Products of the Surface Oxidation of Methyl Linoleate

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ABSTRACT: Methyl linoleate with 5% by weight of methyl palmitate as an internal standard was deposited as a monolayer (20% by weight) on Silica gel H and oxidized at 7, 25, and 40°C. Oxidation was followed by iodometric PV practiced directly on aliquots of the silica gel. Lower temperatures gave higher PV maxima but after longer times. Oxidation of methyl linoleate at all three temperatures followed first-order kinetics, and the energy of activation was 15.0 kcal/mol. The products recovered from the chloroform–acetic acid layer of the peroxide determination were analyzed by GC and identified by EI– and CI–MS. Calculation based on methyl palmitate as an internal standard showed that the total peak area decreased to about 40% that of the original methyl linoleate when the residual methyl linoleate was reduced to less than 2%. The chief nonscission products (NSP) of linoleate oxidation were epoxy, hydroxy, hydroxy–epoxy, dihydroxy, and trihydroxy methyl esters. The greatest NSP concentrations were obtained about the time of the greatest PV, and the yield at 40°C was greater than those at 7 and 25°C. Scission products (SP) increased rapidly until the greatest PV was reached. After this time, SP declined slightly and plateaued at 40°C, but at 7 and 25°C, SP continued to increase slowly with further oxidation.

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Lipid oxidation at ambient temperatures in the bulk liquid state is too slow, especially in the presence of antioxidants, to be considered as a commercial process for producing oxygenated products. At elevated temperatures oxidation is accompanied by rapid decomposition of the peroxides into a variety of products. Various studies (1–8) have shown that the rate of oxidation is greatly accelerated when FA molecules are adsorbed on silica gel at a monolayer concentration, which for silica gel is about 20% by weight of oil. The rate for monolayer oxidation is 8–10 times greater than that in bulk, and this accelerated rate has been attributed to the favorable alignment of the FA chain and ready access to oxygen (3). Monolayers of pure linoleic acid have been reported to oxidize without an observable induction period, but the addition of antioxidants greatly reduces the rate of oxygen uptake and causes an induction period (3,7). Oxidation kinetics of PUFA in monolayers follow first-order kinetics (4,6), but the addition of substantial amounts of saturated FA, especially lauric, myristic, and palmitic acids, decreased the rate of disappearance of linoleic acid. Pure oleic acid hardly oxidized as a monolayer.

The oxidation products of pure linoleic acid monolayers at 60°C were quite different from those of bulk-phase oxidation. The main products of monolayer oxidation were 9,10- and 12,13-epoxyoctadecenoic acids in a ratio of about 1.3:1 based on the size of the m/z 213 to 217 peaks in the mass spectra of trimethylsilyl derivatives (6). The epoxy groups were about 89% *cis* and 11% *trans* based on GC retention times. The unepoxidized double bond was regarded as *cis*. Epoxide concentrations were reduced and the concentration of hydroxy–epoxides was increased by including increasing proportions of palmitic acid in the mixture (8).

Surface oxidation of FA and their methyl esters on silica gel has been studied mostly as a model of oxidation in biological membranes; this technique has never been studied seriously as a means of producing oxygenated fats and oils. However, economically useful products could be produced on a commercial scale by the surface oxidation of polyunsaturated oils. For example, epoxy FA, which are a major product of surface oxidation, are used as plasticizers and are produced commercially by peracetic acid oxidation of oils. Hydroxy FA might be a substitute for castor oil in surface coatings. The aim of this study was to document and quantify the monolayer products of methyl linoleate oxidation at various temperatures.

MATERIALS AND METHODS

Materials. Methyl linoleate, methyl oleate, methyl palmitate, *cis*-9,10-epoxy-12-hydroxy stearate (EPHS), *cis*-9,10-epoxy stearic acid (*c*EPS), and *trans*-9,10-epoxy stearic acid (*t*EPS) were purchased from Sigma Aldrich Co. (St. Louis, MO). The *c*EPS and *t*EPS were converted to methyl esters with diazomethane by the method of Ngan and Ikesagi (9). Methyl ricinoleate (methyl 12-hydroxy-*cis*-9-octadecenoate) was obtained by TLC purification of methyl esters produced from castor oil using Silica gel GF plates developed with hexane/diethyl ether 8:2 (vol/vol). Methyl vernolate (methyl *cis*-12,13-epoxy octadec-9-enoate) was prepared similarly from the methyl esters of *Vernonia galamensis* seed lipids using hexane/diethyl ether 3:1 (vol/vol). The 9,10-dihydroxystearic and 9,10,12-trihydroxystearic acids were prepared by oxidation of oleic and ricinoleic acids with potassium permanganate (10). Methyl 9-oxo-nanonoate was obtained by ozonization of methyl linoleate (11).

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Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Silica gel H having a grain size of 10–40 μm and no binder were purchased from Sigma Aldrich. Analysis of the silica gel for iron by atomic absorption spectroscopy was performed by the Diagnostic Laboratory at Iowa State University. Sodium methoxide was purchased from Fluka Chemical Corp. (Milwaukee, WI). Silica gel GF plates (500 μm) were purchased from Alltech Associates Inc. (Deerfield, IL). All other chemicals and solvents were analytical grade and purchased from Fisher Scientific (Pittsburg, PA).

Methods. For the preparation of monolayers, 3.00 g of methyl linoleate and 158 mg of methyl palmitate, as an internal standard, were dissolved in 50 mL hexane and added to 12.00 g of Silica gel H (5). After stirring for 2 min, the hexane was evaporated under reduced pressure, and oxidation was performed at 7, 25, or 40°C. During the oxidation, 1-g samples were withdrawn and subjected without extraction to a PV test (12). Silica gel was removed from the chloroform–acetic acid layer by filtration, and the filtrate was washed three times with ~30 mL of distilled water to remove acetic acid. The chloroform layer was dried with anhydrous sodium sulfate.

After reduction with hydriodic acid (HI), the products were injected into a gas chromatograph (Hewlett-Packard 5890 Series II, Palo Alto, CA) fitted with an FID and a 30-m HP-5 capillary column (Chrom Tech, Apple Valley, MN). Helium was the carrier gas at 2 mL/min. The temperature was programmed from 40 to 220°C at 10°C/min and held at 220°C for 20 min. Injector and detector temperatures were programmed at 300°C.

The reduced oxidation products of methyl linoleate were also fractionated on Silica gel GF plates using hexane/diethyl ether 3:1 (vol/vol). EI– and CI–GC/MS determination of the fractions was done with a Micromass GCT (Micromass UK Ltd., Altrincham, United Kingdom) fitted with a 30-m DB-5 column (J&W Scientific, Folsom, CA). The temperature program was similar to that described in the previous paragraph. Ammonia was used as the CI reagent gas.

Trimethylsilyl ether (TMS) derivatives of hydroxy compounds were prepared according to Jensen *et al.* (13) using BSTFA.

RESULTS AND DISCUSSION

The iron content of the Silica gel H was 77 ppm, which is considerably lower than the 200 ppm reported for Silica gel H by Wu and Mead (5). Methyl linoleate containing 5% by weight of methyl palmitate on Silica gel H near monolayer concentration was oxidized at 7, 25, and 40°C. Weight recoveries of the oxidized material from the chloroform layer of the PV determinations were nearly 100% of the expected value. Figure 1 shows the change in PV with time. The PV of the samples reached their maximal values in shorter times as the temperature increased, but lower temperatures gave greater PV maxima, probably because the hydroperoxides survived better at the lower temperatures. Peroxide decomposition after reaching the PV maxima also was slower at lower temperatures. Figure 2 shows that the disappearance of
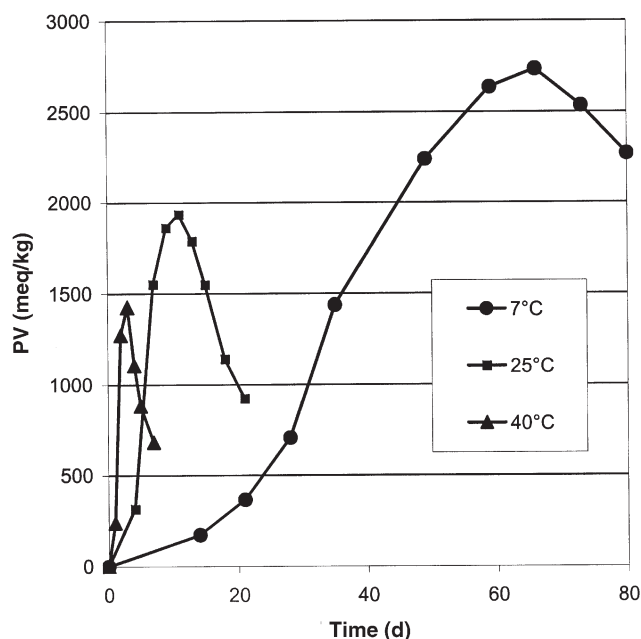

FIG. 1. Change in PV of monolayers of methyl linoleate with time at various temperatures.
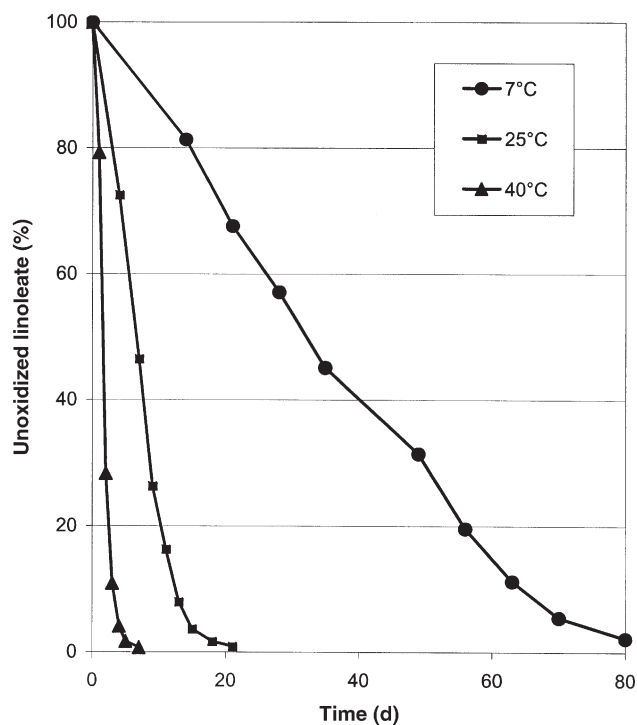

FIG. 2. Decrease in the percentage of methyl linoleate with time at various temperatures during monolayer oxidation.

methyl linoleate was slower the lower the temperature. In contrast to previous reports of oxidation at 60 and 80°C (3,6), there seemed to be a slight induction period, especially at 7 and 25°C, even though no antioxidant was present. The
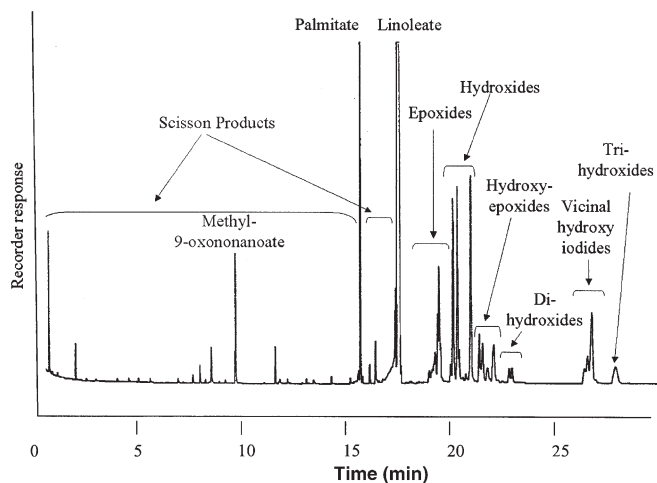
FIG. 3. A typical gas chromatogram of the oxidation products of methyl linoleate produced during monolayer oxidation.

linoleate decrease at all three temperatures fit first-order kinetics, as others have noted (4,6). The rate constants were calculated as 0.046, 0.244, and 0.780 min^{-1} for 7, 25, and 40°C, respectively. The activation energy of the reaction was 15.0 kcal/mol. This value was similar to that for oxidation of oil in bulk (14).

Pure methyl oleate was not noticeably oxidized at 40°C for 60 d, but 7% of the methyl oleate in methyl linoleate oxidized at 7, 25, and 40°C, with the rates of 0.137, 0.141, and 0.174 min^{-1}, respectively.

Oxidation products of methyl linoleate recovered from the chloroform extraction after HI reduction were analyzed by GC; Figure 3 represents a typical chromatogram. Since external standards were not available for many of these compounds, the identifications were based mainly on the M.W. of the compounds obtained by MS, their R_f on TLC, and the GC retention times of similar standards.

From the EI mass spectra of the isomers of epoxy methyl esters (E-FAME) (M.W. 310), a small protonated molecular signal (MH) was obtained at m/z 311. Other fragments were at 293 (M − OH) and 279 (MH − CH$_3$O). The CI spectra gave two strong peaks at m/z 328 (MH + NH$_3$) and 311 (MH). The R_f values of the epoxy isomers on silica gel TLC were at 0.42, and their retention times on GC were between 18.8 and 19.4 min. These findings were comparable to those of the reference standards (cEPS, tEPS, and methyl vernolate).

Three main isomers of hydroxy methyl esters (H-FAME) were obtained after iodide reduction of their hydroperoxides. EI–MS of these gave strong peaks at m/z 292 (M − H$_2$O) and smaller peaks at m/z 293 (M − OH), and there were two strong signals on CI–MS at m/z 310 (M) and 293 (M − OH). Retention times of these isomers on GC were 19.96, 20.16, and 20.74 min. Their R_f on silica TLC was 0.21, comparable to that of methyl ricinoleate. Furthermore, the hydroxy methyl esters reacted with BSTFA to give TMS derivatives with altered GC retention times.

Hydroxy–epoxy FA have been reported as products of the

monolayer oxidation of linoleic acid (8). GC showed four main isomers of the hydroxy–epoxy methyl esters (HE-FAME). EI–MS gave strong peaks at m/z 309 (M − OH), 291 [M − (OH + H$_2$O)], and 277 [M − (H$_2$O + CH$_3$O)]. CI gave three strong peaks at m/z 344 (M + NH$_4$), 326 (M), and 309 (M − OH). The R_f of these compounds on silica TLC was 0.08, and retention times on GC were between 20.95 and 21.96 min. EPHS was used as a reference standard to interpret the CI–MS, GC, and TLC data of HE-FAME. The HE-FAME also reacted with BSTFA and gave altered retention times.

Dihydroxy FAME (DH-FAME) were more polar than HE-FAME and barely eluted above the origin on TLC (R_f 0.03). On EI–MS they gave the same peak as HE-FAME, at m/z 309 (M − OH), and reacted with BSTFA. Their retention times were longer than those of HE-FAME. 9,10-Dihydroxy stearate was used as a reference standard for GC and TLC.

The compounds with penultimate GC retention times were vicinal hydroxy iodides, presumably formed by the reaction of epoxy esters with HI during reduction. Epoxy esters were regained by reacting these compounds with sodium methoxide in methanol. Methyl vernolate, subjected to HI reduction for 1 min under the conditions used for PV determinations, was 90% converted to compounds having similar GC retention times, and 100% of these products were converted back to methyl vernolate by reaction with 1 N NaOCH$_3$ for 1 h at room temperature. In addition, the peaks representing *vic* hydroxy iodides were not found when sodium bisulfite was used to reduce the hydroperoxides instead of iodide.

The last GC peak in Figure 3 is possibly an isomer of trihydroxy esters (TH-FAME). It was obtained from the origin of the TLC plate and gave an altered retention time after reaction with BSTFA. Trihydroxy stearate was used as a reference standard for GC.

Several major scission products (SP) were either identified by using a reference standard such as methyl 9-oxo-nanonoate and/or tentatively identified by the M.W. obtained from their EI– and CI–MS data. They were octanol (M.W. 130), methyl 8-oxooctanoate (M.W. 172), methyl 9-oxononanoate (M.W. 186), methyl 10-oxodecanoate (M.W. 200), and methyl 12-oxo-hydroxydodecenoate (M.W. 242). Most of the SP disappeared after reaction with sodium methoxide, probably because they had a carbonyl group.

Contrary to Wu *et al.* (6), who reported that the oxidation of methyl linoleate was accompanied by its conversion to FFA, we found negligible amounts of FFA among the oxidation products.

Monolayer oxidation products of linoleate at different intervals during oxidation were quantified; the amounts of the chief nonscission products (NSP) (E-, H-, HE-, DH-, and TH-FAME), total NSP, and total SP at 7, 25, and 40°C are presented in Tables 1–3, respectively. NSP comprised compounds having longer retention times than methyl linoleate, whereas SP represented the compounds having shorter retention times than methyl linoleate with the exception of methyl palmitate.

Calculations were made based on the percentage of methyl palmitate, which was originally 5% and increased to 11.03,

TABLE 1
Changes in PV, Percentage of Methyl Linoleate[a], and Percentage of Oxidation Products[a] of Methyl Linoleate During Surface Oxidation at 7°C

	Time (d)									
	0	14	21	28	32	49	59	66	73	80
PV (meq/kg)	—	172	367	707	1437	2239	2635	2736	2535	2268
Linoleate	100	81.31	67.58	57.05	45.10	31.42	19.58	11.26	5.45	2.21
E-FAME[b]	—	1.44	3.44	6.49	6.84	7.75	7.29	7.15	7.23	5.83
H-FAME	—	1.89	3.69	6.67	9.66	13.60	12.37	10.94	6.28	2.71
HE-FAME	—	0.42	0.93	2.46	3.01	5.42	6.29	6.62	6.81	5.25
DH-FAME	—	0.06	0.34	0.43	0.82	0.77	0.67	0.55	0.57	0.47
TH-FAME	—		0.04	0.33	0.82	1.96	3.08	3.63	4.53	2.90
Total chief NSP	—	3.81	8.44	16.38	21.15	29.50	29.70	28.89	25.42	17.60
Total NSP	—	4.22	8.94	17.61	22.56	31.07	31.77	32.02	28.22	20.03
Total SP	—	0.30	0.76	2.60	3.76	8.82	12.11	15.94	18.96	19.92
Total	100	85.83	77.28	77.26	71.42	71.31	63.46	59.22	52.63	42.46
NSP – chief NSP	—	0.41	0.50	1.23	1.41	2.02	2.07	3.13	2.80	2.43
%Chief NSP/total NSP	—	90.30	94.40	93.00	93.80	94.90	93.50	90.20	90.10	87.90

[a]Calculated based on GC area relative to the area and concentration of methyl palmitate. GC correction factors for the oxidation products were 1.17 for epoxy-FAME (E-FAME); 1.58 for hydroxy-FAME (H-FAME); 2.66 for hydroxy–epoxy-FAME (HE-FAME); 2.20 for dihydroxy-FAME (DH-FAME); and 2.63 for trihydroxy-FAME (TH-FAME).
[b]Iodohydroxy compounds were calculated as epoxides. NSP, nonscission products; SP, scission products.

TABLE 2
Changes in PV, Percentage of Methyl Linoleate, and Percentage of Oxidation Products During Surface Oxidation at 25°C[a]

	Time (d)								
	0	4	7	9	11	13	15	18	21
PV (meq/kg)	—	317	1552	1863	1935	1789	1550	1141	923
Linoleate	100	72.25	46.49	26.31	16.30	7.97	3.69	1.73	0.94
E-FAME	—	9.90	8.16	7.45	8.07	8.41	6.62	5.43	4.54
H-FAME	—	6.20	10.38	9.97	7.00	5.23	2.38	1.24	0.91
HE-FAME	—	2.60	6.89	8.12	9.94	11.50	9.93	9.10	7.93
DH-FAME	—	1.50	1.43	1.18	0.96	1.15	1.15	1.07	0.94
TH-FAME	—	0.48	1.84	2.65	3.34	4.79	3.75	3.03	2.18
Total chief NSP	—	20.66	28.70	29.37	29.31	31.08	23.83	19.87	16.50
Total NSP	—	22.66	30.33	31.94	32.40	35.25	27.70	23.96	20.15
Total SP	—	2.33	6.08	9.81	15.63	16.60	17.48	18.71	18.82
Total	100	97.69	82.90	68.06	64.30	59.82	48.87	44.40	39.91
NSP – chief NSP	—	2.00	1.63	2.57	3.09	4.17	3.87	4.09	3.65
%Chief NSP/total NSP	—	91.20	94.60	92.00	90.50	88.20	86.00	82.90	81.90

[a]Footnotes and abbreviations are the same as in Table 1.

11.62, and 11.08% after 80, 21, and 7 d at 7, 25, and 40°C, respectively. Trials showed that methyl palmitate was stable during monolayer oxidation. At all temperatures, the total peak area including unoxidized linoleate, NSP, and SP decreased to about 40% at the end of the oxidation period. This decrease was probably caused by evaporation of some volatile SP, lower detector responses to various NSP, and the possible formation of polymers. However, no polymers could be detected by using the size-exclusion method of White and Wang (15) or by holding the GC at 320°C for 60 min. Detector responses were calculated for each chief NSP relative to methyl palmitate by using cEPH, the hydroxy band from TLC, EPHS, 9,10-dihydroxystearate, and 9,10,12-trihydroxystearate. When these factors were used, the total chief NSP concentrations at the highest points for 7, 25, and 40°C were found, i.e., 54.4, 63.7, and 103.1%.

The highest NSP concentration (63.7%) obtained at 40°C coincided with the maximum PV. The main components at this point were E-FAME (18.7%), HE-FAME (16.1%), and TH-FAME (8.5%). The highest NSP concentrations at 7 and 25°C were 32.0 and 35.2%, about half that observed at 40°C. This difference might have resulted from acceleration of the formation of E- and HE-FAME from hydroperoxides at higher temperatures. The main components at the highest NSP concentrations at 7 and 25°C were H-FAME (10.9%) and HE-FAME (11.5%), respectively.

The concentration of SP increased rapidly at all temperatures until the PV reached its maxima. At 40°C, SP then declined and changed little with further oxidation. At 7 and 25°C, SP continued to increase very slowly after the maximal PV was attained. These differences possibly reflect differences in the volatility of SP at various temperatures.

At all temperatures, the largest NSP concentrations occurred near the time of the greatest PV. The highest percentages of these products were obtained at 40°C except for H-FAME, which reached a higher concentration at 7 than at 40°C.

TABLE 3
Changes in PV, Percentage of Methyl Linoleate, and Percentage of Oxidation Products During Surface Oxidation at 40°C[a]

	Time (d)						
	0	1	2	3	4	5	7
PV (meq/kg)	—	239	1274	1428	1107	883	683
Linoleate	100	79.32	28.35	10.94	4.16	1.71	0.70
E-FAME	—	6.85	15.58	18.74	6.72	6.23	5.13
H-FAME	—	4.10	12.55	6.20	1.44	1.29	1.38
HE-FAME	—	2.88	13.48	16.13	9.42	9.01	7.81
DH-FAME	—	1.21	3.66	2.77	2.06	2.07	2.00
TH-FAME	—	0.33	6.28	8.49	2.61	2.68	2.47
Total chief NSP	—	15.37	51.55	52.33	22.25	21.28	18.79
Total NSP	—	16.77	56.36	63.65	26.04	27.41	25.63
Total SP	—	2.63	13.65	22.71	14.49	14.92	15.13
Total	100	98.72	98.20	97.30	44.69	44.04	41.46
NSP – chief NSP	—	1.40	4.81	11.32	3.79	6.13	6.84
%Chief NSP/total NSP	—	91.70	91.50	82.20	85.40	77.60	73.30

[a]Footnotes and abbreviations are the same as in Table 1.

All the previous studies of monolayer oxidation of which we are aware have used silica gel as a support. However, silica gel may be unique in the products that it forms. Preliminary results at 25°C of the oxidation of methyl linoleate, 25% by weight on bleaching earth and Celite 503 and 20% on activated alumina, were all faster than that on silica gel. Maximal PV were obtained after 12 h on bleaching earth, 3 d on Celite 503, and 4 d on activated alumina. These PV were 1019, 3025, and 1116 meq/kg oil, respectively. Unlike on silica gel, H-FAME was the main component, with small amounts of epoxy esters. Although it is uncertain how close to the monolayer concentration these samples were, their rapid oxidation rates suggest that they were near this value. These faster rates were possibly caused by contamination of the supports with transition metal catalysts, which catalyzed the decomposition of hydroperoxy esters and discouraged the formation of the epoxy ester products. It also is possible that the high yield of epoxy esters with silica gel depended in some way on its unique surface.

ACKNOWLEDGMENTS

REFERENCES

1. Honn, F.J., I.I. Bezman, and B.F. Daubert, Autoxidation of Drying Oils Adsorbed on Porous Solids, *J. Am. Oil Chem. Soc. 28*:129–133 (1951).
2. Porter, W.L., L.A. Levasseur, J.I. Jeffers, and A.S. Henick, UV Spectrophotometry of Autoxidized Lipid Monolayers While on Silica Gel, *Lipids 6*:16–25 (1971).
3. Porter, W.L., L.A. Levasseur, and A.S. Henick, Effects of Surface Concentration, Metals, and Acid Synergists on Autoxidation of Linoleic Acid Monolayers on Silica, *Ibid. 7*:699–709 (1972).
4. Slawson, V., A.W. Adamson, and J.F. Mead, Autoxidation of Polyunsaturated Fatty Esters on Silica, *Ibid. 8*:129–134 (1973).
5. Wu, G.-S., and J.F. Mead, Autoxidation of Fatty Acid Monolayers Adsorbed on Silica Gel I: Nature of Adsorbtion Sites, *Ibid. 12*:965–970 (1977).
6. Wu, G.-S., R.A. Stein, and J.F. Mead, Autoxidation of Fatty Acid Monolayers Adsorbed on Silica Gel II: Rates and Products, *Ibid. 12*:971–978 (1977).
7. Porter, W.L., L.A. Levasseur, and A.S. Henick, Evaluation of Some Natural and Synthetic Phenolic Antioxidants in Linoleic Acid Monolayers on Silica, *J. Food Sci. 42*:1533–1535 (1977).
8. Wu, G.-S., R.A. Stein, and J.F. Mead, Autoxidation of Fatty Acid Monolayers Adsorbed on Silica Gel III: Effects of Saturated Fatty Acids and Cholesterol, *Lipids 13*:517–524 (1978).
9. Ngan, F., and T. Ikesaki, Determination of Nine Acidic Herbicides in Water and Soil by Gas Chromatography Using an Electron-Capture Detector, *J. Chromatogr. 537*:385–395 (1991).
10. Kass, J.P., and S.B. Radlove, The Diastereoisomerism of the 9,10,12-Trihydroxystearic Acids and the Geometric Configurations of Ricinoleic and Ricinelaidic Acids, *J. Am. Chem. Soc. 64*:2253–2257 (1942).
11. Hammond, E.G., W.C. Ault, E. Heftmann, H.K. Mangold, N. Pelick, H. Schlenk, and R.J. Vander Wal, Lipid and Related Compounds, in *Specifications and Criteria for Biochemical Compounds*, edited by D.L. MacDonald, National Academy of Sciences, Washington, DC, 1972, pp. 119–147.
12. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, American Oil Chemists' Society, Champaign, 1989, Method Cd 8-53.
13. Jensen, C.B., B. Jensen, K. Mathiasen, and J. Mollerup, Analysis of Seed Oil from *Ricinus communis* and *Dimorphotheca pluvialis* by Gas and Supercritical Fluid Chromatography, *J. Am. Oil Chem. Soc. 74*:277–284 (1997).
14. Walstra, P., Reaction Kinetics, in *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003, pp. 59–86.
15. White, P.J., and Y.-C. Wang, A High-Performance Size-Exclusion Chromatographic Method for Evaluating Heated Oils, *J. Am. Oil Chem. Soc. 63*:914–920 (1986).