Food Chemistry 169 (2015) 289–296

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Effect of olive storage conditions on Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive oils authenticity

Hazem Jabeur [a], Akram Zribi [a], Ridha Abdelhedi [a], Mohamed Bouaziz [a,b,*]

[a] Laboratoire d'Électrochimie et Environnement, École Nationale d'Ingénieurs de Sfax, Université de Sfax, BP «1173», 3038 Sfax, Tunisia
[b] Institut Supérieur de Biotechnologie de Sfax, Université de Sfax, BP «1175», 3038 Sfax, Tunisia

ARTICLE INFO

*Article history:*
Received 27 July 2013
Received in revised form 20 July 2014
Accepted 25 July 2014
Available online 11 August 2014

*Chemical compounds studied in this article:*
Methyl oleate (PubChem CID: 5364509)
Methyl palmitate (PubChem CID: 8181)
Ethyl palmitate (PubChem CID: 12366)
Methyl stearate (PubChem CID: 8201)
Ethyl stearate (PubChem CID: 8122)
Ethyl oleate (PubChem CID: 5363269)
Methyl linoleate (PubChem CID: 5284421)
Ethyl linoleate (PubChem CID: 5282184)
Methyl heptadecanoate (PubChem CID: 15609)
Lauryl arachidate (PubChem CID: 5214900)

*Keywords:*
Olive fruits
Storage conditions
Extra-virgin olive oil
Mild deodorized olive oil
Fatty acid alkyl esters
Repeated measures analysis

ABSTRACT

The present paper accounts for the study of the storage of Chemlali olive fruits at two conditions of limited aerobiosis: in closed plastic bags and in open perforated plastic boxes for different periods before oil extraction. The ultimate objective is to investigate the effect of the container type of the postharvest fruit storage on the deterioration of the olive oil quality. The results have shown that the oil quality of Chemlali olives deteriorated more rapidly during fruit storage in closed plastic bags than in perforated plastic boxes. Therefore, the use of perforated plastic boxes is recommended for keeping the olives for longer periods of storage. The repeated measures analysis of variance of all parameters analyzed indicated that the olive oil quality is mainly affected by the olives storage conditions (containers type and storage periods). Finally, blends of extra-virgin olive oil and mildly deodorized low-quality olive oils can be detected by their alkyl esters concentrations.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

Virgin olive oil is a valuable vegetable oil extracted from fresh and healthy olive fruits (*Olea europaea* L.) by mechanical processes (pressing or centrifuging) and without heat, solvents or any preliminary refining (Ammar, Zribi, Ben Mansour, et al., 2014; Garcia & Yousefi, 2006). It is practically the only vegetable oil that can be consumed directly in its raw state as well as it contains important nutritional elements (fatty acids, vitamins, sterols, etc.). Extra-virgin olive oil (EVOO) is considered as the best olive oil for its superior organoleptic characteristics (aroma and taste). It has a potential health benefits, remarkable antioxidant properties and chemical composition (Ammar, Zribi, Gargouri, Flamini, & Bouaziz, 2014; Jafari, Kadivar, & Keramat, 2009; Méndez & Falqué, 2007).

It is well-known that the storage period has an influence on the quality of fruit and oil of black-ripe olives. The main factor behind the deterioration of olive oil is accredited to the poor handling of the olives during the time between harvesting and processing. Indeed, the storage of olive fruits that develop all kinds of degenerative processes in a short period of time is carried out by simple heaping in fruit piles, waiting for their processing (Rabiei, Ghorbani, & Hajnajari, 2011).

It is in this context that the present paper lies to study the effect of the storage period of olive fruit (*Olea europaea* cv. Chemlali) in closed plastic bags and in open perforated plastic boxes on the olive oil quality. In fact, oils were extracted and their quality immediately

* Corresponding author at: Laboratoire d'Électrochimie et Environnement, Institut Supérieur de Biotechnologie de Sfax, Université de Sfax, BP «1175», 3038 Sfax, Tunisia. Tel.: +216 98 667 581; fax: +216 74 674 364.
*E-mail address:* mohamed.bouaziz@fsg.rnu.tn (M. Bouaziz).

http://dx.doi.org/10.1016/j.foodchem.2014.07.118
0308-8146/© 2014 Elsevier Ltd. All rights reserved.

evaluated after fruit harvest and at the end of five predetermined temporal storage periods of 3, 6, 11, 17 and 25 days. The chemical quality parameters such as acidity, peroxide value, specific extinction coefficient at 232 and 270 nm, total polyphenols contents, pigment contents and fatty acid alkyl esters (FAAEs) together with sensorial quality determined for 25 days, were analyzed.

The fatty acid methyl esters and fatty acid ethyl esters contents are not only known to be closely related to the health conditions of olive fruits but also obviously higher if olives undergo hydrolytic and fermentative processes, thus increasing the amounts of both free fatty acids and alcohols. What is worthy to note is that oils obtained from fermented fruits are low-quality virgin olive oils, having unpleasant sensorial features that prevent them from being classified as extra-virgin olive oils, thus leading to the decrease in their commercial value.

Unfortunately, EVOO is also easy to falsify. In fact owing to its prominence, it has always been illegally mixed with cheaper and low-quality oils (Harwood & Aparicio, 2000; Jabeur et al., 2014), especially to obtain EVOO sold in supermarkets and discount stores at low cost (Bendini, Cerretani, Salvador, Fregpane, & Lercker, 2009). The so-called lampante low-quality olive oils cannot be used as raw foodstuff for direct human consumption, as they have an acidity level that is too high, and their volatile profile is characterized by 'soft' off-flavours, derived from low-quality olives or from inappropriate procedures during oil extraction or storage.

The newest and most common adulterations of extra-virgin olive oil are the dilution by mild deodorized low quality olive oil. The latter is obtained from lampante virgin oil with an unpleasant flavor, subjected to a mild thermal deodorization, developed under vacuum and at low temperature (100–120 °C) for removing undesired substances that negatively influence its flavor (mainly winey-vinegary, fusty and musty). After correction of the sensorial defects of these oils, is often used to be used for an illegal blending with extra-virgin olive oils (Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo, & Moreda, 2008). In general, such a blending does not produce an easily detectable modification of the chemical composition because of the mild conditions used in the deodorization.

With respect to the second objective of this study, it pertains to the investigation of the effect of incorporating mild deodorized olive oils with variable amounts in extra-virgin olive oil by the determination of fatty acid methyl and ethyl esters contents. Actually, the effectiveness of these chromatographic determinations was examined for the minimum detectable limit of adulterated soft deodorized oil.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Ethanol (⩾99.9%) and n-heptane (99.0%) were obtained from Riedel-de Haën (Steinhein, Germany). Cyclohexane (99.5%), n-hexane (99.0%) and diethyl ether (⩾99.7%) were purchased from Merck KGaA (Darmstadt, Germany). Potassium hydroxide (85.0%) was obtained from CDM (Karlsruhe, Germany) and potassium iodide (>99.0%) was purchased from Chem-Lab (Zedelgem, Belgium). Sodium hydroxide (>99%) was supplied by Scharlau (Chemie, S.A, Spain). Acetic acid (100.0%) and chloroform (>99.1%) were from Prolabo (AnalaR NORMAPUR, France). Folin–Ciocalteu reagent was obtained from Fluka (Buchs, Switzerland). Lauryl arachidate (>99%) and methyl heptadecanoate (>99%) standards were purchased from Sigma–Aldrich (St. Louis, MO, Germany).

### 2.2. Samples

#### 2.2.1. Olive fruits

Black-ripe olives (*Olea europaea*. Chemlali) fruits of good quality were hand harvested from the Sfax region (southern Tunisia) on February 04, 2012 at ripening phase. The maturation index (M.I.) of the used fruits was 5.8. The olive maturity index (MI) was determined according to the method developed by Boskou (1996) on the basis of the evaluation of the olive skin and pulp colors. MI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin). After leaves deletion, washed fruits were mixed to ensure a homogeneous sampling before storage. The olives were randomly divided into batch of 10 kg.

The control sample was processed immediately after harvesting, while the other lots were stored in a room at temperature of 12 ± 2 °C by night and 18 ± 2 °C by day with a relative humidity of 60% during different periods (3, 6, 11, 17 and 25 days) and at two conditions of limited aerobiosis: in closed plastic bags (polyethylene high-density (PE-HD) bags: length = 50 cm and width = 40 cm) and in open perforated plastic boxes (a small format (length = 50 cm, width = 30 cm and height = 40 cm) of perforated polyethylene high-density (PE-HD) boxes).

#### 2.2.2. Oil extraction

Olive oil was extracted using a procedure that imitates the industrial process. Fruits were firstly crushed by a laboratory blender and the resulting paste was mixed for 30 min in the presence of warm (30 °C) distilled water (about 30% of the paste *w/w*) to facilitate oil separation. Olive oil was obtained after the centrifugation of the paste at 3000 rpm for 10 min and stored in dark glass bottles at −20 °C for future analyses.

#### 2.2.3. Deodorized olive oil and blends preparation

Mild deodorized olive oil (DO) was provided by laboratory-scale plant (AGRO-ZITEX, Sfax, Tunisia). It was originally obtained by submitting low-quality oil to a mild refining process at 110 °C under steam stripping for up to 4 h. The small quantity of mild deodorized olive oils was due to the difficulties to find such oils. Blends were prepared in the laboratory, by mixing an extra-virgin olive oil with deodorized olive oil, all of which were produced in 2012, at different increasing amounts: 1, 5, 10, 20, 30, 40 and 50 g/100 g.

### 2.3. Analytical methods

#### 2.3.1. Quality indices determinations

The titratable acidity (free fatty acids) was determined according to the method proposed by ISO660, (1996). Besides, while peroxides were determined according to the method proposed by ISO3960, (2001), UV spectrophtometric constants ($K_{232}$ and $K_{270}$) were carried out according to the analytical methods described by COI (2010). As for the total polyphenols, they were determined according to the previously published protocol, making use of Folin–Ciocalteau methodology described by Zribi et al. (2013). Moreover, the gallic acid was applied as standard reference and the results were expressed as gallic acid equivalents (ppm). Next, carotenoids and chlorophylls (mg/kg of oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values according to the method of Haddada et al. (2008).

#### 2.3.2. Determination of sensory quality

The sensory quality evaluation was determined according to the International Olive Oil Council (COI, 2011a,b) by the Tunisian National Office of Oil panel. The panel, recognized according to IOOC, consists of selected and well-trained olive oil experts monitored in accordance to their skills in the distinction between similar samples by an experienced panel leader.

#### 2.3.3. Determination of fatty acids alkyl esters (FAAEs) and waxes

Fatty acids alkyl esters and waxes were determined by gas chromatography (GC-FID) according to the method reported in

COI (2009) as the "Determination of the content of waxes, fatty acids methyl esters and fatty acids ethyl esters by capillary gas chromatography". A 0.50 ± 0.001 g of the sample was mixed with 250 μL of standard solution of the internal standard (methyl heptadecanoate, C17:0 ME, 0.02% (*w*/*v*) in n-heptane), 100 μL lauryl arachidate (0.05% (*w*/*v*) in n-heptane) and 100 μL of Sudan I dye at 1% in the elution mixture (n-hexane/ethyl ether (99:1, *v*/*v*)) can be added to the sample solution to check visually that the FAAEs waxes were eluted properly. Next, 15 g of silica gel were suspended in n-hexane and settled spontaneously into a glass column for LC (internal diameter 15 mm, length 30–40 cm). The settling was completed by means of an electric shaker to make the chromatographic bed more homogeneous. Then, 30 mL of n-hexane were percolated to remove any impurities. Next, the samples were transferred to the chromatography column using two 2 mL portions of n-hexane. The solvent was allowed to flow to 1 mm above the upper level of the absorbent. The alkyl esters and waxes were then collected eluting 220 mL of a freshly prepared mixture of n-hexane/ethyl ether (99:1, *v*/*v*) at a flow of about 15 drops every 10 s. The elution was stopped when the colored band reached the bottom of the column because Sudan I dye has a similar retention time to TAGs.

The resultant fraction was evaporated in a rotary evaporator at room temperature under vacuum until dry. The fraction containing the alkyl esters and waxes was diluted with 2 mL of n-heptane, and 1 μL of this solution was injected into the gas chromatography (GC). The analysis of esters and waxes was performed on a 7890A Agilent Technologies gas Chromatograph System (Pudong, Shanghai, China) equipped with a flame ionization detector (FID). The column used was a capillary HP-5 (length 30 m, id 0.32 mm and film thickness 0.25 μm). The operating conditions were as follow: oven temperature, 80 °C for 1 min and then increased from 20 °C/min up to 140 °C, then increased from 5 °C up to 335 °C and maintained for 20 min; injector was programmed from 70 to 300 °C; detector temperature was 350 °C. Helium was used as the carrier gas, with a flow through the column of 1 mL/min and 1:50 split ratio.

The method of analysis was focused on determining methyl and ethyl esters of fatty acids and the waxes in olive oil. The fatty acid methyl and ethyl esters are eluted early in the following order: methyl palmitate, ethyl palmitate, methyl hepatdecanoate (internal standard), methyl linoleate, methyl oleate, methyl stearate, ethyl linoleate, ethyl oleate and ethyl stearate. The waxes are eluted in the second half of the chromatogram start with the diterpene esters.

### 2.4. Statistical analysis

The results were expressed as mean ± standard deviation (SD) of 3 measurements for the analytical determination. Significant differences between the values of all parameters were determined at $p < 0.05$ according to the one-way ANOVA: Post Hoc Comparisons (Student Newman–Keuls test).

The estimated marginal means are the means for each factor, adjusted for any other factor or covariate in the model. These means can be obtained by SPSS (GLM: General Linear Model) which correspond to the means of Y for each level of the factor at the mean value of the covariate. If the factor is independent, the marginal means are equal to the means of levels for each factor considered alone. The repeated measures analysis of variance (ANOVA) was applied to show the effect of olive fruits storage conditions on the quality of olive oils according to all the parameters investigated. All analyses were performed using SPSS Statistics 17.0 for Windows (SPSS Inc., 2008).

## 3. Results and discussion

### 3.1. Part I: Influence of olive fruit storage in open perforated plastic boxes and in closed plastic bags on oil quality

#### 3.1.1. Free fatty acids percentage

Virgin olive oil contains about 98% of neutral lipids, mainly triglycerides (96–97%) followed by a small quantity of diglycerides (1–2%) and a variable quantity of free fatty acids which are used as a marker of oil quality (Olias & Garcia, 1997).

Table 1 shows the free fatty acids content variation (FFA) (% C18:1) of the oils obtained from olives stored at different periods in plastic bags and in perforated plastic boxes. The oil acidity of freshly harvested olives was below 0.8% (0.233% ± 0.002), which is the value indicating the classification of olive oil as extra-virgin. Acidity depends on the period of time from harvesting to processing, with the storage temperature being the most important factor in determining its value (Ben Youssef et al., 2012). Besides, being the consequence of hydrolytic enzymes activity in the destroyed cells, acidity increased significantly ($p < 0.001$) after 6 days (>0.8%) in fruits from closed plastic bags, while in open perforated plastic boxes, this was evident after 11 days of storage (>0.8%) ($p < 0.001$).

The increase of oil acidity during storage is certainly related to the increase in storage temperature (Garcia, Gutierrez, Castellano, Perdiguero, & Albi, 1996), and probably the result of fungal lipase activity (Kiritsakis, Nanos, Polymenopoulos, Tomai, & Sfakiotakis, 1998). Gutierrez, Perdiguero, Garcia and Castellano (1992) have reported that the increase in the acidity of the extracted oil from fruits stored at 20 °C was correlated positively with decay incidence. The first action of a parasitic microorganism in an oil-rich tissue is to induce hydrolytic activity by lipase which leads to the release of fatty acids from the triacylglycerol molecules of the oil (Clodoveo, Delcuratolo, Gomes, & Colelli, 2007). The use of perforated plastic boxes is recommended for keeping the olives for longer periods of storage.

#### 3.1.2. Peroxide value

The peroxide value (PV) of oil is an important indicator of oxidation level, which is a measure of primary oxidation. Table 1 shows the changes in the PV (meq $O_2$/kg) of oils obtained from olives stored in closed plastic bags and in open perforated plastic boxes at different periods. The PV of the oils extracted immediately after harvesting was 5.40 ± 0.27 meq $O_2$/kg. In fact, compared to oil extracted from freshly harvested olives, peroxide value of oils obtained from stored olives increased during storage. After 11 days, in relation to the baseline values (Table 1), the PV of the oil obtained from olives stored in closed plastic bags doubled significantly ($p < 0.001$) but PV doubled significantly ($p < 0.001$) after 25 days in the oil obtained from olives stored in open perforated plastic boxes. None of the oils analyzed exceeded the maximum peroxide value for extra-virgin olive oil category (20 meq $O_2$/kg) since the highest PV obtained was on average 16.50 ± 0.98 meq $O_2$/kg for oils extracted from Chemlali olives stored in closed plastic bags for 25 days.

#### 3.1.3. Specific extinction coefficient at 232 nm and 270 nm

UV-specific extinction determination allows for an approximation of the oxidation process in unsaturated oils (Gutierrez, Perdiguero, Gutierrez, & Olias, 1992). The maximum permitted values of $K_{232}$ and $K_{270}$ for extra-virgin olive oils were 2.50 and 0.20, respectively. Table 1 shows the changes in $K_{232}$ and $K_{270}$ of oils obtained from olives stored at different periods in closed plastic bags and in open perforated plastic boxes. Actually, the $K_{232}$ of the fresh oil was on average 1.48 ± 0.09 and the $K_{270}$ was

**Table 1**
Quality indices of Chemlali olive oil extracted after different periods of olives storage in closed plastic bags and open perforated plastic boxes.

| Storage period | FFA (%) | | PV ($meqO_2/kg$) | | $K_{232}$ | | $K_{270}$ | | Total phenol contents (ppm) | | Chlorophylls (ppm) | | Carotenoids (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Bag | Box | Bag | Box | Bag | Box | Bag | Box | Bag | Box | Bag | Box | Bag | Box |
| 0 | 0.233 ± 0.015 | 0.233 ± 0.015 | 5.40 ± 0.27 | 5.40 ± 0.27 | 1.48 ± 0.09 | 1.48 ± 0.09 | 0.074 ± 0.003 | 0.074 ± 0.003 | 202.70 ± 1.71 | 202.70 ± 1.71 | 3.03 ± 0.11 | 3.03 ± 0.11 | 1.27 ± 0.10 | 1.27 ± 0.10 |
| 3 | 0.42 ± 0.01$^{***}$ | 0.38 ± 0.02$^{+++}$ | 6.45 ± 0.24$^{**}$ | 6.20 ± 0.38$^{+}$ | 2.02 ± 0.31$^{*}$ | 1.74 ± 0.23$^{NS}$ | 0.092 ± 0.002$^{**}$ | 0.083 ± 0.002$^{NS}$ | 156.00 ± 1.66$^{***}$ | 181.70 ± 0.96$^{+++}$ | 2.63 ± 0.19$^{*}$ | 2.90 ± 0.09$^{NS}$ | 1.16 ± 0.04$^{NS}$ | 1.22 ± 0.13$^{NS}$ |
| 6 | 1.35 ± 0.02$^{***}$ | 0.70 ± 0.01$^{+++}$ | 9.25 ± 0.23$^{***}$ | 7.30 ± 0.44$^{++}$ | 2.34 ± 0.07$^{***}$ | 2.00 ± 0.11$^{++}$ | 0.113 ± 0.002$^{***}$ | 0.103 ± 0.003 $^{++}$ | 107.40 ± 1.05$^{***}$ | 164.90 ± 0.88$^{+++}$ | 2.24 ± 0.08$^{***}$ | 2.60 ± 0.21$^{+}$ | 1.12 ± 0.09$^{NS}$ | 1.17 ± 0.07$^{NS}$ |
| 11 | 2.61 ± 0.01$^{***}$ | 0.97 ± 0.06$^{+++}$ | 11.30 ± 1.07$^{***}$ | 8.00 ± 0.21$^{+++}$ | 2.62 ± 0.10$^{***}$ | 2.28 ± 0.04$^{+++}$ | 0.173 ± 0.003$^{***}$ | 0.122 ± 0.002$^{+++}$ | 67.20 ± 0.83$^{***}$ | 120.60 ± 1.09$^{+++}$ | 1.97 ± 0.10$^{***}$ | 2.31 ± 0.06$^{+++}$ | 1.07 ± 0.11$^{*}$ | 1.13 ± 0.03$^{+}$ |
| 17 | 5.10 ± 0.01$^{***}$ | 2.72 ± 0.02$^{+++}$ | 14.00 ± 0.91$^{***}$ | 9.80 ± 1.01$^{+++}$ | 2.76 ± 0.14$^{***}$ | 2.52 ± 0.08$^{+++}$ | 0.202 ± 0.004$^{***}$ | 0.154 ± 0.003$^{+++}$ | 52.80 ± 0.31$^{***}$ | 89.60 ± 0.57$^{+++}$ | 1.66 ± 0.24$^{***}$ | 2.04 ± 0.04$^{+++}$ | 1.03 ± 0.08$^{*}$ | 1.06 ± 0.02$^{+}$ |
| 25 | 9.30 ± 0.07$^{***}$ | 4.30 ± 0.03$^{+++}$ | 16.50 ± 0.98$^{***}$ | 12.50 ± 0.51$^{+++}$ | 3.19 ± 0.21$^{***}$ | 2.71 ± 0.17$^{+++}$ | 0.24 ± 0.01$^{***}$ | 0.191 ± 0.003$^{+++}$ | 33.20 ± 0.63$^{***}$ | 66.00 ± 0.21$^{+++}$ | 1.52 ± 0.13$^{***}$ | 1.82 ± 0.08$^{+++}$ | 1.00 ± 0.02$^{**}$ | 1.02 ± 0.06$^{++}$ |

FFA: free fatty acids; PV: peroxide value; $K_{232}$ and $K_{270}$: spectrophotometric indices. Each value represents the mean of three determinations ($n = 3$) ± standard deviation. Significant differences between the extra-virgin olive oil (0 day) and the olive oil obtained from olive fruits stored in closed plastic bags (BAG) groups $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, and between the extra-virgin olive oil and the olive oil obtained from olive fruits stored in open perforated plastic boxes (BOX) groups $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$. NS: no significance between the extra-virgin olive oil and the Chemlali olive oil obtained from olive fruits stored in closed plastic bags groups ($p > 0.05$). NS': no significance between and the extra-virgin olive oil and the Chemlali olive oil obtained from olive fruits stored in open perforated plastic boxes groups ($p > 0.05$).

0.074 ± 0.003. Moreover, for olives stored in open perforated plastic boxes at ambient temperature, it is clear that olive oils behave better since practically none of primary and secondary oxidation product indices exceeded the established limits of extra-virgin category only for $K_{232}$ after 17 days. However, virgin olive oils obtained from olives stored in closed plastic bags lost the extra quality category after 11 days. Indeed, a significant and more drastic increase ($p < 0.001$) was observed in these parameters ($K_{232}$ and $K_{270}$) and these values remained out of the limit for extra-virgin olive oil category after 11 and 25 days of storage, respectively. These results are in agreement with those of Vichi et al. (2009) which suggested that $K_{232}$ and $K_{270}$ progressively increased during the olives storage in particular in oils from olives stored in bags.

### 3.1.4. Total phenol content

Virgin olive oil contains phenolic compounds responsible for its fragrance and peculiar flavor (Servili et al., 2004). Polyphenols are important antioxidants that protect biological systems against oxygen radicals (Bendini et al., 2007). Table 1 shows the changes in the total phenol content (mg of gallic acid/kg of oil) of the oils obtained from olives stored at different periods and in different containers. The loss of phenolic substances was rapid in both cases, but more drastic and significant ($p < 0.001$) in the closed plastic bags.

The losses of phenols could be a result of the rise of fruits oxidative state. In fact, olives contain oxido-reductases, such as polyphenoloxidase and peroxidase that may oxidize polyphenols and impair the health-related qualities and sensory characteristics of olive oil (Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003).

### 3.1.5. Pigment contents

While chlorophylls are responsible for the greenish coloration of certain olive oils, carotenes that are also present in olive oil are responsible for its yellow coloration. The presence of pigments not only determines the color of the product but also plays an important role in the oxidative activity of processed foodstuff, due to their antioxidant nature in the dark and pro-oxidant activity in the light (Oueslati, Anniva, Daoud, Tsimidou, & Zarrouk, 2009). Those pigments are also important in olive oil stability. Table 1 presents the changes in pigment content, mainly the chlorophyll fraction concentration, which decreased gradually with the storage time. Chlorophylls decreased significantly ($p < 0.001$) after 6 days from 3.03 ± 0.11 to 2.24 ± 0.08 ppm in closed plastic bags and after 11 days ($p < 0.001$) from 3.03 ± 0.11 to 2.31 ± 0.06 ppm in open perforated plastic boxes. Nonetheless, the carotenoid content did not change significantly only after 11 days ($p < 0.05$) from 1.27 ± 0.10 to 1.07 ± 0.11 ppm in closed plastic bags and after the same period of storage ($p < 0.05$) from 1.27 ± 0.10 to 1.13 ± 0.03 ppm in open perforated plastic boxes (Table 1).

### 3.1.6. Sensory quality

In storage conditions after 25 days, all kinds of degenerative processes and pathogenic infections develop in the fruit in a short period of time. Oils obtained from these fruits are characterized by an undesirable sensory attributes called "fusty", "musty" and "winey".

The results of the sensory evaluation of olive oils obtained after fruit storage in closed plastic bags and open perforated plastic boxes are summarized in Table 2.

The oil extracted immediately after harvesting had fruity flavor and no negative attributes, and was therefore categorized as extra-virgin olive oil. Yet, the storage of olives in plastic bags or perforated plastic boxes is a common practice during olive fruit processing, but it had negative consequences on the sensory quality of olive oil. Table 2 presents the significant differences between oil extracted from the olives stored in plastic bags and those stored

in perforated plastic boxes in 25 days. The oils obtained from the olives stored in perforated plastic boxes for 6 days had a median of defect less than 3.5 and a median of fruitiness above zero (>0) within the limit of the virgin olive oil category (the median is the midpoint of an ordered set of odd numbers, or the mean of two midpoints of an ordered set of even numbers (COI, 2011a)). Regarding the period of 11 days, the oil becomes unsuitable for consumption and is designated as lampante virgin olive oil.

By contrast, the oil extracted after 6 days from the olives kept in plastic bags had a median of defect up to 3.5 and a median of fruitiness not above zero (= 0). This oil was classified as lampante virgin olive oil that cannot be commercialized for human consumption without previous refining.

Therefore, the holes in the perforated plastic boxes are very important to allow the ventilation of the olives, delaying the fermentation processes in the stored fruit. Hence, perforated plastic boxes of olive fruits may be beneficial, possibly increasing oil yield and moderating the sensory quality of the oils.

### 3.1.7. FAAEs and waxes

Fatty acid alkyl esters (FAAEs) mainly ethyl (FAEEs) and methyl esters (FAMEs) are a family of natural neutral lipids present in olive oils and formed by the esterification of free fatty acids (FFAs) with low molecular alcohols, such as methanol and ethanol. They can easily occur in an acid medium and catalyzed by the presence of certain enzymes. The method is based on solid–liquid chromatography (LC) by traditional glass column for isolating the fraction containing alkyl esters and waxes, with the aim to assign the evaluated sample to the commercial category of EVOO. Indeed, for EVOO, the concentration of the sum of FAMEs and FAEEs [Σ (FAMEs + FAEEs)] cannot exceed 75 mg $kg^{-1}$. If Σ (FAMEs + FAEEs) is between 75 and 150 mg $kg^{-1}$, the oil can be considered as EVOO only if the ratio of FAEEs/FAMEs (RFF) is ⩽1.50 (COI, 2009). It is interesting to underline that the European law (EEC, 2011) permits a same amount of alkyl esters for EVOO (between 75 and 150 mg $kg^{-1}$) and RFF is lower or equal to 1.50, as the FAMEs are typically formed by the technological transformation of overripe olive fruits (Biedermann, Bongartz, Mariani, & Grob, 2008). The concentration of the sum of wax esters cannot exceed 250 mg $kg^{-1}$ in EVOO.

The experimental results of the average content of FAEEs, FAMEs, FAAEs and FAEEs/EAMEs in the oil extracted immediately after harvesting (fresh olives) and in the oils obtained from olives stored at different periods in plastic bags and in perforated plastic boxes are summarized in Table 3.

The control sample (oil extracted immediately after harvesting) was characterized by low concentrations of FAMEs and FAEEs (FAAEs = 14.00 ± 0.57 ppm). The main fatty acid alkyl esters (FAAEs) found in olive oils are those corresponding to palmitic, oleic, and linoleic acids. In the genuine high quality extra-virgin oils, the methyl esters are similar to the corresponding ethyl esters although only methyl palmitate, ethyl palmitate, methyl oleate and ethyl oleate are clearly observed and quantified (Pérez-Camino et al., 2008). The total amount of FAAEs found in this sample does not exceed the quantity of 75.00 mg/kg (maximum acceptable limit by COI). With respect to the oils obtained from olives stored in perforated plastic boxes, the content of FAAEs showed low values which were within the limit of the extra-virgin category between the period of the beginning of storage and 6 days.

On the other hand, the increase of FAAEs level observed in the oils obtained from olives stored in plastic bags was faster and more accentuated than those of oils extracted from olives packed in perforated plastic boxes. The content of FAAEs of oils obtained from olives stored in plastic bags showed low values that were within the limit of the extra-virgin category only before 6 days of storage (FAAEs = 57.60 ± 1.31 ppm) (Table 3).

Concerning the period of 11 days, both cases were classified as lampante olive oil, but the changes of FAAEs contents that occurred in plastic bags are more emphasized. There are clear differences between oil from perforated boxes and plastic bags (Table 3). These differences are accredited to the higher values of FFAs formed by lipolysis of triglycerides and higher values of short chain alcohols by the degradation of the pectins by endogenous pectin-methyl-esterases (methanol) and the aerobic metabolism of micro-organisms (ethanol) (Biedermann et al., 2008). The consequence of fermentation activity was formed by the action of micro-organisms, producing more FAAEs in the samples from plastic bags.

In the lampante virgin olive oils, the FAEEs and FAMEs were much more abundant than in the extra-virgin olive oils, and esters of oleic, palmitic, and linoleic acids were always detected. As opposed to extra-virgin olive oils, the amounts of ethyl esters found in lampante oils were greater than those of methyl esters. Thus, the increase in FAEEs/FAMEs ratio of oil during storage is positively related to the health conditions of olive fruits and is obviously higher if olives undergo hydrolytic and fermentative processes.

The straight chain wax esters are shown to be useful indicators for the quality of olive oil. They are located in the waxy surface layer of the olive and are poorly extracted by the oil derived from fruit pressing. The values of the waxes esters concentrations were very variable, but they remained within the limits of extra quality (Σ Waxes esters after 28 days of storage ⩽250 ppm, maximum acceptable limit by COI) (COI, 2011a). Thus, oils of inferior quality (lampante oils) often contain these wax esters at increased concentrations.

### 3.1.8. Statistical analysis

A highly significant storage time effect was observed for all the studied variables ($p < 0.001$ and $p < 0.01$) except for acidity ($p < 0.05$).

There is also a significant difference in the trends of evolution in the two containers ($p < 0.05$) (Fig. 1) except for some parameters

**Table 2**
Sensory evaluation of olive oils obtained from olive fruits stored in closed plastic bags and open perforated plastic boxes.

| | | EVOO | BAG-3 | BAG-6 | BAG-11 | BAG-17 | BAG-25 | BOX-3 | BOX-6 | BOX-11 | BOX-17 | BOX-25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Positive attributes | Fruity | 5.20 ± 0.02 | 2.90 ± 0.01$^{***}$ | – | – | – | – | 3.73 ± 0.01$^{+++}$ | 1.51 ± 0.02$^{+++}$ | – | – | – |
| | Bitter | 3.50 ± 0.01 | 1.55 ± 0.02$^{***}$ | – | – | – | – | 2.02 ± 0.02$^{+++}$ | 1.00 ± 0.02$^{+++}$ | – | – | – |
| | Pungent | 3.00 ± 0.01 | 1.52 ± 0.02$^{***}$ | – | – | – | – | 1.50 ± 0.02$^{+++}$ | 1.03 ± 0.02$^{+++}$ | – | – | – |
| Negative attributes | Fusty | – | – | 2.03 ± 0.01 | 3.53 ± 0.01 | 5.03 ± 0.01 | 8.20 ± 0.02 | – | – | – | 3.03 ± 0.01 | 6.04 ± 0.01 |
| | Musty | – | 3.03 ± 0.01 | 3.80 ± 0.02 | 6.22 ± 0.01 | 7.50 ± 0.01 | 7.53 ± 0.01 | 1.53 ± 0.01 | 3.83 ± 0.02 | 4.54 ± 0.01 | 5.60 ± 0.01 | 6.20 ± 0.02 |
| | Winey-Vinegary | – | – | – | 2.50 ± 0.01 | 4.03 ± 0.01 | 4.00 ± 0.01 | – | – | 1.02 ± 0.01 | 2.03 ± 0.02 | 4.03 ± 0.02 |

Each value represents the mean of three determinations ($n = 3$) ± standard deviation. Significant differences between the extra-virgin olive oil (EVOO) and the olive oil obtained from olive fruits stored in closed plastic bags (BAG) groups $^{***}p < 0.001$, and between the EVOO and the olive oil obtained from olive fruits stored in open perforated plastic boxes (BOX) groups $^{+++}p < 0.001$.

**Table 3**
Fatty acid alkyl esters and waxes of olive oils obtained from olives stored in closed plastic bags and open perforated plastic boxes.

| | EVOO | BAG-3 | BAG-6 | BAG-11 | BAG-17 | BAG-25 | BOX-3 | BOX-6 | BOX-11 | BOX-17 | BOX-25 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FAMEs (ppm) | 9.00 ± 0.31 | 30.70 ± 0.44$^{***}$ | 62.80 ± 1.22$^{***}$ | 102.00 ± 1.48$^{***}$ | 150.20 ± 1.63$^{***}$ | 232.30 ± 1.56$^{***}$ | 15.80 ± 0.81$^{+++}$ | 36.30 ± 1.08$^{+++}$ | 57.80 ± 1.13$^{+++}$ | 83.90 ± 1.22$^{+++}$ | 149.30 ± 1.56$^{+++}$ |
| FAEEs (ppm) | 5.00 ± 0.26 | 26.90 ± 0.87$^{***}$ | 65.40 ± 1.07$^{***}$ | 115.00 ± 0.78$^{***}$ | 240.20 ± 1.31$^{***}$ | 442.00 ± 2.42$^{***}$ | 11.80 ± 1.17$^{+++}$ | 37.50 ± 1.26$^{+++}$ | 76.20 ± 0.81$^{+++}$ | 137.30 ± 1.04$^{+++}$ | 287.40 ± 1.15$^{+++}$ |
| Total FAAEs (ppm) | 14.00 ± 0.57 | 57.60 ± 1.31$^{***}$ | 128.20 ± 2.29$^{***}$ | 217.00 ± 2.26$^{***}$ | 390.40 ± 2.94$^{***}$ | 674.30 ± 3.98$^{***}$ | 27.60 ± 1.98$^{+++}$ | 73.80 ± 2.34$^{+++}$ | 134.00 ± 1.94$^{+++}$ | 221.20 ± 2.26$^{+++}$ | 436.70 ± 2.71$^{+++}$ |
| FAEEs/FAMEs | 0.55 | 0.87 | 1.04 | 1.12 | 1.59 | 1.90 | 0.74 | 1.03 | 1.31 | 1.63 | 1.92 |
| Waxes (ppm) | 81.60 ± 0.98 | 94.90 ± 1.37$^{***}$ | 106.40 ± 1.51$^{***}$ | 117.60 ± 0.57$^{***}$ | 130.70 ± 1.55$^{***}$ | 148.00 ± 1.78$^{***}$ | 86.40 ± 0.94$^{++}$ | 90.70 ± 1.73$^{+++}$ | 103.40 ± 1.44$^{+++}$ | 112.50 ± 1.05$^{+++}$ | 124.30 ± 0.96$^{+++}$ |

FAMEs: fatty acid methyl esters; FAEEs: fatty acid ethyl esters; FAAEs: fatty acid alkyl esters. Each value represents the mean of three determinations ($n = 3$) ± standard deviation. Significant differences between the extra-virgin olive oil (EVOO) and the olive oil obtained from olive fruits stored in closed plastic bags (BAG) groups $^{***}p < 0.001$, and between the EVOO and the olive oil obtained from olive fruits stored in open perforated plastic boxes (BOX) groups $^{++}p < 0.01$; $^{+++}p < 0.001$.

($p > 0.05$) such as acidity, FAEEs and for some sensorial attributes (fruity, bitter and winey-vinegary).

These $p$-values show that the loss in oil quality is mainly related to the storage time effect rather than to that of the container type. Besides, the analysis of variance of all the analyzed parameters of Chemlali olive oils obtained after different periods of stored olives in the two studied containers are shown in Fig. 1. It indicates that the quality of olive oil obtained from the olives stored in open perforated plastic boxes showed better physicochemical and organoleptic characteristics than that of the olive oil obtained from the olives storage in closed plastic bags.

### 3.2. Part II: blends of extra-virgin olive and deodorized low-quality olive oils

The blend between extra-virgin olive oil and mild thermal deodorized oil, do not produce easily-detectable modifications of the chemical composition. Indeed, the parameters usually checked as quality indicators, such as triacylglycerols composition, sterols, and newly formed steroid hydrocarbons are not appreciably altered by the blending (Saba, Mazzini, Riffaelli, Mattei, & Salvadori, 2005). The most reliable technique seems to be the determination of fatty acid methyl and ethyl esters (fatty acid alkyl esters, FAAEs) as methyl esters of fatty acid (FAMEs) and the ethyl esters of fatty acids (FAEEs) which are present in the waxy fraction of olive oils (Biedermann et al., 2008; Mariani & Bellan, 2008). In good quality EVOOs, FAMEs and FAEEs are present in very small amounts, yet they are present in higher amounts in virgin, lampante olive oils (Mariani & Bellan, 2011) and in the second olive processing oil (the so-called repaso) (Cerratini et al., 2011).

This study sheds the light on the effect of the incorporation of mild deodorized olive oils with variable amounts in extra-virgin olive oil by the determination of fatty acid methyl and ethyl esters contents. Moreover the effectiveness of these chromatographic determinations is investigated to examine the minimum detectable limit for adulterated soft deodorized oil.

#### 3.2.1. Initial compositions

In Table 4, the analytical characteristics of the oils used for making the blends in the experimental work are reported. This Table presents the results obtained from the analysis of the initial

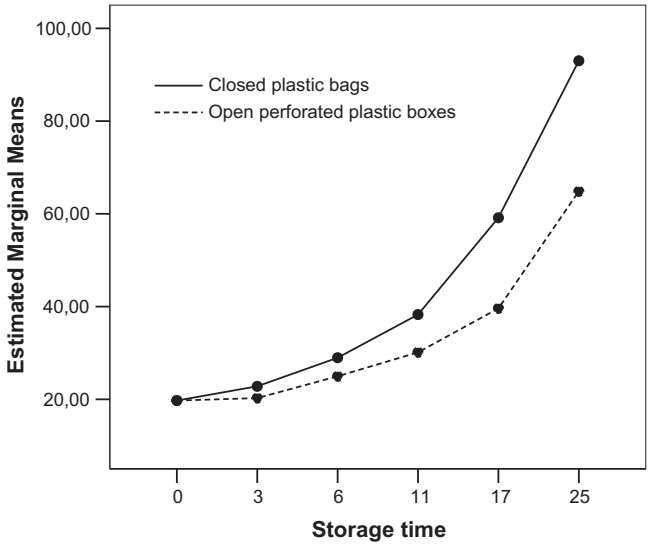


**Fig. 1.** Repeated measures analysis of variance (ANOVA) of all parameters analyzed of Chemlali olive oils obtained after different periods of olives storage in closed plastic bags and open perforated plastic boxes.

compositions of the extra-virgin olive oil and mild deodorized olive oil samples used for this study. FFA, PV, conjugated dienes and secondary oxidation products (carbonylic compounds, aldehydes, and ketones) were measured by extinction value at 232 and 270 nm, while the total of FAAEs was quantified by GC.

The values of the Conventional analyses (acidity, peroxide value and specific extinction coefficient at 232 and 270 nm) of the studied extra-virgin olive oil are below the maximum levels established by the standards of the International Olive Oil Council (COI, 2011a).

In the genuine high quality extra-virgin oils, the methyl esters are similar to the corresponding ethyl esters although only palmitic and oleic are clearly observed and quantified (Pérez-Camino et al., 2008). Table 4 shows low concentrations of the total amount of FAAEs (11.80 ± 0.82 mg/kg) and the average in the FAEEs/FAMEs ratio arising from this extra-virgin olive oil was 0.73. A so-called soft or mild deodorized olive oil employing low temperatures (100–120 °C), is often used to correct the sensorial defects of lampante virgin olive oil, obtained from fermented fruits and have unpleasant sensorial features. The FAEEs and FAMEs were much more abundant than in the extra-virgin olive oil, and esters oleic, palmitic, and linoleic acids were always detected in the low quality oil. Contrary to extra-virgin olive oil, the amounts of ethyl esters found in mild deodorized olive oil were greater than those of methyl esters and present an average ratio of FAEEs/FAMEs over 1.50 (2.50).

### 3.2.2. Identification of EVOO adulteration with mild deodorized olive oil

In order to evaluate the possibility of detecting extra-virgin olive oil (EVOO), the adulteration with low cost oils, binary mixtures containing a 99, 95, 90, 85, 80, 70,60 and 50% of EVOO with a 1, 5, 10, 15, 20, 30, 40 and 50% (*w/w*), respectively of mild deodorized low quality olive oil were prepared. The total amount of methyl and ethyl esters of fatty acids (FAAEs) and the ratio between ethyl and methyl esters (Ratio of FAEEs/FAMEs) of the adulterated extra-virgin olive oil mixed with 1–50% (*w/w*) quantities of mild deodorized olive oil were summarized in Table 5.

As stated above, the profile of fatty acids alkyl esters is almost determinative in clarifying the contamination of extra-virgin olive oil with mild deodorized low quality olive oil. Fatty acids alkyl esters increase significantly from 16.00 ± 0.84 to 187.60 ± 2.17 ppm with the increase in the percentage of adulteration of deodorized olive oil (from 1% to 50%). The addition of more than 30% of soft deodorized olive oil to extra-virgin olive oil would be detected by the amount of FAAEs and the ratio of FAEEs/FAMEs. However, the concentration of the sum of FAMEs and FAEEs is between 75.00 and 150.00 mg/kg (122.10 ± 1.24 mg/kg) but the ratio of FAEEs/FAMEs is >1.50 (1.55): maximal value limited by the COI. Therefore, FAAEs can be considered as a good marker of

**Table 4**
Analytical characteristics of the oils used for making the blends.

| Parameters | EVOO | DO |
|---|---|---|
| FFA (% C18:1) | 0.32 ± 0.01 | 1.69 ± 0.01*** |
| PV (meq/kg) | 6.12 ± 0.08 | 8.20 ± 0.11*** |
| $K_{232}$ | 1.66 ± 0.05 | 3.86 ± 0.09*** |
| $K_{270}$ | 0.12 ± 0.02 | 0.36 ± 0.06*** |
| FAMEs | 6.80 ± 0.61 | 108.30 ± 1.23*** |
| FAEEs | 5.00 ± 0.21 | 271.70 ± 0.86*** |
| Total FAAEs (ppm) | 11.80 ± 0.82 | 380.00 ± 2.09*** |
| FAEEs/FAMEs | 0.73 | 2.50 |

FFA: free fatty acids; PV: peroxide value; $K_{232}$ and $K_{270}$: spectrophotometric indices; FAMEs: Fatty acid methyl esters; FAEEs: Fatty acid ethyl esters; FAAEs: Fatty acid alkyl esters. Each value represents the mean of three determinations ($n$ = 3) ± standard deviation. Significant differences between the extra-virgin olive oil (EVOO) and deodorized oil (DO) groups ***$p$ < 0.001.

**Table 5**
Content of FAAEs in the mixtures of extra-virgin olive oil (EVOO) with deodorized olive oil (DO).

| | g of DO added to EVOO in 100 g of oil mixture | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 100 | |
| FAMEs (ppm) | 6.80 ± 0.61 | 8.20 ± 0.34* | 16.20 ± 0.58*** | 21.00 ± 0.36*** | 30.30 ± 0.27*** | 35.20 ± 0.42*** | 47.80 ± 0.55*** | 53.70 ± 0.77*** | 66.40 ± 0.51*** | 108.30 ± 1.23*** | |
| FAEEs (ppm) | 5.00 ± 0.21 | 7.80 ± 0.50*** | 18.00 ± 0.37*** | 26.40 ± 0.67*** | 41.70 ± 1.09*** | 50.00 ± 0.31*** | 74.30 ± 0.69*** | 87.60 ± 0.41*** | 121.20 ± 1.66*** | 271.70 ± 0.86*** | |
| Total FAAEs (ppm) | 11.80 ± 0.82 | 16.00 ± 0.84** | 34.20 ± 0.95*** | 47.40 ± 1.03*** | 72.00 ± 1.36*** | 85.20 ± 0.73*** | 122.10 ± 1.24*** | 141.30 ± 1.18*** | 187.60 ± 2.17*** | 380.00 ± 2.09*** | |
| FAEEs/FAMEs | 0.73 | 0.95 | 1.11 | 1.25 | 1.37 | 1.42 | 1.55 | 1.63 | 1.82 | 2.50 | |
| Classification | EVOO | EVOO | EVOO | EVOO | EVOO | EVOO | VOO | VOO | VOO | DO | |

FAMEs: fatty acid methyl esters; FAEEs: fatty acid ethyl esters; FAAEs: fatty acid alkyl esters; VOO: virgin olive oil. Each value represents the mean of three determinations ($n$ = 3) ± standard deviation. Significant differences between the extra-virgin olive oil (EVOO) and the mixtures of extra-virgin olive oil with deodorized olive oil (DO) groups *$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001.

low quality olive oil subjected to soft deodorization, and to detect adulteration of EVOO with DO higher than 30%. These results are in accordance with the results obtained by Caponioa et al. (2011). An investigation was carried out to evaluate the use of High performance size-exclusion chromatography (HPSEC) of polar compounds of refined, mild deodorized, extra virgin olive oils as well as of their blends. They classified mixtures containing up to 25 g/100 g of DO as EVOO; all other mixtures (those containing more than 25 g/100 g of DO) were classified as deodorized oils.

## 4. Conclusion

Inappropriate practices during the olive oil extraction process and bad quality of the olive fruits promote the formation of hydrolytic and oxidative deteriorations confirmed by the high quality indices values, concentrations of FAAEs and sensory characteristics defects. It is obvious that the fruits storage in open perforated plastic boxes prevent the fast alteration that is produced in oils extracted from fruits stored in closed plastic bags. Indeed, the holes in the boxes are very important to allow for ventilation of the olives, delaying the fermentation processes in the stored fruit. Moreover, the use of boxes for the storage of olives intended for oil would mean a significant improvement in quality for the olive oil industry.

The determination of the total amount of methyl and ethyl esters of fatty acids could be used as a parameter for the detection of fraud of extra-virgin olive oil with 30% of mild deodorized low-quality olive oil.

## Acknowledgements

We would like to thank the ''Ministère de l'Enseignement Supérieur et de la Recherche Scientifique'' and the ''Ministère de l'Agriculture'' (ONH-Sfax), Tunisia, for their support of this research work. We are also grateful to Professor Ahmed REBAÏ, Head of the Microorganisms and Biomolecules Laboratory, at the Centre of Biotechnology of Sfax (CBS), Tunisia, for his help with statistical analyses. We also wish to extend our thanks to Mrs. Leila MAHFOUDHI, an English teacher at the Sfax Faculty of Science, Tunisia, for having proofread this paper.

## References

Ammar, S., Zribi, A., Ben Mansour, A., Ayadi, M., Abdelhedi, R., & Bouaziz, M. (2014). Effect of processing systems on the quality and stability of Chemlali olive oils. *Journal of Oleo Science, 63*, 311–323.

Ammar, S., Zribi, A., Gargouri, B., Flamini, G., & Bouaziz, M. (2014). Effect of addition of olive leaves before fruits extraction process to some monovarietal Tunisian extra-virgin olive oils using chemometric analysis. *Journal of Agricultural and Food Chemistry, 62*, 251–263.

Bendini, A., Cerratini, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A. M., Segura-Carretero, A., Fernandez-Gutierrez, A., et al. (2007). Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. *Molecules, 12*, 1679–1719.

Bendini, A., Cerretani, L., Salvador, M. D., Fregpane, G., & Lercker, G. (2009). Stability of the sensory quality of virgin olive oil during storage: An overview. *Italian Journal of Food Science, 21*, 389–406.

Ben Youssef, N., Ouni, Y., Dabbech, N., Baccouri, B., Abdelly, C., & Zarrouk, M. (2012). Effect of olive storage period at two different temperatures on oil quality of two tunisian cultivars of *Olea europea*, Chemlali and Chétoui. *African Journal of Biotechnology, 11*, 888–895.

Biedermann, M., Bongartz, A., Mariani, C., & Grob, K. (2008). Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. *European Food Research and Technology, 228*, 65–74.

Boskou, D. (1996). *Olive oil: Chemistry and technology*. Champaign, IL: AOCS Press, pp. 85–127.

Caponioa, F., Summoa, C., Bilanciaa, M. T., Paradisoa, V. M., Sikorskab, E., & Gomesa, T. (2011). High performance size-exclusion chromatography analysis of polar compounds applied to refined, mild deodorized, extra virgin olive oils and their blends: An approach to their differentiation. *LWT – Food Science and Technology, 44*, 1726–1730.

Cerratini, L., Bendini, A., Valli, E., Morchio, G., Chiavaro, E., & Lercker, G. (2011). Chemical characterization of refined olive oils and second extraction olive oils (repaso oils) available on the national and international markets. *Rivista Italiana Delle Sostanze Grasse, 88*, 82–88.

Clodoveo, M. L., Delcuratolo, D., Gomes, T., & Colelli, G. (2007). Effect of different temperatures and storage atmospheres on coratina olive oil quality. *Food Chemistry, 102*, 571–576.

COI/T.15/NC No 3/Rev. 6 (2011a). Trade standard applying to olive oils and olive-pomace oils.

COI/T.20/Doc. No 15 /Rev. 4 (2011b). Sensory analysis of olive oil: Method of the organoleptic assessment of virgin olive oil.

COI/T.20/Doc. No 19 /Rev. 3 (2010). Method of analysis spectrophotomètric investigation in the ultraviolet.

COI/T.20/DOC. No 28 (2009). Determination of the content of waxes fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography.

EC Commission Regulation (EEC) (2011). No. 61/2011 of 24 January 2011 amending Regulation No. 2568/91 on the characteristics of olive oil and olive pomace oil and on the relevant methods of analysis. *Official Journal of the European Communities, L 23*, 1–14.

Garcia, J. M., Gutierrez, F., Castellano, J. M., Perdiguero, S., & Albi, M. A. (1996). Influence of storage temperature on fruit ripening and olive oil quality. *Journal of Agricultural and Food Chemistry, 44*, 264–267.

Garcia, J. M., & Yousefi, K. (2006). The postharvest of mill olives. *Grasas y Aceites, 57*, 16–24.

Gutierrez, F., Perdiguero, S., Garcia, J. M., & Castellano, J. M. (1992). Quality of oils from olives stored under controlled atmosphere. *Journal of the American Oil Chemists' Society, 69*, 1215–1218.

Gutierrez, F., Perdiguero, S., Gutierrez, R., & Olias, J. M. (1992). Evaluation of bitter taste in virgin olive oil. *Journal of the American Oil Chemists' Society, 69*, 394–395.

Haddada, F. M., Krichène, D., Manai, H., Oueslati, I., Daoud, D., & Zarrouk, M. (2008). Analytical evaluation of six virgin olive oils from Northern Tunisia. *European Journal of Lipid Science and Technology, 110*, 905–913.

Harwood, J., & Aparicio, R. (2000). *Handbook of olive oil: Analysis and properties*. Gaithersburg, Maryland, USA: An Aspen Publications Inc.

ISO660, International norm, Animal and Vegetable fats and oils (1996). Determination of acid value and acidity (2nd ed.).

ISO3960, International norm, Animal and Vegetable fats and oils (2001). Determination of peroxide value (3rd ed.).

Jabeur, H., Zribi, A., Makni, J., Rebai, A., Abdelhedi, R., & Bouaziz, M. (2014). Detection of Chemlali extra-virgin olive oil adulteration mixed with soybean oil, corn oil, and sunflower oil by using GC and HPLC. *Journal of Agricultural and Food Chemistry, 62*, 4893–4904.

Jafari, M., Kadivar, M., & Keramat, J. (2009). Detection of adulteration in Iranien olive oil using instrumental (CG, NMR, DCS) methods. *Journal of the American Oil Chemists' Society, 86*, 103–110.

Kiritsakis, A., Nanos, G. D., Polymenopoulos, Z., Tomai, T., & Sfakiotakis, E. M. (1998). Effect of fruit storage conditions on olive oil quality. *Journal of the American Oil Chemists' Society, 75*, 721–724.

Mariani, C., & Bellan, G. (2008). Detection of low quality oils in extra virgin olive oils. *Rivista Italiana Delle Sostanze Grasse, 85*, 3–20.

Mariani, C., & Bellan, G. (2011). On the possible increase of the alkyl esters in extra virgin olive oil. *Rivista Italiana Delle Sostanze Grasse, 88*, 3–10.

Méndez, A. I., & Falqué, E. (2007). Effect of storage time and container type on the quality of extra-virgin olive oil. *Food Control, 18*, 521–529.

Pérez-Camino, M. C., Cert, A., Romero-Segura, A., Cert-Trujillo, R., & Moreda, W. (2008). Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. *Journal of Agricultural and Food Chemistry, 56*, 6740–6744.

Olias, J. M., & Garcia, J. M. (1997). Olive. In *Postharvest physiology and storage of tropical and subtropical fruits* (pp. 229–243). Wallingford, UK: CAB International.

Oueslati, I., Anniva, C., Daoud, D., Tsimidou, M. Z., & Zarrouk, M. (2009). Virgin Olive Oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the arid Tataouine zone. *Food Chemistry, 112*, 733–741.

Rabiei, V., Ghorbani, S., & Hajnajari, H. (2011). Effect of temperature and storage period of olive (*Olea europaea cv. Zard*) fruit on olive quality. *Journal of Food Agriculture and Environment, 9*, 74–77.

Saba, A., Mazzini, F., Riffaelli, A., Mattei, A., & Salvadori, P. (2005). Identification of 9 (E), 11 (E)- 18:2 fatty acid methyl ester at trace level in thermal stressed olive oils by GC coupled to acetonitrile CI-MS and CI-MS/MS, a possible marker for adulteration by addition of deodorized olive oil. *Journal of Agricultural and Food Chemistry, 53*, 4867–4872.

Servili, M., Selvaggini, S., Esposto, A., Taticchi, G., Montedoro, G. F., & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A, 1054*, 113–127.

Servili, M., Selvaggini, R., Taticchi, G., Esposto, S., & Montedoro, G. F. (2003). Volatile compounds and phenolic composition of virgin olive oil: Optimization of temperature and time of exposure of olive pastes to air contact during the mechanical extraction process. *Journal of Agricultural and Food Chemistry, 51*, 7980–7988.

Vichi, S., Romero, A., Gallardo-Chacón, J., Tous, J., López-Tamames, E., & Buxaderas, S. (2009). Influence of olives' storage conditions on the formation of volatile phenols and their role in off-odor formation in the oil. *Journal of Agricultural and Food Chemistry, 57*, 1449–1455.

Zribi, A., Gargouri, B., Jabeur, H., Rebaï, A., Abdelhedi, R., & Bouaziz, M. (2013). Enrichment of pan-frying refined oils with olive leaf phenolic-rich extract to extend the usage life. *European Journal of Lipid Science and Technology, 115*, 1443–1453.