the performance of the

- 145 system, was purchased by Sigma-Aldrich.
- 146

147 2.2. Sampling

Five extra virgin olive oil samples (different brands) were randomly purchased from the retail market. Despite the low number of samples, they covered about 50% of commercial extra virgin olive oils brands marketed in Tunisia. Such oils are produced by mills which export most of their products and sell only small quantities in the local market. On the other hand, Tunisian people mostly consumes oil produced from olives grown in their family olive groves. Olive samples (11)
were collected by hand in different sites during the crop season 2014/2015. They were all from the
olive variety Chemlali, which is the most diffused in Tunisia, covering more than 60% of the total
Tunisian olive tree growing area.

156 Table 1 resumes some characteristics of the sampling sites (information on the area, number of inhabitants, distance from the main road and level of vehicular traffic). To facilitate a rough 157 158 estimation of exposure to urban emission, the sites were classified as urban, semi-urban and rural, 159 depending on dimension and density of the populated area. Based on these data and type of road 160 (main or secondary road), roads were classified into three groups (with low, medium and high 161 vehicular traffic). All sites, except that corresponding to sample OF1, which was very close to an 162 industrial area (production of stones for house construction), were far from sources of industrial 163 emission (> 2 km). Use of pesticides was also reported in table 1. As known, mineral oils are 164 commonly used as fungistats and insecticides. Since many pesticides exhibit limited water solubility, they are often used as carrier to deliver the actives to plants or pests. 165

166 **Table 1.**

167 Characteristics of the sampling sites

Sample code	Site location	Municipality inhabitants*	Type of site	Distance from road/ vehicular traffic	Use of pesticide
OF1	Teboulba-Monastir	37485	Semi-rural	150 m/ medium	no
OF2	Sousse (centre)	221530	Urban	1-5 m/ high	no
OF3A	Monastir (centre)	93306	Urban	1-5 m/ high	no
OF3B	Monastir (centre)	93306	Urban	100 m/ high	no
OF4	Hammam Sousse	42937	Semi-rural	200 m/ medium	yes
OF5A	Bekalta-Monastir	17850	Rural	1-5 m / low	yes
OF5B	Bekalta-Monastir	17850	Rural	200 m/ low	yes
OF5C	Bekalta-Monastir	17850	Rural	400 m/ low	yes
OF6	Moknine-Monastir	57111	Semi-rural	300 m / medium	no
OF7	Menzel-Kamel-Monastir	8432	Rural	300 m / low	no
OF8	Kasserine	83534	Semi-rural	>300 m / low	yes
* 2014 Tı	inisian census data				

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2.3. PAH analysis

170 2.3.1. Olive oil extraction

Oil extraction was performed as described by Issaoui, Dabbou, Brahmi, Ben Hassine, Hajayej Ellouze & Hammami (2009), with an Abencor extractor composed by a hammer crusher, a mixer and a pulpe centrifuge. The olive paste obtained after crushing about 2 kg of olives was mixed into a mixing jar for 30-45 min with the addition of 100 mL of warm water. Mixing allows the combination of small droplets of oil, that are released by the milling process, into larger ones that can be more easily separated. This is followed by centrifugation (for 3-4 min at 3500 rpm) to separate the solid residue from the liquid phase and decantation of the oil.

178 2.3.2. Sample preparation

179 PAH extraction was performed according to the method developed by Moret & Conte (2002). 180 Briefly, 2.5 g \pm 0.001 g of oil were weighted into a 10 mL volumetric flask and diluted to volume with *n*-hexane); then 1.0 mL of the sample solution was loaded onto a 5 g silica SPE cartridge 181 (Mega Bond Elut, 20 mL, Varian, Palo Alto, CA, USA) previously washed with 20 mL of 182 183 dichloromethane, dried completely by means of vacuum, and conditioned with 20 mL of *n*-hexane. PAHs were eluted with a mixture of *n*-hexane and dichloromethane 70:30 (v/v). The first 8 mL of 184 185 eluate was discharged, and the following 8 mL fraction, containing the PAH fraction, was collected in a conical-shaped vial. The collected fraction was concentrated under a nitrogen stream to about 186 187 30μ L, allowing the residual solvent to evaporate spontaneously, at room temperature, in order to 188 minimize volatile PAH losses. The residue was then dissolved in 100 µL of acetonitrile and injected 189 into the HPLC apparatus.

190 2.3.3. HPLC determination

PAH determination was carried out with a Varian model 9010 HLPC gradient pump equipped with a Rheodyne 7161 injector with a 20 μ L loop. The column was a C18 reversed phase, 250x3 mm ID, 5 μ m particle size (Supelcosil LC-PAH, Supelco) thermostatted at 38 °C with a column heater (model L 7350, LaChrom, Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile and water at a flow rate of 1 mL/min. The gradient elution program started with 40% acetonitrile (isocratic for 5 min), going to 100% of acetonitrile in 40 min. PAHs detection was carried out with a Jasco spectrofluorometer (model FP 1520, Cremalla, Como, Italy); wavelength changes and
method performance are reported elsewhere (Moret & Conte, 2002).

199

200 2.4. MOSH and MOAH determination

201 2.4.1. Oil extraction

202 Olive oil extraction was performed in the laboratory by both physical mean (with the Abencor 203 extractor, as described in 2.3.1.) and with solvent. For solvent extraction, olive samples (about 100 204 g) were ground with a mortar and a pestel and then reduced to a paste with an IKA homogenier. To 205 speed-up oil extraction mantaining low the volume of organic solvent employed, solvent extraction 206 was performed by applying microwave assisted extraction (MAE). The apparatus used was a Mars 207 X (CEM Corporation, Matthews, NC) able to process up to 14 samples simultaneously. An aliquot 208 of the olive paste sample (5 g) was directly weighted into a Teflon-lined vessel (Green Chem plus, 209 CEM), added with 5 µL of the internal standard mixture used for MOH determination, 20 mL of an 210 hexane/ethanol 1:1 (v/v) mixture, and heated at 120 °C for 20 min. After cooling, about 40 mL of 211 milli Q water was added (without mixing) into the vessel and the sample was left to rest for about 20 min at -20 °C to facilitate phase separation between the ethanol/water phase and hexane. The 212 213 hexane phase was then taken to dryness and the residual fat, which was weighted to estimate the 214 extraction yield, was further used for MOSH and MOAH analysis.

215 2.4.2. POSH extraction from plastic caps

About 20 mg of the plastic cap (part not came in contact with the oil) were directly weighted into an autosampler vial, extracted for 2 minutes into an ultrasonic bath (in 1 mL of hexane) and directly injected into the LC-GC (10 μ L) after 1 h of contact. The GC profile so obtained was then compared to that obtained by oil analysis.

220 2.4.3. LC-GC-FID analysis

Olive oil samples were analysed according to the method described by Biedermann Fieseler & Grob
(2009), which was later object of an International collaborative study (Lacoste, 2016). Virgin olive

223 oils from the market and oils extracted by physical mean and with solvent underwent direct HPLC-GC analysis: 300 mg of the oil were weighted into an autosampler vial, added with 10 µL of the 224 225 internal standard mixture, diluted to 1 mL with hexane and injected directly into the on-line LC-GC 226 apparatus. To eliminate the interference due to squalene, MOAH analysis was preceeded by 227 epoxidation (Biedermann Fieseler & Grob, 2009). The LC-GC apparatus (LC-GC 9000, Brechbühler, Zurich, Switzerland) consisted of a PAL LHS2-xt Combi PAL autosampler (Zwingen, 228 229 Switzerland), a Phoenix 40 three syringe LC pump with four switching valves (injection, backflush, 230 transfer and additional valve) and an UV-2070 Plus detector (Jasco, Japan). The LC column was a 231 25 cm × 2.1 mm i.d Lichrospher Si 60, 5 µm (DGB, Schlossboeckelheim, Germany). The GC was a 232 Trace GC Ultra from Thermo Scientific (Milan, Italy). A gradient, starting with 100% hexane (0.1 233 min) and reaching 30% of dichloromethane (at 300 µL/min) in 0.5 min, was used to elute the 234 MOSH (from 2.0 to 3.5 min) and the MOAH (from 4.0 to 5.5 min).

235 A 10 m \times 0.53 mm i.d. uncoated, deactivated precolumn was connected by a steel T-piece union to the solvent vapour exit (SVE) and a 15 m \times 0.25 mm i.d. separation column coated with a 0.15 μ m 236 237 film of PS-255 (1% vinyl, 99% methylpolysiloxane) (Mega, Legnano, Italy). A rapid oven gradient (40 °C/min) starting from 55 up to 350 °C was used for GC analysis (Barp, Purcaro, Moret & 238 239 Conte, 2013). The FID and the SVE were heated at 360 and 140 °C, respectively. After the transfer, 240 the LC column was backflushed with dichloromethane and reconditioned prior to the subsequent 241 injection. The data were acquired and processed by ExaChrom software (Brechbühler, Switzerland). The MOSH area was determined by the integration of the whole hump of largely unresolved peaks, 242 243 subtracted from the endogenous *n*-alkanes. Quantification was based on internal standards. Method 244 performance was periodically checked by analysing blank and standard mixtures. To assess 245 linearity, calibration curves were constructed both in solvent (Barp et al., 2013) and in the matrix 246 (oil solution) at concentrations ranging from 1 to 250 mg/kg. The least squares method was applied to estimate the regression lines. Regression coefficients (R^2) of 0.999 were obtained in both cases. 247 By running a *t*-test at the 5% significance level it was demonstrated that the slope of the regression 248

249 lines obtained in the matrix did not differ significantly from that obtained in the solvent. When
250 exploiting the maximimun capacity of the LC column (20 mg of oil), the limit of quantification
251 (calculated as ten times the S/N) was around 1 mg/kg (it depended on the hump width).

Accuracy was verified by analysing spiked extra virgin olive oils. Recoveries verified at different fortification levels were practically quantitative. Repeatability, assessed by replicate analyses (n=6) of the same sample gave relative standard deviation (RSD) lower than 6%. Quality assurance involved the partecipation of our laboratory to an International collaborative trial on MOSH and MOAH determination in vegetable oils and fats (Lacoste, 2016).

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- 258

259 **3. Results and discussions**

260

261 *3.1. Polycyclic aromatic hydrocarbons*

Table 2 reports PAH concentrations ($\mu g/kg$ oil) found in extra virgin olive oils from the market and in oil extracted from olive samples collected from sites with different exposure to environmental contamination, as reported in Table 1.

265

266 **Table 2.**

PAH concentration (µg/kg) in extra virgin olive oils from the market and in oils extracted fromolives by physical mean

	F	Ра	Α	FI	Р	BaA	Ch	BbF	BkF	BaP	DBahA	BghiP	IP	light PAH	PAH4	PAH8
EVOO1	0.5	8.2	0.2	4.8	2.2	tr	0.7	0.1	0.1	tr	tr	0.1	tr	15.9	0.7	1.0
EVOO2	0.2	2.8	tr	1.7	0.8	tr	0.2	tr	tr	tr	tr	0.1	0.1	5.5	0.2	0.4
EVOO3	0.3	10.6	0.1	8.0	3.4	tr	0.5	0.1	0.1	tr	0.1	tr	0.2	22.4	0.5	0.9
EVOO4	0.1	8.4	tr	8.9	2.6	tr	0.3	0.1	0.1	tr	0.1	0.1	0.1	20.1	0.4	0.8
EVOO5	0.5	7.8	0.2	7.1	3.0	tr	0.5	0.1	0.1	tr	tr	tr	0.2	18.6	0.6	0.8
OF1	tr	18.4	0.5	4.7	7.5	0.1	0.9	0.2	0.1	tr	tr	0.1	tr	31.1	1.3	1.4
OF2	0.4	11.8	0.4	6.8	3.1	0.1	tr	0.4	tr	tr	tr	tr	tr	22.5	0.5	0.5
OF3A	1.5	16.5	0.7	10.0	6.7	0.2	1.2	0.5	0.1	tr	0.1	0.1	0.1	35.4	1.9	2.3
OF3B	0.6	8.4	0.1	6.2	2.6	0.1	0.6	0.1	0.1	tr	tr	0.1	tr	17.9	0.8	1.0
OF4	0.1	2.6	tr	1.8	0.8	tr	0.1	0.1	tr	tr	tr	tr	tr	5.4	0.2	0.2
OF5A	1.7	10.5	0.5	4.4	4.5	tr	0.1	0.4	0.1	tr	0.1	tr	tr	21.7	0.5	0.6
OF5B	0.5	4.2	0.2	1.6	1.8	tr	tr	0.4	tr	tr	tr	tr	tr	8.3	0.4	0.4
OF5C	0.6	5.4	0.2	1.6	2.2	tr	0.6	0.1	tr	tr	tr	tr	0.1	10.0	0.7	0.9
OF6	0.3	3.2	0.1	1.2	1.5	tr	0.3	0.2	tr	tr	0.1	0.1	0.1	6.3	0.5	0.8
OF7	1.0	6.2	0.2	2.4	2.2	0.1	0.6	0.2	0.1	tr	tr	0.1	0.1	12.0	0.9	1.2
OF8	0.5	4.6	0.2	2.0	2.0	0.1	0.2	0.1	tr	tr	tr	0.1	0.1	9.3	0.4	0.5

EVOO, extra virgin olive oil; OF, olive fruits; tr, trace (<0.05 µg/kg)

269

Quantified PAHs were divided into "light" (F, Pa, A, Fl, P,) and "heavy" PAHs (PAH8), the latter
being of major concern due to their carcinogenic and genotoxic properties; PAH4 values were also
reported.

PAH contamination can occur either directly during oil processing in the mill, or indirectly, due to
the olive skin contamination by environmental sources (Fromberg, Hojgard & Duedahl-Olesen,
2007; Rodríguez-Acuna, Pérez-Camino, Cert & Moreda, 2008) as result of the deposition of
particle-bound compounds and, more important, of retention of vapour phase PAHs on the waxy
leaf cuticle from which they may partition into the inner tissue (Moret et al., 2007).

278 As reported in Table 2, commercial samples had BaP and PAH4 contents well below the EU legal 279 limits. PAH4 ranged from 0.2 to 0.7 µg/kg (on average 0.5 µg/kg), PAH8 from 0.4 to 1.0 µg/kg (on 280 average 0.8 μ g/kg), while BaP was below the quantification limit of 0.05 μ g/kg in all the samples. 281 Results obtained for extra virgin olive oil extracted with the Abencor extractor, were similar to 282 those found in extra virgin olive oils from the market: PAH4 and PAH8 ranged between 0.2 and 1.9 283 μ g/kg, and between 0.2 and 2.3 μ g/kg, respectively, while BaP was always below the quantification 284 limit. Concerning total light PAHs, concentration ranged from 5.5 to 22.4 (on average 16.5 µg/kg) 285 for oils from the market, and from 5.4 to 35.4 (on average 14.9 µg/kg) for oil extracted in the lab. The most abundant PAH was Pa followed by Fl, P and F. These results are in accordance with those 286 287 previously reported by Moret et al., 2007.

288 Contamination with PAHs was in general very low and this made difficult to appreciate differences 289 among samples differently exposed to potential sources of environmental contamination. Even 290 though the highest PAH load were found in samples collected from sites more exposed to industrial 291 (OF1) or vehicular emission (OF3), no clear correlation between the presence of/distance from 292 potential sources of contamination and the amount of total or heavy PAH in the olives, was 293 observed.

294 Olives collected from the same olive grove at different distance from the road showed in general a 295 higher PAH content in olives collected closer to the road (1-2 m), which decreased considerably 296 already at low distance (100-200 m). For example, sample OF3A (1-2 m from the main road in an 297 urban area) had 35.8 µg/kg of light PAHs and 1.9 µg/kg of PAH4, while olives taken in the same 298 olive grove, at about 100 m from the road (OF3B), had 17.9 µg/kg of light PAHs and 0.8 µg/kg of PAH4. A similar trend, was observed for samples OF5A (21.7 µg/kg of light PAHs and 0.5 µg/kg 299 300 of PAH4) and OF5B (8.3 µg/kg of light PAHs and 0.4 µg/kg of PAH4), collected at increasing 301 distance (1-2, 200 m) from a medium busy road in a rural site, but not for sample OF5C (10.0 µg/kg 302 of light PAHs and 0.7 µg/kg of PAH4) at about 400 m from the road. It's important to underline that variables possibly affecting PAH concentration in olives (i.e. wind direction, rain and other 303 304 metereological conditions, as well as occasional sources of contamination such as fires) were not 305 under control. Also lipid content of olives, that depends on ripening degree reached at harvesting, is 306 expected to influence the PAH load of the oil extracted from olives (Fismes, Perrin-Ganier, Empereur-Bissonnet & Morel, 2002; Kipopoulou, Manoli & Samara, 1999). 307

308 Comparing average PAH concentration of the oil directly extracted from the olives with those of the 309 extra virgin olive oils from the market, no big differences were found, which seems to indicate that 310 most of the contamination is already present in the olive fruit and that olive processing and oil 311 extraction is not responsible for important further contamination.

312 Knowing that the Tunisian household consumes 3.7 kg of olive oil annually, together with 313 significant differences among the regions, average PAH daily intake was calculated by multiplying the average consumption of olive oil by its mean PAH concentration. The calculated intake for PAH4 and PAH8 were respectively 0.08 and 0.13 ng/kg body weight (b.w.) per day (assuming a reference person of 60 kg b.w.), which accounts for less than 1% of the total dietary intake calculated by EFSA (2008).

318

319 *3.2. Mineral oil hydrocarbons (MOH)*

Compared to other edible oils, extra virgin olive oils contain low amount of MOH, in general less than 10 mg/kg of MOSH and no detectable amount of MOAH. Higher MOSH levels have been occasionally found due to leak of lubricating used in the extraction plant, but other contamination sources can be involved (Moret et al., 2003; Moret, Populin & Conte, 2009). Recently Gómez-Coca, Pérez-Camino & Moreda, 2016, found that olive fruits contained on average 3.2 mg/kg of MOSH, and that talc (used as aid to increase the extraction yield) and leaves contributed to the total contamination in the oil with 0.5 and 0.2 mg/kg of MOSH, respectively.

327

328 3.2.2. Olives

329 3.2.2.1 Oil extraction

Some preliminary trials were carried out (in triplicate) to find optimal conditions for rapid 330 331 extraction of the oil from the olive paste. Solvent extraction was optimized in order to obtain high 332 oil yields and efficient MOH extraction. To this purpose, different aliquots of the same olive paste underwent microwave assisted extraction (120 °C x 20 min) using hexane/ethanol (1:1 v/v) or 333 334 hexane as extraction solvent. In the latter case, the sample was previously dried at 60 °C over the weekend and a Carboflon bar (secondary microwave absorber) was added to heat the hexane. 335 336 Hexane extraction on pre-dried sample allowed to obtain quantitative oil extraction, while the 337 hexane/ethanol gave a lower oil yield (80%). Since the two extraction procedures gave comparable MOSH extraction, the hexane/ethanol mixture (which allowed to avoid the drying step) was chosen 338 339 for sample extraction.

340 3.2.2.2. MOSH in olive fruits and in oil extracted by physical mean and with solvent

Fig. 1 reports the MOSH content expressed in mg/kg of fresh olive. Total MOSH levels in olive 341 342 fruits were in the range between 0.4 and 3.2 mg/kg (on average 1.5 mg/kg olives). The main 343 compounds were those ranging from n-C16-35, three samples had detectable contamination in the 344 range *n*-C10-16, and two in the range > n-C35





346

347 *Fig. 1. MOSH concentration (mg/kg) in olives from different sites.*

348

349 Althought a large variability in MOSH contamination was evidenced among different samples, and 350 in general a decreasing MOSH content was observed with the distance from the road (as in the case 351 of PAHs), no clear correlation between the MOSH content and presence of/distance from 352 environmental contamination sources was found. Among the highest contaminated samples there 353 were samples OF3A and OF2 (2.7 and 2.1 mg/kg of olives, respectively) from an urban sites (very close to a trafficated road), but also samples OF5C (3.2 mg/kg olives) and sample OF6 (1.9 mg/kg 354 olives), both from rural or semi-rural areas far from the road. In the case of sample OF5 we cannot 355 exclude a contribution due to the use of mineral oil based pesticides (which usually are not 356 357 uniformely spread in the olive grove).

Table. 3 compares total MOSH (expressed in mg/kg oil) extracted from the olives by physical mean 358 359 (Abencor), with those obtained for the same samples by solvent extraction (using MAE).

360

362 MOSH content (mg/kg) of the oil oil extracted from olives both by physical mean (Abencor) and with solvent

Oil extraction	OF1	OF2	OF3A	OF3B	OF5A	OF5B	OF5C	OF6	OF7
Abencor	1.4	2.4	3.0	1.7	1.8	0.0	4.2	3.6	1.6
Solvent (MAE)	3.9	10.5	16.6	5.4	7.8	2.3	17.0	12.0	3.5
Ratio	2.9	4.4	5.5	3.2	4.3		4.0	3.4	2.2

363

MOSH level found in extra virgin olive oils extracted with the Abencor (on average 2.6 mg/kg oil) represents the background level due to the pre-existing contamination already present in the olives. Olive processing in the oil mill, as well as migration of POSH from plastic food contact materials, could contribute to increase the contamination in the final product.

Mean MOSH concentration in oils extrated with solvent (11.1 mg kg/oil), was about 4 times higher than in oils extracted by physical mean. This well agree with what reported by Moret et al. (2003) who noticed that, with respect to the oil obtained by solvent extraction from the same olive paste, oil obtained by centrifugation had a considerably lower MOSH content, and that less than 25% of the MOSH present in olives was transferred into the virgin oil.

This provides insight into the extractability of MOSH, which are firmly included in solids poorly accessible by oil. Most of the MOSH contained in olives remains in the pomace and will be later extracted with solvent and concentrated into the residual oil giving olive pomace oil with high contamination levels (around 150-250 mg/kg).

377 Fig. 2 shows, for 3 different olive samples (namely samples OF3B, OF5C and OF6), the LC-GC

traces of the oils extracted by physical means (on the left) and with solvent (on the right).



Fig. 2. MOSH profiles of the oil extracted by by physical mean (on the left) and by solvent (on the right) from the same
olive fruit samples

382

379

As visible from the traces of Fig. 2, MOSH resulted enriched in the oil extracted with solvent. The sharp peaks on the top of the MOSH hump mostly represent endogenous *n*-alkanes (*n*-C21-C35), with a prevalence of odd carbon number compounds, and were subtracted from the total area.

All samples, independently on the origin, presented variable amounts of an unresolved complex mixture of hydrocarbons ranging from about n-C20 to n-C38-40 (beyond n-C40 for highly contaminated samples) and centred around n-C27. Such contamination, forming a Gaussian-like

hump, is compatible with that of environmental origin originated by engine lubricating oils from motor vehicle exhausts, observed by Neukom et al. (2002) in plant materials and different edible oils, as well as in air (PM10) and in soil. Nevertheless, based on the results obtained by the present research, we can conclude that further research (on a larger scale) is needed to confirm if the contamination found in olive fruits is all related to peculiar and specific environmental contamination/conditions or if, as hypothesized by some authors (Gómez-Coca et *al.*, 2016) it is, at least partially, of biogenic origin. Three samples had also a little hump ranging from n-C13 to n-C21, centred on n-C17, but, as visible from the traces, MOSH enrichment in solvent extracted oil regarded only the hump centred around n-C27.

399

400 3.2.3. Virgin Olive Oils from the market

401 Table 4 reportes MOSH concentrations (mg/kg oil) found in commercial extra virgin olive oils. The

402 results are divided into three different ranges of volatility (namely MOSH from C_{10} to C_{16} , from C_{16}

403 to C_{35} , and MOSH> C_{35}) and total MOSH. MOAH data are not reported since they were all under

404 the quantification limit (around 1 mg/kg).

405

406 **Table 4.**

407 MOSH concentrations (mg/kg oil) in extra virgin olive oils from the market

	EVOO1	EVOO2	EVOO3	EVOO4	EVOO5
MOSH C10-16	1.6	2.4	2.8	3.9	4.3
MOSH C16-35	8.7	12.3	6.6	10.4	27.0
MOSH >C35	1.2	3.5	0.8	3.3	6.7
MOSH TOT	11.5	18.2	10.3	17.6	38.0

408

409 Olive oils from the market had total MOSH content ranging from 10.3 to 38.0 mg/kg (on average 410 19.1 mg/kg), which were higher than those found in more than 40 extra virgin olive oils from the 411 Italian market which had on average 8 mg/kg of total MOSH (Moret et al., in preparation). The 412 most abundant compounds were those ranging from *n*-C16-35. Except for sample EVOO5, which 413 had a relatively high contamination, other samples did not exceeded the 20 mg/kg.

With respect to the oil extracted from the olive fruits with the Abencor, which contained an average 2.6 mg/kg of oil, MOSH amounts in bottled products from the market were about 8 times higher. These results confirm that most of the MOSH contamination found in the final product comes from other sources, and that contamination already present in the olive fruits account for less than 15% of the total contamination. This was also evident by comparing the LC-GC profiles of the oils 419 extracted with the Abencor (Fig. 2), with those of the bottled samples from the market which had420 different MOSH profiles (Fig. 3).



422



423 Fig. 3. MOSH/POSH profiles of the two extra virgin olive oils from the market and of the respective plastic caps 424

425 Particularly, the LC-GC trace of the highest contaminated oil sample (EVOO5) was characterised 426 by the presence of a large hump with a typical POSH profile in the first part of the trace (Fig. 3). It 427 was packaged into a tin can with a flexible plastic cap which was partially dipped into the oil. The 428 oil was probably contaminated with POSH migrated from the plastic cap, which was a HDPE.

It is important to underline that during routine GC-FID analysis, POSH are analyzed together with MOSH. They cannot be quantitatively separated from MOSH, but, in many cases, are clearly recognisable by their typical GC-FID profile characterised by the presence of clusters of peaks (Biedermann-Brem et al., 2012).

Fig. 3 shows the LC-GC traces of another oil sample (EVOO2) and of the corresponding bottle plastic cap (reducer insert) extracted with solvent. Also in this case, the LC-GC profile suggested a possible contribution from the plastic material. The other 3 samples did not showed evident migration of POSH from the plastic cap. To the best of our knowledge this is the first work reporting evident contamination due to migration of POSH from the plastic closure.

438

439 **4. Conclusions**

440

The quantitative assessment of hydrocarbon contaminants in vegetable oils and their human consumption patterns have recently become a focus of interest, and the methods here applied allows for their accurate and rapid determination. Low PAHs contents were found in both extra virgin olive oils from the market and in the oils extracted from the olives. Most of the contamination was already present in the olive fruits. Based on the data obtained, and in face of the present dietary habit of Tunisian people, extra virgin olive oils do not seem to be an important dietary source of PAHs.

448 Concerning MOSH, it is interesting to observe that all the olive samples, independently of different 449 exposure to environmental sources of contamination, contained variable amounts of hydrocarbons 450 forming a hump centred on *n*-C27. Only a part (about 25%) of these hydrocarbons were found in 451 the oil extracted by physical mean, while the rest remained in the solid residue. It remains unclear if 452 such contamination is completely of environmental origin. Background MOSH level in oil extracted 453 from a number of olive samples from different sites in Tunisia, was evaluated. The knowledge of such data is of great interest also in view of establishing recommended limits for MOH in extra 454 455 virgin olive oil. A new contamination source (POSH migrated from the plastic closure) was 456 evidenced in some bottled oils.

457

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459

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462

463 **4. References**

464

- 465 Barp, L., Purcaro, G., Moret, S., & Conte, L. S. (2013). A high-sample-throughput LC-GC method
- 466 for mineral oil determination. *Journal of Separation Science*, *36*, 3135–3139.
- 467 Barp, L., Kornauth, C., Wuerger, T., Rudas, M., Biedermann, M., Reiner, A., Concin, N., & Grob,
- 468 K. (2014). Mineral oil in human tissues, Part I: concentrations and molecular mass distributions.
- 469 Food Chemical Toxicology, 72, 312-21.
- 470 Brühl, L. (2016). Occurrence, determination, and assessment of mineral oils in oilseeds and 471 vegetable oils. *European Journal of Lipid Science and Technology*, *118*, 361–372.
- 472 Biedermann, M., Fieseler, K., & Grob, K. (2009). Aromatic hydrocarbons of mineral oil origin in
- 473 foods: Method for determining the total concentration and first results. *Journal of the Agricultural*474 *and Food Chemistry*, *57*, 8711–8721.
- Biedermann-Brem, S., Kasprick, N., Simat, T, & Grob, K. (2012). Migration of polyolefin
 oligomeric saturated hydrocarbons (POSH) into food. *Food Additives and Contaminants: Part A*,
 29, 449-460.
- 478 Cejpek, K., Hajslova, J., Kocourek, V., Tomaniová, M., & Cmolík, J. (1998). Changes in PAH
 479 levels during production of rapeseed oil. *Food Additives and Contaminants*, 15(5), 563-574.
- 480 European Commission (2006). Commission Regulation (EC) No. 1881/2006 of 19 December 2006
- 481 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European482 Union, L 364/5.
- European Commission (2011). Commission Regulation (EU) No. 835/2011 of 19 August 2011
 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic
 hydrocarbons in foodstuffs. Official Journal of the European Union, L 215/4.
- 486 EFSA (2008). Scientific Opinion of the Panel on Contaminants in the Food Chain on Polycyclic
 487 Aromatic Hydrocarbons in Food. *The EFSA Journal*, 724, 1-114.
- 488 EFSA (2012). Scientific Opinion of the Panel on Contaminants in the Food Chain on mineral oil
- 489 hydrocarbons in food. *EFSA Journal*, 10(6), 2704 (185 pp.).

- 490 Fismes, J., Perrin-Ganier, C., Empereur-Bissonnet, P., & Morel, J.L. (2002). Soil-to-root transfer
 491 and translocation of polycyclic aromatic hydrocarbons by vegetables grown on industrial
 492 contaminated soils. *Journal of Environmental Quality*, *31*, 1649-1656.
- 493 Fromberg, A., Hojgard, A., & Duedahl-Olesen, L. (2007). Analysis of polycyclic aromatic
 494 hydrocarbons in vegetable oils combining gel permeation chromatography with solid-phase
 495 extraction clean-up. *Food Additives and Contaminants*, 24, 758–767.
- Gharbi, I., Issaoui, M., Mehri, S, Cheraief, I., Sifi, S., & Hammami, M. (2015). Agronomic and
 Technological Factors Affecting Tunisian Olive Oil Quality. *Agricultural Sciences*, *6*, 513-526.
- 498 Gómez-Coca, R.B., Pérez-Camino Mdel, C⁻, & Moreda, W. (2016). Saturated hydrocarbon content
- 499 in olive fruits and crude olive pomace oils. *Food Additives and Contaminants: Part A.*, 33, 391-402.
- 500 Issaoui, M., Dabbou, S., Brahmi, F., Ben Hassine, K., Hajayej Ellouze, M., & Hammami, M.
- 501 (2009). Effect of extraction systems and cultivar on the quality of virgin olive oils. *International*502 *Journal of Food Science and Technology*, 44, 1713–1720.
- 503 Kipopoulou, A.M, Manoli, E., & Samara, C. (1999). Bioconcentration of polycyclic aromatic
- 504 hydrocarbons in vegetables grown in an industrial area. *Environmental Pollution*, *106*, 369-380.
- 505 Lacoste, F. (2016). http://www.iterg.com/IMG/pdf/dgf_lacoste_mineral_oil__2016.03.10_vf.pdf
- 506 Luchetti, F. (2002). Importance and future of olive oil in the world market- An introduction to olive
- 507 oil. European Journal of Lipid Science and Technology, 104, 559-563.
- 508 Moret, S., & Conte, L.S. (2000). Polycyclic aromatic hydrocarbons in edible fats and oils: 509 occurrence and analytical methods. *Journal of Chromatography A*, 882 (1-2), 245–253.
- 510 Moret, S., Dudine, A., & Conte, L.S. (2000). Processing effect on the polyaromatic hydrocarbon
- 511 content of grapeseed oil. Journal of the American Oil Chemists' Society, 77, 1289-1292.
- 512 Moret, S., & Conte, L.S. (2002). A rapid method for polycyclic aromatic hydrocarbon 513 determination in vegetable oil. *Journal of Separation Science*, *25*, 96-100.
- 514 Moret, S., Populin, T., Conte, L.S., Grob, K., & Neukom, H.P. (2003). Occurrence of C15-C45
- 515 mineral paraffins in olives and olive oils. Food Additives and Contaminants, 20, 417-426

- 516 Moret, S., Purcaro, G., & Conte, L.S. (2007). Polycyclic aromatic hydrocarbon (PAH) content of
- 517 soil and olives collected in areas contaminated with creosote released from old railway ties. *Science*
- 518 of the Total Environment, 386, 1-8.
- 519 Moret, S., Populin, T., & Conte, L. S. (2009). La contaminazione degli oli vegetali con oli minerali.
- 520 La Rivista Italiana delle Sostanze Grasse, 84, 3–14.
- 521 Moret, S., Populin, T., & Conte, L.S. (2010). Mineral paraffins in olives and olive oils. In Oxford
- Academic press, Olives and olive oil in health and disease prevention (pp. 499-506). Oxford :
 Elsevier Inc. ISBN : 978-0-12-374420-3
- 524 OfficeNationaldel'huile.(2015).
- 525 <u>http://www.onh.com.tn/index.php/fr/commercialisation/positionnement-sur-le-marche-mondial</u>
- 526 Neukom, H.-P., Grob, K., Biedermann, M., & Noti, A. (2002). Food contamination by C20–C50
- 527 mineral paraffins from the atmosphere. *Atmospheric Environment*, *36*, 4839–4847.
- 528 Poster, D.L., Schantz, M.M., Sander, L.C., & Wise S.A. (2006). Analysis of polycyclic aromatic
- 529 hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic
- 530 (GC) methods. Analytical and Bioanalytical. Chemistry, 386, 859-881.
- 531 Purcaro, G., Moret, S., & Barp, L. (2016). Determination of hydrocarbon contamination in foods. A
 532 review. *Analytical Methods*, 8, 5755-5772.
- 533 Rodríguez-Acuña, R., Pérez-Camino, M.C., Cert, A., & Moreda, W. (2008). Sources of
- 534 contamination by polycyclic aromatic hydrocarbons in Spanish virgin olive oils. Food Additives
- 535 *and Contaminants: Part A*, 25, 115–122.
- 536 Teixeira, V.H., Casal, S., & Oliveira, M.B.P.P. (2007). PAHs content in sunflower, soybean and
- 537 virgin olive oils: Evaluation in commercial samples and during refining process. Food Chemistry,
- 538 *104*, 106–112.

Table 1

Sample code	Site location	Municipality inhabitants*	Type of site	Distance from road/ vehicular traffic	Use of pesticide
OF1	Teboulba-Monastir	37485	Semi-rural	150 m/ medium	no
OF2	Sousse (centre)	221530	Urban	1-5 m/ high	no
OF3A	Monastir (centre)	93306	Urban	1-5 m/ high	no
OF3B	Monastir (centre)	93306	Urban	100 m/ high	no
OF4	Hammam Sousse	42937	Semi-rural	200 m/ medium	yes
OF5A	Bekalta-Monastir	17850	Rural	1-5 m / low	yes
OF5B	Bekalta-Monastir	17850	Rural	200 m/ low	yes
OF5C	Bekalta-Monastir	17850	Rural	400 m/ low	yes
OF6	Moknine-Monastir	57111	Semi-rural	300 m / medium	no
OF7	Menzel-Kamel-Monastir	8432	Rural	300 m / low	no
OF8	Kasserine	83534	Semi-rural	>300 m / low	yes

Characteristics of the sampling sites

* 2014 Tunisian census data

Table 2

PAH concentration (μ g/kg) in extra virgin olive oils from the market and in oils extracted from olives by physical mean

	F	Ра	Α	FI	Ρ	BaA	Ch	BbF	BkF	BaP	DBahA	BghiP	IP	light PAH	PAH4	PAH8
EVOO1	0.5	8.2	0.2	4.8	2.2	tr	0.7	0.1	0.1	tr	tr	0.1	tr	15.9	0.7	1.0
EVOO2	0.2	2.8	tr	1.7	0.8	tr	0.2	tr	tr	tr	tr	0.1	0.1	5.5	0.2	0.4
EVOO3	0.3	10.6	0.1	8.0	3.4	tr	0.5	0.1	0.1	tr	0.1	tr	0.2	22.4	0.5	0.9
EVOO4	0.1	8.4	tr	8.9	2.6	tr	0.3	0.1	0.1	tr	0.1	0.1	0.1	20.1	0.4	0.8
EVOO5	0.5	7.8	0.2	7.1	3.0	tr	0.5	0.1	0.1	tr	tr	tr	0.2	18.6	0.6	0.8
OF1	tr	18.4	0.5	4.7	7.5	0.1	0.9	0.2	0.1	tr	tr	0.1	tr	31.1	1.3	1.4
OF2	0.4	11.8	0.4	6.8	3.1	0.1	tr	0.4	tr	tr	tr	tr	tr	22.5	0.5	0.5
OF3A	1.5	16.5	0.7	10.0	6.7	0.2	1.2	0.5	0.1	tr	0.1	0.1	0.1	35.4	1.9	2.3
OF3B	0.6	8.4	0.1	6.2	2.6	0.1	0.6	0.1	0.1	tr	tr	0.1	tr	17.9	0.8	1.0
OF4	0.1	2.6	tr	1.8	0.8	tr	0.1	0.1	tr	tr	tr	tr	tr	5.4	0.2	0.2
OF5A	1.7	10.5	0.5	4.4	4.5	tr	0.1	0.4	0.1	tr	0.1	tr	tr	21.7	0.5	0.6
OF5B	0.5	4.2	0.2	1.6	1.8	tr	tr	0.4	tr	tr	tr	tr	tr	8.3	0.4	0.4
OF5C	0.6	5.4	0.2	1.6	2.2	tr	0.6	0.1	tr	tr	tr	tr	0.1	10.0	0.7	0.9
OF6	0.3	3.2	0.1	1.2	1.5	tr	0.3	0.2	tr	tr	0.1	0.1	0.1	6.3	0.5	0.8
OF7	1.0	6.2	0.2	2.4	2.2	0.1	0.6	0.2	0.1	tr	tr	0.1	0.1	12.0	0.9	1.2
OF8	0.5	4.6	0.2	2.0	2.0	0.1	0.2	0.1	tr	tr	tr	0.1	0.1	9.3	0.4	0.5

EVOO, extra virgin olive oil; OF, olive fruits; tr, trace (<0.05 μ g/kg)

Table 3.

MOSH content (mg/kg) of the oil oil extracted from olives both by physical mean (Abencor and with solvent)

Oil extraction	OF1	OF2	OF3A	OF3B	OF5A	OF5B	OF5C	OF6	OF7
Abencor	1.4	2.4	3.0	1.7	1.8	0.0	4.2	3.6	1.6
Solvent (MAE)	3.9	10.5	16.6	5.4	7.8	2.3	17.0	12.0	3.5
Ratio	2.9	4.4	5.5	3.2	4.3		4.0	3.4	2.2

Table 4.

MOSH concentrations (mg/kg oil) in extra virgin olive oils from the market

	EVOO1	EVOO2	EVOO3	EVOO4	EVOO5
MOSH C10-16	1.6	2.4	2.8	3.9	4.3
MOSH C16-35	8.7	12.3	6.6	10.4	27.0
MOSH >C35	1.2	3.5	0.8	3.3	6.7
MOSH TOT	11.5	18.2	10.3	17.6	38.0

Figure

Fig. 1





Figure

Fig. 3

