Effect of Olive Ripeness on the Oxidative Stability of Virgin Olive Oil Extracted from the Varieties Picual and Hojiblanca and on the Different Components Involved

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The initial stability of virgin olive oil depends on various factors, among which are the variety and the degree of fruit ripeness. The former, which genetically determines the composition of the olive and its oil, also marks, to some extent, its stability. However, oil stability changes as the olive ripens, so it is obvious that the degree of ripeness is an important factor. The oils were obtained by the Abencor system. Acidity, peroxide index, UV absorption at 232 and 270 nm, sensory analysis, fatty acid composition, tocopherols, phenolic compounds, orthodiphenolic compounds, sterols, pigments, and oxidative stability were determined, and the results were analyzed statistically. During ripening there was a decrease in all of the parameters studied except linoleic acid, Δ-5-avenasterol, and oil content, which increased. Virgin oils showed very good correlation between stability and the concentrations of total phenols, *o*-diphenols, tocopherols, chlorophyll pigments and carotenoids, linoleic and linolenic acids, total sterols, *β*-sitosterol, and Δ-5-avenasterol.

Keywords: *Virgin olive oil; ripening; quality; stability; antioxidant components*

INTRODUCTION

After fruit set, olives grow rapidly for 30 or 40 days, after which time the seed is formed and the pit hardens. From then on, pit weight hardly changes. Pulp weight increases slowly during the summer while irrigation is still available and then more rapidly from mid-September, when structural changes in the fruit indicate the approach of the color change stage ("envero"). Fruit weight continues to increase after the epicarp turns reddish, reaching a maximum when the highest percentage of fruits on the tree are in envero, coinciding approximately with the disappearance of the green fruits.

Various authors (Vázquez et al., 1971; Agramont et al., 1986; de la Torre et al., 1985; Solinas et al., 1987) have studied the structural changes taking place during olive ripening that affect the different components of the fruit and extracted oils. However, they deal with individual components, and there has been no work on oil stability.

The aim of the present work is to investigate the effect of the degree of ripeness of the olive on oxidative stability, on different components (polyphenols, *o*-diphenols, tocopherols, chlorophyll pigments and carotenoids, sterols, and fatty acids), and on sensorial analysis of the oils obtained from fruits in six stages of ripening of the varieties Picual and Hojiblanca (those most commonly grown in Andalusia) and the relationship with oxidative stability.

MATERIALS AND METHODS

Olive Oils. Samples used were fruits of two olive varieties, Picual and Hojiblanca, in six stages of ripening, campaña 95−96. The samples of the variety Picual came from the experimental farm Venta del Llano in Mengibar (Jaén); those of the variety Hojiblanca were supplied by the farm Casablanca in Antequera (Málaga).

The olives were picked in their different harvest periods and sent at once to the Instituto de la Grasa. Their ripeness indices were determined, and they were immediately subjected to the Abencor extraction process (Frias et al., 1991). This method determines the industrial yield of the olive, reproducing at laboratory scale the industrial process and following the same phases: milling, beating, centrifuging, and decanting. The apparatus consists of three essential elements, the mill, the thermobeater, and the pulp centrifuge, and a number of auxiliary ones.

Analytical Methods. *Maturity Index (MI).* This was determined according to the proposals of the Estación de Olivicultura y Elaioctenia, Jaén, defining the ripeness index as a function of fruit color in both skin and pulp (Hermoso et al., 1991). Samples of olives, 100 for each variety, were taken at random, classified into the categories below, and homogenized prior to extraction:

0, olives with epidermis intense green or dark green.

1, olives with epidermis yellow or yellowish green.

2, olives with epidermis yellowish, with reddish spots or areas.

3, olives with epidermis reddish or light violet.

4, olives with epidermis black and pulp totally white.

5, olives with epidermis black and pulp violet to the midpoint.

6, olives with epidermis black and pulp violet almost to the pit.

7, olives with epidermis black and pulp totally dark.

With $a-h$ being the number of fruits in each category, the MI is

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

It can be seen that the ripeness index values are between 0 and 7.

Acidity value, peroxide index, UV light absorption (K_{232} and K_{270}), fatty acid, and sterols were determined, and sensory analysis was carried out, following the analytical methods described in Regulations EEC/ 2568/91 and EEC/1429/92 of the European Union Commission.

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Acidity value, expressed as percent of oleic acid, was determined by titration of a solution of oil in ethanol/ether 1:1 with ethanolic potash.

Peroxide value, given in milliequivalents of active oxygen per kilogram of oil (mequiv/kg), was determined as follows: a mixture of oil and chloroform/acetic acid 3:2 was left to react in darkness with saturated potassium iodide solution; the free iodine was then titrated with a sodium thiosulfate solution.

K_{232} and K_{270} extinction coefficients (absorption of 1% solution in cyclohexane at 232 and 270 nm, respectively, with 1 cm of pass length) were measured using a UV spectrophotometer (Beckman DU 640).

For the determination of the fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potash. The methyl esters were analyzed by gas chromatography on an HP Innowax column (30 m × 0.25 mm i.d.) (Regulation EEC/2568/91).

Sensory Analysis. The samples were evaluated by an analytical panel of the Instituto de la Grasa. The panel comprised 12 selected and trained tasters and followed Annex XII of Regulation EEC/2568/91 of the European Union Commission.

Tocopherols were evaluated following IUPAC Standard Method 2432 (1992), modified by the use of Δ-tocopherol as internal standard (F. Gutiérrez and M. Albi, unpublished data). A solution of oil in hexane was analyzed by high-performance liquid chromatography (LCD Analytical model 3200) on a silica gel column (Merck, Superspher Si 60, particle size 4 m, 250 mm × 4 mm i.d.), eluting with hexane/2-propanol 99.3:0.7 at a flow rate of 1 mL/min. A fluorescence detector (Jasco 821-FP) with excitation wavelength at 290 nm and emission wavelength at 330 nm was used.

Phenolic and orthodiphenolic compounds were isolated by extraction of an oil-in-hexane solution with water/methanol 60:40 three times. To suitable aliquots of the combined extracts were added sodium molybdate 5% in ethanol 50% and commercial Folin−Ciocalteu reagent. The absorption of the solution at 725 nm (phenolic compounds) and 370 nm (orthodiphenolic compounds) was measured using a spectrophotometer (Hewlett-Packard 8450 A UV−vis). Results are given as milligrams of caffeic acid per kilogram of oil (Vázquez et al., 1973).

Chlorophyll Pigments and Carotenoids. For the procedure, 7.5 g of oil was weighed exactly, dissolved in cyclohexane, and taken to a final volume of 25 mL. The chlorophyll and carotenoid fractions in the absorption spectrum were determined at 670 and 472 nm, respectively (Minguez et al., 1991), using a spectrophotometer (Hewlett-Packard 8450 A). Results are given as milligrams per kilogram of oil.

Stability was evaluated from the oxidation induction time, measured with the Rancimat apparatus (Metrohm CH 9100). A flow of air (10 L/h) was bubbled through the oil heated at 100 °C and collected in cold water, increasing the water conductivity. The time taken to reach a fixed level of conductivity was measured (Gutiérrrez, 1989).

Statistical Analysis. The assays were carried out in duplicate. The results are shown as graphs [drawn using Sigma Plot, version 1.2 (Jandel Scientific Corp.)]) or tables of mean values. The discussion of the results is based on the analysis of variance applied to each variety and parameter or component, performed with the program Costat 2.10 (CoHort Software, Barkeley) using the test of Duncan to compare means and thereby the effects of the ripeness index. The constancy or otherwise of the values with ripening is discussed with respect to the significance of the differences between them according to the test, which is not shown in the graphs or tables. The same program (Costat) was used in the correlation study.

RESULTS AND DISCUSSION

Maturity Index. The olives used for oil extraction had the following ripeness indices: (Picual) 0.66, 1.67, 2.68, 3.70, 4.79, 5.70; (Hojiblanca) 0.78, 1.67, 2.35, 3.40, 4.22, 4.95.

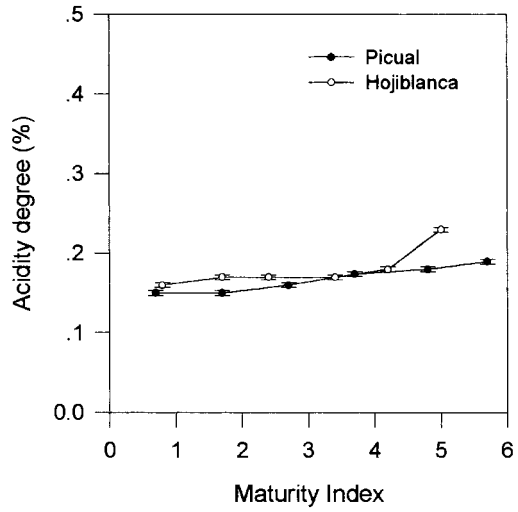

Figure 1. Changes in free acidity as oleic acid (percent) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

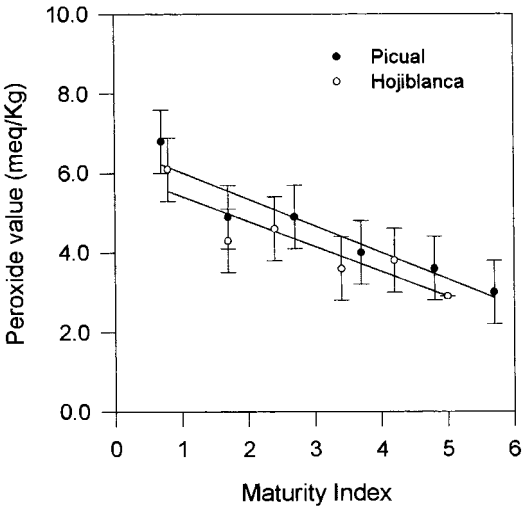

Figure 2. Changes in peroxide value in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

Change in Acidity. Figure 1 shows the change in acidity of the samples during ripening. Although there were no statistically significant differences between values in the variety Picual, and in Hojiblanca only the last was statistically higher, in both cases there was a slight rise during ripening. These results are in agreement with those of Kiritsakis and Tsipeli (1992). The rise in acidity while the fruit remains on the tree is caused by the activation of lipolytic enzymes present in the fruit (Mártinez Suarez, 1973).

Change in the Peroxide Index. The changes in peroxide index were similar in the two varieties: a marked decrease during ripening (Figure 2). This behavior can be explained by a decrease in the activity of the enzyme lipoxygenase in both varieties. These results are in accord with those of Uceda et al. (1992). The peroxide values obtained are far below the limit of 20 mequiv/kg at which the oils lose the category "extra" (Regulation EEC/2568/91).

Change in the Coefficients of Specific Extinction K_{232} and K_{270}. The values of K_{232} (Figure 3A) in the variety Picual tended to fall during ripening, whereas those in Hojiblanca remained practically constant. K_{270} (Figure 3B) behaved similarly to the peroxide

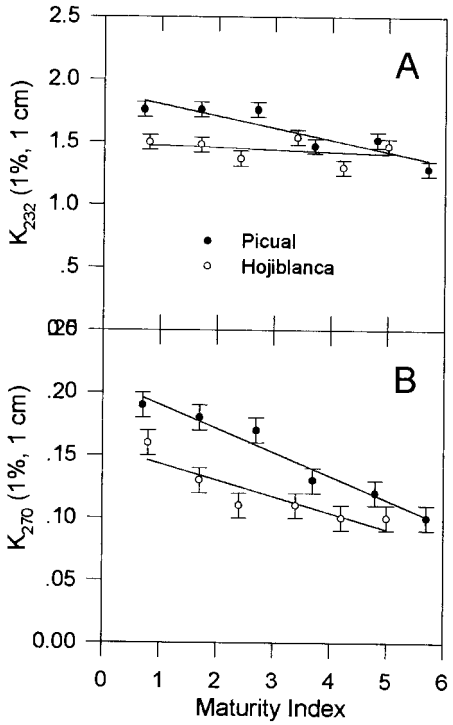

Figure 3. Changes in the K_{232} coefficient (A) and in the K_{270} coefficient (B) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

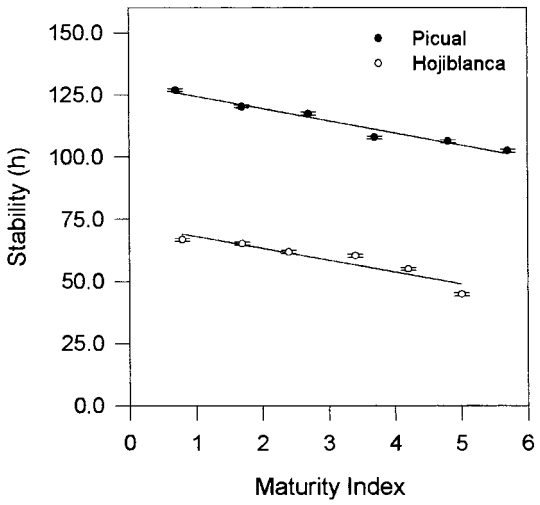

Figure 4. Changes in the stability in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

index: values fell gradually in both varieties throughout ripening. In no case in either variety did these coefficients exceed 2.50 and 0.20, the respective limits for "extra" virgin olive oils (Regulation EEC/2568/91).

Change in Stability. In both varieties, oil stability decreased during ripening (Figure 4). The decrease followed parallel paths but was proportionally greater in the variety Hojiblanca (32.3%) than in Picual (19.3%). This decrease in stability is explained by the loss of natural antioxidants (phenols, *o*-diphenols, and tocopherols), as shown later. These results are in accord with those of Gutiérrez et al. (1977).

Change in Organoleptic Score. The organoleptic score remained practically constant throughout ripening. Only the last score for the variety Hojiblanca was significantly different, with a slight decrease as a result

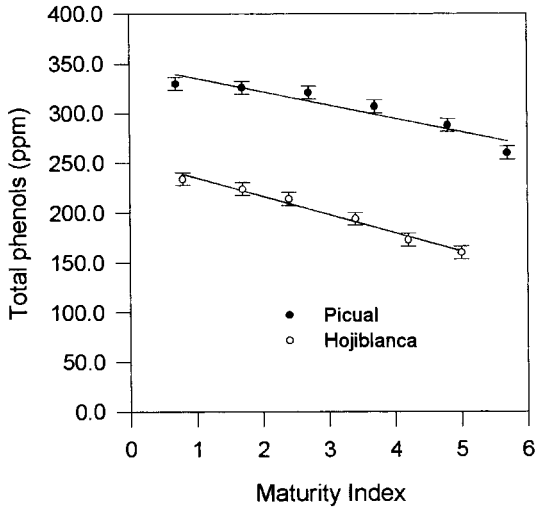

Figure 5. Changes in the amount of phenols (parts per million) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

of some loss of balance in its positive attributes (fruity, bitter, and pungent). All samples maintained the category "extra" (mean score > 6.5) (Regulation EEC/2568/91). These results show that for sensorial quality, it is not necessary to harvest the fruit with the ripeness index of 3.5 recommended by the industry.

Change in Total Phenols. Figure 5 shows the change in total phenols. In both varieties, the phenols decreased during ripening, in accord with the results obtained by Vázquez et al. (1971), following almost parallel paths, as in the case of stability. In Picual, the decrease was 21.2%, similar to the loss in stability. In Hojiblanca, the decrease was somewhat greater at 31.5%, which again is very similar to the loss in stability. These results demonstrate that a ripeness index of ~3.5 is appropriate for oxidative stability (high stability) of the oil and thus for its longer shelf life.

Change in *o*-Diphenols. The change in the *o*-diphenols was parallel to that in the total phenols and similarly showed a good correlation with stability. The losses in *o*-diphenols during ripening were 20.7 and 28% in Picual and Hojiblanca, respectively, practically the same as those for stability and total phenols.

Change in α-Tocopherol. α-Tocopherol (vitamin E) is the major tocopherol in virgin olive oil. Figure 6 shows the change in content of the samples studied. The two varieties behaved differently. In Picual there was a decrease of 33% throughout ripening, coinciding with the results obtained by Agramont et al. (1986) in Italian varieties. In Hojiblanca, the α-tocopherol content remained practically constant in the first five stages of ripening and then fell in the last stage to a value significantly lower than the rest. The total decrease was 10%, one-third that for the variety Picual. Thus, the α-tocopherol content is less affected by ripening in Hojiblanca, despite the lower (1.6 lower) initial content. These results could not be contrasted in the literature because there has been no other study on the topic.

Change in Chlorophyll Pigments and Carotenoids. The content of chlorophyll pigments (Figure 7A) and carotenoids (Figure 7B) decreased markedly during ripening in both varieties; the losses of chlorophyll pigments were 97.6 and 82.4% and those of the carotenoids 82.2 and 46.9% for Picual and Hojiblanca, respectively. The color change to "spotted olive" that

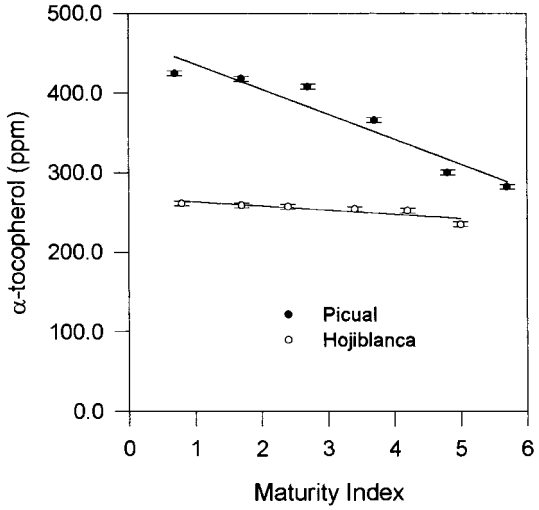

Figure 6. Changes in the amount of α-tocopherol (parts per million) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

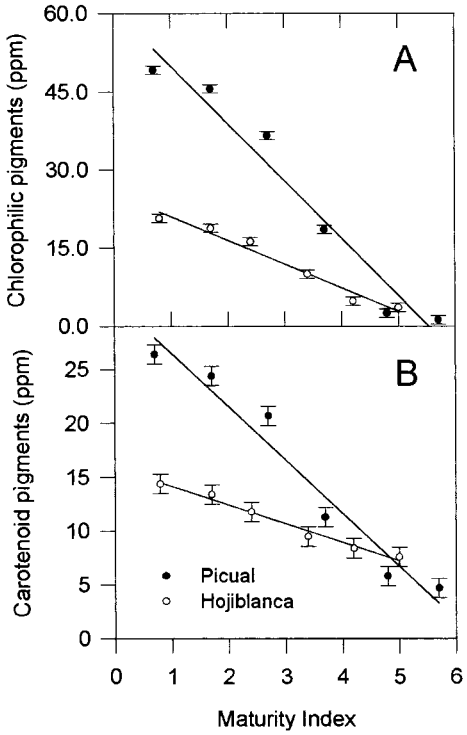

Figure 7. Changes in the amount of chlorophyllic pigments (A) and carotenoid pigments (B) (parts per million) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

occurs during the stage of ripening 2.5−3 is explained not only by the sharp drops in these two pigment types but also by the formation of other colored compounds, such as anthocyanins (Vázquez et al., 1971).

In the last stages of ripening, the yellow fraction is ∼70% and the green ∼30%. These results are in accord with those of Minguez and Garrido (1986) and Minguez et al. (1991). As in the case of α-tocopherol, the loss of these pigments is greater in the variety Picual.

Change in Fat Content. Figure 8 shows the change in fat content expressed as dry matter of olives at different ripeness stages. In both varieties, fat content rose during ripening, with values of 15% in Picual and 12% in Hojiblanca. This indicates the continuation of the triglyceride-forming biosynthetic pathway which,

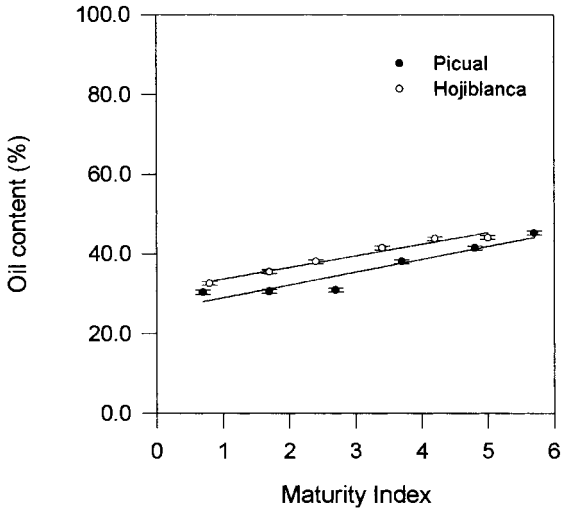

Figure 8. Changes in the oil fraction extracted from the olive varieties Picual and Hojiblanca at different stages of maturity expressed as percent dry weight.

Table 1. Fatty Acid Composition (Percent) of the Virgin Olive Oil from the Variety Picual at Different Stages of Olive Maturity

MI	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0
0.66	12.1[a]	0.93	0.04	0.09	3.18	79.69	2.73	0.96	0.42	0.27	0.14
	0.14[b]	0.02	0.01	0.01	0.01	0.12	0.01	0.0	0.01	0.0	0.01
1.67	11.95	0.91	0.04	0.08	3.14	79.62	2.89	0.93	0.42	0.26	0.14
	0.11	0.02	0.01	0.01	0.01	0.13	0.02	0.0	0.01	0.0	0.01
2.68	11.77	0.90	0.04	0.08	3.13	79.49	3.17	0.89	0.42	0.26	0.14
	0.11	0.02	0.01	0.01	0.01	0.13	0.02	0.0	0.01	0.0	0.01
3.70	11.03	0.88	0.04	0.07	3.11	79.32	3.63	0.66	0.42	0.24	0.14
	0.09	0.02	0.01	0.01	0.02	0.13	0.03	0.0	0.01	0.0	0.01
4.79	10.45	0.83	0.04	0.07	3.09	79.24	4.34	0.72	0.42	0.24	0.13
	0.07	0.02	0.01	0.01	0.02	0.13	0.03	0.01	0.01	0.0	0.01
5.70	10.12	0.82	0.04	0.07	3.02	79.22	4.78	0.67	0.41	0.24	0.12
	0.05	0.02	0.01	0.01	0.02	0.10	0.07	0.01	0.01	0.0	0.01

[a] Mean. [b] Standard deviation.

Table 2. Fatty Acid Composition (Percent) of the Virgin Olive Oil from the Variety Hojiblanca at Different Stages of Olive Maturity

MI	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0
0.78	11.40[a]	0.52	0.18	0.27	3.19	77.18	5.68	1.06	0.43	0.32	0.12
	0.14[b]	0.01	0.01	0.0	0.01	0.10	0.01	0.02	0.01	0.0	0.01
1.67	10.80	0.51	0.16	0.26	3.16	77.10	6.24	1.00	0.43	0.32	0.12
	0.12	0.01	0.01	0.0	0.01	0.10	0.01	0.02	0.01	0.0	0.01
2.35	10.41	0.51	0.15	0.25	3.12	77.04	6.73	0.95	0.43	0.30	0.12
	0.13	0.01	0.01	0.0	0.01	0.10	0.01	0.02	0.01	0.0	0.01
3.40	9.79	0.49	0.14	0.24	3.11	76.92	7.34	0.84	0.42	0.30	0.12
	0.13	0.01	0.01	0.0	0.01	0.09	0.02	0.02	0.01	0.0	0.01
4.22	9.62	0.49	0.14	0.24	3.08	76.84	8.61	0.68	0.42	0.29	0.10
	0.12	0.01	0.01	0.0	0.02	0.08	0.02	0.02	0.01	0.0	0.01
4.95	9.21	0.47	0.13	0.23	3.05	76.85	9.32	0.54	0.41	0.28	0.09
	0.12	0.01	0.01	0.0	0.02	0.08	0.02	0.02	0.01	0.0	0.01

[a] Mean. [b] Standard deviation.

according to Cimato (1988) and Sánchez (1994), finishes 30 weeks after flowering (October−November). Our fruits were picked from October−November 1995 until January−February 1996. This is the first report of such measurement and explains the rise in linoleic acid during ripening mentioned but not explained by various authors (Fiorino and Petruccioli, 1977; Frias et al., 1975). At the end of the study period, the activity seemed to stop in the variety Hojiblanca, which presented some 5% more fat that Picual.

Change in Acid Composition. Tables 1 and 2 show the mean values of palmitic (16:0), palmitoleic (16:1), margaric (17:0), margaroleic (17:1), stearic (18:0), oleic

Table 3. Sterol Composition (Parts per Million and Percent) of the Virgin Olive Oil from the Variety Picual at Different Stages of Maturity

MI	total	cholesterol	campesterol	campestanol	stigmasterol	clerosterol	β-sitosterol	Δ-5-avenasterol	Δ-5,24-stigmastadienol	Δ-5-stigmasterol	Δ-7-avenasterol
0.66	1807[a]	0.23	2.89	0.34	0.62	1.56	90.38	3.27	0.36	0.17	0.34
	160.2[b]	0.10	0.98	0.12	0.20	0.65	8.15	1.01	0.12	0.06	0.11
1.67	1654	0.16	2.77	0.38	0.60	1.52	90.37	3.34	0.29	0.23	0.24
	145.2	0.09	0.96	0.14	0.20	0.65	8.25	1.01	0.11	0.08	0.09
2.68	1536	0.13	2.68	0.38	0.64	1.88	89.86	3.46	0.41	0.26	0.24
	138.2	0.09	0.96	0.14	0.22	0.67	8.15	1.10	0.15	0.08	0.09
3.70	1529	0.16	3.18	0.41	0.60	1.77	88.71	4.37	0.26	0.20	0.44
	135.2	0.06	0.99	0.14	0.20	0.57	8.07	1.13	0.08	0.06	0.19
4.79	1274	0.13	3.03	0.48	0.53	1.12	87.58	6.08	0.31	0.31	0.24
	121.2	0.04	0.89	0.14	0.18	0.37	8.01	1.18	0.09	0.08	0.12
5.70	1128	0.16	3.12	0.40	0.60	1.47	86.28	7.35	0.28	0.21	0.36
	103.2	0.05	0.89	0.12	0.19	0.39	8.01	1.20	0.09	0.06	0.14

[a] Mean. [b] Standard deviation.

Table 4. Sterol Composition (Parts per Million and Percent) of the Virgin Olive Oil from the Variety Hojiblanca at Different Stages of Maturity

MI	total	cholesterol	campesterol	campestanol	stigmasterol	clerosterol	β-sitosterol	Δ-5-avenasterol	Δ-5,24-stigmastadienol	Δ-5-stigmasterol	Δ-7-avenasterol
0.78	2282[a]	0.10	2.54	0.31	0.54	1.26	92.32	2.36	0.20	0.20	1.19
	210.3[b]	0.02	0.89	0.11	0.17	0.47	8.97	0.92	0.09	0.08	0.38
1.67	2260	0.14	2.79	0.28	0.58	0.94	91.51	3.07	0.36	0.13	0.20
	207.3	0.02	0.91	0.11	0.18	0.23	8.57	0.96	0.10	0.04	0.13
2.35	2230	0.09	3.00	0.32	0.62	1.17	90.42	3.82	0.25	0.10	0.18
	202.3	0.02	0.93	0.13	0.19	0.28	8.37	0.98	0.08	0.04	0.13
3.40	2205	0.09	2.82	0.32	0.65	0.98	89.90	4.56	0.35	0.10	0.19
	200.8	0.02	0.95	0.13	0.21	0.18	8.03	1.01	0.09	0.04	0.13
4.22	2186	0.06	3.06	0.26	0.64	0.89	89.17	5.38	0.30	0.14	0.22
	200.1	0.02	0.98	0.16	0.21	0.18	8.01	1.13	0.09	0.06	0.14
4.95	2053	0.13	2.77	0.29	0.49	1.32	85.56	8.37	0.43	0.12	0.18
	196.1	0.08	0.92	0.16	0.17	0.22	7.83	1.21	0.12	0.06	0.12

[a] Mean. [b] Standard deviation.

(18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), gadoleic (20:1), and behenic (22:0) acids of the two varieties during ripening.

It can be seen that with the exception of palmitic, linoleic, and linolenic acids, the fatty acid content did not vary during ripening. Palmitic acid content fell during ripening, possibly as a result of a dilution effect. Its absolute quantity is constant, but the level of oleic acid increases by the active triglyceride biosynthesis. Thus, the palmitic acid percentage, as well as the linolenic acid percentage, is lowered. The increase in linoleic acid content is due to the fact that, besides the continuing biosynthesis of triglycerides, with the formation of oleic acid, the enzyme oleate desaturase is active, transforming oleic acid into linoleic. The net result is that the former remains constant while linoleic increases. This is the first time that this observation has been explained.

This work also shows that the ratio between monounsaturated and polyunsaturated fatty acids tends to decrease during olive ripening. These results coincide with those obtained by Uceda et al. (1990).

Change in Sterol Content. Tables 3 and 4 show the mean content of total (parts per million) and percent individual sterols. Figure 9 shows the change in total sterols, and Figure 10 the content (percent) of actual β-sitosterol and Δ-5-avenasterol for the two varieties during ripening.

The tables show that during ripening the sterol fraction does not vary substantially, except total sterols, β-sitosterol, and Δ-5-avenasterol, which differ significantly. These results are in accord with those of de la Torre et al. (1985) and Mariani et al. (1991). Total sterols (Figure 9) decrease less in the variety Hojiblanca,

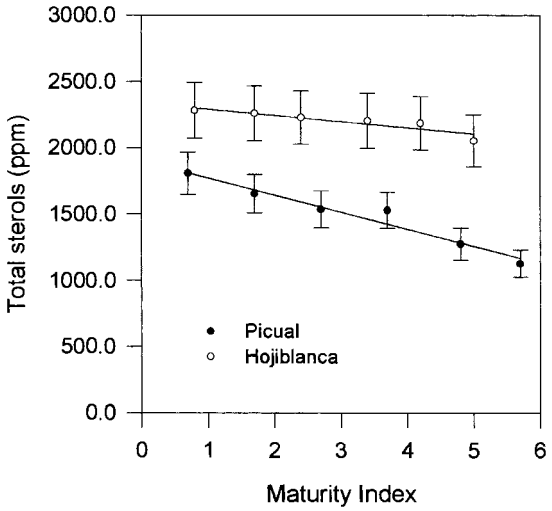

Figure 9. Changes in the amount of sterols (parts per million) in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

as in the case of the tocopherols and pigments. This could be due to the variety's being less affected by ripening. The total sterol content decreased significantly during ripening in the variety Picual (37.6%), but in Hojiblanca, only 10%, and the decrease was significant only in the last stage of ripening. The explanation for the decrease in total sterols is that these form in the first phases of ripening; as the oil content increases during this period, the sterols are diluted. These results coincide with those obtained by Tiscornia et al. (1978).

The content in β-sitosterol ($C_{29}H_{50}O$), the major sterol (Figure 10A), decreased significantly during ripening in

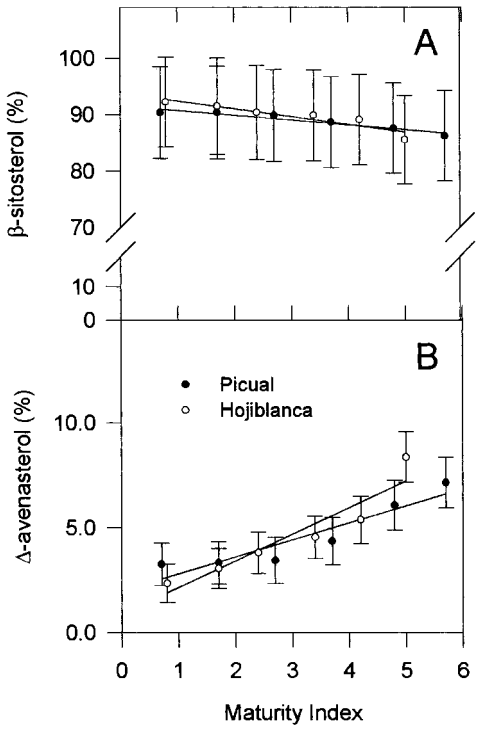

Figure 10. Changes in the proportion of β-sitosterol (A) and Δ-5-avenasterol (B) as a percent of total sterols in the virgin olive oil extracted from the olive varieties Picual and Hojiblanca at different stages of maturity.

Table 5. Correlation of Chemical Composition between the Varieties Picual and Hojiblanca

chemical component and stability	Picual		Hojiblanca	
	r	*p*	*r*	*p*
total phenols	0.983	xxx	0.992	xxx
o-diphenols	0.991	xxx	0.982	xxx
α-tocopherol	0.957	xx	0.876	x
chlorophyll pigments	0.977	xxx	0.987	xxx
carotenoids	0.973	xxx	0.993	xxx
palmitic acid	0.973	xx	0.994	xxx
linoleic acid	0.979	xxx	0.996	xxx
linolenic acid	0.979	xxx	0.990	xxx
total sterols	0.977	xxx	0.915	x
β-sitosterol	0.961	xx	0.920	xx
Δ-5-avenasterol	0.932	xx	0.936	xx

both varieties (4 and 7%, respectively, for Picual and Hojiblanca). The decrease in β-sitosterol was exactly the same as the increase in Δ-5-avenasterol ($C_{29}H_{49}O$) (Figure 10B), suggesting the presence of a desaturase enzyme that transforms β-sitosterol into Δ-5-avenasterol.

Study of the Correlations of the Different Chemical Components and Stability with Ripening. Table 5 shows the values of the correlation coefficients (*r*) and significance (*p*) for the two varieties.

It can be seen that for the chemical components there is a very good correlation, from significant (x, $p < 0.05$) to extremely significant (xxx, $p < 0.01$), between the absolute or percentage content in the virgin olive oil and the ripeness index of the source olive in all cases. This indicates a functional change in the oil with ripening of the fruit. The results could not be contrasted, as this is the first such study. In the rest of the components studied, no statistically significant correlation was found.

During the 1996−1997 season, we have obtained similar results, corroborating the trend described in this paper.

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