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Comparative Study of Virgin Olive Oil Behavior under Rancimat Accelerated Oxidation Conditions and Long-Term Room Temperature Storage

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Oxidative stability should be one of the most important quality markers of edible oils; nevertheless, it is not recognized as a legal parameter. The results reported in this study highlight the differences in the olive oil oxidation process under Rancimat accelerated conditions with respect to long-term storage at room temperature and clearly show the lack of correlation between shelf life and the Rancimat induction period. A better correlation, although not yet satisfactory, was found when the same oxidation end-point was used in both assays. The parameter K_{270}, a marker of secondary oxidation products, was the first index to reach the established upper legal limit under Rancimat conditions, whereas at 25 °C it was an index of primary oxidation products (K_{232}). Furthermore, the ratio of oxidation rate at Rancimat conditions to oxidation rate at 25 °C was more than double for secondary oxidation products compared with primary ones. Notable differences were also observed in degradation rates of the different unsaturated fatty acids and in rates of formation of polar oxidation compounds. Moreover, under the Rancimat conditions antioxidants such as *o*-diphenols and α-tocopherol rapidly depleted, and when they had practically disappeared, there was a sharp increase in oxidation indices, such as peroxide value, and in oxidation products. At 25 °C, on the other hand, the depletion was much lower.

KEYWORDS: Rancimat induction period; accelerated oxidation; long-term storage; virgin olive oil

INTRODUCTION

The main components of virgin olive oil are triacylglycerols, the unsaturated fatty acids of which are susceptible to autoxidation. Nevertheless, it also contains minor compounds with antioxidant activities that provide resistance to oxidative degradation (*1–4*) but increase the complexity of the oxidation process, thus making prediction and mathematical modeling more difficult.

Oxidative stability (OS), determined under standardized conditions, should be one of the most important quality markers of edible oils, and it has commonly been evaluated by accelerated methods employing relatively high temperatures and an air flow supply (e.g., Rancimat, active oxygen method, oxidative stability instrument) (*1, 5–9*). These rapid tests are very useful for practical purposes and give a good correlation with concentrations of phenolic compounds (*10–12*), but OS is not recognized as a legal parameter due to the unsatisfactory relationship between the results of these accelerated assays and the real shelf life of such food products (*13–16*). This is probably because the oxidation mechanism is significantly different at temperatures above 60 °C; moreover, at higher temperatures the oxidation reaction becomes more dependent on oxygen concentration because oxygen solubility decreases. As a result, the oxidation process under these accelerated conditions causes the formation of oxidation products, in particular volatile compounds (i.e., volatile acids), which are not produced in significant amounts under normal storage conditions (*14*).

The purpose of this research was to study the oxidation process of several virgin olive oils, with different contents of natural antioxidants, and of an olive oil lipidic matrix lacking in pro- and antioxidant compounds, under accelerated oxidation conditions using the Rancimat apparatus as compared to long-term storage at room temperature. This paper reports and discusses the evolution of primary and secondary oxidation products and the effect of antioxidant content in the course of oxidation under the given conditions with a view to define the applicability and limitations of the accelerated methodology employed to determine the stability and predict the shelf life of virgin olive oils.

MATERIALS AND METHODS

Virgin Olive Oil (VOO). Five extra virgin olive oil samples (Cornicabra cultivar) that differed mainly in terms of phenolic compounds and tocopherol content were employed. One of these

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samples (A) was produced using the Abencor system (Comercial Abengoa, S.A., Sevilla, Spain), whereas the other four samples (B–E) were virgin olive oils collected from quality-assured industrial oil mills located in the Toledo and Ciudad Real areas (Castilla-La Mancha, Spain). All samples were filtered with anhydrous Na_2SO_4 and stored in darkness at 8 °C until analysis using amber glass bottles without headspace.

Purified Olive Oil (POO) Preparation. A typical Cornicabra VOO was stripped of pro- and antioxidants and trace metals by adsorption chromatography (*17*). A glass column (40 × 2.5 cm i.d.) was packed with 70 g of alumina (type 507c, neutral, Fluka, Buchs, Switzerland), previously activated for 4 h at 180 °C, and suspended in *n*-hexane. VOO (100 g) was dissolved in 1000 mL of distilled *n*-hexane and passed through the column. The column and the collector flask were wrapped in aluminum foil to prevent light-induced oxidation. Finally, *n*-hexane was removed under vacuum and nitrogen stream.

Accelerated Oxidation Experiments. The oxidative stability (OS) of each VOO and the POO was previously determined as the induction period (IP, hours) recorded by a Rancimat 679 apparatus using 3.5 g of oil sample, at 100 °C with an air flow of 10 L/h (*18*). Then, 14 g of each VOO and 21 g of POO were distributed among four and six glass tubes, respectively (3.5 g/tube), and oxidized under the above-cited conditions for periods of time corresponding to 20, 40, 60, 80, and 100% of their respective Rancimat IPs. The oil samples used to determine oxidative stability were also analyzed, giving an oxidation time longer than the induction period (110–120% IP).

Oxidation at Room Temperature (RT). Fourteen samples of 40 mL (36.6 g) of each VOO and of POO were stored in darkness at 25 °C in 125 mL open amber glass bottles (i.d. = 4.2 cm; surface area exposed to air = 13.85 cm^2). One bottle was withdrawn from the incubator for analysis at scheduled times.

Analytical Determinations. All reagents used were of analytical, HPLC, or spectroscopic grade and were supplied by Merck (Darmstadt, Germany).

Peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (mequiv of O_2/kg) and K_{232} and K_{270} extinction coefficients, calculated from absorption at 232 and 270 nm, were measured following the analytical methods described in European Regulation EC 2568/91 (*19*) and later amendments. *p*-Anisidine value (AnV) was determined following the AOCS official method (Cd 18-90), using an Agilent 8453 UV–visible spectrophotometer.

Phenolic Compounds. Two hundred and fifty microliters of a solution of the internal standard (syringic acid in methanol, 15 mg/L) was added to a sample of VOO (2.5 g), and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 mL of *n*-hexane, and a diol-bonded phase cartridge (Supelco Co., Bellefonte, PA) was used to extract the phenolic fraction. The cartridge was conditioned with methanol (6 mL) and *n*-hexane (6 mL), and then the oil solution was applied and the SPE column was washed with *n*-hexane (2 × 3 mL) and *n*-hexane/ethyl acetate (85:15, v/v; 4 mL). Finally, the phenols were eluted with methanol (15 mL), and the solvent was removed with a rotary evaporator at 30 °C under vacuum until dry. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL) (*20*).

HPLC analysis was performed using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven, and a diode array UV detector. A Spherisorb S3 ODS2 column (250 × 4.6 i.d. mm, 5 μm particle size) (Waters Co., Milford, MA) was used, maintained at 30 °C. The injection volume was 20 μL and the flow rate 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B), and acetonitrile (C) from 95% (A)–2.5% (B)–2.5% (C) to 34% (A)–33% (B)–33% (C) in 50 min. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (*20*).

Tocopherols were evaluated following AOCS Method Ce 8-89. A solution of oil in *n*-hexane was analyzed in an Agilent Technologies HPLC (1100 series) on a silica gel Lichrosorb Si-60 column (particle size = 5 μm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain), which was eluted with *n*-hexane/2-propanol (98.5:1.5) at a flow rate of 1 mL/min. A fluorescence detector (Thermo-Finnigan FL3000) was used with excitation and emission wavelengths set at 290 and 330 nm, respectively.

Fatty Acid Composition (European Regulations EEC 2568/91 (*19*) and Following Amendments, Corresponding to AOCS Method Ch 2-91). To determine 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 0.5 mL of 2 N methanolic potassium hydroxide and analyzed by GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor) was used. The carrier gas was helium, at a flow rate of 1 mL/min. The injector and detector temperatures were set at 250 °C, and the oven temperature was set at 210 °C. The injection volume was 1 μL. The loss of unsaturated fatty acids due to oxidation was quantified on the basis of the ratio between each fatty acid and the palmitic acid peak areas, because saturated fatty acids are practically unaltered by autoxidation (*21*). In samples submitted to Rancimat oxidation, 2 mL of a methyl heptadecanoate in heptane (10 mg/mL) solution was added to the oils as internal standard to allow an absolute quantification (*21, 22*) .

Polar Compounds. The altered triacylglycerol (TG) compounds constituting the polar fraction of the oxidized oil were separated into TG dimers and polymers, oxidized TG, diacylglycerols (DG), and free fatty acids (FFA) by high-performance size exclusion chromatography (HPSEC) according to the method of Márquez-Ruiz et al. (*23*). An HPLC (Agilent Technologies, 1100 series) was used, equipped with a refractive index detector working at 35 °C and two serially connected PLgel columns (300 × 7.5 mm, 5 μm particle, and 100 and 500 Å pore sizes, respectively; Agilent, Winnersh, U.K.). The mobile phase was tetrahydrofuran (THF) at 1 mL/min; the injection volume was 20 μL; and monoolein (Sigma Chemical Co., St. Louis, MO) was added as internal standard.

All experiments and analytical determinations were carried out at least in duplicate.

Statistical Analysis. The experimental data set consisted of two individual batches of samples for each of the two oxidation conditions studied (Rancimat and room temperature); moreover, duplicate measurements were carried out for each sample taken at scheduled time intervals. Statistical analysis was performed with the SPSS 14 statistical software (SPSS Inc., Chicago, IL). One-way ANOVA was carried out using the Duncan test. Means were considered to be statistically different at $p < 0.05$. Least-squares linear regression analyses were performed, and the best equations were selected on the basis of statistical parameters of the fitting (R, p). Oxidation rates and antioxidant degradation rates were calculated from the slopes of the respective concentration versus time curves.

RESULTS AND DISCUSSION

As shown in **Table 1**, all of the VOO samples presented comparably low initial oxidation levels, as denoted by the initial peroxide value (PV) and the UV characteristics K_{232} and K_{270}. Lipid matrices were very similar because all samples came from monovarietal VOO grown in the same area (Cornicabra cultivar in Castilla-La Mancha). Nevertheless, there were some expected statistically significant differences in the unsaturated fatty acid contents of samples, especially in linoleic acid (C18:2). On the contrary, as the authors intended, there were notable differences in the types and concentrations of natural antioxidants, phenolic compounds, and tocopherols (**Table 1**). Data relating to the characteristics of the purified olive oil (POO) sample used in this study are also shown.

Evolution of the Oxidation Process. The evolution of the PV in the VOO and POO samples in the course of oxidation under Rancimat conditions (100 °C and 10 L/h air flow) is depicted in **Figure 1**.

PV and K_{232} (data not shown for the latter) both appeared to be suitable indices for monitoring the progress of oxidation and for determining the IP in VOOs under Rancimat conditions,

Table 1. Initial Composition and Quality Indices of Olive Oil Samples

	virgin olive oil samples[a]					
	A	B	C	D	E	POO[b]
PV (mequiv of O_2/kg)	6.0 ± 0.1 a	8.5 ± 0.4 b	8.6 ± 0.3 b	8.0 ± 0.1 b	8.5 ± 0.1 b	ND
K_{232}	1.89 ± 0.03 b	2.02 ± 0.01 c	1.86 ± 0.05 ab	2.02 ± 0.01 c	1.80 ± 0.01 a	0.91 ± 0.01
K_{270}	0.15 ± 0.01 c	0.17 ± 0.01 d	0.13 ± 0.01 b	0.13 ± 0.01 b	0.11 ± 0.01 a	0.02 ± 0.01
C18:1[c]	823.19 ± 0.60 d	799.52 ± 2.04 c	794.45 ± 0.50 b	791.18 ± 1.77 a	795.89 ± 0.13 b	839.13 ± 0.02
C18:2[c]	36.68 ± 0.03 a	38.52 ± 0.11 b	52.71 ± 0.04 d	53.23 ± 0.11 e	49.08 ± 0.01 c	53.24 ± 0.01
C18:3[c]	5.82 ± 0.07 b	6.28 ± 0.02 c	5.81 ± 0.01 b	5.50 ± 0.01 a	5.39 ± 0.07 a	5.51 ± 0.01
C18:1/C18:2	22.45 ± 0.04 e	20.75 ± 0.01 d	15.08 ± 0.02 b	14.86 ± 0.01 a	16.22 ± 0.01 c	16.70 ± 0.01
α-tocopherol[d]	0.46 ± 0.01 c	0.56 ± 0.02 d	0.42 ± 0.01 b	0.41 ± 0.01 b	0.34 ± 0.01 a	ND
o-diphenols[d]	1.48 ± 0.05 d	1.21 ± 0.03 c	0.54 ± 0.01 b	0.56 ± 0.01 b	0.42 ± 0.03 a	ND
total phenols[d]	2.64 ± 0.09 d	1.99 ± 0.0629 c	1.10 ± 0.01 ab	1.13 ± 0.01 b	1.00 ± 0.03 a	ND
o-diphenols/total phenols	0.56 ± 0.01 c	0.61 ± 0.01 d	0.49 ± 0.01 b	0.48 ± 0.01 b	0.41 ± 0.01 a	ND
complex/simple phenols	9.36 ± 0.39 d	6.99 ± 0.16 c	7.38 ± 0.12 c	3.14 ± 0.03 b	0.57 ± 0.17 a	ND
Rancimat (h)	140.3 ± 3.3 d	118.7 ± 1.7 c	65.7 ± 1.0 a	72.2 ± 1.9 b	65.1 ± 2.0 a	4.0 ± 0.1

[a] Mean values with different letters in the same row are statistically different ($p \leq 0.05$). [b] POO, purified olive oil; ND, not detected. [c] Expressed as g/kg of methyl heptadecanoate. [d] Expressed as mmol/kg.

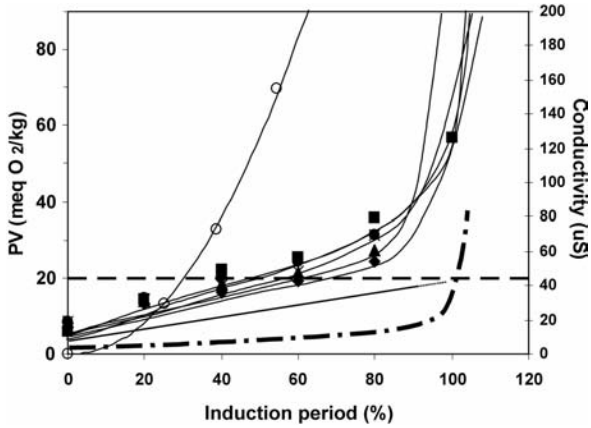

Figure 1. Evolution of peroxide value under Rancimat conditions (100 °C and air flow of 10 L/h). Samples: ■, A; ●, B; ▲, C; ♦, D; × , E; ○, POO. Average PV trend at 25 °C (—) and Rancimat conductivity record (– · –) are illustrated.

rising linearly until the Rancimat assay end-point, when a sudden increment was observed (**Figure 1**). In all VOOs, in line with previous findings by our group and other authors (*16*, *24*), the PV increased linearly up to about 80–90% of the IP following a pseudo-zero-order kinetic ($R^2 > 0.964$). In contrast, the PV increase for POO was considerably greater from the very first (*25*), fitting a second-grade polynomial equation ($R^2 = 0.999$). Under these accelerated oxidation conditions both primary and secondary oxidation indices K_{232}, K_{270}, and *p*-anisidine value followed a similar trend (data not shown).

The average PV trend observed at 25 °C for the same VOO samples (*16*) is also shown in **Figure 1** as a straight line ascending to the established end-point (20 mequiv of O_2/kg, the maximum value established for the extra virgin category) in 96–167 weeks (**Table 2**). However, as expected, under Rancimat conditions VOO samples reached this PV in just 28–56 h, which is about 40–60% of the IP, in accordance with reports by other authors (*24*).

Under the Rancimat conditions the UV characteristic K_{270}, a marker of secondary oxidation products, was the first oxidation index to reach the legal limit established for the extra virgin olive oil category (*26*), whereas at 25 °C it was K_{232}, an index of primary oxidation products (**Table 2**). This means that under these accelerated conditions (100 °C and 10 L/h air flow) oxidation was very fast and hydroperoxides decomposed to secondary oxidation products much more quickly than at 25 °C. In fact, the ratio of the oxidation rates measured under the Rancimat and at 25 °C conditions was more than double in the case of the secondary oxidation products (445 to 798, measured by the parameter K_{270}) than for primary products (124 to 285, measured by the K_{232}), as depicted in **Table 3**.

As **Figure 2** clearly shows, no correlation was found between the stability at room temperature, measured as the time taken by the first oxidation index to reach the legal upper limit (K_{232} as previously mentioned) and Rancimat oxidative stability ($R^2 = 5 \times 10^{-6}$) in seven comprehensively studied VOOs, five samples from the present study and two more from our previous work (*16*). A similar plot was obtained using the time taken to reach PV = 20 as the end-point of the assay ($R^2 = 0.15$). This means that the Rancimat test cannot be used to predict the real shelf life of VOOs, probably because of the different oxidation mechanisms that take place under these diverse conditions. A better correlation, although not yet satisfactory ($R^2 = 0.55$, $p = 0.01$), was found between stability at 25 °C and under the Rancimat conditions, using the time required to reach PV = 20 as end-point in both cases (**Table 2**). It could be helpful to investigate this matter further to see whether the Rancimat assay could be modified to provide more relevant information regarding the stability and shelf life of VOO under normal storage conditions.

During oxidation under the Rancimat conditions, triacylglycerol unsaturated fatty acids (UFA: oleic, linoleic, and linolenic) remained virtually constant throughout the induction period while protected by antioxidant *o*-diphenols and suddenly decreased when the latter had practically disappeared, as **Figure 3** clearly shows. The ratio between fatty acid losses under the Rancimat conditions and at 25 °C was higher in the case of linoleic acid (4.1–5.3), followed by linolenic (2.3–4.1) and oleic (1.1–4.5) acids; this shows that the effect of the Rancimat conditions on oxidizability was greater in the case of the di-unsaturated fatty acid, although sample B presented similar rates for all three fatty acids (**Table 3**). One possible explanation is that the highest linolenic acid content in this sample produced more linolenyl peroxyl radicals at 100 °C, which also accelerate the oxidation of oleic and linoleic acids (*14*). No significant differences were observed in the ratio between the percentages of loss of C18:2 and C18:1 with respect to C18:3/C18:2 at 25 °C, indicating that the presence of an additional double bond produces a proportional change in oxidizability. The same did not apply under the Rancimat conditions, when the ratio of percent loss of C18:2/C18:1 was generally higher, and the ratio of loss of C18:3/C18:2 was even lower than at 25 °C. These

Table 2. VOO Stability under Experimental Oxidation Conditions Expressed as Rancimat Induction Period and as the Time Taken To Reach the Legal Limits of Some Quality Indices

	Rancimat conditions				25 °C		
sample	IP (h)	PV = 20 mequiv/kg (h and IP%)	K_{232} = 2.5 (h)	K_{270}= 0.22 (h)	PV = 20 mequiv/kg (h)	K_{232} = 2.5 (h)	K_{270}= 0.22 (h)
A	140.3	55.7	39.7	62.3	22.9	167[a]	103[a]
B	118.7	49.8	41.9	44.7	15.7	103[a]	54
C	65.7	32.7	49.7	28.9	20.4	96[a]	53
D	72.2	44.2	61.2	26.4	24.4	122[a]	63
E	65.1	27.9	42.8	31.3	24.8	100[a]	56
POO	4.0	1.2	29.8	1.8	3.8	6.8	7.7

[a] Times calculated by extrapolation.

Table 3. Ratios of Some Oxidation Parameters under Rancimat Conditions and Room Temperature Storage

	oxidation rate		reduction (% loss) in unsaturated fatty acid							oxidized polar compounds[a] (%)			
	Rancimat/25 °C		Rancimat/25 °C			25 °C		Rancimat conditions		Rancimat/25 °C			
sample	K_{232}	K_{270}	C18:1	C18:2	C18:3	C18:2/C18:1	C18:3/C18:2	C18:2/C18:1	C18:3/C18:2	oxTGs	DTGs + PTGs	DGs	FFAs
A	285	704	1.5	4.5	2.5	2.80	2.68	8.34	1.50	3.57	3.76	1.15	0.66
B	124	445	4.5	4.8	4.1	2.21	2.78	2.37	2.37	1.45	4.91	0.91	0.62
C	204	656	1.1	4.1	3.2	2.15	2.17	7.86	1.68	2.01	2.75	0.88	0.72
D	201	505	1.5	5.3	2.7	3.11	2.58	11.22	1.31	1.09	1.87	0.83	0.41
E	272	798	2.8	4.3	2.3	3.83	2.55	5.82	1.34	1.27	2.14	0.66	0.57

[a] oxTGs, oxidized triacylglycerol monomers; DTGs + PTGs, dimeric and polymerized TGs; DGs, diacylglycerol monomers; FFAs, free fatty acids.

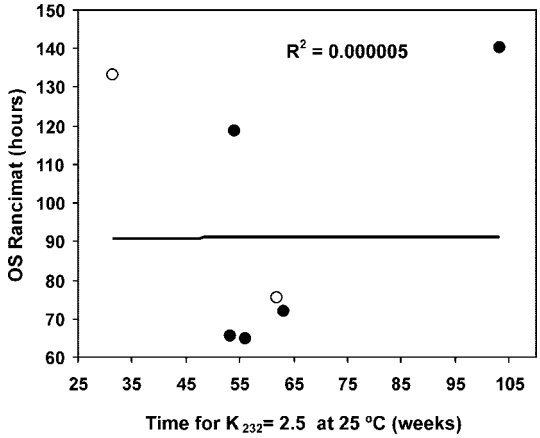

Figure 2. Correlation between VOO shelf life and Rancimat oxidative stability: ●, samples from the present study; ○, samples from a previous study.

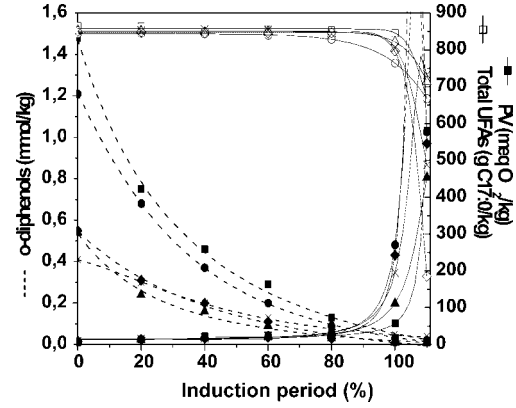

Figure 3. Behavior of *o*-diphenols versus the evolution of PV and UFAs during oxidation in Rancimat. Samples: ■, □, A; ●, ○, B; ▲, △, C; ◆, ◇, D; ×, ×, E. Solid symbols represent *o*-diphenols and PV; open symbols represent total UFAs.

ratios indicate that temperature and oxygen influenced the stability of linoleic acid more than the other unsaturated fatty acids.

Differences concerning the formation of polar oxidation compounds were also evaluated. The formation of these compounds drastically increased close to the Rancimat IP, causing a large analytical error in their quantification. At about 80% of the IP, the sum of dimers and polymers of TGs (DTGs + PTGs), formed mainly at high temperatures as a consequence of thermal oxidation, rose by between 1.9 and 4.9 times more than with oxidation at 25 °C, and the oxidized triacylglycerol monomers (oxTGs) increased by between 1.1 and 3.6 times (**Table 3**). In contrast, diacylglycerols and free fatty acid contents were lower than under oxidation at room temperature (0.66–1.15 for diacylglycerols and 0.41–0.72 for free fatty acids) due to further oxidation, thermal decomposition, and side reactions (polymerization and cleavage) that become significant at 100 °C (*14*).

Behavior of Natural Antioxidants. *o*-Diphenols are the most active antioxidants in VOOs (*5*, *10*, *12*), which was one of the main criteria used in selecting the samples employed in this study, along with the ratios between the concentrations of the different types of phenolic compounds. Hence, samples C–E, with similar total phenol, *o*-diphenol, and α-tocopherol contents, differed considerably in their complex/simple phenols ratio. Moreover, samples A and B possessed the highest antioxidant content; at the same time, however, the former possessed the highest total phenol and *o*-diphenol contents and complex/simple phenols ratio, whereas the latter had the highest α-tocopherol content and *o*-diphenols/total phenols ratio (**Table 1**).

Under the Rancimat conditions *o*-diphenols rapidly became depleted from the outset. Once they had practically disappeared, the oxidation indices, such as the peroxide value, and the oxidation products, increased sharply (**Figure 3**). The *o*-diphenols were considerably depleted in all samples: depletion of their initial content reached 90% before 80% of the IP; on the other hand, the depletion of tyrosol and its derivatives was less pronounced (70–86%). In contrast, the *o*-diphenol loss was much smaller in the same samples at 25 °C (*16*), ranging between 54 and 71%, whereas that of tyrosol and derivatives was between 33 and 58% (**Figure 4**). Under both oxidation conditions, the behavior of hydroxytyrosol and its secoiridoid

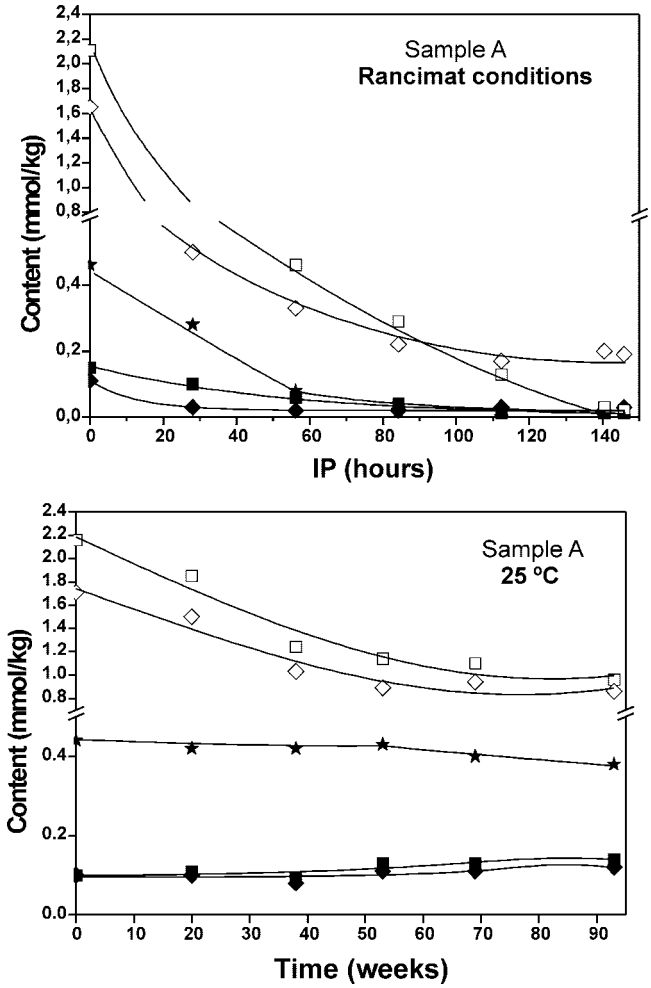

Figure 4. Behavior of phenolic compounds and α-tocopherol under Rancimat conditions and at room temperature: ■, hydroxytyrosol; □, hydroxytyrosol + secoiridoids of hydroxytyrosol (*o*-diphenols); ◆, tyrosol; ◇, tyrosol + secoiridoids of tyrosol; ★, α-tocopherol.

derivatives (*o*-diphenols) appeared to follow a pseudo-first-order kinetics: Ln $C = kt$ + Ln C_0; $0.89 \leq R^2 \leq 0.96$ at 25 °C and $0.97 \leq R^2 \leq 0.99$ under the Rancimat conditions. Taking into account the known lack of antioxidant activity of tyrosol and its derivatives (*5*, *8*), their elevated loss at 100 °C was presumably caused by thermoxidative degradation, which may of course also have occurred in the other phenolic compounds.

With respect to simple phenols, as an example in the case of sample A, **Figure 4** shows that hydroxytyrosol and tyrosol remained practically constant at 25 °C, probably due to a rate of hydrolysis of secoiridoid derivatives (*27*), releasing simple phenols, similar to the rate of their oxidation. In contrast, under Rancimat conditions decomposition appeared to occur at a higher rate than formation from complex secoiridoids; moreover, the complex phenols also decompose more quickly, which means there is a smaller pool able to give simple phenols. As a result, concentrations of simple phenols fell from the beginning.

Under the Rancimat conditions α-tocopherol also decreased rapidly and almost linearly from the very first, being reduced about 99% of its initial content at 80% of the IP (**Figure 4**). The decrease of this compound was much less pronounced at 25 °C, at which depletion was no higher than 30% in any sample. As **Figure 4** shows, in the case of sample A, this compound remained nearly constant during >40 weeks of storage at 25 °C; under the Rancimat conditions, however, α-tocopherol content dropped from the very first. The rapid decrease observed under the Rancimat conditions could be due mainly to the very high susceptibility of this molecule to oxidation to α-tocopherolquinones at high temperatures (*28*), which diminishes the protection of unsaturated fatty acids against oxidation.

The synergistic effect of *o*-diphenols and α-tocopherol studied by some authors is dependent on the relative concentration of both antioxidants and the oxidation level of the lipid matrix (*29*). Moreover, according to the results of this study it would be strongly related to the oxidation conditions used because it is considerably different at ambient and accelerated conditions. Therefore, the antioxidant capacity determined under accelerated oxidation conditions (*1*, *5*, *8*) could probably not be ascribed as well at room temperature.

On the basis of the existing literature and the results reported in this study, which highlight how the oxidation process under Rancimat accelerated conditions (similar to AOM and OSI) differs from oxidative stability at room temperature, it is confirmed that no correlation exists between the stability under normal storage conditions and the Rancimat induction period (**Figure 2**).

Therefore, although these rapid assays are very useful in measuring a relative oxidative stability index for virgin olive oils and other edible oils, moreover providing a good correlation with concentrations of phenolic compounds as we know, they cannot be used to extrapolate the shelf life of these food products because this correlation has not been confirmed at room temperature (*16*, *30*).

LITERATURE CITED

(1) Mateos, R.; Trujillo, M.; Pérez-Camino, M. C.; Moreda, W.; Cert, A. Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. *J. Agric. Food Chem.* **2005**, *53*, 5766–5771.

(2) Vázquez Roncero, A.; Janer del Valle, C.; Janer del Valle, M. L. Natural polyphenols and stability of olive oil. *Grasas Aceites* **1975**, *26*, 14–18.

(3) Blekas, G.; Psomiadou, E.; Tsimidou, M.; Boskou, D. On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 340–346.

(4) Servili, M.; Montedoro, G. Contibution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 602–613.

(5) Baldioli, M.; Servili, G.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73* (11), 1589–1593.

(6) 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.

(7) Koski, A.; Psomiadou, E.; Tsimidou, M.; Hopia, A.; Kefalas, P.; Wähälä, K.; Heinonen, M. Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. *Eur. Food Res. Technol.* **2002**, *214*, 294–298.

(8) Mateos, R.; Domínguez, M. M.; Espartero, J. L.; Cert, A. Antioxidant effect of phenolic compounds, α-tocopherol and other minor components in virgin olive oil. *J. Agric. Food Chem.* **2003**, *51*, 7170–7175.

(9) Abaza, L.; Taamalli, W.; Temime, S. B.; Daoud, D.; Gutiérrez, F.; Zarrouk, M. Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. *Riv. Ital. Sostanze Grasse* **2005**, *82*, 12–18.

(10) Gutfinger, T. Polyphenols in olive oil. *J. Am. Oil Chem. Soc.* **1981**, *58*, 966–968.

(11) Salvador, M. D.; Aranda, F.; Gómez-Alonso, S.; Fregapane, G. Cornicabra virgin olive oil: a study of five crop seasons. Composition, quality and oxidative stability. *Food Chem.* **2001**, *74*, 267–274.

(12) Gómez-Alonso, S.; Salvador, M. D.; Fregapane, G. Phenolic compounds profile of Cornicabra virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 6812–6817.

(13) Kaya, A.; Tekin, A. R.; Öner, D. Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage. *Lebensm.-Wiss. -Technol.* **1993**, *26*, 464–468.

(14) Frankel, E. N. In *Lipid Oxidation*; Frankel, E. N., Ed.; Oily Press: Dundee, Scotland, 1998.

(15) Velasco, J.; Dobarganes, C. Oxidative stability of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 661–676.

(16) Gómez-Alonso, S.; Mancebo-Campos, V.; Salvador, M. D.; Fregapane, G. Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. *Food Chem.* **2007**, *100*, 36–42.

(17) Marinova, E. M.; Yanishlieva, N. V. Antioxidative activity of phenolic acids on triacylglycerols and fatty acid methyl esters from olive oil. *Food Chem.* **1996**, *56*, 139–145.

(18) Gutiérrez, F. Determinación de la estabilidad oxidativa de aceites de oliva vírgenes: Comparación entre el método del Oxígeno Activo (A.O.M.) y el método Rancimat. *Grasas Aceites* **1989**, *40*, 1–5.

(19) European Regulation (EC) no. 2568/91. On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Communities* 1991, *L248*, 1.

(20) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185–2192.

(21) Dobarganes, M. C.; Perez-Camino, M. C. Fatty acid composition: a useful tool for the determination of alteration level in heated fats. *Rev. Fr. Corps Gras* **1988**, *75*, 67–70.

(22) *Productos derivados de aceites y grasas. Ésteres metílicos de ácidos grasos (FAME). Determinación de los contenidos de éster y de éster metílico de ácido linoleico*; AENOR: Madrid, Spain, 2002; UNE-EN 14103.

(23) Márquez-Ruiz, G.; Jorge, N.; Martín-Polvillo, M.; Dobarganes, M. C. Rapid, quantitative determination of polar compounds in fats and oils by solid-phase extraction and size exclusion chromatography using monostearin as internal standard. *J. Chromatogr., A* **1996**, *749*, 55–60.

(24) Gutiérrez, F.; Villafranca, M. J.; Castellano, J. M. Changes in the main components and quality indices of virgin olive oil during oxidation. *J. Am. Oil Chem. Soc.* **2002**, *79*, 669–676.

(25) Gómez-Alonso, S.; Mancebo-Campos, V.; Salvador, M. D.; Fregapane, G. Oxidation kinetics in olive oil triacylglycerols under accelerated shelf-life testing (25–75 °C). *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 369–375.

(26) European Regulation (EC) no. 1989/2003 of 6 November 2003 amending Regulation (EC) no. 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis. *Off. J. Eur. Communities* 2003, *L295*, 57.

(27) Brenes, M.; García, A.; García, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycones during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609–5614.

(28) Verleyen, T.; Kamal-Eldin, A.; Dobarganes, C.; Verhe, R.; Dewettinck, K.; Huyghebaert, A. Modelling of α-tocopherol loss and oxidation products formed during thermoxidation in triolein and tripalmitin mixtures. *Lipids* **2001**, *36*, 719–726.

(29) Blekas, G.; Tsimidou, M.; Boskou, D. Contribution of α-tocopherol to olive oil stability. *Food Chem.* **1995**, *52*, 289–294.

(30) Cinquanta, L.; Esti, M.; Di Matteo, M. Oxidative stability of virgin olive oils. *J. Am. Oil Chem. Soc.* **2001**, *78*, 1197–1202.

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