Thermal Oxidation of Synthetic Triglycerides[1,2]
I. Composition of Oxidized Triglycerides

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Tripalmitin, 1- and 2-lauryl dipalmitin and 1- and 2-oleyl dipalmitin were subjected to thermal oxidation at 200C in the presence of air for various lengths of time. The triglycerides showed a loss in weight, and an increase in carbonyl, hydroxyl and acid values. The I.V. increased in the case of saturated triglycerides and decreased in the case of unsaturated triglycerides.

Hydrolysis of the ester linkage between glycerol and fatty acid was found to occur during thermal oxidation of the triglycerides. The hydrolysis occurred irrespective of the type and position of the fatty acid in the triglyceride molecule. The fatty acids released from the triglyceride by hydrolysis were found either to be oxidized further to short chain fatty acids, or were oxygenated with the introduction of a carbonyl or hydroxyl group in the molecule. Moreover, the unsaturated fatty acid in the triglyceride molecule was found to be oxidized more readily than the saturated fatty acid.

A hydroxy fatty acid with a carbon number of 13.5 on a diethylene glycol succinate column was isolated from oxidized tripalmitin and was also found to occur in the free fatty acid fraction of oxidized tripalmitin, 1-lauryl, 2-3 dipalmitin, and 1-oleyl, 2-3 dipalmitin. The presence of lauric or oleic acid in the 2-position of the triglyceride prevented the formation of this acid, which suggested that it is an oxidation product of palmitic acid.

WHEN FATS are exposed to high temperatures in the presence of air or oxygen, they undergo chemical and structural changes. Most of the work carried out in different laboratories has been based on the use of naturally occurring fats which are complex mixtures of glycerol esters of fatty acids (1–13). Data from studies of a particular natural fat or oil cannot be extrapolated to predict the results of oxidation of another fat because of the varied and complex nature of different fats. In addition, the kind and position of a fatty acid in a glyceride molecule with respect to another fatty acid in the molecule might well have an effect on the course of oxidation. The present study was, therefore, undertaken to determine the effect of the nature and the position of a fatty acid in a glyceride molecule upon its stability to thermal oxidation.

Experimental Procedures and Data

Tripalmitin was obtained commercially (Eastman Organic Chemicals) and purified by crystallization from acetone. 1-Lauryl dipalmitin, 2-lauryl dipalmitin, 1-oleyl dipalmitin, and 2-oleyl dipalmitin were synthesized according to the method of Hartman (14). Palmitoyl chloride and lauroyl chloride were obtained commercially (Eastman Organic Chemicals) and used without further purification. Oleyl chloride was prepared by refluxing pure oleic acid with an equal weight of oxalyl chloride in petroleum ether (40–60C) (1 g./5 ml.) for 2 hr. on a steam bath. The excess of oxalyl chloride was distilled off under vacuum. The analytical values and melting points of the pure synthetic triglycerides with theoretical and literature values are given in Table I.

TABLE I

Analysis of Synthetic Triglycerides and Comparison with Theoretical Values

	Iodine value		Melting point °C.	
	Theory	Observed	Observed	Lit.
Tripalmitin	0.00	0.00	63-64	63.5
1-Lauryl, 2-3 dipalmitin	0.00	0.00	51-52	54.0
2-Lauryl, 1-3 dipalmitin	0.00	0.00	52-53	53.5
1-Oleyl, 2-3 dipalmitin	30.5	30.8	34-35	34.5
2-Oleyl, 1-3 dipalmitin	30.5	30.5	37-38	37.5

The purity of the synthesized glycerides (Table II) was determined by the pork pancreatic lipase hydrolysis procedure of Ast and Vander Wal (15); the free fatty acids were isolated according to the method of Hornstein *et al.* (16). Methyl esters were prepared directly on the ion exchange resin and analyzed by gas chromatography. The systematic multiple fractional extraction procedure of Bush and Densen (17) was used to separate monoglycerides from di- and triglycerides (Table III). A 4 x 4 completion of squares process, with 80% ethanol and Skellysolve F as solvent pair, was used. The monoglycerides were refluxed for 2 hr. with 10 ml. of absolute methanol saturated with anhydrous HCl to convert the fatty acids to their methyl esters; the methyl esters were analyzed by gas chromatography.

The triglycerides were oxidized in a 50-ml. round-bottomed, glass stoppered flask fitted with a 14/35 ground glass sidearm. A full length 14/35 ground glass tube which almost touched the bottom of the oxidation flask was so positioned that air was forced to pass down into and then up through the oxidizing glyceride. Efficient mixing between air and glyceride was obtained. In order to follow the oxidation of the glycerides and to insure trapping of condensable and noncondensable volatile products, the system in Figure 1 was employed. The reaction flask (E) was placed in the constant temperature bath (G) maintained at 200±1C. Air was freed of moisture, carbon dioxide, and carbon monoxide by means of purification Train I. This train consisted of a Vycor tube (A) packed with copper oxide heated to red heat in order to convert carbon monoxide to carbon dioxide

TABLE II

Hydrolysis of Triglycerides by Pancreatic Lipase

	% composition monoglyceride			% composition of free fatty acids		
	C_{12}	C_{16}	Oleic	C_{12}	C_{16}	Oleic
1-Lauryl, 2-3 dipalmitin	8.3	91.7	.....	47.2	52.8	.....
2-Lauryl, 1-3 dipalmitin	90.4	9.6	.....	6.3	93.7	.....
1-Oleyl, 2-3 dipalmitin	.....	91.9	8.1	.....	49.9	50.1
2-Oleyl, 1-3 dipalmitin	.....	7.9	92.1	.....	90.8	9.2

TABLE III

Fatty Acid Composition of Synthetic Triglycerides

	% based on diethylene glycol succinate column			
	Lauric	Palmitic	Stearic	Oleic
Tripalmitin	.....	98.2	1.2	.....
1-Lauryl, 2-3 dipalmitin	32.7	65.2	1.1	.....
2-Lauryl, 1-3 dipalmitin	34.0	65.3	0.7	.....
1-Oleyl, 2-3 dipalmitin	.....	65.6	0.4	34.0
2-Oleyl, 1-3 dipalmitin	.....	66.3	1.0	32.7

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and hydrogen to water, and absorption tubes of magnesium perchlorate (B) and ascarite (C) to remove water and carbon dioxide. Absorption Train II consisted of tubes which were similar to Train I; all tubes in Train II were accurately weighed. It was possible to calculate the amounts of carbon dioxide, carbon monoxide, and hydrogen formd during the oxidation with this system. After passing through Train II the gas was bubbled through mineral oil which acted as a seal to the system.

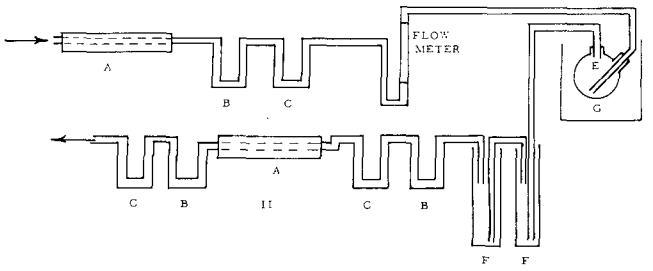

FIG. 1. Apparatus for thermal oxidation.

The procedure for oxidation of the triglycerides was as follows: The Vycor tubes containing copper oxide were heated to dull red, and the constant temperature bath was brought to 200C. The two traps (F) were filled with a dry ice trichloroethylene mixture to maintain a steady −75C. Approximately 6 g. of the glyceride were accurately weighed into the reaction flask and the flask was then connected to the cold traps and absorption trains. Air was supplied at a rate (in ml. per min.) equal to 15 times the weight of the glyceride oxidized. Air was metered through a manostat type predictability flowmeter, No. G-9143-B.

The glycerides were oxidized for periods of 3, 8, 15, and 24 hr. Condensable and noncondensable products were collected from 0–3 hr., 3–8 hr., 8–15 hr., 15–24 hr., and 0–24 hr. Upon completion of the reaction period, air to the reaction flask was stopped, the absorption tubes closed, the reaction flask removed, wiped free of bath oil, and allowed to cool. The cold traps were removed from the −75C bath and the contents immediately taken up in carbonyl-free methanol.

The short connection between the cold traps and reaction flask was rinsed with (2 x 5 ml.) carbonyl-free methanol, and the cold traps also washed with (3 x 10 ml.) carbonyl-free methanol. The washings were combined, transferred to a 50-ml. volumetric flask and made up to volume with methanol. Aliquots of this methanolic solution were used for further analysis of the condensable vapor phase. The gain in weight of the absorption tubes was recorded. The contents of the reaction flask were transferred to a glass stoppered bottle, flushed with nitrogen, and stored at a low temperature until the contents were analyzed.

The acid value and I.V. were determined by the official A.O.C.S. Methods except that a smaller sample was used (18). The acetyl chloride-pyridine method of Smith and Shriner was used in the determination of the hydroxyl group (19), a correction was applied to the individual samples for their F.F.A. content.

The carbonyl value in thermally oxidized fats was determined by a method developed in our laboratory. Fifty ml. of normal octyl alcohol containing 0.25 ml. of pyridine and 15 ml. of a 3.5% solution of hydroxylamine hydrochloride in carbonyl-free ethyl alcohol was pipetted into a 250-ml. glass stoppered Erlenmeyer flask and approximately 100 mg. of the sample to be analyzed was added to the flask. The flask was stoppered, gently rotated to effect a clear solution, and left in the dark overnight at room temperature. A blank which contained all the reagents was also set aside. At the end of the reaction, the contents of the flask were quantitatively transferred to a 250-ml. beaker with 10 ml. of carbonyl-free alcohol. The pH of the blank was recorded. The reaction mixture containing the fat sample was potentiometrically titrated against 0.1 *N* NaOH in methanol to the same pH as that of the blank. The solution was gently stirred during the titration. Free fatty acids were not found to interfere in the determination.

$$\text{Carbonyl value} = \frac{\text{ml. NaOH} \times \text{normality NaOH}}{\text{wt. of sample in g.}}$$
$$= \text{millimoles per g. of sample}$$

The peroxide value was determined according to the method of Lundberg and Chipault (22).

The free fatty acids were isolated from thermally oxidized glycerides according to the method of Hornstein *et al.* (16), and the methyl esters prepared by transesterification. Approximately 1 g. of sample was placed in a glass stoppered flask; 10 ml. of anhydrous ethyl ether and 5 ml. of a 0.5% sodium methoxide solution were added. Sodium methoxide solution was prepared by dissolving 0.5 g. of sodium in 200 ml. of anhydrous methanol. The reaction was allowed to proceed for 24 hr. Upon completion of the reaction, the mixture was acidified with dilute HCl, and extracted three times with portions of ethyl ether. The ether extracts were combined, washed free of mineral acid with water and dried over anhydrous sodium sulfate. Solvent was removed on a hot water bath with the aid of a stream of nitrogen. All esters were stored under nitrogen at 3C until analyzed.

The methyl esters were run on two columns with different properties as described by Mirva *et al.* (23), namely a 10-ft., ¼-in. diameter diethylene glycol succinate column (DEGS), and a 5-ft., ¼-in. diameter Apiezon L column. The numerical constants obtained were the "carbon numbers" as described by Woodford and Van Gent (24).

An Aerograph A-90-C with a 1-mv. Daystrom-Weston recorder was used. The DEGS column was maintained at 215C; helium pressure at 10 psig. The Apiezon L column was maintained at 225C; helium pressure at 15 psig. Filament current in both cases was maintained at 200 ma. A standard consisting of the methyl esters of C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} saturated acids and methyl oleate, and a standard consisting of C_5, C_6, and C_{10} saturated dicarboxylic methyl esters, was run each day. The composition of each sample was calculated on the basis of the area under each peak, as found by the triangulation method.

Infrared spectra were run on a Beckman IR-7 recording spectrophotometer, using a 10% solution of the sample in chloroform.

TABLE IV

Chemical Analysis of Thermally Oxidized Triglycerides

	Time in hr.	Tripalmitin	1-Lauryl 2-3 dipalmitin	2-Lauryl 1-3 dipalmitin	1-Oleyl 2-3 dipalmitin	2-Oleyl 1-3 dipalmitin
Wijs iodine	0-3	0.15	0.13	0.05	1.16	0.94
	0-24	0.24	0.24	0.10	0.82	0.78
Carbonyl value	0-3	0.42	0.30	0.30	0.39	0.25
	0-24	0.88	0.51	0.78	0.48	0.63
Acid value	0-3	0.08	0.07	0.11	0.04	0.05
	0-24	0.16	0.13	0.23	0.11	0.15
Hydroxyl value	0-3	0.16	0.68	0.22	0.31	0.77
	0-24	0.25	0.98	1.56	1.77	0.83

Results and Discussion

The thermal oxidation of pure synthetic triglycerides at 200C led to the formation of products which differed both physically and chemically from the original triglycerides. All glycerides were white solids at room temperature, and turned into dark brown or red semisolids after 24 hr. of oxidation. Data after 3 and 24 hr. of oxidation periods only have been reported in this paper.

The apparent I.V. (Table IV) increased for saturated glycerides and decreased for the unsaturated glycerides as oxidation progressed. The decrease in I.V. for unsaturated glycerides indicated that disruption of the double bond of oleic acid had occurred and was in agreement with the decrease in the oleic acid content of these triglycerides (Table V). The apparent I.V. of thermally oxidized saturated triglycerides indicated the presence of unsaturation in the molecules. Part of the apparent I.V. may have been due to the presence of carbonyl compounds. These results are in agreement with those reported by Ramanathan *et al.* (26) on the thermal oxidation of methyl laurate and methyl stearate. An increase in the carbonyl and hydroxyl value of all oxidized triglycerides was observed (Table IV). The increase in hydroxyl value could have been due to the fixation of molecular oxygen or to hydrolysis of the ester linkage between glycerol and fatty acid forming a free hydroxyl group. The increase in the acid value indicated that hydrolysis of the ester linkage with the formation of free fatty acids had occurred. None of the oxidized samples were found to have a peroxide value.

The fatty acid composition of the residues from the thermally oxidized triglycerides after the removal of free fatty acids on an ion-exchange resin are given in Table V. In the case of oxidized tripalmitin, fatty acids consisted essentially of palmitic acid and a fraction which had a carbon number of 13.5 on a DEGS column. A sufficient quantity of this fraction was isolated by gas chromatography and analyzed by infrared spectroscopy. This compound exhibited a strong absorption at 3640 $cm.^{-1}$, the characteristic region of hydroxy group. On the basis of infrared analysis this fraction was tentatively identified as a hydroxy acid. Due to a lack of sufficient amount of material, further characterization of the chain length and of the position of the hydroxyl group in the chain was impossible. Small amounts of fatty acids with carbon number 16.5 and 17.5 were also found to be present in the tripalmitin oxidized for 3 hr. However, these fractions disappeared on further oxidation. The results indicated that these fractions were further oxidized and broken down into small chain fatty acids and removed from the oxidizing triglycerides as volatile products. For 1-lauryl dipalmitin and 2-lauryl dipalmitin, the fatty acids consisted mainly of lauric and palmitic acid; for 1-oleyl and 2-oleyl dipalmitin there was approximately a 10% loss of oleic acid after 3 hr. and about 30% loss after 24 hr. of oxidation.

The composition of the free fatty acids isolated from the various thermally oxidized triglycerides is given in Table VII. The composition of the free fatty acids was found to be much more complex than the fatty acid composition of the oxidized triglycerides. Palmitic acid was found to be the main constituent of the free fatty acids of all the thermally oxidized triglycerides. Lauric acid was found to be one of the main constituents of the free fatty acids in thermally oxidized 1-lauryl and 2-lauryl dipalmitin. Similarly, oleic acid was found to be one of the constituents of the free fatty acids in thermally oxidized triglycerides containing oleic acid. These results definitely indicated that hydrolysis of the ester linkage was one of

TABLE V

Fatty Acid Composition of Residue from Thermally Oxidized Glycerides—Free Fatty Acids Removed

Carbon number on DEGS	Carbon number on Apiezon L	Tentative identification	% Composition Based on DEGS									
			Tripalmitin		1-Lauryl 2-3 dipalmitin		2-Lauryl 1-3 dipalmitin		1-Oleyl 2-3 dipalmitin		2-Oleyl 1-3 dipalmitin	
			0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.
12.0	12.0	C_{12} saturated	.....	.....	30.5	35.4	29.3	29.6	.....	.....	.....	.....
13.5	12.4	Hydroxy acid	13.1	15.8	.....	.....	.....	.....	.....	.....	.....	.....
14.0	14.0	C_{14} saturated	1.5	Trace	Trace	Trace	2.3	4.0	1.3	Trace	.....	.....
15.0	15.0	C_{15} saturated	Trace	Trace	Trace	Trace	.....	Trace	Trace	Trace	.....	.....
16.0	16.0	C_{16} saturated	78.4	84.2	69.5	64.6	66.7	66.4	68.1	87.9	75.5	88.6
16.5	.....	Unidentified	4.2	.....	.....	.....	.....	.....	3.2	Trace	.....	.....
17.5	.....	Unidentified	2.7	.....	.....	.....	.....	.....	1.6	1.5	.....	.....
18.0	18.0	C_{18} saturated	Trace	.....	Trace	Trace	1.6	Trace	.....	.....	.....	.....
18.3	17.7	C_{18} *monoenoic*	.....	.....	.....	.....	.....	.....	25.8	10.6	24.5	11.4

TABLE VI

Composition of Free Fatty Acids of Residue from Thermally Oxidized Glycerides

Carbon number on DEGS	Carbon number on Apiezon L	Tentative identification	% composition based on DEGS									
			Tripalmitin		1-Lauryl 2-3 dipalmitin		2-Lauryl 1-3 dipalmitin		1-Oleyl 2-3 dipalmitin		2-Oleyl 1-3 dipalmitin	
			0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.	0-3 hr.	0-24 hr.
10.0	10.0	C_{10} saturated	.....	.....	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
11.0	11.0	C_{11} saturated	.....	Trace	Trace	Trace	Trace	Trace	Trace	Trace	.....	.....
11.8	.....	Unidentified	.....	.....	.....	.....	.....	.....	.....	.....	18.8	.....
12.0	12.0	C_{12} saturated	.....	Trace	32.4	16.8	21.4	4.9	Trace	Trace	.....	.....
13.0	13.0	C_{13} saturated	0.8	1.4	.....	.....	1.2	Trace	Trace	Trace	3.4	0.6
13.5	12.4	Hydroxy acid	12.6	0.7	20.1	13.8	0.5	Trace	10.9	14.3	.....	.....
14.0	14.0	C_{14} saturated	0.4	2.7	1.5	2.0	2.6	1.5	1.8	3.0	1.6	2.8
15.0	15.0	C_{15} saturated	6.1	2.5	2.6	4.9	6.9	1.8	2.6	3.9	1.9	5.9
16.0	16.0	C_{16} saturated	65.2	66.4	44.7	52.1	49.2	59.0	60.0	52.0	58.4	78.1
16.5	.....	Unidentified	.....	.....	.....	6.2	.....	.....	7.2	8.2	Trace	1.0
17.0	.....	Unidentified	5.6	3.7	Trace	2.9	2.8	4.0	2.9	6.1	.....	.....
18.0	18.0	C_{18} saturated	4.6	4.5	.....	1.6	.....	5.1	.....	.....	.....	.....
18.3	17.6	C_{18} monoenoic	.....	.....	.....	.....	.....	.....	14.6	7.8	15.7	5.0
19.0	.....	Unidentified	2.4	3.7	.....	.....	.....	.....	.....	.....	.....	.....
19.4	.....	Unidentified	2.4	4.1	.....	.....	1.9	4.3	.....	.....	.....	.....
20.8	.....	Unidentified	Trace	3.5	.....	.....	1.9	4.9	.....	.....	.....	.....
21.0	.....	Unidentified	Trace	2.7	.....	.....	2.2	3.1	.....	.....	.....	.....
22.3	.....	Unidentified	.....	4.1	.....	.....	4.0	10.5	.....	.....	.....	.....

the main reactions taking place during thermal oxidation of the triglycerides. A number of fatty acids with a carbon number greater than 16 were observed but not identified. These are probably oxygenated short chain fatty acids since the addition of a functional group such as hydroxyl or carbonyl is known to increase the carbon number. The hydroxy acid with carbon number of 13.5 was also observed as a component of the free fatty acids of oxidized tripalmitin, 1-lauryl dipalmitin and 1-oleyl dipalmitin.

There was a distinct difference between the composition of the residue fatty acids from 1-lauryl and 2-lauryl dipalmitin. For 1-lauryl dipalmitin the fatty acids consisted essentially of lauric, palmitic, and the hydroxy acid (C-13.5), whereas in the case of 2-lauryl dipalmitin the fatty acids consisted essentially of lauric and palmitic with the remainder consisting of acids with carbon numbers greater than 16. The pattern for 1-oleyl and 2-oleyl dipalmitin was similar to the lauric acid glycerides. With oleic acid in the 1 position, the hydroxy acid (C-13.5) was identified in addition to oleic and palmitic acid. However, with 2-oleyl dipalmitin no hydroxy acid (C-13.5) was observed, but both oleic and palmitic acids were present in the free fatty acids. These results seem to indicate that palmitic acid in the 2 position of the triglyceride was the precursor for the formation of the hydroxy acid with carbon number of 13.5, and that lauric or oleic acid in 2 position of the triglycerides prevented the formation of this acid. The absence of free fatty acids with carbon number more than 18 in 1-lauryl dipalmitin and 1- and 2-oleyl dipalmitin indicates that these fatty acids, if formed during thermal oxidation, are immediately broken down to short chain fragments and removed from the oxidizing triglyceride. This was further confirmed from the analysis of the volatile condensable products, which were found to contain more of short chain fatty acids.

The results of this experiment indicated that there are a number of reactions which occur during the thermal oxidation of triglycerides. These are simultaneous and probably competitive. The attack of oxygen at high temperatures causes dehydrogenation of saturated triglycerides and formation of unsaturation in the molecule. Subsequent attack of oxygen produced hydroperoxides and intermediates which contained hydroxyl, carbonyl, and carboxyl groups. In the case of triglycerides containing oleic acid, attack of oxygen may take place directly at the double bond. Disruption of the double bond of oleic acid contained in 1- and 2-oleyl dipalmitin was found to occur easily as evidenced by more than a 30% loss of oleic acid from the triglyceride molecule after 24 hr. of oxidation and a significant drop in I.V.

Hydrolysis of the ester linkage between glycerol and the fatty acid also occurred during thermal oxidation. Hydrolysis was found to occur whether the acid was a long chain saturated such as palmitic, a short chain saturated such as lauric, or a long chain unsaturated such as oleic acid.

A significant decrease in lauric acid in the free fatty acid portion of the 1- and 2-lauryl glycerides and oleic acid in the 1- and 2-oleyl glycerides indicated that oxygen attack also took place on the products of hydrolysis of the triglycerides during thermal oxidation. Lauric and oleic acids were probably broken down into short chain fragments and removed along with the volatile condensable products.

Small amounts of short chain fatty acids, namely C_{11} to C_{15}, were also found in the free fatty acids of the oxidized triglycerides. These fatty acids were probably formed from free palmitic acid by stepwise removal of one carbon from the molecule by oxidation. The following mechanism may explain the formation of C_{15} acid from palmitic acid:

$$CH_3(CH_2)_{14}COOH \longrightarrow CH_3(CH_2)_{13}\overset{O}{\overset{\|}{C}}COOH \longrightarrow$$

$$CH_3(CH_2)_{13}CHO \xrightarrow{O_2} CH_3(CH_2)_{13}COOH$$

Attack of oxygen at the alpha carbon of palmitic acid may form alpha keto palmitic acid, which was very unstable and released carbon dioxide to form an aldehyde. This aldehyde on further oxidation probably formed a C_{15} acid. The formation of other shorter chain fatty acids may be explained in the same way. It was, however, observed that the amounts of C_{13}, C_{12} (except in the case of lauryl glycerides), and C_{11} acids were much lower as compared to the C_{15} acid. These results indicated that short chain fatty acids are less susceptible to oxygen attack than long chain fatty acids. Nobori (25) and Ramanathan (26) have also pointed out that long chain methyl esters such as methyl stearate are more susceptible to oxygen attack than short chain esters. The isolation of a hydroxy acid with carbon number of 13.5 and the observation of acids with carbon number greater than 16 indicate that oxygenation of the fatty acids also occurred during thermal oxidation. This is in agreement with the results of other workers (27–30).

The following three reactions have therefore been found to occur during the thermal oxidation of a triglyceride: (1) oxygenation of the molecule, (2) hydrolysis of the ester linkage between glycerol and the fatty acid, and (3) oxidation of the free fatty acids.

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