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Volatile profiles of extra virgin olive oil, olive pomace oil, soybean oil and palm oil in different heating conditions

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(Article begins on next page)

1 **Volatile profiles of extra virgin olive oil, olive pomace oil, soybean oil and palm**
2 **oil in different heating conditions**

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15

16 **ABSTRACT**

17 Extra virgin olive oil from Calabria Region (South Italy), pomace olive oil, palm oil and
18 soybean oil were heated at 180 and 220°C for 30, 60 and 120 mins. Emission of
19 volatile organic compounds produced during the heat treatment was evaluated by the
20 solid phase micro-extraction of the head space technique and analysed by gas
21 chromatography with mass spectrometric detector. The results after oil heating showed
22 a significant variation in the volatile composition with temperature and time increasing.
23 Twenty-five compounds have been identified, and alkanals, alkenals, and alkadienals
24 were the most represented classes. Results from volatile profile evolution suggest that
25 specific aldehyde compounds can be used as markers both for the evaluation of the
26 organoleptic properties and for the health characteristics of the studied oils.

27

28 **Keywords**

29 Deep-fried oil, volatile organic compounds, SPME, GC-MS.

30

31

32 1. Introduction

33 One of the many uses of an edible vegetable oil is in deep-frying processes in food
34 preparation. Deep-frying is one of the oldest cooking methods (Jaarin & Kamisah,
35 2012) which give a characteristic taste to the food. It is highly valued by the consumers
36 and easy and fast to do. When a vegetable oil is heated, changes in physical
37 appearance occur such as darkening in colour and increasing in viscosity due to the
38 alteration of the fatty acid composition (Rani et al., 2010). Heating causes some
39 chemical reactions in the oil such as oxidation, hydrolysis and polymerization (Choe &
40 Min, 2007). During these processes, many oxidative products such as hydroperoxides
41 and aldehydes are produced, which can be absorbed into the fried food (Choe & Min,
42 2006).

43 In Italy, one of the main agricultural crops is represented by the olive tree, mainly for
44 extra virgin olive oil (EVOO) production. The flavour in an olive oil is very important and
45 it was widely studied (Vichi et al., 2003; Cavalli et al., 2004; Baccouri et al., 2007;
46 Baccouri et al., 2008;). It is characterised by a large number of carbonyl substances,
47 which includes linear saturated and unsaturated aldehydes and alcohols, esters and
48 hydrocarbons (Flath et al., 1973; Esposto et al., 2009;). This composition can be
49 affected by geographical origin (Ouni et al., 2011), by ripening degree (Konavouras et
50 al., 2005) and by extractive processing method (Dabbou et al., 2012). Olive pomace is
51 the most important by-product in the olive oil extraction industry, in particular when the
52 three-phase technology is used (Nasopoulou et al., 2011). The three phases system
53 (oil, olive pomace and olive mill waste water) produces an olive pomace with a low
54 moisture content which facilitates the pomace olive oil (PO) solvent extraction by *n*-
55 hexane (Ciftci et al., 2009; Mele et al., 2018). Palm oil (P) and soybean oil (SO) are

56 rich in antioxidants such as tocopherols and tocotrienols (Kamisah et al., 2005;
57 Clemente & Cahoon, 2009) and are the most commonly used vegetable oils in the
58 household and industrial deep frying purposes. Each oil produces different volatile
59 emissions in different cooking methods (Peng et al., 2017). Many studies were
60 conducted on these and others vegetable oils about the variation in the chemical
61 composition during heating, in particular on its oxidative stability (Saad et al., 2007;
62 Maggio et al., 2011; Jaarin & Kamisah, 2012; Poyato et al., 2014; Qing et al., 2016;
63 Giuffrè et al., 2017a; Giuffrè et al., 2017b; Giuffrè et al., 2018). A minor number of
64 studies exist on their volatile profile after heating (Katragadda et al., 2010; Peng et al.,
65 2017).

66 The most rapid and accurate technique to study the volatile fraction of an oil is the
67 SPME (Song et al., 1997) introduced since 90s for the aroma analysis of various food
68 products (Arthur & Pawliszyn, 1990). It is based both on the capacity of the silica fibre
69 to absorb the analytes released in head space from the sample and to desorb the
70 same molecules in an instrument, such as gas chromatography with mass
71 spectrometric detector (GC-MS).

72 VOCs produced by cooking were also found to be an indoor pollutant both in
73 household and in food factory, in particular if the ventilation is not enough (Jones,
74 1999). Outdoor cooking is a problem in volatile organic compounds (VOCs) emission
75 even if lower than for indoor cooking (Edwards et al., 2017). Studies on Chinese
76 women have demonstrated that the exposure to fumes of cooked oils is associated
77 with the lung cancer (Zhao et al. 2018). For this reason is important to know the
78 emission composition in VOCs during oil frying.

79 The aim of this work was to study the effect of temperature and heating duration on the
80 volatile organic compound (VOC) composition of extra virgin olive oil, olive pomace oil,
81 soybean oil and palm oil.

82 **2. Materials and method**

83 **2.1. Materials**

84 **EVOO and PO** were produced in the Calabrian region (South of Italy) in the harvest
85 year 2016-2017. **SO and P** were purchased in a supermarket **EVOO** was obtained by a
86 mechanical extraction with a three phase apparatus. **PO, SO and P** were obtained by
87 solvent (*n*-hexane) extraction.

88 Nine 1L bottles of the each oil were randomly taken for analysis. After this, three
89 representative batches each one composed by the mixed content of nine bottles were
90 prepared for each oil to analyse VOCs in triplicate. At this point, from each batch were
91 prepared 7 glass pyrex beakers, containing 150 g oil each one; 6 out of 7 contained the
92 oil to be heated for the six different treatments: 180 °C (**30, 60** and 120 min), 220°C
93 (**30, 60** and 120 min) and 1 out of 7 contained the oil to be analysed as a fresh oil
94 (control). Beakers containing oils were placed on a heating plate and temperature was
95 adjusted and controlled by a thermometer.

96 **2.2. Solid phase micro-extraction of the head space (HS-SPME)**

97 The most rapid and accurate technique to study the volatile fraction of an oil is the HS-
98 SPME (Song et al., 1997) introduced since 90s for the aroma analysis of various food
99 products (Arthur & Pawliszyn, 1990). A SPME fibre coated with poly-dimethylsiloxane
100 (PDMS, 100 µm) from Supelco (Bellafonte, PA, USA) was used to absorb the VOC's
101 with the procedure described by Temime et al. (2006) modified as follow. A 10 g aliquot
102 of each heated and unheated (fresh) oil was placed in a 20 ml glass vial with septum

103 cup and provided with a magnetic stirrer. The fibre was conditioned before using at
104 270°C for 30 mins as recommended by the manufacturer. Sample was placed in a
105 thermostatic bath at 45°C in stirring (500 rpm). The fibre was inserted into the vial's HS.
106 Absorption was stopped after 20 mins and fibre was thermally desorbed for 5 mins in a
107 gas chromatographer in split-less mode. All SPME operations were conducted
108 manually.

109 **2.3. GC-MS analysis**

110 A GC Thermo Trace 1310 apparatus (Waltham, MA, USA) equipped with Single
111 Quadrupole Mass Spectrometer ISQ LT system and a fused-silica capillary columns
112 (30 m length, 0.25 mm i.d., 0.25 µm film thickness, Thermo Scientific, Waltham, MA,
113 USA), TG-5MS 5% phenyl phase was used for GC-MS analysis. The oven
114 temperature was programmed at 60°C held for 10 mins, then from 60°C to 220°C at
115 5°C/mins, and held isothermally at 220°C for 10 mins. Injector, MS transfer line and ion
116 source temperatures were respectively 250, 270 and 260°C. Mass range was from 45
117 to 500 atomic mass unit (amu). Gas carrier was helium at 1.5 mL/mins flow rate. The
118 tentative identification of the VOCs was based on the comparison of spectra with those
119 of NIST/EPA/NIH Mass spectral library Version 2.0.

120 **2.4. Statistical analyses**

121 Each sample was analysed in triplicate and volatile profiles aroma data were submitted
122 to analysis of variance (ANOVA) using SPSS 20.0 software for Windows (SPSS Inc.,
123 Chicago, IL, U.S.A.). Wherever F values were significant, Tukey's test was used to
124 separate the mean effects (different temperatures for each oil type). Significance was
125 defined at $P < 0.001$. Graphics were done using Excel 2010 software for Windows
126 (Microsoft Corp., Redmond, WA, USA).

127 **3. Results and discussion**

128 **3.1. Volatile organic compounds profile**

129 The highest number of compounds, i.e., twenty-one was found in EVOO (Table 1). A
130 similar result was obtained both by Temime et al. (2006) in Tunisian extra virgin olive
131 oils, in which were found twenty-five compounds and by Cavalli et al. (2004) in nine
132 virgin olive oils from France and Spain, in which were found a number of VOCs ranging
133 between 23 and 25. A lower number of compounds (twenty) was found in three
134 Algerian EVOOs (Cherfaoui et al., 2018). It has to be pointed out that other authors, in
135 different cultivars, have identified a greater number of compounds with the same
136 technique (Hammouda et al., 2017) or with a different technique (Lukić et al., 2019).

137 The E-2-hexenal has been found to be the most represented constituent (28.3% in
138 EVOO) (Table 1), in fact it is considered as a quality fresh-marker of an olive oil
139 (Cavalli et al., 2004), it gives the characteristic sensory properties of sweet, fragrant,
140 almond, green and leafy to the olive oil (Kanavouras et al., 2005). During heating a
141 very high significant decrease in E-2-hexenal content was observed to the lowest value
142 (1.7%) in the EVOO 220°C-120 min sample. The same decreasing trend in E-2-
143 hexenal was found in almost all the studied oils with the exception of PO 180°C-30 min
144 (Tables 1-4). The higher the heating temperature and the longer the heating duration,
145 the lower the E-2-hexenal content. Other compounds characterising the volatile profile
146 of EVOO were α -farnesene (15.3%), α -cubebene (14.4%) and nonanal (8.9%) (Table
147 1). They are characteristic for an EVOO composition and it was demonstrated to be
148 affected by the olive cultivar (Reboredo-Rodríguez et al., 2012) and the olive growing
149 area (Temime et al., 2006). Some aldehyde compounds such as Z-2-heptanal, E-2-
150 octenal, 2-nonenal and 2-decenal (Z), which were not in EVOO, were formed during

151 heating, due to oleic acid oxidation (Belitz et al., 2009), and could be used as indicators
152 of the oil heating treatment. The Z-2-decenal alkenal was not detected in EVOO,
153 however it was found in high quantity in all the heated samples, and increased
154 constantly with heating to 16.3% in EVOO 180°C-30 min and to 30.8% in EVOO
155 220°C-30 min (Table 1). Other minor compounds were not in fresh EVOO but they
156 were identified after heating: butyl-cyclopentane (found in EVOO heated at 220°C), Z-
157 2-heptenal, 1-heptanol, 1-octen-3-ol (in EVOO 220°C-60 min), 5-hepten-2-one, 6-
158 methyl- (2.0 and 2.2% in both EVOO heated at 180°C-60 min and 180°C-120 min), E-
159 2-octenal (2.5 and 2.6% in EVOO 180°C-120 min and 220°C-120min respectively), 1-
160 octanol (in highest quantity, 1.6%, in EVOO 220°C-60 min and in EVOO 220°C-120
161 min), 2-nonenal which was mainly produced at 220°C after 120 min heating. This was
162 due to the oxidation mechanism of the oil, accelerated by the heating (Fullana et al.,
163 2004; Lin & Liou, 2000), that causes a chemical primary oxidation and the consequent
164 production of hydroperoxides (Choe & Min, 2006), and its successive degradation to
165 different class of compounds (aldehyde, ketons, alcohols, etc.).

166 Aldehydes can possess toxic properties, in particular, many studies have been
167 conducted on E,E-2,4 decadienal (Chang et al., 2005; Chang & Lin, 2008; Halvorsen &
168 Blomhoff, 2011), considered either carcinogens or suspected carcinogens. It was
169 categorized into group 2A by the International Agency of Research on Cancer, which
170 estimated a daily limit in human diet to 5 mg/kg of body weight (Conklin et al., 2010;
171 Wang et al., 2008).

172 The major constituent detected in the PO volatile fraction was decanal (42.54%),
173 followed by E-2-hexenal (16.01%), 2-undecenal (15.70%) and nonanal (12.43%) (Table
174 2). In PO there was a smaller decrease in E-2-hexenal content than in EVOO, which

175 was lowest (7.76%) in PO180°C-120 min. Interesting was the highly significant
176 decrease in decanal in all the heated samples, which achieved a value of 2.24% in PO
177 220°C-120 min and which can be taken into account as the oil temperature stress
178 index parameter. A similar conclusion, but in reverse, can be done for Z-2-decenal
179 passed from 5.02% in PO to 21.67% in PO 220°C-120 min.

180 In P were found seven volatiles compounds: the highest was nonanal (32.75%) and the
181 lowest was Z-2-decenal (6.82%) (table 4). In P heated samples, heptanal, octanal and
182 decanal showed a highly significant decrease. These compounds passed respectively
183 from 14.41%, 13.12% and 10.84% to 1.37% (P 180°C-120 min), 1.92% (P 220°C-60
184 min and P 220°C-120 min) and 1.47% (P 220°C-60 min). Nine new compounds were
185 formed during P heating. The E,E-2,4-decadienal was the most represented in all the
186 heated samples, mainly in P180°C-120 min (32.35%), followed by Z-2-heptenal in P
187 180°C-30 min (8.68%) and E,E-2,4-decadienal in P 180°C-120 min (6.44%), (Table
188 3). The formation of these compounds is due to the oxidation after heating of the
189 unsaturated fatty acids: the higher the unsaturated fatty acid content, the higher the
190 aldehydes formation (Peng et al., 2017). This because poly-unsaturated fatty acids
191 such as linoleic and linolenic acids are more susceptible to oxidation because of
192 presence of multi double-bonds (Schauer et al., 2002). In fact, SO, considered a
193 linolenic acid-rich oil showed higher concentration in the aldehyde compounds, with
194 E,E-2,4-dodecadienal found as the highest (58.73%) in SO 180°C-120 min (Table 4).
195 Nonanal (34.97%) and decanal (34.19%) were the most represented VOCs in fresh
196 SO. Decanal constantly decreased with heating and with heating duration, whereas
197 nonanal showed an initial increase at 180°C-30 min and a subsequent decrease with
198 heating and with heating duration (Table 4). Furthermore, fresh SO was the one with

199 the lowest number of identified compounds, moreover it showed a greater number of
200 VOCs in the heated samples than in the fresh ones, confirming the high instability of
201 this vegetable oil under heating (Table 4). The SO instability was also confirmed by the
202 lowest total phenolic content (15 mg gallic acid/kg) and by the lowest antioxidant
203 activity quantified with both the lowest values in the ABTS assay (51.8 μ M TE/100g)
204 and the lowest values in the DPPH hydrophilic assay (6.6 μ M TE/100g) measured in
205 the same samples studied by Giuffrè et al., (2018).

206 **3.2. Total alkanals, alkenals and alkadienal**

207 The identified aldehyde compounds have been grouped as alkanals, alkenals and
208 alkadienals to study the relationship between the heating temperatures, the heating
209 time and their production in the studied vegetable oils.

210 The total aldehydes rate varied significantly during heating of all of the four studied oils
211 both at 180°C and 220°C (Figure 1a,b). This was due to the presence of some alkans
212 (α -cubebene, α -muurolene and α -farnesene) (Table 1) in the EVOO, the only oil
213 obtained from a mechanical process which minimally alters the olive original volatile
214 profile, while they are totally absent in all the other three studied unheated vegetable
215 oils obtained by solvent extraction. In figures 1a and 1b a significant increment is
216 shown by the total alkenals, which increased from 32.1% in EVOO to 60.9% and 68.8%
217 respectively in EVOO 180°C-120 min and EVOO 220°C-120 min, confirming how the
218 formation of these compounds is strictly correlated to the oleic acid oxidation, the major
219 acid constituent in olive oil.

220 The PO heated at 180°C showed a significant difference in the total aldehydes with
221 respect to PO control with a decrease at 180°C-30 min and a tendency increase at
222 180°C-60 min and 180°C-120 min (Figure 2a), this was probably due to an initial

223 degradation of some aldehydes and a subsequent formation of new ones, while the
224 220°C heated samples showed a significant difference after 30 mins heating with a
225 decrement in total alkanals (-57.1%) and an increment in total alkenals (+58.6%) after
226 120 mins heating (Figure 2b).

227 The total aldehyde profile of P showed a similar trend in both heating temperature, with
228 a significant increment in total alkadienal compounds, from 0% in P to 38.79% and
229 25.40% respectively in P 180°C-120 min and P 220°C-120 min, and a decrement in
230 total alkanal compound, from 71.13% in P to 29.81% and 28.16% respectively in P
231 180°C-120 min and P 220°C-120 min (Figure 3a,b). Total alkanals and total alkenals of
232 P varied significantly with heating and with heating duration (Figure 3a,b).

233 The total aldehydes in SO showed a trend similar to P, in fact total alkanals decreased
234 when total alkadienal increased during heating time at each heating temperature (figure
235 4a,b). A very significant difference was found in the sample before heating and after
236 heating at 180°C for 60 mins, with a decrement in alkanals (-80.0%) and an increment
237 in alkadienal of +867.2%; while the sample heated at 220°C showed the highly
238 difference from its unheated oil immediately after 30 mins, -90.1% and +916.2%
239 respectively in total alkanals and total alkadienals.

240 **4. Conclusion**

241 Results showed that temperature and heating duration have a very high significant
242 influence in the volatile organic compounds content of edible vegetable oils. EVOO
243 showed the highest number of components with E-2-hexenal as the highest in quantity
244 in fresh oil and Z-2-decenal and 2-undecanal as the highest in quantity in heated extra
245 virgin olive oils. PO (control) contained decanal in higher quantity and after heating Z-2-
246 decenal and 2-undecanal prevailed as for extra virgin olive oil in addition to nonanal.

247 Nonanal prevailed in both fresh P and SO whereas E,E-2,4-dodecadienal prevailed in
248 both P and SO oils after their heating. Heating completely varied the volatile organic
249 compounds content of four of the edible vegetable oils mostly used in the world. By the
250 results of this study, the oil user can decide the temperature and the heating duration to
251 be applied to preserve flavours and to reduce off-flavours which are produced during
252 heating in the EVOO, PO, P and SO.

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