Influence of Fruit Ripening and Crop Yield on Chemical Properties of Virgin Olive Oils from Seven Selected Oleasters (Olea europea L.)

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Abstract: The aim of the present research is to investigate the effect of harvest date and crop year on the chemical composition and quality of virgin olive oils extracted from fruits of seven selected oleasters. This study showed that changes in olive oil characteristics are due mainly to fruit maturity while crop season have minor incidence. The majority of the analytical parameters, i.e., peroxide value, UV absorption at 270 nm, phenols, pigments, decreased during ripening, whereas oil content, free acidity and linoleic acid level increased. In general, the antioxidants and the related parameters decreased as olive fruit ripened.

Key words: Virgin olive oil, maturity index, oxidative stability, quality, crop year

INTRODUCTION

Olive oil is obtained from the fruit of olive trees (Olea europaea L.) and is a genuine fruit juice with excellent nutritional, sensorial and functional properties. Today, its biological, nutritional and healthful effects are universally acknowledged (Servili et al., 2004; Morrello et al., 2005).

Olive oil quality is influenced by a great number of factors among which the geographical production area (altitude, soil composition, latitude), the cultivar chosen, the harvest period and extraction procedure, as well as the climatic conditions prevalent in the year of production (Abaza et al., 2005; Ben Temime et al., 2006; Baccouri et al., 2007). During the ripening, several metabolic processes take place in olives with subsequent variations on profiles of some compounds. These changes are reflected on the quality grade, sensorial characteristics, oxidative stability and nutritional value of the obtained product.

Beltrán et al. (2005) suggest that the majority of olive oil produced is not of the best commercial quality, as the fruit has not been picked at the optimal harvest time. This illustrates the need to determine an appropriate maturation stage before processing.

In a previous study, we have evaluated olive oils composition of 150 oleasters from six populations throughout Tunisia. The obtained results showed the performance of seven cultivars yielding high quality oils. So a further study is required for their complete characterization.

The purpose of this study was to investigate the influence of the stage of fruit ripening and crop year on analytical parameters which determine oil quality, in an attempt to establish an optimum harvesting time for the seven selected oleasters growing under Tunisian conditions. The study identifies and explains the changes that take place during maturity stages in olive fruit from the onset of ripening through to full ripeness. A wide range of chemical quality characteristics was assayed as olive oil quality: fatty acid profiles, total phenols, total chlorophylls and carotenoids, free fatty acids, peroxide values and stability. In addition to, oil yield, maturity index, were measured at each harvest time, providing a useful picture of the impact of harvest timing on olive oil quality and yield.

MATERIALS AND METHODS

Plant materials: Seven oleasters were selected among wild tree populations (150 trees) originating from different regions of Tunisia (Mateur, Ichkeul, Enfidha, Grombalia, Sers and Nebeur) after agronomic and chemical evaluations. Olives were collected at biweekly intervals from July when the ripening index was near to zero. Only healthy fruits, without any kind of infection or physical damage, were processed. The period of maintenance in the Experimental Station of Tunis Biotechnology Center (36° 42’ 38.54"N-10° 25’ 49.97"E) (Fig. 1) is 5 years and the age of trees is 6 years. Olive samples studied were harvested at crop seasons 2003/04, 2004/05 and 2005/06.

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Oil extraction: The olives were washed and defoliated, crushed with a hammer crusher and the paste mixed at 25°C for 30 min, centrifuged without addition of warm water and then transferred into dark glass bottle. All samples were stored at 4°C in darkness using amber glass bottles without headspace until analysis.

Maturity index: The maturity index was determined on 100 randomly selected olives in each sample to obtain a numerical value for the olive sample appearance. Olives were cut into half to expose the internal flesh and to permit grading.

The olives were sorted into categories using the following parameters:

0 = Skin is a deep or dark green colour.
1 = Skin is a yellow or yellowish-green colour.
2 = Skin is a yellowish colour with reddish spots.
3 = Skin is a reddish or light violet colour.
4 = Skin is black and the flesh is completely green.
5 = Skin is black and the flesh is a violet colour halfway through.
6 = Skin is black and the flesh is a violet colour almost through to the stone.
7 = Skin is black and the flesh is completely dark.

The total number of olives in each category was counted and recorded. The following equation was then applied to determine the maturity index:

\[ \text{Maturity Index (MI)} = (0 \times n_0) + (1 \times n_1) + \ldots + (7 \times n_7) / 100 \]

Where \( n \) is the number of fruits in each category from dark green to dark black (Boskou, 1996). The ripeness index values range from 0 to 7 were classified.

Oil content: Forty-grams of olive fruits were dried in an oven at 80°C to constant weight. The dried olives were crushed and extracted with hexane using a Soxhlet apparatus. The results were expressed as percentage of Dry Matter (DM).

Free acidity, peroxide value and specific ultraviolet absorbance (K232 and K270): Determination of free acidity, peroxide value and ultraviolet absorbance was carried out following the analytical methods described in European Union Commission (EEC) Regulation (2568/91) and later modification of the European Union. Free acidity, given as percent of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with ethanolic potassium. Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq kg⁻¹), was determined as follows: a mixture of oil and chloroform-acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution. K232 and K270 extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with a spectrophotometer.
Rancimat assay: oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metromm Ω), using an oil sample of 3.6 g warmed to 100°C and an air flow of 10 L h⁻¹.

Pigment contents: Oil (7.5 g) was accurately weighted and dissolved in cyclohexane up to final volume of 25 mL. Chlorophylls and carotenoids contents were calculated from the absorption spectra of the oils. The absorption at 670 nm was usually considered to be related to the chlorophylls fraction, pheophytin a being its major component, the dominant pigment in the carotenoids fraction was lutein and the absorption was measured at 470 nm. Thus chlorophylls and carotenoids contents were expressed as mg of pheophytin a and lutein per kg of oil, respectively (Minguez-Mosquera et al., 1991).

Total phenols and ortho-diphenols contents: Total phenols and ortho-diphenols amounts were quantified colorimetrically. Oil (10 g) was dissolved in 25 mL hexane and extracted with 10 mL of a 60:40 (v/v) ethanol water mixture. The extraction process was repeated three times. The Folin-Ciocalteau reagent was added to a suitable aliquot of the combined extracts and the absorption of the solution at 725 nm was measured. Values were given as mg of caffeic acid per kg of oil (Vázquez et al., 1973).

Ortho-diphenols were measured colorimetrically at 370 nm after adding 5% (w/v) sodium molybdate in 50% ethanol to the extract (Vázquez et al., 1973). Results were given as milligrams of caffeic acid per kilogram of oil.

Fatty acid composition: The analytical methods for the determination of fatty acid composition were described in regulation EEC 2568/91. Fatty acids were converted to fatty acid methyl esters before analysis by shaking off a solution of 0.2 g oil and 3 mL of hexane with 0.4 mL of 2N methanolic potassium hydroxide and analyzed by a Hewlett-Packard (HP 4890D) chromatograph, equipped with a capillary column (Supelcowax: 30 μm; 0.25 μm), an injector split-splitless, and a FID detector. The carrier gas was nitrogen with a flow rate of 1 mL min⁻¹. The temperatures of the injector, the detector and the oven were held at 230, 250 and 210°C, respectively. The injection volume was 1 μL.

Statistical analysis: All analyses were carried out in triplicate and the results are reported as mean values. Data were compared on the basis of SD of the mean values. In addition, Duncan's multiple range tests were used to determine significant differences among data. Statistical analysis was performed using the Statistica 5.0 package (StatSoft '97 edition).

RESULTS AND DISCUSSION

Effect of olive ripeness on olive oil quality

Change in oil content during fruit ripening: The evolution of oil content during ripening of olives was similar for all cultivars with the amount of oil content tending to increase according to increased Maturity index. It reached a maximum at mid maturity and then decreases (Bacchetti et al., 2007). There was a general trend at the end of the season for the oil content to decrease (Fig. 2), probably due to the degradation of the oil that would be occurring at this stage of the season. Numerous studies show that during the ripening period, oil percentage increases dramatically during early fruit ripening and declines slightly as fruit becomes over ripe (Salvador et al., 2001).

Change in acidity during fruit ripening: An increase was observed in free acidity as ripening progressed (Fig. 3A). The same behaviour has been observed for other varieties, such as Arbequina (Garcia et al., 1996), Picual (Gutiérrez et al., 1999) and Correggiolo (Rotondi and Magli, 2004).

The acidity increased from 0.1-0.15 at a Maturity index of about 1 to 0.2-0.3 at a Maturity index close to 5. In all samples studied, the free acidity was much lower than the upper limit of 0.8 (IOOC, 2003) established for the best commercial quality olive oil, designed extra virgin. Olives

![Fig. 2: Changes in oil content of olives during fruit ripening. Error bars show the variations of three determinations in terms of SD](image-url)
at a later stage of ripening undergo an increase in enzymatic activity, especially by lipolytic enzyme (Gutiérrez et al., 1999) and are more sensitive to pathogenic infections and mechanical damage. The relatively low free acidity observed in the oil samples studied, is due to healthy fruits and to their rapid processing.

Change in the peroxide values during fruit ripening: The oils from olives at more advanced stages of maturity showed lower peroxide values (Fig. 2B). The changes in peroxide index were similar in all cultivars: A marked decrease during ripening (Fig. 3B). These results are in agreement with those of Matos et al. (2007).

Change in the coefficients of specific extinction $K_{232}$ and $K_{270}$ during fruit ripening: The specific extinction coefficient at 232 nm wavelength, $K_{232}$, is related to the primary oxidation of oil and is an indication of conjugation of polyunsaturated fatty acids, whereas $K_{270}$ is an indication of carbonylic compounds (aldehydes and ketones) in olives and is related to the secondary oxidation products (Boskou, 1996). UV-specific extinction determination permits an approximation of the oxidation process in unsaturated oils (Gutiérrez et al., 1999). The values of these parameters were all good in oils analyzed and were below the limits established for extra virgin olive oils (IOOC, 2003). The low values of $K_{232}$ and $K_{270}$ confirmed the good overall quality of these oils at each olive ripening stage. The values of $K_{132}$ and $K_{270}$ extinction coefficients showed a slight decrease during ripening (Fig. 3C and D). These results are in accordance with those reported by Matos et al. (2007) for other varieties, such as Madural and Verdeal transmontana.

Change in fatty acid composition during fruit ripening: Changes in fatty acids levels during maturation process showed very similar trends across all cultivars (Fig. 4).
Oleic acid, quantitatively the most important fatty acid, increased dramatically in the first weeks of development and then plateaued at high Maturity index values in all cultivars. Linoleic acid content was high at the beginning of fruit development followed by a rapid decrease and then shows a gradual increase until the end of the fruit ripening, as described in general, for several olive varieties (Salvador et al., 2001; Baccour et al., 2007). The increase in linoleic acid content is due to the fact that, besides the continuing biosynthesis of triglycerides, with the formation of oleic acid, the enzyme oleate desaturase is active, transforming oleic acid into linoleic (Gutierrez et al., 1999).

At the beginning of fruit development, palmitic acid content was high, followed by an extreme decrease, then it remained unchanged until the end of fruit development. The drop in the percentage of palmitic acid observed during ripening is in accordance with results described for all olive cultivars. The fatty acid composition of olive oil is an important parameter in the length of shelf life that is quantitatively affected by two main factors: The olive variety used in the production of the oil and the ripening stage at which the olives are harvested (Beltran et al., 2005).

**Change in total phenols during fruit ripening:** Phenols and tocopherols are recognised as antioxidant compounds and their presence in olive oils has been related to their general properties, improving stability, nutritional value and sensorial properties (Servili et al., 2004). The studied oils had very high levels of phenolic compounds at early maturity which decreased as the fruit matured. For each cultivar, there was virtually a linear reduction with time of ripening (Fig. 5A). These results are in accordance with those reported by other authors (Rotondi et al., 2004; Beltran et al., 2005).

**Change in O-diphenols content during fruit ripening:** The O-diphenol family can be identified as the main source of the overall antioxidant activity of extra virgin olive oils (Servili et al., 2004). Figure 5B shows how O-diphenols content decrease during ripening, in agreement with the results obtained by Rotondi and Magli (2004). The change in the O-diphenols was parallel to that in the total phenols and similarly showed a good correlation with stability. Total O-diphenols decreased, as ripening of the olives progressed. The components of this fraction may therefore play a major role in ensuring the preservation of the oils and influencing their organoleptic characteristics.

**Change in oxidative stability during fruit ripening:** Oxidative stability is an important parameter to evaluate the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative degeneration, the main cause of their alteration. In all cultivars, oil stability decreased during ripening (Fig. 6). The decrease was greater in the cultivar H3 than for the other cultivars. This decrease in stability is explained by the loss of natural antioxidants, as shown later. These results are in accordance with those of Beltran et al. (2005). The highest stability was shown by the oils extracted from the cv. H3 followed by the oils from cv. ZI2, SB12 and ZI1. The oils
from cv. MAT7 and MAT10 occupied, in that order; an intermediate position and the lowest mean value was shown by the oils from the cv. MAT22, although the oil extracted from black fruits of this variety showed the poorest stability. The resistance to oxidative deterioration is usually attributed to two main reasons: (i) the fatty acid composition that, in the case of olive oil, is characterized by a high monounsaturated/polyunsaturated fatty acid ratio (Abaza et al., 2005) and (ii) the pool of minor compounds of powerful antioxidant activity which, in this case, is constituted mainly by tocopherols and polyphenols but also by chlorophylls and carotenoids. Antioxidants act in scavenging free radicals and also chelating metal ions that initiate free radical reactions (Leonardis et al., 2002).

**Change in chlorophylls and carotenoids pigments during fruit ripening:** In addition to their antioxidant activities, the pigments are responsible for the oil colour, which is one of the factors that influence selection by consumers. The content of chlorophyll pigments (Fig. 7A) and carotenoids (Fig. 7B) decreased markedly during ripening in all cultivars. These results agree with the findings of other authors (Beltran et al., 2005). The colour change to spotted olive that occurs during the stage of ripening 2.5-3 is explained not only by the sharp drops in these two pigment types but also by the formation of other coloured compounds, such as anthoeyanins (Leonardis et al., 2002).

Selection of new olive cultivars with a good oil quality among wild olives may be considered as a way to diversify our olive genetic resources and to select new cultivars for oil production under Tunisian conditions. Although the study was carried out in Tunisia, the methodology might be applied to other countries with wild olive trees, in order to contrast productivity and oil quality with those resulting from native cultivars and transplanted foreign varieties.

As the maturation process continues, a number of changes, both physical and chemical, occur within the fruit (Beltran et al., 2005). The rate of these changes varies according to cultivar. They include changes in the fatty acid profile, declining phenolic compound levels, changing phenolics profiles and reducing pigments levels. Changes in these components are commercially relevant as they have a significant effect on the stability (shelf life potential) and sensory characteristics of the oil (Leonardis et al., 2002).

The oil content, as expected, increased in a sigmoidal fashion, with a slow and continuous increase toward the end of maturity. The point at which the rapid increase in
oil levels out provides a good indicator for determining optimum time of harvest. The maximum amount of oil was found in the second period (end of November). Therefore, given that at this stage oil quality has also reached a very good level, the results suggest that the end of November (Maturity index: 3-4.5) is the best harvest time because it allows the combination of the highest production quantity and quality.

Results showed that ripening process had no significant influence on analytical parameters (free acidity, peroxide values and ultraviolet absorbance), which are basically affected by factors causing damage to the fruits (e.g., olive fly attacks or improper systems of harvesting, carriage and storage of olives) (Salvador et al., 2001, 2003; Rontondi and Magli, 2004).

These findings are valuable in determining acceptable times of harvest to ensure fatty acids are within the IOOC limits for extra virgin olive oil. Maturity is often a compromise between oil quality and quantity but is a key factor in determining the style of oil produced. It can even have a greater influence on oil quality of the variety. For each oleaster, there is about 3 or 4 weeks of ideal harvest period to reach its best quality.

Influence of crop yield on olive oil quality: Mean data for the three crop years studied are shown in Table 1. All the oils produced and analysed (Table 1) showed very low values for the regulated physicochemical parameters evaluated (acidity 6.08; peroxide index ≤ 20 meq O2/kg; K270 ≤ 0.22; K222 ≤ 2.5), with all of them falling within the extra virgin category, as stated by Regulation (IOOC, 2003).

The fatty acid composition has previously been used by a number of authors as a parameter for oil classification (Rotondi and Magli, 2004; Bacceux et al., 2007). It is an essential aspect of the qualitative assessment of olive oil. The extra virgin olive oils of the seven analysed samples present a very good fatty acid composition (Table 1) which respect the established limits (IOOC, 2003). The results obtained confirmed the variability of the fatty acid composition of virgin olive oils; this variability is well known and was attributed to varietal factor.

The Rancimat method is an accelerated stability test that provides very useful information about the resistance of oil to oxidation. The oxidative stability of the studied olive oil samples varies widely according to the cultivar (Table 1). It ranged from a minimum of 31 h to a maximum of 85 h. Aparicio et al. (1999) studied the levels of contribution of different olive oil constituents on stability and they deduced that the contribution of phenolic compounds was around 51%, fatty acids 24% and in less percentage α-tocopherol, carotenoids and chlorophyll. Although some authors have used the main fatty acid levels to differentiate olive oils according to the year of production (Uceda and Hermoso, 2001; Beltrán et al., 2005).

Olive oil is the only vegetable oil that contains appreciable amounts of phenols acting as antioxidant substances and conferring to it a greater stability against oxidation during storage since they exert a scavenger effect on peroxyl radicals (Servili et al., 2004). H3 oil has the highest contents for phenols and O-diphenols (435 and 217 mg kg⁻¹, respectively), whereas MAT22 oil has the lowest ones (186 and 105 mg kg⁻¹, respectively).

The studied oleasters produce olive oils with good quality characteristics in terms of natural antioxidants, oxidative stability and fatty acids. Results showed that analytical parameters are sharply influenced by olive genotype. While the crop season is not a critical variable, the chemical composition seem unchanged from one
Table 1: Quality indices of studied virgin olive oils from crop seasons 2003/04 to 2005/06

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<tr>
<th>Oilers</th>
<th>Palmitic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Phenol content</th>
<th>e-Alpha Oleanol</th>
<th>Cationic stability (%)</th>
<th>Oxidative stability (%)</th>
<th>Chlorophyll a (mg/L)</th>
<th>Carotenoid (mg/L)</th>
<th>Furanic values</th>
<th>Activity</th>
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<td>ID3</td>
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<td>Z12</td>
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Error bars show the variation of three determinations in terms of SE. The numbers followed by the same letter(s) are not significantly different at 0.05%.

This research describes chemical composition of monovarietal virgin olive oils, establishing changes due to ripening process and crop year, the main agronomical factors. The major variation in oil quality and oil yield between the studied cultivars is due to fruit maturity. On the basis of the behaviour of the analytical parameters studied, in particular free acidity, peroxide value, stability, oil and phenol contents, the best stage of maturity of the 7 new olive cultivars for processing would be to ripen index lower than 3 and lower 4.5. These results improve our knowledge about the effect of ripening and crop year on olive oil quality that could help to establish the optimum fruit harvesting date according to the nutritional, sensorial and commercial advantages.

CONCLUSIONS

REFERENCES


