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Influence of Harvest Date and Crop Yield on the Fatty Acid Composition of Virgin Olive Oils from Cv. Picual

GABRIEL BELTRÁN,*,† CARMEN DEL RIO,‡ SEBASTIÁN SÁNCHEZ,§ AND LEOPOLDO MARTÍNEZ#

Estación de Olivicultura y Elaiotecnia, CIFA 'Venta del Llano', Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Junta de Andalucía, P.O. Box 50, E-23620 Mengíbar, Jaén, Spain; CIFA 'Alameda del Obispo', IFAPA, P.O. Box 50, E-14081 Córdoba, Spain; Departamento de Ingeniería Química, Ambiental y de los Materiales, Universidad de Jaén, Paraje 'Las Lagunillas' s/n, E-23071 Jaén, Spain; and Departamento de Ingeniería Química, Facultad de Ciencias, Universidad de Granada, Avenida Fuentenueva s/n, E-18071 Granada, Spain

In this study was analyzed the effect of crop year and harvesting time on the fatty acid composition of cv. Picual virgin olive oil. The study was carried out during the fruit ripening period for three crop seasons. The mean fatty acid composition of Picual oils was determined. The oils contained palmitic acid (11.9%), oleic acid (79.3%), and linoleic acid (2.95%). The content of palmitic acid and saturated fatty acids decreased during fruit ripening while oleic and linoleic acids increased. The amount of stearic and linolenic acids decreased. The amount of saturated acids, palmitic and stearic, and the polyunsaturated acids linoleic and linolenic was dependent on the time of harvest, whereas the amount of oleic acid varied with the crop year. The differences observed between crop years for both palmitic and linoleic acid may be explained by the differences in the temperature during oil biosynthesis and by the amount of summer rainfall for oleic acid content. A significant relationship was observed between the MUFA/PUFA ratio and the oxidative stability measured by the Rancimat method.

KEYWORDS: Virgin olive oil; Picual; ripening; crop year; harvesting date; fatty acids

INTRODUCTION

Virgin olive oil is characterized by its sensorial and nutritional properties, which are different from those of other edible oils. Its health benefits are due to both its fatty acid composition and minor compounds, tocopherols, polyphenols, sterols, and carotenoids (*1*).

Olive oil contains mainly monounsaturated fat. The main fatty acid is oleic acid, which can represent between 55 and 83% of the total fat. It also contains a moderate amount of linoleic acid (3.5−21%) (*2*, *3*). Fatty acid composition of olive oil is strongly affected by several agronomical factors such as cultivar, fruit ripeness, crop yield, and growing medium (*4*−*9*). Uceda and Hermoso (*5*), in a preliminary olive germplasm bank evaluation, concluded that the cultivar was the main source of variability for the major fatty acids.

During ripening olive fruit changes its chemical composition, with activation and inhibition of different enzymatic activities. These changes also affect the oil composition. Changes in fatty acids have been described during olive ripening (*5*, *10*−*12*). In general, the content of oleic acid remains constant or shows a slight increase. The saturated fatty acids decrease. The content of palmitic acid (C16:0) decreases, whereas linoleic acid increases. Another parameter that changes is the ratio between monounsaturated and polyunsaturated fatty acids (MUFA/PUFA), which falls as the fruit ripens.

The fatty acid composition of virgin olive oil has great importance from a health point of view. Several studies have shown the dietary importance of fatty acid composition of lipids (*13*). Diets rich in monounsaturated fatty acids and lower in saturated fatty acids lowered low-density lipoprotein (LDL) cholesterol and total cholesterol without altering the beneficial high-density lipoprotein cholesterol levels (*14*, *15*). Aviran and Eias (*16*) noted that LDLs incubated with oleic acid were less oxidized than others with linoleic and arachidonic acid. In other work (*17*) it has been demonstrated that a diet rich in virgin or refined olive oil protected LDL particles from oxidation. Similarly, LDL particles rich in oleic acid and poor in polyunsaturated fatty acids become more resistant to oxidation (*18*). The MUFA/PUFA ratio or the ratio of unsaturated and saturated should show a value of 2 (*19*). In addition, the beneficial effects of dietary monounsaturated fats in preventing

* Address correspondence to this author at the Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, P.O. Box 226, Reading RG6 6AP, U.K. [telephone +44(0) 1183786218; fax +44(0)1189310080; e-mail gbelt@eresmas.com].
† Estación de Olivicultura y Elaiotecnia, IFAPA.
‡ CIFA 'Alameda del Obispo', IFAPA.
§ Universidad de Jaén.
# Universidad de Granada.

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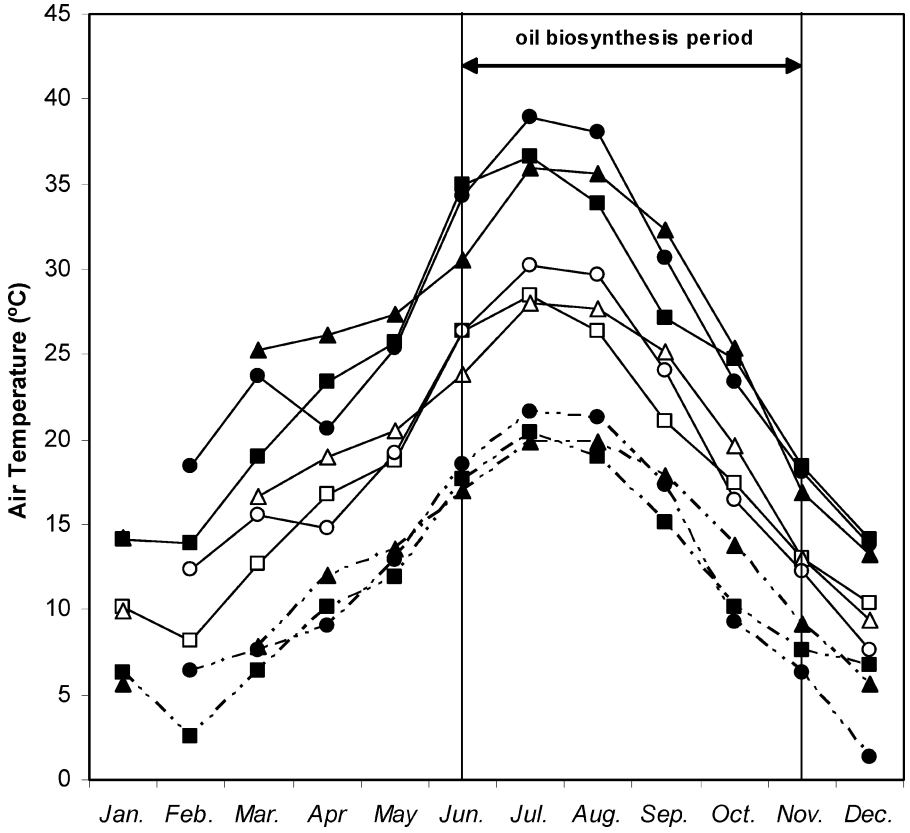

Figure 1. Air temperatures: maximum (black symbols on solid lines), minimum (black symbols on broken lines), and mean (open symbols) registered in Mengibar, Jaén, for the years (■) 1996, (▲) 1997, and (●)1998.

glycemia (*20*), obesity (*21*), and colorectal (*22*), breast, ovary, and prostate cancer have been reported (*23*).

Oxidation depends on the chemical structure and degree of unsaturation of the fatty acid. The relative rates of autoxidation have been determined for oleate/ linoleate/linolenate (1:12:25) (*24*). Monounsaturated acids are more resistant to oxidation. In virgin olive oils, the MUFA/PUFA or oleic/linoleic ratios are responsible for oxidative stability as well as the natural antioxidant content (*12*, *25*, *26*).

Cv. Picual is the most important Spanish olive cultivar for oil extraction purposes and represents ~860 000 ha, located in Jaén and other Andalusian areas. It is also now being grown in different Spanish regions and other countries (*27*). It has a high oil content and produces a virgin olive oil characterized by a high oxidative stability and a strong sensorial personality (*28*). Although Picual is one of the most important olive cultivars in the world, its composition has not been widely studied, and there have not been studies on the effect of the main agronomical factors on its composition. In the current study the effects of fruit ripeness and crop yield on the fatty acid composition of Picual virgin olive oil are examined.

MATERIALS AND METHODS

Plant Material. For this work 12 olive (*Olea europaea L.*) trees of the Picual cultivar were selected. The 16-year-old olive trees were grown in the experimental farm of Estación de Olivicultura y Elaiotecnia in Mengibar (Jaén, Spain) and were cultivated using standard growing techniques. The study was carried out during three consecutive crop seasons: 1996/1997, 1997/1998, and 1998/1999. These crop years showed important differences of registered rainfall (**Table 1**): 1996/1997, 880 mm; 1997/1998, 596 mm; and 1998/1999, 348.9 mm. The monthly temperatures (mean, maximum, and minimum) are shown in **Figure 1**. Olives were collected at biweekly intervals from the middle of September until the autumn frosts. For each crop year, samples were harvested on nine consecutive dates: 1996/1997, September 16 and 30, October 15 and 30, November 15, December 2 and 20, and January 10 and 30; 1997/1998, September 15 and 30, October 15 and 30, and November 14; and 1998/1999, September 16, October 2 and 16, and November 3 and 20. Two samples weighing 2.5 kg were harvested from olive trees in each group and brought to the laboratory for analysis. The ripening index for olives was determined according to the method of Estación de Olivicultura y Elaiotecnia of Spain (*29*).

Table 1. Monthly Rainfall (Millimeters) Registered in Mengibar, Jaén, for the Years 1996, 1997, and 1998

month	1996	1997	1998
January	167.7	191.2	43.8
February	57.7	0	63.6
March	49.6	0	13.2
April	61.7	84.7	62.4
May	75	74.9	84.4
June	6	43.4	17.2
July	0	0	0
August	32.5	0	0
September	89.1	36.1	29.1
October	27	57	9.1
November	118.4	254.2	26.1
December	195.9	138.5	0

Oil Extraction. Oil extraction was performed using an Abencor laboratory oil mill (Abengoa, Spain) equipped with a hammer mill, a thermobeater, and a paste centrifuge (*30*). The extraction was carried out at 28 °C with kneading for 30 min. The oily must was decanted, filtered, and stored at −24 °C until analysis.

Fatty Acid Methyl Esters (FAMEs) Analysis. The FAMEs were prepared as described by the EU official method (*31*). The chromato-

Table 2. Seasonal Changes in Fatty Acid Composition of Picual Virgin Olive Oil during the Ripening Process for Three Different Crop Seasons [Data Are Mean ± Standard Deviation (SD)]

harvest date	crop yield										
	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
						1996/1997					
1	13.18 ± 0.07	0.85 ± 0.07	0.04 ± 0.00	0.08 ± 0.00	1.68 ± 0.04	79.11 ± 0.04	2.42 ± 0.04	0.76 ± 0.06	0.30 ± 0.04	0.25 ± 0.01	0.09 ± 0.01
2	12.44 ± 0.08	0.91 ± 0.01	0.04 ± 0.00	0.07 ± 0.00	1.79 ± 0.04	80.01 ± 0.16	2.37 ± 0.03	0.65 ± 0.06	0.30 ± 0.03	0.24 ± 0.01	0.08 ± 0.00
3	11.59 ± 0.13	0.88 ± 0.03	0.04 ± 0.00	0.07 ± 0.00	1.92 ± 0.06	81.01 ± 0.06	2.41 ± 0.01	0.57 ± 0.04	0.30 ± 0.00	0.22 ± 0.01	0.09 ± 0.01
4	11.54 ± 0.03	0.89 ± 0.03	0.04 ± 0.00	0.07 ± 0.00	2.15 ± 0.07	80.51 ± 0.13	2.77 ± 0.35	0.55 ± 0.08	0.32 ± 0.03	0.23 ± 0.01	0.10 ± 0.00
5	11.12 ± 0.14	0.86 ± 0.03	0.04 ± 0.00	0.08 ± 0.00	2.48 ± 0.08	80.24 ± 0.16	3.26 ± 0.10	0.51 ± 0.04	0.33 ± 0.03	0.22 ± 0.01	0.09 ± 0.01
6	10.74 ± 0.34	0.85 ± 0.03	0.04 ± 0.00	0.08 ± 0.00	2.76 ± 0.08	80.24 ± 0.20	3.45 ± 0.21	0.49 ± 0.00	0.33 ± 0.01	0.21 ± 0.01	0.10 ± 0.01
7	10.53 ± 0.10	0.82 ± 0.03	0.04 ± 0.00	0.08 ± 0.00	2.59 ± 0.04	79.68 ± 0.25	3.89 ± 0.30	0.50 ± 0.01	0.34 ± 0.01	0.22 ± 0.01	0.10 ± 0.00
8	9.89 ± 0.16	0.74 ± 0.03	0.04 ± 0.00	0.08 ± 0.00	2.54 ± 0.08	81.02 ± 0.27	3.42 ± 0.13	0.49 ± 0.03	0.35 ± 0.04	0.23 ± 0.01	0.11 ± 0.01
9	9.67 ± 0.13	0.73 ± 0.01	0.04 ± 0.00	0.08 ± 0.00	2.63 ± 0.07	80.84 ± 0.08	3.73 ± 0.07	0.53 ± 0.01	0.36 ± 0.03	0.24 ± 0.01	0.10 ± 0.00
						1997/1998					
1	13.57 ± 0.03	0.85 ± 0.01	0.04 ± 0.00	0.08 ± 0.00	1.89 ± 0.03	77.76 ± 0.01	2.72 ± 0.03	0.89 ± 0.01	0.36 ± 0.01	0.28 ± 0.01	0.12 ± 0.00
2	13.40 ± 0.13	0.90 ± 0.06	0.04 ± 0.00	0.07 ± 0.00	1.88 ± 0.03	78.02 ± 0.13	2.70 ± 0.03	0.88 ± 0.01	0.35 ± 0.00	0.27 ± 0.00	0.12 ± 0.00
3	12.97 ± 0.06	1.00 ± 0.01	0.03 ± 0.00	0.07 ± 0.00	1.86 ± 0.01	78.73 ± 0.08	2.84 ± 0.03	0.69 ± 0.01	0.34 ± 0.01	0.26 ± 0.01	0.13 ± 0.01
4	12.74 ± 0.04	1.02 ± 0.01	0.04 ± 0.00	0.08 ± 0.00	2.00 ± 0.11	78.31 ± 0.07	3.48 ± 0.06	0.64 ± 0.00	0.34 ± 0.01	0.26 ± 0.01	0.11 ± 0.01
5	12.56 ± 0.03	1.11 ± 0.01	0.03 ± 0.00	0.08 ± 0.01	1.94 ± 0.01	78.47 ± 0.08	3.64 ± 0.03	0.61 ± 0.00	0.33 ± 0.01	0.24 ± 0.00	0.11 ± 0.00
						1998/1999					
1	12.83 ± 0.18	0.90 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	1.70 ± 0.03	79.94 ± 0.03	2.31 ± 0.13	0.79 ± 0.01	0.32 ± 0.00	0.23 ± 0.00	0.11 ± 0.00
2	12.34 ± 0.14	0.90 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	1.79 ± 0.08	80.40 ± 0.06	2.38 ± 0.03	0.75 ± 0.04	0.32 ± 0.00	0.23 ± 0.00	0.10 ± 0.01
3	12.17 ± 0.08	0.94 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	1.90 ± 0.01	80.08 ± 0.10	2.51 ± 0.10	0.70 ± 0.01	0.32 ± 0.00	0.24 ± 0.00	0.11 ± 0.01
4	11.85 ± 0.11	0.98 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	2.14 ± 0.03	79.97 ± 0.28	2.96 ± 0.21	0.62 ± 0.01	0.33 ± 0.01	0.23 ± 0.00	0.10 ± 0.01
5	11.52 ± 0.03	0.98 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	2.31 ± 0.03	80.40 ± 0.06	2.75 ± 0.03	0.57 ± 0.00	0.34 ± 0.00	0.23 ± 0.00	0.10 ± 0.01

graphic separation was carried out using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer, Spain) equipped with an autosampler, a split/splitless injector, and a flame ionization detector (FID). A fused silica capillary column BPX70 (50 m length × 0.25 mm i.d. and 0.25 μm film thickness, SGE Scientific Pty. Ltd., Australia) was used. Helium was used as the carrier gas, and the oven temperature was maintained at 198 °C. The injector and detector temperatures were 235 and 245 °C, respectively. The results were expressed as relative area percent of the total.

Oxidative Stability. Oxidative stability was measured as induction time using a Rancimat 764 instrument (Methrom, Switzerland). Oil, 2.5 g, was heated at 98 °C, and air was bubbled through it at a flow rate of 10−12 L/min. The oxidative stability was measured twice for each sample, and the results are expressed as induction time in hours (*32*).

Statistical Analysis. ANOVA analysis was used to evaluate the effect of the fruit ripeness, expressed as harvesting time, and the crop year on fatty acid composition and to establish differences between mean values (Tukey's test). For ANOVA analysis were used the five common harvest dates. Statistical analyses were carried out using Statistix 1.0 for Windows software.

RESULTS AND DISCUSSION

The ripeness index of olives used for oil extraction during the three crop years oscillated between 0.18 and 3.38 (**Figure 2**).

The results of seasonal changes during ripeness in fatty acid composition of cv. Picual virgin olive oils are shown in **Table 2**. To simplify the analysis and discussion of the results, only the main fatty acids will be discussed: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3).

The major saturated fatty acid in virgin olive oils is palmitic acid, and its mean value in Picual oils was 11.93%. This value is similar to that found by Uceda and Hermoso (*5*). To establish the sources of its variability, an ANOVA analysis was applied (**Table 3**). The variability for palmitic acid can be explained, mainly, by the harvesting time (50.84%) and, then, the crop year (42.37%). The drop in the percent of palmitic acid observed during ripening coincides with the results described for all olive cultivars (*5*). This fall was explained by a dilution effect because its absolute quantity is constant (*12*). During the crop year 1997/1998 the mean content in palmitic acid was higher, whereas the lowest was found in the oils obtained during 1996/1997, obtaining significant differences between the three crop years ($p = 0.01$). These differences may be explained because of the effect of growth temperature on the plant lipid metabolism (*33*). It has been reported that reduced growth temperatures increase membrane lipid unsaturation in order to maintain membrane fluidity at low temperatures. The lower temperatures reduced fatty acid biosynthesis during the June−November period in 1996 (**Figure 1**).

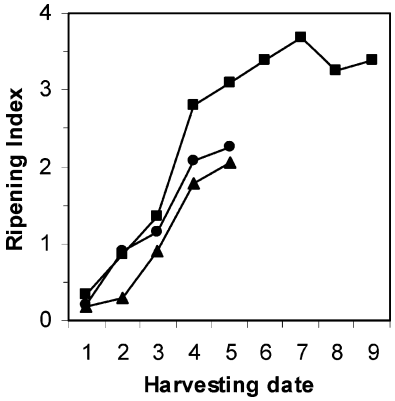

Figure 2. Variation of fruit ripening index for the crop years (■) 1996/1997, (▲) 1997/1998, and (●) 1998/1999.

The elongation of palmitoyl-ACP to stearoyl-ACP is carried out by the condensing enzyme β-ketoacyl-ACP synthase II (KAS II). This step is considered to be very important because it determines the C16/C18 ratio that is directly related to the degree of unsaturation of the final oil product (*34*). The highest C16/C18 ratio mean value was obtained for oils from the 1997/1998 period, whereas the lowest was observed in 1998/1999. The higher value of the 1997/1998 oils is due to their high palmitic acid content, and the lower percent of C18 fatty acids as a consequence of a lower KAS II activity until the first sample collection (September) may be caused by the higher summer rainfalls registered during 1997 (although there was rainfall registered in August 1996, it was from a summer storm), as

Table 3. Variability Expressed as Percent of the Total Sum of the Squares for Fatty Acids and Related Parameters of Picual Virgin Olive Oils[a]

parameter	yield	harvest date	yield × harvest date	error
C16:0	43.27***	50.84***	5.87***	0.91
C16:1	30.11***	32.37***	29.96***	7.56
C17:0	67.86***	10.71	21.43**	0.00
C17:1	74.21***	11.23***	11.93***	2.63
C18:0	3.08**	68.61***	25.41***	2.90
C18:1	80.62***	12.20***	6.34***	0.84
C18:2	28.72***	58.73***	8.31*	4.24
C18:3	22.38***	67.11***	5.45	5.06
C20:0	36.44*	4.59	22.91	36.03
C20:1	53.58***	18.63**	15.10	12.68
C22:0	59.41***	6.73	15.56	18.29
saturated	57.39***	35.17**	5.62**	1.81
monounsaturated	77.35***	15.20***	6.53***	0.92
polyunsaturated	47.88***	38.10***	9.84*	4.18
MUFA/PUFA	54.27***	31.36***	9.46*	4.91
unsaturated/saturated	56.34***	35.96***	6.08***	1.62
oleic/linoleic	52.97***	35.73***	6.30	5.00
oleic/palmitic	49.51***	42.68***	6.91***	0.91

[a] Significance level at *, $p = 0.05$; **, $p = 0.01$; and ***, $p = 0.001$.

described by Romero et al. (*35*). This may explain the unusually greater palmitic content for 1997/1998 oils.

Stearic acid has been observed in Picual at lower values than those found in the literature: 3.25% (*5*) and 4.7% (*12*). The crop year did not show significance as a variability source (3.08%); however, the main factor was the harvesting time, which explains 58.73% of its variation. During olive ripening its content increased; this trend is different from other studies on different olive varieties (*12*, *36*, *37*), although it has been reported that stearic acid does not accumulate (*34*).

Picual oils show high oleic acid content (~77%) as described by Uceda and Hermoso (*5*). The mean value found in this work was 79.10%, within the range of 77.76−81.02%. The variation in the oleic content is due to the crop year, mainly (80.62%), and the harvesting time (12.2%). Thus, it is the fatty acid with the greatest dependence on crop year; significant differences have been found between 1997/1998 oils, with the lower values, and the oils obtained in the other crop years ($p = 0.001$). Different authors have observed that wet summers produce lower levels in oleic acid (*35*, *38*) and higher C16/C18 ratios; for the 1997/1998 crop year, during the June−August period was registered the higher accumulated rainfall, producing the oils with lower oleic content. Because of the low rainfalls registered during 1998, a higher oleic content should be found as described for oils from olives growing in water stress conditions (*39*); although for the first harvesting date the value was higher, significant differences were not found with respect to 1996/1997 oils. As the ripening process goes on, the oleic acid content rises for all crop years. However, differences can be observed in the rise for each year; their increase rates might be related to the initial oleic acid content. Whereas the 1997/1998 yield, characterized by oils with a lower initial content (77.76%), showed the fastest increase, during 1996/1997 was observed the lowest rate because of the initial high oleic content. The rise obtained does not agree with the decrease observed by different authors (*5*, *12*), although it coincided with the results found by Cimato et al. (*11*), who observed the oleic percent to remain constant or rise.

Like cv. Cornicabra, cv. Picual shows one of the lower levels in linoleic acid among the olive cultivars studied (*5*). From the results, the mean value obtained was 2.95%, similar to that described by Gutierrez et al. (*12*), although its content ranged between 2.31 and 3.89%. For this fatty acid the most important source of variability was the harvesting time (58.72%), whereas the crop yield represented 28.72%, although significant differences ($p = 0.001$) have been found between 1997/1998 oils and those obtained during the other crop years. During the ripening process the relative level of linoleic acid increases, as described in general, for several olive varieties (*12*, *36*). The desaturation of oleate to linoleate takes place via phosphatidylcholine substrates on the cytoplasmatic reticulum; for oilseeds it has been observed that the desaturase activity is regulated by the growth temperature, obtaining for lower temperatures higher levels of unsaturation in the fatty acids (*39*, *40*). The lower linoleic mean content was obtained for 1998/1999, which may be explained by the effect on the desaturase activity of higher temperatures registered during this year (**Figure 1**). From the first sample collection (mid-September), the linoleic content increased at a different rate for each crop year; for the 1998/1999 year it showed an increase of 16%, and for the other crop years it was ~25%.

In olive oil, the highest degree of unsaturation is shown by linolenic acid, which achieves a mean value of 0.64% for Picual oils, less than that reported for this cultivar (*5*, *12*). The crop year showed the greater influence on this fatty acid (22.38%), explaining the differences observed between years ($p = 0.05$). As described for linoleic acid, the influence of temperature may be responsible of the higher content observed for 1996/1997 oils. During maturation linolenic content dropped, although a constant value was observed previously.

The fatty acid composition has been studied considering the general groups saturated, unsaturated, monounsaturated, and polyunsaturated. The seasonal changes for fatty acid groups are shown in **Figure 3**. Of the factors that influence saturated fatty acids can be highlighted the crop yield, which explains 57.39% of the variability found, whereas the harvesting time explains only 35.17%. The decrease observed for this fatty acid is due to those observed in palmitic acid; the inverse relationship found between saturated and unsaturated acids indicates a trend to unsaturated forms of de novo synthesis (**Figure 4**). Saturated fatty acids show a behavior similar to that described for palmitic acid and therefore are influenced by the temperature.

The monounsaturated fatty acids comprise the largest group in virgin olive oils; for Picual oils a mean value of 81.31% was achieved, one of the greater of the olive cultivars. The variability observed for these fatty acids may be explained by the ANOVA, where the main variation source is the crop year above the effect of the harvesting time (**Table 3**). Monounsaturated fatty acids show the same seasonal changes during olive fruit ripening that oleic acid does and thus, increases were found for all of the crop yields monitored. Significant differences were observed between the crop years 1996/1997 and 1998/1999.

Polyunsaturated acids correspond to, for the whole of the oils analyzed, 3.59% of the total content, which can be considered as one of the lower among the olive cultivars. For these acids the crop yield had a great importance of 47.88%, as can be observed in the differences found between years. The increase observed during the ripening process is similar for the three crop yields.

The seasonal variation of the parameters related with fatty acid composition, MUFA/PUFA, oleic/linoleic, oleic/palmitic, and unsaturated/saturated, are shown in **Figure 5**. The ratio MUFA/PUFA has great importance because of the effects on nutritional properties and oxidative stability of olive oils. This cultivar achieved a mean value of 22.91, within a wide range

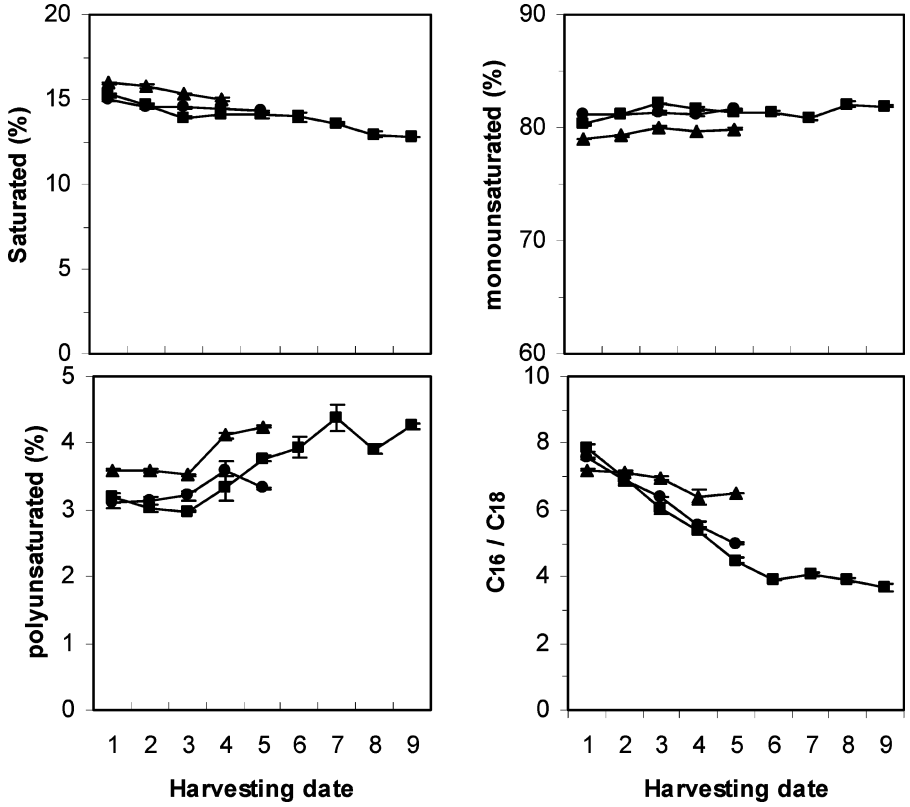

Figure 3. Seasonal changes in fatty acid groups of Picual virgin olive oil during the fruit ripening process for the crop years (■) 1996/1997, (▲) 1997/1998, and (●) 1998/1999.

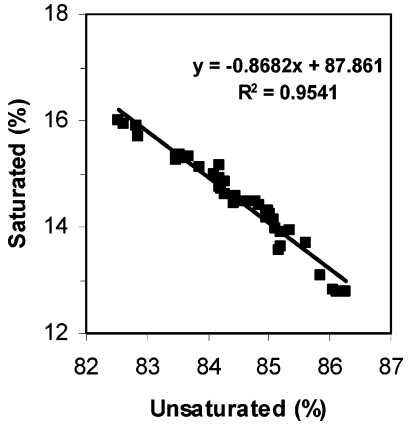

Figure 4. Regression plot between saturated and unsaturated fatty acids during the fruit ripening process for the crop years 1996/1997, 1997/1998, and 1998/1999.

of 18.46−26.91, due to the high oleic acid content and the low linoleic acid level. The differences observed between years are explained by the results of analysis of variance, where the crop yield is mainly responsible for its variation and after the harvesting period (**Table 3**). For the first three harvest dates the value of this parameter showed constant values; however, a drop was observed in its value later. In crop yield for 1996/1997, for the three first harvest dates an increase could be observed; this behavior is caused by the slight decrease of the linoleic acid content. However, the general trend was to decrease during fruit ripening as a result of the slight increase of oleic acid and the greater rise of linoleic acid. This explanation differs from that given by Uceda and Hermoso (*5*), who established that the decrease is attributable to the constant value of oleic acid and the increase of linoleic acid content.

Table 4. Seasonal Changes in Oxidative Stability (Hours) of Picual Virgin Olive Oils during the Fruit Ripening Pocess for the Crop Yields 1996/1997, 1997/1998, and 1998/1999 (Data Are Mean ± SD)

harvest date	crop yield 1996/1997	crop yield 1997/1998	crop yield 1998/1999
1	146.9 ± 4.5	146.5 ± 13.8	193.3 ± 1.3
2	161.0 ± 3.1	169.3 ± 0.9	187.3 ± 2.8
3	192.0 ± 8.1	178.6 ± 8.7	182.3 ± 7.1
4	191.1 ± 4.4	168.9 ± 6.4	183.6 ± 19.2
5	174.8 ± 1.3	169.6 ± 7.8	181.4 ± 3.1
6	175.6 ± 5.5		
7	150.3 ± 1.1		
8	164.9 ± 0.3		
9	137.6 ± 1.1		

The oxidative stability values for the Picual oils are shown in **Table 4**. The mean oxidative stability for Picual olive oils was 171.3 h. The parameter MUFA/PUFA has been directly related with oxidative stability for virgin olive oils (*12*, *26*); for the oils analyzed in this work has been applied a linear regression analysis, obtaining a relationship between this parameter and oxidative stability with an adjusted R^2 of 0.24, which is significant for $p = 0.0001$ (**Table 5**). Other parameters related with the nutritional aspects are the unsaturated/saturated ratio, which for Picual oil shows a mean value of 6.28 because of the high oleic content. The effect of the different factors studied on this ratio is similar to that described for saturated.

The ratio of oleic/linoleic is another parameter with importance for both nutritional studies and oxidative stability. The evolution during ripening is similar to MUFA/PUFA (**Figure 5**) and, thus, shows a similar behavior. Like MUFA/PUFA, this ratio has been described as being responsible for virgin olive oils' stability (*25*), obtaining a relationship with the oxidative stability with a value of $R^2 = 0.21$ for a confidence level of

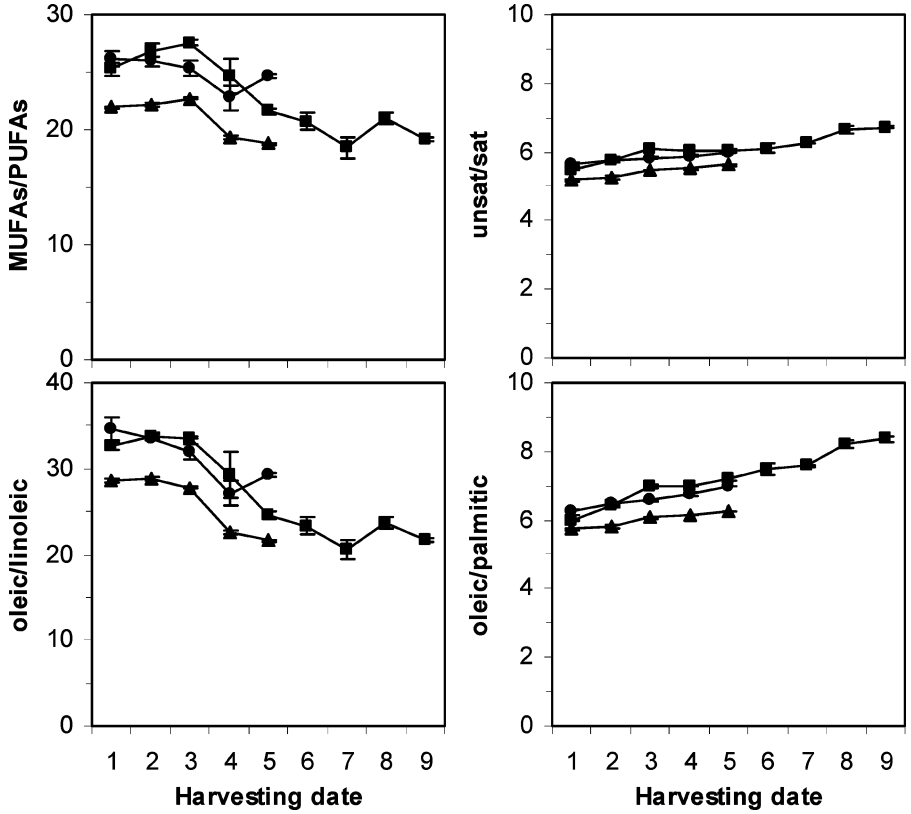

Figure 5. Seasonal variation of fatty acid related parameters of Picual oils during the fruit ripening process for the crop years (■) 1996/1997, (▲) 1997/1998, and (●) 1998/1999.

Table 5. Linear Regression between MUFA/PUFA Ratio and the Oxidative Stability, Measured by Rancimat, of Picual Virgin Olive Oils[a]

variable	coefficient	STD error	Student's *t*	*p*
constant	101.83	19.45	5.24	0.0000
MUFA/PUFA	3.03	0.84	3.6	0.0009

[a] Adjusted $R^2 = 0.244$; F (1, 36): 12.97 significant at $p = 0.001$.

99.9%. Finally, the parameter oleic/palmitic increased during fruit maturation; significant differences between years were observed, showing the same behavior as the unsaturated/saturated ratio.

Picual virgin olive oil shows one of the higher oleic contents and lower linoleic levels among olive cultivars; the fatty acid composition and related parameters (MUFA/PUFA, oleic/linoleic, oleic/palmitic and unsaturated/saturated) presented mean values very interesting from a nutritional point of view. A highly significant relationship has been found between the fatty acid composition (MUFA/PUFA, oleic/linoleic) and the oxidative stability measured by Rancimat. The effects of the agronomic factors are different for each fatty acid; the fatty acids influenced mainly by the crop yield, air temperatures, and rainfall registered during the oil biosynthesis and summer, respectively, were palmitic and oleic acids. The harvesting period, and thus the ripening stage, affected palmitic, steraric, linoleic, and linolenic acids. The results can be used to determine the optimal harvesting time, because the variation described for fatty acid composition may be useful to determine the origin and identity of Picual virgin olive oils.

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LITERATURE CITED

(1) Martínez de Victoria, E.; Mañas, M. El aceite de oliva en la dieta y salud humanas. In *ElCultivo del Olivo*; Barranco, D., Fernández-Escobar, R., Rallo, L., Eds.; MundiPrensa: Madrid, Spain, 2001; pp 663−684.

(2) Commission of Codex Alimentarius. Revised Norm for olive oil. CI 1993/15-FO, 1993.

(3) Consejo Oleicola Internacional (COI). Resolución RES-4/75-IV/96 del 20 de noviembre de 1996, 1996.

(4) Hermoso, M.; Uceda, M.; Frías, L.; Beltrán, G. Maduración. In *El Cultivo del Olivo*; Barranco, D., Fernández-Escobar R., Rallo, L., Eds.; MundiPrensa: Madrid, Spain, 2001; pp 153−169.

(5) Uceda, M.; Hermoso, M. La calidad del aceite de oliva. In *El Cultivo del Olivo*; Barranco, D., Fernández-Escobar R., Rallo, L., Eds.; MundiPrensa: Madrid, Spain, 2001; pp 589−614.

(6) Fiorino, P.; Alessandri, S.; Cert, A.; Dikmen, I.; Rahmani, M. Técnicas agronómicas y características del aceite de oliva. In *La Enciclopedia del Olivo*; COI Plaza & Janés: Barcelona, Spain, 1996; pp 195−222.

(7) Mousa, Y. M.; Gerasopoulos, D.; Metzidakis, I.; Kiritsakis, A. Effect of altitude on fruit and oil quality characteristics of mastoides olives. *J. Sci. Food Agric.* **1996**, *71*, 345−349.

(8) Osman, M.; Metzidakis, I.; Gerasopoulos, D.; Kiritsakis, A. Qualitative changes in olive oil collected from trees grown at two altitudes. *Riv. Ital. Sostanze Grasse* **1994**, *71*, 187−194.

(9) Tiscornia, E.; Fiorino, N.; Evangelisti, F. Chemical composition of olive oil and variations induced by the refining. *Riv. Ital. Sostanze Grasse* **1982**, *59*, 519−524.

(10) García, J. M.; Mancha, M. Evolución de la biosíntesis de lípidos durante la maduración de las variedades Picual y Gordal. *Grasas Aceites* **1992**, *43*, 277−280.

(11) Cimato, A.; Modi, G.; Mattei, A.; Niccolai, M.; Alessandri, S. *La Caratterizzazione de'll Olio Tipico Toscano. II Anno di Ricerca*; CROEVOTT: Firenze, Italy, 1991.

(12) Gutiérrez, F.; Jiménez, B.; Ruíz, A.; Albi, M. A. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and different components involved. *J. Agric. Food Chem.* **1999**, *47*, 121−127.

(13) Aguilera, M. C.; Ramírez-Tortosa, M. D.; Mesa, A. Gil. Do MUFA and PUFA have beneficial effects on development of cardiovascular disease? In *Recent Research Developments in Lipids (Advances in Lipid Research)*; Pandalai, S. G., Ed.; Transworld Research Network: Trivandrum, India, 2000; pp 369−390.

(14) Matson, F. M.; Grundy, S. M. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* **1985**, *26*, 194−202.

(15) Mensink, R. P.; Katan, M. B. Effect of dietary fatty acids on serum lipids and lipoproteins. *Arterioscler., Thromb. Vasc. Biol.* **1992**, *12*, 911−919.

(16) Aviram, M.; Eias, K. Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. *Ann. Nutr. Metab.* **1993**, *37*, 75−84.

(17) Ramírez-Tortosa, M. C.; Aguilera, C. M.; Quiles, J. L.; Gil, A. Influence of dietary lipids on lipoprotein composition and LDL Cu^{2+} induced oxidation in rabbits with experimental atherosclerosis. *Biofactors* **1998**, *8*, 79−85.

(18) Baroni, S. S.; Amelio, M.; Sangiorfi, Z.; Gaddi, A.; Battino, M. Solid monunsaturated diet lowers LDL unsaturation trait and oxidability in hypercholesterolemic (type IIb) patients. *Free Radical Res.* **1999**, *30*, 275−280.

(19) U.S. National Research Council. *Diet and Health: Implications for Reducing Chronic Disease Risk*; National Academic Press: Washington, DC, 1989.

(20) Garg, A.; Bonanone, A.; Grundy, S. M.; Zhang, Z.; Unger, R. H. Comparison of high carbohydrate diet with a high monounsaturated-fat in patients with non-insulin-dependent diabetes mellitus. *New Engl. J. Med.* **1988**, *319*, 829−834.

(21) Katan, M. V.; Aravanis, C.; Mensink, R. P. Serum lipoproteins in cretan boys and men consuming a high olive oil diet. *Circulation* **1987**, *76*, 530−536.

(22) Tuyns, A.; Haelterman, M.; Kaaks, M. Colorectal cancer and the intake of nutrients: oligosacharides are a risk factor, fats are not. A case control study in Belgium. *Nutr. Cancer* **1987**, *10*, 81−85.

(23) Weisburger, J. Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants and carotenoids. *Am. J. Clin. Nutr.* **1991**, *53*, S226−S237.

(24) Frankel, E. N. Free radical oxidation. In *Lipid Oxidation*; The Oily Press: Dundee, Scotland, 1998; pp 13−22.

(25) Aparicio, R.; Roda, L.; Albi, M. A.; Gutiérrez, F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.

(26) Beltrán, G. Influence of ripening process in *Olea europaea* L. fruits on the physicochemical characteristics of the oils. Ph.D. Thesis, Universidad de Jaén, Spain, 2000.

(27) Barranco, D.; Cimato, A.; Fiorino, P.; Rallo, L.; Touzani, A.; Castañeda, C.; Serafini, F.; Trujillo, I. *Catalogo Mundial de Variedades del Olivo*; Consejo Oleicola Internacional: Madrid, Spain, 2000.

(28) Uceda, M.; Aguilera, M. P.; Beltrán, G.; Jiménez, A. Aceites de Oliva Vírgenes Extra. *Calidad y Diversidad*; PROEDI: Zaragoza, Spain, 2000.

(29) Uceda, M.; Frias, L. Harvest dates. Evolution of the fruit oil content, oil composition and oil quality. In *Proceedings of the Segundo Seminario Oleicola Internacional*; International Olive Oil Council: Cordoba, Spain, 1975; pp 125−130.

(30) Martínez, J. M.; Muñoz, E.; Alba, J.; Lanzón, A. Informe sobre la utilización del analizador de rendimientos Abencor. *Grasas Aceites* **1975**, *26*, 379−385.

(31) European Union Commission. Regulation EEC 2568/91 on the characteristics of olive oil and olive pomace and their analytical methods. *Off. J. Eur. Communities* **1991**, L248.

(32) Gutiérrez, F. Determinación de la estabilidad oxidativa de los aceites de oliva vírgenes: comparacion entre el método del oxigeno activo (AOM) y el método Rancimat. *Grasas Aceites* **1989**, *40*, 1−5.

(33) Harwood, J. L.; Jones, A. L.; Perry, H. J.; Rutter, A. J.; Smith, K. L.; Williams, M. Changes in plant lipids during temperature adaptation. In *Temperature Adaption of Biological Membranes*; Cossins, A. R., Ed.; Portland Press: London, U.K., 1994; pp 107−118.

(34) Salas, J. J.; Sanchez, J.; Ramli, U. S.; Manaf, A. M.; Willians, M.; Harwood, J. L. Biochemistry of lipid metabolism in olive and other oil fruits. *Prog. Lipid Res.* **2000**, *39*, 151−180.

(35) Romero, M. P.; Tovar, M. J.; Ramo, T.; Motilva, M. J. Effect of crop season of the composition of virgin olive oil with protected designation of origin 'Les Garrigues'. *J. Am. Oil Chem. Soc.* **2003**, *80*, 423−430.

(36) Modi, G.; Nizzi, F.; Fiorino, P. *Crescita, Maturatione dei Frutti e Caratteri dell'Olio in Cultivar Toscane*; Proceedings of the Symposium "Olive oil quality"; Firenze, Italy, 1992.

(37) Fiorino, P.; Nizzi-Griffi, F. Maturazione delle olive e variación di alcuni componenti dell'olio. *Olivae* **1991**, *35*, 25−31.

(38) Angerosa, F.; Di Giacinto, L.; Basti, C.; Serraiocco, A. Influenza della variabile 'ambiente' sulla composizione degli oli vergini di oliva. *Riv. Ital. Sostanze Grasse* **1996**, *63*, 461−467.

(39) Salas, J.; Pastor, M.; Castro, J.; Vega, V. Influencia del riego sobre la composición y las características organolépticas del aceite de oliva. *Grasas Aceites* **1997**, *48*, 74−82.

(40) Martinez-Rivas, J. M.; García-Diaz, M. T.; Mancha, M. Temperature and oxygen regulation of microsomal oleate desaturase (FAD2) from sunflower. *Biochem. Soc. Trans.* **2000**, *28*, 890−892.

(41) García-Diaz, M. T.; Martinez-Rivas, J. M.; Mancha, M. Temperature and oxygen regulation of oleate desaturation in developing sunflower (*Helianthus annuus*) seeds. *Physiol. Plant.* **2002**, *114*, 13−20.

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