Thermal Degradation of Single Methyl Oleate Hydroperoxides Obtained by Photosensitized Oxidation

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ABSTRACT: A peroxidation mixture containing methyl 9- and 10-hydroperoxy-trans-octadecenoates (MOHP) was obtained by singlet oxygen oxidation of methyl oleate. The two hydroperoxides were collected by solid phase extraction and purified separately by high-performance liquid chromatography. Identification and single-isomer purity evaluations were carried out by comparing the chromatographic and gas chromatography–mass spectrometry parameters of the corresponding reduced hydroxy derivatives. Each purified MOHP was thermally degraded and new reaction mechanisms were proposed from the identification of the degradation products. Thermal rearrangement of each hydroperoxide isomer involved an allylic 3-carbon intermediate before further degradation steps. The two MOHP isomers obtained from singlet oxygen oxidation produced all eight hydroperoxide isomers by thermal degradation in the condensed phase at high temperature (200°C). This result supports the assumption of singlet oxygen as a promoter of the first steps of oxidation of food lipids and also reconsiders the Khan mechanism. *JAOCS 75,* 1115–1120 (1998).

KEY WORDS: GC–MS, HPLC, lipid photooxidation, methyl oleate hydroperoxide oxidation mechanisms, thermal degradation.

Lipid peroxidation has important effects *in vivo* because the free radicals formed by lipid hydroperoxide decomposition can lead to cellular damage and consequent aging. The autoxidation of organic substances is a complex process activated by free radicals through a chain-reaction mechanism. Although the oxidation of unsaturated substances has been the subject of numerous investigations, some uncertainties still remain in relation to the mechanisms of formation and decomposition of hydroperoxides.

Hydroperoxides are the first products of fatty acid oxygenation that can be analytically isolated. The role of hydroperoxides as intermediates in the formation of volatile off-flavors has been reviewed recently by Frankel (1) and Grosch (2). During thermal oxidation of a natural lipid system or a model system, such as methyl oleate (3,4), hydroperoxides are formed and further degraded, giving rise to volatile products.

The study of hydroperoxide structure could help to elucidate the reaction mechanisms involved in the first steps of oxidation processes. One of the most studied monounsaturated model systems is methyl oleate (3,4). A total of eight isomeric methyl oleate hydroperoxides (MOHP) are generated by oxidation. Four of these positional isomers of the hydroperoxy group have a *trans* configuration of the double bond; the other four have a *cis* one. The MOHP obtained from photosensitized oxidation (singlet oxygen oxidation) (5), methyl 9-hydroperoxy-Δ^{10}, *trans*-octadecenoate (9-OOH) and methyl 10-hydroperoxy-Δ^{8}, *trans*-octadecenoate (10-OOH), are produced by a nonradical mechanism (double-bond addition). On the other hand, a nonradical mechanism leads to the formation of the same hydroperoxy isomers produced by the radical mechanism proposed by Khan (6,7); the main difference between these mechanisms is that the nonradical one involves singlet oxygen produced with chlorophyll or hematoporphyrines as catalysts.

In order to understand the reaction mechanisms and the decomposition processes of hydroperoxides that give rise to volatile and nonvolatile compounds, two high-purity hydroperoxide isomers obtained by photosensitized oxidation of methyl oleate were subjected to thermal degradation (Fig. 1). The resulting products from single hydroperoxides were analyzed by high resolution gas chromatography and gas chromatography–mass spectrometry (GC–MS). The study of the molecular structures of these thermal degradation products was also carried out on the mixture of the two hydroperoxides.

EXPERIMENTAL PROCEDURES

Materials and reagents. Reagents and solvents were analytical or high-performance liquid chromatography (HPLC) grade, supplied by Carlo Erba (Milan, Italy). Methyl oleate standard was purchased from Nu-Chek-Prep, Inc. (Elysian, MN); it was purified by passing through an alumina (neutral) liquid chromatographic column (2 cm × 1 cm i.d.), using *n*-hexane as the elution solvent, and analyzed by capillary gas chromatography (CGC) as suggested by Bortolomeazzi *et al.* (8).

MOHP preparation and isolation. The two isomeric hydroperoxides (MOHP) (9-OOH and 10-OOH) were produced by photosensitized oxidation as reported in (5); a 1:1 mixture of methyl oleate/extra-virgin olive oil (containing chlorophyll) was exposed to a tungsten lamp (100 watt, 15 cm distance), for 6–7 h (5). The polar oxidation products were then

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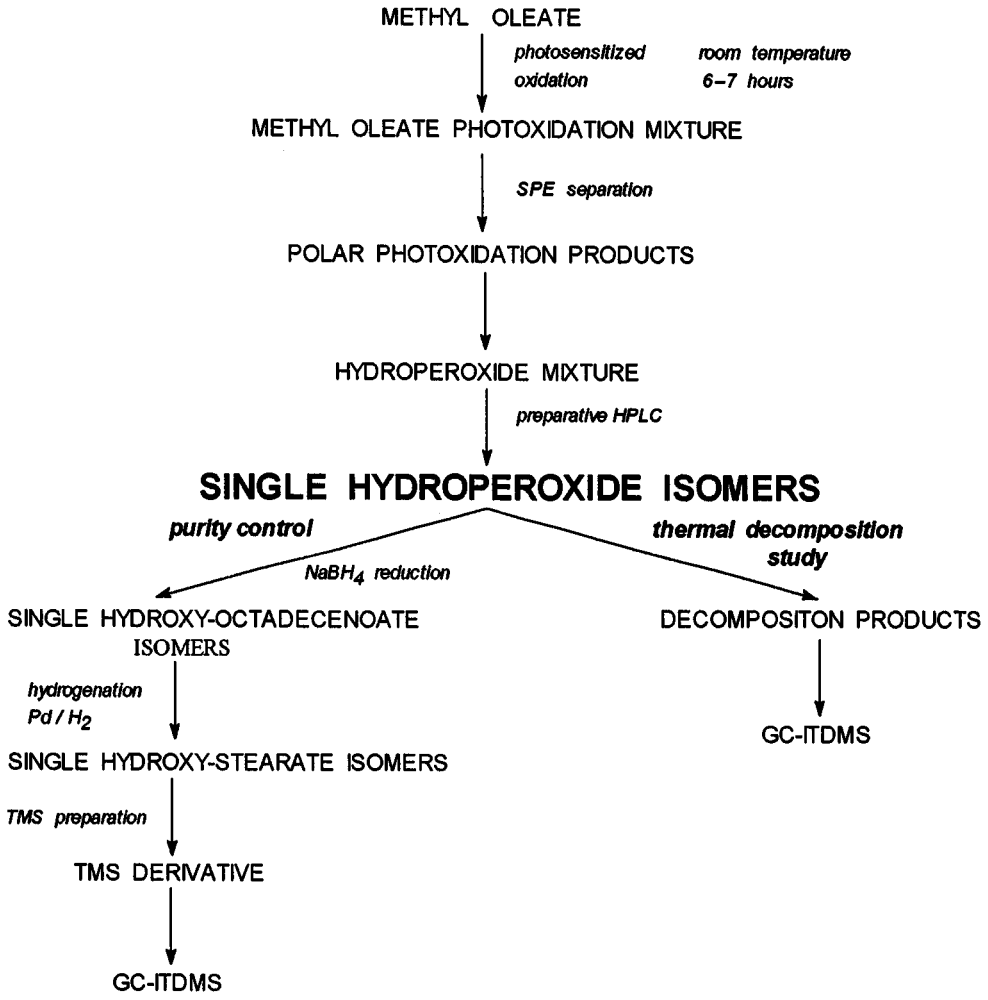

FIG. 1. Flow diagram of the preparation/purification and thermal degradation of methyl oleate hydroperoxides (MOHP).

separated from the photooxidation mixture by solid phase extraction (SPE) (8). To obtain the single hydroperoxide isomers, the polar mixture containing hydroperoxides was subjected to preparative HPLC. See Figure 1.

The MOH obtained by $NaBH_4$ reduction and the thermal decomposition products of these isomers were analyzed by CGC and gas chromatography–ion trap mass spectrometry (GC–ITDMS) (8).

SPE of hydroperoxides. The photoxidation mixture (about 100 mg) was dissolved in 1 mL *n*-hexane and loaded onto a SPE silica column (500 mg), which was preconditioned with *n*-hexane (5 mL). The cartridge was then washed with 10 mL *n*-hexane and eluted with 5 mL of *n*-hexane/diethyl ether, 1:1, vol/vol. The last SPE fraction contained MOHP and polar products. See Reference 8.

HPLC. The liquid chromatograph was a Varian 5040 equipped with a Varian CDS 401 data system and a Varian UV 50 detector set at 212 nm; the column was a 3-µm Spherisorb CN (15 cm × 4.6 mm i.d.) (Phase Separations Ltd., Deeside, United Kingdom). The analyses were performed under isocratic conditions at a flow rate of 1.5 mL/min, using 0.3% anhydrous ethanol in *n*-hexane as the mobile phase. See Reference 8.

Purity control of the single MOHP and isomeric determination of their thermal degradation products. (i) MOHP reduction and hydrogenation. Each MOHP, as well as the mixture of the two isomers, was reduced in methanol to the corresponding MOH by using $NaBH_4$ (8). The single hydroxy octadecenoate isomers, as well as the mixture of the two isomers, were hydrogenated to methyl hydroxy stearate (MHS) with hydrogen gas, using charcoal-supported Pd as catalyst.

(ii) Trimethylsilyl (TMS) preparation. The single hydroxy–stearate isomers were silylated, as well as the mixture of the two isomers, with about 0.1 mL of 5:2:1 pyridine/hexamethyldisilazane/tri-methylchlorosilane vol/vol/vol, according to Sweeley *et al.* (9). The TMS derivatives were then analyzed by GC–ITDMS.

Thermal decomposition study. Thermal degradation of the individual hydroperoxide compounds (0.5 µg/injection) and

the mixture of two hydroperoxides was performed by using the glass injection port (7 cm × 0.3 cm i.d.) of a Varian 3400 capillary GC, equipped with a DB 5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25-μm film thickness) (J&W, Folsom, CA) and coupled to a Varian Saturn ion trap detector (GC–ITDMS). The oven temperature was programmed from 50 to 300°C with a rate of 5°C/min. Injector, transfer line and manifold temperatures were 300°C. The filament emission current was 10 μA and the electron impact energy was 70 eV.

Thermal degradation in the condensed phase of the mixture of the two hydroperoxides was carried out in a test tube sealed with a screw teflon cap by placing it in an oven at 200°C for 10 min.

GC–ITDMS determinations. The determinations were carried out with a Varian 3400 capillary GC equipped with the same type of capillary column as the one used for the GC analysis. The GC experimental conditions were the same as those described in the thermodegradation step.

RESULTS AND DISCUSSION

The isomeric compositions of the hydroperoxide mixtures collected by silica SPE and those of the individual hydroperoxides obtained by purity analytical control are listed in Table 1. Figure 2 (A and B) shows the reconstructed GC-ITDMS profiles of the thermal degradation products (volatiles and nonvolatiles) from 10-OOH and 9-OOH. The GC-ITDMS traces are printed with different relative peak intensities, in order to make evident the presence of minor components in MOHP thermal degradation. Accordingly, some peaks are out of scale, but the relative intensities among the original peaks are not modified.

Thermal degradation products from individual hydroperoxides were identified by comparing the mass spectra from GC–ITDMS analyses with those identified previously in a MOHP mixture (3,4). These products are listed in Table 2; the results confirm the presence of some degradation products that have also been identified by other authors (1,2). The main decomposition products of the 10-OOH isomer (Fig. 2A) correspond to those obtained from the degradation of the 10-

TABLE 1
Isomer Compositions of Purified Methyl Oleate Hydroperoxides

Positional isomer	MOHP from photosensitized oxidation[a] Mean (cv%)	MOHP after HPLC purification[b]
8-OOH Δ^9	6.8% (30.8)	—
9-OOH Δ^{10}	42.7% (8.6)	48.9%
10-OOH Δ^8	44.3% (4.7)	51.1%
11-OOH Δ^9	6.2% (33.8)	—

[a]Mean of six replicates, calculated by characteristic MS fragments ratio after reduction, hydrogenation and trimethylsilyl derivatization. The amounts of *cis* and *trans* isomers determined by direct GC analysis were 2 and 98.0%, respectively.
[b]Determined by high-performance liquid chromatography (HPLC). Abbreviations: cv, coefficient of variation; MOHP, methyl oleate hydroperoxides; GC, gas chromatography; MS, mass spectrometry.

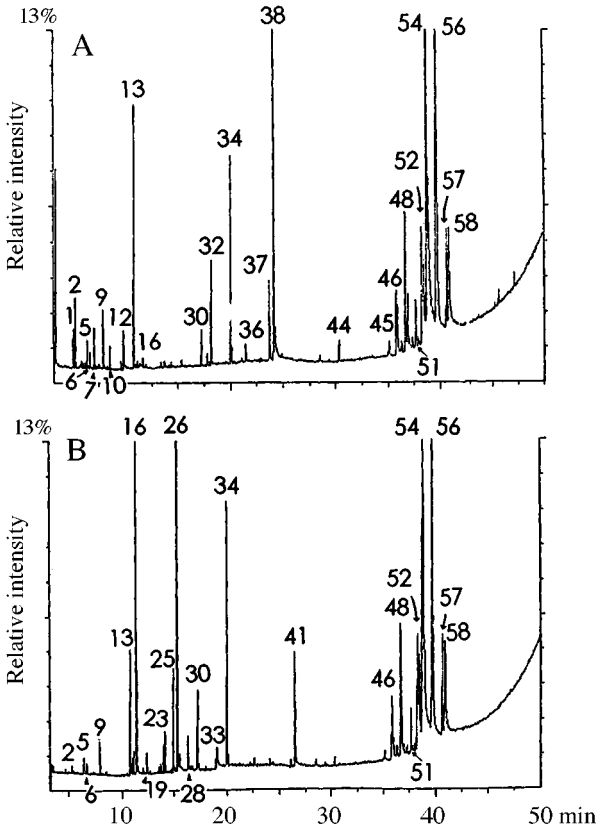

FIG. 2. Gas chromatography–ion trap mass spectrometric (GC–ITDMS) profiles of thermal decomposition of the single hydroperoxide isomers. GC peak numbers correspond to those given in Table 2. A: 10-OOH isomer and B: 9-OOH isomer.

TABLE 2
Thermal Degradation Products of Purified Methyl Oleate Hydroperoxides: Decomposition Products

GC peak	Identification	Isomer[a] 9-OOH	Isomer[a] 10-OOH
9	Octanal	0.9	1.5
10	Methyl heptanoate	trace[b]	0.7
12	1-Octanol (tentative)		1.2
13	Nonanal	4.2	10.4
15	Methyl octenoate	0.7	
16	Methyl octanoate	12.5	trace
18	2,4-Nonadienal	trace	
19	2-Nonenal	0.7	
25	2,4-Decadienal	3.5	
26	2-Decenal	32.0	trace
30	Methyl 8-oxo-octanoate	0.9	
32	2-Undecenal		2.7
33	Methyl 8-hydroxy-octanoate	0.9	
34	Methyl 9-oxo-nonanoate	10.4	4.4
38	Methyl 10-oxo-8-decenoate		50.0
41	Methyl 11-oxo-9-undecenoate	4.1	
56	Methyl keto-octadecenoates mix	100.0	100.0
45–58	Oxidation "evolution" products	166.0	166.0

[a]The amounts were calculated by assigning 100 to total ion current of the main GC peak, n. 56. All other values are reported as relative area of peak compared to the area of peak n. 56.
[b]trace = <0.2. See Table 1 for abbreviation.

TABLE 3
Thermal Degradation Products of Purified Methyl Oleate Hydroperoxides: "Evolution" Products[a]

GC peak	Identification (methyl esters)	Isomer	
		9-OOH	10-OOH
45	Octadecenoate isomers	0.1	0.4
46	Octadecadienoate isomers	4.3	3.8
48	Epoxy-octadecanoate isomers	6.0	4.0
49	Epoxy-octadecanoate isomers	1.5	trace[b]
50	Unknown	0.4	0.1
51	Unknown	1.3	1.3
52	Unknown	8.2	7.3
53	Unknown	2.7	1.6
54	Hydroxy-octadecenoate isomers	42.8	40.5
55		0.7 (9 and 11 isomers)	0.7 (8 and 11 isomers)
56	Oxo-octadecenoate isomers	100.0 (9 and 11 isomers)	100.0 (8 and 10 isomers)
57	Oxo-epoxy-octadecanoate isomers	3.3 (9 and 11 oxo-isomers)	3.0 (8 and 10 oxo-isomers)
58	Hydroxy-epoxy-octadecanoate isomers	6.2 (9 and 11 isomers)	6.2 (8 and 10 isomers)

[a]The amounts were calculated by assigning 100 to total ion current of the main GC peak, n. 56. All other values are reported as relative area of peak compared to the area of peak, n. 56.
[b]trace = <0.2. See Table 1 for abbreviations.

OOH and the methyl 8-hydroperoxy-Δ^{10}-octadecenoate (8-OOH) isomers (3,4). Similarly, the decomposition products of the 9-OOH isomer correspond to those obtained from 9-OOH and methyl 11-hydroperoxy-Δ^{9}-octadecenoate (11-OOH) (Fig. 2B), by decomposition of their corresponding hydroperoxy (4) and alkoxy radicals (3,4). These results indicate an extensive isomerization of each hydroperoxy isomer that can be explained by 1,3-migration of the hydroperoxy or peroxy groups, followed by the production of an hydroperoxy isomer or an alcohoxy isomer and the fragmentation of the corresponding hydroperoxy and alkoxy radicals. This assumption agrees, in fact, with those proposed by Frankel *et*

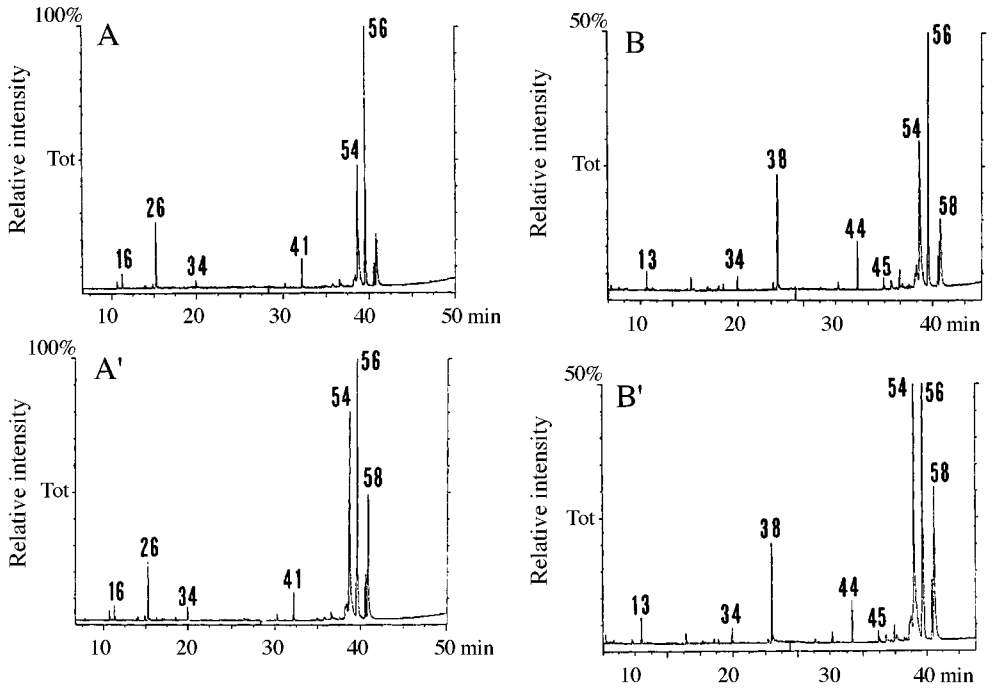

FIG. 3. GC-ITDMS profiles of thermal decomposition of 10-OOH and 9-OOH dissolved in methanol (A and B, respectively) and in *n*-hexane (A' and B', respectively), under the same experimental conditions. Peak numbers correspond to those shown in Figure 2 and Table 2. See Figure 2 for abbreviation.

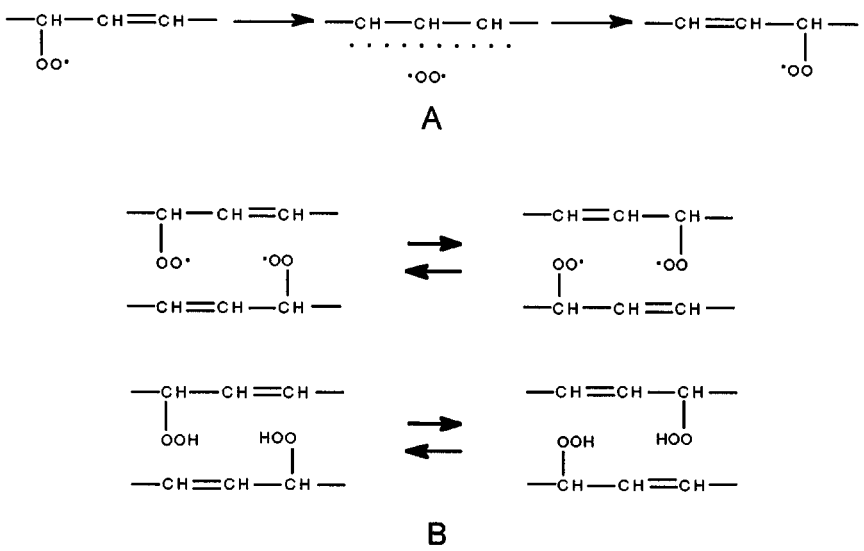

FIG. 4. A, reaction mechanisms proposed in the literature for the allylic isomerization of MOHP. B, reaction mechanisms proposed in this paper for the allylic isomerization of MOHP. See Figure 1 for abbreviation.

al. (10,11), Garwood *et al.* (12), Porter and Zuraw (13), and Grosch (2) for the thermal degradation of MOHP and by Chan *et al.* (14) for the thermal degradation of individual methyl linoleate hydroperoxides obtained via enzymatic oxidation of linoleic acid.

Table 3 reports the identification of the oxidation products having the same dimension of the original molecule of substrate (so-called "evolution" products), obtained by thermal degradation of the single hydroperoxides (Fig. 2). Like the decomposition products, the evolution products have molecular structures that correspond to those of the hydroperoxide isomers.

Figure 3 shows the GC-ITDMS profiles of the thermal decomposition of pure 10-OOH and 9-OOH dissolved in two solvents of opposite polarity (A and B, methanol; A' and B', *n*-hexane); the peak numbers are the same as those used in Figure 2 and Table 2. Peaks 54, 56, 57, and 58 correspond to the hydroxy-octadecenoate isomers, oxo-octadecenoate isomers, oxo-epoxy-octadecenoate isomers, and hydroxy-epoxy-octadecenoate isomers, respectively. These reaction products are, in fact, the monomeric components of hydroperoxide degradation (15–17). The amounts of these peaks in *n*-hexane were two times higher than those found in the methanol system, due to a solvent effect during thermal decomposition. This indicates that these components might be formed by a bimolecular mechanism, because no solvent effect is observed in those components that are generated by a monomolecular mechanism, such as the volatile fraction (peaks 16, 26, 34, and 41).

Thermal rearrangement of oleate hydroperoxides involving the allylic 3-carbon intermediates has been suggested by some authors (1,10–13) (Fig. 4, A). To explain the thermal rearrangement, the same 1–3 migration is assumed; however, interaction between two hydroperoxide molecules or radicals must occur (Fig. 4B).

The presence of such dimeric hydroperoxides can explain the formation of some oxidation evolution products (14–17), as indicated by the Russell's mechanism (18). At first, each purified MOHP seems to generate a considerable amount of the 1,3-isomer of the hydroperoxy group (5–20%, in these condi-

TABLE 4
Thermal Degradation Products (in condensed phase)[a] of the Two Purified MOHP: Residual Hydroperoxides and "Evolution Products"

	1 %	2 %	3 %	Mean %	Standard deviation
Hydroperoxide					
11-OOH Δ^9	8.6	25.3	21.6	18.5	8.8
10-OOH Δ^8	27.0	23.5	27.8	26.1	2.3
9-OOH Δ^{10}	24.4	30.8	27.1	27.4	3.2
8-OOH Δ^9	39.9	20.4	23.1	27.8	10.6
Total *cis* isomers	14.4	11.6	—[b]	13.0	2.0
Total *trans* isomers	85.6	88.3	—[b]	87.0	1.9
Hydroxy derivative					
11-OH Δ^9	26.6	28.3	19.7	24.9	4.6
10-OH Δ^8	24.3	26.6	33.5	28.1	4.8
9-OH Δ^{10}	27.9	38.3	37.0	34.4	5.7
8-OH Δ^9	21.1	6.9	9.8	15.9	4.7
Total *cis* isomers	21.3	14.8	11.8	16.0	4.9
Total *trans* isomers	78.3	85.2	88.2	83.9	5.1
Keto derivative					
11=O Δ^9	18.7	23.5	17.7	20.0	3.1
10=O Δ^8	27.5	20.7	27.6	25.3	4.0
9=O Δ^{10}	33.2	38.7	34.5	35.5	2.9
8=O Δ^9	20.4	17.1	20.2	19.2	1.9
Total *cis* isomers	3.3	—[b]	8.5	5.9	3.7
Total *trans* isomers	96.4	—[b]	91.5	94.0	3.5

[a]The amounts were calculated with the HPLC areas from the corresponding fraction analyses.
[b]Due to the small component amount, it was not possible to make a correct evaluation of the percentage. See Table 1 for abbreviation.

tions) by bimolecular isomerization; thereafter, the two isomers thermo-degrade to form all the identified degradation products (Table 4). Furthermore, the two pure MOHP isomers originated by singlet oxygen oxidation produced, *via* thermal degradation at high temperature in the condensed phase, generated all eight isomeric MOHP (Table 4). The amount of each positional isomer was similar to those obtained from thermal oxidation of methyl oleate. These results and the presence of *cis* and *trans* configurations support the assumption of singlet oxygen as a promoter of the first steps of the oxidation of food lipids.

ACKNOWLEDGMENT

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REFERENCES

1. Frankel, E.N., Volatile Lipid Oxidation Products, *Prog. Lipid Res. 22:*1–33 (1982).
2. Grosch, W., Reactions of Hydroperoxides—Products of Low Molecular Weight, in *Autoxidation of Unsaturated Lipids,* edited by H.W.-S. Chan, Academic Press, London, 1987, pp. 95–139.
3. Selke, E., E.N. Frankel, and W.E. Neff, Thermal Decomposition of Methyl Oleate Hydroperoxides and Identification of Volatile Components by Gas Chromatography–Mass Spectrometry, *Lipids 13:*511–513 (1978).
4. Lercker G., P. Capella, and L.S. Conte, Thermal Decomposition of Methyl Oleate Hydroperoxides, *Riv. Ital. Sostanze Grasse 61:*623–627 (1984).
5. Lercker, G., R. Bortolomeazzi, L. Pizzale, and S. Vichi, Thermal Degradation of Single Cholesteryl Acetate Hydroperoxide, *Chromatographia 42:*29–33 (1996).
6. Khan, N.A., Autoxidation, Part XXVIII, Hydroperoxide Isomers in Autoxidation of Methyl Oleate, *Oléagineux 19:*397–401 (1964).
7. Khan, N.A., Hydroperoxidation in Autoxidation: Part I. Simultaneous Oxygen Attack on Alpha Methylenic Group and Double Bond and Process of Hydroperoxidation, *Ibid. 20:*683–687 (1965).
8. Bortolomeazzi, R., L. Pizzale, and G. Lercker, Chromatographic Determination of Position and Configuration Isomers of Methyl Oleate Hydroperoxides, *J. Chromatogr. 626:*109–116 (1992).
9. Sweeley, C.C., R. Bentley, M. Makita, and W.W. Wells, Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances, *J. Am. Chem. Soc. 85:*2497–2507 (1963).
10. Frankel, E.N., W.E. Neff, and T.R. Bessler, Analysis of Autoxidized Fats by Gas Chromatography–Mass Spectrometry: V. Photosensitized Oxidation, *Lipids 14:*961–967 (1979).
11. Frankel, E.N., W.E. Neff, and E. Selke, Analysis of Autoxidized Fats by Gas Chromatography–Mass Spectrometry: VII. Volatile Thermal Decomposition Products of Pure Hydroperoxides from Autoxidized and Photosensitized Oxidized Methyl Oleate, Linoleate and Linolenate, *Ibid. 16:*279–285 (1981).
12. Garwood, R.F., B.P.S. Kambay, B.C.L. Weedon, and E.N. Frankel, Allylic Hydroperoxides from the Autoxidation of Methyl Oleate, *J. Chem. Soc. Chem. Comm.* 364 (1977).
13. Porter, N., and P. Zuraw, The Allylic Rearrangement of Hydroperoxides: Oxygen Entrapment of the Proposed Carbon Radical Intermediate, *Ibid.* 1472–1473 (1985).
14. Chan, H.W.-S., G. Levett, and J.A. Mathew, The Mechanism of the Rearrangement of Linoleate Hydroperoxides, *Chem. Phys. Lipids 24:*245–256 (1979)
15. Lercker, G., P. Capella, and L.S. Conte, Thermo-Oxidative Degradation Products of Methyl Oleate, *Riv. Ital. Sostanze Grasse 61:*337–344 (1984).
16. Capella, P., M.F. Caboni, G. Bonaga, and G. Lercker, Meccanismi di Formazione dei Prodotti di Evoluzione Degli Idroperossidi Monoinsaturi: Idrossi-e Cheto-Esteri, *Ibid. 65:*629–631 (1988).
17. Gardner, H.V., Reactions of Hydroperoxides—Products of High Molecular Weight, in *Autoxidation of Unsaturated Lipids,* edited by H.W.-S. Chan, Academic Press, London, 1987, pp. 51–93.
18. Russell, G.A., Deuterium-Isotope Effects in the Autoxidation of Aralkyl Hydrocarbons. Mechanism of the Interaction of Peroxy Radicals, *J. Am. Chem. Soc. 79:*3871–3877 (1957).

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