

Volatile Components from Tristearin Heated in Air¹

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ABSTRACT

Tristearin was heated to 192 C in air, and its volatile oxidation products were collected directly on a cooled (-60 C) gas liquid chromatography column. Subsequently, the volatile products were separated by temperature programming up to 250 C and identified by mass spectrometry. Methyl ketones and aldehydes were the major degradation products along with minor amounts of monobasic acids, n-hydrocarbons, primary alcohols, and γ -lactones. Qualitative results indicated all the fatty acid methylene carbon atoms are susceptible to oxidation. Quantities of aldehydes and ketones were found to be in excess of their taste threshold concentrations, suggesting thermally oxidized saturated fatty acids may be precursors of some odors and flavors associated with heated lipids.

INTRODUCTION

Saturated fatty acids and their derivatives are relatively stable to oxidation at low temperatures (<60 C) (1). As the temperature is increased to 100-200 C, oxidation does occur but still less than corresponding unsaturated isologues (2). Consequently, thermal oxidation of saturated lipid materials has not been as extensively studied as mono- and polyunsaturated systems. From the limited published material, two pertinent papers have been found relative to thermal (<200 C) oxidation of saturated triglycerides. Crossley, et al., (3) heated tricaproin to 190 C in air, collected the volatiles and identified monobasic acids, methyl ketones and a trace of decanal. Caproic acid and methyl heptyl ketone were the principal products. Endres, et al., (4) reported that aldehydes and monobasic acids were produced, besides traces of methyl ketones, upon subjecting tripalmitin to 200 C temperatures in air. As in the former study, the dominant decomposition products had carbon chain lengths near or equal to the parent fatty acid in the triglyceride. In other studies, C 12-22 saturated fatty acids and their esters were oxidized at various temperatures (100-200 C) in air or oxygen (5-8). Saturated aldehydes, methyl ketones, monobasic acids, and dibasic acids (fatty acids were the starting materials [6]) were the volatile compounds isolated and identified. To that list, Ramanathan, et al., (5) also added lactones. However, in each of the studies, the reported decomposition products included only one or two classes of compounds, generally one-two carbon atoms less than the parent fatty acid. Some preference for the formation of odd or even carbon atoms compounds of a homologous series was noted. These incongruities may reflect the investigators' methods used to isolate, separate, or analyze collected volatile oxidation products.

This present study was undertaken in a continuing investigation to identify volatiles and their precursors derived from heated soybean oil. Since palmitic and stearic acids are the two main saturated fatty acids in soybean oil, "tristearin" was chosen for this particular study. Other studies on mixtures of stearin-olein, stearin-linolein, and stearin-linolenin have been performed and, upon further refinement, will comprise subsequent reports.

EXPERIMENTAL PROCEDURES

Tristearin was obtained commercially (Anderson Clayton Foods, Richardson, Tex.) and was purified by crystallizing three times in acetone. Analysis of the purified tristearin showed it to contain no free fatty acids and its glycerol fatty acid composition to be 88.9% stearic and 11.1% palmitic acids with no unsaturated acids detected. Just prior to thermal oxidation, tristearin was deodorized for 1½ hr at 7.5 mm pressure between 180-215 C.

Duplicate experiments were performed. In each of these runs, 2 cc tristearin was placed in a stainless steel container, previously described as a microroom (9), then capped, and heated to 192 C. Hot oil volatiles were swept out of the container by air onto a gas liquid chromatography (GLC) column which was cooled with dry ice. Subsequently, the condensed volatiles were separated by temperature-programmed GLC. The column effluent was split three ways to provide gas chromatograms (flame ionization detector [FID]), intensiograms (smelling at the GLC exit port and recording psychological responses), and mass spectra of the eluting compounds. Specific details of the analytical scheme have been previously reported (9).

The GLC column used to condense and separate hot oil volatiles was a 14 ft x 4 mm inside diameter glass column packed with 80-100 mesh Chromosorb GHP coated with 5% OV-17 purchased from Supelco, Bellefonte, Pa.

A T-connection in the GLC exit port line allowed a portion of the GLC effluent to enter directly into the ionization region of the mass spectrometer (MS). The spectrometer magnet was set to scan every 9 sec from m/e 10-450. Output of the MS detector was connected electrically to a computer which stored the data on magnetic tape for later processing. With the use of the MS-computer system, data on the magnetic tape were processed to give: (A) total ion plots (10,11), (B) mass chromatograms (11), (C) the mass spectra of individual scans, and (D) a comparison search (12) of unknown volatile mass spectra against 12,000 known mass spectra on magnetic tape purchased from John Wiley & Sons, N.Y., and U.K. Atomic Energy Authority, Reading, England, for compound identification.

The mass chromatogram was particularly useful in identifications when two or more compounds were unresolved under a single GLC peak. It also allowed an estimation of the contribution of each component to the total peak area. Specifically, the mass chromatogram was used to estimate the relative areas of penta- and hexanoic acids, γ C-5 and C-10 lactones which eluted simultaneously with heptanal, 2-octanone, octanol, and tridecanol, respectively. Mass spectral compound identifications were confirmed further by comparing elution temperatures of identified volatile compounds and available authentic compounds.

Integration and relative area percent calculations were performed by computer. This permitted integration of the nine off-scale peaks seen in Figure 1. In the duplicate experiments, the integrated corresponding peak areas of the methyl ketones and n-aldehydes agreed within 10% of each other. Computer relative area percents were converted to wt percents by multiplying each peak area by the identified compound mol wt, dividing by ionizable carbon atoms of the compound, then renormalizing all values. This is a closer approximation of quantities than area percents when homologous series of compounds are present (13).

To calculate the μ g of methyl ketones and aldehydes produced from heated tristearin, authentic compounds

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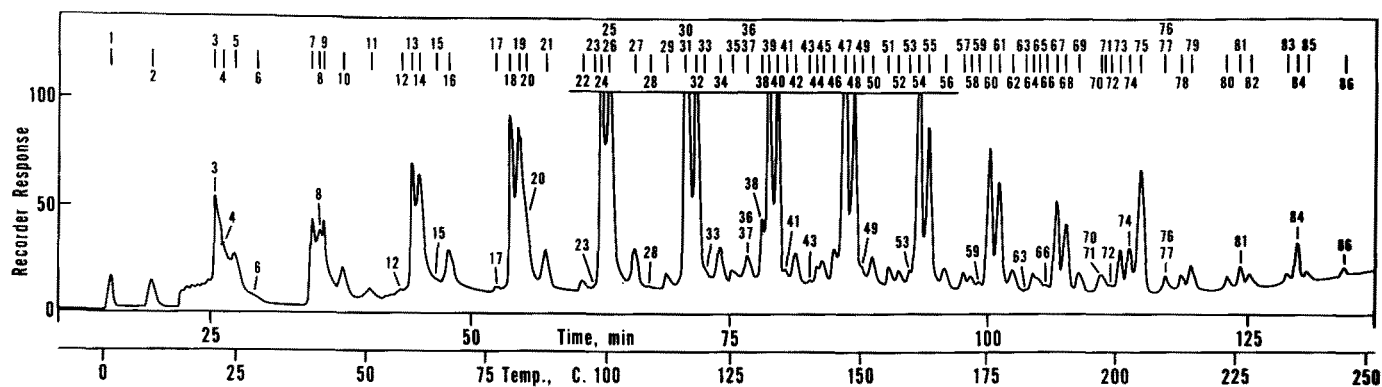


FIG. 1. Gas chromatogram of volatiles from heated (192 C) tristearin. Compound identification of numbered peaks are listed in Table I.

were used to determine their absolute FID responses. For this objective, five different concentrations of equal wt mixtures of methyl ketones, C 3-8, 13, were prepared so that upon GLC analysis peak areas were both less and greater than corresponding methyl ketones peak areas produced from heated tristearin. The individual compound FID responses then were calculated in terms of integration units/ μg for each particular compound injected. Those values then were calculated statistically to give average response factors for C 3-18 methyl ketones. Response factors for the ketones not included in the test mixtures were obtained by extrapolating plotted data. The standard error of the predicated FID response in integration units/ μg was $\pm 20\%$. In an analogous manner, seven different concentrations of equal wt mixtures containing C 5-12 n-aldehydes were used to calculate absolute response factors for C 3-20 n-aldehydes with a standard error of the predicated FID response in integration units/ μg of $\pm 14\%$.

RESULTS AND DISCUSSION

Approximate composition of the volatile thermal oxidation products from tristearin was methyl ketones, 38%; aliphatic aldehydes, 36%; n-hydrocarbons, 9%; monobasic aliphatic acids, 10%; γ -lactones, 4%; and primary alcohols, 2.7% plus 0.2% of unknowns. A total of 77 compounds were identified. As shown in Figure 1 and Table I, each class of compounds was represented by a homologous series, thus indicating no specific oxidative attack occurs in the triglyceride; rather, each methylene carbon in the fatty acyl group appears to be susceptible to oxidation with preferential attack toward the center of the molecule. This agrees with a similar conclusion drawn by Brodnitz (14) who oxidized methyl palmitate with oxygen at 150 C and chemically determined the locations of oxygen attack and, by GLC, determined the extent of oxidation at each location.

Aldehydes and methyl ketones are the principal volatile degradation products produced from heated tristearin. Formation of the former can be attributed to the thermal (190 C) decomposition of monohydroperoxides (15) which are proposed to be the initial products of thermally oxidized saturated fats (14). As seen in Table I, the homologous series of aldehydes constitute 36% of the volatiles identified and range from C 3 through C 17 in carbon chain length.

Formation of methyl ketones from heated saturated systems is attributed to β -oxidation (to the carboxyl) of the carbon chain followed by decarboxylation (3,4,7). In all these studies, the authors suggested the shorter chain fatty acids originate sequentially from the parent acid in step-wise degradation, losing one carbon atom/degradation step. The three reactions, degradation, β -oxidation, and decarboxylation, occurring simultaneously were proposed to account for the homologous series of methyl ketones. How-

ever, the ketones present in the greatest quantity contained only one or two carbon atoms less than the parent material. Data in Table I indicate some methyl ketones could have formed in the manner described above. Note the sequential decrease of the amounts of 2-hepta-, 2-hexa-, and 2-pentadecanone. However, since the quantities of these ketones are considerably less than those in the C 6-11 chain lengths, the fatty acid precursors originate by a different path than sequential degradation of the parent acid, viz stearic. Apparently, further studies are required to elucidate the mechanism(s) for methyl ketone formation from thermally oxidized saturated fatty acids and their derivatives.

Two other classes of compounds identified from heated tristearin were n-hydrocarbons and primary alcohols. To our knowledge, they have not been reported previously as decomposition products from thermally (150-200 C) oxidized saturated acids or their derivatives, though they have been identified in volatiles derived from commercial stearic acid aged for 8 months at 31 C (16). Presence of n-hydrocarbons can be explained by the reaction of alkyl-free radicals, which are products of thermally decomposed hydroperoxides (15) with hydrogen-free radicals. Both Ramanathan, et al., (5) and Endres, et al., (4) identified hydrogen ($\text{H}\cdot + \text{H}\cdot \rightarrow \text{H}_2$) as a main gaseous product from heated methyl esters of laurate, palmitate, and stearate. The conspicuous amount of heptadecane may be formed by decarboxylation of stearic acid formed by hydrolysis of the triglyceride under the experimental conditions. Appearances of primary alcohols can be explained in an analogous manner as hydrocarbons where hydroxyl-free radicals react with the alkyl-free radicals to produce primary alcohols.

Aliphatic acids appear to be the most ubiquitous of volatiles isolated from heated saturated systems. Of the studies reviewed (1,3-8), the acids always were included among the volatiles identified. Table I shows the acids identified from heated tristearin. Their distribution pattern shows a similarity to the distribution pattern of the aldehydes in that both classes of compounds peak at the C 6 carbon atom position. This could indicate that the various acids may be formed via oxidation of the corresponding carbon chain aldehydes. This conclusion was also postulated in two other studies on the identification of volatiles associated with heated lipids (5,17).

γ -Lactones, though more characteristic of dairy fats, have been isolated from vegetable oils. Smouse and Chang (18) and Kawada, et al., (19) have identified γ -lactones in collected volatiles from refined and hydrogenated soybean oil, respectively. In the latter case, the lactones were attributed to oxidized linolenic acid. Fioriti, et al., (20) isolated γ -lactones from highly oxidized soybean oil. They surmised that reduction of hydroperoxides in heated oils promotes lactone formation. In a recent publication, Cheng and Chang (16) identified γ -lactones in volatiles from commercial stearic acid which was aged at 31 C for 8 months. They

TABLE I

Compounds ^a Identified in Collected Volatiles from Heated (192 C) Tristearin		
Peak no. in chromatogram (Fig. 1)	No. carbon atoms	Corrected ^{b,c} wt. %
Primary n-Alcohols		
12	C-4	0.1
17	C-5	0.2
22	C-6	0.3
29	C-7	0.3
37	C-8	0.4
45	C-9	0.5
52	C-10	0.4
58	C-11	0.3
65	C-12	0.1
71	C-13	0.1
77	C-14	Trace
		2.7
γ Lactones		
35	C-4	0.7
36	C-5	0.6
44	C-6	0.4
51	C-7	0.5
57	C-8	0.4
64	C-9	0.4
70	C-10	0.3
76	C-11	0.3
80	C-12	0.2
83	C-13	0.2
86	C-14	0.1
		4.1
n-Hydrocarbons		
1	C-4	0.4
2	C-5	0.5
5	C-6	0.2
10	C-7	0.5
16	C-8	0.6
21	C-9	0.9
27	C-10	0.9
34	C-11	0.6
42	C-12	0.5
50	C-13	0.6
56	C-14	0.4
62	C-15	0.4
69	C-16	0.2
75	C-17	2.1
		8.8
n-Acids		
11	C-2	0.1
15	C-3	Trace
20	C-4	2.4
26	C-5	2.4
31	C-6	2.5
38	C-7	1.1
46	C-8	0.7
53	C-9	0.3
59	C-10	0.1
66	C-11	0.1
72	C-12	Trace
		9.7
n-Aldehydes		
4	C-3	0.4
9	C-4	1.8
14	C-5	3.6
19	C-6	4.8
25	C-7	4.8
32	C-8	4.6
40	C-9	4.4
48	C-10	4.0
55	C-11	3.2
61	C-12	2.1
68	C-13	1.2
74	C-14	0.7
79	C-15	0.3
82	C-16	0.1
85	C-17	0.1
		36.1

Methyl Ketones		
3	C-3	2.6
7	C-4	1.9
13	C-5	2.4
18	C-6	3.6
24	C-7	4.6
30	C-8	4.3
39	C-9	4.9
47	C-10	4.5
54	C-11	3.8
60	C-12	2.6
67	C-13	1.5
73	C-14	0.6
78	C-15	0.2
81	C-16	0.3
84	C-17	0.6
		38.4
	Unknown ^d	0.2 ^e

^aAll identified compounds are straight chain and saturated.

^bCorrected for $\frac{\text{mol wt}}{\text{ionizable carbon atoms}}$

^cGas liquid chromatography peak no. 8 is HCCl_3 , a solvent used to clean the deodorization apparatus. Its area is not included in the wt % calculations.

^dUnknowns include integrated gas liquid chromatography peaks nos. 6, 23, 28, 33, 41, 43, 49, and 63 and the area just prior to the acetone peak.

^eThis value is relative area percent.

estimated each of the C 6-11 lactones to be in the 0.006-0.53 ppm range. In a study, more closely related to this report, Ramanathan et al. (5) mentioned that γ -lactones were just detected in volatiles from heated methyl esters of saturated fatty acids. Our study confirms γ -lactones as thermal degradation products from tristearins and shows that saturated fats are at least one of their precursors. Table I lists the γ -lactones identified from heated tristearin. The lactones also are present as a homologous series, but their distribution pattern shows no correlation to any of the other homologous series in Table I, which might have suggested their precursors.

Compounds other than those listed in Table I were present in the isolated volatiles from tristearin. Their presence was noted in some instances by strong odors while smelling at the GLC exit port and in others by small GC peaks. In either case, their concentrations were insufficient to give identifiable mass spectra.

The prominence of the aldehyde and methyl ketone GLC peaks and the strong odor intensity ratings given to these compounds as they were sniffed when emerging from the GLC exit port gave impetus to determining their absolute concentrations. Our premise was that, if their concentrations were significantly above their taste threshold levels, and since it is generally accepted that odor thresholds are lower than taste thresholds, then conceivably they could contribute to odors and flavors associated with heated lipids. Consequently, aldehyde and ketone concentrations were determined by the absolute FID calibration technique and are shown in Table II. Also shown are the μg concentrations converted to ppm values, plus reported taste threshold values for aldehydes (21) and ketones (22), and those subthreshold quantities (ppm) of each ketone in a mixture which demonstrated a synergistic behavior (22). From the data, it is apparent that all the aldehydes and half of the ketones do, indeed, equal or exceed their reported taste threshold values. In some instances, the aldehydes and ketones surpass their reported levels by factors of 300 and 20, respectively. The latter multiplication factor is even greater when ketone concentrations are compared to corresponding subthreshold levels necessary to compositely impart an oxidized flavor. Thus, the data indicate heated saturated fatty acids may form some volatiles in sufficient quantities to be potential contributors, be it minor, to

TABLE II

Concentrations of Methyl Ketones and n-Aldehydes Isolated from 2 cc of Heated Tristearin and Comparison of Those Levels with Reported Taste Threshold Values

Carbon number	Methyl ketones				n-Aldehydes		
	Concentrations ^a		Threshold ^b		Concentrations ^c		Threshold, ^d Individual, ppm
	μg	ppm	Individual, ppm	Mixture, ppm	μg	ppm	
C-3	10.9	6.3			2.8	1.6	1.6
C-4	8.1	4.7	79.5	0.13	10.9	6.3	0.02
C-5	10.0	5.8	8.4	0.20	17.6	10.2	0.15
C-6	14.6	8.5	0.4		20.9	12.1	0.15
C-7	17.8	10.3	0.7	0.38	17.4	10.1	0.04
C-8	16.7	9.7	0.5		14.4	8.4	0.46
C-9	18.0	10.4	3.5	0.18	12.6	7.3	0.32
C-10	16.0	9.3			10.5	6.1	1.0
C-11	12.9	7.5	15.5	0.20	7.5	4.4	0.1
C-12	8.6	5.0			4.7	2.3	0.46
C-13	4.9	2.8	18.4	0.46	2.4	1.4	
C-14	2.0	1.2			1.3	0.8	
C-15	0.5	0.3			0.8	0.5	
C-16	1.0	0.6			0.4	0.2	
C-17	1.7	1.0			0.1	0.1	

^aStandard error ± 20%.

^bTaste threshold values in milk reported by Langler and Day (22): individual compounds and a mixture of methyl ketones at sublevels giving a synergistic effect.

^cStandard error ± 14%.

^dTaste threshold values in oil reported by Kinsella (21): individual aldehydes.

odors and flavors from heated lipids. Data in Table II and inferences drawn from them may not necessarily be applicable, at least in magnitude, to systems which include substantial amounts of unsaturated fatty acids. However, results reported by Wishner and Keeney (23) from a comparative study of carbonyl compounds formed from various heated (200 C) fats showed quantities of 2-alkanones, and alkanals increased as the saturated fatty acid content of the fats increased from 16-48%.

Unpublished results at our Laboratory showed that the tristearin volatile GLC pattern was quite apparent in volatiles which were derived from a heat (192 C) glyceride mixture containing tristearin and triolein.

The above observations indicate tristearin can be oxidized at elevated temperatures and can produce a complex mixture of compounds, including methyl ketones, aldehydes, and monobasic acids. Saturated n-hydrocarbons, primary alcohols, and γ-lactone also were confirmed as oxidation products. The distribution of the compounds indicates all methylene carbon atoms of the parent acid are susceptible to oxidation with preferential oxygen attack near the center of the molecule. If sequential degradation of the parent fatty acid does occur, it does not appear to be the major mechanism whereby the various volatiles originate. Thermal oxidation of tristearin can produce sufficient quantities of methyl ketones and aliphatic aldehydes conceivably to contribute to odors and flavors of heated lipids.

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