# OLIVE OIL QUALITY AND RIPENING IN SUPER HIGH DENSITY ARBEQUINA ORCHARD

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Running title: Olive oil and ripening in hedgerow Arbequina orchard

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## ABSTRACT

BACKGROUND: The aim of this work was to evaluate the evolution of the quality of extra virgin olive oil obtained from a super-high density Arbequina orchard, under a drip irrigation system, throughout the ripening process. For this objective, physico-chemical, nutritional and sensorial parameters were studied. In addition, oxidative stability, pigment content, and the evolution of olive oil color has been analyzed during the ripening process. RESULTS: Free acidity increased slightly along the ripening process, while the peroxide values and extinction coefficients decreased. There was a similar trend between total phenol content and oxidative stability increasing at the beginning of ripeness up to a maximum and then decreasing. The content of  $\alpha$ -tocopherol and the pigment content decreased with ripening leading to changes in the color ordinates. Sensorial parameters were related to the total phenol content following a similar trend along the maturity process. CONCLUSION: Using weekly sampling and monitoring the ripeness index, it would be possible to determine an optimum harvesting time for olives, according to the industrial yield and the physicochemical, nutritional, and sensorial properties of the olive oil.

Keywords: high density, Arbequina, olive oil, quality, ripening

#### **INTRODUCTION**

The olive tree (Olea europaea L.) is one of the most important crops in Mediterranean countries, especially Spain, Italy, and Greece. Olive oil is a genuine fruit juice with excellent nutritional, sensory, and functional quality obtained from the fruit of olive trees. The olive oil benefits for human health have been associated with its well-balanced fatty acid composition, from of which oleic acid is the main component, and with the presence of minor biomolecules such as vitamins and natural antioxidants <sup>1</sup>. The chemical composition and quality of virgin olive oil are influenced by a many different factors such as the geographical production area (altitude, soil quality, latitude)<sup>2,3</sup> climatic conditions prevailing in the production year, cultivar, extraction process<sup>4-6</sup> and different production systems such as traditional system, intensive<sup>7-9</sup> or superintensive systems<sup>10</sup>.

Intensive cultivation systems, usually characterized by high  $(200-600 \text{ trees ha}^{-1})$  or super-high plant density  $(1200-2500 \text{ trees ha}^{-1})$ , have been introduced in recent years in traditional olive-producing countries (in southern Europe, northern Africa, and the Middle East) and in countries such as Argentina, Australia, and South Africa, where the olive tree is a crop of recent introduction within the local agricultural systems. This new cultivation strategy has driven a revolution in olive oil production and affected the selection of cultivars that are better adapted to the new orchards. The most commonly intensive planted variety around the world is Arbequina, though other varieties such as Arbosana, Koroneiki, Sikitita, and Tosca are also used.

Traditional rain-fed olive groves are characterized by low plantation density, which allows the exploitation by the root system of an adequate soil volume, minimizing competition for water among plants. The increase in plant density determines the intensification of the trees' competition for water and nutrients, so irrigation is recommended to obtain, throughout the years, a high and as steady as possible production of olives.

During ripening, important chemical changes occur inside the drupes that are related to the synthesis of organic substances, especially triglycerides, and to other enzymatic activities<sup>11</sup> that may affect virgin olive oil quality<sup>12</sup>. The composition of fatty acids and the levels of polyphenols, tocopherols, and pigments change with maturation<sup>13</sup>. These changes are of great commercial importance, as they dramatically affect the sensory characteristics of the oils, as well as its shelf life. Many studies have investigated the changes in olive oil quality from traditional orchards during fruit maturity<sup>14-17</sup>.

The increasing proportion of intensive orchards and the development of rapid tools for mechanized harvesting have brought about the need to determine the effects of harvest time and fruit maturity on yield and quality oil in relation to cultivar, environmental conditions, and agronomic practices. Some studies have investigated the evolution of olive oil quality during the ripening of olives from intensive<sup>9, 18, 19</sup> and superintensive crops<sup>10, 20, 21</sup>. However, these studies were performed on a single index of maturity or on specific dates of sampling but not along the ripening process, allowing evidence of small changes due to climatic and environmental conditions. Thus, the aim of this work was to improve knowledge about the effect of fruit ripening on olive oil quality from an Arbequina superintensive orchard, monitoring the ripeness index and analytical parameters with weekly sampling and then to determine an optimum harvesting time for olives according to the results obtained for the ripeness index, the industrial yield and the physicochemical, nutritional, and sensorial properties.

#### EXPERIMENTAL

## 2.1 Olive fruit sampling

The trial was carried out during the crop seasons from 2008 to 2010 in an experimental superintensive orchard (Olea europea L. cv. Arbequina) located at the Aula Dei Experimental Station-CSIC in Zaragoza (Spain), planted in 2002 with a frame of 4 x 2 m and a density reaching 1250 trees ha<sup>-1</sup>. The orchard was irrigated through a drip irrigation system that was buried at a depth of 30 cm. The irrigation schedule was designed in accordance with the FAO method<sup>22</sup>. The crop fertilization in the irrigation application, attempted to cover a contribution of 40-20-48 U/ ha of N-P-K respectively. The climate classification of the area is Mediterranean-continental, with rainy winters and quite dry summers.

Two Samples from Arbequina trees were usually handpicked once a week, from the middle of September to beginning of December. Each sample consisted of 1200 olives from 60 trees in perfect sanitary conditions. After harvest, the maturity index was determined and olive oil was extracted immediately.

# 2.2 *Maturity index*

The olive ripeness index (RI) was determined according to the method developed by the Agronomic Station of Jaén<sup>23</sup> based on the evaluation of the olive skin and pulp colors. RI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

## 2.3 Oil extraction process and industrial oil yield

Oil extraction was performed using an Abencor laboratory oil mill (MC2 Ingenierías y Sistemas, Sevilla, Spain) according to the method described by Martínez et al.<sup>24</sup>. Olives of the Arbequina variety were cleaned of leaves and crushed with a hammer crusher, and the paste was mixed at a temperature of about 26° C for 30 min and then

centrifuged at 3500 rpm for 1 min. After filtration, the olive oil samples were stored at -18° C in darkness using amber glass bottles without headspace prior to analysis.

The industrial oil yield was expressed as a percentage of fresh olive paste weight using the following equation:

Oil yield = (V x d / W) x 100

Where V is the volume of olive oil obtained (mL), d is the density of the olive oil (0.915 g/mL), and W is the weight of olive paste used.

## 2.4 Analytical determinations

Determinations of the regulated physicochemical quality parameters (free acidity, peroxide value, and UV absorption characteristics,  $K_{270}$  and  $K_{232}$ ) were carried out following the analytical methods described in Regulation EEC/2568/91 of the Commission of the European Union<sup>25</sup>.

# 2.4.1 Determination of fatty acids

The fatty acid composition of the olive oil samples was determined by gas chromatography using the fatty acid methyl ester (FAME) method as described by Frega and Bocci<sup>26</sup>. About two drops of oil were dissolved in six drops of a solution of 2 N KOH in methanol and then 2 ml of n-hexane were added. The mixture was vigorously shaken with a vortex for 2 min, sodium sulfate was added and the mixture was shaken again. Chromatographic analyses were performed using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector and a split/splitless injector. The experimental conditions used were as follows: DB- 225 column (30 m x 0.25mm i.d. x 0.15 mm film thickness) (J & W Scientific, Agilent). The injector and detector temperatures were maintained at 250 °C. The oven temperature was programmed from 190 °C (1 min) to 210 at 4 °C/min and maintained for 5 min, then

heated to 215 at 3 °C/min and, finally, an isotherm was used for 18 min; the carrier gas was nitrogen.

Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times.

## 2.4.2 $\alpha$ -tocopherol determination

A solution of 1 g of oil dissolved in 10 ml of hexane was analyzed by HPLC (HP series 1100) with a Zorbax SB-C18 phase reverse column (Agilent), which was eluted with acetonitrile/water (98:2 v/v) at a flow rate of 1 mL/min and at 25 °C of temperature. A photodiode matrix detector (G1315B, series 1100) was used. Chromatograms were registered at 295 nm.

## 2.4.3 Total phenol content

To extract the phenols from the olive oil, we followed the method described by Favati et al.<sup>27</sup>. The phenols were extracted by SPE using Isolute C18 columns of 1g of sample and 6 ml volume. Columns were conditioning with 2 x 5 ml of methanol and 2 x 5ml of hexane. After sample loading (1g of oil in 10 ml oh hexane) column was washing with 3 x 5 ml of hexane. Phenols were eluted with 2 x 5 ml of methanol. The extract was brought to dryness in a rotary evaporator and the residue was dissolved in 5ml methanol. For the colorimetric determination of total phenols we used the method described by Vázquez Roncero et al.<sup>28</sup> 2.5 ml of extract, from the 5 ml in methanol, was mixed with 1.25 ml of Folin-Ciocalteau reagent, and after 3 min, 2.5 ml of 20 % sodium carbonate was added. The solutions were left for 1 hour in dark. After this time, the absorption of the solution was measured at 725 nm. The results were expressed as mg equivalents of gallic acid per kg of oil.

## 2.4.4 Oxidative stability

Stability was expressed as the oxidation induction time (hours) measured with a Rancimat 743 apparatus (Metrohm, Switzerland) using an oil sample of 3 g warmed to 120° C with 20 l h-1 air flow. The induction time is the time needed to reach the break point of this curve representing the increase in conductivity versus time. The break point is designated as the intersection point of the two extrapolated straight parts of the curve<sup>29</sup>.

## 2.4.5 Determination of chlorophyll and carotenoid compounds

Chlorophyll and carotenoid were calculated from the absorption spectra of the virgin olive oil spectra for each sample (7.5 g) dissolved in cyclohexane (25 ml) following the method of Minguez-Mosquera et al.<sup>30</sup>. The maximum absorption is related to the chlorophyll fraction at 670 nm and to the carotenoid fraction 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 of pheophytin and lutein per Kg, respectively.

## 2.4.6 Color measurement

The CIELAB color space<sup>31</sup> was studied with a spectrophotometer (Avantes Ava Spec 1024) after the spectra was 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).

## 2.4.7 Sensory analysis

The sensory analyses of the samples were carried out by 10 selected and trained panelists from Aragon's accredited panel and the Zaragoza Faculty of Veterinary Science according to the method described in Regulation EEC/640/2008<sup>32</sup>. 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 nonstructured, 10 cm scale anchored by its origin.

#### 2.5 Statistical analysis

Statistical analysis was performed using the Graph Pad Prisma 5.0 program (GraphPad Software, Inc., USA) and Statgraphics Plus 5.1 program (Statgraphics Software, Inc., USA). Results were expressed as mean values  $\pm$  standard deviation of three independent experiments and LS mean values  $\pm$  95% confidence intervals. Significant differences between the samples were determined using the ANOVA and Fisher's least significant difference (LSD) procedures. The correlations between parameters were calculated using the Pearson two-tailed correlation with a 95% confidence level.

#### **RESULTS AND DISCUSSION**

The study was carried out from the middle of September, when the fruit color change began; the ripening index was near to zero. The values of the ripening index and harvesting dates for the three crop years are shown in Table 1. The evolution of the ripening index along the dates of harvesting was similar between the three studied years. The industrial yield is shown in Figure 1. It began to maximize in the middle of October, but it was greatly influenced by climatic conditions (data shown in Gracia et al.<sup>33</sup>). The maximum value (17.76%) was reached at the end of harvesting period in 2008 due to the frost that occurred during this period. The effects of freeze injures in olive fruit are a consequence of cell dehydration and important cell destruction caused by ice crystals forming inside parenchyma cells, which allows greater intracellular oil output<sup>34</sup>. The increase in the industrial yield at the end of November was due to the decrease of olive humidity during this period coinciding with the purple pigmentation of olives, as observed in previous works (Gracia et al.,<sup>33</sup>).

3.1 Free acidity, peroxide value, and UV spectrophotometric indexes

The physicochemical parameters (Figures 2, 3, 4, and 5) of all studied olive oils fell broadly within the estimated limits of EEC Reg.  $1989/2003^{35}$ , so the oils could be classified in the category of extra virgin olive oil.

A slight rise in free acidity values was observed as ripening progressed in all studied samples (Fig. 2) except for those from 2008 in that there is not a clear trend in free acidity. An increase in free acidity has been observed in the traditional Arbequina cultivar,<sup>36, 15</sup> in the Picual and Hojiblanca varieties<sup>37, 14</sup> and in superintensive Spanish and Greek olive cultivars<sup>10</sup>. Olives at a later stage of ripening give oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially by lipolitic enzymes<sup>38</sup>. Moreover, the acidity values obtained during the ripening period were much lower than those obtained by Desouky et al.<sup>39</sup> in Arbequina, Koroneiki and Bouteillan olive oils cultivated by intensive system.

The changes in the peroxide index were similar in 2009 and 2010: a decrease during the ripening process (Fig. 3) was observed. In 2008, we observed an initial decrease of peroxide values and an increase of these values on the final dates because the olive fruits were frozen on the trees. In general, the extra virgin olive oil obtained from olives at more advanced stages of maturity showed lower peroxide values. This phenomenon can be explained by a decrease in the activity of the enzyme lipoxygenase. A decrease in peroxide values during the ripening process were also obtained by Tovar et al.,<sup>8</sup> Salvador et al.<sup>14</sup> and Baccouri et al.<sup>19</sup> in virgin olive oils obtained from intensive Arbequina and the traditional Cornicabra and Chetoui cultivars, respectively.

The measurements of absorbance at specific wavelengths in the UV region are used to provide information on the quality of olive oil (Figs. 4 and 5). The specific extinction coefficient at 232 nm is related to the primary oxidation of oil, and it is an indication of

the conjugation of polyunsaturated fatty acids, whereas  $K_{270}$  is an indication of carbonylic compounds (aldehyds and ketones) in olives and is related to the secondary oxidation products. The spectrophotometric absorption of  $K_{232}$  and  $K_{270}$  behaved similarly to the peroxide index, decreasing at a later ripeness index according to the peroxide value trend in the three studied crop seasons. The low values of  $K_{232}$  and  $K_{270}$  also confirmed the good overall quality of these oils at each olive ripening stage.

All of the analyzed oils showed very low mean values for quality parameters (acidity < 0.8%; peroxide index < 20 meq O<sub>2</sub> kg–1;  $K_{270} < 0.22$ ;  $K_{232} < 2.5$ ); they belonged to the "extra virgin" category according to Regulation EC/1989/2003<sup>35</sup>. Note that low values for these parameters indicate a high quality of oil because of the use of only healthy fruits in the experiment and their rapid processing.

## Fatty acid composition

The fatty acids identified were: palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), and gadoleic (C20:1) acids. The palmitic, stearic, oleic, and linoleic acids were measured as major fatty acids (Table 2). In all the samples, the oleic acid was always the most abundant compound, never comprising less than 69% of the total fatty acid. It can be seen that, with the exception of the palmitic, stearic, linoleic, and linoleic, and linolenic acids, the fatty acid content did not vary during the maturation process.

During maturation, as the fruit ripened, the palmitic acid content decreased slightly in the three studied crop seasons: between 14.56% and 12.02% in 2008; 14.96% and 13.29% in 2009, and 14.33% and 12.58% in 2010. The contents of palmitic acid obtained in olive oils under study were significantly lower than those described by Allalout et al.<sup>10</sup> who obtained percentages close to 18% for the same variety grown in Tunis. The palmitic acid level fell during the ripening process, possibly as a result of a

dilution effect<sup>37</sup>. Gutierrez et al.<sup>37</sup> related this decline to a dilution effect, caused by a constant quantity of palmitic acid but rising levels of total fatty acids resulting in a reduction in the relative amount of palmitic acid bound to the triglycerides.

In the course of fruit ripening, the oleic and linoleic acids showed different trends in the studied samples. In fact, the oleic acid remained constant (with the exception of the 2008 crop, in which an increase was observed), whereas the linoleic acid levels increased as the fruit ripened. Similar results were observed by Cimato et al.<sup>40</sup>, who reported constant relative amounts of oleic acid during the maturation process.

The increase in linoleic acid content could be due to the transformation of oleic acid into linoleic acid by oleate desaturase activity, which is active during the triacylglycerol biosynthesis<sup>41</sup>. The products of the complex "fatty acid synthase enzyme" are saturated chains of 16 and 18 carbon atoms, however, stearate does not accumulate, but the main product in the "novo synthesis" of the fatty acid is oleic acid. While continuing lipid biosynthesis along maturation, oleate desaturase enzyme would be active, transforming oleic acid into linoleic and the net result would be an almost constant content of oleic acid and linoleic acid increased<sup>18, 42, 43</sup>.

Hence, the changes observed from the first harvest to the last harvest in the oleic/linoleic acid ratio showed a decreasing trend during the maturity process, which was confirmed by a good negative correlation between this ratio and the ripeness index in the 2009 and 2010 crop (r = -0.80, P < 0.01 and r = 0.86, P < 0.01, respectively) (Table 3).

Furthermore, it is well-known that, fatty acid composition could be affected not only by the maturity stage but also by environmental factors such as rainfall and geographical origin<sup>44</sup>.

## a -tocopherol content

In all the seasons studied, the content of  $\alpha$ -tocopherol decreased in the oils as ripening progressed, with significant differences between years (P < 0.0001) and between dates of harvest (P < 0.001). Higher content was observed at the beginning of the fruit maturation process in 2008: 496.4 mg  $\alpha$ -tocopherol per Kg of oil. Lower values were observed at the end of the ripening process (182.0 mg/Kg) (Figure 6). These results confirm those previously reported by several authors<sup>36,37</sup>. Thus, in this sense, considering only vitamin-E content, the nutritional value of the olive oils could decrease with the maturation level of the fruits. However, the content of  $\alpha$ -tocopherol in olive oils obtained in this study was higher than that obtained by other authors from the Arbequina variety along the harvesting period<sup>15</sup>.

## Total phenol content

Phenols are recognized as important antioxidant compounds that protect the oil against auto-oxidation, at the cellular level, against oxygen radicals. Furthermore, phenolic substances not only affect virgin olive oil stability but also contribute to oil pungency, flavor, and aroma, especially to the typical bitter taste of olive oil<sup>45</sup>.

The total phenol levels increased progressively until they reached a maximum at an RI between 1.7 and 2.3, after which they decreased (Fig. 7). These results match those observed for the Chetoui<sup>19, 46</sup> and Cornicabra varieties<sup>14</sup>. However, this trend is not the same in the three crop seasons. There were significant differences between years (P < 0.0001). Although, in the three crop seasons, the total phenol content first increased and then decreased, in 2009, the maximum content was reached, earlier than in 2008 and 2010. Similar results were observed for the Chetoui Tunisian variety<sup>46</sup> reaching the maximum phenol content at RI 2.05. Also, the phenol content this year is higher than in

the other years with a maximum of 379.1 mg of gallic acid per Kg of oil at the beginning of October, probably due to the lower rainfall that occurred this year.

In fact, several authors<sup>47, 48</sup> have shown that the presence of rainfall during the growth and maturation of olive fruits affects the phenolic content of oils obtained. The theory which may explain the particular trend in total phenolic content in the superintensive systems under linear-irrigation, might be based on that while the activity of the enzyme responsible for the synthesis of phenolic compounds in olive, phenylalanine ammonia lyase (PAL), decreases during the ripening process<sup>49</sup>, the extraction ability of the phenolic compounds during processing of olive oil increases as the olive loses moisture (which coincides with ripeness index of 2-3). Afterwards, with advancing fruit maturity, the activity of the enzyme polyphenol oxidase (PPO) increases coinciding with a sharp decrease in the concentration of oleuropein in olive.

Phenolic compounds are more soluble in water than in oil, thus in the olive paste having a higher percentage of humidity (as in the case of those originating in the first harvest dates), most of these compounds would be dragged from the oil phase during the extraction process, due to the oil partition coefficient between two immiscible liquids. The content of phenolic compounds in the oil is also affected by the extraction process. In fact, it has been reported that the concentration of these compounds in the extra virgin olive oil is strongly affected by the processing conditions<sup>50</sup>.

Moreover, total phenol contents obtained in this study were slightly higher to those obtained by other authors in olive oil from Arbequina variety cultivated by intensive system<sup>10,51,52</sup>.

## Oxidative stability

The stability of the olive oils with respect to oxidation at different stages of ripening, measured by the Rancimat method, is shown in Figure 8. Hence, as observed for the total phenol content, the oxidative stability values of the three studied seasons increased progressively until it reached a maximum at the between 1.7 and 2.3, respectively, after which it decreased. In fact, the statistical data analysis evidenced positive correlations between the values of oxidative stability and the total phenol content (r = 0.85, 0.89; P < 0.001 in 2009 and 2010, respectively). Youssef et al.<sup>46</sup> observed that olive oils with the highest stability values (RI between 2 and 3) also had the highest levels of major phenolic compounds. Oxidative stability, although not considered a standard parameter of quality, is useful in providing information on the oil's hypothetical shelf-life because it reveals the resistance of the product to the beginning of the oxidation process, characterized by free radical reactions<sup>53</sup>.

## Pigment content

Pigments are responsible for the oil color, which is one of the factors that influence selection by consumers. The stage of olive maturity is very important for pigment, chlorophyll, and carotenoid concentrations in virgin olive oil. In the three studied crop seasons, the chlorophyllic pigments are between 63.01 and 4.46 mg/Kg since the carotenoid fraction is between 33.13 and 5.04 mg/Kg, indicating significant differences between years (P < 0.001) (Fig. 9 and 10). Oils with higher pigment content were obtained during 2009, while the content was lower in 2008 due to the rainfall that occurred during 2008. Pastes obtained from 2008 season were more fluid because of their higher water contents. During the milling, a great part of them rapidly crossed the hammer-crush sieves, suffering less tissue crushing. This might result in a reduction in the extraction of pigments mainly located in the epicarp of the fruit<sup>54</sup>.

During the ripening process, there was a decrease in chlorophyll and carotenoid content, as described previously by Garrido et al.<sup>55</sup> and Gutierrez et al.<sup>37</sup>, but the decrease was different between years. The losses in chlorophyll content between the middle of September and the beginning of December were 78.34%, 86.67%, and 71.33% in 2008, 2009, and 2010 respectively. On the other hand, the losses in carotenoid content were 71.42%, 76.80%, and 53.50% in 2008, 2009, and 2010, respectively. In fact, there is a high negative correlation between the maturity index and pigment content (Table 3). Similar results were obtained in Manzanilla and Hojiblanca olive oils with a high correlation between the pigment content and the ripening index<sup>56</sup>. However, Motilva et al.<sup>18</sup> observed that oil pigment content remained relatively constant over time, while a gradual decrease, mainly in the first stages of ripening in olive oils from traditional Arbequina orchards.

## Color measurement

The values of the chromatic ordinates L\*, a\*, and b\* obtained from the absorption spectra of the oils were used to evaluate the effect of picking data on color (Table 4). There were significant differences (P < 0.001) in the color ordinates between years due to the different chlorophyll and carotenoid content. Olive oils obtained from 2009 and 2010 are significantly more green than those obtained from 2008, with significantly more negative values of a\* (red-green) and the highest values of b\* (yellow-blue). The L\* values increased as the fruit ripened and decreased the of pigment content. In fact, a high negative correlation was found between the L\* values and pigment content (Table 4). Usually, this coordinate increases with the reduction in the pigment content of the oils, as the pigments would capture part of the light instead of transmitting it. A decrease in the ordinate b\* values with maturity progress has been described (Mínguez-Mosquera et al.,<sup>30</sup>); however, we observed an initial increase in the values of ordinate b\*

at the beginning of the maturity process, followed by a decrease at the end of the study. This trend could be related to the increase in the higher pigment transference from olive paste to the oil with the more advanced ripening stage of the olive fruit, independent of the pigment content<sup>54</sup>. Similarly, the ordinate a\* became more negative in the middle of the ripening process, increasing at the end of the harvesting period.

# Sensory analysis

The results of the sensory evaluation are reported in Table 5. The studied oils showed no defects in the sensory analysis, so they were considered extra virgin olive oils under Regulation CEE 640/2008. The fruity score ranged from 6.0 to 3.0 in 2008, from 6.4 to 4.4 in 2009, and from 6.7 to 4 in 2010. Pungency and bitterness, two positive attributes of virgin olive oil, ranged from 3.5 to 1.2 in the case of bitterness and from 5.1 to 2.1 in the case of pungency. The oils obtained from the studied Arbequina superintensive orchard were more pungent than bitter, obtaining high scores for the first parameter. The evolution of the positive sensorial attributes throughout the maturation process in the three studied crop seasons was the same as the trend of the total phenol content. The fruity, bitterness, and pungency scores increased up to a maximum coinciding with the maximum total phenol content and then decreased. It is known that penolic compounds are responsible for the bitterness and pungency in oils<sup>57, 58, 59</sup>.

To establish the optimum date for olive harvesting to obtain high-quality extra virgin olive oil, the chemical parameters established by regulations<sup>25, 35</sup> have to be broadly within estimated limits; the total phenol content in this period has to be at a maximum, which leads to high scores in the positive fruity, bitterness, and pungency attributes, and there must be a good ratio between the oleic and linoleic acids. We suggest that, in our study conditions, for the production of optimal extra virgin olive oil, Arbequina olives grown under a super-high density system should be harvested from the end of October

to the middle of November, coinciding with an RI between 2 and 3. In regard to olive yield, the highest values were produced after November 10. If we focus on olive oil yield, the best date for harvesting to conserve olive oil quality could be the middle of November, around the 15<sup>th</sup>, as observed in our previous works concerning olive oil yield<sup>33</sup>, establishing this data as the limit for harvesting because of the risk of significant frost, which in our area increases linearly as November progresses, reducing the quality of obtained virgin olive oil.

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**Fig.1.** Industrial yield values obtained from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.

(a) Data are expressed as mean values ±SD of three independent experiments

(b) LSMeans of industrial yield values of three years and 95% confidence interval

(c) LSMeans of industrial yield values of the different data and 95% confidence interval

**Fig.2.** Free acidity values obtained from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.



Means and 95% LSD intervals



(a) Data are expressed as mean values ±SD of three independent experiments
(b) LSMeans of free acidity values of three years and 95% confidence interval
(c) LSMeans of free acidity values of the different data and 95% confidence interval

**Fig.3.** Peroxide values obtained from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.







**Fig.4.**  $K_{232}$  values obtained from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.



Means and 95% LSD intervals



(a) Data are expressed as mean values  $\pm$ SD of three independent experiments (b) LSMeans of K<sub>232</sub> of three years and 95% confidence interval (c) LSMeans of K<sub>232</sub> of the different data and 95% confidence interval

**Fig.5.**  $K_{270}$  values obtained from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.





(a) Data are expressed as mean values  $\pm$ SD of three independent experiments (b) LSMeans of K<sub>270</sub> of three years and 95% confidence interval (c) LSMeans of K<sub>270</sub> of the different data and 95% confidence interval

**Fig.6.**  $\alpha$ -tocopherol content from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.





(a) Data are expressed as mean values  $\pm$ SD of three independent experiments (b) LSMeans of  $\alpha$ -tocopherol content of three years and 95% confidence interval (c) LSMeans of  $\alpha$ -tocopherol content of the different data and 95% confidence interval

**Fig.7.** Total phenol content from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.



 Means and 95% LSD intervals

 400
 350

 300
 I

 250
 I

 1
 I

 1
 I

 1
 I

 100
 I

 50
 I

 100
 I

 50
 I

 0
 I

 50
 I

(a) Data are expressed as mean values ±SD of three independent experiments
(b) LSMeans of total phenol content of three years and 95% confidence interval
(c) LSMeans of total phenol content of the different data and 95% confidence interval

**Fig.8.** Evolution of olive oil stability (h) in relation to picking date of superintensive Arbequina cultivar for the crop years 2009 and 2010.







(a) Data are expressed as mean values ±SD of three independent experiments
(b) LSMeans of olive oil stability of three years and 95% confidence interval
(c) LSMeans of olive oil stability of the different data and 95% confidence interval

**Fig. 9.** Carotenoid contents from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.



(a)



(a) Data are expressed as mean values ±SD of three independent experiments(b) LSMeans of carotenoid content of three years and 95% confidence interval(c) LSMeans of carotenoid content of the different data and 95% confidence interval

**Fig. 10.** Chlorophyll contents from olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.







(a) Data are expressed as mean values ±SD of three independent experiments
(b) LSMeans of chlorophyll content of three years and 95% confidence interval
(c) LSMeans of chlorophyll content of the different data and 95% confidence interval

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	I	Ripeness Index (RI	)
	2008	2009	2010
15 september	$0.0 \pm 0.00$	0 ± 0.00	-
23 september	$0.3 \pm 0.02$	$0.3 \pm 0.04$	$0.2 \pm 0.01$
27 september	$0.5 \pm 0.03$	$0.5 \pm 0.01$	$0.3 \pm 0.01$
6 october	$0.6 \pm 0.14$	0.6 ± 0.03	0.5 ± 0.00
14 october	$1.5 \pm 0.01$	$1.0 \pm 0.04$	$1.0 \pm 0.04$
20 october	$1.6 \pm 0.11$	1.7 ± 0.03	1.2 ± 0.04
27 october	1.7 ± 0.17	1.9 ±0.11	1.5 ± 0.17
3 november	2.3 ± 0.08	2.3 ± 0.04	2.3 ± 0.35
10 november	3.0 ± 0.28	2.5 ± 0.03	2.8 ± 0.11
17 november	3.4 ± 0.08	3.2 ± 0.06	$3.1 \pm 0.11$
24 november	3.5± 0.17	3.6 ± 0.07	3.5 ± 0.08
1 december	3.8 ± 0.09	3.8 ± 0.04	-

**Table 1.** Ripeness indexes (RI) obtained from olives in relation to picking date ofsuperintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.

Table 2. Fatty acid composition (%) of studied olive oils in relation to picking date of superintensive Arbequina cultivar for the crop years 2008,

2009 and 2010.

	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	MUFAS	PUFAS	SFAS
							2008						
15-sep	$14.47 \pm 0.01^{X;H}$	$0.91 \pm 0.01^{X;D}$	$0.16 \pm 0.03^{X;BC}$	$0.27 \pm 0.02^{X;E}$	$2.07 \pm 0.11^{X;BC}$	$72.66 \pm 0.03^{\text{Y;D}}$	$7.81 \pm 0.06^{X;A}$	0.89± 0.01 <sup>Y;G</sup>	$0.42 \pm 0.00^{X;DE}$	$0.34 \pm 0.02^{X;CD}$	$74.18 \pm 0.00^{Y;C}$	8.71 $\pm 0.07^{X;A}$	17.11 ± 0.07 <sup>X;H</sup>
23-sep	14.56 $\pm 0.19^{XY;H}$	$0.94 \pm 0.01^{X;E}$	$0.13\pm0.00^{\text{Y;ABC}}$	$0.27 \pm 0.00^{Y;E}$	$1.98 \pm 0.05^{Y;AB}$	$72.32 \pm 0.28^{Y;BC}$	$8.30 \pm 0.05^{Y;B}$	$0.78 \pm 0.02^{Y;F}$	$0.39 \pm 0.02^{X;BCD}$	$0.32 \pm 0.01^{X;B}$	$73.86 \pm 0.29^{Y;B}$	$9.08 \pm 0.07^{Y;B}$	$17.07 \pm 0.22^{Y;FG}$
27-sep	14.19 $\pm 0.06^{X;G}$	$0.82 \pm 0.00^{X;C}$	$0.17 \pm 0.01^{Z;C}$	$0.34 \pm 0.04^{Y;F}$	$2.15 \pm 0.23^{Y;C}$	$71.82 \pm 0.12^{X;A}$	$8.93 \pm 0.04^{Z;D}$	$0.87 \pm 0.06^{Y;G}$	$0.36 \pm 0.03^{X;A}$	$0.36 \pm 0.00^{Y;E}$	$73.34 \pm 0.08^{X;A}$	9.80± 0.02 <sup>Z;F</sup>	$16.87 \pm 0.12^{Y;F}$
06-oct	$13.72 \pm 0.07^{X;EF}$	$0.79 \pm 0.01^{X;B}$	$0.14\pm0.00^{Z;ABC}$	$0.27 \pm 0.00^{\text{Y;CDE}}$	$2.03 \pm 0.03^{Y;ABC}$	$72.34 \pm 0.02^{Y;C}$	$9.27 \pm 0.07^{Z;F}$	$0.68 \pm 0.04^{X;E}$	$0.42 \pm 0.01^{^{Y;DE}}$	$0.35\pm0.00^{\text{X;DE}}$	$73.75 \pm 0.01^{Y;B}$	9.94± 0.03 <sup>Z;G</sup>	$16.31 \pm 0.03^{Y_{;E}}$
14-oct	$13.87 \pm 0.09^{X;F}$	$1.07 \pm 0.02^{Y;G}$	$0.14\pm0.01^{\text{Y;ABC}}$	$0.26\pm0.01^{\text{Y;BCDE}}$	$1.96 \pm 0.03^{Y;AB}$	$72.23 \pm 0.19^{Y;BC}$	$9.21 \pm 0.01^{_{Y;F}}$	$0.61\pm0.04^{\text{XY;CD}}$	$0.36 \pm 0.01^{X;AB}$	$0.29 \pm 0.01^{X;A}$	$73.85 \pm 0.15^{Y;B}$	$9.83 \pm 0.03^{Y;F}$	$16.33 \pm 0.10^{X;E}$
20-oct	$13.82 \pm 0.02^{X;F}$	$1.15 \pm 0.02^{X;H}$	$0.14 \pm 0.05^{X;ABC}$	$0.27 \pm 0.03^{Y;DE}$	$1.96 \pm 0.04^{Y;AB}$	$72.05 \pm 0.03^{Y;AB}$	$9.20 \pm 0.04^{X;F}$	0.64 ±0.01 <sup>Z;DE</sup>	$0.42\pm0.00^{\text{X;ABC}}$	$0.34 \pm 0.00^{Y;D}$	$73.88 \pm 0.08^{Y;B}$	$9.84 \pm 0.03^{Y;F}$	16.29 ± 0.10 <sup>X;E</sup>
27-oct	13.58 ± 0.06 <sup>X;E</sup>	$0.94 \pm 0.00^{X;E}$	$0.15 \pm 0.04^{X;ABC}$	0.23±0.01 <sup>Y;ABCD</sup>	$2.02 \pm 0.05^{\text{Y;ABC}}$	72.30± 0.06 <sup>Y;BC</sup>	$9.48 \pm 0.01^{Y_{G}}$	$0.56 \pm 0.01^{_{Y;B}}$	$0.43 \pm 0.00^{Y;DE}$	$0.32 \pm 0.00^{\text{XY;B}}$	$73.79 \pm 0.07^{Y;B}$	$10.04 \pm 0.00^{Y;H}$	$16.17 \pm 0.07^{Y_{;E}}$
03-nov	$13.10 \pm 0.03^{X;D}$	$0.90 \pm 0.00^{X;D}$	$0.11 \pm 0.00^{Y;AB}$	$0.22 \pm 0.00^{Y;AB}$	$1.94 \pm 0.01^{\text{Y;AB}}$	$72.95 \pm 0.06^{Z;E}$	$9.45 \pm 0.03^{X;G}$	$0.57 \pm 0.00^{Z;BC}$	$0.40\pm0.00^{\text{XY;DE}}$	$0.32 \pm 0.00^{X;BC}$	74.40± 0.05 <sup>Y;C</sup>	10.02± 0.03 <sup>Y;GH</sup>	15.58 ± 0.01 <sup>X;D</sup>
10-nov	$13.09 \pm 0.06^{X;D}$	$1.05 \pm 0.01^{X;G}$	$0.14{\pm}0.01^{\text{Y;ABC}}$	$0.22 \pm 0.00^{X;A}$	$1.93 \pm 0.04^{\text{Y;AB}}$	$73.39 \pm 0.01^{Z;F}$	$8.95 \pm 0.00^{X;D}$	$0.53 \pm 0.00^{X;AB}$	$0.39 \pm 0.00^{Y;CD}$	$0.31 \pm 0.00^{X;B}$	$74.98 \pm 0.01^{Y;D}$	9.47±0.01 <sup>x;D</sup>	$15.55 \pm 0.02^{Y;D}$
17-nov	$12.63 \pm 0.03^{X;C}$	$1.02 \pm 0.00^{X;F}$	$0.10 \pm 0.00^{\text{Y;A}}$	$0.22 \pm 0.02^{Y;AB}$	$1.88 \pm 0.00^{Y;A}$	$74.15 \pm 0.06^{Z;G}$	$8.75 \pm 0.01^{X;C}$	$0.53 \pm 0.00^{Y;AB}$	$0.39\pm0.00^{\text{Y;BCD}}$	$0.31 \pm 0.01^{X;B}$	75.70± 0.01 <sup>Z;E</sup>	9.29± 0.02 <sup>x;c</sup>	15.01 ± 0.03 <sup>Y;C</sup>
24-nov	12.02 $\pm 0.03^{X;A}$	$0.76 \pm 0.01^{X;A}$	$0.11 \pm 0.00^{Y;AB}$	0.23± 0.02 <sup>X;ABC</sup>	$2.01{\pm}0.01^{\text{Z;ABC}}$	$74.69 \pm 0.06^{Z;H}$	$8.91 \pm 0.05^{X;D}$	$0.49 \pm 0.00^{X;A}$	$0.44 \pm 0.01^{Z;E}$	$0.34 \pm 0.00^{\text{Y;CD}}$	$76.01 \pm 0.07^{Z;F}$	$9.41 \pm 0.06^{X;D}$	14.58 ± 0.01 <sup>X;A</sup>
1-dec	$12.20 \pm 0.02^{X;B}$	$0.84 \pm 0.00^{X;C}$	$0.14 \pm 0.04^{X;ABC}$	0.24±0.01 <sup>X;ABCD</sup>	$1.96 \pm 0.01^{X;AB}$	$74.28 \pm 0.04^{Y;G}$	$9.09 \pm 0.03^{X;E}$	$0.54 \pm 0.01^{X;AB}$	$0.41 \pm 0.01^{X;DE}$	$0.31 \pm 0.00^{X;B}$	$75.66 \pm 0.03^{Y;E}$	9.64 ± 0.03 <sup>x;E</sup>	14.71 ± 0.06 <sup>X;B</sup>
							2009						
15-sep	$14.85 \pm 0.01^{\text{y;GH}}$	$0.94 \pm 0.03^{X;A}$	0.15 ± 0.01 <sup>X;F</sup>	$0.29 \pm 0.00^{X;G}$	$2.03 \pm 0.02^{X;E}$	71.62 ± 0.02 <sup>X;F</sup>	8.51 ± 0.01 <sup>X;A</sup>	$0.83 \pm 0.02^{Y;G}$	$0.44 \pm 0.00^{Y;DE}$	0.34± 0.00 <sup>X;EF</sup>	73.19 ± 0.05 <sup>x;E</sup>	$9.33 \pm 0.01^{Y_{;B}}$	$17.47 \pm 0.04^{Y;H}$
23-sep	14.86 ± 0.08 <sup>y;GH</sup>	$0.98 \pm 0.01^{Y;AB}$	$0.14 \pm 0.01^{\rm Y;E}$	$0.27 \pm 0.00^{Y;E}$	$2.00 \pm 0.01^{X;DE}$	71.64 ± 0.08 <sup>X;F</sup>	8.59 ± 0.02 <sup>Z;C</sup>	$0.75 \pm 0.00^{Y;F}$	$0.44 \pm 0.02^{Y;DE}$	0.33±0.01 <sup>X;DE</sup>	$73.21 \pm 0.08^{X;E}$	$9.35 \pm 0.02^{Z;B}$	17.44 $\pm 0.10^{Y;H}$
27-sep	$14.73 \pm 0.04^{\text{y;EFG}}$	$0.96 \pm 0.00^{Y;AB}$	$0.14 \pm 0.00^{\rm Y;E}$	0.28± 0.00 <sup>XY;F</sup>	$1.99 \pm 0.02^{X;CD}$	$71.85 \pm 0.01^{\rm Y;G}$	$8.55 \pm 0.01^{Y;B}$	$0.73 \pm 0.01^{X;F}$	$0.44 \pm 0.01^{X;CD}$	0.34 ±0.01 <sup>X;EF</sup>	73.43± 0.02 <sup>X;F</sup>	$9.28 \pm 0.01^{Y;A}$	$17.30 \pm 0.01^{Z;FG}$
06-oct	$14.79 \pm 0.04^{Z;FG}$	1.07± 0.01 <sup>Y;ABC</sup>	$0.13 \pm 0.00^{\rm Y;D}$	$0.26 \pm 0.00^{\rm Y;D}$	1.97 ± 0.02 <sup>X;BC</sup>	$71.49 \pm 0.05^{X;E}$	$8.85 \pm 0.00^{\rm Y;D}$	0.69± 0.02 <sup>X;E</sup>	$0.43 \pm 0.00^{\text{Y;BCD}}$	$0.33 \pm 0.00^{X;E}$	$73.15 \pm 0.04^{Z;E}$	$9.54 \pm 0.01^{Y;C}$	17.31 $\pm 0.03^{Z;G}$
14-oct	$14.96 \pm 0.02^{Z;H}$	$0.95 \pm 0.01^{X;A}$	$0.13 \pm 0.00^{\text{Y;DE}}$	$0.25 \pm 0.01^{\text{Y;BC}}$	$1.96 \pm 0.01^{X;B}$	69.99 ± 0.06 <sup>X;A</sup>	10.29± 0.03 <sup>Z;I</sup>	$0.68 \pm 0.01^{\rm Y;E}$	$0.44 \pm 0.00^{Y;DE}$	$0.35 \pm 0.00^{Y;F}$	71.54± 0.05 <sup>X;A</sup>	10.97± 0.04 <sup>Z;G</sup>	$17.49 \pm 0.01^{Y;H}$
20-oct	$14.66 \pm 0.02^{Z;EF}$	1.24± 0.06 <sup>XY;CD</sup>	$0.13 \pm 0.00^{X;CD}$	$0.25 \pm 0.00^{XY;CD}$	$1.96 \pm 0.01^{X;B}$	$70.67 \pm 0.02^{X;C}$	$9.77 \pm 0.02^{Y;E}$	$0.61 \pm 0.00^{Y;D}$	$0.44 \pm 0.01^{\text{Y;DE}}$	0.31±0.01 <sup>X;BC</sup>	72.47± 0.03 <sup>X;C</sup>	$10.38 \pm 0.02^{Z;D}$	$17.18 \pm 0.01^{Y;EF}$
27-oct	$14.59 \pm 0.02^{Y;E}$	$1.19 \pm 0.01^{\text{Y;BCD}}$	$0.12 \pm 0.01^{X;BC}$	$0.24 \pm 0.00^{Y;AB}$	$1.95 \pm 0.00^{X;B}$	$70.36 \pm 0.01^{X;B}$	$10.24 \pm 0.03^{Z;GH}$	$0.61 \pm 0.01^{Z;D}$	$0.40\pm0.00^{Y;\text{ABC}}$	$0.31 \pm 0.00^{X;AB}$	$72.09 \pm 0.00^{X;B}$	$10.84 \pm 0.03^{Z;F}$	$17.06 \pm 0.03^{Z;E}$
03-nov	$14.19 \pm 0.13^{Y;D}$	$1.40 \pm 0.36^{X;D}$	$0.12 \pm 0.00^{Y_{;B}}$	$0.24 \pm 0.00^{Z;A}$	$2.00 \pm 0.01^{X;DE}$	$70.77 \pm 0.08^{X;C}$	$10.19 \pm 0.00^{ m Y;F}$	0.53±0.00 <sup>Y;AB</sup>	$0.47 \pm 0.05^{Y;E}$	$0.34 \pm 0.00^{Y;EF}$	$72.75 \pm 0.28^{X;D}$	$10.72 \pm 0.00^{Z;E}$	$16.78 \pm 0.07^{Y;D}$
10-nov	$14.27 \pm 0.02^{y;D}$	$1.22 \pm 0.01^{\text{Y;CD}}$	$0.12 \pm 0.00^{Y_{;B}}$	$0.25 \pm 0.00^{X;BC}$	$1.91 \pm 0.00^{X;A}$	$70.71 \pm 0.07^{X;C}$	$10.23 \pm 0.01^{Z;G}$	$0.59 \pm 0.03^{Y;D}$	$0.40 \pm 0.00^{Y;AB}$	0.31±0.00 <sup>X;ABC</sup>	72.47± 0.06 <sup>X;C</sup>	$10.83 \pm 0.04^{Z;F}$	$16.69 \pm 0.02^{Z;D}$
17-nov	$14.02 \pm 0.10^{\text{Y;C}}$	$1.22 \pm 0.02^{Y;CD}$	$0.11 \pm 0.00^{Z;AB}$	$0.24 \pm 0.00^{Z;AB}$	$1.89 \pm 0.01^{X;A}$	$71.00 \pm 0.08^{X;D}$	$10.27 \pm 0.02^{Z;HI}$	$0.57 \pm 0.00^{Z;C}$	$0.38 \pm 0.00^{Y;A}$	$0.30 \pm 0.01^{X;A}$	$72.75 \pm 0.06^{X;D}$	$10.84 \pm 0.02^{Z;F}$	$16.41 \pm 0.08^{Z;C}$
24-nov	$13.54 \pm 0.08^{Z;B}$	$1.15\pm0.03^{\text{Y;ABC}}$	$0.11 \pm 0.00^{Y;A}$	$0.23 \pm 0.01^{X;A}$	$1.91 \pm 0.01^{X;A}$	$71.60 \pm 0.10^{X;EF}$	$10.21 \pm 0.01^{\text{Y;FG}}$	$0.55 \pm 0.00^{Y;BC}$	$0.39 \pm 0.01^{Y;AB}$	0.31 ±0.01 <sup>X;BC</sup>	73.29 ±0.07 <sup>X;EF</sup>	$10.76 \pm 0.02^{Y;E}$	$15.95 \pm 0.06^{Y;B}$
1-dec	$13.29 \pm 0.10^{Y;A}$	1.01± 0.02 <sup>Y;ABC</sup>	$0.11 \pm 0.00^{X;AB}$	$0.23 \pm 0.01^{X;A}$	$1.96 \pm 0.01^{X;B}$	$71.62 \pm 0.08^{X;EF}$	$10.52 \pm 0.01^{ m Y;J}$	$0.52 \pm 0.01^{X;A}$	$0.41\pm0.00^{X;\text{ABCD}}$	0.32 ±0.01 <sup>X;CD</sup>	73.18± 0.09 <sup>X;E</sup>	$11.04 \pm 0.00^{\text{Y;H}}$	$15.78 \pm 0.09^{Y;A}$

							2010						
15-sep	-	-	-	-	-	-	-	-	-	-	-	-	-
23-sep	14.32 $\pm 0.06^{X;G}$	$1.00 \pm 0.01^{Y;A}$	$0.11 \pm 0.00^{X;E}$	$0.25 \pm 0.01^{X;E}$	$1.68 \pm 0.00^{X;B}$	$73.78 \pm 0.09^{Z;E}$	$7.40 \pm 0.02^{X;A}$	$0.71 \pm 0.01^{X;H}$	$0.39 \pm 0.03^{X;D}$	$0.37 \pm 0.00^{Y;E}$	75.39 ± 0.06 <sup>Z;H</sup>	$8.12 \pm 0.01^{X;A}$	$16.39 \pm 0.06^{X;F}$
27-sep	14.33 $\pm 0.03^{X;G}$	$1.01 \pm 0.00^{Z;A}$	$0.09 \pm 0.00^{X;CD}$	$0.24\pm0.01^{\text{Z;DE}}$	$1.68 \pm 0.02^{X;B}$	73.29± 0.13 <sup>Y;D</sup>	$7.89 \pm 0.02^{X;B}$	$0.69 \pm 0.01^{X;G}$	$0.41 \pm 0.03^{\text{Y;D}}$	$0.38 \pm 0.00^{Y;F}$	$74.92 \pm 0.13^{Y_{;E}}$	$8.58 \pm 0.03^{X;B}$	$16.40 \pm 0.07^{X;F}$
06-oct	14.10 $\pm 0.03^{Y;F}$	$1.19 \pm 0.01^{Z;C}$	$0.10\pm0.01^{\text{X;DE}}$	0.23 ±0.01 <sup>X;CDE</sup>	$1.69 \pm 0.01^{Y;B}$	$73.33 \pm 0.08^{Z;D}$	$8.03 \pm 0.08^{X;C}$	$0.61 \pm 0.02^{X;F}$	$0.38 \pm 0.01^{X;CD}$	$0.35 \pm 0.01^{X;D}$	75.10± 0.06 <sup>X;FG</sup>	$8.64 \pm 0.10^{X;B}$	$16.17 \pm 0.04^{X;E}$
14-oct	14.31 $\pm 0.01^{Y;G}$	$1.46 \pm 0.00^{Z;C}$	$0.09 \pm 0.00^{X;BCD}$	$0.21\pm0.01^{\text{Y;BC}}$	$1.65 \pm 0.01^{Y;A}$	$72.25 \pm 0.03^{Y;A}$	$8.78 \pm 0.00^{X;E}$	$0.56 \pm 0.01^{X;E}$	$0.35 \pm 0.01^{X;AB}$	$0.33 \pm 0.01^{\text{Y;BC}}$	$74.25 \pm 0.01^{Z;B}$	$9.34 \pm 0.02^{X;D}$	$16.31 \pm 0.00^{X;F}$
20-oct	$14.13 \pm 0.00^{Y;F}$	$1.34 \pm 0.01^{\rm Y;D}$	$0.09 \pm 0.00^{X;BCD}$	$0.20\pm0.00^{\text{Y;AB}}$	$1.68 \pm 0.01^{Y;B}$	$72.21 \pm 0.00^{Y;A}$	$9.12 \pm 0.00^{X;F}$	$0.54 \pm 0.00^{X;DE}$	$0.35 \pm 0.01^{X;BC}$	$0.33 \pm 0.00^{Y;C}$	$74.09 \pm 0.00^{Z;A}$	$9.66 \pm 0.01^{X;E}$	$16.16 \pm 0.01^{X;E}$
27-oct	$13.6 \pm 0.06^{X;E}$	$1.25 \pm 0.00^{Z;D}$	$0.09 \pm 0.00^{X;BCD}$	$0.21\pm0.00^{\text{X;AB}}$	$1.73 \pm 0.01^{\rm Y;C}$	$73.36 \pm 0.10^{Z;D}$	$8.56 \pm 0.03^{X;D}$	$0.52 \pm 0.01^{X;CD}$	$0.35 \pm 0.00^{X;BC}$	0.33±0.00 <sup>Y;BC</sup>	$75.14 \pm 0.10^{Z;G}$	$9.08 \pm 0.04^{X;C}$	$15.68 \pm 0.06^{X;D}$
03-nov	13.37 ± 0.06 <sup>X;D</sup>	$1.33 \pm 0.02^{X;E}$	$0.09{\pm}0.00^{\text{X;ABCD}}$	$0.21\pm0.00^{\text{X;AB}}$	$1.70 \pm 0.00^{Y;B}$	72.75 ±0.04 <sup>Y;B</sup>	$9.39 \pm 0.05^{X;G}$	$0.50 \pm 0.01^{X;BC}$	$0.34 \pm 0.00^{X;AB}$	$0.32 \pm 0.00^{X;B}$	$74.60 \pm 0.01^{\rm Y;D}$	$9.89 \pm 0.05^{X;F}$	$15.42 \pm 0.06^{X;C}$
10-nov	$13.09 \pm 0.05^{x;c}$	$1.36 \pm 0.03^{Z;E}$	$0.08 \pm 0.00^{X;A}$	$0.22 \pm 0.03^{X;BCD}$	$1.68 \pm 0.01^{Y;B}$	$73.09 \pm 0.01^{\rm Y;C}$	$9.36 \pm 0.05^{Y_{G}}$	$0.48 \pm 0.00^{X;AB}$	$0.34 \pm 0.00^{X;AB}$	$0.31 \pm 0.00^{X;A}$	74.96± 0.03 <sup>Y;EF</sup>	$9.84 \pm 0.05^{Y;F}$	$15.11 \pm 0.06^{X;B}$
17-nov	12.71 $\pm 0.013^{X;B}$	$1.24 \pm 0.01^{_{Y;E}}$	$0.08 \pm 0.01^{X;AB}$	$0.19 \pm 0.00^{X;A}$	$1.69 \pm 0.01^{Y;B}$	$73.78 \pm 0.04^{Y;E}$	$9.21 \pm 0.11^{Y;F}$	$0.47 \pm 0.00^{X;A}$	$0.32 \pm 0.02^{X;A}$	$0.30 \pm 0.00^{X;A}$	$75.52 \pm 0.06^{\text{Y;H}}$	$9.68 \pm 0.11^{Y_{;E}}$	14.72 $\pm 0.04^{X;A}$
24-nov	$12.58 \pm 0.04^{Y;A}$	$1.13 \pm 0.01^{\rm Y;F}$	$0.09 \pm 0.00^{X;ABC}$	$0.20\pm0.00^{X;AB}$	$1.73 \pm 0.01^{\rm Y;C}$	$72.81 \pm 0.06^{Y;B}$	$10.32 \pm 0.07^{Y;H}$	$0.49 \pm 0.02^{X;AB}$	$0.36 \pm 0.00^{X;BC}$	$0.30 \pm 0.00^{X;A}$	$74.44 \pm 0.06^{\text{Y;C}}$	10.81± 0.09 <sup>Y;G</sup>	14.67 $\pm 0.03^{X;A}$
1-dec	-	-	-	-	-	-	-	-	-	-	-	-	-

 $^{X-Z}$  different letters between three columns for the same parameters indicated statistical differences (P $\leq$ 0.05) between years for the same data.

<sup>A-J</sup> different letters indicated statistical differences between consecutive dates at each year.

		2008		2009	2	010
Correlations r ( P≤ 0.05)	r	Р	r	Р	r	Р
RI /(oleic/linoleic)	ns	0.55	-0.80**	0,0016	-0.86**	0.0013
RI /(mufa/pufa)	ns	0.84	-0.77**	0.0032	-0.86**	0.0015
RI / Carotenoid	-0.85***	0.0005	-0.85***	0.0005	ns	0.0645
RI /Chorophyll	-0.83***	0.0009	-0.90***	< 0.0001	-0.65*	0.0416
RI /Total pigments	-0.83***	0.0007	-0.88***	0.0001	-0.64*	0.0467
L*/Carotenoid	-0.97***	< 0.0001	-0.96***	< 0.0001	-0.83**	0.0029
L*/Chlorophyll	-0.98***	< 0.0001	-0.97***	< 0.0001	-0.83**	0.0038
L*/ Total pigments	-0.98***	< 0.0001	-0.97***	< 0.0001	-0.82**	0.0035
Oxid. Stab/ Total phenols	-	-	0.85***	0.0005	0.89***	0.0006

**Table 3.** Statistical correlations ( $P \le 0.05$ ) between some studied analytical parameters.

For the significance level: 0.05

(ns) if p >0.05 (Not significant)

1 (\*) if p= 0.01 to 0.05 (Significant)

2 (\*\*) if p= 0.001 to 0.01 (Very significant)

3 (\*\*\*) if p < 0.001 (Extremely significant)

5 Table 4. Color coordinates of studied olive oils in relation to picking date of

		L*			a*		b*			
	2008	2009	2010	2008	2009	2010	2008	2009	2010	
15-sep	70.24 ± 0.23 <sup>Y;C</sup>	61.22 ± 0.07 <sup>X;A</sup>	-	0.63 ± 0.06 <sup>Y;G</sup>	-15.63 ± 0.08 <sup>X;D</sup>	-	112.72± 0.10 <sup>Y;H</sup>	$101.75 \pm 0.12^{X;A}$	-	
23-sep	70.58 ± 0.04 <sup>Y;D</sup>	69.61 ± 0.29 <sup>X;B</sup>	83.43 ± 0.09 <sup>Z;A</sup>	2.91 ± 0.04 <sup>Z;H</sup>	$-18.49 \pm 0.09^{X;A}$	$-13.80 \pm 0.03^{Y;G}$	113.99± 0.08 <sup>Y;I</sup>	$110.11 \pm 0.38^{X;D}$	116.81 ± 0.17 <sup>Z;J</sup>	
27-sep	$58.38 \pm 0.04^{X;A}$	78.07 ± 0.59 <sup>Y;C</sup>	$83.42 \pm 0.03^{Z;A}$	- 4.91±0.00 <sup>Z;A</sup>	-15.01 ± 0.15 <sup>Y;F</sup>	$-15.39 \pm 0.03^{X;C}$	$101.00 \pm 0.03^{X;C}$	118.76 ± 0.95 <sup>Z;G</sup>	112.29 ± 0.28 <sup>Y;H</sup>	
06-oct	62.75 ± 0.01 <sup>X;B</sup>	78.05 ± 0.81 <sup>Y;C</sup>	$87.15 \pm 0.38^{Z;B}$	-2.14± 0.01 <sup>Z;C</sup>	$-13.24 \pm 0.16^{_{Y;H}}$	$-17.01 \pm 0.04^{X;B}$	$105.91 \pm 0.73^{X;E}$	$119.63 \pm 1.02^{Z;H}$	$113.45 \pm 0.65^{Y;I}$	
14-oct	$80.24 \pm 0.01^{X;E}$	$83.80 \pm 0.04^{\text{Y};\text{E}}$	91.78± 0.070 <sup>Z;G</sup>	$0.52 \pm 0.01^{Z;G}$	-12.25 ± 0.06 <sup>Y;I</sup>	$-12.78 \pm 0.03^{X;J}$	$120.53 \pm 0.40^{X;K}$	$125.97 \pm 0.14^{Z;K}$	$81.01 \pm 0.57^{Y;A}$	
20-oct	$87.48 \pm 0.04^{Y;J}$	81.68 ± 0.05 <sup>X;D</sup>	$88.16 \pm 0.14^{Z;C}$	-0.85± 0.05 <sup>Z;E</sup>	$-15.56 \pm 0.06^{X;D}$	$-13.10 \pm 0.03^{Y;I}$	$102.43 \pm 0.19^{\text{Y;D}}$	$123.89 \pm 0.11^{Z_{; }}$	$84.46 \pm 0.13^{X;B}$	
27-oct	85.63 ± 0.02 <sup>x;H</sup>	$87.08 \pm 0.02^{Y;F}$	89.91 ± 0.12 <sup>Z;E</sup>	-1.04±0.02 <sup>Z;DE</sup>	$-14.11 \pm 0.04^{Y;G}$	$-14.16 \pm 0.03^{X;F}$	$115.10 \pm 0.52^{\text{Y;J}}$	$125.31 \pm 0.15^{Z;J}$	94.34 ± 0.17 <sup>X;D</sup>	
03-nov	84.78 ± 0.01 <sup>X;G</sup>	92.90 ± 0.05 <sup>Z;H</sup>	$91.41 \pm 0.14^{Y;F}$	$0.48 \pm 0.06^{Z;G}$	$-16.96 \pm 0.02^{X;C}$	$-14.33 \pm 0.06^{Y;D}$	$111.33 \pm 0.48^{Y;G}$	$113.53 \pm 0.30^{Z;F}$	98.53 ± 0.24 <sup>X;F</sup>	
10-nov	$88.61 \pm 0.01^{X;L}$	89.20 ± 0.09 <sup>X;G</sup>	$93.99 \pm 0.11^{\text{Y;H}}$	-1.50± 1.17 <sup>Z;D</sup>	$-18.46 \pm 0.06^{X;A}$	$-13.75 \pm 0.03^{\text{Y;H}}$	$100.54 \pm 0.18^{\text{Y;C}}$	$123.71 \pm 0.18^{Z_{;}I}$	86.32 ± 0.13 <sup>X;C</sup>	
17-nov	$88.36 \pm 0.01^{Y;K}$	86.67 ± 0.02 <sup>X;F</sup>	93.93 ± 0.26 <sup>z;H</sup>	$-2.78 \pm 0.03^{Z;B}$	$-17.27 \pm 0.04^{X;B}$	$-14.21 \pm 0.05^{\text{Y;E}}$	$108.93 \pm 0.53^{\text{Y;F}}$	112.52 ± 0.38 <sup>Z;E</sup>	95.88 ± 0.35 <sup>X;E</sup>	
24-nov	$82.50 \pm 0.15^{X;F}$	$87.91 \pm 0.07^{Y;F}$	$89.15 \pm 0.25^{Z;D}$	-0.14± 0.11 <sup>Z;F</sup>	$-17.33 \pm 0.03^{Y_{;B}}$	$-17.78 \pm 0.03^{X;A}$	98.57 ± 0.27 <sup>x;B</sup>	$109.02 \pm 0.39^{\text{Y:C}}$	$109.00 \pm 0.45^{Y;G}$	
1-dec	$86.47 \pm 0.01^{x;i}$	92.08 ± 0.05 <sup>Y;H</sup>	-	-1.46± 0.01 <sup>Y;DE</sup>	-15.26 ± 0.01 <sup>X;E</sup>	-	91.41 ± 0.14 <sup>X;A</sup>	$106.34 \pm 0.17^{Y;B}$	-	

6 superintensive Arbequina cultivar for the crop years 2008, 2009 and 2010.

<sup>X-Z</sup> different letters between three columns for the same parameters indicated statistical differences ( $P \le 0.05$ ) between years for the same data.

 $^{A\text{-L}}$  different letters indicated statistical differences (P≤0.05) between consecutive dates at each year.

Table 5. Scores of fruity, bitterness and pungency of olive oils from olive oils in
relation to picking date of superintensive Arbequina cultivar for the crop years 2008,

19 2009 and 2010.

	Fruity				Pungency				
	2008	2009	2010	2008	2009	2010	2008	2009	2010
15-sep	3.20 <sup>X; B</sup>	5.65 <sup>Y; H</sup>	-	1.60 <sup>Y; D</sup>	1.20 <sup>x; c</sup>	-	3.70 <sup>X; G</sup>	4.45 <sup>Y; J</sup>	-
23-sep	4.10 <sup>X; D</sup>	4.90 <sup>Y; C</sup>	5.40 <sup>Z; D</sup>	2.60 <sup>Z; H</sup>	1.30 <sup>X; E</sup>	2.40 <sup>Y; E</sup>	3.50 <sup>Z; E</sup>	3.35 <sup>Y; G</sup>	2.40 <sup>X; A</sup>
27-sep	4.60 <sup>Y; F</sup>	4.60 <sup>Y; B</sup>	4.00 <sup>X; A</sup>	2.20 <sup>Y; G</sup>	1.55 <sup>x; g</sup>	3.50 <sup>Z; J</sup>	3.60 <sup>Y; F</sup>	2.95 <sup>x; d</sup>	4.60 <sup>Z; G</sup>
06-oct	3.50 <sup>x; c</sup>	6.40 <sup>Z; J</sup>	4.90 <sup>Y; C</sup>	1.50 <sup>x; c</sup>	2.60 <sup>Z; L</sup>	1.80 <sup>Y; B</sup>	3.50 <sup>Y; E</sup>	5.10 <sup>Z; K</sup>	3.20 <sup>x; c</sup>
14-oct	4.30 <sup>X; E</sup>	6.05 <sup>Y; I</sup>	6.70 <sup>Z; I</sup>	2.80 <sup>Y; I</sup>	1.90 <sup>x; J</sup>	1.90 <sup>x; c</sup>	4.10 <sup>Z; I</sup>	4.00 <sup>Y; H</sup>	2.40 <sup>X; A</sup>
20-oct	3.00 <sup>X; A</sup>	5.55 <sup>Y; G</sup>	6.30 <sup>Z; G</sup>	1.70 <sup>X; E</sup>	2.40 <sup>Y; K</sup>	3.30 <sup>Z; H</sup>	3.10 <sup>X; A</sup>	4.40 <sup>Z; I</sup>	4.20 <sup>Y; E</sup>
27-oct	6.00 <sup>Y; I</sup>	5.10 <sup>X; E</sup>	6.10 <sup>Z; F</sup>	1.30 <sup>X; B</sup>	1.60 <sup>Y; H</sup>	2.30 <sup>Z; D</sup>	3.20 <sup>Y; B</sup>	3.10 <sup>X; E</sup>	4.30 <sup>Z; J</sup>
03-nov	5.00 <sup>x; g</sup>	5.55 <sup>Y; G</sup>	5.60 <sup>Z; E</sup>	1.20 <sup>X; A</sup>	1.75 <sup>Y; I</sup>	2.90 <sup>Z; F</sup>	4.50 <sup>z; J</sup>	3.15 <sup>x; f</sup>	4.00 <sup>Y; D</sup>
10-nov	5.00 <sup>x; g</sup>	5.05 <sup>Y; D</sup>	6.40 <sup>Z; H</sup>	3.00 <sup>Y; J</sup>	0.90 <sup>X; A</sup>	3.20 <sup>Z; G</sup>	3.30 <sup>Y; C</sup>	2.45 <sup>X; B</sup>	4.00 <sup>Z; D</sup>
17-nov	5.00 <sup>Z; G</sup>	4.40 <sup>X; A</sup>	4.70 <sup>Y; B</sup>	1.70 <sup>Y; E</sup>	1.50 <sup>X; F</sup>	3.40 <sup>Z; I</sup>	3.90 <sup>Z; H</sup>	2.85 <sup>Y; C</sup>	2.70 <sup>X; B</sup>
24-nov	5.20 <sup>Z; H</sup>	5.10 <sup>Y;E</sup>	4.90 <sup>x; c</sup>	1.80 <sup>Z; F</sup>	1.25 <sup>X; D</sup>	1.40 <sup>Y; A</sup>	3.40 <sup>Z; D</sup>	2.10 <sup>x; A</sup>	3.20 <sup>Y; C</sup>
1-dec	-	5.15 <sup>F</sup>	-	-	1.00 <sup>B</sup>	-	-	2.1 <sup>A</sup>	-

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 $^{X-Z}$  different letters between three columns for the same parameters indicated statistical differences (P $\leq$ 0.05) between years for the same data.

 $^{\text{A-L}}$  different letters indicated statistical differences (P $\!\leq\!\!0.05$ ) between consecutive dates at each year.

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