Influence of roasting olive fruit on the chemical composition and polycyclic aromatic hydrocarbon content of olive oil

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Abstract – Alwana olive oil is a special product of the people from the mountains of Morocco. To produce this oil, prior to the pressing procedure, olives are roasted at up to 130 °C, which leads to the formation of a typical roasty and nutty aroma. It can be expected that this process, which strongly differs from the usual production technic of virgin olive oil, impact the oil chemical composition. We observed that the roasting process has no influence on the fatty acid and sterol composition of Alwana olive. Concentration in α-tocopherol underwent a slight decrease (from 93 to 91%) whereas γ-tocopherol, polyphenol, and chlorophyll content were increased by the roasting process. Polycyclic aromatic hydrocarbon content was found to be 50 μg kg⁻¹.

Keywords: oil / Alwana olive / roasting / oxidative stability / polycyclic aromatic hydrocarbons

1 Introduction

The fruit of the Olive tree (Olea europaea L.) is undoubtedly one of the most important and typical fruit throughout the Mediterranean Basin (Mataix et al., 2008). Olive oil, derived from the oily juice of the olive, is considered as a health food, especially when consumed under its crude form, thus preserving fatty acids, vitamins and phenolic compounds of nutritional importance (Stark and Madar, 2002).

Olive oil fatty acid composition is characterized by a good balance between saturated, monounsaturated, and polyunsaturated acids. Besides, olive oil has been recommended and used since ancient times for the prevention of cardiovascular diseases and for its anti-oxidative capacity (Allalout et al., 2009; Djebali et al., 2012).

Generally speaking, the ideal objective of any oil production method is to extract the largest possible amount of oil without altering its original quality (Gharby et al., 2013). However, to ascertain olive oil quality, the Codex Alimentarius, the international olive council (IOC), and EC regulations consider virgin olive oil as being “the oil obtained from the...
fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, excluding other treatment than washing, decantation, centrifugation, or filtration. This excludes using solvents or using adjuvants having a chemical or biochemical action”. However, in Morocco there is know-how to produce a different olive oil called “Alwana oil” (AO). Characteristic of some Moroccan regions, AO is the culmination of a procedure of four consecutive stages following the harvest of the olives: roasting in a hot oven (kiln), grinding, kneading and pressing the fruit. The process has some similarities with the Millennium extraction process of argan oil (El Alaoui, 2007).

In detail, the traditional preparative procedure of AO is performed as follows. Fresh ripe olives (generally 5 kg) hand-picked by women are spread out carefully in a closed oven overnight (about twelve hours) most often beforehand used for cooking traditional bread (Fig. 1). After this heating period, the dehydrated – dark colored – olives are rapidly placed in a clay bowl and women crushed the still hot olives between two stones (Fig. 2). The resulting coarse paste, consisting of pulp and olive debris, is called olive paste. These 2 steps take at least 90 minutes.

Then, the olive paste is heated in a clay bowl on a brazier powered by oak charcoal before to be kneaded while adding a small volume of hot water. At the end, the olive paste is introduced into round baskets, called scourtins, letting out the liquid while retaining the pulp and pits. To extract the maximum of oil, the baskets loaded with this paste (one or two) are placed on the lower plate of the bin of the press. By manual and progressive tightening of the press-nuts, the pressure plate is lowered onto the baskets. The press is then inclined against a wall until all oil is collected without human intervention. About 75 mL of oil can be extracted from 5 kg of fresh olives (El Alaoui, 2007).

To the best of our knowledge, chemical quality of Moroccan AO has only been shallowly studied, especially its oxidative stability. The objective of this study was to identify the impact of olive roasting on AO:
– fatty acid, sterol, polyphenol, and tocopherol composition;
– oxidative stability by the Rancimat test;
– polycyclic aromatic hydrocarbon content.

2 Materials and methods

2.1 Olive sample preparation

Greenish olive fruit (Picholine, 10 kg) was harvested near Taounate, a city located in northern Morocco, 67 km in the north from Fes. Roasting was performed in a traditional oven at a temperature not exceeding 130°C for 12 hours. Roasted olives were crushed using two stones. Crushed olives were placed in a clay container which was set on fire and then the olives were further crushed by hand. Small amounts (500 mL) of hot water were then added gradually until obtaining a paste that was poured into a traditional sieve made of weaved palm tree, and pressed for 3 hours using a traditional press. From 10 kg of fruit, 1.4 L of AO were collected.

2.2 Chemicals reagents

All the reagents were of analytical or HPLC grade. 2,2,4-trimethylpentane, heptane and isopropanol used in chromatography and cyclohexane used for extinction coefficient determination were purchased from Professional Labo (Casablanca, Morocco).

2.3 Analytical determination

All analytical determination was performed in triplicate and results are expressed as mean value and standard deviation.

2.3.1 Physical and chemical oil parameters

Free fatty acid content (FFA), peroxide value (PV), extinction coefficients ($K_{232}$ and $K_{270}$), refractive index, and iodine value were determined according to European
Commission Regulation EEC/2568/91 (2003). FFA content was expressed as weight percent of oleic acid. PV was expressed as Meq of active oxygen per kilogram of oil (Meq O₂/kg oil), and extinction coefficients as the specific extinction of a 1% (w/v) solution of oil in cyclohexane at a 1 cm path length, using a CARY 100 Varian UV spectrometer. Carotenoid and chlorophyll compounds were determined in cyclohexane at 470 and 670 nm, respectively, using the specific extinction coefficients, according to the method of Mínguez-Mosquera et al. (1991). The values of specific extinction coefficients (E0) were 613 for phyclophytin as major component in the chlorophyll fraction and 2000 for lutein as major component in the carotenoid fraction. Pigment contents were expressed as mg of pheophytin “a” and lutein per kg of oil, respectively.

2.3.2 Fatty acid composition

Before analysis, lipids containing fatty acids (FAs) were converted to fatty acid methyl esters (FAMEs) by shaking for 25 min a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2N methanolic potassium hydroxide. FAMEs were analyzed by gas chromatography using a Varian CP-3800 (Varian Inc.) chromatograph equipped with a FID and a CP-Wax 52CB column (30 m × 0.25 mm id.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium and the flow rate was 1 mL/min. The initial and final column temperatures were 170°C and 230°C, respectively, and the temperature was increased at a rate of 4°C/min. The injector and detector temperature was 230°C. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Results were expressed as the relative percentage of each individual FA present in the sample. Iodine value (IV) was computed from FAME percentages using the formula: IV = (% palmitoleic × 1.001) + (% oleic × 0.899) + (% linoleic × 1.814) + (% linolenic × 2.737).

2.3.3 Sterol composition

After isolation from the thin layer chromatography plate, the sterol fraction was prepared by trimethylsilylation according to the standard EN ISO 6799. Sterol composition was determined by gas chromatography using a Varian 3800 instrument equipped with a VF-1 ms column (30 m & 0.25 mm id.) and using helium (flow rate 1.6 mL/min) as carrier gas. Column temperature was isothermal at 270°C, injector and detector temperature was 300°C. Injected volume was 1 μL. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

2.3.4 Tocopherol composition

For the analysis of the tocopherol content, high performance liquid chromatography (HPLC) was used, using a solution of 250 mg of oil in 25 mL of n-heptane. Tocopherols were analyzed by HPLC using Shimadzu CR8A instrument (Champ sur Marne, France) equipped with a C18 column (25 cm × 4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent was a 99:1 isoctane/isopropanol (v/v) mixture (flow rate 1.2 mL/min).

2.3.5 Colorimetric determination of polyphenols

Phenolic compounds were isolated by triple extraction of a solution of oil in hexane with a water/methanol mixture (60:40, v/v). Total phenols were determined by adding the Folin-Ciocalteu reagent to a suitable aliquot of the combined extracts, and measuring the absorbance at 725 nm 2 h later using a spectrophotometer (Model Cary 100 Varian UV).

2.3.6 Benzo[a]pyrene content

Benzo[a]pyrene (B[a]P) content was determined following NF EN ISO 15302:2007 standard. Briefly, 2 g of oil were weighed (precision of 0.1 mg), then dissolved in petroleum ether (total volume 10 mL). The mixture was homogenized by manual shaking. In a glass column (300 mm × 15 mm) fitted with a sintered-glass filter, 22 g of aluminum oxide were introduced and topped with anhydrous sodium sulfate (30 mm) and the column was tapped to pack it. After conditioning the column by passing 30 mL of petroleum ether without allowing it to dry, 2 mL of oil solution were charged on the top of the column, and then eluted with an additional 5 mL of petroleum ether. The initial eluted fraction (20 mL) was discarded. The column was then eluted with additional petroleum ether and a 60 mL fraction was collected in a 100-mL conical flask. The solvent was evaporated at 35°C under reduced pressure to approximately 0.50 mL and then dried under a nitrogen flow. For HPLC analysis, the residue was dissolved in 50 μL of acetonitrile by shaking. An aliquot of 10 μL was injected into the HPLC using an auto-sampler (ProStar 410, Varian Inc., and Walnut Creek, USA) with pick-up injection mode to avoid cross-contamination. HPLC system used was ProStar HPLC equipped with a Varian C18 column (2 cm × 4.6 mm), and a ChromSep guard cartridge (Varian Chrompack, Varian Inc., Middelburg, The Netherlands). The column temperature was set at 20°C and a mixture of acetonitrile/water (7:1) was used as mobile phase at a flow rate of 1.2 mL/min. A Varian ProStar 363 fluorimetric detector was used with fluorescence emission at 306 nm and 408 nm, data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, USA).

2.3.7 Oxidative stability

The oxidative stability was evaluated by the Rancimat method. Stability was expressed as the oxidative induction period (IP, hours) measured at 110°C on a Rancimat 743 (Mettashc Co, Basel) apparatus using 3 g of oil sample with an air flow of 20 L/h. Volatile oxidation products were stripped from the oil and dissolved in cold water, whose conductivity increased progressively. The time taken to reach a level of conductivity was measured.

3 Results and discussion

To evaluate the impact of olive roasting, we decided to analyze AO and olive oil prepared from the same batch of fruit without roasting them.
3.1 Effect of roasting of olive fruit on physicochemical characterization of olive oil

Roasting of olives resulted in a significantly lower moisture content in AO compared to oil obtained from unroasted olives through the same preparation procedure (0.08% vs. 0.18%, Tab. 1).

The FFA content, here referred as “acidity”, is an important quality parameter used for classifying olive oil within a range commercial grades (COI, 2013). The higher percentage limit has been established internationally at 0.8% for FFAs (as oleic acid) in extra-virgin olive oil. Oils extracted from fresh healthy fruits and crushed according to best practices present a very low acidity (Gutierrez et al., 2000; Gharby et al., 2013). The acidity of the AO and olive oil from unroasted olive fruits was 0.18 ± 0.01 and 0.21 ± 0.02%, respectively (Tab. 1). Although both oils displayed acidity much lower than the allowed limit for extra virgin olive oil (0.8%; COI, 2013), our results show that roasting slightly increases the global acidity (Tab. 1). This likely results from easy known heat-induced hydrolysis of the triglycerides (Cossignani et al., 1998). This is also consistent with our observations on argan oil (Harhar et al., 2011) and those of Yoshida et al. (1995, 2003; Fukuda, 1990). In all cases, FFA content increased with roasting time.

Peroxide value (PV) evaluates the hydroperoxide content and provides a measure of lipid oxidation. The PV of our reference olive oil was found to be 2.8 Meq O₂/kg of oil whereas AO displayed a lower value (0.8 Meq O₂/kg of oil) (Tab. 1). Therefore, heating of the olives in the oven may result in the accelerated degradation of hydroperoxides leading to a lower PV because of the formation of secondary oxidation products.

For the further evaluation of the influence of the roasting process on the oxidative stability, the specific extinction at 232 and 270 nm was determined immediately for the oil from roasted and unroasted olive fruits (Tab. 1). UV absorption at 232 nm (K₂32) is useful to evaluate the presence of primary oxidation products. K₂32 of AO was found to be lower than that of regular olive oil (Tab. 1). This can be explained by the formation of secondary oxidation products. Indeed, UV absorption at 270 nm (K₂70), which is one of the markers used to monitor secondary oxidation products, was found to be twice higher in AO compared to olive oil (Tab. 1). Such high value signs the formation of secondary oxidation products, and is consistent with the low PV and reduced K₂32 absorption.

We evaluated the oxidative stability of AO using the Rancimat method at 110°C. Olive oil prepared by pressing unroasted fruits presented an induction time of 26 h, while AO had an induction time of 34 h. These data confirm the transfer of Maillard reaction products developed during the roasting process into the olive oil and their preservative properties against oxidation (Morales and Babbel, 2002).

3.2 Effect of roasting of olive fruits on fatty acid composition

Fatty acid (FA) composition is an essential indicator for the nutritional value of the oil. Almost no significant differences were observed between the fatty acid composition of olive oils prepared with and without roasting of the olives before pressing (Tab. 2). Olive oil from unroasted olives contained the following acids; 10.3 ± 0.57% palmitic, 2.56 ± 0.06% stearic, 75.2 ± 0.98% oleic, and 9.21 ± 0.05% linoleic acids. AO consisted of 11.0 ± 1.41% palmitic, 2.35 ± 0.21% stearic, 75.3 ± 2.75% oleic, and 8.85 ± 1.06% linoleic acids. Lack of influence of roasting parameters on FA composition is not surprising and has already been reported for other oils (Lee et al., 2004, Anjum et al., 2006). Our results confirm the good stability of olive oil fatty acids.

3.3 Effect of roasting of olive fruits on sterol composition of olive oil

Sterols are also important constituents of olive oils because they relate to the quality of the oil and are widely used to check authenticity. Sterol composition of the oils is reported in Table 3. Both olive oil samples analyzed showed high β-sitosterol content. Olive oil is known to be particularly rich in β-sitosterol (Gharby et al., 2012). This high content is likely to be responsible of olive oil excellent preservation properties as well as of most of its physiological properties (Moreau et al., 2002). In the present study, similar sterol levels were determined for olive oil prepared from roasted or unroasted
Table 3. Sterol composition of different olive oils (%).

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Olive oil</th>
<th>Alwana</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>2.75 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>&lt; 4.0</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.95 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>&lt; Campesterol</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>88.9 ± 0.7</td>
<td>88.55 ± 0.1</td>
<td>&gt; 93</td>
</tr>
<tr>
<td>Δ5-Avenasterol</td>
<td>5.45 ± 0.5</td>
<td>5.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Δ7-Stigmasterol</td>
<td>0.25 ± 0.1</td>
<td>0.15 ± 0.1</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Δ7-Avenasterol</td>
<td>0.25 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

All values are the mean of three replicates ± standard deviation of the mean.

Table 4. Natural antioxidant content of olive oils.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Olive oil</th>
<th>Alwana</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>93.72 ± 4</td>
<td>91.70 ± 5</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>2.81 ± 0.3</td>
<td>4.50 ± 0.5</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>3.47 ± 0.2</td>
<td>3.78 ± 0.2</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>226 ± 5.5</td>
<td>293 ± 5.5</td>
</tr>
<tr>
<td>β-Carotene (mg/kg)</td>
<td>4.96 ± 0.1</td>
<td>7.11 ± 0.2</td>
</tr>
<tr>
<td>Chlorophyll (mg/kg)</td>
<td>9.1 ± 2.3</td>
<td>24.1 ± 2.4</td>
</tr>
</tbody>
</table>

All values are the mean of three replicates ± standard deviation of the mean.

fruit (Tab. 3). Even after roasting, β-sitosterol content in olive oil was found to be very high (Tab. 3). Δ5-avenasterol is known to act as an antioxidant and antipolymerization agent in frying oils (Giocametti and Milin, 2001, Gordon and Magos, 1983). During roasting, nor Δ5-avenasterol neither campesterol and stigmasterol changed significantly. The results show that the sterol content of olive oil was not sensitive to fruit roasting.

3.4 Effect of roasting of olive fruits on natural antioxidants contents of olive oil

Tocopherols are important components of the unsaponifiable fraction in vegetable oils. This family of compounds is particularly important in preventing lipid oxidation processes in olive oils (Woollard and Indyk, 2003). Contradictory results have been reported regarding the influence of heating on the tocopherol content (Yoshida et al., 1995; Yen, 1990; Lane et al., 1997; Kim et al., 2002). We observed that the content of α-tocopherol in olive oil decreased after roasting. It was 93.7 ± 4 mg/kg in olive oil from unroasted fruits, whereas the content of α-tocopherol for AO was 91.7 ± 5 mg/kg. Conversely, an increase of γ-tocopherol was observed (from 2 to 4%). However, there were no significant differences in the contents of δ-tocopherol. We also observed the absence of the β-tocopherol in both oils.

Changes in the concentration of chlorophylls and carotenoids in the olive oil samples during roasting for 12 h are shown in Table 4. The concentration of chlorophylls increased from 9.1 ± 2.3 mg/kg to 24.1 ± 2.4 mg/kg, whereas that of carotenoids increased from 4.96 ± 0.1 to 7.11 ± 0.2 mg/kg during the roasting process. During the extraction process, a proportion of the native chlorophyls is transformed into pheophytins when the central Mg2+ ion of the porphyrin ring is substituted by H+. This reaction is visually very striking, because it directly affects the chromophore group of the chlorophyll, and the color changes from bright green to olive brown. Only a part of the original chlorophyll content of the fruit remains intact in the oil. In the carotenoid fraction, the acidity of the medium causes isomerization of the 5,6-epoxide groups to 5,8-furanoids.

3.5 Effect of roasting of olive fruits on polycyclic aromatic hydrocarbons (PAH) of olive oil

PAH belong to a large class of organic compounds originated from incomplete combustion of organic matter, known or suspected to be carcinogenic and genotoxic to mammals. Benzo[a]pyrene is the most studied PAH and is used as a marker. The main sources of PAH in foods are the environmental contamination and the food processing (such as smoke curing, cooking over charcoal and roasting). Results for Alwana oil samples are presented in Table 5. Similarly as for Rodriguez-Acuña et al. (2008), BaA, Chrysene, BaP and BbF were the main PAHs, with concentrations ranging from 2.19 ± 0.2 to 31.3 ± 0.3, 2.44 ± 0.1 to 14.85 ± 0.4, 0.51 ± 0.01 to 1.59 ± 0.02 and 0.26 ± 0.02 to 2.92 ± 0.1 μg/L, respectively. For all compounds, an increase was noticeable for olive oil from roasted fruits. The highest increase was found for benz[a]anthracene, and the lowest was for BaP.

B[a]P frequently occurs in roasted material. In a previous and preliminary study performed at a roasting temperature of 100 °C, it was demonstrated that roasting of argan kernels did not induce the formation of B[a]P (Harhar et al., 2011). Nevertheless, we took advantage of this study to reinvestigate B[a]P content because of the known toxicity of this compound (Powrie et al., 1986).

The EU has set a maximum level of 2.0 μg/kg for BaP in oils and fats intended for direct consumption or used as an ingredient in foods (European Commission, 2005, 2006). Prior to that, some countries (Spain, Italy, Portugal and Greece) have established limits for the concentration of the following eight heavy PAH: BaA, BeP, BbF, BkF, BaP, DhA, BgP, IcP. The values established were a maximum limit of 2 μg/kg for each single PAH and 5 μg/kg for the sum of the referred eight heavy PAH (Teixeira et al., 2007). Some organizations establish their
own recommendations, such as the German Society for Fat Science (GSFS), who suggested that the total PAH level in edible oils should not surpass 25 μg/kg and heavy PAH should be below 5 μg/kg (Cejpek et al., 1998). For our result, we found that the sum surpass 50 μg/kg.

4 Conclusions

In conclusion, PAHs increase significantly during roasting. B (a)A and Chrysene were the major PAHs in the olive oil samples analyzed in this study. Even though they were present in olive oil from unroasted fruits, their content increased during the roasting process under elevated temperatures (about 130°C), up to 15–50 μg/kg. Strong roasting conditions led to significant levels of chrysene and benzo[a]anthracene (near 13 μg/kg), whereas low levels of anthracene, benzo[b]fluoranthene, and benzo[a]pyrene were also noted in those samples.

The avoidance of these contaminants in the diet is highly desirable and special attention must be given to the intake of roasted foods since considerable amount of PAHs can be ingested in a single meal.

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