Oxidative Stability of Sunflower Oils Differing in Unsaturation Degree During Long-Term Storage at Room Temperature

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ABSTRACT: The objective of this work was to study the evolution of oxidation in sunflower oils differing in unsaturation degree during long-term storage at room temperature. For this purpose, a combination of adsorption and size-exclusion chromatographies was used for quantification of oxidized triacylglycerol (TG) monomers, dimers, and polymers. Conventional sunflower oil, genetically modified high-oleic sunflower oil, and a 1:1 mixture of the two were used. Results showed that oxidized TG monomers were the only group of oxidation compounds increasing during the early oxidation stage, and an excellent correlation was found between amounts of oxidized TG monomers and PV during the induction period, independently of the degree of oil unsaturation. Both the rate of formation and the amount of oxidized TG monomers accumulated at the end of the induction period increased as the unsaturation degree of the oils tested was higher. The end of the induction period was marked by the initiation of polymerization and exhaustion of tocopherol. Therefore, the concomitant determination of oxidized TG monomers and polymerization compounds provided a complete picture of the oxidation process.

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KEY WORDS: Dimers, HPSEC, induction period, oxidation, oxidized triacylglycerols, peroxide value, polymers, storage, sunflower oils, tocopherol.

Lipid oxidation is the cause of important deteriorative changes in edible oils and fatty foods since it affects their chemical, sensory, and nutritional properties (1,2) and hence is often the decisive factor determining their useful storage life. However, studies on shelf life or storage of edible oils at room temperature, which may approximate real storage conditions, are scarce in contrast with the abundance of studies carried out under accelerated oxidative conditions. The main drawbacks of studies under ambient conditions include the prolonged experiments required, the difficulties in drawing general conclusions from the limited number and specific composition and quality of the oils tested, and the multitude of possible combinations of variables involved in the oil shelf life, such as light exposure, type of packaging materials, availability of oxygen, and addition of antioxidants.

The influence of the degree of unsaturation on oxidative stability of oils has been a subject of utmost interest. In this context, an important approach has been evaluation of purified oil TG for the purpose of examining the influences of FA composition, TG composition and structure (3), and FA rearrangement after randomization (4) on oxidative stability. In general, Neff and coworkers (3) found that the rates of peroxide and total headspace volatiles formation were dependent on the degree of unsaturation and on the contents of linoleic and linolenic acids at the 2-position of the glycerol moiety. However, the influence of the location of unsaturated fatty acyl groups in the TG molecule on oxidative stability is still controversial (5–7).

Overall, the literature reflects the main problems encountered in the evaluation of oil oxidation. First, more than one measurement must be made to obtain a complete picture of the oxidation process. Normally, the analytical methods used provide only partial information on the oxidation state (8); hence, it it usual to find opposite results for similar substrates and experimental conditions, depending on the method selected. Second, the end point of the assays is normally predetermined, and it is the same for all samples regardless of the great differences that may exist in their evolution of oxidation. In this respect, following the progress of oxidation until the accelerated stage is reached seems to be a better approach to establishing valid and reliable comparisons between oils. Finally, minor compounds having prooxidant or antioxidant activity can vary between apparently similar oils; hence, their content and changes during oxidation must be evaluated because of their decisive contribution to the course of oxidation.

Recently, the kinetics of oxidation in trilinolein model systems was studied (9). The analytical approach used consisted of a combination of adsorption and size-exclusion chromatographies, which provided concomitant quantification of primary and secondary oxidation compounds (10–12). This approach has been found useful for the evaluation of oil-containing foods oxidized at low temperature (13,14). Kinetic results on trilinolein samples showed that the amounts of primary oxidation compounds accumulated during the induction period decreased at higher temperatures, 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.

The objective of the present work was to study the evolution of oxidation in sunflower oils differing almost exclusively in degree of unsaturation during long-term storage at room temperature. Experiments were prolonged until the advanced

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oxidation stage was reached, to compare duration of induction periods. It is important to note that using oils instead of trilinolein model systems for shelf-life studies involves (i) an increase of complexity in TG composition, (ii) the occurrence of minor compounds that, even at very low concentrations, may exert considerable pro-oxidant, antioxidant, or synergistic actions (15), and (iii) the possible presence of degradation compounds due to differences in initial quality or refining processes (16). For these reasons, genetically modified seed oils are normally used to evaluate the influence of FA composition in oxidation studies (17).

In the present work, the values found for the oxidation compounds formed were compared with the results obtained through two analytical indexes of wide application to evaluate primary and secondary oxidation products, i.e., peroxide value (PV) and UV absorption at 270 nm (K_{270nm}), respectively.

EXPERIMENTAL PROCEDURES

Samples. Conventional sunflower oil (SO), genetically modified high-oleic sunflower oil (HOSO), and a 1:1 mixture of the two (SO/HOSO) were used. SO and HOSO were supplied by Medeol (Neuilly sur Seine, France). Forty 20-mL samples of each oil tested were placed in open beakers (surface-to-volume ratio of 0.35 cm^{-1}) and stored at 25°C in the dark for different periods of time.

Analytical methods. (i) Determination of FA and TG compositions. FA composition was determined by GLC following transesterification with sodium methoxide and hydrochloric acid/methanol (18), using a SUPELCOWAX 10 fused-silica capillary column (Supelco, Bellefonte, PA), 30 m long and 0.32 mm i.d., at 180°C. TG composition was determined by GLC (19) using an Rtx 65TG (Restek, Bellefonte, PA) fused-silica capillary column, 30 m long and 0.25 mm i.d. The column temperature was set at 350°C for 1 min, then programmed to 360°C at 0.5°C/min and kept at 360°C for 6 min.

(ii) Determination of minor components. α-Tocopherol was determined by HPLC and fluorescence detection (19). Fe and Cu traces were determined by graphite furnace atomic absorption spectrometry (20). Sterol composition was determined in the unsaponifiable fraction through separation by TLC and analysis by GLC (18) using an HP 5 (Hewlett-Packard, Avondale, PA) fused-silica capillary column, 25 m long and 0.32 mm. i.d., at 275°C.

(iii) Evaluation of physicochemical characteristics. FFA content (as determined by titration), PV (as determined by iodometric assay), K_{270nm}, and unsaponifiable matter were evaluated according to standard methods (18). Oil stability index was determined at 100°C using a 679 Rancimat apparatus (Metrohm, Herisau, Switzerland) following the AOCS Method (21).

(iv) *Quantification of total polar compounds and distribution in oxidized and hydrolytic compounds.* Nonpolar and polar fractions were separated from 1 g of oil sample by silica column chromatography (20 g silica/H_2O, 95:5 w/w). The nonpolar fraction containing unoxidized TG was eluted with 150 mL of *n*-hexane/diethyl ether (90:10, vol/vol). A second fraction, comprising total polar compounds, was eluted with 150 mL of diethyl ether. Efficiency of the separation by adsorption chromatography was checked by TLC using hexane/diethyl ether/acetic acid (80:20:1, by vol) for development of plates and exposure to iodine vapor to reveal the spots. After evaporation of solvents, both fractions were weighed and dissolved in diisopropyl ether (25 mg/mL) for analysis by high-performance size-exclusion chromatography, using a Rheodyne 7725i injector with 10-μL sample loop, a Waters 510 pump (Waters, Milford, MA), an HP 1037 A refractive index detector, and an HP 3392 A integrator (Hewlett-Packard). The separation was performed on two 100 and 500 Å Ultrastyragel columns (25 cm × 0.77 cm i.d.) packed with porous, highly cross-linked styrene-divinylbenzene copolymers (film thickness: 10 μm) (Hewlett-Packard) connected in series, with THF (1 mL/min) as the mobile phase (10). The groups of compounds separated were TG polymers (TGP), TG dimers (TGD), oxidized TG monomers (oxTGM), diacylglycerols (DG), monoacylglycerols (MG), and FFA.

(v) Statistical analysis. SigmaStat and SigmaPlot (Systat, Point Richmond, CA) software packages were used for the kinetic study and plots.

RESULTS AND DISCUSSION

Tables 1 to 3 show the evaluation of the major and minor components and physicochemical characteristics of the starting sunflower oils. The main difference between oils was observed in the FA and TG compositions (Table 1). Thus, the global levels of PUFA were 67.7, 42.3, and 16.8%, those of monounsaturated FA were 20.3, 46.4, and 72.5%, and those of saturated FA were 11.1, 10.4, and 9.7% for SO, SO/HOSO, and HOSO, respectively. However, no significant differences were found in the amounts of those minor components that may have an influence on their behavior toward oxidation,

TABLE 1
FA and TG Compositions of the Starting Sunflower Oils[a,b]

	SO	SO/HOSO	HOSO
FA			
16:0	6.4	5.5	4.0
18:0	4.7	4.5	4.3
18:1	21.0	46.7	72.4
18:2	67.7	42.3	16.8
Others	0.2	1.0	2.5
TG			
LLL	26.4	17.0	5.6
OLL	25.6	17.4	7.5
PLL	10.9	7.3	2.2
OOL	8.1	8.7	9.5
POL + SLL	12.9	9.1	4.0
OOO	1.7	25.2	51.1
POO + SOL	4.7	5.8	8.7
SOO	1.6	3.7	7.9
Others	5.0	2.5	0.7

[a]Abbreviations: SO, sunflower oil; HOSO, high-oleic sunflower oil; SO/HOSO, 1:1 mixture of sunflower oil and high-oleic sunflower oil; L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid.
[b]wt% of oil.

TABLE 2
Minor Components Initially Present in Sunflower Oils[a]

	SO	SO/HOSO	HOSO
α-Tocopherol (mg/kg)	625	630	651
Fe (μg/kg)	<4.4	<4.4	<4.4
Cu (μg/kg)	<1.3	<1.3	<1.3
Polar compounds (wt% of oil)			
Total	3.0	2.6	2.3
oxTGM	0.9	0.6	0.5
TGD	0.6	0.3	0.2
TGP	<0.1	<0.1	<0.1
DG	0.9	0.9	1.2
FFA	0.5	0.4	0.4
Sterols (wt% of sterol fraction)			
Campesterol	9.9	9.0	8.3
Stigmasterol	8.1	8.4	8.9
Δ_7-Campesterol	2.6	2.7	2.8
β-Sitosterol	59.2	59.0	59.0
Δ_7-Stigmastenol	14.5	13.9	13.3
Δ_7-Avenasterol	4.6	4.5	4.3
Others	1.1	2.5	3.4

[a]Abbreviations: oxTGM, oxidized TG monomers; TGD, TG dimers; TGP, TG polymers. For other abbreviations see Table 1.

TABLE 3
Physicochemical Characteristics of the Starting Sunflower Oils[a]

	SO	SO/HOSO	HOSO
FFA (g oleic acid/100 g oil)	0.02	0.02	0.02
PV (meq O_2/kg)	8	6	3
K_{270nm}	0.465	0.448	0.419
Unsaponifiable matter (wt% of oil)	1.01	1.00	0.97
Oil stability index (h)	7.4	10.1	20.1

[a]For abbreviations see Table 1.

i.e., naturally occurring α-tocopherol, Fe and Cu traces, sterols, and minor oxidized, polymerized, and hydrolytic compounds (Table 2). Therefore, the distinct values of oil stability index, which provides an estimation of oil shelf life, found for the three oils (Table 2) can be attributed to differences in unsaturation degree. As to the indexes commonly used to evaluate initial quality of vegetable oils, i.e., free acidity, PV, K_{270nm}, and unsaponifiable matter content (Table 3), similar values were found for the three sunflower oils. These results supported the selection of such sunflower oils as excellent substrates to study the influence of unsaturation degree on oil oxidation since the possible influence of other variables associated with differences in initial quality and/or presence of antioxidant or prooxidant minor compounds could be avoided.

Tables 4 to 6 show the evolution of oxidation at 25°C in SO, SO/HOSO, and HOSO samples, respectively. For each sampling point, total polar compounds and their distribution in oxidation compounds (oxTGM, TGD, and TGP) and hydrolytic products (DG and FFA) were quantified, although only oxidation compounds were included in the tables since, as expected, hydrolytic products did not increase during storage. Results corresponding to duplicate experiments showed good reproducibility with respect to the progress of oxidation and the distribution of specific groups of compounds under the conditions used (the CV was lower than 8% for total polar compounds). Also, the tables list levels of α-tocopherol and the values found for two common analytical indexes evaluated in parallel, PV and K_{270nm}.

To illustrate the general profiles of oil oxidation obtained through quantification of oxTGM, TGD, and TGP, evolution of these groups of oxidation compounds is represented for all samples in Figure 1, along with changes in α-tocopherol contents.

The process of autoxidation is widely known to proceed *via* a free radical mechanism that gives rise to a wide variety of volatile and nonvolatile compounds. Of greatest nutritional significance are the nonvolatile oxidation compounds because they are retained in the food and hence digested (22). The analytical methodology applied in this study permits quantification of three groups of nonvolatile oxidation compounds, i.e., oxTGM, TGD, and TGP, which provide a complete picture of the oxidation progress. On one hand, since oxTGM are

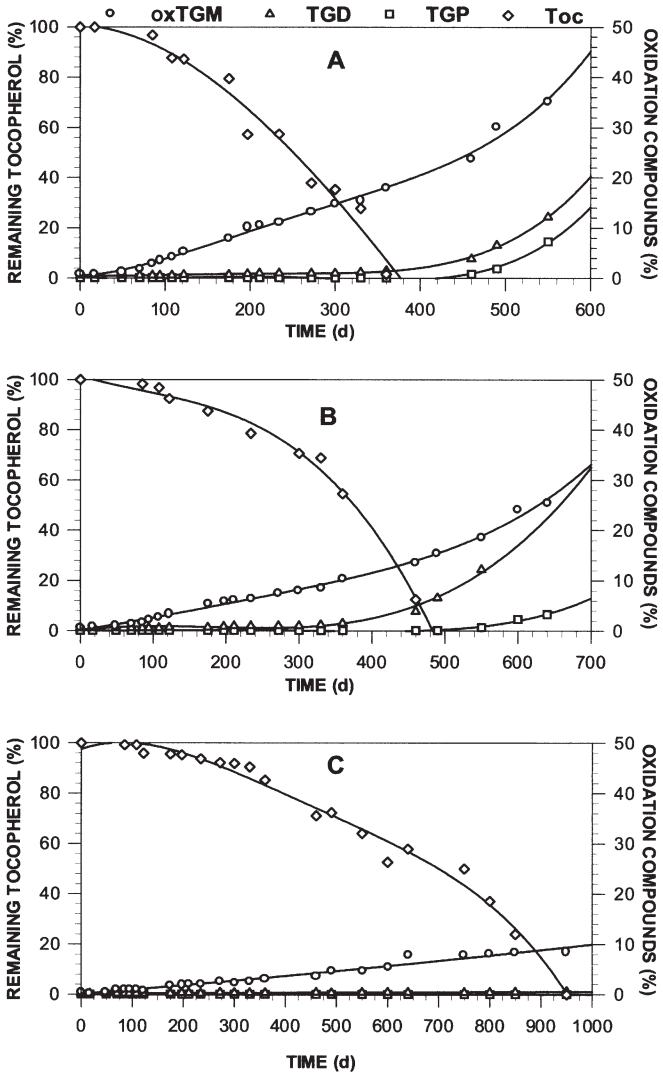

FIG. 1. Evolution of oxidized TG monomers (oxTGM) (○), TG dimers (TGD) (△), TG polymers (TGP) (□), and tocopherol (Toc) contents (◇) in sunflower oil (A), a 1:1 mixture of sunflower oil and high-oleic sunflower oil (B), and high-oleic sunflower oil (C) stored at 25ºC.

TABLE 4
Evolution of Oxidation in SO Samples Stored at 25°C

	Oxidation compounds (wt% of oil)[a]			Oxidation indexes[a]		α-Toc[a]
Days	oxTGM	TGD	TGP	PV (meq O_2/kg)	K_{270nm}	(mg/kg)
0	0.9	0.6	ND	8	0.465	625
49	1.8	0.6	ND	15	0.492	624
85	2.9	0.6	0.1	51	0.500	605
108	4.3	0.6	0.1	104	0.508	548
122	5.3	0.7	0.1	114	0.515	545
175	8.0	0.7	0.1	181	0.575	497
197	10.2	0.9	0.1	226	0.629	358
234	11.1	1.0	0.1	265	0.674	359
272	13.2	1.0	0.1	305	0.712	237
300	14.9	1.0	0.1	351	0.788	221
330	15.5	1.2	0.1	367	0.795	174
360	18.0	1.5	0.1	435	0.869	10
460	23.8	3.9	0.8	676	1.456	0
490	30.2	6.6	1.9	858	2.400	0
550	35.1	12.2	7.3	1202	5.139	0

[a]Abbreviations: α-Toc, α-tocopherol; ND, not detected; oxTGM, oxidized TG monomers; TGD, TG dimers; TGP, TG polymers; for other abbreviation see Table 1.

monomeric TG that contain at least one oxygenated function, whether peroxide groups or epoxy, keto, or hydroxy functions, their quantification is useful to detect both primary and secondary oxidation products. On the other hand, quantification of polymerization compounds (TGD and TGP) is useful to detect the onset of the accelerated oxidation stage since they are characteristic products of advanced oxidation.

Figure 1 shows that oxTGM were the only group of oxidation compounds increasing during the early oxidation stage. At a certain point, oxidation was accelerated, as shown by the sharp increase of oxTGM and the development of polymerization reactions, denoted by a significant rise in TGD. Therefore, as already observed in trilinolein model systems with added α-tocopherol (9), two oxidation stages could be clearly distinguished: first, a period characterized by slow progress of oxidation or an induction period, and second, an accelerated oxidation stage. The end of the induction period, defined as the time point when a notable shift in oxidation rate is observed, was clearly characterized by exhaustion of antioxidants and significant formation of polymerization products. In general, increases of about 1% in dimer concentrations indicated the start of the accelerated phase, as was previously found at all temperatures tested in trilinolein model systems (9).

The end of the induction time occurred between 360 and 460 d in SO, between 460 and 490 d in SO/HOSO, and between 850 and 950 d in HOSO. Results showed, as expected, that the induction period length depended on the unsaturation degree, although the oil mixture prepared using equal amounts of SO and HOSO showed values markedly closer to those found for the most unsaturated oil. Overall, two important findings stood out. First, the amounts of oxTGM accumulated at the end of the induction period increased as the oil unsaturation degree was higher, being more than double for SO as compared with HOSO. Second, the relation between

TABLE 5
Evolution of Oxidation[a] in 1:1 SO/HOSO Samples Stored at 25°C

	Oxidation compounds (wt% of oil)			Oxidation indexes		α-Toc
Days	oxTGM	TGD	TGP	PV (meq O_2/kg)	K_{270nm}	(mg/kg)
0	0.6	0.3	ND	6	0.448	630
49	1.0	0.4	ND	19	0.462	626
85	1.7	0.5	0.1	32	0.477	619
108	2.8	0.4	0.1	61	0.485	610
122	3.4	0.4	ND	65	0.474	582
175	5.4	0.7	ND	118	0.575	551
197	5.8	0.6	ND	129	0.534	545
234	6.4	0.6	0.1	145	0.550	495
272	7.5	0.7	0.1	175	0.565	479
300	8.0	0.6	0.1	198	0.594	445
330	8.6	0.7	ND	205	0.605	434
360	10.4	0.7	ND	238	0.626	344
460	13.6	1.1	ND	332	0.794	79
490	15.5	1.4	0.1	378	0.910	0
550	18.5	3.1	0.7	517	1.345	0
600	24.1	5.9	2.3	704	1.873	0
640	25.4	7.0	3.3	840	2.770	0

[a]For abbreviations see Tables 1 and 4.

TABLE 6
Evolution of Oxidation[a] in HOSO Samples Stored at 25°C

	Oxidation compounds (wt% of oil)			Oxidation indexes		α-Toc
Days	oxTGM	TGD	TGP	PV (meq O_2/kg)	K_{270nm}	(mg/kg)
0	0.5	0.2	ND	3	0.419	651
49	0.3	0.2	ND	7	0.426	650
85	0.9	0.2	ND	13	0.439	645
108	1.0	0.2	ND	26	0.446	645
122	0.7	0.2	ND	23	0.439	623
175	1.8	0.2	ND	44	0.454	621
197	1.9	0.3	ND	44	0.452	620
234	2.0	0.3	ND	43	0.466	609
272	2.6	0.4	ND	44	0.471	600
300	2.3	0.3	ND	60	0.480	597
330	2.6	0.3	ND	57	0.472	588
360	3.0	0.3	ND	65	0.472	554
460	3.6	0.4	ND	89	0.511	462
490	4.6	0.3	ND	101	0.524	470
550	4.8	0.4	ND	116	0.520	416
640	7.8	0.4	ND	186	0.607	376
750	7.9	0.4	ND	197	0.594	325
800	8.1	0.4	ND	210	0.589	241
850	8.3	0.5	ND	216	0.608	156
950	8.5	0.6	ND	227	0.612	0

[a] For abbreviations see Tables 1 and 4.

the induction periods obtained at room temperature was similar to that between the oil stability indexes as determined by Rancimat at 100°C (Table 3), thus reflecting the utility of this determination to foresee the shelf life of the oil (9).

The results obtained using the analytical methodology were compared with the data provided through determination of PV and K_{270nm}, both of which are commonly used to evaluate primary and secondary oxidation products, respectively. PV determination is widely applied to evaluate the extent of oxidation in fats, oils, and food lipids. As a measurement of hydroperoxide formation, it has been recognized as a useful index for early stages of oxidation. PV reaches a maximum during the progress of oxidation followed by a decrease, when the rate of decomposition of hydroperoxides exceeds the rate of their formation at more advanced stages, which varies according to the degree of unsaturation and storage conditions (1). Good correlations have been reported beween PV and headspace oxygen content and between PV and sensory scores for various commercial fats and oils during the initial stages of oxidation (23). In this study, results obtained for PV were compared with those of oxTGM, which, as commented on above, are basically composed of hydroperoxides during the early stage of oxidation. The relationship between both determinations is represented in Figure 2 for all oil samples within the early oxidation stage (up to TGD concentrations of about 1%), given that no significant differences ($P < 0.05$) were found between values for the slope when oils were considered separately (0.040 ± 0.001, 0.040 ± 0.001, and 0.038 ± 0.002, for SO, SO/HOSO, and HOSO, respectively). Thus, an excellent correlation was found during the induction period independently of the degree of oil unsaturation. Once oxidation accelerated, this relationship was complex since secondary oxidation products were formed and, as a consequence, TG containing oxygenated functions other than hydroperoxide (epoxy, keto, hydroxy, etc.) start contributing to the amount of oxTGM, whereas hydroperoxide functions not only were present in primary oxidation compounds but also were involved in dimeric linkages of polymerization compounds (24).

UV absorption at 270 nm constitutes a measurement of conjugated trienes as well as ethylenic diketones and conjugated ketodienes produced from polyunsaturated lipids (1). Even though this index does not provide quantitative data, it has been traditionally used to evaluate secondary oxidation products. A maximum limit of K_{270nm} has been established for virgin olive oils, but not for refined oils since K_{270nm} values depend on the degree of oil unsaturation, refining conditions, and the presence of minor compounds that absorb at wavelengths close to 270 nm. In this study, this index was evaluated to determine its utility as a rapid oxidation test, provided that starting oils gave low and similar values. As reflected in Tables 4–6, slight

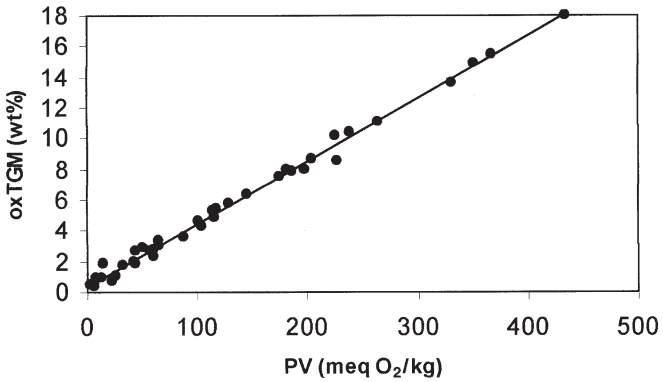

FIG. 2. Correlation between PV and oxTGM during the induction period. $Y = 0.336 + 0.041X$, $r = 0.997$ ($n = 40$). For abbreviation see Figure 1.

TABLE 7
Kinetic Parameters for Formation of oxTGM[a] at 25°C

Oils	I.P. (h)	*k*	*r*
SO	8,640	$(2.12 \pm 0.07)\cdot10^{-3}$	0.993
SO/HOSO	11,040	$(1.25 \pm 0.04)\cdot10^{-3}$	0.993
HOSO	22,800	$(0.35 \pm 0.09)\cdot10^{-3}$	0.994

[a]Abbreviations: I.P., induction period; *k*, rate constant for formation of oxidized TG monomers; *r*, correlation coefficient; for other abbreviations see Tables 1 and 4.

changes in K_{270nm} were detected during the induction period, and only once oxidation accelerated and tocopherol was exhausted was a significant increase observed, which was parallel to the formation of polymerization compounds. These results were not unexpected, considering data obtained through PV and oxTGM that indicated the low formation of secondary oxidation compounds during the induction period.

The results obtained in this study showed that evolution of oxidation in the sunflower oils tested was very similar to that observed earlier in trilinolein model systems; thus, similar kinetics considerations were applied (11). Briefly, since oxTGM are, in practice, the only products formed during the induction period and it is assumed that they do not participate in other side reactions during this period, the relation between oxTGM concentrations and *t* (time) during the early stages of oxidation can be expressed as follows:

$$[\text{oxTGM}]^{(1-n)} = [\text{oxTGM}]_0^{(1-n)} + (1 - n)\, kt \qquad [1]$$

where $[\text{oxTGM}]_0$ is the initial concentration of oxTGM, *k* is the rate constant for oxTGM formation, and *n* is the reaction order. Table 7 lists *k* values and the correlation coefficients (*r*) calculated from the experimental data, considering $n = 0$ since the increase of oxTGM was linear during the induction period. As expected, an increment of *k* was found as the degree of unsaturation increased. *k* was also found to be directly related to temperature when trilinolein model systems were oxidized at 25, 60, and 100°C (9).

The results obtained in this study clearly indicate that both the rate of formation and the amount of primary oxidation compounds accumulated at the end of the induction period are higher as the degree of unsaturation increases. Overall, it is important to remark that the concomitant determination of oxTGM and polymerization compounds is of great utility for oxidation kinetics studies and for oxidative stability evaluation.

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