The evolution of Arbequina olive oil quality during ripening in a commercial super-high density orchard in north-east Spain

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The objective of this work was to follow the maturity of the olives in a commercial Arbequina super-high density orchard and to study the evolution of the olive oil quality.
For this objective, the physicochemical, nutritional and sensory parameters were studied.
The free acidity was found to be similar during ripening whereas the peroxide index and K_{232}, K_{270} decreased.
The total phenol content and oxidative stability showed a similar trend. First, it increased and then decreased.
α-tocopherol, saturated fatty acid and pungency decreased.
The polyunsaturated content increased while the MUFAS/PUFAS ratio decreased.
In 2010, the crop maturity was quicker, and the maturity indexes were higher than in the 2009 crop for the same picking date. Even in the same orchard, the maturity is changing in each crop season. For this reason, it is necessary to choose the optimal harvesting date.
Keywords: super-high density, Arbequina, olive oil, quality, ripening, commercial orchard.
Running title: Olive oil in commercial high density orchard

Evoluzione, durante la maturazione, della qualità dell'olio d'oliva Arbequina in un frutteto commerciale di super-alta densità del nord-est della Spagna.

L'obiettivo di questo lavoro è stato quello di seguire la maturazione delle olive Arbequina in un frutteto commerciale di super-alta densità e di studiare l'evoluzione della qualità dell'olio d'oliva.
Per questo obiettivo, sono stati studiati i parametri chimico-fisici, nutrizionali e sensoriali.
L'acidità libera è risultata essere simile durante la maturazione mentre indice di perossido, K_{232} e K_{270} sono diminuiti.
Il contenuto totale di fenoli e la stabilità ossidativa hanno mostrato una tendenza simile. Prima sono aumentate e poi diminuite.
α-tocoferolo, acidi grassi saturi e piccante sono diminuiti.
Il contenuto di polinsaturi è aumentato mentre il rapporto MUFAS/PUFAS è diminuito.
Nel 2010, la maturazione del raccolto era più veloce, e gli indici di maturazione delle colture erano più alti che nel 2009 nella stessa data di raccolta.
Nello stesso frutteto, la maturazione sta cambiando ad ogni stagione di raccolto. Per questo motivo, è necessario scegliere la data ottimale di raccolta.
Parole chiave: densità super-alta, Arbequina, olio di oliva, qualità, maturazione, frutteto commerciale.

INTRODUCTION

Olive oil is a fruit juice with great nutritional, sensory and commercial qualities. It is a healthy food because of its high levels of unsaturated fatty acids (mainly oleic acid) and the presence of minor components, such as the natural antioxidant α- tocopherol [1].

The quality and chemical composition of virgin olive oil are affected by many factors. These factors include: the cultivar and extraction process [2], the climatic conditions during the production year and the geographic production area [3, 4], the production systems—traditional, intensive [5] or super intensive systems [6]. The intensive cultivation systems characterized by high (200-600 trees ha^{-1}) or super-high-plant-density orchards (1200–2500 trees ha^{-1}), have been introduced in producer countries. The most used cultivar for these types of orchards around the world is Arbequina. However, other varieties, such as Arbosana and Koroneiki, are also used.

During maturity (one of the most important factors affecting olive oil quality), some chemical changes occur inside the drupes, and they will affect the olive oil chemical composition. Subsequently, the fatty acids profile and the levels of polyphenols, tocopherols, and pigments will change with maturation [7]. These changes will also affect the sensory characteristics and shelf life of the olive oil. Several studies have investigated the evolution of olive oil quality from traditional orchards during the ripening stage [8, 9, 10, 11]. Other studies have investigated the changes of the olive oil quality during the ripening of olives from intensive and super intensive crops [12, 13, 14]. However, these studies were done on a single index of maturity [15, 16], on specific dates of sampling or every two weeks but not throughout the maturity process [17] and in other studies, experimental orchards were used [17, 18, 19].

The objective of this study was to follow the maturity of the olives in a commercial Arbequina super-high-plant-density and to see the influence on the olive oil quality in order to choose the appropriate harvest time [20]. To reach this objective, we studied two crop seasons using weekly sampling by monitoring the ripeness index and analytical parameters.

EXPERIMENTAL PART

OLIVE FRUIT SAMPLING

The trial was implemented during the crop seasons 2009 and 2010 in a super-high-plant-density commercial orchard (*Olea europaea L.* cv. Arbequina) located at 220 m altitude in Zaragoza (Spain) with 650 hectares. During the 2010 crop season more rainfall than in 2009 was recorded. In the 2010 crop season the lowest temperature was reached (-5°C). The average temperature was almost 16°C in 2009 and 14°C in the year 2010 crop season (Table I). The orchard (with a frame of 5 m $\times$ 2 m) was irrigated through a drip irrigation system buried at a depth of 45 cm. The irrigation schedule was designed following the FAO method [21]. This variety is very important in this geographic area specially in the high density orchards. Samples from Arbequina trees were handpicked once a week; the samples were taken from the middle of

September to the beginning of December. Each sample consisted of 1,400 olives from 70 trees in perfect sanitary conditions. After the harvest, the weight and maturity indexes of 100 olives were determined, and the olive oil was extracted immediately. The olive weight during the crop season of 2009 was 1.53±0.13 g and in the crop season of 2010 was 2.20±0.15 g.

MATURITY INDEX

The olives were carefully blended and a hundred of each cultivar were randomly taken for determining the maturity index (MI) according to the fruit color of both the skin and pulp [22]. In this system a group of 100 olives was separated in different categories according to the color of the skin and pulp: 0-Deep green; 1-Yellow green; 2-Turning color with reddish spots; 3-Turning color with red or light purple color in all the fruit; 4-Black, without color under the skin; 5-Black, with color in less than the half of the pulp; 6-Black, with color in more than half, but not in the stone; 7-Black, with color in all the pulp. After that the MI was calculated as follows:

$$MI = (ax0+bx1+cx2+dx3+ex4+fx5+gx6+hx7)/100$$

Where a, b, c, d, e, f, g and h are fruit numbers of each category.

OIL CONTENT

A sample of olives was processed in a hammer mill to obtain an olive paste. The oil and moisture content of the olive paste were determined by a near-infrared system, using a NIR FOSS™ OliveScan. Two replicates per sample were performed.

OIL EXTRACTION PROCESS

Oil extraction was performed using Abencor™ laboratory equipment (MC2 Ingenierías y Sistemas, Sevilla, Spain), following the method described by Martínez et al., [23]. The olives of the Arbequina variety were crushed with a hammer mill, and the paste was malaxated at 26°C/30 min and then centrifuged at 1370 g/1 min. After filtration, the samples were stored at -18°C, using amber glass bottles without headspace prior to analysis.

ANALYTICAL DETERMINATIONS

Determinations of the physicochemical parameters (free acidity, peroxide value, and UV absorption coefficients K_{270} and K_{232}) were made, following the methods described in Regulation EEC/2568/91 of the Commission of the European Union [24].

Determination of fatty acids

The fatty acid profile of samples was determined by gas chromatography using a modified fatty acid methyl esters (FAMEs) method, as described by Frega and Bocci [25]. The FAMEs were obtained by shaking a solution of each olive oil sample in n-hexane and 2N methanolic potassium hydroxide. Chromatographic analyses were done, using a Hewlett Packard 5890 gas chromatograph, equipped with a flame ionization detector and a split/splitless injector. The

experimental conditions were: DB-225 column (30 m × 0.25 mm i.d. × 0.15 mm film thickness) (J & W Scientific, Agilent). The injector and detector temperatures were maintained at 250°C. The oven temperature ranged from 190°C (1 min) to 210 at 4°C/min and was maintained for 5 min, then heated to 215 at 3°C/min. Finally, an isotherm was used for 18 min, and nitrogen was used as the carrier gas. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times.

α-Tocopherol determination

A sample of oil in hexane was analyzed by HPLC (high pressure liquid chromatography) with a Zorbax SB-C18 phase reverse column (Agilent), eluted with acetonitrile/water (99:1 v/v), using a flow rate of 1 ml/min. A photodiode matrix detector was used. Chromatograms were registered at 295 nm. The results were expressed as mg of α-tocopherol/Kg oil.

Total phenol content

For the extraction from the olive oil, we used the method described by Favati et al., [26]. The phenols were extracted by Solid Phase Extraction (SPE), using Isolute™ C18 cartridges. The extract was dried in a rotary evaporator, and the residue was dissolved in 5 ml of methanol. For the colorimetric determination, 2.5 ml of extract was mixed with 1.25 ml of Folin-Ciocalteau reagent. Then, after 3 min, 2.5 ml of sodium carbonate was added. The absorption of the solution was measured at 725 nm. The results were expressed as mg gallic acid/Kg oil.

Individual phenols

Phenolic compounds were extracted from the virgin olive oil, following the method of Gutfinger [27]. A Hewlett Packard (Agilent-Series 1100) chromatograph was used for the analysis. The column was a Zorbax SB-C18 (3.5 µm, 150 × 4.6 mm I.D., Agilent Technologies). The HPLC analysis was performed by following the procedure of Montedoro et al., [28]. The eluents were 0.2% aqueous acetic acid (pH 3.1) and methanol. The flow rate was 1.5 ml/min, and the injection volume was 20 µl. The total run time was 60 min. The initial composition was 95% aqueous acetic acid and 5% methanol. The gradient changed as follows: The concentration of methanol was maintained at 2 min. Then, it was increased to 25% in 8 min. Finally, the methanol percentage was increased to 40, 50, and 100% for 10 min at each of the percentages. Initial conditions were reached in 15 min. Chromatograms were obtained at 280 nm and 339 nm. Phenolic compounds were tentatively identified on the basis of their retention times, compared to those of the standard compounds. The quantitative determination was performed using external standards.

Oxidative stability

It was expressed as the oxidation induction time (hours), measured with a Rancimat™ 743 instrument (Metrohm, Switzerland), using 3 g of oil, warmed to 120°C with 20 l h^{-1} air flow. The

induction time is the time it took to reach the break point of the curve. The break point is the intersection point of the two extrapolated straight parts of the curve [29].

Colour coordinates

The CIELAB color space (CIE) was studied with an AvaSpec-1024 spectrophotometer after the spectra were obtained. Illuminant D65 was chosen, along with observer CIE64. The following color coordinates were determined: lightness (L*), redness (a*, red/green), and yellowness (b*, yellow/blue).

Determination of chlorophyll and carotenoid compounds

Chlorophyll and carotenoid contents were obtained from the absorption spectra of the virgin olive oil for each sample (7.5 g) and were dissolved in cyclohexane (25 ml), according to the method of Minguez-Mosquera et al., [30]. The maximum absorption is related to the chlorophyll at 670 nm and to the carotenoid at 470 nm. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin, as a major component in the chlorophyll fraction, and E0=2000 for lutein, as a major component in the carotenoid fraction. The concentrations of chlorophyll and carotenoids were expressed as mg pheophytin and lutein/Kg of oil, respectively.

Sensory analysis

The sensory analysis of the samples were carried out by 10 selected and trained panelists from Aragon's accredited panel and the Zaragoza Faculty of Veterinary Science following the method described in Regulation EEC/640/2008 [31]. The intensities of the positive (fruity, bitter, and pungent) and negative (fusty, winey, musty, muddy, rancid, metallic, and other) attributes were evaluated for each oil sample on a non-structured, scale of 10 cm, anchored by its origin.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statgraphics Plus 5.1. The results were expressed as a mean ± standard deviation of three experiments and as a least squares mean ± 95% confidence interval. The significant differences between the samples were determined by an analysis of variance (One way ANOVA) and Multiple Range Test.

RESULTS AND DISCUSSION

The ripeness index of the olives for the two crop seasons is shown in Table II. In the first date of sampling, this index is near 0, that means a green color for the olives. This value increased until the last sampling in the 2009 crop season which reached a 3.33 value (olives with skin with red spots all over the fruit). In the 2010 crop season, the ripeness index increased, nearly reaching a plateau in the three last samplings at approximately a 5 value (olives with black skin and color

in the pulp for at least half of the fruit). In most of the cases, the significant differences were found between the same picking dates in the two crop seasons. In 2010, the crop maturity was quicker, and the maturity indexes were higher, in comparison to the 2009 crop season for the same picking date. For this reason at the end of the sampling these indexes were higher in the 2010 crop season. For the crop season also significant differences were observed between the different picking dates, as a consequence of the maturity along the time period. The oil content (Table II) along the sampling dates increased reaching in 2009 a 52% oil in the last sampling. For the 2010 crop season, this value also increased, and in the last sampling, the value reached a plateau at approximately 49%. In most of the cases, significant differences were noted between picking dates. Small differences were observed between crop seasons and the % oil at the end of sampling was always between 49% and 52%. Similar values were described by other authors in this olive variety [32].

The regulated parameters (acidity, peroxide value, K_{232} and K_{270}) (Table II) were below the limits established by European regulations for the extra virgin olive oil (EEC, 1991). The acidity was similar during the ripening on the different picking dates. The peroxide index decreased during maturity whereas K_{232} and K_{270} had a similar profile [10,12, 33].

The results obtained for the fatty acid composition is shown in Table III. As expected, the most abundant fatty acid was the monounsaturated oleic acid, which amounted to more than 67% of the total in all of the cases. The MUFAS increased along ripening in the 2009 crop season and decreased in the 2010 crop season. The same behavior was observed for oleic acid. This probably is due to the environmental conditions during the development and maturity of the fruit, since oleic acid is the main fatty acid in olive fruit [32]. In previous works different behavior was observed for the evolution of oleic acid during maturity. For some authors, this content decreases [12, 34, 35]. For other authors, there are no changes [33]. Finally, some authors observed an increase [36]. The temperature during olive oil accumulation has a negative correlation with the oleic acid percentage [37,38]. In some previous works [19] we observed different behaviour in the three studied crop seasons. The PUFAS content increased during sampling, and the MUFAS/PUFAS ratio decreased during maturity, as other authors have described it [39]. SFA decreased during ripening.

The α- tocopherol content is shown in Figure 1. For the first sampling dates, the content was 511 mg/Kg oil in 2009 and 474 mg/Kg oil in 2010. This content decreased drastically during ripening and reached a plateau on the three last sampling dates at approximately 238 mg/Kg oil. No significant differences were observed during the two crop seasons. These results agree with other previous works [19].

The total phenol content for the olive oils during ripening is shown in Table IV. In both crop seasons, this value first increased and then decreased. The maximum value was reached on November 4 during the 2009 crop season (464 mg gallic acid/Kg oil, maturity index 1.67). For the 2010 crop season, the maximum was reached on October 28 (554 mg gallic acid/Kg oil, maturity index 3.17). Significant differences were found for the same picking dates for different

years. In other previous studies, similar results were described but the highest values for total phenol content in the present study was obtained later [18, 19].

The results for the individual phenols are in Table V. Secoiridoids derivatives of hidroxytyrosol (3,4 DHPEA-EDA), tyrosol (p-HPEA-EDA), oleuropein aglicone (3,4 DHPEA-EA) and 3,4 DHPEA-AC follows the same tendency like total phenols (first increases, reaches a maximum and then decreases). 3,4 DHPEA-EDA is the most abundant (590 mg/Kg in the 2010 crop season and 463 mg/Kg in the 2009 crop season). In the 2010 crop season all of them reached the maximum on October 28 (like total phenols). In the 2009 crop season, due to a later maturity, the maximum was not reached on the same date but on the second and third weeks of November. During the ripening, the levels of tyrosol, luteolin, and apigenin generally increased during both crop seasons. In terms of vanillin, coumaric acid and lignans, the overall tendency was that the levels decreased. The others individual phenols did not indicate a clear tendency. A few similar results were described in previous works [40] for Arbequina olive oil harvested in three ripening degrees. Different trends were described in an experimental super-high-density Arbequina orchard [18].

The oxidative stability (Table IV) followed a pattern similar to total phenol content. The induction time increased and then decreased. In other previous studies [18, 19] similar results were described but the highest values for oxidative stability in the present study was obtained later as we had described before for the total phenols content since these compounds contribute to the oxidative stability. Another component affecting this stability is the α-tocopherol content.

In Table VI, the correlation between the ripeness index and pigments are shown. Also, the pigment content is correlated with lightness. The correlation between pigments (carotenoid and chlorophyll) and ripeness index was negative (-0.8 in 2009 and -0.88 in 2010) since along the ripening pigments content they were decreasing drastically [7]. Also, when pigments content decrease lightness increases and for this reason correlations are negative. The highest correlations were obtained in the 2010 crop season. This behavior is due to the capture of the light for the pigments [33].

The results for the sensory analysis are shown in Table VII. In terms of fruitiness, a clear tendency was not observed in the 2009 crop season. A maximum value was observed in the middle of October in the 2010 crop season. In terms of bitterness, during the two crop seasons, there was no clear tendency. However, in the last sampling, the oils were less bitter. The pungency decreased during maturity in the two crop seasons. This trend was different to other previous works [19].

CONCLUSIONS

In this work a complete description of the maturity during a period of approximately three months in a commercial super-high density olive orchard is presented. The progress of the

maturity was followed by the olive oil quality characterization in two crop seasons with 12 samplings.

The different behavior in the different crop seasons is shown. Depending on the climatic conditions of the season the olive maturity changes and as a consequence the olive oil quality is different even in the same orchard.

According to the results obtained, it can be proposed that the conditions in early November are an indication that it is a good harvesting period to obtain high-quality extra virgin olive oil. These days, the oils have the highest phenols content and stability. Also during this period the olive oils have medium scores for the positive attributes (fruitiness, bitterness and pungency), high content of α-tocopherol and a good ratio between MUFAS and PUFAS. The percentage of oil and dry matter is quite high, and the ripeness index has values between 2 and 3 (olives with epidermis with reddish spots or reddish or light violet).

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Table 1. Temperature and rainfall data across the sampling dates during 2009 and 2010 crops.

	Average temperature (°C)	Minimun temperature (°C)	Maximum temperature (°C)	Total rainfall (mm)
2009	15.8	-1.0	35.2	53.8
2010	14.0	-5.3	36.1	90.3

Table 2. Ripeness index, % oil/dry matter and physicochemical parameters of olives and olive oils across the sampling dates during 2009 and 2010 crops.

Picking date	Ripeness index	% Oil / dry matter	Acidity (% acid oleic)	Peroxide value (meq O_2active/Kg oil)	K_{232} (Abs 232 nm)	K_{270} (Abs 270 nm)
2009						
16 Sept	$0.00\pm0.00^{X;A}$	$35.62\pm1.32^{X;A}$	$0.12\pm0.01^{X;DE}$	$7.68\pm0.09^{Y;E}$	$1.57\pm0.03^{Y;DE}$	$0.18\pm0.02^{Y;D}$
23 Sept	$0.36\pm0.08^{X;B}$	$36.96\pm2.65^{X;A}$	$0.11\pm0.00^{X;CDE}$	$6.71\pm0.06^{Y;D}$	$1.58\pm0.02^{Y;E}$	$0.16\pm0.00^{Y;D}$
30 Sept	$0.73\pm0.01^{X;C}$	$39.85\pm0.57^{X;B}$	$0.11\pm0.01^{X;CDE}$	$5.49\pm0.57^{Y;C}$	$1.51\pm0.08^{Y;CDE}$	$0.12\pm0.01^{X;C}$
07 Oct	$1.03\pm0.13^{X;D}$	$42.03\pm0.72^{X;BC}$	$0.11\pm0.01^{X;BCD}$	$4.77\pm0.28^{Y;C}$	$1.42\pm0.13^{X;ABC}$	$0.11\pm0.01^{X;ABC}$
14 Oct	$1.02\pm0.03^{X;D}$	$43.46\pm1.26^{X;C}$	$0.10\pm0.00^{X;ABC}$	$2.77\pm0.68^{X;AB}$	$1.45\pm0.10^{X;BCD}$	$0.10\pm0.01^{X;ABC}$
21 Oct	$1.28\pm0.03^{X;E}$	$43.91\pm0.40^{X;CD}$	$0.11\pm0.00^{X;CDE}$	$2.43\pm0.38^{X;AB}$	$1.30\pm0.03^{X;A}$	$0.09\pm0.01^{X;AB}$
28 Oct	$1.91\pm0.10^{X;G}$	$46.58\pm0.21^{X;E}$	$0.11\pm0.00^{X;CDE}$	$2.11\pm0.38^{X;A}$	$1.32\pm0.06^{X;AB}$	$0.09\pm0.01^{X;A}$
04 Nov	$1.67\pm0.01^{X;F}$	$46.11\pm0.66^{X;DE}$	$0.11\pm0.00^{X;CDE}$	$2.66\pm0.68^{X;AB}$	$1.35\pm0.04^{X;AB}$	$0.11\pm0.02^{Y;ABC}$
11 Nov	$2.31\pm0.21^{X;H}$	$48.08\pm0.31^{X;EF}$	$0.11\pm0.01^{X;BCD}$	$2.09\pm0.37^{X;A}$	$1.39\pm0.07^{X;ABC}$	$0.11\pm0.00^{Y;ABC}$
18 Nov	$2.97\pm0.01^{X;I}$	$49.95\pm0.43^{X;FG}$	$0.09\pm0.01^{X;A}$	$2.99\pm0.67^{X;B}$	$1.39\pm0.05^{X;ABC}$	$0.12\pm0.01^{Y;C}$
25 Nov	$3.15\pm0.01^{X;IJ}$	$51.47\pm0.89^{X;GH}$	$0.09\pm0.02^{X;AB}$	$2.22\pm0.40^{X;AB}$	$1.41\pm0.12^{X;ABC}$	$0.10\pm0.03^{X;ABC}$
02 Dec	$3.33\pm0.13^{X;J}$	$52.32\pm0.46^{Y;H}$	$0.12\pm0.02^{X;E}$	$2.46\pm0.37^{X;AB}$	$1.33\pm0.07^{X;AB}$	$0.11\pm0.01^{Y;BC}$
2010						
16 Sept	$0.73\pm0.04^{Y;A}$	$37.53\pm1.04^{X;A}$	$0.18\pm0.02^{Y;D}$	$5.66\pm0.02^{X;F}$	$1.39\pm0.01^{X;CD}$	$0.11\pm0.00^{X;H}$
23 Sept	$0.92\pm0.11^{Y;AB}$	$42.29\pm0.12^{X;B}$	$0.12\pm0.01^{X;ABC}$	$3.10\pm0.19^{X;BC}$	$1.40\pm0.01^{X;D}$	$0.10\pm0.01^{X;FG}$
30 Sept	$1.09\pm0.13^{X;B}$	$42.22\pm0.61^{X;B}$	$0.12\pm0.01^{X;AB}$	$3.23\pm0.18^{X;C}$	$1.37\pm0.02^{X;BC}$	$0.10\pm0.01^{X;G}$
07 Oct	$1.78\pm0.07^{Y;C}$	$43.79\pm0.85^{X;C}$	$0.13\pm0.01^{Y;C}$	$3.33\pm0.02^{X;C}$	$1.50\pm0.00^{X;E}$	$0.10\pm0.01^{X;G}$
14 Oct	$2.37\pm0.10^{Y;D}$	$45.64\pm0.54^{X;D}$	$0.12\pm0.01^{Y;AB}$	$3.78\pm0.19^{X;D}$	$1.39\pm0.02^{X;CD}$	$0.09\pm0.00^{X;F}$
21 Oct	$2.59\pm0.10^{Y;E}$	$47.96\pm0.59^{Y;EF}$	$0.11\pm0.01^{X;A}$	$4.21\pm0.18^{Y;E}$	$1.39\pm0.01^{Y;CD}$	$0.08\pm0.00^{X;E}$
28 Oct	$3.17\pm0.13^{Y;F}$	$48.77\pm0.81^{X;FG}$	$0.11\pm0.00^{X;A}$	$3.22\pm0.20^{Y;C}$	$1.39\pm0.02^{X;CD}$	$0.10\pm0.00^{X;FG}$
04 Nov	$3.61\pm0.06^{Y;G}$	$47.46\pm0.19^{X;E}$	$0.11\pm0.01^{X;A}$	$2.92\pm0.11^{X;AB}$	$1.31\pm0.03^{X;A}$	$0.04\pm0.00^{X;A}$
11 Nov	$4.75\pm0.14^{Y;H}$	$50.34\pm0.14^{Y;H}$	$0.11\pm0.00^{X;A}$	$2.78\pm0.19^{Y;A}$	$1.30\pm0.01^{X;A}$	$0.05\pm0.00^{X;AB}$
18 Nov	$4.60\pm0.07^{Y;H}$	$48.95\pm0.06^{X;FG}$	$0.11\pm0.00^{Y;A}$	$2.93\pm0.13^{X;AB}$	$1.34\pm0.02^{X;B}$	$0.07\pm0.01^{X;D}$
25 Nov	$5.25\pm0.07^{Y;J}$	$49.61\pm0.44^{X;GH}$	$0.13\pm0.01^{Y;C}$	$3.93\pm0.12^{Y;D}$	$1.37\pm0.01^{X;CD}$	$0.06\pm0.01^{X;BC}$
02 Dec	$4.98\pm0.11^{Y;I}$	$49.10\pm0.77^{X;FGH}$	$0.13\pm0.00^{X;BC}$	$2.71\pm0.07^{X;A}$	$1.39\pm0.02^{X;CD}$	$0.06\pm0.00^{X;CD}$

$^{X-Y}$ For each attribute, different letters for the same picking date indicate statistically significant differences ($p \leq 0.05$) between years.

$^{A-L}$ For each attribute, different letters for the same year indicate statistically significant differences ($p \leq 0.05$) among picking dates.

Table 3. Fatty acid composition of olive oils across the sampling dates during 2009 and 2010 crops.

Picking date	% Oleic Acid	MUFAS	PUFAS	MUFAS/PUFAS	SFA
2009					
16 Sept	69.89±0.08$^{X;A}$	71.59±0.07$^{X;A}$	10.04±0.04$^{X;B}$	7.14±0.03$^{X;D}$	18.38±0.04$^{Y;H}$
23 Sept	70.42±0.02$^{Y;ABCD}$	72.14±0.03$^{Y;BCD}$	9.74±0.02$^{X;A}$	7.41±0.02$^{Y;E}$	18.12±0.01$^{X;FGH}$
30 Sept	70.65±0.11$^{Y;BCD}$	72.40±0.11$^{Y;BCD}$	9.78±0.01$^{X;A}$	7.41±0.00$^{Y;E}$	17.83±0.13$^{X;EFGH}$
07 Oct	70.19±0.80$^{X;AB}$	71.98±0.78$^{X;AB}$	9.76±0.11$^{X;A}$	7.38±0.00$^{Y;E}$	18.27±0.89$^{X;GH}$
14 Oct	70.40±0.04$^{Y;ABCD}$	72.17±0.04$^{Y;BCD}$	10.12±0.02$^{X;C}$	7.13±0.01$^{Y;D}$	17.71±0.07$^{X;EFG}$
21 Oct	70.20±0.05$^{Y;ABC}$	71.95±0.03$^{Y;AB}$	10.49±0.04$^{X;DE}$	6.87±0.02$^{Y;B}$	17.57±0.06$^{X;DEF}$
28 Oct	70.27±0.13$^{Y;ABC}$	72.09±0.11$^{Y;ABC}$	10.64±0.04$^{X;G}$	6.78±0.01$^{Y;A}$	17.27±0.15$^{X;CDE}$
04 Nov	70.74±0.21$^{Y;CDE}$	72.52±0.19$^{Y;CD}$	10.45±0.01$^{X;D}$	6.94±0.03$^{Y;C}$	17.03±0.17$^{X;CD}$
11 Nov	70.85±0.03$^{Y;DE}$	72.66±0.04$^{Y;DE}$	10.54±0.03$^{X;EF}$	6.90±0.02$^{Y;B}$	16.80±0.07$^{X;BC}$
18 Nov	71.27±0.06$^{Y;EF}$	73.06±0.05$^{Y;EF}$	10.66±0.01$^{X;G}$	6.86±0.00$^{Y;B}$	16.29±0.06$^{X;AB}$
25 Nov	71.70±0.14$^{Y;FG}$	73.41±0.13$^{Y;FG}$	10.66±0.01$^{X;G}$	6.89±0.02$^{Y;B}$	15.95±0.13$^{X;A}$
02 Dec	72.00±0.03$^{Y;G}$	73.65±0.04$^{Y;G}$	10.58±0.02$^{X;FG}$	6.96±0.02$^{Y;C}$	15.77±0.01$^{X;A}$
2010					
16 Sept	70.12±0.02$^{X;I}$	71.84±0.03$^{Y;H}$	9.96±0.00$^{X;A}$	7.22±0.01$^{X;G}$	18.20±0.02$^{X;F}$
23 Sept	68.98±0.02$^{X;H}$	70.73±0.01$^{X;G}$	10.64±0.01$^{Y;B}$	6.66±0.01$^{X;F}$	18.65±0.02$^{Y;I}$
30 Sept	68.74±0.25$^{X;G}$	70.57±0.22$^{X;FG}$	10.92±0.03$^{Y;C}$	6.46±0.04$^{X;E}$	18.52±0.19$^{X;HI}$
07 Oct	67.81±0.10$^{X;D}$	69.84±0.05$^{X;D}$	11.77±0.03$^{Y;D}$	5.94±0.02$^{X;D}$	18.40±0.02$^{X;GH}$
14 Oct	66.38±0.18$^{X;A}$	68.66±0.15$^{X;A}$	12.68±0.14$^{Y;GH}$	5.41±0.07$^{X;A}$	18.67±0.00$^{Y;I}$
21 Oct	67.05±0.00$^{X;B}$	69.23±0.03$^{X;B}$	12.44±0.03$^{Y;E}$	5.57±0.01$^{X;BC}$	18.33±0.06$^{Y;FG}$
28 Oct	67.36±0.03$^{X;C}$	69.54±0.00$^{X;C}$	12.49±0.02$^{Y;EF}$	5.57±0.01$^{X;BC}$	17.98±0.02$^{Y;E}$
04 Nov	67.89±0.07$^{X;D}$	70.01±0.06$^{X;D}$	12.45±0.07$^{Y;EF}$	5.63±0.04$^{X;C}$	17.55±0.00$^{X;D}$
11 Nov	68.19±0.03$^{X;E}$	70.25±0.05$^{X;E}$	12.57±0.04$^{Y;FG}$	5.59±0.01$^{X;BC}$	17.18±0.09$^{Y;C}$
18 Nov	68.46±0.06$^{X;F}$	70.53±0.04$^{X;F}$	12.70±0.07$^{Y;H}$	5.56±0.03$^{X;B}$	16.78±0.04$^{Y;B}$
25 Nov	68.59±0.02$^{X;FG}$	70.60±0.02$^{X;FG}$	12.74±0.03$^{Y;H}$	5.54±0.02$^{X;B}$	16.67±0.01$^{Y;B}$
02 Dec	68.78±0.06$^{X;GH}$	70.74±0.04$^{X;G}$	12.80±0.06$^{Y;H}$	5.53±0.02$^{X;B}$	16.47±0.10$^{Y;A}$

MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids, SFA, saturated fatty acids.

$^{X-Y}$ For each attribute, different letters for the same picking date indicate statistically significant differences ($p \leq 0.05$) between years.

$^{A-L}$ For each attribute, different letters for the same year indicate statistically significant differences ($p \leq 0.05$) among picking dates.

Table 5. Individual phenols of olive oils across the sampling dates during 2009 and 2010 crops.

Picking date	Hydroxytyrosol	Tyrosol	Vanillic acid	Vanillin	Coumaric acid	3.4-DHPEA-AC	3.4-DHPEA-EDA	p-HPEA-EDA	Lignans	3.4-DHPEA-EA	Luteolin	Apigenin
2009												
16 Sept	-	-	-	-	-	-	-	-	-	-	-	-
23 Sept	-	-	-	-	-	-	-	-	-	-	-	-
30 Sept	0.78±0.05$^{Y;F}$	0.61±0.04$^{X;A}$	0.42±0.00$^{Y;B}$	1.42±0.01$^{X;F}$	1.25±0.00$^{Y;J}$	22.28±0.36$^{X;A}$	361.77±0.41$^{X;E}$	20.34±0.30$^{X;G}$	64.83±0.20$^{X;J}$	20.70±0.70$^{Y;B}$	2.25±0.06$^{Y;A}$	1.47±0.02$^{Y;A}$
07 Oct	0.66±0.01$^{Y;BC}$	0.70±0.04$^{X;B}$	0.42±0.01$^{Y;B}$	1.45±0.00$^{X;G}$	1.15±0.00$^{X;H}$	30.81±0.10$^{X;B}$	313.23±0.43$^{X;A}$	19.85±0.17$^{X;EF}$	59.33±0.15$^{X;I}$	18.25±0.07$^{Y;A}$	2.51±0.04$^{Y;B}$	1.61±0.00$^{Y;B}$
14 Oct	0.63±0.01$^{Y;B}$	0.77±0.03$^{X;C}$	0.46±0.01$^{Y;C}$	1.40±0.01$^{X;E}$	1.08±0.01$^{X;F}$	41.70±0.50$^{X;D}$	318.40±0.09$^{X;B}$	19.16±0.20$^{X;D}$	57.56±0.20$^{X;G}$	20.24±0.29$^{X;B}$	3.09±0.01$^{Y;C}$	2.02±0.01$^{Y;C}$
21 Oct	0.75±0.02$^{Y;EF}$	0.88±0.01$^{X;D}$	0.48±0.01$^{Y;D}$	1.33±0.00$^{X;B}$	1.11±0.01$^{X;G}$	39.13±0.45$^{X;C}$	404.59±0.36$^{X;F}$	19.54±0.35$^{X;E}$	54.80±0.17$^{X;E}$	24.06±0.67$^{X;C}$	3.76±0.01$^{Y;D}$	2.45±0.02$^{Y;D}$
28 Oct	0.57±0.00$^{Y;A}$	0.89±0.02$^{X;D}$	0.37±0.00$^{Y;A}$	1.36±0.01$^{X;CD}$	1.20±0.00$^{X;I}$	75.51±0.43$^{X;H}$	420.03±0.48$^{X;H}$	21.07±0.10$^{X;H}$	58.32±0.21$^{X;H}$	28.48±0.63$^{Y;D}$	4.13±0.09$^{Y;E}$	2.53±0.02$^{Y;E}$
04 Nov	0.64±0.02$^{Y;B}$	1.25±0.03$^{Y;E}$	0.56±0.01$^{Y;F}$	1.35±0.00$^{X;C}$	0.77±0.01$^{X;D}$	56.67±0.24$^{X;E}$	354.70±0.63$^{X;D}$	19.90±0.10$^{X;F}$	53.18±0.04$^{X;D}$	31.28±0.34$^{Y;EF}$	4.64±0.01$^{Y;F}$	2.66±0.01$^{Y;F}$
11 Nov	0.71±0.02$^{Y;DE}$	1.24±0.03$^{X;E}$	0.55±0.01$^{Y;F}$	1.37±0.02$^{X;D}$	0.79±0.01$^{X;E}$	101.32±0.33$^{X;J}$	411.40±0.37$^{X;G}$	18.00±0.14$^{X;B}$	48.14±0.02$^{X;B}$	35.00±0.40$^{Y;G}$	5.86±0.07$^{Y;H}$	2.83±0.01$^{Y;H}$
18 Nov	0.68±0.01$^{Y;CD}$	1.23±0.02$^{X;E}$	0.48±0.01$^{X;D}$	1.32±0.01$^{Y;B}$	0.73±0.01$^{X;C}$	88.47±0.31$^{Y;I}$	461.27±0.56$^{Y;I}$	20.66±0.18$^{X;G}$	55.88±0.12$^{X;F}$	31.89±0.39$^{Y;F}$	6.07±0.03$^{Y;I}$	2.90±0.01$^{Y;I}$
25 Nov	0.82±0.01$^{Y;G}$	1.23±0.00$^{X;E}$	0.53±0.00$^{Y;E}$	1.24±0.01$^{X;A}$	0.70±0.00$^{X;B}$	63.68±0.27$^{X;F}$	463.96±0.63$^{Y;J}$	18.36±0.11$^{X;C}$	52.55±0.20$^{X;C}$	34.73±0.55$^{Y;G}$	6.89±0.05$^{Y;J}$	3.15±0.01$^{Y;J}$
02 Dec	0.69±0.01$^{Y;CD}$	1.46±0.02$^{Y;F}$	0.56±0.01$^{Y;F}$	1.24±0.01$^{Y;A}$	0.46±0.01$^{X;A}$	68.38±0.14$^{Y;G}$	343.34±0.41$^{X;C}$	16.47±0.06$^{X;A}$	45.72±0.24$^{X;A}$	30.56±0.21$^{Y;E}$	5.60±0.04$^{Y;G}$	2.80±0.01$^{Y;G}$
2010												
16 Sept	0.51±0.01^{E}	0.71±0.01^{A}	0.21±0.00^{A}	1.66±0.01^{K}	1.33±0.00^{I}	24.87±0.37^{A}	239.25±0.40^{A}	20.04±0.35^{A}	69.85±0.13^{E}	13.71±0.38^{A}	0.89±0.01^{A}	0.94±0.01^{A}
23 Sept	0.46±0.01^{B}	0.70±0.01^{A}	0.24±0.01^{B}	1.59±0.00^{I}	1.47±0.00^{J}	27.42±0.38^{B}	323.53±1.13^{B}	24.99±0.33^{D}	86.19±0.14^{I}	14.08±0.60^{A}	1.46±0.06^{B}	1.09±0.01^{B}
30 Sept	0.45±0.01$^{X;A}$	0.87±0.00$^{Y;C}$	0.31±0.00$^{X;E}$	1.62±0.00$^{Y;J}$	1.08±0.01$^{X;E}$	29.98±0.40$^{Y;C}$	371.87±0.52$^{Y;C}$	26.07±0.21$^{Y;E}$	92.10±0.27$^{Y;J}$	17.54±0.48$^{X;B}$	1.85±0.01$^{X;C}$	1.22±0.01$^{X;C}$
07 Oct	0.47±0.00$^{X;B}$	0.79±0.01$^{Y;B}$	0.22±0.01$^{X;A}$	1.50±0.01$^{Y;G}$	1.17±0.01$^{Y;F}$	60.18±0.18$^{Y;E}$	440.54±0.74$^{Y;H}$	20.45±0.39$^{X;A}$	76.34±0.38$^{Y;F}$	17.25±0.30$^{X;B}$	1.98±0.02$^{X;D}$	1.26±0.02$^{X;D}$
14 Oct	0.46±0.00$^{X;B}$	1.08±0.01$^{Y;E}$	0.28±0.00$^{X;D}$	1.52±0.01$^{Y;H}$	1.46±0.01$^{Y;J}$	91.28±0.59$^{Y;H}$	431.32±1.03$^{Y;G}$	26.00±0.20$^{Y;E}$	76.80±0.25$^{Y;G}$	23.12±0.42$^{Y;D}$	2.69±0.06$^{X;E}$	1.48±0.01$^{X;E}$
21 Oct	0.47±0.00$^{X;B}$	1.12±0.00$^{Y;F}$	0.30±0.00$^{X;E}$	1.41±0.01$^{Y;E}$	1.30±0.00$^{Y;H}$	92.95±0.37$^{Y;I}$	536.25±1.16$^{Y;K}$	27.20±0.47$^{Y;F}$	78.39±0.17$^{Y;H}$	23.08±0.34$^{X;D}$	3.64±0.07$^{X;F}$	1.61±0.01$^{X;F}$
28 Oct	0.48±0.01$^{X;CD}$	1.27±0.02$^{Y;G}$	0.34±0.00$^{X;F}$	1.43±0.01$^{Y;F}$	1.22±0.01$^{Y;G}$	123.23±0.15$^{Y;L}$	590.13±0.58$^{Y;L}$	27.29±0.35$^{Y;F}$	78.39±0.20$^{Y;H}$	25.86±0.28$^{X;F}$	3.66±0.05$^{X;F}$	1.68±0.01$^{X;H}$
04 Nov	0.48±0.00$^{X;C}$	1.01±0.03$^{X;D}$	0.26±0.01$^{X;C}$	1.38±0.00$^{Y;D}$	0.89±0.01$^{Y;D}$	116.45±0.35$^{Y;J}$	442.90±0.69$^{Y;I}$	22.82±0.26$^{Y;B}$	61.41±0.15$^{Y;A}$	20.53±0.23$^{X;C}$	4.13±0.04$^{X;G}$	1.64±0.01$^{X;G}$
11 Nov	0.55±0.00$^{X;F}$	1.46±0.02$^{Y;H}$	0.40±0.01$^{X;G}$	1.37±0.00$^{X;D}$	0.85±0.01$^{Y;C}$	118.47±0.23$^{Y;K}$	474.37±0.51$^{Y;J}$	23.88±0.32$^{Y;C}$	64.28±0.35$^{Y;BC}$	24.35±0.92$^{X;E}$	4.62±0.04$^{X;H}$	1.72±0.02$^{X;I}$
18 Nov	0.56±0.00$^{X;G}$	1.96±0.00$^{Y;I}$	0.51±0.01$^{Y;J}$	1.23±0.00$^{X;B}$	0.84±0.01$^{Y;C}$	84.35±0.33$^{X;G}$	393.37±0.69$^{X;D}$	22.59±0.06$^{Y;B}$	63.96±0.28$^{Y;B}$	24.63±0.61$^{X;E}$	4.70±0.02$^{X;I}$	1.68±0.02$^{X;H}$
25 Nov	0.57±0.00$^{X;H}$	2.01±0.01$^{Y;J}$	0.48±0.00$^{X;I}$	1.30±0.01$^{Y;C}$	0.71±0.00$^{X;B}$	71.85±0.30$^{Y;F}$	397.46±0.27$^{X;E}$	23.02±0.38$^{Y;B}$	64.59±0.11$^{Y;C}$	23.95±0.38$^{X;E}$	5.11±0.05$^{X;J}$	1.73±0.02$^{X;I}$
02 Dec	0.49±0.01$^{X;D}$	1.28±0.02$^{X;G}$	0.43±0.01$^{X;H}$	1.21±0.00$^{X;A}$	0.69±0.01$^{Y;A}$	45.79±0.40$^{X;D}$	422.94±0.24$^{Y;F}$	22.85±0.05$^{Y;B}$	65.79±0.23$^{Y;D}$	21.05±0.53$^{X;C}$	5.51±0.01$^{X;K}$	1.73±0.01$^{X;I}$

3.4-DHPEA-AC, 4-(acetoxyethyl)-1.2-dihydroxybenzene; 3.4-DHPEA-EDA, dialdehydric form of elenolic acid linked to hydroxytyrosol; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3.4-DHPEA-EA, oleuropein aglycone.

$^{X-Y}$ For each attribute, different letters for the same picking date indicate statistically significant differences ($p \leq 0.05$) between years.

$^{A-L}$ For each attribute, different letters for the same year indicate statistically significant differences ($p \leq 0.05$) among picking dates.

Table 4. Oxidative stability ant total phenol content of olive oils across the sampling dates during 2009 and 2010 crops.

Picking date	Oxidative stability (hours)		Total phenols (mg gallic acid/Kg oil)	
	2009	2010	2009	2010
16 Sept	12.64±0.16$^{X;A}$	15.14±0.11$^{Y;A}$	300.28±5.42$^{X;A}$	380.46±2.12$^{Y;A}$
23 Sept	14.01±0.23$^{X;B}$	15.09±0.22$^{Y;A}$	317.56±4.81$^{X;B}$	388.47±6.12$^{Y;B}$
30 Sept	18.71±0.24$^{X;CD}$	18.24±0.14$^{X;DE}$	324.15±2.76$^{X;B}$	427.49±4.87$^{Y;E}$
07 Oct	18.53±0.01$^{X;C}$	18.64±0.01$^{Y;EFG}$	334.60±4.73$^{X;C}$	493.36±5.06$^{Y;H}$
14 Oct	18.86±0.22$^{Y;CD}$	17.79±0.06$^{X;CD}$	409.38±4.47$^{X;FG}$	476.30±1.39$^{Y;FG}$
21 Oct	19.47±0.08$^{X;EF}$	18.96±0.46$^{X;FGH}$	405.90±5.06$^{X;F}$	472.95±3.50$^{Y;F}$
28 Oct	19.70±0.08$^{X;FG}$	19.45±0.16$^{X;H}$	462.93±1.41$^{X;H}$	554.53±4.20$^{Y;I}$
04 Nov	20.10±0.20$^{Y;GH}$	19.13±0.23$^{X;GH}$	464.92±1.42$^{X;H}$	495.15±3.56$^{Y;H}$
11 Nov	21.21±0.16$^{Y;J}$	18.40±0.01$^{X;EF}$	413.68±5.72$^{X;G}$	479.33±1.45$^{Y;G}$
18 Nov	20.60±0.18$^{Y;I}$	16.31±0.54$^{X;B}$	345.28±1.91$^{X;D}$	413.36±1.27$^{Y;D}$
25 Nov	20.28±0.25$^{Y;HI}$	16.70±0.40$^{X;B}$	343.33±4.90$^{X;D}$	403.09±3.84$^{Y;C}$
02 Dec	19.04±0.29$^{Y;DE}$	17.51±0.26$^{X;C}$	389.40±3.45$^{Y;E}$	376.01±2.66$^{X;A}$

$^{X-Y}$ For each attribute, different letters for the same picking date indicate statistically significant differences ($p \leq 0.05$) between years.

$^{A-L}$ For each attribute, different letters for the same year indicate statistically significant differences ($p \leq 0.05$) among picking dates.

Table 6. Correlations (*r*) among ripeness index, pigments and lightness.

Correlation	2009	2010
Ripeness index/carotenoid	-0.78	-0.90
Ripeness index /chlorophyll	-0.81	-0.86
Ripeness index /total pigments	-0.80	-0.88
Lightness/carotenoid	-0.97	-0.99
Lightness/chlorophyll	-0.99	-0.99
Lightness/total pigments	-0.99	-0.99

Table 7. Sensory analysis of olive oils across the sampling dates during 2009 and 2010 crops.

Picking date	Fruitiness		Bitterness		Pungency	
	2009	2010	2009	2010	2009	2010
16 Sept	$6.0^{Y;F}$	$4.2^{X;B}$	$2.5^{X;F}$	$3.4^{Y;E}$	$4.3^{X;I}$	$4.7^{Y;G}$
23 Sept	$6.0^{Y;F}$	$4.5^{X;E}$	$3.4^{Y;H}$	$2.9^{X;C}$	$4.2^{X;H}$	$4.6^{Y;F}$
30 Sept	$5.1^{Y;B}$	$4.4^{X;D}$	$2.5^{X;F}$	$4.3^{Y;I}$	$4.2^{X;H}$	$5.0^{Y;H}$
07 Oct	$5.4^{Y;C}$	$4.7^{X;F}$	$2.5^{X;F}$	$3.4^{Y;E}$	$4.1^{X;G}$	$4.5^{Y;E}$
14 Oct	$6.2^{X;G}$	$6.2^{X;I}$	$3.4^{X;H}$	$3.9^{Y;H}$	$3.7^{X;E}$	$4.0^{Y;C}$
21 Oct	$5.6^{Y;D}$	$5.2^{X;H}$	$2.1^{X;D}$	$3.7^{Y;F}$	$3.8^{X;F}$	$4.6^{Y;F}$
28 Oct	$5.8^{Y;E}$	$4.9^{X;G}$	$1.6^{X;A}$	$3.7^{Y;F}$	$2.8^{X;B}$	$4.5^{Y;E}$
04 Nov	$4.6^{Y;A}$	$4.1^{X;A}$	$1.7^{X;B}$	$3.2^{Y;D}$	$2.7^{X;A}$	$4.1^{Y;D}$
11 Nov	$6.0^{Y;F}$	$4.2^{X;B}$	$2.6^{X;G}$	$2.7^{Y;B}$	$2.8^{X;B}$	$3.6^{Y;B}$
18 Nov	$6.2^{Y;G}$	$4.3^{X;C}$	$2.3^{X;E}$	$3.8^{Y;G}$	$3.1^{X;D}$	$3.4^{Y;A}$
25 Nov	$6.3^{Y;H}$	$4.4^{X;D}$	$2.0^{X;C}$	$3.4^{Y;E}$	$2.9^{X;C}$	$3.6^{Y;B}$
02 Dec	$5.4^{Y;C}$	$4.4^{X;D}$	$1.6^{X;A}$	$2.5^{Y;A}$	$2.9^{X;C}$	$3.4^{Y;A}$

$^{X-Y}$ For each attribute, different letters for the same picking date indicate statistically significant differences ($p \leq 0.05$) between years.

$^{A-L}$ For each attribute, different letters for the same year indicate statistically significant differences ($p \leq 0.05$) among picking dates.

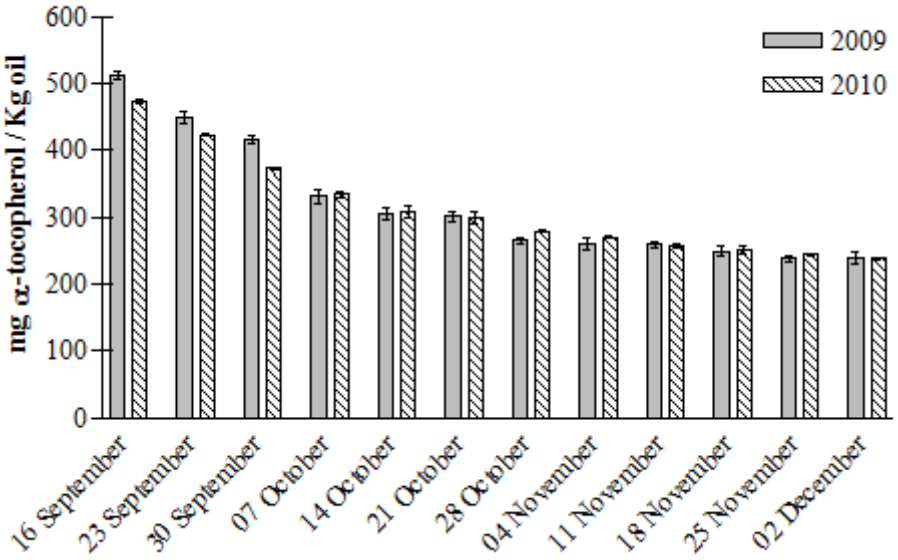

Figure 1. Evolution of α-tocopherol in olive oils across the sampling dates during 2009 and 2010 crops.