

Evolution of the Oxidation Process in Olive Oil Triacylglycerol Under Accelerated Storage Conditions (40–60°C)

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ABSTRACT: This paper presents an analysis of oxidation in olive oil TAG at different temperatures within a range of 40–60°C in darkness, as measured by the rate of formation of hydroperoxides, their decomposition products, and sensory and chemical flavor deterioration. At 60°C, the oxidation process was relatively fast, and all the indexes of the extent of oxidation behaved in a very similar way. The behaviors of the rate of formation of conjugated dienes (measured as the K_{232}), of oxidized TAG (determined by HPLC), and of carbonyl compounds (measured as anisidine value) were very similar to that of the PV. The induction periods (IP) of the four indexes were less than 36 h. Nevertheless, the IP for K_{270} and the sum of oxidized TAG dimers and polymers were 5 d. At 50 and 40°C, the rate of formation of secondary oxidation products was lower. Residual linolenic acid or PUFA as determined by GC showed correlation coefficients higher than 0.999 with the Totox index at all of the assayed temperatures. The rancid recognition threshold corresponded to lower values of the oxidation indexes at lower temperature. Moreover, at all assayed temperatures, the rancidity threshold apparently coincided with the IP for the kinetics of 2,4-decadienal formation.

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KEY WORDS: Olive oil, oxidation, rancidity, sensory, triacylglycerols, volatiles.

Lipid oxidation is one of the most fundamental reactions in lipid chemistry. When unsaturated FA are exposed to air, molecular oxygen reacts with them by a free radical chain mechanism yielding hydroperoxides, which decompose to a complex variety of secondary oxidation products. Finally, volatile compounds are formed, causing rancidity and decreasing the quality of edible oils and fat-containing food (reviewed in Ref. 1). Oxidation is therefore one of the major causes of virgin olive oil quality deterioration.

Oxidative stability, which may be defined as the resistance of lipids to oxidation, is not recognized as a standard quality index, mainly owing to an unsatisfactory relationship between the commonly used stability assays (e.g., Rancimat or active oxygen method) and real oil shelf life at room temperature (2). The more relevant reasons for that discrepancy are that the autoxidation mechanism is significantly different at temperatures higher than 60°C, and that oxidation becomes more dependent

on oxygen concentration at higher temperatures, whereas oxygen solubility decreases. As a result, oxidation in the Rancimat test causes the formation of volatile acids that are not produced in significant quantities under normal storage conditions.

The goal of this medium-term research project is therefore to develop an accelerated stability test at relatively low temperatures (40–60°C), the results of which correlate satisfactorily with storage at room temperature and the potential shelf life of virgin olive oil. This entails a study of the oxidation process at different temperatures, preferably between 40 and 60°C, and the use of more than one method for measuring both the initial products of lipid oxidation (hydroperoxides or conjugated dienes) and their decomposition products (carbonyl compounds) to determine the real extent of oxidation (3).

Determination of volatile compounds is considered one of the best methods to evaluate the extent of oxidation in edible fats and oils and hence the consumer acceptability of the food product (4). Furthermore, several authors have attempted to establish a relationship between the analytical determination of volatile compounds and the results obtained by sensory analysis (5,6). In the case of seed oils, total volatiles, hexanal, or pentane contents are often used to determine the degree of alteration (7,8). However, virgin olive oil is a special case since it is consumed without refining; therefore, some of its positive sensory attributes are related to the volatile compounds that it contains, such as hexanal (9). A study of the evolution of volatile compounds during the course of the oxidation process is therefore required to establish what compounds are to be taken as indexes of oxidation (10).

This paper reports the first stage of the study, consisting of an analysis of the evolution of the oxidation process in olive oil TAG stored in darkness at different temperatures, ranging from 40 to 60°C, in the absence of pro- and antioxidant compounds to avoid confounding effects, as measured by the rate of formation of hydroperoxides and their decomposition products. Finally, to address the loss of sensory quality of purified olive oil and the relationship between analytical determinations and organoleptic quality, flavor stability is assessed by a trained tasting panel, the best means of determining product quality as perceived by consumers.

MATERIALS AND METHODS

Purified olive oil (POO) preparation. Cornicabra virgin olive oil was stripped of pro- and antioxidants and trace metals by

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adsorption chromatography (11). Virgin olive oil (100 g) in 1000 mL distilled hexane was passed through a column (2 cm i.d.) filled with 70 g alumina (type 507c, neutral; Fluka, Buchs, Switzerland) that was activated for 4 h at 180°C, and collected in darkness.

Oxidation experiments. Twelve 36.6-g (40-mL) samples of POO were stored in darkness at different temperatures (40, 50, and 60°C) in 125-mL open amber glass bottles (i.d., 4.2 cm; surface area exposed to the atmosphere, 13.85 cm²). One bottle was taken 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).

PV and UV absorption characteristics (K_{232} and K_{270}) were measured following the analytical methods described in European Regulation EEC 2568/91. PV were expressed as milliequivalents of active oxygen per kilogram of oil (meq O₂/kg); K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively. *p*-Anisidine value (AnV) was determined following the AOCS official method (Cd 18-90; Ref. 12), using a UV-vis spectrophotometer.

Polar compounds. The altered TAG compounds that constitute the polar fraction of the oxidized oil were separated into TAG dimers and polymers, oxidized TAG, DAG, and FFA by high-performance size-exclusion chromatography (HPSEC) according to Márquez-Ruiz *et al.* (13). A high-performance liquid chromatograph equipped with a refractive index detector operating at 35°C, and two serially connected PLgel columns (300 × 7.5 mm; 5 μm particle, and 100 and 500 Å pore size, respectively; Agilent, West Lothian, United Kingdom) at 25°C were used. The mobile phase was THF at 1 mL/min, the injection volume was 20 μL, and monoolein (Sigma Chemical Co., St. Louis, MO) was added as internal standard.

FA composition [European Regulations EEC 2568/91 and following amendments, corresponding to AOCS Method Ch 2-91 (12)]. To determine FA 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 potassium hydroxide and analyzed by GC with an FID detector. A fused-silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Madrid, Spain) was used. The carrier gas was helium at a flow through the column of 1 mL/min. The temperature of the injector and detector was set at 250°C and the oven temperature at 210°C. The injection volume was 1 μL.

The loss in the unsaturated FA due to oxidation was quantified on the basis of the ratio between each FA and the palmitic acid peak areas, since saturated FA are not altered by autoxidation (14).

Volatile compounds [adapted from Jelen *et al.* (15)]. Three grams of oil sample was placed in a 10-mL headspace vial and kept at 28°C for 1 h. The TeflonTM-lined septum covering the vial was pierced with a solid phase micro-extraction (SPME) needle, and a 100 μm divinylbenzene/carboxene/poly(dimethylsiloxane) (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA) fiber was exposed to the oil headspace for 20 min. The fiber was

then retracted into the needle and immediately transferred and desorbed for 5 min in the injection port of a gas chromatograph equipped with an FID. Compounds were resolved on an HP-5 fused-silica column (30 m × 0.32 mm × 0.25 μm; Agilent Technologies, Palo Alto, CA) under the following conditions: injection port temperature 240°C; helium flow 2 mL/min; oven temperature ramp: 35°C for 5 min, 4°C/min up to 100°C and then 15°C/min up to 220°C (maintained for 5 min). Volatile compounds were identified by comparison with standard substances. The following reference compounds were used: hexanal from Sigma Chemical Co.; heptanal, octanal, nonanal, *t*-2-hexenal, *t*-2-heptenal, *t*-2-octenal, and *t,t*-2,4-decadienal from Aldrich Chemie (Steinheim, Germany); and *t*-2-decenal from Fluka Chemie.

Sensory analysis. POO samples were assessed, for aroma changes only, at 12 different stages of the oxidation process by a sensory panel of eight assessors from the University of Castilla-La Mancha and from the Protected Designation of Origin "Montes de Toledo" Foundation (Toledo, Spain).

The purpose of the sensory analysis was to determine the recognition threshold of rancid defect and to correlate it with the chemical composition of the oil at that stage of the oxidation process. The recognition threshold is defined as the level of a stimulus at which the specific stimulus can be recognized and identified. A rank probability plot is a useful tool for testing whether a set of individual thresholds is normally distributed. The graph also serves to locate the group threshold of the stimulus that corresponds to 75% correct answers by the panel. Assessors were therefore asked to evaluate differences between the aroma of the fresh POO (reference oil) and the 12 samples of POO removed from the incubator at different times, and to mark in an appropriate form those oil samples in which they could recognize the defect (Method of Investigating Sensitivity of Taste; ISO 3972:1996, corresponding to Spanish UNE 87003-2000) (16).

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

Statistical analysis. Statistical analyses were performed using SPSS 11 statistical software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The initial characteristics of the POO used for the accelerated oxidation experiments are reported in Table 1. The POO presented very low levels of both primary (FA hydroperoxides, measured as, PV, K_{232} , and oxidized TAG, oxTAG) and secondary oxidation products (FA hydroperoxide decomposition products, reflected as K_{270} , AnV, and dimers and polymers of TAG, DTAG + PTAG). FA analysis of the olive oil used showed a high content of oleic acid and low PUFA.

Figures 1 to 3 depict the effect of storage temperature (range 40–60°C) on the evolution of the POO oxidation process as measured by the rate of formation of primary and secondary oxidation products.

At 60°C (Fig. 1) the oxidation process was relatively fast, and all the indexes of the extent of the oxidation behaved in a

TABLE 1
Initial Characteristics of Purified Olive Oil^a (POO)

Analytical determination	Mean \pm SD	Analytical determination	Mean \pm SD
PV (meq/kg)	ND	FA composition (%)	
K_{232}	0.91 \pm 0.01	Palmitic acid, C _{16:0}	9.02 \pm 0.01
K_{270}	0.017 \pm 0.003	Stearic acid, C _{18:0}	3.62 \pm 0.01
AnV	0.09 \pm 0.01	Oleic acid, C _{18:1}	80.21 \pm 0.02
Polar compounds (%)		Linoleic acid, C _{18:2}	4.80 \pm 0.01
oxTAG	0.23 \pm 0.02	Linolenic acid, C _{18:3}	0.50 \pm 0.01
DTAG + PTAG	<0.01		

^aND, not detected; K_{232} , absorbance at 232 nm; K_{270} , absorbance at 270 nm; AnV, anisidine value; oxTAG, oxidized TAG; DTAG + PTAG, dimers and polymers of TAG.

very similar way. The formation rate of conjugated dienes, measured as K_{232} , and of oxTAG, measured by HPLC, showed behavior very similar to the PV. Moreover, at this temperature part of the FA hydroperoxides readily decomposed, producing aldehydes as indicated by the AnV kinetics (17), which apparently lacked an induction period (IP), as observed for primary oxidation products. Nevertheless, for K_{270} and the sum of oxidized DTAG + PTAG, the approximate mean IP was 5 d.

During the accelerated oxidation experiments at 50°C (Fig. 2), the behavior was similar to that observed at 60°C. However, at this lower temperature, the rate of formation of secondary oxidation products was lower. A comparison of Figures 1 and 2 shows that at similar levels of primary oxidation products, e.g., PV = 150 meq/kg, K_{232} = 7.3, and oxTAG = 6.70%, the content of secondary oxidation products was lower, K_{270} = 0.226 and AnV = 18.7 at 50°C as compared with K_{270} = 0.305 and AnV = 21.1 at 60°C. At 50°C the observed IP was 2 d for

the primary oxidation product indexes and about 1 wk for the secondary oxidation product indexes.

As expected, the difference in the rate of formation of the primary and secondary oxidation products was higher at 40 than at 50°C (Fig. 3), and the corresponding IP were about 6 and 12 d, respectively. The observed behavior at the three assayed storage temperatures showed that, as expected, the activation energy was higher on decomposition than on formation of FA hydroperoxides (3).

Although DTAG and PTAG were formed in oils heated at elevated temperatures, e.g., during frying, they were also found at low temperatures (Figs. 1–3), in agreement with reports by other authors (18,19).

There was a high correlation among the three indexes of the primary oxidation products determined (PV, K_{232} , and oxTAG) at the assayed temperatures (Table 2). Therefore, the determination of one of these indexes should be adequate to measure

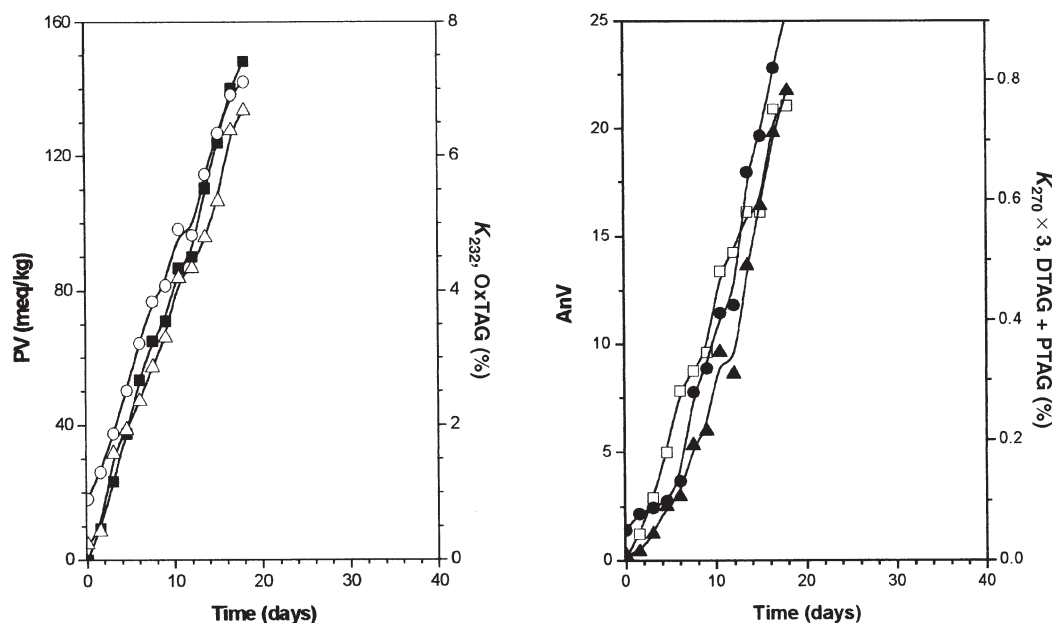


FIG. 1. Evolution of purified olive oil (POO) oxidation at 60°C. ■, PV; ○, K_{232} ; △, oxTAG; □, AnV; ●, K_{270} ; ▲, DTAG + PTAG. K_{232} , absorbance at 232 nm; oxTAG, oxidized TAG; AnV, anisidine value; K_{270} , absorbance at 270 nm; DTAG + PTAG, dimers and polymers of TAG. Note: In Figures 1–3 K_{270} values are multiplied by 3 to allow both analytical parameters to appear in a clear form in the same axis.

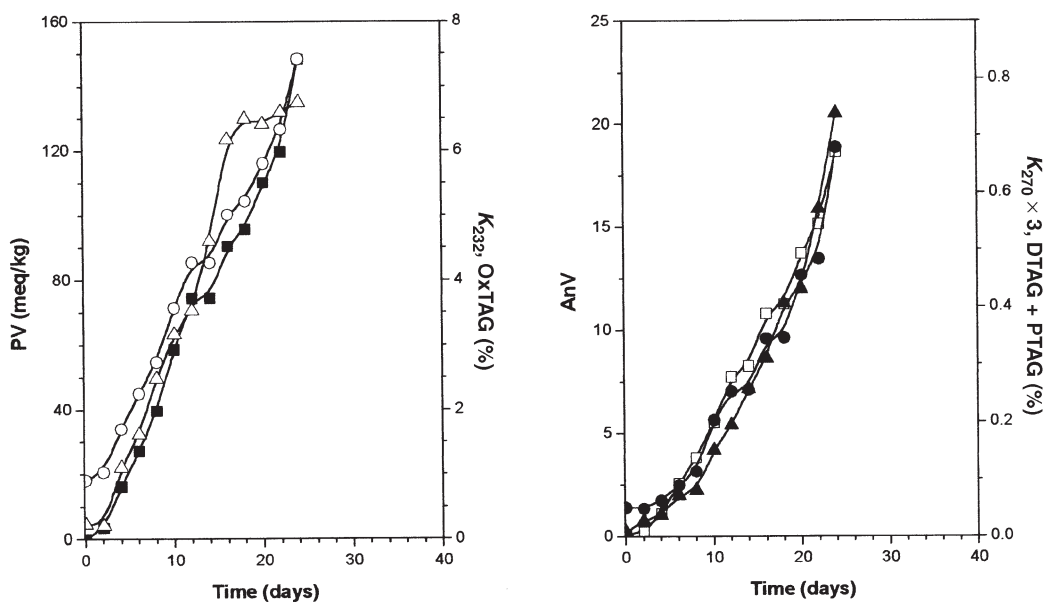


FIG. 2. Evolution of POO oxidation at 50°C. ■, PV; ○, K_{232} ; △, oxTAG; □, AnV; ●, K_{270} ; ▲, DTAG + PTAG. For abbreviations, see Figure 1.

the extent of the formation of hydroperoxides. Moreover, the correlation coefficients found were higher than those reported by Crapiste *et al.* (19) for sunflower oil, in particular, the coefficient between PV and oxTAG by HPLC, which in this study was higher than 0.99 for all the temperatures assayed.

On the other hand, the behavior of the secondary oxidation products index AnV was different from what was expected and what has been reported for highly polyunsaturated oils, such as sunflower and rapeseed oils (19,20). In fact, at higher tempera-

tures the correlation coefficients and IP shown by the AnV index were closer to those for the formation of primary than of secondary oxidation products (Table 2; Figs. 1–3). The trend in evolution of K_{270} and DTAG + PTAG was similar, although at lower temperature the correlation between them was lower.

Trend of unsaturated FA during oxidation. The decrease in GC-determined total unsaturated FA (UFA) in the course of oxidation correlated by more than 0.98 with both PV and K_{270} at all the temperatures assayed (data not shown).

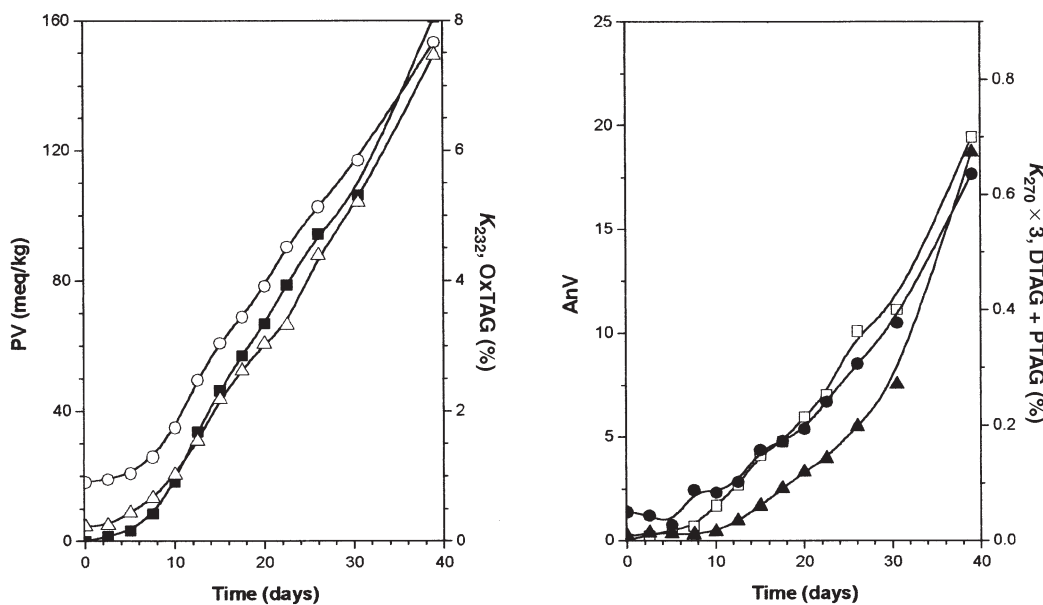


FIG. 3. Evolution of POO oxidation at 40°C. ■, PV; ○, K_{232} ; △, oxTAG; □, AnV; ●, K_{270} ; ▲, DTAG + PTAG. For abbreviations, see Figure 1.

TABLE 2
Correlation Coefficients Between Oxidation Indexes^a

	60°C					50°C					40°C				
	PV	K ₂₃₂	oxTAG	AnV	K ₂₇₀	PV	K ₂₃₂	oxTAG	AnV	K ₂₇₀	PV	K ₂₃₂	oxTAG	AnV	K ₂₇₀
K ₂₃₂	0.999					1.000					0.998				
oxTAG	0.995	0.994				0.990	0.968				0.997	0.995			
AnV	0.994	0.993	0.995			0.995	0.993	0.959			0.988	0.978	0.992		
K ₂₇₀	0.972	0.965	0.965	0.963		0.979	0.974	0.918	0.990		0.976	0.965	0.984	0.994	
DTAG + PTAG	0.972	0.964	0.968	0.963	0.996	0.965	0.961	0.906	0.982	0.990	0.919	0.896	0.930	0.965	0.975

^aFor abbreviations, see Table 1.

TABLE 3
Unsaturated FA (UFA) Decrease^a (%) at PV of 150 meq/kg with Respect to the Initial Percentage in POO

	C _{18:1}	C _{18:2}	C _{18:3}	UFA	PUFA
40°C (39 d)	1.44 ^a (1.16)	18.2 ^{a,b} (0.87)	32.3 ^a (0.16)	2.56 ^a (2.19)	19.5 ^{a,b} (1.03)
50°C (24 d)	1.50 ^a (1.20)	18.0 ^a (0.87)	32.2 ^a (0.16)	2.61 ^a (2.23)	19.4 ^a (1.03)
60°C (18 d)	1.67 ^b (1.34)	18.9 ^b (0.91)	33.7 ^b (0.17)	2.83 ^b (2.42)	20.3 ^b (1.08)

^aExpressed as the percentage decrease of the FA with respect to its initial content or to the initial total FA content (in parentheses). Mean values with different superscript letters are statistically different ($P \leq 0.05$). For abbreviation, see Table 1.

Oleic acid decreased at all the temperatures assayed (Table 3). This decrease was greater than the decrease in linoleic and linolenic acids in absolute terms because the oleic acid content of the POO was much higher. This observation contradicts those of other authors (21–23), who have suggested that at low temperatures oleate behaves as an inert diluent for linoleate. The quantity of each FA degraded for a given PV, e.g., 150 meq/kg reached in 39, 24, and 18 d at 40, 50, and 60°C, respectively, for the data reported in Table 3, was similar at the different temperatures studied, although there were some statistically significant differences. In fact, at 60°C, significantly more UFA were oxidized to reach the same PV, since more secondary oxidation products were naturally formed at higher temperatures (Figs. 1–3).

The observed IP decreased from 12 to 7 d at 40 and 50°C, respectively, for oleic acid and from 6 to 2 d at 40 and 50°C, respectively, for PUFA; at 60°C the behavior of oleic, linoleic, and linolenic acids was more alike, although minor differences were observed. A possible explanation for the difference in observed

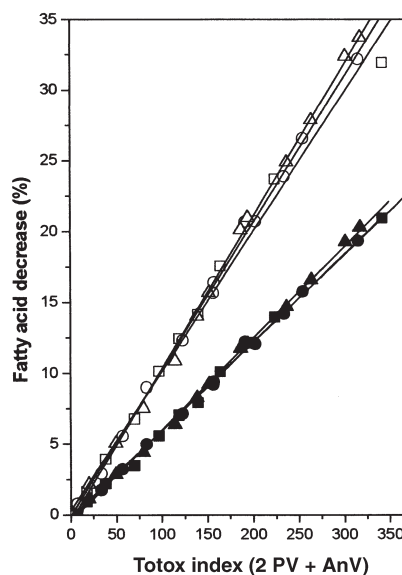


FIG. 4. Decrease in linolenic acid (C_{18:3}) and PUFA content vs. the Totox index in the course of POO oxidation at 40, 50, and 60°C. C_{18:3}: □, 40, ○, 50, and △, 60°C. PUFA: ■, 40, ●, 50, ▲, 60°C. AnV, anisidine value; for other abbreviations see Figure 1.

TABLE 4
Content^a of Volatile Compounds in POO at PV of 150 meq/kg

Compound	Fresh POO	40°C (39 d)	50°C (24 d)	60°C (18 d)
Hexanal	19.9 ± 3.0	341.5 ± 57.2	644.9 ± 121.2	817.7 ± 37.4
<i>t</i> -2-Hexenal	0.5 ± 0.1	11.3 ± 1.8	19.8 ± 1.2	50.4 ± 1.8
Heptanal	0.5 ± 0.1	9.66 ± 2.48	23.8 ± 5.0	38.0 ± 1.2
<i>t</i> -2-Heptenal	1.0 ± 0.1	99.36 ± 11.36	147.0 ± 10.3	231.7 ± 9.4
Octanal	1.2 ± 0.1	14.41 ± 2.33	21.8 ± 3.2	36.2 ± 2.5
<i>t</i> -2-Octenal	0.6 ± 0.1	53.80 ± 6.08	54.1 ± 3.8	90.7 ± 5.7
Nonanal	0.8 ± 0.3	13.25 ± 2.44	18.2 ± 2.3	32.6 ± 2.4
<i>t</i> -2-Decenal	ND	8.66 ± 0.67	10.1 ± 1.0	23.2 ± 1.8
<i>t,t</i> -2,4-Decadienal	ND	0.78 ± 0.07	1.06 ± 0.16	2.5 ± 0.1
Sum	24.4 ± 3.6	552.8 ± 83.4	940.7 ± 128.4	1323.1 ± 60.2

^aConcentration expressed as arbitrary peak area units. For abbreviations, see Table 1.

IP between oleic acid and PUFA is that the linoleyl radical could be implicated in the initiation of the oleate oxidation, as suggested by Kamal-Eldin *et al.* (23).

It has been suggested that the loss of PUFA in the course of oxidation is not sensitive enough to serve as an index of oxidative degradation of oils (24). Nevertheless, this study clearly shows that the GC determination of residual linolenic acid or PUFA correlates by more than 0.999 with the Totox index at any of the assayed temperatures (Fig. 4). As known, the Totox index (2PV + AnV) is currently the only simple oxidation index that takes into account both primary and secondary oxidation products. Moreover, GC determination of residual FA is not affected by the decomposition or evaporation of part of the products measured as the PV or volatile compounds since it measures the loss of the oxidizing substrate in a very simple and straightforward way.

Volatile compounds. Table 4 reports the content of the main volatile compounds found in the fresh and oxidized POO stored

at different temperatures until PV reached 150 meq/kg. The major volatile compounds found in the oxidized POO were hexanal, *t*-2-heptenal, and *t*-2-octenal, decomposition products of linoleate hydroperoxides (25), whereas 2,4-decadienal, also formed from linoleic acid, was the least abundant.

The content of volatile aldehydes in the oxidized POO at any given level of a primary oxidation products index, e.g., PV of 150 meq/kg as reported in Table 4, was very different at the three assayed temperatures, clearly confirming that the degradation rate of hydroperoxides is strongly affected by the temperature, as already known (26). As expected, the correlation coefficients of the sum of the volatile compounds vs. any of the secondary oxidation product indexes were greater than 0.98 at all the assayed temperatures (data not shown).

Sensory analysis. Table 5 reports the values obtained by interpolation of the data for the oxidation indexes considered, corresponding to the rancid group threshold determined by sensory analysis (75% of correct answers in the recognition thresh-

TABLE 5
Interpolated Values of the Oxidation Indexes at Sensory Rancidity Threshold in POO

	40°C (14.1 d)	50°C (10.2 d)	60°C (8.3 d)
PV (meq/kg)	41.86 ± 0.70 ^a	60.60 ± 0.78 ^b	68.06 ± 0.46 ^c
<i>K</i> ₂₃₂	2.84 ± 0.02 ^a	3.66 ± 0.03 ^b	3.96 ± 0.03 ^c
<i>K</i> ₂₇₀	0.046 ± 0.003 ^a	0.070 ± 0.003 ^b	0.100 ± 0.002 ^c
AnV	3.64 ± 0.06 ^a	5.79 ± 0.07 ^b	9.17 ± 0.04 ^c
oxTAG (%)	1.95 ± 0.12 ^a	3.21 ± 0.03 ^b	3.08 ± 0.09 ^b
DTAG + PTAG (%)	0.05 ± 0.00 ^a	0.16 ± 0.02 ^b	0.20 ± 0.02 ^c
C _{18:1} (%) ^a	0.27 ± 0.09 ^a	0.38 ± 0.03 ^a	0.74 ± 0.02 ^b
C _{18:2} (%) ^a	4.39 ± 0.45 ^a	6.83 ± 0.02 ^b	8.09 ± 0.03 ^c
C _{18:3} (%) ^a	8.92 ± 0.55 ^a	12.70 ± 0.11 ^b	14.84 ± 0.11 ^c
Hexanal ^b	34.64 ± 8.21 ^a	162.65 ± 47.66 ^b	211.61 ± 8.36 ^c
<i>t</i> -2-Hexenal ^b	4.04 ± 0.44 ^a	12.03 ± 0.66 ^b	19.21 ± 1.02 ^c
Heptanal ^b	2.89 ± 0.29 ^a	10.15 ± 1.82 ^b	11.32 ± 1.06 ^b
<i>t</i> -2-Heptenal ^b	34.75 ± 3.56 ^a	104.22 ± 6.54 ^b	94.60 ± 1.34 ^c
Octanal ^b	2.64 ± 0.30 ^a	9.50 ± 2.21 ^b	10.00 ± 1.12 ^b
<i>t</i> -2-Octenal ^b	2.11 ± 0.26 ^a	8.46 ± 0.40 ^b	9.97 ± 0.73 ^c
Nonanal ^b	3.40 ± 0.66 ^a	7.81 ± 1.84 ^b	8.58 ± 0.37 ^b
<i>t</i> -2-Decenal ^b	2.53 ± 0.22 ^a	2.49 ± 0.18 ^a	5.17 ± 0.24 ^b
<i>t,t</i> -2,4-Decadienal ^b	0.25 ± 0.04 ^b	0.20 ± 0.02 ^a	0.58 ± 0.02 ^c
Total volatiles ^b	90.46 ± 3.67 ^a	314.49 ± 62.28 ^b	373.51 ± 9.12 ^c

^aDecrease with respect to the initial content.

^bConcentration expressed as arbitrary peak area units. Mean values with different superscript letters are statistically different ($P \leq 0.05$). For abbreviations see Table 1.

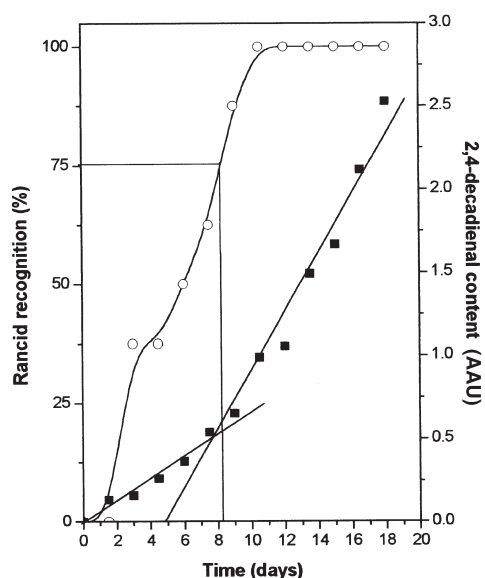


FIG. 5. Changes in 2,4-decadienal content and rancid recognition threshold in the course of the oxidation process at 60°C. ○, Correct answers of rancid recognition; ■, *t,t*-2,4-decadienal content. AAU, arbitrary area unit.

old test) in the course of the oxidation process in POO at the different experimental temperatures. According to the indexes, there were statistically significant differences in values at each of the three temperatures, and the rancid recognition threshold in POO corresponded to lower oxidation indexes values at lower temperature. Moreover, identification of the rancid defect by the sensory panel did not correspond to the total or individual content of any volatile compound in the oxidized oil, and may be due to a relationship between different substances.

Further analysis of the relationship between the rancid threshold and the content of SPME-GC volatile compounds suggested that 2,4-decadienal was a very relevant compound in identifying the relationship between these two methods, as suggested by other authors in reference to seed oils (27). In fact, Figure 5 shows that the rancidity threshold apparently coincided with the IP for the kinetics of 2,4-decadienal formation. Figure 5 shows this behavior at 60°C, but the same effect was observed at 40 and 50°C, so that measurement of the IP of 2,4-decadienal could be a key point as regards the relationship between sensory perception of oxidized olive oil and analytical chemistry methods.

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REFERENCES

1. Dobarganes, M.C., and J. Velasco, Analysis of Lipid Hydroperoxides, *Eur. J. Lipid Sci. Technol.* 104:420–428 (2002).
2. Kaya, A., A.R. Tekin, and M.D. Öner, Oxidative Stability of Sunflower and Olive Oils: Comparison Between a Modified

- Active Oxygen Method and Long-Term Storage, *Lebensm. Wiss. Technol.* 26:464–468 (1993).
3. Frankel, E.N., *Lipid Oxidation*, The Oily Press, Dundee, Scotland, 1998.
4. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends Food Sci. Technol.* 4:220–225 (1993).
5. Warner, K., E.N. Frankel, and T.L. Mounts, Flavor and Oxidative Stability of Soybean, Sunflower, and Low Erucic Acid Rapeseed Oils, *J. Am. Oil Chem. Soc.* 66:558–564 (1989).
6. Coppin, E.A., and O.A. Pike, Oil Stability Index Correlated with Sensory Determination of Oxidative Stability in Light-Exposed Soybean Oil, *Ibid.* 78:13–18 (2001).
7. Warner, K., and T. Nelsen, AOCS Collaborative Study in Sensory and Volatile Compound Analyses of Vegetable Oils, *Ibid.* 73:157–166 (1996).
8. Andersson, K., and H. Lingnert, Influence of Oxygen and Copper Concentration on Lipid Oxidation in Rapeseed Oil, *Ibid.* 75:1041–1046 (1998).
9. Aparicio, R., M.T. Morales, and M.V. Alonso, Relationship Between Volatile Compounds and Sensory Attributes of Olive Oil by the Sensory Wheel, *Ibid.* 73:1253–1264 (1996).
10. Morales, M.T., J.J. Rios, and R. Aparicio, Changes in the Volatile Composition of Virgin Olive Oil During Oxidation: Flavors and Off-Flavors, *J. Agric. Food Chem.* 45:2666–2673 (1997).
11. Yanishlieva, N.V., and E.M. Marinova, Effects of Antioxidants on the Stability of Triacylglycerols and Methyl Esters of Fatty Acids of Sunflower Oil, *Food Chem.* 54:377–382 (1995).
12. *Official Methods and Recommended Practices of the AOCS*, 5th edn., AOCS Press, Champaign, 1998.
13. Márquez-Ruiz, G., N. Jorge, M. Martín-Polvillo, and M.C. Dobarganes, 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* 749:55–60 (1996).
14. Dobarganes, M.C., and M.C. Pérez-Camino, Fatty Acid Composition: A Useful Tool for the Determination of Alteration Level in Heated Fats, *Rev. Franç. Corps Gras* 75:67–70 (1988).
15. Jelen, H.H., M. Obuchowska, R. Zawirska-Wojtasiak, and E. Waśowicz, Headspace Solid-Phase Microextraction Use for the Characterization of Volatile Compounds in Vegetable Oils of Different Sensory Quality, *J. Agric. Food Chem.* 48:2360–2367 (2000).
16. UNE 87-003, in *Análisis Sensorial*, AENOR, Madrid, Spain, 1997, pp. 189–196.
17. White, P.J., Conjugated Diene, Anisidine Value, and Carbonyl Value Analyses, in *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, edited by K. Warner and N.A.M. Eskin, AOCS Press, Champaign, 1995, pp. 159–178.
18. Dobarganes, M.C., M.C. Pérez-Camino, and G. Márquez-Ruiz, High-Performance Size Exclusion Chromatography of Polar Compounds in Heated and Non-heated Fats, *Fat Sci. Technol.* 90:308–311 (1988).
19. Crapiste, G.H., M.I.V. Bredvan, and A.A. Carelli, Oxidation of Sunflower Oil During Storage, *J. Am. Oil Chem. Soc.* 76:1437–1443 (1999).
20. Lampi, A.M., V. Piironen, A. Hopia, and P. Koivistoinen, Characterization of the Oxidation of Rapeseed and Butter Oil Triacylglycerols by Four Analytical Methods, *Lebensm. Wiss. Technol.* 30:807–813 (1997).
21. Romero, A.J., and I. Morton, A Kinetic Study of the Competitive Oxidation of Oleic Acid–Linoleic Acid Mixtures, *J. Sci. Food Agric.* 26:1353–1356 (1975).
22. Romero, A.J., and I. Morton, Competitive Oxidation of Fatty Acid. Effects of the Degree of Unsaturation, *Ibid.* 28:916–920 (1977).

23. Kamal-Eldin, A., J. Velasco, and C. Dobarganes, Oxidation of Triolein and Trilinolein at Elevated Temperatures, *Eur. J. Lipid Sci. Technol.* 105:165–170 (2003).
24. Gordon, M.H., Measuring Antioxidant Activity, in *Antioxidants in Food: Practical Applications*, edited by J. Pokorny, N. Yanishlieva, and M. Gordon, Woodhead Publishing, Cambridge, 2001, pp. 71–84.
25. Frankel, E.N., Chemistry of Autoxidation: Mechanism, Products and Flavor Significance, in *Flavor Chemistry of Fats and Oils*, edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, 1985, pp. 1–37.
26. Nawar, W.W., Chemistry of Thermal Oxidation of Lipids, *Ibid.*, pp. 39–60.
27. Przybylski, R., and N.A.M. Eskin, Methods to Measure Volatile Compounds and the Flavor Significance of Volatile Compounds, in *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, edited by K. Warner and N.A.M. Eskin, AOCS Press, Champaign, 1995, pp. 107–133.

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