Effect of Temperature and Addition of α-Tocopherol on the Oxidation of Trilinolein Model Systems

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ABSTRACT: The effects of temperature and addition of α-tocopherol were evaluated in trilinolein model systems through quantification of oxidized TAG monomers, dimers, and polymers following oxidation at different temperatures. Samples of trilinolein without and with 250 and 500 mg/kg α-tocopherol added were stored at 25, 60, and 100°C. Quantification of oxidized monomers, dimers, and polymers by a combination of adsorption and exclusion chromatography provided a useful measurement for studying the evolution of oxidation. Results showed that the amounts of primary oxidation compounds (trilinolein oxidized monomers) that accumulated during the induction period decreased as the temperature increased, indicating that the slope of the initial linear stage of oxidation depended on temperature. The end of the induction period was marked by a sharp increase in the levels of total oxidation compounds, the initiation of polymerization, and the loss of α-tocopherol. Addition of α-tocopherol did not prevent, but rather delayed, formation of trilinolein oxidized monomers and the initiation of polymerization.

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The complexity of the reactions involved in lipid oxidation and the wide range of compounds produced cause great difficulties in evaluating alterations in oxidation and justify the need for new analytical procedures with general application. The methods currently in use have been reviewed by several authors, who have generally concluded that, in spite of the multitude of assays available, no universal method allows the extent of oxidation to be evaluated throughout the entire process (1–7). Therefore, the need arises for improving methodologies to reevaluate aspects of particular concern, such as the effectiveness of antioxidants and, in general, the influence of different variables that modify the rate of oxidative reactions.

Previously, we developed and applied a methodology based on a combination of adsorption and size-exclusion chromatographies that enables quantification of oxidized and polymeric compounds, as well as hydrolytic products, i.e. DAG and FA (8,9). Application of this procedure has proved to be of great utility for quantification of oxidation compounds in fats and fatty foods at low temperatures (10–15). This analytical approach offers the great advantage of providing a good measurement for early and advanced stages of oxidation by concomitantly evaluating primary and secondary oxidation products. A modification of this methodology was introduced in which small quantities of samples and solvents were used together with an internal standard for purposes of quantification (16). The high reproducibility achieved for samples with low alteration levels added further possibilities for applying the methodology to initial stages of oxidation.

In a previous publication (17), we reported the value of this methodology for oxidation studies. Early stages of oxidation were characterized by a significant increase in monomeric oxidation compounds, whereas the presence of polymerization compounds in significant amounts indicated the end of the induction period (IP). The objective of the present study was to obtain complete quantitative data on the main groups of compounds formed during oxidation of trilinolein (LLL), used as model system, in order to gain insight into the effects of temperature and added α-tocopherol on oxidation kinetics. Unfortunately, in most of the oxidation studies published, the effects of tocopherol have been evaluated at a fixed level of oxidation, sometimes at very low oxidation points, e.g., at PV of 10–20 meq/kg; in fact, the degree of oxidation should be determined at appropriate time intervals, making one data point insufficient (18). Additionally, it is generally recommended that the formation of both primary and secondary products be measured together with the stability of tocopherols (7).

LLL has been used in this work as a model unsaturated TAG to avoid interference from potential prooxidant or antioxidant effects of minor compounds that could otherwise be present in oils. Linoleyl was selected as the FA constituent of the model system because this fatty acyl group is the most susceptible to oxidation in most natural fats and oils. α-Tocopherol was selected because it is the most important natural antioxidant in fats and oils. The concentrations of α-tocopherol used were 250 and 500 mg/kg, which are within the range naturally occurring in commercial seed oils (19). Experiments were undertaken at 25°C, and accelerated oxidative assays were carried out at 60 and 100°C, the latter two being temperatures commonly used in standard accelerated tests directed toward estimating the shelf life of oils (20,21).

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Abbreviations: DIM, trilinolein dimers; HPSEC, high-performance size-exclusion chromatography; IP, induction period; LLL, trilinolein; LLL-250, trilinolein with 250 mg/kg α-tocopherol added; LLL-500, trilinolein with 500 mg/kg α-tocopherol added; oxMON, oxidized monomers; POL, trilinolein polymers.

EXPERIMENTAL PROCEDURES

Samples and treatments. LLL was purchased from Nu-Chek-Prep (Elysian, MN), and α-tocopherol was obtained from Aldrich Chemical Co. (Milwaukee, WI). A solution of α-tocopherol (10 mg/mL) was prepared by weighing 50 mg of α-tocopherol into a 5-mL volumetric flask using diethyl ether stabilized with ethanol (Romil, Cambridge, United Kingdom). Samples of LLL with 500 (LLL-500) and 250 mg/kg (LLL-250) α-tocopherol added were prepared by adding 1 and 0.5 mL of α-tocopherol solution, respectively, to 20 g LLL. Samples were homogenized using a magnetic stirrer, purged of solvents at 30°C with a stream of nitrogen, and maintained at –40°C until the experiments were conducted. Samples of LLL, LLL-250, and LLL-500 were placed in open beakers (surface-to-volume ratio of 10 cm^{-1}) to ensure their accessibility to air and then either heated in an oven at 60 or 100°C, or stored at 25°C in the dark for different periods of time. Experiments were repeated at each temperature tested.

Analytical determinations. (i) Separation of polar fractions by adsorption chromatography. LLL samples were fractionated using silica cartridges for solid-phase extraction (Sep-Pak columns supplied by Waters Associates, Milford, MA). The methodology is described in detail (including precision, accuracy, and recovery data) in a previous publication (16). Briefly, 2 mL of the sample solution in *n*-hexane, containing 50 mg of sample and 1 mg of monostearin used as internal standard (Nu-Chek-Prep), was placed on the column and the solvent was passed through while the sample was retained on the column. Next, the nonpolar fraction was eluted with 15 mL of petroleum ether/diethyl ether (90:10). A second fraction containing polar compounds and the internal standard was eluted with 15 mL of diethyl ether. Nonpolar and polar fractions were evaporated under reduced pressure and redissolved in 1 mL of THF for further analyses by TLC (to check the efficiency of the separation) and high-performance size-exclusion chromatography (HPSEC).

(ii) HPSEC. Fractions of polar compounds from the LLL samples, obtained as outlined above, were analyzed by HPSEC using a Rheodyne 7725y injector with a 10-μL sample loop pump (Waters Associates), an HP 1037 A refractive index detector (Hewlett-Packard, Avondale, PA), and an HP 3392 A integrator. The separation was performed on two 100- and 500-Å Ultrastyragel columns (25 × 0.77 cm i.d.; Hewlett-Packard) packed with a porous, highly cross-linked styrene-divinylbenzene copolymer (film thickness < 10 μm) connected in series, with THF (1 mL/min) as the mobile phase (16). The groups of oxidized compounds separated were LLL oxidized monomers (oxMON), LLL dimers (DIM), and LLL polymers (POL).

(iii) α-Tocopherol content. α-Tocopherol levels were quantified by HPLC with fluorescence detection (22).

Statistical analysis. SigmaStat and SigmaPlot software packages (SPSS Science, Chicago, IL) were used for the kinetic study and plots.

RESULTS AND DISCUSSION

Figure 1 presents a representative HPSEC chromatogram of the polar fraction of an LLL sample stored at 60°C showing the groups of compounds quantified through the analytical procedure used. At that point of oxidation, the LLL sample showed a significant increase in oxMON (17.7% on total sample) and DIM (1.4% on total sample), and even POL could be detected (retention time: 11.4 min). The oxMON peak comprises a large number of monomeric LLL molecules containing one or more oxidized fatty acyl groups, either peroxide groups or other oxygenated functions such as epoxy, keto, or hydroxy groups. Quantification of this group of compounds can therefore be of great utility not only to detect the oxidation products initially formed, even before rancidity, but also to follow oxidation during further stages. Polymerization compounds, here separated into dimeric LLL molecules (DIM) and higher oligomeric LLL molecules (POL), are characteristic of advanced oxidation. The number of possibilities of different structures for the compounds formed during oxidation is enormous, even starting from LLL as a model compound, and this number increases exponentially with higher M.W. Therefore, great difficulties are still encountered in elucidating and quantifying the structures of such compounds, especially of DIM and POL (23). In this context, the main advantage provided by the methodology used in this study is that three groups of compounds, which include initial and decomposition oxidation products, can be quantified concomitantly. Thus, it is possible to determine the degree of oxidation at any time during the course of oxidation (17).

Tables 1 to 3 show the evolution of oxidation in LLL samples without and with added α-tocopherol at 25, 60, and

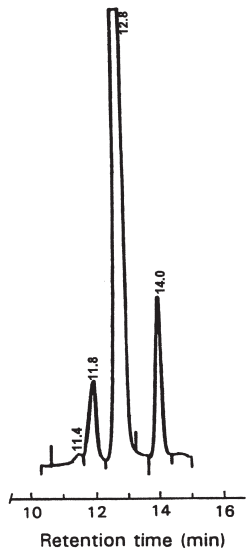

FIG. 1. High-performance size-exclusion chromatogram of a representative polar fraction isolated from trilinolein (LLL) oxidized at 60°C. Retention times (min): 11.4, LLL polymers; 11.8, LLL dimers; 12.8, LLL oxidized monomers; and 14.0, monostearin (internal standard).

TABLE 1
Evolution of Oxidation in Trilinolein (LLL) Samples at 25°C

Model system	Days	Oxidation compounds (%)				Remaining α-tocopherol (%)
		Total	oxMON	DIM	POL	
LLL	1	1.1	1.1	ND	ND	—
	2	2.2	2.2	ND	ND	—
	3	5.7	5.6	0.1	ND	—
	4	10.8	10.5	0.3	ND	—
	5	18.8	18.2	0.6	ND	—
	6	27.9	26.6	1.1	0.2	—
	8	39.7	34.9	3.8	1.0	—
	10	46.9	40.1	5.2	1.6	—
	13	65.4	47.4	11.1	6.9	—
	17	84.2	55.9	15.7	12.6	—
LLL-250	6	1.5	1.5	ND	ND	92.5
	12	2.9	2.8	0.1	ND	92.2
	17	4.0	3.6	0.4	ND	73.5
	23	8.8	8.6	0.2	ND	60.8
	26	8.9	8.7	0.2	ND	57.1
	27	9.2	9.1	0.1	ND	51.5
	29	9.7	9.6	0.1	ND	38.1
	33	9.0	8.8	0.2	ND	31.3
	41	10.9	10.5	0.4	ND	7.5
	44	12.0	11.6	0.4	0.1	ND
	47	16.3	15.4	0.7	0.2	—
	51	61.3	41.9	8.1	2.3	—
LLL-500	10	2.1	2.0	0.1	ND	92.1
	17	3.7	3.6	0.1	ND	52.7
	23	4.1	4.0	0.1	ND	48.4
	26	5.8	5.7	0.1	ND	38.1
	29	6.5	6.4	0.1	ND	31.3
	33	7.8	7.6	0.2	ND	28.5
	41	8.8	8.7	0.1	ND	24.0
	47	10.6	10.3	0.3	ND	4.5
	51	11.9	11.7	0.2	0.1	ND
	54	14.3	13.2	0.8	0.3	—
	58	43.4	34.5	7.2	1.7	—
	60	69.1	43.8	16.4	8.9	—

[a]LLL-250, trilinolein with 250 mg/kg α-tocopherol added; LLL-500, trilinolein with 500 mg/kg α-tocopherol added; oxMON; oxidized monomers; DIM, dimers; POL, polymers; ND, not detected.

100°C, respectively. For each sampling point, values for the total oxidation compounds and their distribution in oxMON, DIM, and POL are included, and levels of α-tocopherol are expressed as the percentage of remaining α-tocopherol. Oxidation compounds were not detected in samples at the start of the experiments. Results of duplicate experiments showed good reproducibility for the oxidation progress and distribution of specific groups of compounds under the conditions used (CV < 8% for total oxidation compounds).

To illustrate the general LLL oxidation profiles, total oxidation compounds are represented for all samples in Figure 2. Regardless of the temperature used, there was a rapid increase in total oxidation products in LLL samples without tocopherol from the beginning, whereas the presence of the antioxidant led to a considerable delay in the formation of oxidation compounds and allowed two stages to be clearly distinguished. The first period was characterized by the slow progression of oxidation and the second was characterized by an accelerated oxidation. The end of the induction period could therefore be defined in samples with added antioxidants as the time when a notable shift in oxidation rate was observed. For example, at 25°C the end of the induction period occurred between 47 and 51 d in LLL-250, corresponding to an increase in oxidation compounds from 16.3 to 61.3%. In LLL-500 it occurred between 54 and 58 d, corresponding to an increase in oxidation compounds from 14.3 to 43.4%. At 60°C, the end of the induction period for LLL-250 and LLL-500 was observed between 95 and 102 h, and between 111 and 114 h, respectively, at changes from 5.4 to 23.5% and from 8.1 to 17.7% oxidation compounds. At 100°C, the end of the induction period occurred between 8 and 10 h and between 10 and 12 h, respectively, in LLL-250 and LLL-500, corresponding to increases in oxidation compounds from 4.8 to 12.5% and from 5.2 to 8.8%. Also, regardless of temperature, the end of the induction period was indicated by the total loss of α-tocopherol in all samples (Tables 1 to 3). In other words, once the antioxidants were exhausted, the course of oxidation entered a second accelerated phase.

TABLE 2
Evolution of Oxidation in LLL Samples at 60°C

Model system	Hours	Oxidation compounds (%)				Remaining α-tocopherol (%)
		Total	oxMON	DIM	POL	
LLL	3	3.0	3.0	ND		—
	6	4.4	4.3	0.1	ND	—
	9	10.9	10.4	0.5	ND	—
	11	16.4	15.4	0.9	0.1	—
	13	19.4	17.7	1.5	0.2	—
	15	29.0	26.1	2.5	0.4	—
	17	34.0	29.7	3.6	0.7	—
	19	40.7	34.6	4.9	1.2	—
	21	46.0	37.5	6.4	2.1	—
	23	56.5	44.3	8.8	3.4	—
LLL-250	24	1.3	1.3	ND	ND	76.1
	46	2.1	2.1	ND	ND	60.6
	54	2.8	2.8	ND	ND	53.1
	71	3.4	3.4	ND	ND	38.9
	79	3.8	3.7	0.1	ND	24.3
	95	5.4	5.2	0.2	ND	ND
	102	23.5	20.9	2.2	0.4	—
	105	40.7	34.6	4.9	1.2	—
	108	56.1	44.1	8.4	3.6	—
	111	65.3	46.2	11.9	7.2	—
LLL-500	24	1.5	1.5	ND	ND	81.0
	46	2.8	2.8	ND	ND	68.6
	54	3.3	3.3	ND	ND	63.8
	71	4.4	4.4	ND	ND	53.8
	79	4.8	4.8	ND	ND	43.0
	95	5.4	5.3	0.1	ND	27.2
	102	6.1	5.9	0.2	ND	17.4
	108	7.2	6.9	0.3	ND	5.0
	111	8.1	7.7	0.4	ND	ND
	114	17.7	16.5	1.1	0.1	—

[a]For abbreviations see Table 1.

It is also interesting to note the profile of oxidation at 100°C. As can be observed, the increase in oxidation compounds in the accelerated phase was slower than that at 25 or 60°C in spite of the more rapid oxidation at 100°C. An explanation for these different profiles could be that the quantity of air was limited during the oxidation process at 100°C. Oxygen solubility decreases when the temperature increases, and, even at the high surface-to-volume ratio used in this study, it is possible that the availability of oxygen was reduced at 100°C. In fact, when oxygen was bubbled at 100°C in the Rancimat apparatus under the conditions specified for measuring the oil stability index (24), no induction period was found for LLL. The sample was more rapidly oxidized and hence dependent on the availability of oxygen. In contrast, IP obtained with the Rancimat apparatus for LLL-250 and LLL-500 were similar to those shown in Figure 2C for LLL-250 and LLL-500 (8.9 and 10.3 h, respectively). Furthermore, the lines obtained with the Rancimat apparatus for the accelerated stage of oxidation rose more sharply with time than those in Figure 2C, suggesting that oxygen had been limited in the latter case.

On the other hand, the main effect of the increase in temperature was, as expected, a decrease in the induction period. An additional important observation was the influence of temperature on the amount of oxidation compounds that accumulated at the end of the IP, which decreased as the temperature increased. This was probably related to the effect of temperature on antioxidant degradation.

From the results obtained at each temperature, it is clear that oxidation still proceeded, although it was delayed, in the presence of α-tocopherol. Especially at 25°C, considerable amounts of oxidation compounds were compatible with substantial levels of α-tocopherol remaining. For example, at 27 d, LLL with 250 mg/kg α-tocopherol added (Table 1) contained 9.2% total oxidation compounds, and approximately 50% of α-tocopherol was still present. We also concluded that the increase in antioxidant levels from 250 to 500 mg/kg did not substantially modify the stability against oxidation of LLL. This is not strange given the results obtained in numerous studies on the effect of antioxidant concentration, some of which have even indicated the prooxidant action of α-tocopherol at high concentrations (25–27).

Specific quantification of oxMON, DIM, and POL provided complementary information of great utility for elucidating oxidation kinetics (Tables 1 to 3). In general, the only group of compounds that increased during the early oxidation stage,

TABLE 3
Evolution of Oxidation in LLL Samples at 100°C

Model system	Hours	Oxidation compounds (%)				Remaining α-tocopherol (%)
		Total	oxMON	DIM	POL	
LLL	1.5	3.4	3.1	0.3	ND	—
	3.0	6.8	6.3	0.5	ND	—
	4.5	10.9	10.4	1.1	ND	—
	6.0	13.2	11.5	1.7	ND	—
	7.0	17.1	14.4	2.5	0.2	—
	8.0	22.8	18.6	3.8	0.4	—
	10.0	27.9	21.1	6.0	0.8	—
	12.0	33.0	23.5	8.0	1.5	—
	14.0	38.3	24.8	9.9	3.6	—
	16.0	46.1	27.2	12.8	6.1	—
	18.0	56.4	32.4	14.4	9.6	—
	20.0	76.5	40.3	19.5	16.7	—
	22.0	93.4	47.0	22.4	24.0	—
LLL-250	3.0	1.9	1.8	0.1	ND	65.7
	6.0	3.4	3.0	0.4	ND	39.2
	7.0	4.0	3.6	0.4	ND	22.5
	8.0	4.8	4.3	0.5	ND	8.8
	10.0	12.5	10.6	1.6	0.3	ND
	12.0	18.4	15.5	2.6	0.3	—
	14.0	24.4	19.2	4.6	0.6	—
	16.0	33.0	23.8	7.4	1.8	—
LLL-500	3.0	2.2	2.0	0.2	ND	82.5
	6.0	3.5	3.2	0.3	ND	75.7
	7.0	4.1	3.7	0.3	ND	62.4
	8.0	4.3	3.9	0.4	ND	45.6
	10.0	5.2	4.5	0.7	ND	19.9
	12.0	8.8	7.4	1.4	ND	3.1
	14.0	13.4	12.7	2.5	0.2	ND
	16.0	20.0	16.0	3.6	0.4	—

[a]For abbreviations see Table 1.

independent of temperature or the presence of antioxidants, was the group of oxMON, mainly composed of hydroperoxides during early oxidation (28). As can be observed in the tables, concentrations of oxMON were practically identical to those of total oxidation compounds before oxidation accelerated, independent of temperature and the amount of antioxidant. At the end of the induction period, oxidation was accelerated, as shown by the sharp increase in oxMON and the development of polymerization reactions, denoted by a significant rise in dimers. In general, increases of about 1% in dimer concentrations indicated the start of the accelerated phase at all temperatures tested.

However, the oxMON concentration at the end of the IP depended on temperature and was much lower as the temperature increased. For example, in LLL-500 samples, the end of the IP occurred when samples contained between 13.2 and 34.5%, 8.5 and 16.5%, and 4.3 and 7.4% oxMON at 25, 60, and 100°C, respectively. These results indicate that polymerization started at very different levels of primary oxidation products, depending on temperature. Such differences were clearly reflected in the ratio oxMON-to-polymerization compounds (DIM + POL) obtained at 25, 60, and 100°C. For example, for similar levels of total oxidation compounds (27.9–29.0%), that ratio was approximately 20:1, 9:1, and 3:1, respectively. On the other hand, overall results showed that, independent of temperature, initiation of accelerated oxidation was clearly marked by a rise in polymerization compounds and a total loss of antioxidants. The general oxidation pattern found for samples without and with antioxidants is illustrated in Figure 3.

Kinetic considerations. Since oxidized TAG monomers are, in practice, the only products formed during the early stages of oxidation, we can write

$$\mathrm{LLL} + \mathrm{O_2} \rightarrow \mathrm{oxMON} \qquad [1]$$

Assuming that oxMON do not participate in other side reactions during this period,

$$d[\mathrm{oxMON}]/dt = k[\mathrm{oxMON}]^n \qquad [2]$$

where k is the rate constant and n is the reaction order. Rearrangement and integration lead to the following equation (29):

$$[\mathrm{oxMON}]^{(1-n)} = [\mathrm{oxMON}]_0^{\ (1-n)} + (1-n)kt \qquad [3]$$

which represents the relationship between [oxMON] and t (time) during the early stages of oxidation, where $[\mathrm{oxMON}]_0$ is the initial concentration of oxMON, that is, 0.

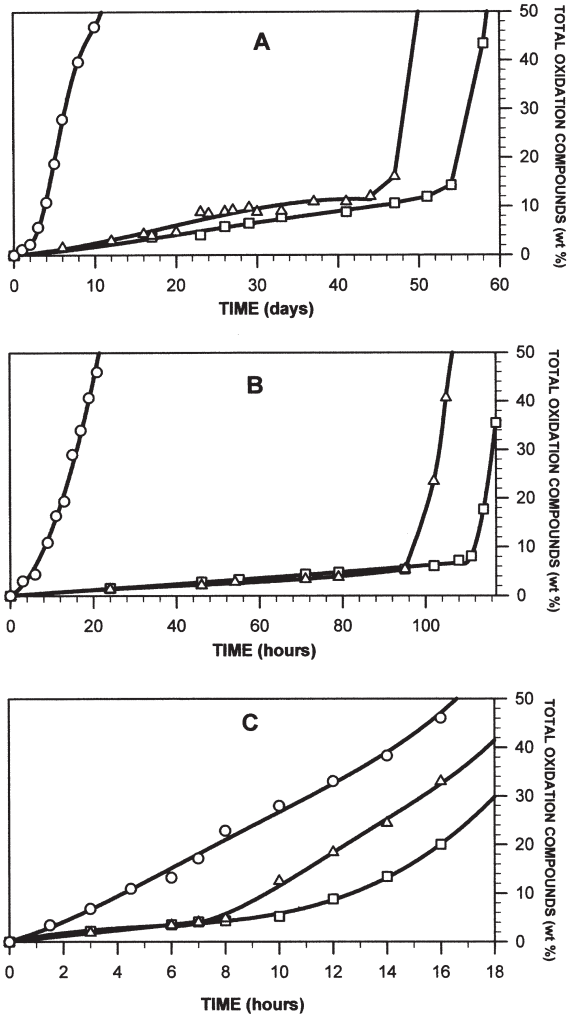

FIG. 2. Evolution of total oxidation compounds in samples of LLL without (-○-) and with the addition of 250 (-Δ-) and 500 (-□-) mg/kg α-tocopherol at 25 (A), 60 (B), and 100°C (C). For abbreviation see Figure 1.

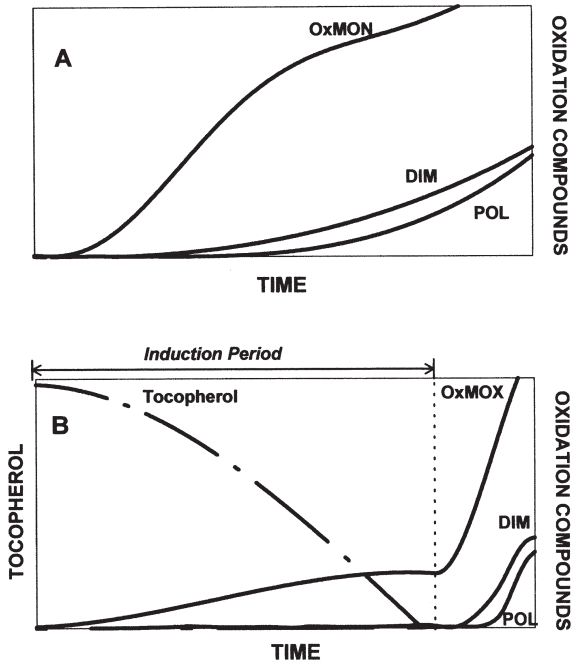

FIG. 3. General oxidation profile found for LLL without (A) and with added tocopherol (B). oxMON, oxidized monomers; DIM, dimers; POL, polymers. For other abbreviation see Figure 1.

Kinetic parameters were calculated from the experimental data by the least squares method. For experiments without α-tocopherol, no clear IP were obtained, and data corresponding to dimer concentrations lower than 1% were considered for kinetic studies.

Table 4 lists the values corresponding to the rate constants for oxMON formation (k), the reaction order (n), and the correlation coefficients (r). Values found for the reaction order were not significantly different from 0 when the antioxidant was present, thus indicating that the increase in oxMON was linear during the induction period. On the contrary, the reaction orders for LLL without antioxidant were different from 0 and close to 0.5 at 25 and 60ºC, as previously reported (30). As already mentioned, the lower value for the order of reaction obtained at 100ºC for LLL without antioxidant could be attributed to oxidation under conditions of limited air.

The influence of temperature on the oxidation rate during the IP can also be examined on the basis of the Arrhenius law:

$$k = A \exp(-E_a/RT) \quad [1]$$

where k is the rate constant, A is a pre-exponential factor, E_a is the activation energy, T is the absolute temperature, and R = 8.314 J/mol K.

A plot of ln k vs. T^{-1} should thus yield a straight line:

$$\ln k = \ln A - (E_a/RT) \quad [5]$$

Experimental values obtained for E_a were 41.8 ± 9.1, 46.1 ± 8.1, and 51.1 ± 2.0 kJ/mol, for LLL, LLL-250, and LLL-500, respectively. Such similarity in values would indicate that there was no substantial change in E_a when tocopherol was added.

On the other hand, k and the IP are inversely proportional and, consequently, ln IP would be directly proportional to E_a/RT. Values for the experimental IP were 8, 95, and 1128 h at 100, 60, and 25ºC, respectively, for LLL containing 250 α-tocopherol and were 10, 111, and 1296 h at 100, 60, and 25°C, respectively, for LLL containing 500 mg/kg α-tocopherol. The correlation coefficients obtained between ln k and ln IP were 0.994 and 0.999 for LLL containing 250 and 500 mg/kg α-tocopherol, respectively.

Figure 4 shows the linear relationship between ln IP and $1/T$ in samples with added α-tocopherol at 25, 60, and 100°C. Thus, assays at 60 or 100°C could be used to predict the IP at room temperature. Also, similar oxidation mechanisms for different α-tocopherol contents can be deduced from the parallel lines corresponding to 250 and 500 mg/kg α-tocopherol.

TABLE 4
Kinetic Parameters[a] for Formation of LLL Oxidized Monomers at 25, 60, and 100°C

T (°C)	α–Tocopherol (mg/kg)	*k*	*n*	*r*
25	0	$(67.4 \pm 6.6)\cdot10^{-3}$	0.568 ± 0.019	0.999
	250	$(12.2 \pm 1.7)\cdot10^{-3}$	0.005 ± 0.105	0.964
	500	$(7.8 \pm 0.5)\cdot10^{-3}$	0.108 ± 0.049	0.994
60	0	$(74.3 \pm 0.5)\cdot10^{-2}$	0.441 ± 0.087	0.991
	250	$(4.9 \pm 0.2)\cdot10^{-2}$	0.094 ± 0.090	0.992
	500	$(5.9 \pm 0.9)\cdot10^{-2}$	0.067 ± 0.089	0.989
100	0	1.96 ± 0.08	0.125 ± 0.040	0.999
	250	0.53 ± 0.02	0.0 ± 0.1	0.996
	500	0.49 ± 0.04	0.0 ± 0.2	0.982

[a]Mean values ± SE.

It is important to note that the results obtained here do not agree with those establishing that the mechanism of oil oxidation changes from ambient temperature to 100ºC (31) and that, consequently, oil shelf life cannot be deduced from accelerated tests. Such a conclusion could be easily assumed; however, it is important to point out two important considerations. First, oil shelf life may vary greatly depending on external parameters other than temperature. Oil storage in light or dark conditions and differences in availability of oxygen caused by packing and storage conditions are important enough to expect considerable differences in resistance of the oil to oxidation. Thus, we should define the specific conditions needed to predict the shelf life of the oil.

Second, deducing oil shelf life from accelerated tests also involves differences in the oxidation level measured and in the analytical method used, i.e., evaluating the IP by a sudden change in conductivity or oxygen depletion in the automated methods, the appearance of rancidity in the Schaal oven test, or the time to reach a certain PV beyond which the oil cannot be marketed as edible, in the case of room temperature tests.

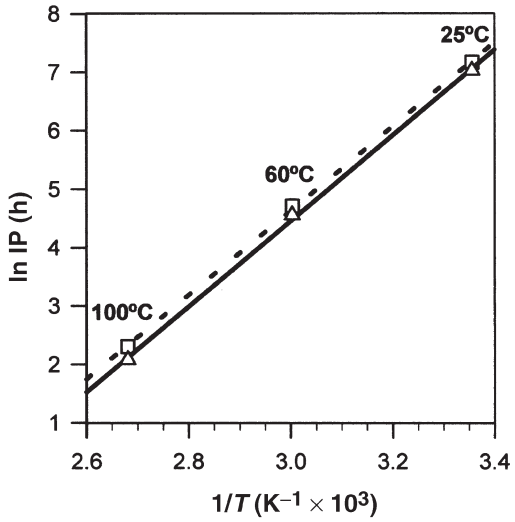

FIG. 4. Plot of ln induction period (IP) vs. 1/*T* in samples of LLL with the addition of 250 (-Δ-) and 500 (-□-) mg/kg α-tocopherol at 25, 60, and 100°C.

Nevertheless, with the exception of temperature, the same conditions of darkness and adequate air supply and the same criterion for evaluating oxidation (development of advanced oxidation either by the formation of polymerization compounds or the loss of antioxidants) were applied in this study. The results obtained indicate that temperature is not a variable that contributes significantly to changes in the reaction mechanism of oxidation under the conditions applied. Therefore, it would be useful in future studies to define whether the different oxidation mechanisms claimed are due to differences in variables other than temperature.

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