palm oil (6-8) and also found to stabilize β′-form in hydrogenated rapeseed oil (9,10). The literature on their effect in other fats, especially confectionery hard butters, is scanty.

The above results show that diglycerides of sal fat either inhibited or delayed the phase transition of all the crystal forms of TG from their lower melting crystal forms to the next higher melting forms. The delay in phase transition and stability of lower melting crystal forms was more pronounced with the increase in DG level from 5 to 15%. The effect of DG in delaying the phase transition of forms I → II → III was more pronounced at higher rates of heating. DG at 5 and 10% levels also were found to delay the solid-solid transition of lower melting crystal forms (I, II, III) to higher melting crystal form IV at supercooled temperatures (0 C), while at the 15% level, they virtually inhibited the phase transition of form I to higher polymorphs of TG. DG also delayed the transition of form IV to V even after tempering. DG, therefore, could have a beneficial effect in producing a smooth consistency in margarine, whereas they have a somewhat deleterious effect in the manufacture of chocolate because they affect the quality of the product.

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REFERENCES

1. Bhambhani, T.R., A.D. Shitole and J.G. Kane, *J. Oil Technol. Assoc. India 4*:3 (1972).
2. *J. Oil Technol. Assoc. India 6*:32 (1974).
3. Mani, V.V.S., and G. Lakshminarayana, *Fette, Seifen, Anstrichm. 72*:434 (1970).
4. Yella Reddy, S., and J.V. Prabhakar, *J. Am. Oil Chem. Soc. 62*:1126 (1985).
5. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th Edition, Association of Official Analytical Chemists, Washington, DC, 1980, p. 461.
6. Okiy, D.A., *J. Sci. Food Agric. 28*:955 (1977).
7. Okiy, D.A., *Oleagineux 33*:625 (1978).
8. Berger, K.G., in *Lipids*, Vol. 2, edited by G.J. Paoletti and G. Porcellati, Raven Press, New York, 1979, p. 323.
9. Hernquist, L., B. Herslof, K. Larsson and O. Podlaha, *J. Sci. Food Agric. 32*:1197 (1981).
10. Hernquist, L., and K. Anjou, *Fette, Seifen, Anstrichm. 85*:64 (1983).
11. Bringi, N.V., F.B. Padley and R.E. Timms, *Chem. Ind. 805* (1972).

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Radiolysis of Lipids in Monolayers. I. Saturated Fatty Acids

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In order to study the effect of molecular orientation on the behavior of lipids when exposed to high energy radiation, model systems of palmitic acid or ethyl palmitate adsorbed as monolayers on silica were irradiated with ^{60}Co at 25 Mrad under vacuum, and the volatile products compared with those of control samples irradiated in bulk. Major quantitative differences were observed. More of the C_{n-1} alkane relative to the shorter-chain members of the homologous series were formed in bulk samples as compared to samples in monolayer. The C_{n-2} alkene and C_n aldehyde also were formed in greater quantities in bulk. These observations are explained on the basis of a reduced preferential cleavage near the carbonyl group and a restricted mobility of free radical intermediates, in the case of the monolayers.

The effects of irradiation on fatty acids, esters, triacylglycerols, natural fats and fat-containing foods have been reported earlier (1-4). General mechanisms were deduced largely from irradiation of bulk lipids in the liquid phase where the molecules are much less organized than in the solid state or in biological membranes. It has been proposed that the primary event in the radiolysis of oxygen-containing compounds is the loss of a non-bonding electron from an oxygen atom, with the result that the unpaired electron is highly localized on the oxygen atom (5). The products which arise from irradiation of the lipids in complex foods are qualitatively similar to those formed from the irradiation of natural fats in bulk (6).

In biological systems, the lipid molecules usually exist with a high degree of order as, for example, the bilayer arrangement in cell membranes. The question arises whether products resulting from irradiation of bulk liquids differ qualitatively or quantitatively from those in the ordered state. An understanding of the specific mechanisms by which these differences may arise would be necessary in the extrapolation of results from one situation to another. Since the study of ordered lipid molecules as they exist in biological membranes is extremely complex, pure fatty acid and fatty acid esters adsorbed on silica were used as a model system to investigate the effect of molecular orientation on the radiolysis of lipids. The nature of adsorption of lipid molecules on silica has been reported in an earlier publication (7).

MATERIALS AND METHODS

Materials. The substrates palmitic acid and ethyl palmitate were purchased in the highest available purity from Sigma Chemical Co., St. Louis, Missouri. These were used without further purification. Silica gel G was purchased from Applied Science Laboratories, State College, Pennsylvania. It had a particle size of 10-40 μ.

Preparation of monolayers. Lipid monolayers were prepared according to the procedures described by Porter and coworkers (8), with minor modifications. More than

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the saturation amount of each substrate (2 g 16:0 acid and 16:0 EE) in 100 ml pentane was added to 6 g silica and stirred for 1 hr to achieve equilibrium. Substrate concentration in the supernatant was determined gravimetrically after solvent evaporation. From the initial and final concentration, the amount of substrate adsorbed per g silica was determined.

The specific surface area for the silica gel G used in this study was 335 m/g (determined by Pacific Sorption Service, Chico, California). The coverage ration for ethyl palmitate was 0.41 mmoles/g silica.

Irradiation treatment. One-gram samples of substrate, either adsorbed on silica or in bulk, were sealed in glass tubes (1.2 cm i.d. × 30 cm) under vacuum (0.01 torr), and then irradiated with gamma rays from a ^{60}Co source. Each sample received a dose of 25 Mrad (10 rads/hr) at 20 C. This dosage, which far exceeded the amount usually used in the irradiation of food, was used to facilitate the detection and identification of the small amounts of radiolytic products produced. Preliminary data showed that there is practically no qualitative difference between radiolytic compounds obtained at low and high doses (9). After irradiation, the samples were stored at −20 C until analyzed.

Collection of volatiles. Volatiles were collected by cold-finger distillation as described by Nawar et al. (10). Each sample was distilled under vacuum for 1 hr at 80 C. To minimize peak overlap in subsequent GC analysis, the distillate was fractionated into polar and nonpolar fractions. The cold finger was rinsed with 25 ml of pentane and the solution added to 5 g of silica gel which had been activated at 110 C. The mixture was stirred for 25 min, then filtered using a Buchner funnel with a scintered glass plate. The silica was washed with 10 ml of pentane and the filtrates combined. This filtrate contained the nonpolar compounds. Polar compounds were removed by washing with 25 ml of diethyl ether after rinsing with 100 ml of pentane to remove any traces of nonpolar compounds. In some experiments, the polar fractions were obtained by extraction with acetone. Both fractions were concentrated slowly under nitrogen to about 0.1 ml for further analysis.

Analysis of volatile decomposition products: Gas chromatography (GC). The gas chromatograph used was a Perkin-Elmer model 3920 B equipped with a flame ionization detector. The temperature was programmed from 60 C to 200 C at 4 C/min. A carbowax 20M capillary column (500 ft × 0.02 i.d.) with a carrier gas flow rate of 8 ml/min was used. Quantitation of the major peaks was carried out by the use of internal standard. Radiolytic products were identified by gas chromatography-mass spectrometry (GC-MS). Heptadecane and 10-undecenal were used as internal standards for the quantitation of the major peaks. Twenty μl of a 1% solution of each were added after irradiation and before distillation. The recovery of these compounds was approximately 95%.

RESULTS AND DISCUSSION

The major volatile radiolytic decomposition products from ethyl palmitate in both bulk and monolayers (Tables 1 and 2) were series of alkanes and alkenes, A C_n aldehyde, n being the number of carbon atoms in the substrate fatty acid, and a series of short-chain ethyl esters, as typical of radiolytic decomposition (1). Because the major differences between monolayers and bulk samples were observed in the nonpolar fractions, for palmitic acid only the nonpolar fractions were quantitated (Table 3). In the case of the bulk samples, the C_{n-1} alkane was the major hydrocarbon, the C_{n-2} alkene was the most abundant of the alkenes and the C16 aldehyde was the only major aldehyde. In monolayers, however, striking quantitative differences can be observed. First, less of the C_{n-1} alkane, relative to the shorter-chain members of the homologous series, were formed in monolayer as compared to bulk samples. Second, much less of the C_{n-2} alkene and C_n aldehyde were formed in monolayer compared to bulk samples. Third, more shorter-chain alkanes

TABLE 1

Nonpolar Compounds (μmol/g) Produced by Irradiation of Ethyl Palmitate at 25 Mrad

	In bulk		Adsorbed on silica	
	Mean[a]	AD[b]	Mean	AD
n-Alkanes				
C9	3.8	0.15	6.3	0.80
C10	1.9	0.15	4.6	0.25
C11	1.6	0.05	4.0	0.20
C12	1.1	0.05	2.6	0.10
C13	0.7	0.05	1.2	0.05
C14	0.8	0.05	1.5	0.05
C15	18.6	1.20	12.7	0.65
1-Alkenes				
C14	2.6	0.20	0.5	0.05
C15	0.9	0.05	0.2	0.05

[a]Mean from 2 determinations.
[b]Average deviation from the mean.

TABLE 2

Polar Compounds (μmol/g) Produced by Irradiation of Ethyl Palmitate at 25 Mrad

	In bulk		Adsorbed on silica	
	Mean[a]	AD[b]	Mean[a]	AD[b]
Ethyl esters				
C4	0.4	0.05	0.3	0.04
C5	0.4	0.06	0.4	0.07
C6	0.3	0.05	0.3	0.07
C7	0.4	0.02	0.3	0.04
C8	0.4	0.01	0.4	0.03
C9	0.4	0.02	0.3	0.02
C10	0.5	0.07	0.4	0.18
C11	0.4	0.06	0.3	0.05
C12	0.4	0.07	0.3	0.04
C13	0.4	0.07	0.4	0.07
C14	0.8	0.10	0.6	0.07
C15	0.3	0.04	0.6	0.13
Aldehydes				
C6	—	—	1.0	0.18
C16	5.0	1.08	1.2	0.21

[a]Mean from 2 determinations.
[b]Average deviation from the mean.

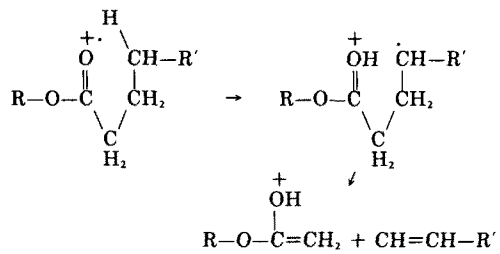
TABLE 3

Nonpolar Compounds (μmol/g) Produced by Irradiation of Palmitic Acid at 25 Mrad

	In bulk		Adsorbed on silica	
	Mean	AD	Mean	AD
n-Alkanes				
C9	1.6	0.30	5.7	0.50
C10	1.1	0.10	3.9	2.20
C11	0.4	0.10	2.1	0.80
C12	0.5	0.10	1.3	0.30
C13	0.6	0.30	0.5	0.05
C14	2.1	1.05	1.2	0.15
C15	41.9	9.95	12.0	2.10
1-Alkenes				
C14	4.6	1.10	0.6	0.20
C15	2.0	0.60	0.4	0.10

were produced in monolayer than in bulk samples. All of the above quantitative data seem to point toward a reduced preferential cleavage (i.e., near the carbonyl group) in the case of radiolysis in monolayers.

As reported earlier, the major products formed by irradiation are those resulting from preferential cleavage near the ester carbonyl group (1,4). For ethyl palmitate and palmitic acid, the C16 aldehyde (C_n), the C15 alkane (C_{n-1}) and the C14 alkene (C_{n-2}) were in the greatest quantities. Among the alkenes, it is the C_{n-2} and not the C_{n-1} which is the most abundant. This is presumed to be the result of a mechanism similar to that responsible for the MacLafferty rearrangement ion at m/e 60 in the mass spectra of fatty acids (4):



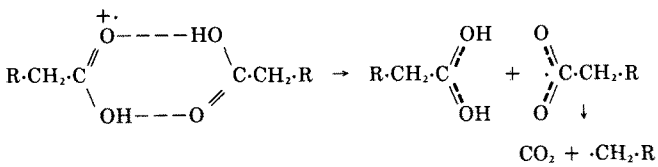
SCHEME 1

In addition to preferential cleavage near the carbonyl group, random cleavage of carbon-carbon bonds along the hydrocarbon chain produces the alkane and alkene homologous series with chain lengths shorter than C_{n-2}. These, however, are usually produced in relatively small quantities.

For palmitic acid and ethyl palmitate, hydrogen bonding between the non-bonding electrons of the carbonyl oxygen and silanol group on the silica surface plays a major role in monolayer adsorption (11). Such hydrogen-bonding produces a high degree of order, similar to that in crystals, in the portion of the monolayer chains closer to the silica surface (i.e., the carboxyl end of the molecule). Rates of diffusion in this region are relatively low. Complexation to silica lowers the yields of the C_{n-1} alkane and C_n aldehyde, most likely because radical pairs resulting from radiolysis do not diffuse away from one another so rapidly, thus allowing some recombination.

The lower yield of the C_{n-2} alkene in the case of monolayers is probably due to steric hindrance. The chains are confined to extended conformations, and a McLafferty type rearrangement is difficult. The fact that the acid and the ester produced similar amounts of the C_{n-2} alkene, in spite of the fact that the former binds more strongly to silica, indicates that the structure of the monolayer (i.e., packing of the chains) is similar for acid and ester, hence the similar steric effect. The reduced mobility of the substrate molecules on silica would not be expected to reduce the formation of short chain decomposition products which presumably result from random homolytic cleavage along the hydrocarbon chains. The shorter-chain alkanes were in fact produced more abundantly in the monolayer. On the other hand, the homologous series of ethyl esters was present in approximately the same amounts in both monolayer and bulk samples. It should be pointed out that an exact quantitative balance of the shorter-chain radiolytic products is extremely difficult to rationalize in view of the numerous possibilities of secondary radiolysis (12), free-radical recombination (13), and simultaneous formation of such compounds via more than a single mechanism.

Radiolytic decarboxylation of free fatty acids may be enhanced by the carboxyl-carboxyl associative dimeric condition which facilitates intermolecular hydrogen atom transfer to the electron-deficient oxygen atom, as proposed by Howton and Wu (14).



SCHEME 2

For ethyl palmitate, the lack of a donatable hydrogen atom inhibits the carboxyl-carboxyl dimer formation; thus, less radiolytic decarboxylation of ethyl palmitate can occur. This may explain why the amount of n−1 alkane formed from palmitic acid is much more than that of ethyl palmitate in bulk (Tables 1 and 3). In monolayers, however, no major quantitative differences were observed between ethyl palmitate and palmitic acid. This indicates that for the adsorbed substrates (acid or ester), carboxyl-carboxyl association is no longer of primary importance in the production of the C_{n-1} radiolytic product.

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REFERENCES

1. Dubravcic, M.D., and W.W. Nawar, *J. Am. Oil Chem. Soc. 45*:656 (1968).
2. Champagne, J.R., and W.W. Nawar, *J. Food Science 34*:335 (1969).

3. Nawar, W.W., *J. Agric. Food Chem. 26*:21 (1978).
4. LeTellier, P.R., and W.W. Nawar, *Ibid. 20*:129 (1972).
5. Williams, T.F., *Nature 194*:348 (1962).
6. Merritt, C. Jr., J.T. Walsh, M.L. Bazinet, R.F. Kramer and S.R. Bersnick, *J. Am. Oil Chem. Soc. 42*:57 (1965).
7. Hau, L.B., and W.W. Nawar, *J. Am. Oil Chem. Soc. 62*:1596 (1985).
8. Porter, W.L., L.A. Lavasewr and A.S. Henick, *Lipids 7*:699 (1972).
9. Vajdi, M., W.W. Nawar and C. Merritt Jr., *J. Am. Oil Chem. Soc. 59*:38 (1982).
10. Nawar, W.W., J. Champagne, M. Dubravcic and P. LeTellier, *J. Agric. Food Chem. 17*:645 (1969).
11. Marshall, K., and C.H. Rochester, *J. Chem. Soc. Faraday Trans. I. 71*:1754 (1975).
12. LeTellier, P.R., and W.W. Nawar, *J. Agric. Food Chem. 22*:693 (1974).
13. LeTellier, P.R., and W.W. Nawar, *J. Am. Oil Chem. Soc. 49*:259 (1972).
14. Howton, D.R., and G.S. Wu, *J. Am. Chem. Soc. 89*:516 (1967).

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High Performance Size Exclusion Chromatography of Fatty Acids, Mono-, Di- and Triglyceride Mixtures

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A high performance size exclusion chromatographic (HPSEC) method is described for the separation and quantitation of fatty acids, mono-, di- and triglyceride mixtures. The various lipid components were separated on two columns packed with 5 μm styrene/divinylbenzene copolymer and connected in series. Toluene was employed as eluant, and components were monitored by refractometry. A formula derived for calculation of total weighted correction factors (WCF) for the various lipid classes based on known values of correction factors of simple lipid components and the fatty acid composition of the sample allowed quantitation of lipid mixtures containing a variety of different molecules. The precision of the experiments is such that the relative standard deviation for each lipid component was 1-5%, and a component could be detected at 0.05% level.

Analytical methods based on liquid, thin layer and gas liquid chromatography are used to analyze mixtures of fatty acids and acylglycerols, in emulsifiers, polymer additives and in many other areas of chemistry and biochemistry. These methods permit satisfactory separation of such lipids, but quantitation is often tedious and inaccurate. In recent years high performance liquid chromatographic methods have been developed using polar column packings and nonaqueous gradient elution systems. However, the requirement for special types of detectors (1,2) as well as poor quantitation of unsaturated components (3) has limited their use.

The development of high resolution size exclusion chromatographic columns has allowed simple and rapid separation of low molecular weight compounds with relatively small differences in molecular weight. In this study we report the development of a method for the separation of methyl esters, mono-, di- and triglycerides by high performance size exclusion chromatography (HPSEC).

EXPERIMENTAL

High performance size exclusion chromatography: Apparatus. The chromatographic system consisted of a Tracor 995 Isochromatographic Pump (Tracor, Inc., Austin, Texas); a Rheodyne 7120 syringe loading sample injector with a 20 μl loop (Rheodyne, Berkeley, California), and a Waters Model 401 Differential Refractometer (Waters Associates, Framingham, Massachusetts). Chromatograms were recorded and peak areas determined using an HP 3390 A Integrator (Hewlett-Packard, Avondale, Pennsylvania).

Analyses were performed on a pair of LiChrogel PS_4 and LiChrogel PS_1 columns connected in series with the LiChrogel PS_4 column placed first (EM Science, Gibbstown, New Jersey). The columns were 25 cm × 0.7 cm ID, packed with spherical, styrene/divinylbenzene copolymer beads with an average particle size of 5 μm. The upper molecular weight exclusion limit was 5.10^3 daltons and 2.10^3 daltons for LiChrogel PS_4 and LiChrogel PS_1, respectively; 100 daltons was the lower exclusion limit for both columns.

Materials and reagents. Standards used for chromatographic studies were methyl esters, mono-, di- and triglycerides containing C-12:0 to C-18:2 fatty acids (Nu-Chek Prep., Inc., Elysian, Minnesota); Myverol 18-00 (Eastman Chemical Products, Inc., Kingsport, Tennessee), and safflower oil (Hollywood Health Foods, Los Angeles, California). The purity of standards was >99% as determined by GLC. Chromatographic solvents, toluene (A.C.S. grade, Fisher Scientific Co., Fair Lawn, New Jersey), tetrahydrofuran and dichloromethane (A.C.S. grade, MBC Manufacturing Chemists, Inc.,Cincinnati, Ohio) had been distilled in glass.

The sample concentration was 25 mg/ml in either toluene or tetrahydrofuran; 0.5 mg were injected onto the column with a 20 μl sample loop. All samples and eluants were pre-cleaned by passing them through a filter (<2 microns). Toluene was used as the eluant at a flow rate of 0.5 ml/min.

Quantitation. Actual correction factors were calculated for methyl esters, mono-, di- and triglycerides of C-16:0, C-18:0, C-18:1 and C-18:2 fatty acids. Monolaurin was used as the internal standard for mixtures containing components with C-16:0 to C-18:2 fatty acids. A mixture of methyl ester, mono-, di- and triglyceride of each acid was prepared gravimetrically, and a known amount of internal standard added. Each mixture was analyzed three times by the HPSEC method. Correction factors were

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