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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.



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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We confirm that the paper is unpublished and has not been submitted for publication elsewhere.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 <http://www.break-thru.com/product/break-thru/Documents/Brochure-Oil-Enhancers-EN-web.pdf>)

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 from 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 polycyclic 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 (repeatability 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 abbreviations (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 representative 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 preceded 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 µg/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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24

25 **Abstract**

26

27 The present paper investigated on the presence of some hydrocarbon contaminants, namely
28 polycyclic aromatic hydrocarbons (PAHs), mineral oil hydrocarbons (MOH) comprising saturated
29 (MOSH) and aromatic (MOAH) compounds, and polyolefin oligomeric saturated hydrocarbons
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
36 levels never exceeding the EU legal limits. All olive samples showed similar MOSH profiles, but
37 not clear correlation between the variable contamination levels and considered sources of
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.

44

45

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

48

49 **1. Introduction**

50

51 Extra virgin olive oil is a staple food largely consumed in the Mediterranean Countries. Its benefits
52 to health have prompted an increased demand worldwide (Luchetti, 2002). In Tunisia, olive oil
53 production plays an important role in the agronomy and economy (Gharbi, 2015). Accounting for
54 more than 4% of the world olive oil production, Tunisia is holding an important position in the
55 olive oil market; it exports about 75% of its production and is ranked as the second largest exporter
56 after the European Union with an average of 115 000 tons per year over the last five years (ONH,
57 2015).

58 Edible oils can be contaminated with polycyclic aromatic hydrocarbons (PAHs) and mineral oil
59 hydrocarbons (MOH) which are both environmental and processing contaminants (EFSA, 2008,
60 EFSA, 2012). Due to their similarity to MOH, some concerns also derive by possible migration of
61 polyolefin oligomeric saturated hydrocarbons (POSH) (Biedermann-Brem, Kasprick, Simat &
62 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
64 temperature during incomplete combustion of organic matter, mainly combustion of fossil fuels,
65 motor vehicle exhausts and industrial emission. Forest fires, volcanoes or hydrothermal processes
66 are natural sources of PAHs (Poster, Schantz, Sander & Wise, 2006). Only heavy PAHs (4-6
67 benzene rings) are genotoxics and carcinogenic, while light PAHs (2-4 benzene rings) may act as
68 synergists (EFSA, 2008). The necessity for a legislation arose in 2001 after the finding of a highly
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
73 not a suitable marker, and suggested to use PAH8, sum of benz[a]anthracene (BaA), chrysene (Ch),
74 benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, dibenz[a,h]anthracene (DBahA),

75 benzo[g,h,i]perylene (BghiP) and indeno [1, 2, 3-cd] pyrene (IP), or PAH4 (sum of BaA, Ch, BbF,
76 and BaP). Regulation 835/2011 (European Commission 2011) fixed a limit of 2 mg/kg for BaP and
77 10 mg/kg for PAH4 in vegetable oils and fats. No legal limit has been established for total PAHs,
78 even though the German Society of Fat Science considered a maximum acceptable level of 25
79 µg/kg for the sum of 16 PAHs (PAH8 plus 8 light PAHs) indicated as priority by the Environmental
80 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
85 represent less than 1-5% of total hydrocarbons). Neukom, Grob, Biedermann & Noti (2002),
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
88 heating and diesel oils, engine lubricating oils, and road tar debris.

89 Depending on their composition and molecular range, mineral oil fractions may have different
90 bioaccumulation potential and toxicity. MOSH from *n*-C16 to *n*-C35 accumulate in several tissues
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)
96 on the presence of MOSH and MOAH in food, as consequence of migration from recycled
97 paperboard, established a limit of 2 mg/kg for MOSH *n*-C20-35 and 0.5 mg/kg for MOAH *n*-C10-
98 35, and, recently, very restrictive limits have been requested in extra virgin olive oil by large scale
99 distribution in German.

100 Virgin olive oil is extracted from the olive fruit exclusively by mechanical processes.
101 Environmental contamination generally proceeds via atmospheric deposition on growing crops.
102 Thus, oil extracted from olive fruits is expected to contain a background contamination reflecting
103 the contamination of the environment where the olive grows. High PAH concentrations were
104 occasionally reported in oil obtained from olive collected in an olive-grove in a rural area with piles
105 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
111 PAH load (Moret, Dudine & Conte, 2000). Storage of pomace under inadequate conditions
112 contributes to increase the MOH load (Moret, Populin, Conte, Grob, Neukom, 2003). Use of
113 mineral oil based pesticide, mechanical harvesting, contact with lubricatings used for maintenance
114 of extraction plant, transport in jute bags, are some of the possible sources of contamination for
115 MOH (Moret & Conte, 2000; Brühl, 2016).

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

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

125

126 **2. Material and Methods**

127

128 *2.1. Reagents and standards*

129 All solvents used (Sigma-Aldrich, Milan, Italy) were of HPLC grade. Ultra pure water was obtained
130 with a MilliQ filter system (Millipore, Bedford, MA, USA). To avoid contamination during sample
131 preparation, all the glassware was carefully washed and rinsed with clean solvents (acetone and
132 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 µg/mL), phenanthrene (Pa) (100 µg/mL), DBahA (200 µg/mL), IP (100 µg/mL)
138 and pyrene (P) (100 µg/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-
142 methyl-naphthalene (1-MN, 0.30 mg/mL), 2-methyl-naphthalene (2-MN, 0.30 mg/mL), tritert-
143 butylbenzene (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*

148 Five extra virgin olive oil samples (different brands) were randomly purchased from the retail
149 market. Despite the low number of samples, they covered about 50% of commercial extra virgin
150 olive oils brands marketed in Tunisia. Such oils are produced by mills which export most of their
151 products and sell only small quantities in the local market. On the other hand, Tunisian people

152 mostly consumes oil produced from olives grown in their family olive groves. Olive samples (11)
 153 were collected by hand in different sites during the crop season 2014/2015. They were all from the
 154 olive variety Chemlali, which is the most diffused in Tunisia, covering more than 60% of the total
 155 Tunisian olive tree growing area.

156 Table 1 resumes some characteristics of the sampling sites (information on the area, number of
 157 inhabitants, distance from the main road and level of vehicular traffic). To facilitate a rough
 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
 165 solubility, they are often used as carrier to deliver the actives to plants or pests.

166 **Table 1.**

167 Characteristics of the sampling sites

| <i>Sample code</i> | <i>Site location</i> | <i>Municipality inhabitants*</i> | <i>Type of site</i> | <i>Distance from road/ vehicular traffic</i> | <i>Use of pesticide</i> |
|--------------------|-----------------------|----------------------------------|---------------------|--|-------------------------|
| 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 Tunisian census data

168

169 2.3. PAH analysis

170 2.3.1. Olive oil extraction

171 Oil extraction was performed as described by Issaoui, Dabbou, Brahmi, Ben Hassine, Hajajej
172 Ellouze & Hammami (2009), with an Abencor extractor composed by a hammer crusher, a mixer
173 and a pulpe centrifuge. The olive paste obtained after crushing about 2 kg of olives was mixed into
174 a mixing jar for 30-45 min with the addition of 100 mL of warm water. Mixing allows the
175 combination of small droplets of oil, that are released by the milling process, into larger ones that
176 can be more easily separated. This is followed by centrifugation (for 3-4 min at 3500 rpm) to
177 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, $2.5 \text{ g} \pm 0.001 \text{ g}$ of oil were weighted into a 10 mL volumetric flask and diluted to volume
181 with *n*-hexane); then 1.0 mL of the sample solution was loaded onto a 5 g silica SPE cartridge
182 (Mega Bond Elut, 20 mL, Varian, Palo Alto, CA, USA) previously washed with 20 mL of
183 dichloromethane, dried completely by means of vacuum, and conditioned with 20 mL of *n*-hexane.
184 PAHs were eluted with a mixture of *n*-hexane and dichloromethane 70:30 (v/v). The first 8 mL of
185 eluate was discharged, and the following 8 mL fraction, containing the PAH fraction, was collected
186 in a conical-shaped vial. The collected fraction was concentrated under a nitrogen stream to about
187 30 μL , 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

191 PAH determination was carried out with a Varian model 9010 HLPC gradient pump equipped with
192 a Rheodyne 7161 injector with a 20 μL loop. The column was a C18 reversed phase, 250x3 mm ID,
193 5 μm particle size (Supelcosil LC-PAH, Supelco) thermostatted at 38 °C with a column heater
194 (model L 7350, LaChrom, Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile
195 and water at a flow rate of 1 mL/min. The gradient elution program started with 40% acetonitrile
196 (isocratic for 5 min), going to 100% of acetonitrile in 40 min. PAHs detection was carried out with

197 a Jasco spectrofluorometer (model FP 1520, Cremalla, Como, Italy); wavelength changes and
198 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 μ L 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
212 20 min at -20 °C to facilitate phase separation between the ethanol/water phase and hexane. The
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

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

220 2.4.3. LC-GC-FID analysis

221 Olive oil samples were analysed according to the method described by Biedermann Fieseler & Grob
222 (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-
224 GC analysis: 300 mg of the oil were weighted into an autosampler vial, added with 10 μ L of the
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 preceded by
227 epoxidation (Biedermann Fieseler & Grob, 2009). The LC–GC apparatus (LC–GC 9000,
228 Brechbühler, Zurich, Switzerland) consisted of a PAL LHS2-xt Combi PAL autosampler (Zwingen,
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 \times 2.1 mm i.d Lichrospher Si 60, 5 μ m (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 μ L/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
236 the solvent vapour exit (SVE) and a 15 m \times 0.25 mm i.d. separation column coated with a 0.15 μ m
237 film of PS-255 (1% vinyl, 99% methylpolysiloxane) (Mega, Legnano, Italy). A rapid oven gradient
238 (40 $^{\circ}$ C/min) starting from 55 up to 350 $^{\circ}$ C was used for GC analysis (Barp, Purcaro, Moret &
239 Conte, 2013). The FID and the SVE were heated at 360 and 140 $^{\circ}$ 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).
242 The MOSH area was determined by the integration of the whole hump of largely unresolved peaks,
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
247 to estimate the regression lines. Regression coefficients (R^2) of 0.999 were obtained in both cases.
248 By running a *t*-test at the 5% significance level it was demonstrated that the slope of the regression

249 lines obtained in the matrix did not differ significantly from that obtained in the solvent. When
250 exploiting the maximum 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).

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

257

258

259 **3. Results and discussions**

260

261 *3.1. Polycyclic aromatic hydrocarbons*

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

265

266 **Table 2.**

267 PAH concentration ($\mu\text{g}/\text{kg}$) in extra virgin olive oils from the market and in oils extracted from
268 olives by physical mean

| | F | Pa | A | Fl | P | 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 | tr | 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

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

273 PAH contamination can occur either directly during oil processing in the mill, or indirectly, due to
 274 the olive skin contamination by environmental sources (Fromberg, Hojgard & Duedahl-Olesen,
 275 2007; Rodríguez-Acuna, Pérez-Camino, Cert & Moreda, 2008) as result of the deposition of
 276 particle-bound compounds and, more important, of retention of vapour phase PAHs on the waxy
 277 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 µg/kg), while BaP was below the quantification limit of 0.05 µg/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 µg/kg, and between 0.2 and 2.3 µg/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.
 286 The most abundant PAH was Pa followed by Fl, P and F. These results are in accordance with those
 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
299 PAH4. A similar trend, was observed for samples OF5A (21.7 µg/kg of light PAHs and 0.5 µg/kg
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
303 that variables possibly affecting PAH concentration in olives (i.e. wind direction, rain and other
304 meteorological 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,
307 Empereur-Bissonnet & Morel, 2002; Kipopoulou, Manoli & Samara, 1999).

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

314 the average consumption of olive oil by its mean PAH concentration. The calculated intake for
315 PAH4 and PAH8 were respectively 0.08 and 0.13 ng/kg body weight (b.w.) per day (assuming a
316 reference person of 60 kg b.w.), which accounts for less than 1% of the total dietary intake
317 calculated by EFSA (2008).

318

319 3.2. Mineral oil hydrocarbons (MOH)

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

327

328 3.2.2. Olives

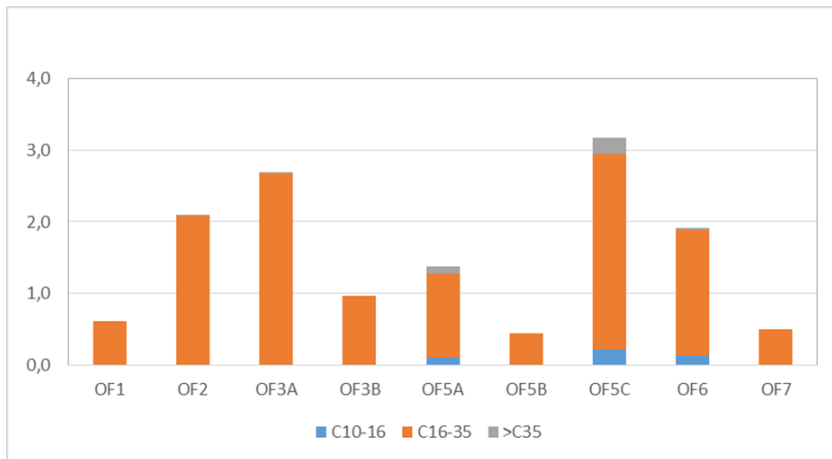
329 3.2.2.1 Oil extraction

330 Some preliminary trials were carried out (in triplicate) to find optimal conditions for rapid
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
333 underwent microwave assisted extraction (120 °C x 20 min) using hexane/ethanol (1:1 v/v) or
334 hexane as extraction solvent. In the latter case, the sample was previously dried at 60 °C over the
335 weekend and a Carboflon bar (secondary microwave absorber) was added to heat the hexane.
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
338 MOSH extraction, the hexane/ethanol mixture (which allowed to avoid the drying step) was chosen
339 for sample extraction.

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

341 Fig. 1 reports the MOSH content expressed in mg/kg of fresh olive. Total MOSH levels in olive
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

345



346

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

348

349 Although 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
354 close to a trafficated road), but also samples OF5C (3.2 mg/kg olives) and sample OF6 (1.9 mg/kg
355 olives), both from rural or semi-rural areas far from the road. In the case of sample OF5 we cannot
356 exclude a contribution due to the use of mineral oil based pesticides (which usually are not
357 uniformly spread in the olive grove).

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

360

361 **Table 3**

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 |
|-----------------------|------------|------------|-------------|-------------|-------------|-------------|-------------|------------|------------|
| <i>Abencor</i> | 1.4 | 2.4 | 3.0 | 1.7 | 1.8 | 0.0 | 4.2 | 3.6 | 1.6 |
| <i>Solvent (MAE)</i> | 3.9 | 10.5 | 16.6 | 5.4 | 7.8 | 2.3 | 17.0 | 12.0 | 3.5 |
| <i>Ratio</i> | 2.9 | 4.4 | 5.5 | 3.2 | 4.3 | | 4.0 | 3.4 | 2.2 |

363

364 MOSH level found in extra virgin olive oils extracted with the Abencor (on average 2.6 mg/kg oil)

365 represents the background level due to the pre-existing contamination already present in the olives.

366 Olive processing in the oil mill, as well as migration of POSH from plastic food contact materials,

367 could contribute to increase the contamination in the final product.

368 Mean MOSH concentration in oils extrated with solvent (11.1 mg kg/oil), was about 4 times higher

369 than in oils extracted by physical mean. This well agree with what reported by Moret et al. (2003)

370 who noticed that, with respect to the oil obtained by solvent extraction from the same olive paste,

371 oil obtained by centrifugation had a considerably lower MOSH content, and that less than 25% of

372 the MOSH present in olives was transferred into the virgin oil.

373 This provides insight into the extractability of MOSH, which are firmly included in solids poorly

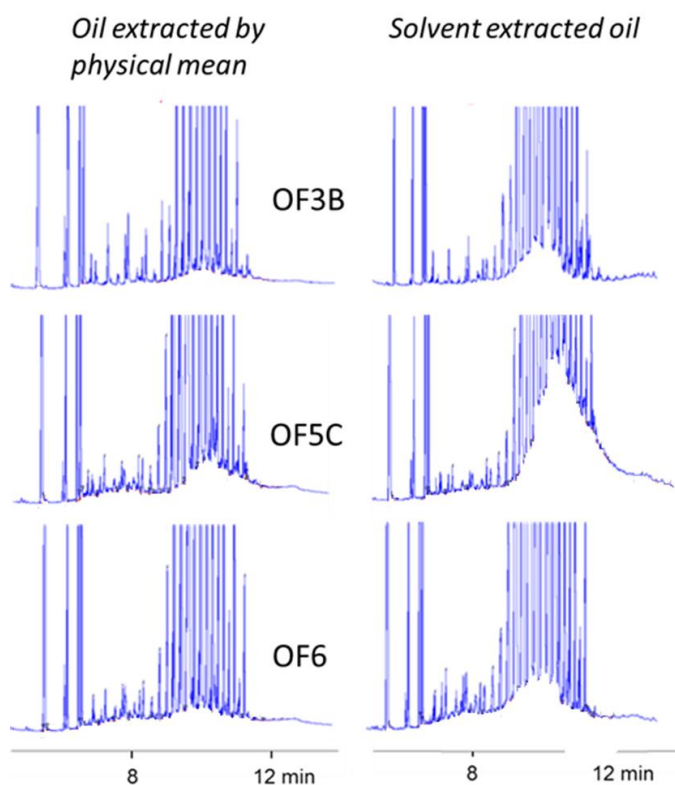
374 accessible by oil. Most of the MOSH contained in olives remains in the pomace and will be later

375 extracted with solvent and concentrated into the residual oil giving olive pomace oil with high

376 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

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



379

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

382

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

386 All samples, independently on the origin, presented variable amounts of an unresolved complex
 387 mixture of hydrocarbons ranging from about *n*-C20 to *n*-C38-40 (beyond *n*-C40 for highly
 388 contaminated samples) and centred around *n*-C27. Such contamination, forming a Gaussian-like
 389 hump, is compatible with that of environmental origin originated by engine lubricating oils from
 390 motor vehicle exhausts, observed by Neukom et al. (2002) in plant materials and different edible
 391 oils, as well as in air (PM10) and in soil. Nevertheless, based on the results obtained by the present
 392 research, we can conclude that further research (on a larger scale) is needed to confirm if the
 393 contamination found in olive fruits is all related to peculiar and specific environmental
 394 contamination/conditions or if, as hypothesized by some authors (Gómez-Coca et al., 2016) it is, at
 395 least partially, of biogenic origin.

396 Three samples had also a little hump ranging from *n*-C13 to *n*-C21, centred on *n*-C17, but, as
397 visible from the traces, MOSH enrichment in solvent extracted oil regarded only the hump centred
398 around *n*-C27.

399

400 3.2.3. Virgin Olive Oils from the market

401 Table 4 reports 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 C₁₀ to C₁₆, from C₁₆
403 to C₃₅, and MOSH>C₃₅) 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

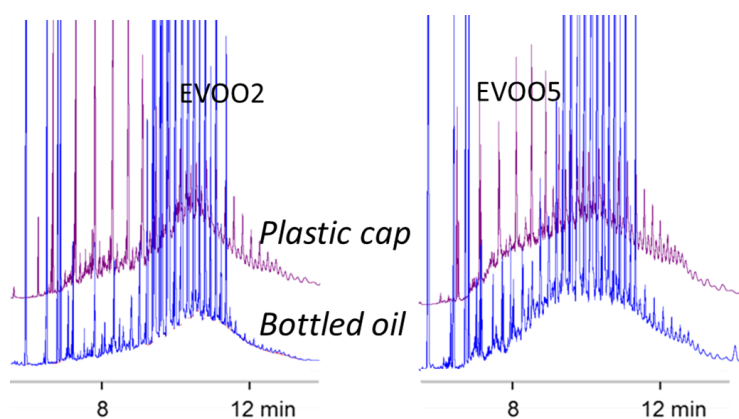
409 Olive oils from the market had total MOSH content ranging from 10.3 to 38.0 mg/kg (on average
410 19.1 mg/kg), which were higher than those found in more than 40 extra virgin olive oils from the
411 Italian market which had on average 8 mg/kg of total MOSH (Moret et al., in preparation). The
412 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.

414 With respect to the oil extracted from the olive fruits with the Abencor, which contained an average
415 2.6 mg/kg of oil, MOSH amounts in bottled products from the market were about 8 times higher.

416 These results confirm that most of the MOSH contamination found in the final product comes from
417 other sources, and that contamination already present in the olive fruits account for less than 15% of
418 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 had
420 different MOSH profiles (Fig. 3).

421



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.

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

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

438

439 **4. Conclusions**

440

441 The quantitative assessment of hydrocarbon contaminants in vegetable oils and their human
442 consumption patterns have recently become a focus of interest, and the methods here applied allows
443 for their accurate and rapid determination. Low PAHs contents were found in both extra virgin olive
444 oils from the market and in the oils extracted from the olives. Most of the contamination was
445 already present in the olive fruits. Based on the data obtained, and in face of the present dietary
446 habit of Tunisian people, extra virgin olive oils do not seem to be an important dietary source of
447 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
454 such data is of great interest also in view of establishing recommended limits for MOH in extra
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

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464

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Table 1

Characteristics of the sampling sites

| <i>Sample code</i> | <i>Site location</i> | <i>Municipality inhabitants*</i> | <i>Type of site</i> | <i>Distance from road/ vehicular traffic</i> | <i>Use of pesticide</i> |
|--------------------|-----------------------|----------------------------------|---------------------|--|-------------------------|
| 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 Tunisian census data

Table 2PAH concentration ($\mu\text{g}/\text{kg}$) in extra virgin olive oils from the market and in oils extracted from olives by physical mean

| | F | Pa | A | Fl | P | 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 ($<0.05 \mu\text{g}/\text{kg}$)

Table 3.

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

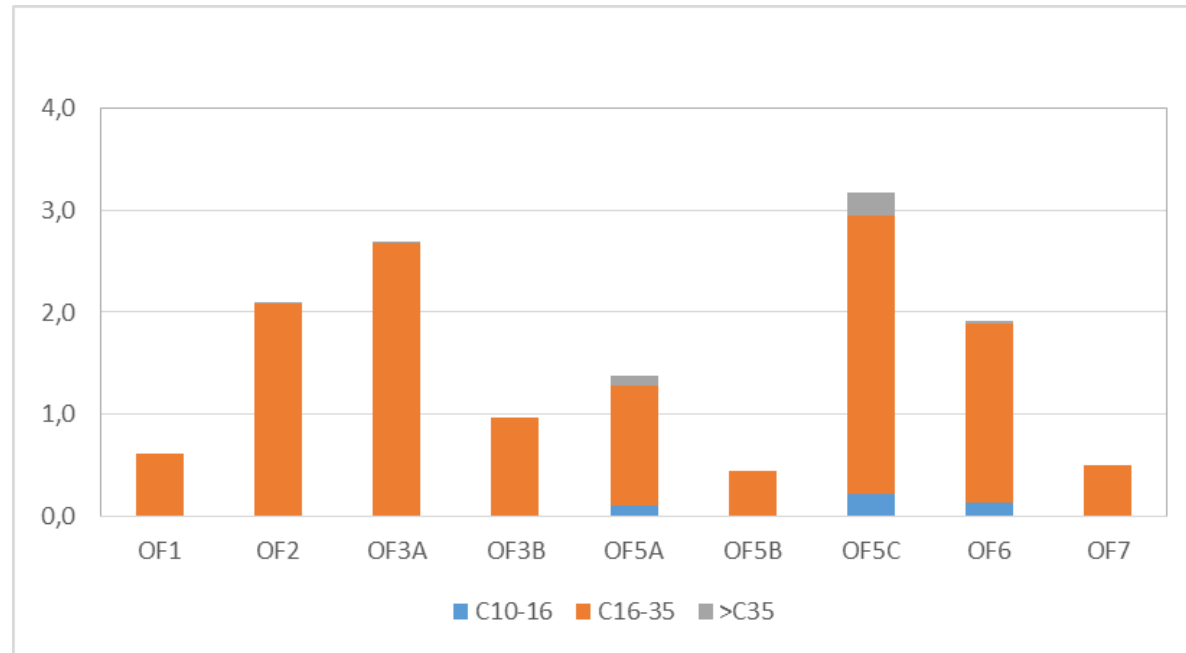
| Oil extraction | OF1 | OF2 | OF3A | OF3B | OF5A | OF5B | OF5C | OF6 | OF7 |
|-----------------------|------------|------------|-------------|-------------|-------------|-------------|-------------|------------|------------|
| <i>Abencor</i> | 1.4 | 2.4 | 3.0 | 1.7 | 1.8 | 0.0 | 4.2 | 3.6 | 1.6 |
| <i>Solvent (MAE)</i> | 3.9 | 10.5 | 16.6 | 5.4 | 7.8 | 2.3 | 17.0 | 12.0 | 3.5 |
| <i>Ratio</i> | 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 |

Fig. 1



Figure

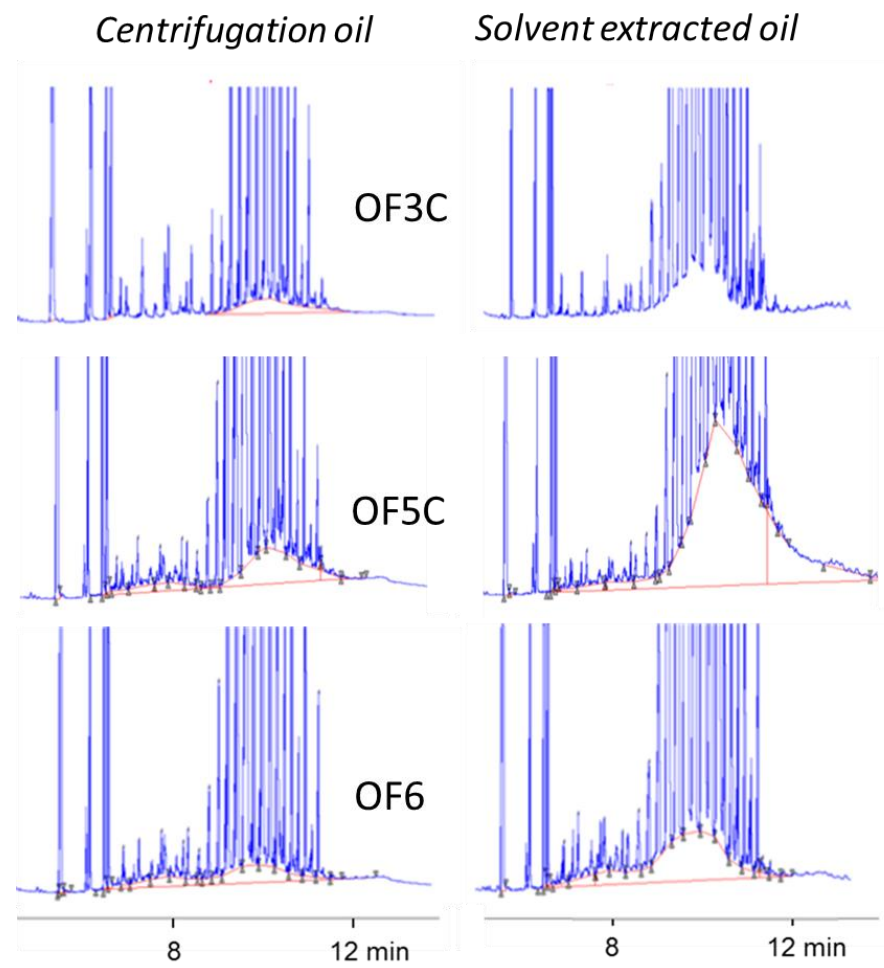


Fig. 3

