Changes in the Main Components and Quality Indices of Virgin Olive Oil During Oxidation

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ABSTRACT: This work reports on changes in the major and minor components of virgin olive oil during oxidation, details modifications found in the standardized quality indices, and analyzes the most significant relationships between the components of the oil and its oxidative stability. During the induction period or slow phase of oxidation, polyphenols, tocopherols, and pigments undergo the most important alterations. Other compounds, such as FA or volatiles, suffer significant modifications only during the rapid or exponential phase of oxidation when the natural antioxidant systems fall to minimal values. Among the quality indices, PV and the specific extinction coefficients K_{232} and K_{270} increase markedly from the earliest stages of oxidation, whereas titratable acidity does not change appreciably during the induction period. The evolution of the different compounds and parameters analyzed suggests that the tocopherol and orthodiphenol contents are the best indices to determine the average life of the oils.

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KEY WORDS: Antioxidant components, oxidation, quality, stability, virgin olive oil.

Oils are oxidized by atmospheric oxygen. This alteration is characterized by physicochemical changes, a marked decrease in the nutritional value, an unpleasant flavor called "rancid," and even some toxicity (1). The process is complex because of the influence of multiple factors, such as light, temperature, enzymes, and metals. However, it always takes place by the same mechanism: chain reactions involving free radicals (autooxidation). The unsaturated FA are the main substrates in the autooxidation process, with the allyl hydroperoxides their primary products (2). Furthermore, any remaining double bonds in the FA molecule may change their position and/or configuration with respect to the original form. The hydroperoxides can subsequently be substrates of different processes, giving rise to secondary oxidation products. Other unsaturated substrates, such as carotenes and α-tocopherol, may undergo similar oxidative modifications with loss of vitamin activity and color, and thus of nutritive value. Carotenes, tocopherols, and polyphenols are natural antioxidants that delay the oxidation of lipids and the production of undesirable volatile compounds.

The FA of virgin olive oil are mainly monounsaturated. This fact and the presence of α-tocopherol, carotenoids pigments, and phenolic compounds make virgin olive oil more stable than other edible oils (3). This paper reports on the changes in the major and minor components of virgin olive oil, the quality indices (acidity, PV, K_{232}, $K_{270,}$ sensory analysis), and stability during oxidation. It also shows the correlations of stability with contents of orthodiphenols, tocopherols, chlorophylls, and carotenoids and with the quality indices.

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MATERIAL AND METHODS

Olive oils. Extra virgin oil samples of three olive cultivars (Picual, Hojiblanca, and Arbequina) grown in the crop year 1996–1997 and extracted by the three-phase continuous system were used. Samples of the Picual cultivar came from the experimental mill Venta del Llano in Mengibar (Jaén, Spain); those of Hojiblanca were supplied by the Agrupación Hojiblanca from Antequera (Málaga, Spain), whereas Arbequina oil was supplied by Olis de Cataluña (Reus, Tarragona, Spain). These cultivars were chosen as being representative of Spanish olive oil production and because they have different compositions and quality attributes. Their initial chemical characteristics are given in Table 1.

Oxidation of the oils. To achieve samples with different oxidation stages, the stability of each oil was initially determined by the Rancimat method (4) in an independent assay (Table 1). Subsequently, 102 g of oil was distributed among six tubes and bubbled with air at 10 L/h in a Rancimat apparatus at 100°C. Eight different oxidation times were chosen for sampling: Six were shorter than the length of the induction period (stability value in Table 1) and two were longer. Each batch of six tubes was mixed to give a single sample for each oxidation time. A series of three independent and identical oxidative treatments were carried out for each olive cultivar.

Analytical methods. (i) Titratable acidity. Acidity, expressed as percentage of oleic acid, was determined by titration of a solution of oil (20 g) in 125 mL of a mixture consisting of equal volumes of ethanol and diethyl ether with a 0.1 N potassium hydroxide solution (5).

(ii) PV. An oil sample (5 g) was dissolved in 30 mL of a 3:2 (vol/vol) chloroform/acetic acid mixture. Subsequently, 0.5 mL of a saturated KI solution was added, and the mixture was allowed to stand for 1 min in darkness. Free iodine produced was then titrated with a 0.1 N sodium thiosulfate solution. Results are expressed as milliequivalents of active oxygen per kilogram of oil (meq/kg) (5).

(iii) K_{232} *and* K_{270} *specific extinction coefficients.* Absorp-

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TABLE 1
Initial Characteristics of Olive Oils

	Oil variety		
	Picual	Hojiblanca	Arbequina
Acidity (% oleic acid)	0.15	0.20	0.25
PV (meq/kg)	6.60	4.30	7.50
K_{232} (1%, 1 cm)	1.41	1.64	1.93
K_{270} (1%, 1 cm)	0.11	0.11	0.11
Sensory evaluation (global score)[a]	7.70	7.90	8.10
Stability (h)	103.9	79.2	49.9
FA (%)			
16:0	12.44	9.64	14.04
16:1	0.86	0.46	1.27
17:0	0.05	0.13	0.11
17:1	0.10	0.23	0.24
18:0	2.28	2.93	1.79
18:1	79.67	77.35	71.88
18:2	3.06	7.65	9.39
18:3	0.81	0.76	0.54
20:0	0.36	0.39	0.34
20:1	0.26	0.32	0.29
22:0	0.12	0.15	0.12
Phenols (ppm caffeic acid)	219.00	217.00	85.3
Orthodiphenols (ppm caffeic acid)	18.92	18.67	3.99
α-Tocopherol (ppm)	253.00	217.00	156.00
β-Tocopherol (ppm)	1.80	1.91	0.40
γ-Tocopherol (ppm)	6.23	10.97	1.54
Chlorophylls (ppm)	22.80	51.23	14.03
Carotenoid pigments (ppm)	12.35	21.72	7.30

[a]Global score was evaluated on a 9-point scale, where 1 is assigned for very poor quality and 9 for optimal quality. A virgin olive oil is classified as "extra quality" when its global score is ≥6.5.

tion at 232 and 270 nm (1 cm path length) of a 1% (wt/vol) solution of oil in cyclohexane was measured using a Beckman DU 640 UV spectrophotometer (Beckman, Fullerton, CA) (5).

(iv) FA composition. The methyl esters of the FFA were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of a 2 N methanolic potassium hydroxide solution. The methyl esters were analyzed in a Hewlett-Packard gas chromatograph (HP 6890 Series) equipped with an HP INNOWax column (30 m × 0.25 mm i.d.) and an FID, according to Regulations EC/2568/91 (5). The oven temperature was held at 140°C for 10 min, then was increased to 200°C at 2°C/min and held for 20 min. The split ratio was 1:60, and the sample volume 1 µL.

(v) Sensory analysis. Sensory analysis was carried out by four trained virgin olive oil tasters following Regulation EC/2568/91 (5).

(vi) Tocopherols. Tocopherols were evaluated according to the IUPAC Standard Method No. 2432 (1992) (6). An aliquot of a solution of oil in hexane (175 mg in 10 mL) was analyzed by HPLC in an LCD apparatus (LCD Analytical, Riviera Beach, FL) Model 3200, equipped with a silica gel column (Superspher Si 60, particle size 4 µm, 250 × 4 mm i.d.) (Merck, Darmstadt, Germany) and a Jasco 821-FP fluorescence detector (Jasco International, Easton, MD) with excitation wavelength at 290 nm and emission wavelength at 330 nm. Elution was performed with hexane/2-propanol (99.3:0.7 vol/vol) at a flow rate of 1 mL/min.

(vii) Phenolic compounds. Oil (10 g) was dissolved in 25 mL hexane and extracted with 10 mL of a 60:40 (vol/vol) methanol/water mixture. The extraction process was repeated three times. To an aliquot of the combined extracts, Folin–Ciocalteau reagent and 5% sodium molybdate in 50% ethanol were added. Absorption at 725 (phenols) and 370 nm (orthodiphenols) was measured using a Beckman DU 640 UV/VIS spectrophotometer. Results are given as mg of caffeic acid/kg of oil (7).

(viii) Chlorophylls and carotenoids. Oil (7.5 g) was accurately weighed and dissolved in cyclohexane up to a final volume of 25 mL. Chlorophylls and carotenoids were then determined by their absorption at 670 and 472 nm, respectively, using a Beckman DU 640 UV/VIS spectrophotometer (8). Results are expressed as mg/kg of oil.

(ix) Stability. In a Rancimat apparatus (Metrohm CH 9100, Herisau, Switzerland) the oil (3.5 g) was heated at 100°C and bubbled with air at 10 L/h. The effluent gases were collected in cold water. Stability was determined as the time taken for the water to reach an electrical conductivity of 25 µS (4).

(x) Oxidized TG. The IUPAC method used for frying fats (9) can be summarized as follows: Oil (1.0 g) was accurately weighed, dissolved in 20 mL of a hexane/diethyl ether mixture (87:13 vol/vol), and transferred to a chromatographic column packed with 25 g of silica. Unaltered TG eluted in a first fraction with 150 mL of hexane/diethyl ether (87:13 vol/vol), whereas polar compounds subsequently eluted with 150 mL of diethyl ether. Both fractions were tested by TLC and, after evaporating the solvent, were gravimetrically determined. Results were expressed as mg/g of oil.

Separation of different polar compounds was carried out by the procedure described by Dobarganes *et al.* (10). The polar fraction was taken to dryness and redissolved in 5 mL of THF. An aliquot of 10 µL (15–20 mg/mL) was injected in a Waters liquid chromatograph equipped with two Ultrastyragel columns of 100 and 500 Å pore diameter in series (30 × 0.75 cm i.d., Merck), and a Hewlett-Packard model 1037-A refractive index detector. Elution was performed with THF at a flow rate of 1 mL/min. Concentrations of the different compounds (TG polymers and dimers, oxidized TG, DF, and FFA) were calculated from the areas of the chromatogram and expressed as percentage of the total polar fraction.

(xi) Volatile compounds. Volatile compounds were isolated by a dynamic headspace technique following the method of Morales *et al.* (11). A 0.5-g sample of virgin olive oil was stirred and heated at 40°C. Volatile components were swept by a nitrogen stream (flow rate 12 L/h) for 30 min and trapped on a Tenax TA trap (Chrompack, Middelburg, The Netherlands). Subsequently, they were analyzed by GC–MS. A Hewlett-Packard 5890 series II gas chromatograph coupled with a MS 30/70 mass spectrometer (VG Analytical, Manchester, UK) and a VG Model 11/250 data system were used for mass spectrometric analysis. A DB-Wax (J&W Scientific, Folsom, CA) fused-silica capillary column (60 m × 0.25 mm

i.d., 0.25 μm film thickness) was employed. The column temperature was held at 40°C for 15 min and then was increased to 220°C at 1°C/min. The carrier gas (helium) flow rate was 1 mL/min. The end of the fused-silica column was inserted directly into the source block. The spectra were recorded at an ionization voltage of 70 eV and an ion source temperature of 200°C.

Statistical analysis. All determinations were carried out with six replicates. Discussion of the results is based on the ANOVA applied to each variety and parameter or component, and Duncan's test was used for comparison of means. Analysis of data (including correlation studies) was performed with the software program Costat 2.10 (CoHort Software, Berkeley, CA).

RESULTS AND DISCUSSION

When the evolution of both the chemical components and the standardized quality indices of olive oil is observed during oxidation of the oil, two well-differentiated periods can be distinguished. Initially, the changes are slow, but after some time the rate of the processes suddenly increases (exponential phase). The period of time up to the beginning of the exponential phase is designated as the "induction period."

Resistance to autooxidation dropped as the time over which air treatment was applied increases. The stability decrease was linear during the induction period, and no significant differences were found among the slopes of the three cultivars (Fig. 1), despite their different oxidative stabilities (Table 1). Since the amounts of natural antioxidants, tocopherols, and polyphenols were similar in Picual and Hojiblanca oils, the higher stability of Picual oil may be explained by its higher oleic/linoleic ratio (26.0, against 10.1 for Hojiblanca). In the case of the Arbequina variety, the explanation for its lower stability may lie both in its significantly lower content of phenolics and pigments and in its low oleic/linoleic ratio (7.6).

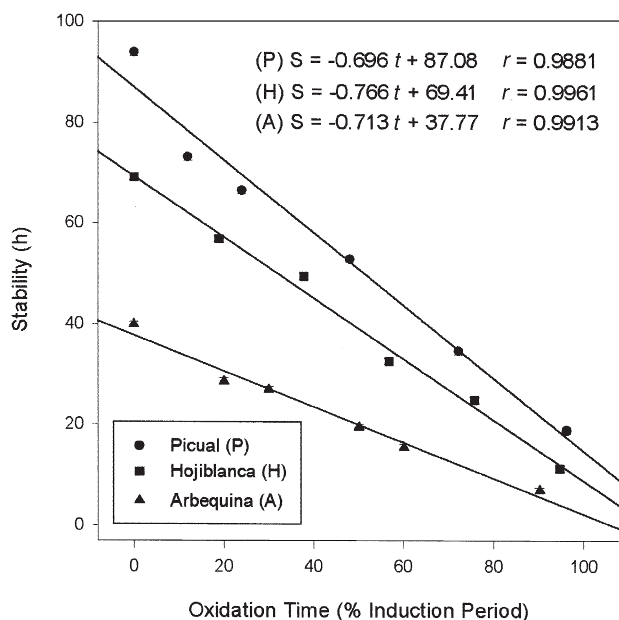

FIG. 1. Changes in the stability of virgin olive oils during oxidation. Linear regression equations during the induction period are included. Results are expressed as mean ± SD.

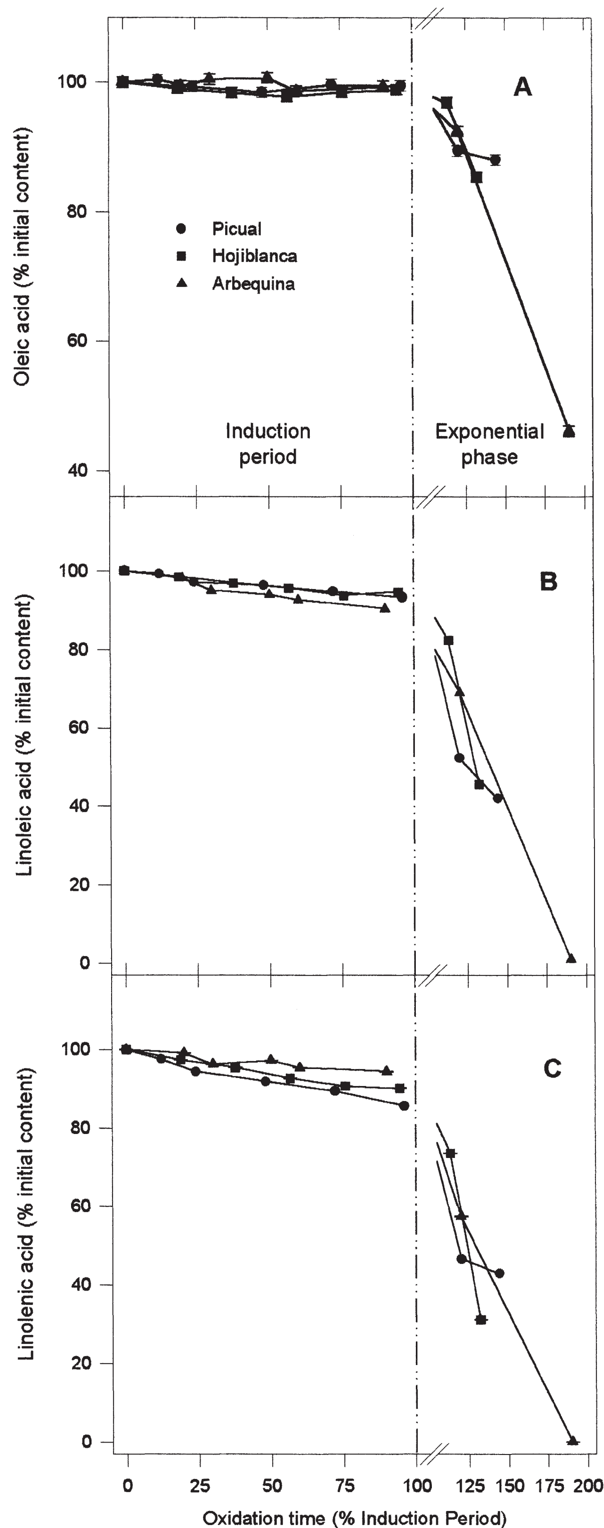

FIG. 2. Evolution of residual oleic, linoleic, and linolenic acid contents during oxidative treatment. Results are expressed as mean ± SD.

Figure 2 indicates that oleic acid content remained practically constant during the induction period, then subsequently decreased, losing at the end of the oxidation process around 12, 15, and 60% of its initial contents for Picual, Hojiblanca, and Arbequina cultivars, respectively. On the other hand, during the induction period linoleic (L) and linolenic (Ln) acids decreased moderately (5–15%). The regression equations describing this were quite satisfactory:

Picual	$\%L = -6.57 \times 10^{-2}\, t + 99.71$	$r = -0.9893$	[1]
Hojiblanca	$\%L = -8.04 \times 10^{-2}\, t + 99.58$	$r = -0.9886$	[2]
Arbequina	$\%L = -1.42 \times 10^{-2}\, t + 99.65$	$r = -0.9885$	[3]
Picual	$\%Ln = -1.35 \times 10^{-1}\, t + 99.06$	$r = -0.9885$	[4]
Hojiblanca	$\%Ln = -1.38 \times 10^{-1}\, t + 99.55$	$r = -0.9893$	[5]
Arbequina	$\%Ln = -1.42 \times 10^{-1}\, t + 99.65$	$r = -0.9885$	[6]

where %L and %Ln indicate the residual contents of the L and Ln acids with respect to their initial values. A comparison between the slopes for L and Ln acids indicates that there is a significant difference ($P < 0.05$) between them. These data agree with the results obtained by Frankel (2), who worked with pure linolenate and linoleate. At the end of the oxidation process, the contents of L and Ln acids were reduced by as much as 60–70% from their initial levels in Picual and Hojiblanca, whereas in Arbequina these FA were practically deleted.

Polyphenol and orthodiphenol degradation is well fitted to first-order kinetics (Fig. 3) with excellent correlation coefficients throughout the oxidation process ($P < 10^{-7}$). Although the Folin–Ciocalteau reagent is quite specific for the colorimetric determination of polyphenols, other compounds such as FFA may interfere (12,13). This could explain why total polyphenol contents for the two oxidation times in the exponential phase were higher than zero. Chimi *et al.* (1) also established that polyphenol degradation followed first-order kinetics, but their study was carried out with refined oils to which exogenous polyphenols were added. Figure 3 also shows that the α-tocopherol level dramatically decreased ($P < 0.05$) during the oxidation process with excellent fitting to a linear regression. The lowest degradation rate was found in the Arbequina cultivar followed by Hojiblanca and Picual, and their slopes were significantly ($P < 0.05$) different. Similar behavior was found with β- and γ-tocopherols, as was expected (data not shown). The higher degradation rate of tocopherols relative to polyphenols and orthodiphenols indicates that the former compounds were destroyed earlier during oxidation. Chlorophylls and carotenoids were also degraded throughout the oxidation (Fig. 4). The best correlation coefficients were found for first-order kinetics. This result agrees with those obtained by Fatima *et al.* (14) in leaves and purée of spinach. Sensory analysis (data not shown) clearly detected the incidence of rancidity at the end of the induction period in oils from the three cultivars, with an average intensity of 4.8 points in a 5-point structured scale (where the zero value indicates the absense of the attribute; 1 = nearly imperceptible; 2 = slight; 3 = middle; 4 = great; and 5 = extreme intensity).

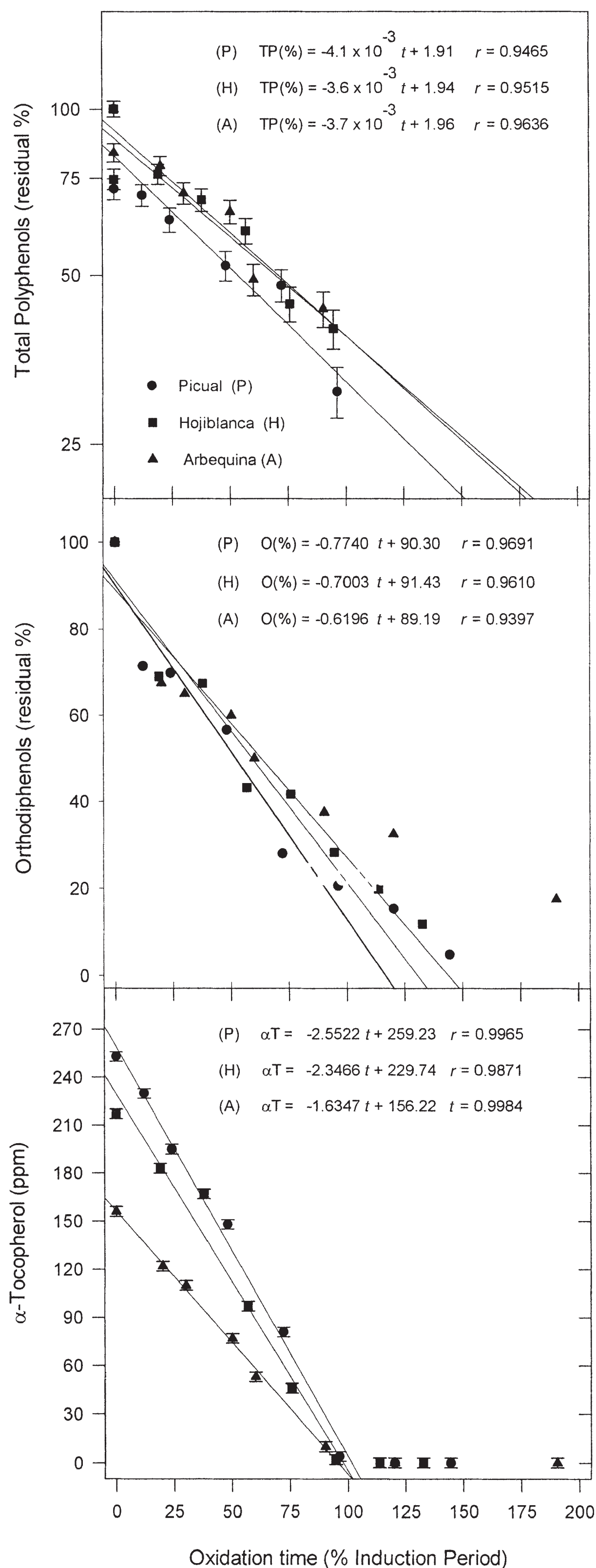

FIG. 3. Changes in phenolic compounds (expressed as residual percentage) throughout the oxidation process of virgin olive oils. Linear regression equations during the induction period are included. TP, total polyphenol; O, orthodiphenol; αT, α-tocopherol. Results are expressed as mean ± SD.

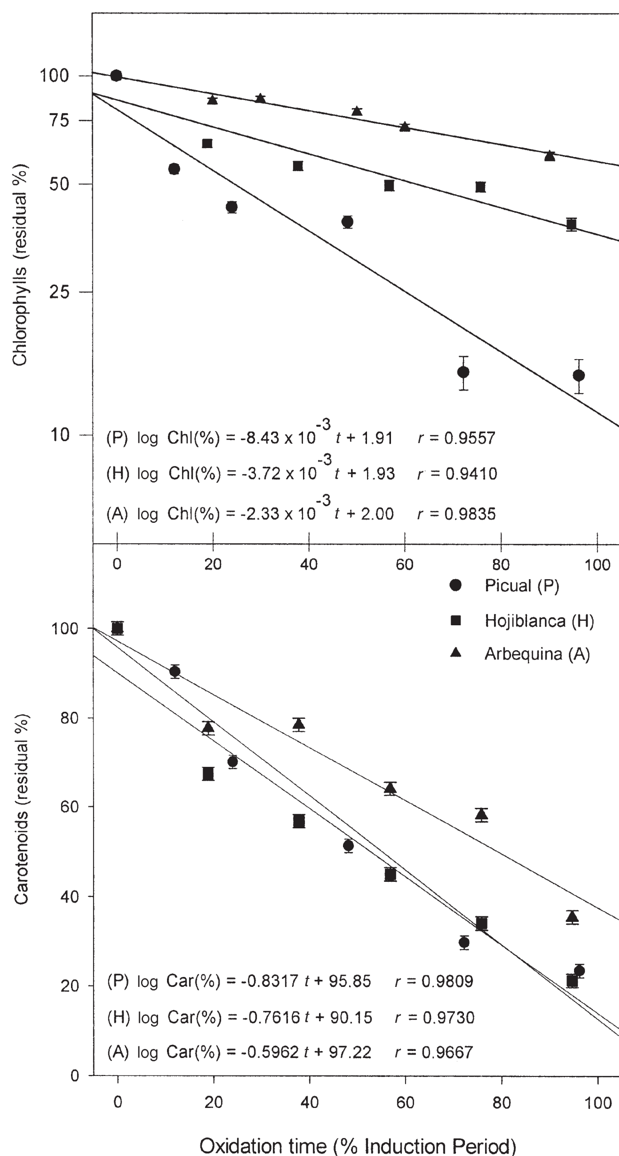

FIG. 4. Residual contents of the photosynthetic pigments in virgin olive oils during oxidation. Linear regression equations during the induction period are included. Chl, chlorophyll; Car, carotenoid. Results are expressed as mean ± SD.

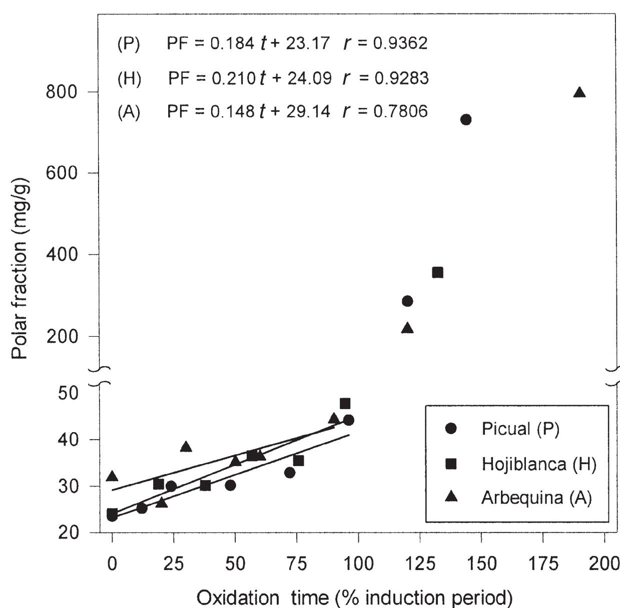

FIG. 5. Evolution of polar fraction (PF) during oxidation. Linear regression equations during the induction period are included.

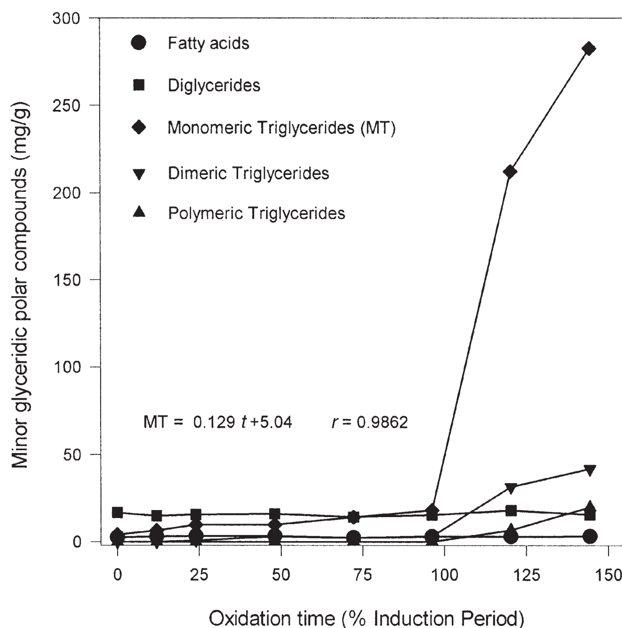

FIG. 6. Evolution of different polar compounds during the oxidative treatment of virgin olive oils. The linear regression equation for monomeric oxidized TG is included.

The polar fraction comprises all those minor glyceridic compounds with polarity higher than TG. This fraction increased slightly but not significantly during the induction period, and very good correlations with time were obtained for the first 100 h (Fig. 5). The slopes for the Hojiblanca and Arbequina cultivars were not different, whereas that for the Picual cultivar was significantly ($P < 0.05$) lower. The greater rise of the polar fraction during the exponential phase in Hojiblanca and Arbequina oils might be a consequence of their higher initial level of unsaturation (10). Exclusion chromatography is able to distinguish the following groups of compounds from the polar fraction: oxidized polymeric, dimeric, and monomeric TG, DG, and FA. Figure 6 shows the evolution of the polar fractions during Picual oil oxidation. Only the oxidized monomeric TG increased during the induction period, whereas the other compounds remained practically constant. At the end of this period, the monomer level was nearly 20 mg/g, a value similar to that reported by Márquez-Ruíz *et al.* (15). During the exponential phase, significant ($P < 0.05$) increases in monomers and dimers were observed, coincident with the exhaustion of oil antioxidants. A similar behavior was found with Hojiblanca and Arbequina oils (data not shown).

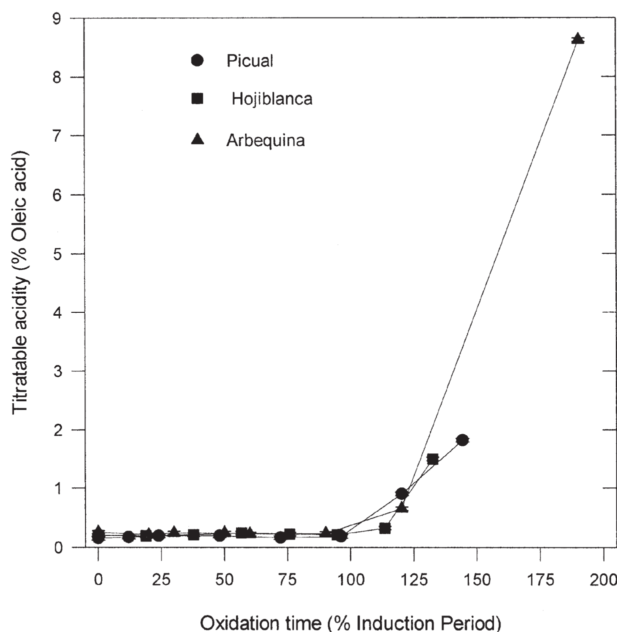

FIG. 7. Evolution of titratable acidity during oxidation.

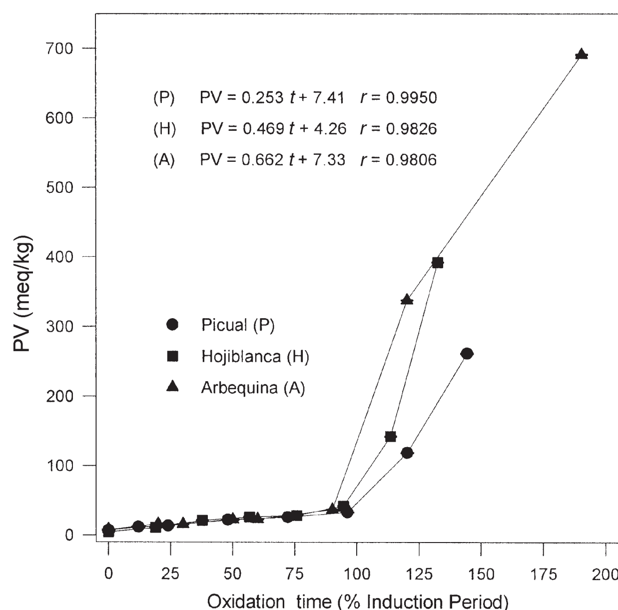

FIG. 8. Effect of oxidation time on PV. Linear regression equations during the induction period are included.

As noted above, some volatile compounds with great importance in oil flavor originate as a result of oxidation. Their quantitative determination is extremely difficult owing to the complexity of the mixture, which includes compounds of very different M.W. and polarity. Moreover, the sampling procedure may contribute to loss of the less-volatile compounds. However, the changes in the chromatographic profile during oxidation allowed a semiquantitative study. Many of the volatile compounds are responsible for the oil flavor, and most are produced *via* the enzymatic lipoxygenase pathway during oil extraction. They are always present in the headspace of virgin olive oils, although in different concentrations depending on the cultivar (16). When oil samples were subjected to the oxidative treatment, the initial flavor disappeared in a few hours along with the overproduction of newly formed volatiles. A considerable increase in hexanal, octanal, nonanal, and volatile organic acids was observed (data not shown).

The titratable acidity remained practically constant during the induction period, but a sharp increase was observed throughout the exponential phase of oxidation (Fig. 7). Because FFA did not change appreciably during the treatment, the rise in acidity is better explained by the active production of volatile acids originating through decomposition of hydroperoxides and oxidation of aldehydes.

The PV increased with time through the induction period (Fig. 8), with excellent correlation coefficients ($P < 10^{-8}$) for samples of the three cultivars. The slopes were significantly ($P < 0.05$) different, with the highest value for the Arbequina oil, followed by Hojiblanca and Picual. The official limit of PV for extra virgin olive oil (20 meq/kg) was reached at the approximate midpoint of their respective induction periods. Increases in PV during the exponential phase were the consequence of the higher oxidation of unsaturated FA and the increase in the polar compounds. Our results agree with those described by Márquez-Ruíz *et al.* (15). Excellent correlations among PV and TG content were found, supporting the concept that TG containing hydroperoxides in some of their acyl groups are the primary oxidation products.

Picual	PV = 1.891 TG – 2.07	$r = 0.9845$	[7]
Hojiblanca	PV = 1.858 TG – 4.18	$r = 0.9765$	[8]
Arbequina	PV = 1.796 TG – 1.73	$r = 0.9857$	[9]

Changes in the specific extinction coefficient at 232 nm were parallel to those in the PV (Fig. 9). Excellent correlations among K_{232} and time were found during the induction period for oils of the three cultivars. The slopes were significantly ($P < 0.05$) different, with the greatest value found for the Arbequina cultivar. This result is consistent with the higher amount of L + Ln acids in Arbequina oil and reflects both the formation of hydroperoxides and the conjugation of double bonds. The limit of 2.50 for extra virgin olive oils was exceeded after 75 h in the Picual oil, at 45 h in Hojiblanca, and at 30 h in Arbequina. The specific extinction coefficient at 270 nm also increased linearly during the induction period (Fig. 9), reaching the limit of 0.20 for extra virgin olive oils after 75 h in Picual, at 45 h in Hojiblanca, and between 45 and 60 h in Arbequina.

Finally, correlations between stability and the compounds and quality indices considered in this study are shown in Table 2. Analysis of data indicates that the compounds most related to oxidative stability are the phenolic compounds and pigments. They underwent the most appreciable alterations

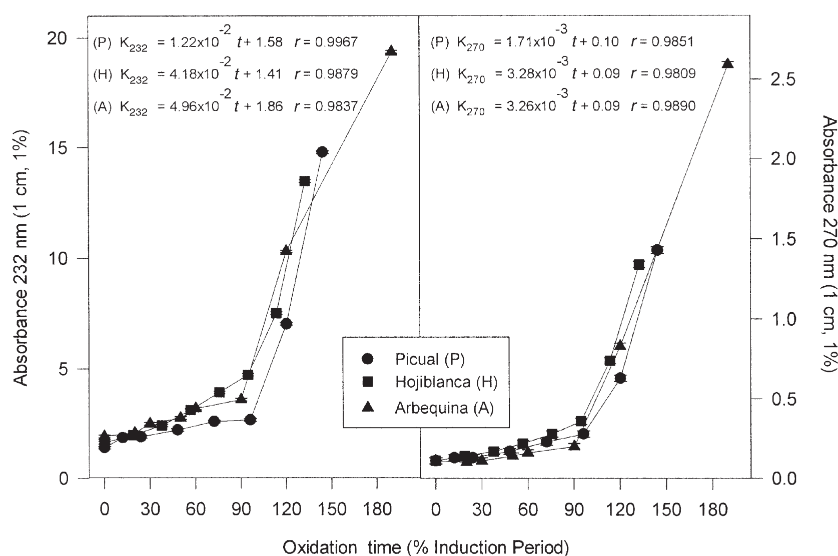

FIG. 9. Evolution of the specific extinction coefficients K_{232} and K_{270} during oxidation. Linear regression equations during the induction period are included.

TABLE 2
Correlations of Different Compounds and Quality Parameters with Stability

	a[a]	b[b]	r[c]	*P*[d]
Stability/polyphenols				
Picual	–28.4	0.64	0.9549	2.8×10^{-8}
Hojiblanca	–28.4	0.48	0.9505	1.5×10^{-7}
Arbequina	–26.3	0.77	0.9968	3.2×10^{-12}
Stability/orthodiphenols				
Picual	4.56	4.77	0.9911	2.1×10^{-11}
Hojiblanca	–6.60	4.35	0.9708	5.3×10^{-9}
Arbequina	–0.30	13.13	0.9726	6.9×10^{-9}
Stability/tocopherols				
Picual	16.8	0.23	0.9722	3.1×10^{-8}
Hojiblanca	10.7	0.23	0.9910	1.8×10^{-10}
Arbequina	3.8	0.21	0.9922	1.1×10^{-12}
Stability/chlorophylls				
Picual	32.8	2.95	0.9872	2.8×10^{-8}
Hojiblanca	11.8	1.73	0.9883	2.9×10^{-9}
Arbequina	–44.2	5.96	0.9906	5.1×10^{-13}
Stability/carotenoids				
Picual	–2.4	7.27	0.9869	1.5×10^{-8}
Hojiblanca	–0.1	3.48	0.9684	2.3×10^{-7}
Arbequina	–13.3	7.17	0.9924	9.5×10^{-12}
Stability/PV				
Picual	107.0	–2.73	–0.9884	2.3×10^{-8}
Hojiblanca	75.2	–1.58	–0.9790	1.6×10^{-7}
Arbequina	44.1	–1.09	–0.9662	1.9×10^{-7}
Stability/K_{232}				
Picual	113.6	–55.28	–0.9880	1.8×10^{-8}
Hojiblanca	94.0	–17.95	–0.9828	1.1×10^{-8}
Arbequina	69.0	–17.20	–0.9872	2.3×10^{-8}
Stability/K_{270}				
Picual	122.8	–382.16	–0.9828	3.2×10^{-8}
Hojiblanca	88.9	–226.33	–0.9816	3.7×10^{-8}
Arbequina	58.1	–263.60	–0.9808	4.5×10^{-8}

[a]Origin ordinates, i.e., y value when x = 0.
[b]Slopes.
[c]Correlation coefficients.
[d]Probability that $r = 0$.

during the induction period, or slow phase of oxidation. Because tocopherols and orthodiphenols were the first compounds to be degraded, we suggest that their measurement is a useful way to establish the average life of oils subjected to oxidation.

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