TABLE II

Richest Sources of Petroselinic Acid

Species family	% 18:1^6 in	% Oil	% 18:1^6 in seed
Bifora radians (Umbelliferae)	75	50	37
Bifora testiculata (Umbelliferae)	81	42	34
Aralia spinosa (Araliaceae)	71	46	33
Trachymene caerulea (Umbelliferae)	76	37	29
Dendropanax trifidus (Araliaceae)	83	34	28
Apium leptophyllum (Umbelliferae)	87	32	28
Garrya fremontii (Garryaceae)	81	34	28
Hedera helix (Araliaceae)	79	35	28
Acanthopanax spinosus (Araliaceae)	70	37	26
Eryngium nudicaule (Umbelliferae)	75	34	26
Crithmum maritimum (Umbelliferae)	67	37	25
Astrantia maxima (Umbelliferae)	53	38	25
Ferula galbaniflua (Umbelliferae)	52	47	25
Actinolema eryngioides (Umbelliferae)	60	40	24

REFERENCES

1. Placek, L.L., JAOCS 40:319 (1963).
2. Hilditch, T.P., and P.N. Williams, "The Chemical Constitution of Natural Fats," 4th Edn., John Wiley & Sons, Inc., New York, NY, 1964.
3. Lotti, G., and E. Bogan, Ric. Sci. 37:798 (1967).
4. Lotti, G., and E. Bogan, Ibid. 37:1005 (1967).
5. Privett, O.S., J. D. Nadenicek, R.P. Weber and F.J. Pusch, JAOCS 40:28 (1963).
6. Chobanov, D., E. Chooparova, A. Popov and D. Kamenova, Fette Seifen Anstrichm. 68:447 (1966).
7. Kleiman, R., V.L. Davison, F.R. Earle and H.J. Dutton, Lipids 2:339 (1967).
8. U.S. Department of Agriculture, Agricultural Statistics, 1979.
9. Manske, R.H.F., "The Alkaloids, Chemistry, and Physiology," Vol. XII, Academic Press, New York, NY, 1970.
10. Earle, F.R., E.H. Melvin, L.H. Mason, C.H. VanEtten, I.A. Wolff and Q. Jones, JAOCS 36:304 (1959).
11. Mikolajczak, K.L., T.K. Miwa, F.R. Earle, I.A. Wolff and Q. Jones, Ibid. 38:678 (1961).
12. Kleiman, R., G.F. Spencer and F.R. Earle, Lipids 4:118 (1969).
13. Miller, R.W., F.R. Earle, I.A. Wolff and Q. Jones, JAOCS 42:817 (1965).
14. Miwa, T.K., Ibid. 40:307 (1963).
15. Gunther, E., "The Essential Oils," Vol. IV, D. van Nostrand Co., Inc., Princeton, NJ, 1965, Chapt. 5.

[Received June 15, 1981]

Effects of Various Parameters on the Formation of Radiolysis Products in Model Systems

M. VAJDI and **W.W. NAWAR**, Department of Food Science, University of Massachusetts, Amherst, MA 01003, and **C. MERRITT, Jr.**, Food Sciences Laboratory, US Army Natick Research and Development Laboratories, Natick, MA 01760

ABSTRACT

Effects of various parameters on the formation of radiolysis products in tripalmitin, palmitic acid, oleic acid, caprylic acid and capric acid were investigated. A greater yield of primary and recombination products was observed in palmitic acid compared to that of oleic acid. In general, higher amounts of recombination products are formed in both acids when irradiated at room temperature than at -45 C. In the short chain acids, the yield of the hydrocarbon recombination product was enhanced in the liquid state. In contrast, the ketone recombination product was formed in greater amounts from solid capric acid than from liquid caprylic acid. In the absence of oxygen, a linear relationship was obtained between the dose and the yield of radiolysis products in tripalmitin. In the presence of oxygen, a considerable increase in the formation of γ-palmitolactone, n 3 and n 2 alkanes was observed at room temperature.

INTRODUCTION

Much of our knowledge regarding radiation chemistry of fats is based on model systems made from known compounds. Such systems have included free fatty acids (1), methyl esters (2) and triglycerides (3,4). The radiolytic behavior of these systems are similar and include such mechanisms as decarboxylation, dehydrogenation, polymerization and cleavage at all points of the alkyl chain.

Most of the early work on the effect of radiation in lipids was done in the presence of oxygen. Consequently, the changes taking place in the fat were partly the result of irradiation-induced oxidation (5). Little difference, however, between the radiolytic compounds identified under vacuum and in the presence of oxygen could be detected if analysis is conducted immediately after radiation. Nevertheless, storage of irradiated items in air has produced a variety of typical decomposition products (4).

In the previous publication, the effects of radiation parameters on the formation of some relatively small molecular weight (MW) volatile compounds have been reported (6). In the present study, the effects of various parameters (dose, temperature, oxygen, chemical nature and physical state) on the formation of the major primary and recombination radiolysis products in tripalmitin, palmitic acid, oleic acid, caprylic and capric acids were investigated.

EXPERIMENTAL PROCEDURES

Triglycerides and acids were purchased from ICN Pharmaceuticals Inc., Plainview, NY, and Applied Science Labs. Inc., State College, PA. Purity was established by gas chromatography/mass spectrometry (GC/MS). The tripalmitin was found to contain some dipalmitin and palmitic acid as impurities. These impurities were measured with the aid of internal standards and appropriate corrections were applied.

Irradiation treatment was done (Co^{60}-7.5 × 10^{4} rads/min)* at temperatures controlled by admitting the appropriate amount of liquid nitrogen gas through the use of a thermocouple sensor. Samples under vacuum and air were prepared at 5 μ and atmospheric pressure, respectively.

Fatty Acids

Experiment I. Analysis of fatty acids was done on 1 g of the irridiated samples. Palmitic and oleic acids were irradiated with a dose of 25 Mrads at (-45 C, VAC), (25 C, VAC) and (25 C, AIR).

Experiment II. Caprylic and capric acids analyses were done on 1-g samples subjected to 25 Mrads under vacuum at 25 C and 10 C, respectively. The samples were introduced to the KOH/silicic acid column chromatograph (7) and the ethyl ether eluate containing the radiolysis products was evaporated for GC/MS analysis.

Triglyceride

Experiment III. Analysis of radiolysis products formed in tripalmitin was done on 1 g of sample. Four samples were irradiated under vacuum and 25 C at doses of 5, 15, 25 and 30 Mrads.

Experiment IV. One-g samples of tripalmitin were subjected to 25 Mrads at (-45 C and VAC), (25 C and VAC) and (25 C and AIR).

A Waters Associates Inc. liquid chromatograph Model 201 equipped with 2-ml injection loop and refractive index detector was used for separation of the major component classes by means of steric exclusion chromatography (8). Appropriate fractions were collected and evaporated for GC/MS analysis. Gas chromatographic and mass spectrometric data were obtained on a 3% Dexil 400 column (5' × 1/8") and a system composed of a PE Model 3920 gas chromatograph coupled to a Dupont Model 21-491 double focusing mass spectrometer. Mass spectral data were acquired and analyzed using a data system employing HP Model 2116 and DEC PDP 15/76 computers.

RESULTS AND DISCUSSION

Effect of Temperature and Oxygen on the Radiolysis of Fatty Acids

This study was designed to determine the effects of temperature and oxygen on the formation of major primary radiolytic compounds and the recombination products of relatively high MW. As formation of long-chain alkanes and ketones is considerably greater for acids than triglycerides, palmitic and oleic acids were chosen for this study (experiment I).

The effect of temperature over a wide range (38 C and -196 C) on the radiolysis of aliphatic carboxylic acids has shown that the solid phase radiolysis of these acids is temperature-dependent (9). A greater yield of CO_2 in samples irradiated at 38 C compared to those at -196 C may suggest variations in the type and extent of bond homolysis (RCOOH) in these samples, respectively. Due to the higher yield of CO_2 (R· & COO) in samples irradiated at higher temperature, one would expect to observe a greater yield of R_2 recombination product. As seen in Table I, a greater increase in the yield of triacontane (R_2) was observed for the sample irradiated at 25 C (G -0.05) compared to that irradiated at -45 C (G =0.02). With respect to the ketone recombination product (RCOR), however, temperature did not play a significant role. The higher yield of 16-hentriacontanone (dipentadecyl ketone) than that of triacontane, from palmitic acid was observed for both temperatures. Similar findings were reported in the study of nongaseous products from the radiolysis of stearic acid (10), as a greater yield of 18-pentatriaconta 9,26-dienone (diheptadecenyl ketone) was observed compared to that of the hydrocarbon recombination product tetratriacontane. The authors suggested that the considerable disparity between the 2 recombination products presumably reflects not so much the relative likelihood of COO H and CO OH homolysis as the chances of both radicals surviving to combine instead of abstracting hydrogen from neighboring α-methylene groups.

Although similar behavior was observed for oleic acid (Table I), the considerable increase in the yield of recombination products such as tetratriacontadiene and diheptadecenyl ketone may not be solely attributed to the effect of temperature, obviously, the physical state of the product (solid/liquid) plays an important role in view of the dependence of free radical mobility on the physical state of the product. Similar behavior was observed in the radiolysis of crystalline oleic acid (11) as an increase in the yield of hydrocarbons greater than heptadecene was observed in the

TABLE I

Radiolysis Products from Fatty Acids (average of 2 trials)

Compound	25 Mrads (-45 C + VAC) (mg/g)	(G)	25 Mrads (25 C + VAC) (mg/g)	(G)	25 Mrads (25 C + AIR) (mg/g)	(G)
			Palmitic acid			
Pentadecane	19.2 ± 0.85	3.6	20.4 ± 0.5	3.8	21.4 ± 1.5	4.0
Triacontane	0.28 ± 0.01	0.02	0.57 ± 0.04	0.05	0.58 ± 0.03	0.05
Pentadecyl ketone	1.05 ± 0.05	0.09	1.22 ± 0.02	0.1	1.04 ± 0.05	0.09
			Oleic acid			
Heptadecane	4.8 ± 0.1	0.8	5.0 ± 0.25	0.84	4.9 ± 0.2	0.8
Tetratriacontadiene	0.13 ± 0.01	0.01	0.28 ± 0.01	0.02	0.3 ± 0.1	0.02
Heptadecenyl ketone	0.34 ± 0.01	0.03	0.74 ± 0.02	0.06	0.72 ± 0.01	0.06

liquid samples (35 C) compared to those of the solid samples irradiated at -78 C.

In contrast to the yield of recombination products, the yield of the primary products in both acids (pentadecane and heptadecene) was not dependent on the temperature or physical state of the product. Although Jones (9) has reported a temperature dependency for CO_2 yield over a wide range of temperature (38 C and -196 C), one would expect to observe less dependence for the yield between samples irradiated at a narrow temperature range. As shown in our study, we did not observe a significant difference in the yield of primary hydrocarbons for the temperature range of 25 C and -45 C. Similar findings were observed in the study of oleic acid (11) as a slight change in the yield of heptadecene was observed between the sample irradiated at temperatures of -78 C and 35 C. It appears that vibrational energy of the carboxyl moiety and ultimately decarboxylation is affected by the temperature of irradiation to the extent that, at very low temperatures, vibrational activity is reduced considerably but increases significantly with the rise in temperature.

In general, a greater yield of primary and recombination products was observed in palmitic acid compared to that of oleic acid (Table I). These results may be rationalized on the basis of the different localization of the electron loss upon irradiation (12).

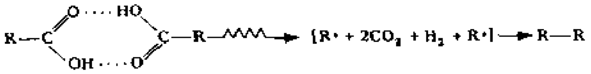
No significant change was observed in the yield of radiolysis products between samples irradiated under vacuum and air (Table I).

Effect of Solid vs Liquid

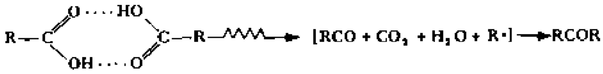
In order to eliminate the temperature effect and chemical differences (saturation and unsaturation) between the 2 acids, caprylic and capric acids were chosen to determine the effect of physical state on the formation of recombination products (experiment II). Because the chemical nature (carbon no.) of these acids is similar, any differences observed in the yield of the recombination products can be attributed mainly to the behavior of solid and liquid states. As shown in Table II 2 major recombination products of interest (alkanes and ketones) were identified and examined. The yield for tetradecane was enhanced considerably in the liquid state compared to octadecane in the solid state. In contrast, the ketone was formed in a greater yield in the solid sample nonadecanone (dinonyl ketone from capric acid) than in the liquid sample pentadecanone (diheptyl ketone from caprylic acid). Furthermore, for capric acid, nonyl ketone was formed in a greater quantity (G=0.19) than octadecane (G=0.09). Different behavior appears for the liquid caprylic acid as a slightly higher yield of tetradecane (G=0.23) was observed compared to that of the ketone (G=0.17).

In general, it is understood that localization of the excess energy in the carboxy or hydrocarbon moiety of the acid determines the fate of decarboxylation of a fatty acid molecule. Homolysis of the covalent bonds involving RCO-OH and RCOO-H has been indicated during radiation of carboxylic acids (13). As a result, the observed products such as R_2 and RCOR appear to be rationalized in terms of homolysis yielding radicals capable of reacting with entities in their immediate vicinity.

It has been suggested (10) that the formation of R_2 from radiolysis of fatty acid probably arises from decarboxylation of an H-bonded pair of fatty acid molecules through a single excitation event:

[A]


Such a sequence might be triggered by COO-H homolysis, followed by the abstraction of hydrogen from the other carboxy group by the resulting high energy hydrogen atom. Likewise, formation of ketones (RCOR) may involve essentially simultaneous creation of 2 juxtapared radicals with high probability of combining within the intact crystal matrix:

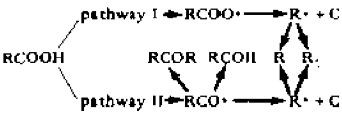
[B]


This complex transformation may well be precipitated by CO-OH homolysis and high energy HO• radical which could effectively abstract hydrogen from the neighboring carboxy group (10). It should be realized that, although in the radiolysis of fatty acids both reaction mechanisms (A&B) are in operation, their reactivities are temperature- and phase-dependent.

On the basis of previous data and our findings, the following observations on the relative yields of radiolysis products as a function of physical state (solid vs liquid) are summarized here for the saturated acids:

Solid		Liquid
R	>	R
R_2	<	R_2
∧		∨
RCOR		RCOR

Furthermore, in view of the data collected, a general scheme has been drawn to explain the effects of phase variations:



In view of these relative yields for the radiolysis compounds and the higher yield of CO_2 from solid phase radiolysis of fatty acids (10) compared to the liquid phase, it may be justified to associate the relative likelihood of pathways I and II with the solid and liquid samples, respec-

TABLE II

Recombination Products from Fatty Acids (average of 3 trials)

	Capric acid			Caprylic acid	
	25 Mrads (10 C + VAC)			25 Mrads (25 C + VAC)	
Compound	(mg/g)	(G)	Compound	(mg/g)	(G)
Octadecane	0.59 ± 0.07	0.09	Tetradecane	1.12 ± 0.1	0.23
Nonyl ketone	1.34 ± 0.14	0.19	Heptyl ketone	0.98 ± 0.08	0.17

tively. Consequently, in the solid state radiolysis of saturated fatty acids, in view of the higher yield of $R\cdot$ radical and lack of mobility, one would expect to observe a greater yield of R and thus a lower quantity of R_2 hydrocarbon. The higher yield of RCOR in solid samples may be explained in terms of the higher stability of $RCO\cdot$ radical in the condensed phase having no alternative than to combine.

In the liquid state, however, in view of the greater free radical mobility, one would expect an opposite behavior leading to lower quantities of $R\cdot$ and a higher yield of R_2 hydrocarbon. The lower yield of RCOR compared to R_2 could be due to the free mobility of $RCO\cdot$ radical ultimately dissociating ($R\cdot + CO$) to contribute to a higher yield of R_2 as indicated in Table II.

In general, on the basis of our findings and results obtained from previous studies, it appears that differences in yield of radiolysis products can be attributed to the combined effects of homolytic behavior with charge localization and the nature of the free radical surviving its environment under the influence of temperature and phase variations to either abstract hydrogen or combine with other radicals to form the corresponding recombination products.

Effect of Dose on the Radiolysis of Tripalmitin

In this study, the amounts of 8 radiolysis products were determined corresponding to irradiation doses ranging from 5 to 30 Mrads (experiment III). Figure 1 presents the yield of radiolytic compounds as a function of the corresponding doses. As shown, the dose/yield relationship follows a linear pattern up to 30 Mrads.

Effect of Temperature and Oxygen

An attempt was made to establish the effect of temperature and oxygen on the formation of radiolysis products in tripalmitin irradiated at 25 Mrads (experiment IV). Table III presents the yield of 10 radiolysis products under various conditions. A greater yield for hexadecanal was observed at low temperature and absence of oxygen. According to a previous study (10), electron capture by the carboxy group in a fatty acid molecule is probably a major mechanism for the aldehyde formation. As a result, the anion radical ($RCO_2H\cdot^-$) formed during radiation is stable at low temperatures and, upon warming, is replaced by the acyl radical leading to the formation of aldehyde. An alternative rationalization for the greater yield of aldehyde is the build-up of an intermediate ($RCO_2{}^+H_2$) at low temperature and capture of a thermal electron by this ion to yield a radical type $R\dot{C}(OH)_2$ believed to be a major aldehyde precursor (10). Perhaps a similar mechanism is also functioning in the case of triglycerides. Furthermore, it is likely that, as the persistence of carbonyl radical ($R\dot{C}O$) is enhanced at low temperature, its mobility is reduced, thus combining with the surrounding hydrogen to form a greater yield of aldehyde at low temperatures. The presence of oxygen, on the other hand, could lead to the formation of carbonyl oxidation products or fatty acids which could ultimately lead to the loss of aldehyde.

As shown in Table III, the effect of oxygen on the yield is considerable for the major alkanes and γ palmitolactone. Tridecane (n-3) and tetradecane (n-2) were increased whereas the major hydrocarbon (pentadecane) was reduced. This may be explained on the basis of free radical localization along the fatty acid molecule. In addition to the radical formation at the carboxy group, free radicals can be also formed at the α-position and to a lesser degree along the chain at the β- or γ-positions. Consequently, in the presence of air, oxy free radicals produced at these positions would lead to the greater formation of n-2 and n-3 alkanes.

In a previous report (14), we suggest a free radical

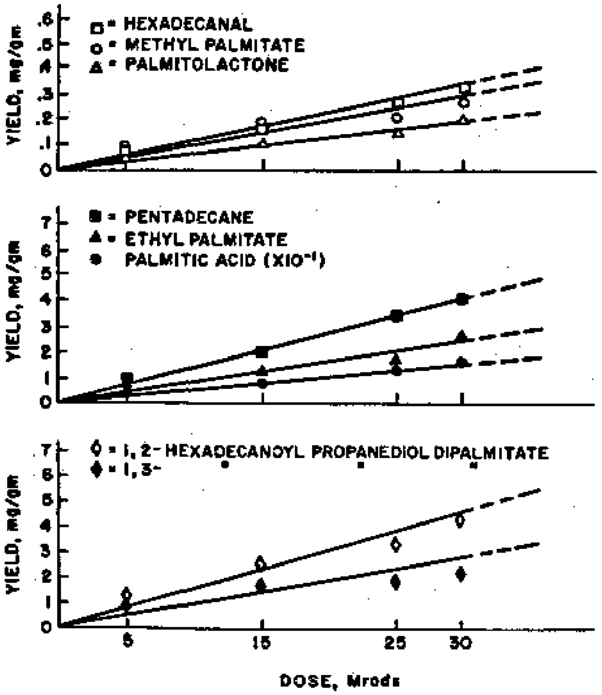


FIG. 1. The yield of radiolysis products from tripalmitin as a function of dose.

TABLE III

Radiolysis Products from Tripalmitin (average of 4 trials)

Compound	25 Mrads (VAC/-45 C) (mg/g)	25 Mrads (VAC/-45 C) (G)	25 Mrads (VAC/25 C) (mg/g)	25 Mrads (VAC/25 C) (G)	25 Mrads (AIR/25 C) (mg/g)	25 Mrads (AIR/25 C) (G)
Tridecane	0	0	0	0	0.26 ± 0.07	0.05
Tetradecane	0.09 ± 0.01	0.02	0.09 ± 0.01	0.02	0.81 ± 0.1	0.16
Pentadecane	2.00 ± 0.1	0.37	3.10 ± 0.1	0.58	2.20 ± 0.2	0.4
Hexadecanal	0.55 ± 0.06	0.09	0.28 ± 0.02	0.04	0.16 ± 0.03	0.03
Methyl palmitate	0.14 ± 0.01	0.02	0.21 ± 0.04	0.03	0.45 ± 0.09	0.06
Ethyl palmitate	0.26 ± 0.02	0.03	1.10 ± 0.1	0.15	1.20 ± 0.1	0.16
Palmitic acid	5.60 ± 1.1	0.80	10.40 ± 1.2	1.60	13.40 ± 1.7	2.0
γ-palmitolactone	0.35 ± 0.06	0.05	0.26 ± 0.02	0.04	1.50 ± 0.1	0.23
1,2-Propanediol dipalmitate	3.10 ± 0.1	0.25	3.40 ± 0.01	0.24	3.20 ± 0.07	0.23
1,3-Propanediol dipalmitate	1.40 ± 0.1	0.11	1.80 ± 0.01	0.12	1.50 ± 0.1	0.1

mechanism for the formation of lactones in samples irradiated under vacuum. However, in samples irradiated in air, formation of γ-palmitolactone was considerably enhanced (Table III). The effect of oxygen on the increased yield of γ palmitolactone could be attributed to the formation of oxy free radicals leading to the formation of the stable 5-member ring lactone.

ACKNOWLEDGMENT

This work was supported in part by a contract from the U.S. Army and University of Massachusetts Experiment Station Project no. 433.

REFERENCES

1. Howton, D.R., and Guy Shuang Wu, JAOCS 89:516 (1967).
2. Merritt, C., Jr., J.T. Walsh, M.L. Bazinet, R.E. Kramer and S.R. Bresnick, Ibid. 42:57 (1965).
3. Nawar, W.W., Prog. Chem. Fats Other Lipids 13:91 (1972).
4. Nawar, W.W., Radiat. Res. Rev. 3:327 (1972).
5. Chipault, J.R., "Lipids and Their Oxidation–Symposium on Foods," edited by M.W. Shultz, The AVI Publishing Co., Inc., Westport, CT, 1962.
6. Merritt, C., Jr., P. Angelini and R.A. Graham, J. Agric. Food Chem. 26:29 (1978).
7. McCarthy, R.D., and A.J. Duthie, J. Lipid Res. 3:117 (1962).
8. Vajdi, M., W.W. Nawar and C. Merritt, Jr., JAOCS 56:611 (1979).
9. Jones, A.R., Radiat. Res. 50:41 (1972).
10. Wu, G.S., and D.R. Howton, Rad. 57:390 (1974).
11. Faucitano, A., P. Locatelli, A. Perotti and F.F. Martinotti, J. Chem. Soc. Perkin Trans. 2 # 1786 (1972).
12. Vajdi, M., W.W. Nawar and C. Merritt, Jr., JAOCS 55:849 (1978).
13. Wu, G.S., and D.R. Howton, Ibid. 61:374 (1975).
14. Vajdi, M., W.W. Nawar and C. Merritt, Jr., JAOCS 56:906 (1979).

[Received May 22, 1981]

Characterization of Minor Constituents in Commercial Oleic Acid[1]

SHERMAN S. LIN[2], YUKINOBU MURASE[3], DAVID B.S. MIN[4], OLIVER A.L. HSIEH and STEPHEN S. CHANG, Department of Food Science, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

ABSTRACT

Minor constituents were isolated from a mixture of commercial oleic acid manufactured from beef tallow by 10 different companies. Silicic acid was used as an adsorbent to isolate the minor constituents. They were first separated into acidic and nonacidic fractions. Each fraction was then separated into numerous subfractions by stepwise gradient elution liquid chromatography, using silicic acid as the adsorbent. The subfractions which had an adverse effect on the color stability of oleic acid during heating were characterized with functional group analysis, elemental analysis and IR spectrophotometry. The minor constituents amounted to ca. 1.18% of the commercial oleic acid. They were complex mixtures with multiple functional groups. Some of the subfractions contained 2-3 times as much oxygen in the molecule as oleic acid. They had molecular weights ranging from 308 to 830 which are from monomers to trimers of oxidized oleic acid. These minor components contained carbonyl, ester and hydroxyl groups and double bonds. Some of the nonacidic minor constituents may contain amide groups. Most of the acidic subfractions were dark red, viscous liquids and the nonacidic subfractions were dark green or greenish-brown, semisolid substances. A relationship was established that the greater the polarity of the minor constituents, the greater is its adverse effect on the color stability of oleic acid during heating.

INTRODUCTION

Commercial oleic acid has a tendency to develop a dark color during heating. Lin et al. (1) reported that this discoloration is partially due to oxidation and partially due to the presence of minor constituents. The effects of the 2 factors are synergistic and thus accentuate the darkening of the commercial oleic acid when it is heated under air. Removal of the minor constituents from commercial oleic acid by silicic acid treatment drastically improved its color stability. The present paper is an attempt to characterize the chemical nature of the minor constituents.

EXPERIMENTAL PROCEDURES

Material Used

The mixed commercial oleic acid (MCOA) used for this investigation was a composite sample of commercial oleic acid manufactured from beef tallow by 10 companies. Each company submitted an oleic acid sample which was a composite of different grades of oleic acid produced by that company.

Isolation and Fractionation of Minor Constituents from MCOA

The procedure for the isolation and fractionation of minor constituents from MCOA is shown in Figure 1, and is essentially the same as that previously reported (2). The sample (29 kg) was passed through 10 parallel chromatographic columns (4.4 × 55 cm), each packed with 300 g of silicic acid (100 mesh, Mallinckrodt 2847). The silicic acid was purified and activated according to the method of Sahasrabudhe and Chapman (3). The oleic acid that passed through the columns was practically colorless and was considered purified oleic acid (POA). The colored substance originally present in MCOA was adsorbed on the silicic acid column. The silicic acid columns which retained the minor constituents of MCOA were first eluted with hexane to remove all the residual free fatty acids. It was then eluted with ethyl

---

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

[2] Present address: Anderson Clayton Foods, PO Box 63, Richardson, TX 75080.

[3] Present address: Asahi Denka Kogyo Co., Ltd., 7-1, Higashioku, Arakawa-ku, Tokyo, Japan.

[4] Present address: Department of Food Science and Nutrition, Ohio State University, Columbus, OH 43210.