1981

Effect of randomization on the oxidation products of corn oil

Flora Yu-man Lau
Iowa State University

LAU, FLORA YU-MAN

EFFECT OF RANDOMIZATION ON THE OXIDATION PRODUCTS OF CORN OIL

Iowa State University PH.D. 1981

Effect of randomization on the oxidation products of corn oil

by

Flora Yu-man Lau

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Food Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1981

TABLE OF CONTENTS

INTRODUCTION

Randomized oils oxidize two to three times faster than nonrandomized oils. Triglyceride structure may affect the susceptibility of fatty acids to oxidation (45b, 10, 47). The specific location of a fatty acid within the triglyceride maybe of particular importance, but little research has been done to investigate this.

The role of triglyceride structure in fat stability is important not only because randomization is an important commercial process. During manufacture, fats and oils from several sources may be blended. Possibly, the stability of the blend depends on the glyceride structure of the components. Also we frequently eat a diet that mixes fats and oils from several sources. A considerable amount of the original glyceride structure is preserved through digestion; and the stability of our depot fat may be affected by diet. Hydrogenation of fats is a more or less random process. Does this change the glyceride structure in undesirable ways? There is considerable interest in altering the fatty acid composition of plant oils by breeding. Is it possible to alter the triglyceride pattern of plant oils by breeding? Until we understand the role of glyceride structure in oil stability, it is impossible to answer these questions.

In this study, corn oil and samples of randomized corn coil and methyl esters of corn oil were prepared, purified and oxidized at 28C. The oxidative rate and the products of the initial state of oxidation were studied. Additional oxidative rate studies were performed to determine the cause of the variation in rates of oxidation.

LITERATURE REVIEW

Fatty Acid Distribution Hypotheses

Properties of triglycerides depend both on fatty acid composition and on the distribution of fatty acids in the triglyceride. Several hypotheses have been proposed to account for the observed distribution of fatty acids. Some of these theories include mathematical fatty acid distribution hypotheses for natural fats that predict the particular triglyceride contributions that occur. These theories assume random combinations occur except for specific restraints that may be imposed by the specific theory. Even or widest distribution (11) and restricted random (34, 35) distributions were proposed to account for the lack of trisaturated glycerides in vegetable oils. 1,2,3-random or unrestricted random distribution was believed to apply to animal fats (44a). 1,3-random-2-random distributions were proposed to account for results obtained by lipase analyses (58). The most widely accepted theory at present is 1-random-2-random-3-random theory first proposed by Tsuda in 1962, as cited by Litchfield (38). It accounts for the results of stereospecific analyses. Tsuda assumed that three different pools of fatty acids were separately distributed to the sn-1, sn-2, and sn-3-positions of all glycerol molecules in fat. Within its respective

position, each pool of acids was distributed at random. The amount of each component triglyceride could be calculated from the general equation:

$$\%\text{sn-XYZ} = \left[\begin{matrix}\text{mole \% X at} \\ \text{sn-1-position}\end{matrix}\right] \left[\begin{matrix}\text{mole \% Y at} \\ \text{sn-2-position}\end{matrix}\right] \left[\begin{matrix}\text{mole \% Z at} \\ \text{sn-3-position}\end{matrix}\right] (10^{-4})$$

Attempts have been made to predict the distribution of particular acyl groups at various positions. Gunstone (23) first suggested that in vegetable oils, oleic, linoleic, and linolenic acids were randomly distributed among the free hydroxy groups of glycerol remaining after palmitic, stearic, and longer chain fatty acids were esterified at the 1,3-positions. But further study of lipolysis results by Gunstone and co-workers (24) indicated that among the C_{18} unsaturated acids, oleic and linolenic acids were more likely to be found in the 1,3-positions while linoleic acid was more frequently encountered on the 2-position. This was taken into account by Evans et al. (15) who proposed the following three rules to calculate the composition of whole oil:

a. Saturated acids and those with chain lengths greater than 18 carbons are first distributed equally at the 1,3-positions.

b. Oleic and linolenic acids are then distributed equally and randomly on the unfilled 1-, 2-, and 3-positions, with excess from the 1- and 3-positions being added to the 2-position.

c. All remaining positions are filled by linoleic acids.

At the time this theory was proposed, there was no method available to distinguish the sn-1 and 3-positions. With the advent of sterospecific analysis by Brockerhoff (8), the fatty acid compositions of the sn-1 and 3-positions were found to differ but to resemble each other more than they resembled that of the 2-position.

Fatty Acid Composition and Positional Distribution in Corn Oil

Although corn is one of the principal crops of the United States, only a small fraction of it is used for obtaining corn oil. Corn oil is a byproduct of wet or dry-milling of corn.

According to Mattil et al. (40), crude corn oil has a dark reddish amber color and even after refining it is somewhat darker than most other vegetable oils. It contains relatively large amounts (1-3%) of phosphatides and other nonoil substances, mainly sterols (often 1% or more); and its free fatty acid content (usually above 15%) is higher than that of other common vegetable seed oils of good quality.

Tocopherol is also an important component (0.1%) of the unsaponifiable fraction, and may contribute to its high stability. Oleic and linoleic acids usually comprise over 80% of the fatty acids in a ratio of 1:2 to 1:3. Linolenic acid is either absent or present in traces. Palmitic acid is the main saturated fatty acid (10%). The triglycerides are mainly di- and triunsaturated. Characteristics and composition of corn oil are given in Table 1.

Table 1. Characteristics and composition of corn oil (3)

Saturated fatty acids, wt %				
Myristic	Palmitic	Stearic	C_{20-22} Saturated	Total
0.2	9.9	2.9	0.2	13.2
Unsaturated fatty acids, wt %				
Hexadececenoic	Oleic	Linoleic	Linolenic	Total
0.5	30.1	56.2	0.0	86.8

Scholfield et al. (49) by using a counter current distribution apparatus reported the major component glycerides of corn oil were trilinolein, dilinoleo-oleins and dioleo-linoleins. Brockerhoff (8) has examined the positional distribution of fatty acids in corn oil

triglycerides with two different methods. In the first method, referred to as the sn-1,3-diglyceride method, deacylation of triglycerides was carried out with a Grignard reagent which was nonspecific in its attack on the triglycerides. The sn-1,2(2,3)-diglycerides were separated from the sn-1,3-diglycerides by preparative thin-layer chromatography (TLC). The sn-1,3-diglycerides were converted to sn-1,3-diacyl-2-phosphatidylphenol using phenyl dichlorophosphate. Treatment with phospholipase A would hydrolyze only the sn-1-acyl group from an sn-2-phosphatide, leaving a lysophosphatide containing sn-3-acyl chain. In the second method, referred to as the sn-1,2(2,3)-diglyceride method, triglycerides were incubated with pancreatic lipase to obtain sn-2-monoglycerides. Either subsequently or simultaneously, the triglycerides were deacylated to representative sn-1,2(2,3)-diglycerides with pancreatic lipase or a Grignard reagent. After isolation of sn-1,2(2,3)-diglycerides by preparative TLC, they were reacted with phenyl dichlorophosphate to produce a mixture of sn-1,2-diacyl-3-phosphatidylphenol and sn-2,3-diacyl-1-phosphatidylphenol. Treatment with phospholipase A liberated the fatty acids from the 2-position of the sn-3-phosphatide but left the sn-1-phosphatide unhydrolyzed. Separation and fatty acids analysis of the various reaction products allowed the determination of the composition of

fatty acids in the sn-1-, sn-2- and sn-3- positions in the original triglyceride. His results summarized in Table 2, showed close agreement between the two methods.

Table 2. Comparison of Brockerhoff's two methods for sterospecific analysis of corn oil triglycerides (8)

Method	Position	Fatty Acid Composition (mole %)					
		16:0	16:1	18:0	18:1	18:2	18:3
sn-1,2(2,3)-diglycerides	1	17.9	0.3	3.2	27.5	49.8	1.2
	2	2.3	0.1	0.2	26.5	70.3	0.7
	3	13.5	0.1	2.8	30.6	51.6	1.0
sn-1,3-diglycerides	1	18.5	0.4	3.5	28.1	48.5	1.0
	2	1.8	0.1	0.2	25.8	71.2	0.9
	3	12.6	0.5	2.2	31.0	52.6	1.1

Transesterification

Transesterification, sometimes called "ester interchange" or "interesterification", changes the distribution of fatty acids among the glycerides of fats or mixtures of fats toward a random distribution. It involves an interchange of acyl groups among triglycerides. Acyl groups may exchange positions within a triglyceride or between triglyceride molecules. Interesterification can be carried to an equilibrium condition, at which point the fatty acids assume an almost random distribution among the triglycerides. The

process also is commonly referred to as randomization. Transesterification requires a catalyst. Screenivasan (50) summarized information on some of the important catalysts and conditions for their use. He also reviewed the possible mechanisms involved in the interchange.

Transesterification affects the physical nature and behavior of fats. It finds application in such fields as shortenings, margarine and confectionary fats where these characteristics are important (50). In the case of lard, unmodified lard shortenings tend to become grainy and produce very coarse crystals. Randomization improves the plastic range of lard and this makes it a better shortening than unmodified fat (13).

Products of Autoxidation of Fats and Oils

Primary products of oxidation

Edible fats and oils contain unsaturated and saturated fatty acids in various ratios. The unsaturated fatty acids autoxidize at a different rate and in addition each unsaturated fatty acid can give rise to different hydroperoxides. From previous work on hydroperoxide theory of autoxidation, it is possible to predict which hydroperoxide will be formed in autoxidation.

Saunders et al. (48) obtained direct experimental evidence that hydroperoxides and other oxygen-containing products were formed even in early stages of autoxidation of methyl oleate. They followed the absorption of oxygen quantitatively over a wide range of oxygen uptakes and then analyzed the methyl oleate hydroperoxides polarographically and iodometrically. They showed that of the total peroxides formed, only 90-95% could be accounted for as hydroperoxides. Swern et al. (56) proposed these nonhydroperoxides could be cyclic peroxides since on reduction they yielded a glycol. Khan et al. (36) reported the presence of α-ketols in autoxidized samples. The α-ketols also were readily reduced to glycols. This question has not been re-examined with more recent techniques and the nature of these nonhydroperoxides is not known.

Numerous investigations have been carried out to isolate and determine the structure of hydroperoxides. Farmer and Sutton (16) isolated methyl oleate peroxides by continuous molecular distillation. These somewhat impure peroxides consisted mainly of mono-hydroperoxide but also contained a little di-hydroperoxide together with some peroxide transformation products. Ross et al. (46) reported that the shifting of double bond occurs in the autoxidation of methyl oleate. They also found the four isomers

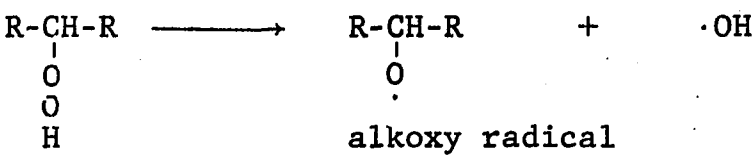
(8,9,10 and 11-hydroperoxides) predicted by Farmer's α-attack theory. According to Frankel (18), the most accepted mechanism for oleate autoxidation involved hydrogen abstraction at carbon-8 and -11. By this mechanism equal amounts of 8-, 9-, 10-, and 11-isomers are expected. However, Frankel et al. (19) found consistently higher concentrations of 8- and 11-hydroperoxides than 9- and 10-hydroperoxides in their investigation of autoxidation products of methyl oleate. They suggested that allyl isomerization might provide an adequate explanation for the uneven distribution of isomers found in their study.

Bergstrom (5) isolated the hydrogenated products from autoxidized linoleate and showed that they contained 9- and 13-hydroxystearins but not 11-hydroxystearins. His results, therefore, left it an open question whether any 11-hydroperoxide or other unconjugated hydroperoxide was formed to any appreciable extent during the autoxidation of methyl linoleate. Bolland and Gee (7) in their studies of the kinetics of autoxidation of ethyl linoleate suggested that there would be little probability of the formation of appreciable amounts of unconjugated 11-hydroperoxide. The absence of 11-hydroperoxide has been reported also by some other workers (6, 26, 59). Bank et al. (4) reported equal amounts of 9- and 13-hydroperoxides. Recently, Frankel and his co-workers (20) studied the co-oxidation of methyl

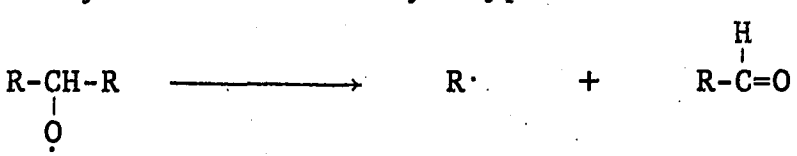
oleate and methyl linoleate by gas-liquid chromatography (GLC) - mass spectrometry (MS) analysis of trimethylsilyl- (TMS) - ether derivatives of the reduced hydroperoxides. In equal mixtures of oleate and linoleate oxidized to different degrees, about 80% of the peroxides originated from linoleate, and with a 9:1 oleate-linoleate mixture, 50% of the hydroperoxides came from linoleate. They found that the 9-hydroxyester came from both oleate and linoleate hydroperoxides, but the 13-hydroxyester came only from linoleate hydroperoxides.

Secondary degradation products

Hydroperoxides are the primary products of autoxidation. The secondary degradation products of lipid oxidation are formed from hydroperoxide decomposition. The first step of hydroperoxide decomposition is visualized as decomposition to the alkoxy and hydroxy free radicals (2).



Then chain scission can occur on either side of the radical to yield an aldehyde and a new alkyl type radical.



Reaction schemes have been published for the production of various carbonyl compounds from oleic and linoleic acids (2, 12). They are presented in Table 3.

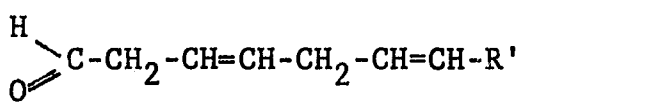
Table 3. Carbonyl compounds resulting from the decomposition of hydroperoxides

Isomeric Hydroperoxides	Carbonyl Compounds
From oleic acid	
11-hydroperoxy 9	C_8 n-aldehyde
9-hydroperoxy 10	C_{10} 2-enal, C_9 n-aldehyde
8-hydroperoxy 9	C_{11} 2-enal, C_{10} n-aldehyde
10-hydroperoxy 8	C_9 n-aldehyde
From linoleic acid	
13-hydroperoxy 9, 11	C_6 n-aldehyde
9-hydroperoxy 10, 11	C_{10} 2,4-dienal, C_9 3-enal and/or C_9 2-enal

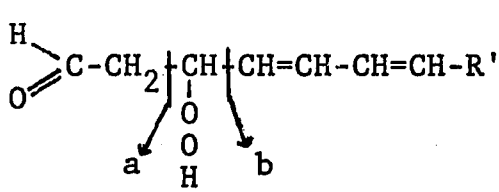
The hydroperoxide theory is used to explain the occurrence of C_8 and C_9 n-aldehyde and C_{10} and C_{11} 2-enals from oxidized oleic acids (2) and triolein (22). Swift et al. (57a) identified C_{11} 2-enal as a carbonyl formed in the decomposition of oleate hydroperoxides. Bading (2) cited a study done by Horikx and Schogt in 1959. Horikx and Schogt studied the carbonyl compounds formed in the initial phases of autoxidation of methyl oleate and triolein. They identified the saturated aldehydes and chain

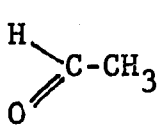
lengths C_5 to C_{11} and C_6 to C_{12} 2-enals. The formation of C_8 and C_9 n-aldehydes and C_{10} and C_{11} 2-enals was in line with the theory of decomposition of four isomeric hydroperoxides of oleic acid. C_6 n-aldehydes and C_{10} 2,4-dienals isolated from a limited autoxidation of ammonium linoleate (1), linoleic acid and esters (22) could be explained by hydroperoxide theory.

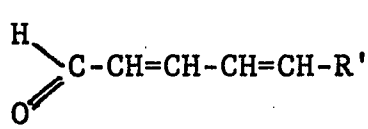
Evans (14) illustrated how the following dialdehydes, unsaturated hydrocarbons, and semialdehyde esters of different chain length could be obtained from secondary oxidation of linolenate hydroperoxide decomposition products through chain scission:

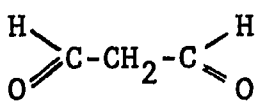


unsaturated aldehyde ester, a linolenate hydroperoxide decomposition product

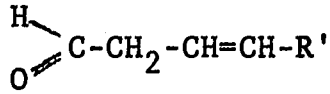


a. 



b. 

$H_2C=CH-CH=CH-R'$



Secondary oxidation of this type could explain the formation of cis-3-hexenal (14), which might be derived from more than one source, and the formation of a large number of dialdehydes. Buss and MacKinney (9) indicated the formation of several dialdehydes during the autoxidation of corn oil for 10 days at 78 to 85C with aeration. The oxidized corn oil was steam distilled and the carbonyls collected in the distillate were converted to 2,4-dinitrophenylhydrazones, which were separated by column chromatography. However, they did not identify the individual components.

Keeney (37) illustrated how the primary alkoxy radical could react to give rise to series of compounds. The mechanisms may be summarized in the following manner:

$$RCH_2O\cdot \longrightarrow CH_2O + R\cdot$$

$$RCH_2O\cdot + R'H \longrightarrow RCH_2OH + R'\cdot$$

$$RCH_2O\cdot + R'\cdot \longrightarrow RCHO + R'H$$

$$RCH_2O\cdot + R'\cdot \longrightarrow RCH_2O - R'$$

$$RCH_2O\cdot + R'O\cdot \longrightarrow RCHO + R'OH$$

Gaddis et al. (22) reported that the major aldehydes in oleate and linoleate were those that might be expected from the scission of monomeric hydroperoxide isomers. The number of minor monocarbonyl compounds increased with the degree of unsaturation. Their route of formation was not clear. Selke et al. (51) investigated the volatile components from triolein and trilinolein heated at an elevated temperature

in air. Results showed that major volatiles were associated with decomposition of the theoretical hydroperoxides. However, other volatiles were also present which were not directly associated with the theoretical decomposition mechanism. There were aliphatic acids, saturated and unsaturated aldehydes, primary alcohols, gamma lactones, hydrocarbons and methyl ketones. Furan and secondary alcohols were also produced from heated trilinolein. Selke et al. (52) also studied the thermal decomposition of methyl oleate hydroperoxides and found the major volatile compounds identified corresponded to those formed from the heated triolein. Henderson et al. (29) were able to isolate and identify the decomposition products of autoxidized linoleate. They reported a large number of cyclic hydrocarbons and alkyl ethers when trilinoleins were heated at a high temperature. Quantitatively, hexanal and aldehyde esters were found in significantly greater amounts in samples heated at a lower temperature, whereas the reverse was true for 2,4-decadienal. Sahasrabudhe and Farn (47) separated the triglyceride fraction and the polymeric fraction from corn oil after heating the oil to 200 C in air. In the polymeric fraction, they found branching in both short chain unsaturated acids and hydroxy acids.

Effect of Fatty Acid Composition and Glyceride Structure on the Rate of Autoxidation in Lipids

The rates of autoxidation are greatly dependent upon the degree of unsaturation of fatty acids. The rate of autoxidation increases in an exponential manner with increasing unsaturation. Comparison of rates of oxidation of purified methyl oleate, linoleate and linolenate has shown these esters oxidized at rates in the ratio of 1:10:20, respectively (25, 31a, 55).

Wong and Hammond (60) measured the rate of oxidation of methyl oleate and linoleate in mixtures by reducing the hydroperoxides to the corresponding alcohols and separated the methyl hydroxyoleate from the methyl hydroxylinoleate by GLC of the TMS-ether derivatives. They found that the relative rates of oxidation of methyl oleate and methyl linoleate seemed to vary with the composition of the mixture, but in general linoleate oxidized about ten times faster than oleate. Frankel et al. (19, 20, 21) also analyzed hydroperoxide mixtures by combined GLC-MS. The TMS-ether derivatives were obtained from oxidized methyl esters after reduction of hydroperoxides and saturation of double bonds. Their data suggested that the relative rates of oxidation of oleate, linoleate and linolenate varied with the composition of the mixture. Recently, Fatemi and Hammond (17) by using the same technique as Wong and Hammond (60) studied the

relative rates of oxidation of fatty acid methyl esters in mixtures. The relative rates of oxidation of methyl oleate, linoleate and linolenate in a mixture were about 1:10.3:21.6. They believed that Wong and Hammond's (60) conclusion that the ratios varied with the composition of the mixtures was caused by artifacts giving high values for oleate hydroperoxide. The results of Frankel et al. (20) supported the conclusion of Wong and Hammond (60), but the experiments of Frankel et al. (20) were done at a high temperature where recoveries of peroxides were low.

The glyceride structure of fats and oils may affect the rates of autoxidation. The specific location of fatty acid within the triglyceride may be of particular importance because the position of a fatty acid affects the susceptibility to oxidation (47). However, relatively little research has been done to investigate the influence of more than one type of fatty acid and its position in the same triglyceride molecule.

Gaddis et al. (22) observed that the amounts and proportions of monocarbonyl compounds produced varied considerably when pork, lamb, beef and butterfat with palm oil were heated at 165 C. These fats in general are similar in unsaturated fatty acid composition but different in triglyceride structure. They suggested that the antioxidants, glyceride structure, fatty acid content or the degree of

oxidation could possibly be the cause of the variation. Raghuveer and Hammond (45b) found that the rate of autoxidation of mixtures of 1.5-2% triunsaturated glycerides in tridecanoin was decreased after randomization. This was attributed to more complete dispersal of the unsaturated fatty acid in the mixture. They noted that most fats decreased in stability on randomization and proposed a theory based on the hexagonal packing of glyceride acyl chains in the molten state. They suggested that the acyl groups at sn-1 and sn-3-positions should oxidize faster than those at sn-2. The concentration of unsaturated fatty acid in the 2 position of a triglyceride should stabilize a fat against oxidation. However, their theory does not sufficiently explain the increased oxidation rate in natural oils and fats after randomization. Fatemi and Hammond (17) reported randomized oils or methyl ester mixtures oxidized three or four times faster than natural oils. The reason is unknown.

Hoffman et al. (30) studied the oxidative stability of synthesized triglycerides containing palmitic, stearic, oleic and linoleic acids. They reported that the oxidative stability of various triglycerides appeared not to be determined solely by the total unsaturation. The 1,3-equiacyl triglycerides were found to be more stable against oxidation than the 1,2-position isomers. The reason is unclear. They

further pointed out that palm oil was more stable than lard. When both fats were randomized (sodium methylate as catalyst, 110 C, 1 hr, N_2 atmosphere), oxidative stability of palm oil decreased even below that of the nonrandomized lard, whereas the stability of randomized lard hardly changed. They suggested the cause might be due to the instability of 1-oleo-2,3-distearin. Randomization of palm oil causes a big increase (7 to 20%) in this triglyceride, whereas randomization of lard causes only a slight change in this triglyceride. Catalino et al. (10) supported this finding. They found cocoa butter and palm oil which were high in 1,3-distearo-2-olein were more stable against oxidation than lard which was high in 1-oleo-2,3-distearin. The stability of cocoa butter and palm oil vanished after randomization. There was little change in the stability of randomized lard. Hammond[1] synthesized 1,3-distearo-2-olein and 1-oleo-2,3-distearin. The triglycerides were purified through an alumina column and oxygen uptake was measured during oxidation. He reported that 1-oleo-2,3-distearin oxidized 2.5 times faster than 1,3-distearo-2-olein.

Fatemi and Hammond (17) reported that the proportions of oleate, linoleate, and linolenate hydroperoxides formed in the oxidation of soybean and olive oils were similar

---

[1]E. G. Hammond, Department of Food Technology, Iowa State University, Ames, IA, 1968.

before and after randomization and similar to that from corresponding methyl ester mixtures. They concluded that glyceride structure did not greatly affect the peroxide types in the autoxidation of natural oils. However, the types of fatty acids and their positional distribution in the triglyceride molecule were not studied. Their results are in contrast to the findings of Raghuveer (45a) and Johnson (33). Raghuveer (45a) and Johnson (33) found that fatty acids in natural oils oxidized in different proportions than that would be predicted from oxidation of the corresponding methyl ester mixtures. In their studies, the method they used for measuring hydroperoxides formation involved the reduction of hydroperoxides in the oxidized mixture to corresponding hydroxyesters; these were acylated with acetic or butyric anhydride and the resulting methyl acyl esters were separated from unoxidized esters by urea fractionation. The oxidized esters were estimated by densitometry on TLC. However, Wong and Hammond (60) found the separation of hydroxyesters of acylated hydroxyesters by TLC was difficult to reproduce. This may account partly for the difference in findings between Fatemi and Hammond (17) and Raghuveer (45a) and Johnson (33).

MATERIALS AND METHODS

Materials

Corn oil was randomized using 0.5% sodium methoxide. The reaction mixture was stirred with a magnetic stirrer for 5 hr. The pressure was kept below 1 Torr and the temperature was maintained at 60 C. After randomization, the mixture was washed with 5% acetic acid, 5% sodium bicarbonate solution, and then with distilled water. The randomized oil was dried over sodium sulfate.

Methyl esters of corn oil were prepared in the presence of either 0.5% sodium methoxide or 2% sulfuric acid as catalyst. Corn oil weighing 200 g and 500 ml methanol were refluxed for 6 hr with sodium methoxide and 6 hr or 18 hr with sulfuric acid.

Triacetin was "randomized and washed" by the same procedure used for corn oil.

Natural and randomized corn oil and methyl esters of corn oil were purified through alumina before oxidation (31) to remove impurities which interfered with the subsequent analyses. The absence of components interfering with the TLC of the oxidation products was ascertained by the same methods used below in the analysis.

To ascertain that the tocopherols present in corn oil were removed by the alumina treatment (31), tocopherol

analysis was performed in natural, randomized and methyl esters of corn oil by TLC-GLC procedure of Meijbroom and Jongenotter (42a). The GLC analysis was carried out on a Varian Model 3700 gas chromatograph. The temperature of the column (1 m, glass, 3.3 mm o.d.) was programmed from 190 to 230 C at 10 deg/min. The flow rates of nitrogen, hydrogen and air were 30, 30 and 300 cc/min, respectively. The column packing was 3% JXR on 100/120 mesh Gas-chrom Q (Applied Science Laboratory, State College, PA).

Castor oil with part of its hydroxy groups converted to TMS-ethers was prepared for a TLC standard by reacting 10 g of oil with 1.06 g of pyridine and 1.55 g of trimethylchlorosilane. The reaction mixture was stirred with a magnetic stirrer at room temperature till the reaction was complete. This was ascertained by TLC. The reaction mixture was washed with an equal volume of distilled water and 0.1 N hydrochloric acid and dried over sodium sulfate.

Methyl ricinoleate prepared by Wong and Hammond (60) was used for a TLC standard.

Methyl azelaic semialdehyde: To synthesize methyl azelaic semialdehyde, ozonolysis of purified corn oil methyl esters was carried out (43). A solution of 20 mg of methyl esters in 1 ml of carbon disulfide was cooled in dry ice-acetone and ozone was passed through it. Sudan red was dissolved directly in the reaction medium as an indicator.

The reaction mixture turned greyish white. When ozone absorption was complete, an excess of triphenylphosphine (100 mg) was added, and the reaction mixture was allowed to warm to room temperature. The triphenylphosphine reduced the ozonides to the corresponding aldehydes. An aliquot of the carbon disulfide solution was applied to TLC plate with methyl ricinoleate as standard. The fraction containing methyl azelaic semialdehyde was isolated and analyzed by infrared spectroscopy (IR), mass spectrometry (MS), and gas-liquid chromatography (GLC).

Azelaic acid methyl esters: 1 M of azelaic acid was reacted with 1.5 M of methanol and 0.5% sulfuric acid. The mixture was incubated overnight at 55 C. Hexane was added and the mixture was heated to solubilize the azelaic acid. Distilled water was added to wash away the sulfuric acid and the hexane layer was evaporated under nitrogen. An aliquot was applied to TLC plate. Further analyses were carried out by IR, MS and GLC.

Technical grade tetrachloroethane was purified by heating the solvent with lauroyl peroxide at 100 C for 1 hr. The solvent was distilled under reduced pressure. The distillate collected was redistilled in the presence of 1,5-diphenylcarbohydrazide.

In experiments in which distilled methyl esters were used, they were distilled through a 48-cm Widmer column at 0.5 Torr.

Oxidation and Analysis of Products

Oxidation conditions

All methyl esters and triglycerides were oxidized in 5-g lots in 125-ml Erlenmeyer flasks without stirring at 28 C.

When samples reached peroxide values (PV) of approximately 30 to 40, samples were withdrawn and peroxides were reduced to alcohols by the iodometric method of the American Oil Chemists' Society (41). The samples were extracted into chloroform, washed with distilled water and twice with 5% sodium bicarbonate solution. After drying the chloroform extract over sodium sulfate, the solvent was removed in a rotary evaporator at reduced pressure while the temperature was kept below 40 C.

Thin-layer chromatography

Plates of Silica Gel G (0.75 mm thick) were used for preparative separations. They were air-dried and activated at 100 C for 1 hr and stored in a dry chamber. To separate the oxidized products from the unoxidized glycerides of methyl esters, a 100 mg sample was applied to a silica gel plate with a sample streaker (Applied Science Laboratory, State College, PA). Methyl ricinoleate was applied to the sides as standard for oxidized corn oil methyl esters. Castor oil TMS-ether derivatives were used as standards

for oxidized triglycerides. The plate was developed in hexane-diethyl ether (60:40 v/v), and after spraying the plate with 0.2% 2',7'-dichlorofluorescein in 95% ethanol, the bands were viewed under ultraviolet light. Bands were scraped off the plate and extracted with 15 ml of ether. The ether was evaporated under a stream of nitrogen. The monohydroxy-triglyceride isolated from oxidized natural and randomized oil was converted to methyl esters by trans-esterification in methanol with sodium methoxide. The esters from the monohydroxy-triglyceride were examined by GLC.

Trimethylsilyl-ether derivatives

Methyl hydroxyesters were dissolved in 1 m of pyridine and 2 ml of hexane, and 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane were added with rapid mixing for 30 seconds. After standing overnight at room temperature, 5 ml of hexane and 5 ml of distilled water were added, and the hexane layer was recovered. The water layer was washed twice more with hexane, and the combined hexane extracts were dried over sodium sulfate and evaporated under a stream of nitrogen.

Gas-liquid chromatography

The GLC analysis for TMS-ether derivatives was carried out on a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. The temperature of the column

(6 ft, 1/8 in. o.d.) was 195 C and the flow rates of the nitrogen, hydrogen and air were 30, 30 and 300 cc/min, respectively. The packing was 10% OV225 cyanopropyl silicone on 100/120 mesh Chromsorb W (HP) (Pierce Chemical Company, Rockford, IL). The analyses of unoxidized methyl esters were performed on a Beckman GC5 gas chromatograph equipped with a flame ionization detector. The packing was 15% EGSX on 100/120 mesh Chromosorb P (Applied Science Laboratory, State College, PA). The flow rates of nitrogen, hydrogen and air were 50, 40 and 300 cc/min, respectively.

Lipase hydrolysis

The procedure of Luddy et al. (39) was used for the deacylation of monohydroxy methyl esters and triglycerides. The product after hydrolysis was quickly separated by preparative TLC on silica gel (0.5 mm thick) containing 6% (w/w) boric acid. The developing system consisted of hexane-diethyl ether (50:50 v/v), and after spraying the plate with 0.2% 2',7'-dichlorofluorescein in 95% ethanol, the spots were viewed under ultraviolet light.

Identification of unknown band

In the separation of oxidized products from the unoxidized triglycerides and methyl esters, an unknown band with a greater R_f than the monohydroxy-triglyceride band or methyl monohydroxy ester was detected in all oxidized samples.

Analyses were conducted in an attempt to identify the unknown band.

Infrared spectroscopy IR spectra were obtained with a Beckman IR12 infrared spectrophotometer fitted with a beam condensor. The analysis was performed in a potassium bromide ultramicro liquid cell.

Gas-liquid chromatography The GLC analysis was performed on a Varian Model 3700 gas chromatograph as before. The temperature was programmed from 50 to 230 C at 10 deg/min.

Mass-spectrometry Mass spectra of the unknown band in all oxidized samples were obtained with a Finnigan Model 400 gas chromatograph-mass spectrometer.

Oxidative Rate Studies

Five grams of ester or triglyceride in a 125-ml Erlenmeyer flask was oxidized at 28 C. Care was taken whenever possible to avoid metal contamination by soaking the glassware in alcoholic potassium hydroxide followed by 1N ammonium hydroxide. The PV was determined by the method of Hamm et al. (27).

RESULTS AND DISCUSSION

Oxidation and Analysis of Products

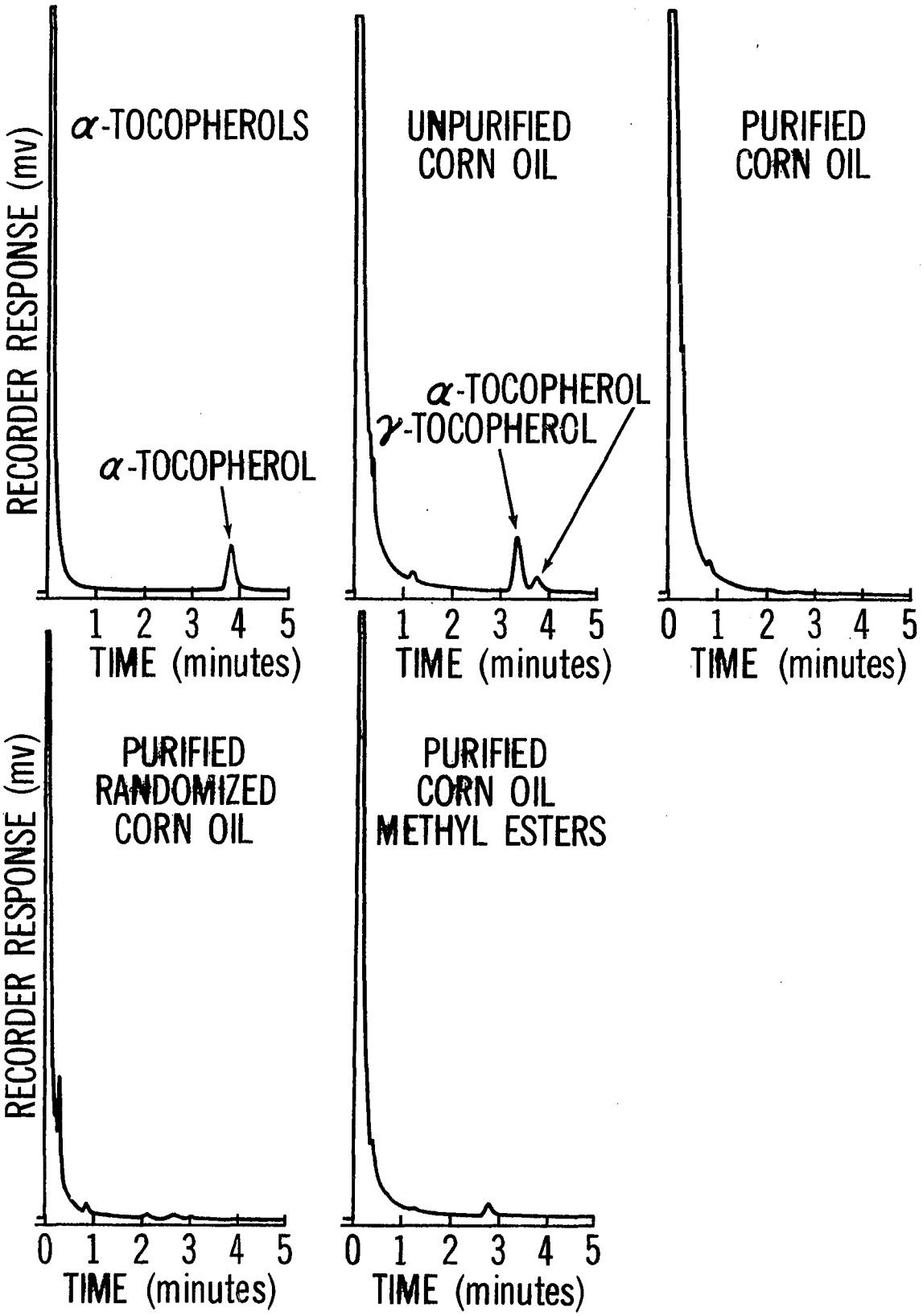
Triglyceride structure may affect the susceptibility of fatty acids to oxidation (45b, 47). To study this, corn oil, randomized corn oil, and corn oil methyl esters prepared with a sodium methoxide catalyst were purified by passage through an alumina column. The fatty acid composition of the oils is given in Table 4.

Table 4. Fatty acid composition of methyl esters of oil by gas-liquid chromatography (GLC) by percent

fatty acids	corn oil	randomized corn oil	corn oil methyl esters
palmitic	10.71	11.19	11.33
stearic	1.35	1.48	1.81
oleic	24.62	25.82	25.43
linoleic	62.55	60.92	60.57
linolenic	0.77	0.55	0.86

Thin-layer chromatography (TLC) analyses on these samples were performed and plates were sprayed with 10% phosphomolybdic acid in 95% ethanol. There were no components with R_f's below the triglyceride or methyl ester bands that would interfere with the TLC of the oxidation products.

Figure 1. Gas-liquid chromatography of tocopherols in corn oil samples and methyl esters of corn oil


RECORDER RESPONSE (mv)
α-TOCOPHEROLS
α-TOCOPHEROL
1 2 3 4 5
TIME (minutes)
UNPURIFIED CORN OIL
α-TOCOPHEROL
γ-TOCOPHEROL
1 2 3 4 5
TIME (minutes)
PURIFIED CORN OIL
0 1 2 3 4 5
TIME (minutes)
RECORDER RESPONSE (mv)
PURIFIED RANDOMIZED CORN OIL
0 1 2 3 4 5
TIME (minutes)
PURIFIED CORN OIL METHYL ESTERS
0 1 2 3 4 5
TIME (minutes)

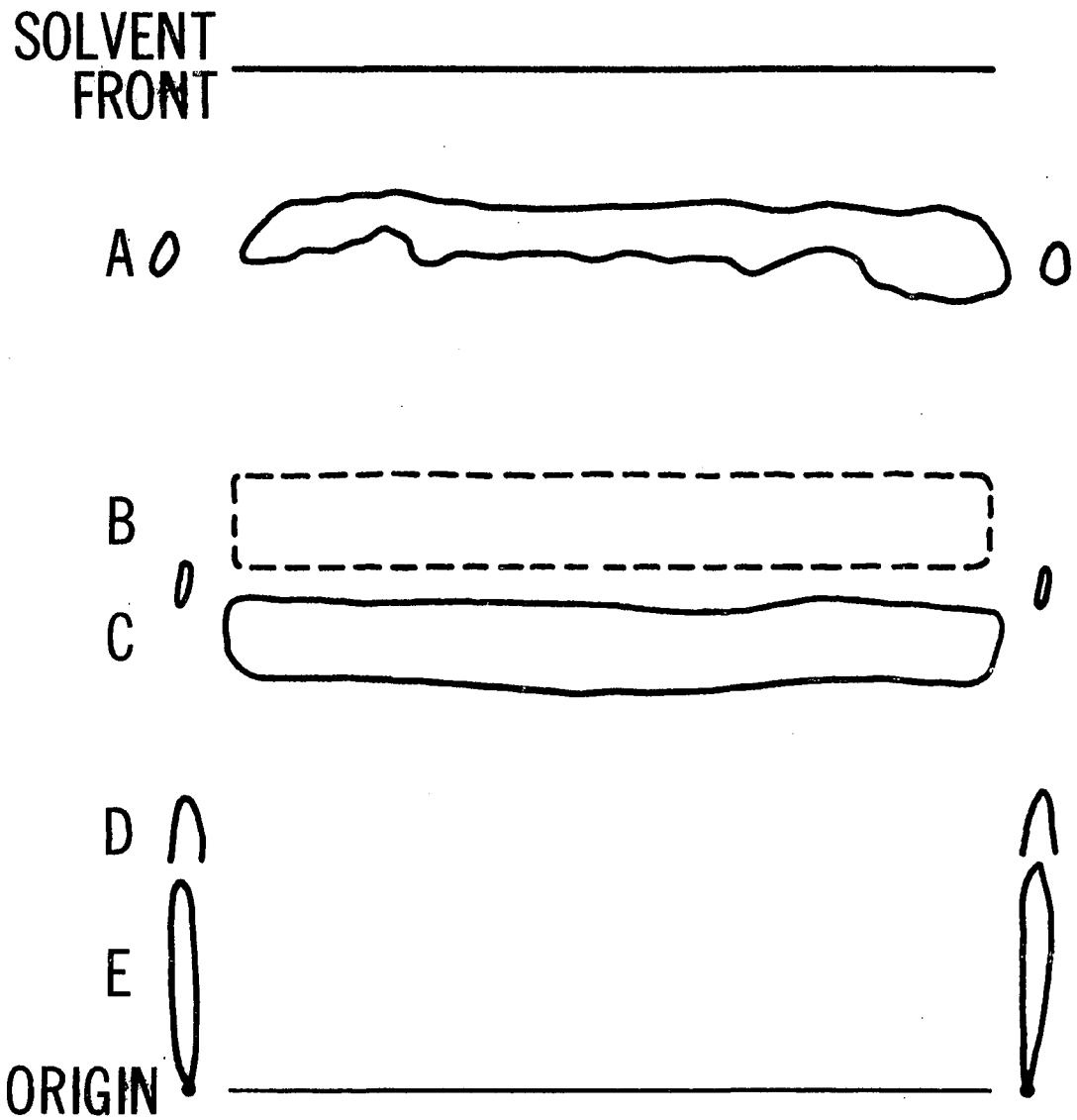
Gas-liquid chromatographic (GLC) analyses for tocopherols in the purified natural and randomized corn oil and methyl esters of corn oil are shown in Figure 1. Obviously, alumina treatment removed tocopherols from the corn oils and methyl esters.

The purified oils were oxidized to peroxide value (PV) of approximately 30 to 40. The kinetics of oxidation will be discussed in a later section, but in accord with earlier studies, I found that randomized oil always oxidized significantly faster than the natural oil, and methyl esters oxidized at a rate similar to the randomized oil.

After reduction of hydroperoxides to the corresponding alcohols, analyses on all oxidized samples were performed. The plate was developed twice in hexane and diethyl ether (70:30 V/V), sprayed with 2'7'-dichloroflourescein in 95% ethanol and viewed under ultraviolet (UV) light. The TLC analysis of the oxidized oil is shown in Figure 2. Two new bands were present with R_f's below those of the unoxidized triglycerides or methyl esters. The two bands had R_f's close to those expected for monohydroxy-triglycerides or monohydroxy-methyl esters. One traveled slightly ahead and the other slightly behind the castor oil standard. Castor oil with part of its hydroxy groups converted to ethers with trimethyl-chlorosilane (TMS) was used as TLC standard for oxidized natural and randomized corn oil. Castor oil is mainly triricinolein. With all of its hydroxy groups blocked by TMS,

Figure 2. Preparative thin-layer chromatography separations of oxidized randomized corn oil on Silica gel G. Solvent system: hexane and diethyl ether (70: 30 V/V)

A unoxidized triglycerides
B unknown band
C monohydroxy-triglycerides
D dihydroxy-triglycerides
E trihydroxy-triglycerides


SOLVENT FRONT
A
B
C
D
E
ORIGIN

castor oil should have a R_f corresponded to unoxidized corn oil triglycerides. When two of its hydroxy groups were blocked, the R_f should correspond to a monohydroxy-triglyceride, and with one hydroxy group converted, the R_f should correspond to the dihydroxy-triglycerides region. The unconverted castor oil triglyceride should remain at the origin. Methyl ricinoleate was used as TLC standard for oxidized corn oil methyl esters.

There was no evidence of the presence of dihydroxy- or trihydroxy-triglycerides or methyl esters.

Band C was converted to methyl esters with methanolic sodium methoxide. The methyl hydroxyesters contained in the C-band from the natural and randomized corn oil and the methyl hydroxyester band from the oxidized methyl ester corn oil were silylated. The GLC analysis of the silylated products gave three partially-separated peaks as shown in Figure 3. These results resemble those reported by Wong and Hammond (60) and Fatemi and Hammond (17). The results of autodation of natural and randomized corn oil and methyl esters of corn oil are given in Table 5. Wong and Hammond (60) reported that the apparent yield by GLC of the TMS-derivatives was 63% for methyl oleate hydroperoxide and 40% for methyl linoleate hydroperoxide based on methyl heptadeconoate as an internal standard. Fatemi and Hammond (17) reported values of 62 and 44% for oleate and linoleate hydroperoxide,

Figure 3. Typical gas chromatograph of TMS derivatives of methyl hydroxyesters of oxidized corn oil

1,3 : unoxidized fatty acid methyl esters
2 : methyl heptadecanoate
4 : TMS-oleate
5,6 : TMS-linoleate

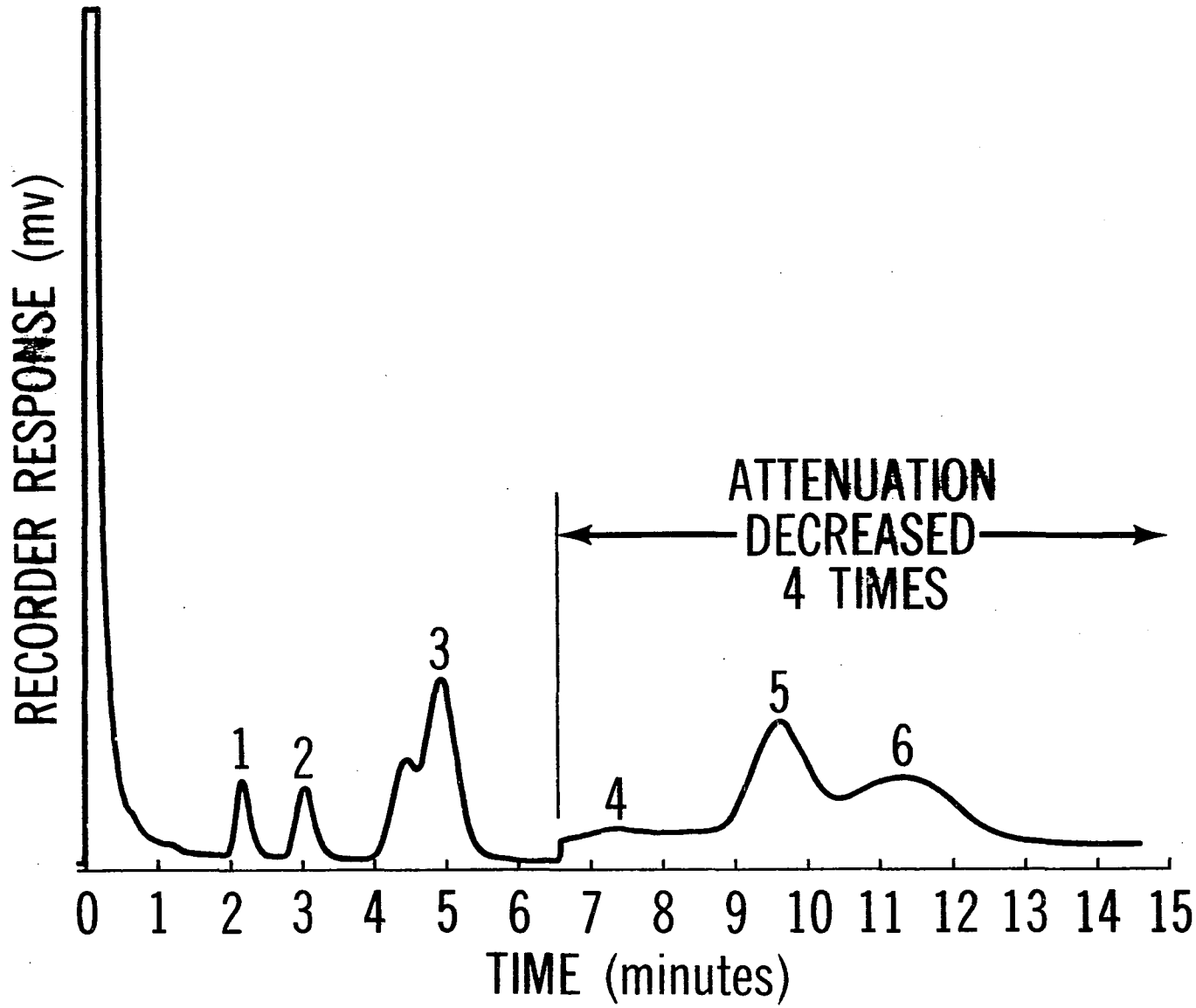

RECORDER RESPONSE (mv)
ATTENUATION
DECREASED
4 TIMES
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (minutes)

Table 5. GLC analyses on the recovery of TMS-derivatives of methyl hydroxyesters of oxidized natural and randomized corn oil and methyl esters of corn oil based on PV

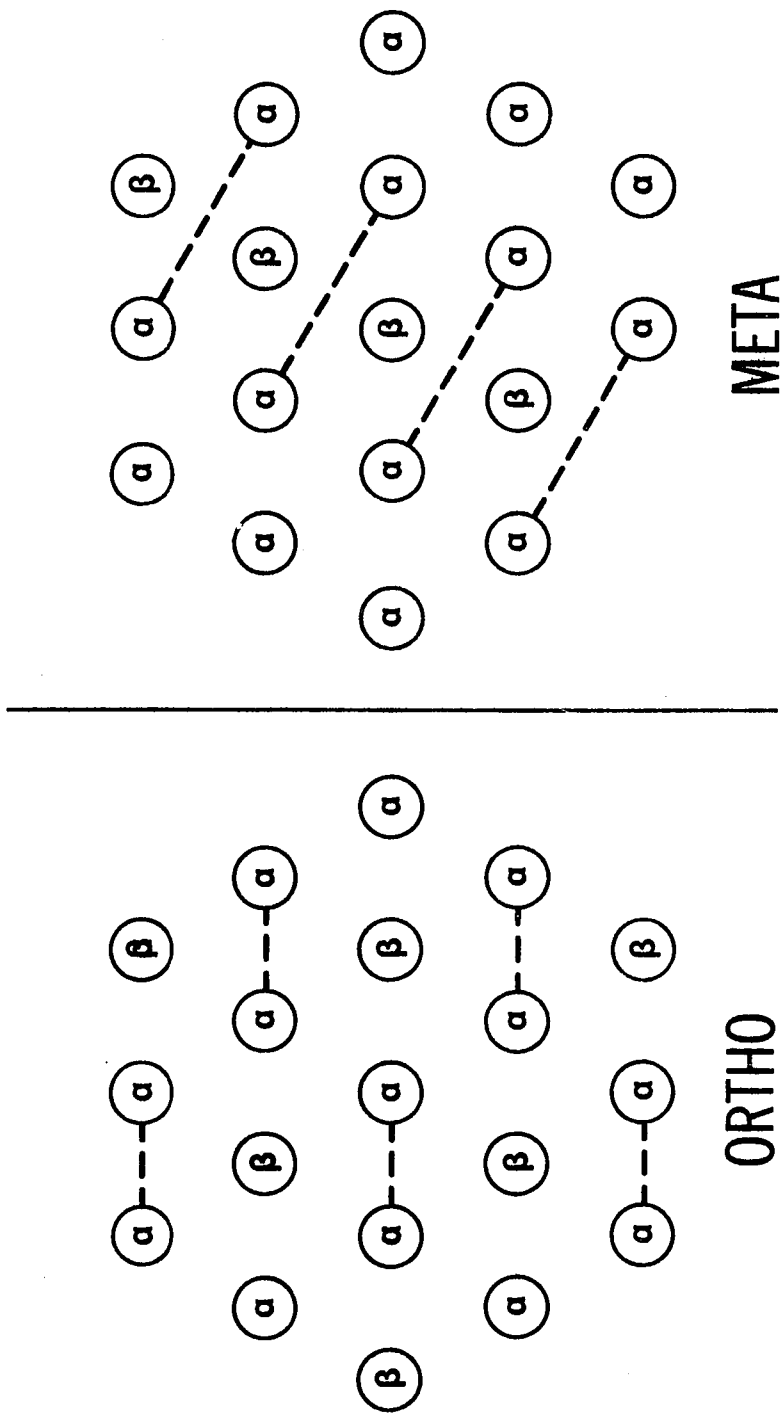
oil	PV	fatty acid % recovery		
		oxidized	unoxidized	ratio oxidized:unoxidized
corn oil[a]	30.54	70.17	60.98	1:1.74
	36.74	71.31	66.55	1:1.87
	43.43	68.24	53.56	1:1.57
randomized[a] corn oil	23.85	68.90	57.89	1:1.68
	30.85	69.44	70.96	1:2.04
	31.11	82.62	57.40	1:1.40
	37.11	82.33	62.12	1:1.53
methyl esters corn oil	26.66	52.64		
	33.95	58.60		
	45.73	56.13		
	45.76	57.23		
	48.66	51.40		

[a]Correction factors of 0.62 for methyl hydroxyoleate (17) and 0.55 for methyl hydroxylinoleate were applied in the calculation.

respectively. Subsequently, their analyses were corrected by these factors. In this study, the apparent yield by GLC of the TMS-derivatives was 55% for corn oil methyl esters which are primarily methyl linoleate hydroperoxide. The apparent yield probably improved because the time allowed for silylation was increased from 4 hr to overnight. The reasons for the large correction factors are not certain. Wong and Hammond (60) found that at least some of the correction factor was a result of the flame ionization detector's response to silyl ethers. Some of the correction might arise from side reaction in the conversion of the hydroperoxides to TMS-hydroxyesters, but they were unable to detect significant amounts of side products.

The TMS-ether derivatives obtained from the C-bands in the oxidized samples of randomized and natural corn oil accounted for 70-80% of the hydroperoxides generated in the early stage of autoxidation. The loss of unoxidized fatty acids is generally about 10% greater than for the hydroxy fatty acids, so probably part of the correction factor used for hydroxy fatty acids is for mechanical losses. The ratio of unoxidized to hydroxy fatty acid is about 1.5-2 to 1. A ratio of 2 to 1 would be expected for monohydroxy-triglycerides, so it can be concluded that the C-band is monodydroxy-triglycerides.

Figure 4. A cross-sectional representation of the ortho and meta hexagonal packing of molten glycerides. The dashed lines indicate alpha chains attached to the same glycerol molecule


META
ORTHO

These results were contradictory to the theory proposed by Raghuveer and Hammond (45b) to account for the effect of glyceride structure on autoxidation. They proposed a theory based on the hexagonal packing of glyceride acyl chains in the molten state. Figure 4 represents a cross-sectional view of this hexagonal packing and shows that there are two possible regular arrays; one in which the two alpha chains from a given molecule (indicated by the dashed line) occupy the ortho positions of the hexagon, and one in which they are in meta positions. They reasoned that when the two alpha chains occupied the ortho positions, they were hexagonal neighbors and should interact rapidly if one contains a peroxyl radical. Therefore, fats with polyene fatty acid concentrated on the β position would be more stable. Dihydroperoxy-triglycerides that yield dihyroxy-triglycerides in this analysis should be a major product according to Raghuveer and Hammond's theory (45b). The preparative TLC results indicated otherwise. There were no detectable dihydroxy-triglycerides in the oxidized oil. This indicates that either oxidation must occur exclusively on the β-chains or the α-chains on the same molecule seldom interact in autoxidation.

The unoxidized methyl esters recovered from the monohydroxy-triglycerides bands were analyzed by gas-liquid

Table 6. Fatty acid composition of unoxidized methyl esters recovered from monohydroxy-triglycerides by percent

corn oil	fatty acids				
	palmitic	stearic	oleic	linoleic	linolenic
natural					
triglycerides	10.71	1.35	24.62	62.55	0.77
monohydroxy-triglycerides	12.22	1.63	23.56	61.74	0.84
randomized					
triglycerides	11.19	1.48	25.82	60.96	0.55
monohydroxy-triglycerides	12.00	1.82	26.89	58.55	0.80

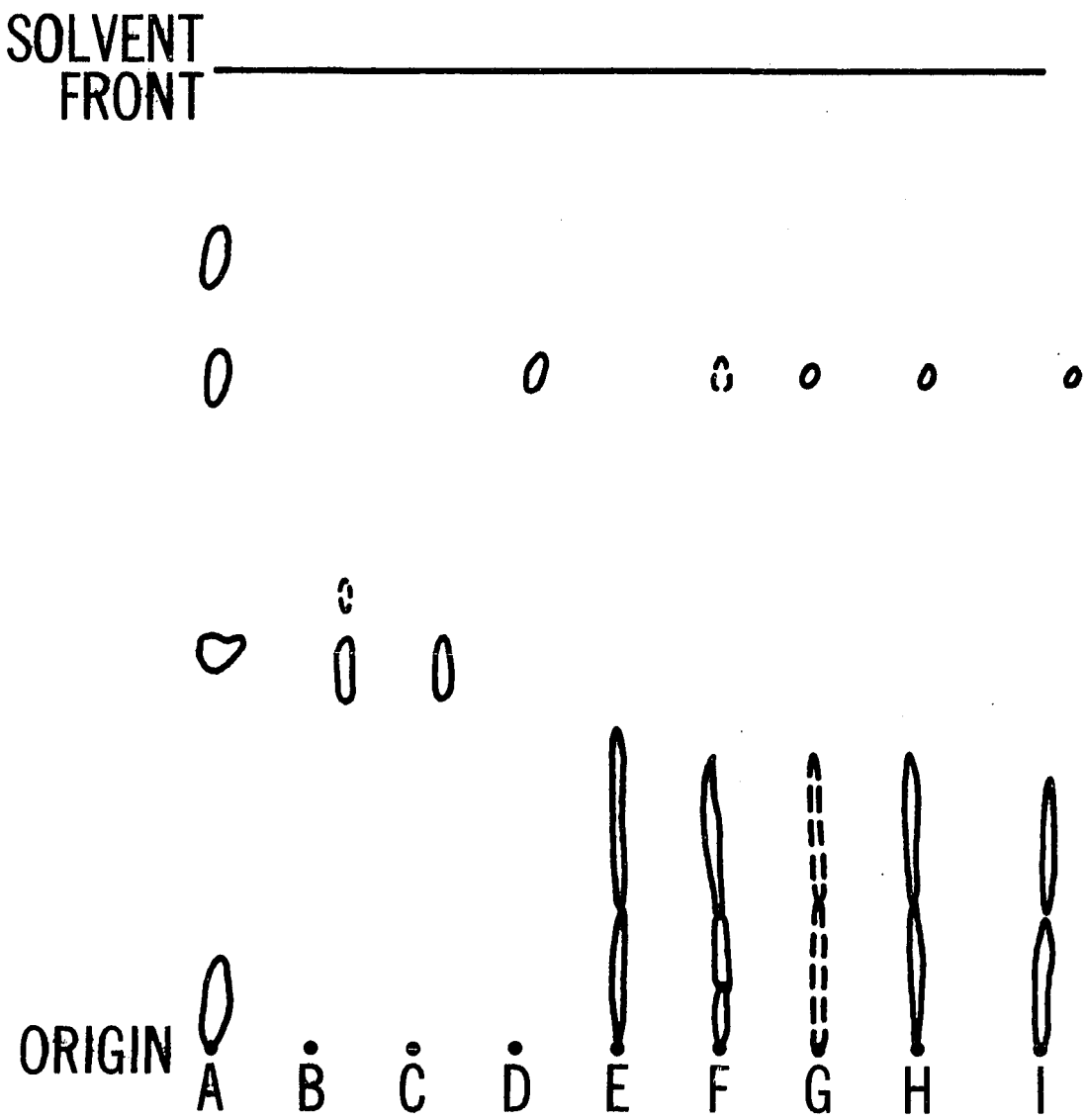
chromatography (GLC) and the results are summarized in Table 6.

The oxidized fatty acid in the monohydroxy-triglycerides is primarily linoleic acid as shown by Figure 3. This is expected because linoleic oxidizes about ten times faster than oleic, and given the composition of corn oil, about 96% of the oxidized acyl groups should be linoleic acid. But if the triglyceride structure were random, the fatty acids associated with the oxidized acyl group should be the same as the fatty acid composition of the unoxidized oil. This is approximately true for both the randomized and natural corn oil. There is a slight tendency for the unoxidized acids to be richer in saturated acyl groups, but this may not be a significant difference. Thus, there is no evidence that triglycerides with particular compositions are singled out for oxidation.

Attempts were made to determine the distribution of the hydroxy group in oxidized triglyceride by lipase hydrolysis. The TLC analysis of lipase-hydrolyzed monohydroxy-triglyceride or methyl-ester is shown in Figure 5. Standards were used to aid in the identification. Normal diglycerides appeared to be absent in the lipase-treated monohydroxy-triglycerides. Any monoglyceride found in the reactions was obscured by spots originating in the lipase blank. This interfered with the interpretation of the results. Moreover, little information

Figure 5. TLC of lipase-hydrolyzed monohydroxy-triglycerides or methyl esters of corn oil

A = lipase hydrolyzed unoxidized corn oil
B = 1,2 (1,3) diolein
C = 1,3 distearin
D = urucic acid
E = lipase blank
F = lipase-hydrolyzed corn oil
G = lipase-hydrolyzed monohydroxy-triglycerides from oxidized natural corn oil PV 32.41
H = lipase-hydrolyzed monohydroxy-triglycerides from oxidized randomized corn oil PV 31.16
I = lipase-hydrolyzed methyl monohydroxyesters from oxidized corn oil methyl esters PV 33.95


SOLVENT FRONT
ORIGIN
A
B
C
D
E
F
G
H
I

is available on the specificity of pancreatic lipase on glyceride acyl chains with hydroxy group attached. It is possible that pancreatic lipase is not a suitable enzyme for this purpose or perhaps, further purification of the enzyme is needed prior to use. However, it is possible that normal 1,2-diglycerides were indeed absent which would indicate that only the fatty acid on the β-position was being oxidized and only hydroxydiglycerides were present after lipase action.

Identification of Band B

A band with a greater R_f than monohydroxy-triglycerides but less than unoxidized triglycerides or methyl esters was detected under ultraviolet (UV) light after spraying with 2'7'-dichlorofluorescein. To enhance detection, plates were sprayed with 10% phosphomolybdic acid and heated at 110C. By visual estimate, with a dichlorofluorescein spray, the intensity of the B-band increased with peroxide value (PV) and appeared about three times more intense in the randomized oil than natural oil when the oils were oxidized to comparable PV's.

To compare the relative amount of the B-band in oxidized natural and randomized corn oil, the B-bands were isolated by thin-layer chromatography (TLC), converted to methyl esters with methanolic sodium methoxide, silylated and

analyzed by gas-liquid chromatography (GLC). The GLC analysis indicated there was only unoxidized methyl esters and no methyl hydroxyesters were detected. The relative amount of unoxidized methyl esters was approximately two times greater in the randomized than in the natural corn oil when they were oxidized to a comparable PV. Thus, the amount of band B produced during oxidation is an important difference between natural and randomized oils.

By visual estimate, with a dichlorofluorescein spray, the intensity of monohydroxy-triglycerides band was about two times more intense than the B-band in oxidized randomized corn oil.

The properties of band B suggested that it might contain a scission product such as azelaic acid semialdehyde; therefore, methyl azelaic semialdehyde and azelaic acid methyl esters were synthesized and purified by TLC. Components in these preparations had TLC R_f's similar to band B of oxidized corn oil methyl esters, using methyl ricinoleate as an R_f guide. These components were scraped off, eluted with ether and compared to band B by GLC, infrared (IR) and mass spectrometry (MS). The mass spectral data of methyl azelaic semialdehyde and azelaic acid methyl esters are listed in Table 7.

The GLC analyses for the B-bands in oxidized natural, randomized corn oil and corn oil methyl esters are shown in Figure 6.

Band B in oxidized corn oil methyl esters

The GLC analysis showed a number of peaks. Peak 1 appears only in oxidized methyl esters. The peak disappeared when band B was treated with methanolic sodium methoxide. The mass spectral data and GLC analysis indicate that peak 1 was probably nonanal. The mass spectral data of peak 1 are in Table 7. Both peaks 2 and 3 also disappeared when band B was treated with methanolic sodium methoxide. They are produced in the oxidation of both methyl esters and triglycerides. They are probably some kind of aldehyde scission product that is polymerized by sodium methoxide. The mass spectral data of the two components were similar and are in Table 7. This scission product has a molecular weight of 152 and probably has a furan ring. It matched closely with values reported for α-propyl furanacetaldehyde (31b). The retention time of peak 4 corresponded to that of methyl azelaic semiadlehyde. The retention time for peak 5 which appeared as a very small peaklet on the GLC, corresponded to the retention time of dimethyl azelate. MS analysis showed strong signals from dimethyl azelaic semiadlehyde.

Figure 6. The gas-chromotograms for B-bands in oxidized corn oil methyl esters and oxidized natural and randomized corn oil

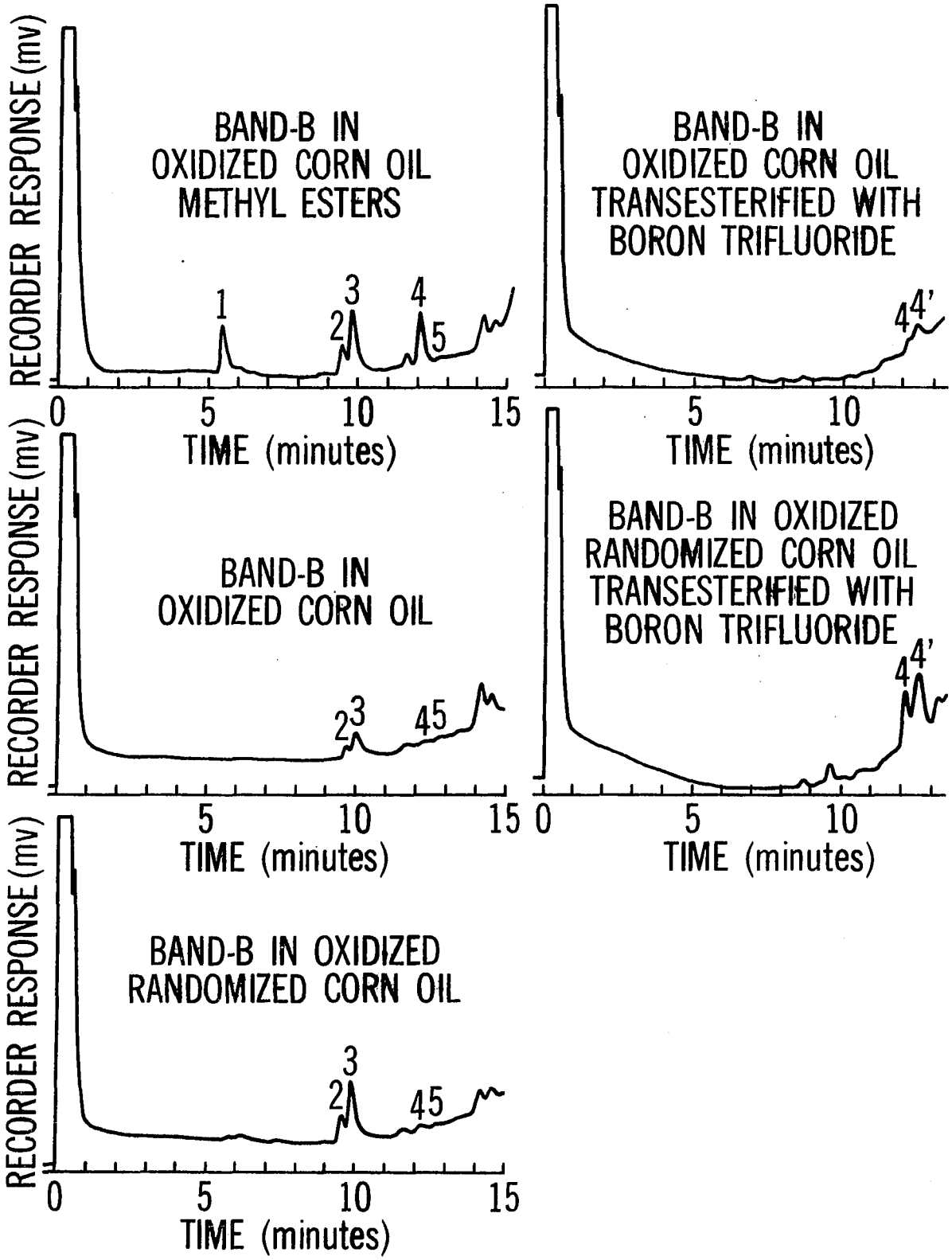

BAND-B IN
OXIDIZED CORN OIL
METHYL ESTERS
1
2
3
4
5
RECORDER RESPONSE (mv)
0
5
10
15
TIME (minutes)
BAND-B IN
OXIDIZED CORN OIL
TRANSESTERIFIED WITH
BORON TRIFLUORIDE
4
4'
5
10
TIME (minutes)
BAND-B IN
OXIDIZED CORN OIL
2
3
45
RECORDER RESPONSE (mv)
5
10
15
TIME (minutes)
BAND-B IN OXIDIZED
RANDOMIZED CORN OIL
TRANSESTERIFIED WITH
BORON TRIFLUORIDE
4
4'
0
5
10
TIME (minutes)
BAND-B IN OXIDIZED
RANDOMIZED CORN OIL
2
3
45
RECORDER RESPONSE (mv)
0
5
10
15
TIME (minutes)

Table 7. Mass spectral data of the scission products present in B-band

Identification	fragments m/e (relative abundance)
methyl azelaic semialdehyde	74(100), 87(49.43), 83(34.83), 111(24.16), 143(15.73), 155(8.43), 98(7.3), 94(5.62), 115(5.62), 158(3.9), 129(1.68)
dimethyl azelate	74(100), 152(62.57), 83(61.45), 111(45.25), 185(44.69), 84(32.96), 87(31.28), 143(25.69), 97(19.55), 124(14.23)
peak 1	71(100), 85(57.71), 70(44.00), 84(23.43), 67(12.00), 99(12.28), 69(10.57), 98(10.71), 142(6.57), 113(5.71)
peaks 2 and 3	81(100), 67(27.87), 65(12.35), 66(12.35), 83(11.20), 79(7.18), 152(2.88), 109(1.44), 123(1.44)

It is difficult to explain the presence of dimethyl azelate as an oxidation product, so its presence in the starting material was tested. Preparative TLC was performed on unoxidized corn oil methyl esters. The region corresponded to the B-band was scraped off, eluted with ether and analyzed by GLC-MS. A little methyl azelaic semialdehyde was detected in the unoxidized methyl esters but no dimethyl azelate. Since monomethyl azelate would come from the scission reaction, the presence of dimethyl azelate in band B of oxidized methyl esters could not be explained. Evans (14) pointed out that it was difficult to remove many breakdown products from oils and it was easy to obtain positive tests for an aldehyde in even the purest distilled fatty esters or refined fats. Some methyl azelaic semialdehyde might have generated during the transesterification step and had not been removed completely by the alumina treatment. The GLC analyses showed the amount present in oxidized methyl esters was about four to five times greater than in unoxidized methyl esters.

The IR spectrum of band B was similar to the spectra of methyl azelaic semialdehyde and dimethyl azelate. There was no indication of a hydroxy group. The CH stretch band (3010, 3040 cm^{-1}) for $-\underset{H}{C} = \underset{H}{C}-$ was missing, suggesting that double bonds were absent.

Band B in oxidized natural and randomized corn oil

The GLC analysis indicated the absence of peak 1 in both natural and randomized samples. Peaks 2 and 3 also disappeared when B-bands in these two samples were transesterified with sodium methoxide or boron trifluoride. These peaks are probably some kind of aldehyde as mentioned earlier. There were several times more of these peaks by GLC in randomized than in natural corn oil when they were at comparable peroxide values. Peaks 4 and 5, which corresponded to the retention time of methyl azelaic semialdehyde and dimethyl azelate, respectively, were also present. These two peaks were not very prominent in the GLC trace. Their presence was confirmed by MS.

A new peak, 4', with retention time immediately after peak 4 was detected when band B was transesterified with boron trifluoride. Several fold more area under peak 4' was produced from randomized than from natural corn oil oxidized to the same peroxide value. To identify peak 4', methyl azelaic semialdehyde was transesterified with boron trifluoride and analyzed by GLC. Dimethylacetal was formed and has a retention time corresponding to peak 4'. The B-band of oxidized randomized corn oil was transesterified with boron trifluoride and analyzed by MS. MS confirmed the presence of dimethylacetal. A mass spectrum of dimethylacetal of methyl azelaic semialdehyde showed characteristic fragments

at mass 157 and 201. The interpretation of the rest of the peaks was speculative. In general, interpretation of the mass spectra of the B-band was complicated because the products in the B-band frequently did not give parent peaks in the MS. The signals from some of the products were very small and obscured by background noise.

The IR spectra of the B-bands from oxidized natural and randomized corn oil were similar to those of corn oil except the relative proportions of the -CH- stretch band at 3010 and 3040 cm^{-1} for unsaturation were reduced, thus, results from GLC, MS and IR indicated the major products in the B-band in oxidized corn oil methyl esters were nonanal, methyl azelaic semialdehyde, dimethyl azelate and an unidentified aldehyde which might be α-propyl furanacetaldehyde. In oxidized oil, the band consisted of a triglyceride containing two fatty acids and one azelaic semialdehyde or methyl azelaic group, or the unidentified aldehyde in peaks 2 and 3.

Oxidative Rate Studies

In the first part of this study, different rates of oxidation were observed for natural and randomized corn oil methyl esters. To explore this variation further, samples of natural, and randomized corn oil and corn oil methyl esters were oxidized at 28 C, and the peroxide values (PV) were determined periodically by either the iodometric method (4) or the method of Hamm et al. (27). The slope of the

logarithm of peroxide value versus time for each sample was calculated. The slope indicates the rate constant of the oxidation. The results are sumarized in Table 8. The r^2 values which are a measure of how well a straight line fits the data, indicate good fit. Randomized corn oil oxidized approximately three to four times faster than natural oil and corn oil methyl esters oxidized approximately as fast as randomized oil.

Table 8. Rate of oxidation of natural and randomized corn oil and corn oil methyl esters

	slope (rate constant of oxidation, $days^{-1}$)	r^2
corn oil	.06	1.00
	.06	1.00
	.08	0.92
	.08	1.00
randomized corn oil	.18	1.00
	.19	0.98
	.21	1.00
	.25	0.96
	.29	0.94
corn oil methyl esters	0.25	0.99
	0.26	0.97
	0.37	0.89

The corn oil methyl esters in Table 8 were prepared with methanolic sodium methoxide. Similar results were obtained if methanolic sulfuric acid was used to make the esters, and if care was taken to allow time for the reaction to go to completion. Corn oil partly converted to methyl esters with a sulfuric acid catalyst sometimes oxidized at rates comparable to natural corn oil and sometimes comparable to randomized corn oil. Rate studies such as these are subject to many sources of error because of trace contamination, so key experiments were replicated several times.

The previous results suggest that the natural glyceride structure of corn oil may affect its rate of oxidation. The most notable changes of randomization is to allow saturated acyl groups on the sn 2-position of triglycerides. Hoffman et al. (30) pointed out 1,3-distearo-2-olein was more stable against oxidation than 1-oleo-2,3-distearin. They attributed the change in stability of palm oil after randomization to changes in concentration of such triglycerides. There is less of such triglycerides in corn oil, but theoretically, corn oil with 1-oleo-2,3-distearin added should oxidize faster than natural corn oil or randomized corn oil with 1,3-distearo-2-olein added should oxidize slower than randomized corn oil. To see if this had any effect, the following samples were prepared: In the first sample, 3% of 1-oleo-2,3-distearin was added to corn oil, and in the second sample,

3% of 1,3-distearo-2-olein was added to randomized corn oil. These two triglycerides were gifts from Dr. Earl Hammond and they were synthesized according to the method of Quinn et al. (44b). The samples were oxidized and the PV was determined over a period of time. The oxidative rates of the two samples are listed in Table 9. There was no difference in oxidation rates of the oil to which the oleyldistearin was added. If glyceride structure in the natural oil has a stabilizing effect against oxidation as speculated, it is unclear what the important structural feature may be.

On the other hand, the presence of either antioxidants or pro-oxidants could cause the difference in oxidative rates in natural and randomized corn oil and corn oil methyl esters. If the variation was caused by an antioxidant, then this antioxidant must be very nonpolar and not removed by alumina treatment as are tocopherols. Such an antioxidant also must be acid and alkali-labile for when corn oil is transesterified with either sodium methoxide or sulfuric acid, the antioxidant activity is lost. If the variation was caused by pro-oxidants, these pro-oxidants were probably produced during transesterification and were not removed by alumina treatment.

Possibly, sodium methoxide and sulfuric acid cause some side reaction with esters of glycerol which produces a pro-oxidant effect. To test this, "randomized triacetin"

produced by treating triacetin with sodium methoxide was added to corn oil in an amount equal to the glycerol content of the corn oil triglycerides. A control was prepared with a similar amount of untreated triacetin. The oxidative rates are also listed in Table 9. There was no difference between the two samples and the rates were similar to natural corn oil. This leads to the conclusion that alteration of the glycerol or the ester group has no role in accelerating the oxidation.

If transesterification produced a pro-oxidant, it might be removed by distillation of the methyl esters. But after distillation through a Widmer column, the methyl esters oxidized as fast as the undistilled methyl esters. The oxidation rates are listed in Table 9.

General Conclusions

The results of these experiments show that randomized corn oil oxidizes three to four times faster than natural corn oil and at a rate comparable to corn oil methyl esters. The oxidation produces only monohydroperoxy-triglycerides in the early stages. Randomized corn oil produces about twice as much chain scission products as natural corn oil.

Raghuveer (45b) suggested that the glyceride structure of natural oils affected the availability of substrate, a factor which should affect the rate of propagation reaction.

Table 9. Oxidative rates of various oil samples

	slope (rate constant of oxidation, day^{-1})	r^2
corn oil + 3% 1-oleo-2,3-distearin	0.05	.95
randomized corn oil + 3% 1,3-distearo-2-olein	0.25	1.00
corn oil + "randomized triacetin"	.06	0.81
corn oil + triacetin	.06	.96
corn oil methyl esters	.27	.97
distillate	.35	1.00

$$RO_2 \cdot + RH \xrightarrow{kp} ROOH + R$$

Fatemi and Hammond (17) found that randomization did not affect the fatty acids oxidized - in the case of corn oil, almost all of it is linoleic acid. This, along with the current finding that only monohydroperoxy-triglycerides are formed, argues against an effect on the propagation reaction, for if a peroxy radical always attack a neighboring molecule and never another fatty acid on the same molecule, the effect of glyceride structure on the availability of substrate must be minimal.

The finding that randomization increases chain scission suggests the acceleration of oxidation by randomization may be an effect of initiation,

$$2ROOH \xrightarrow{ki} \text{free radicals}$$

although an increase in scission does not prove there is an increase in radical formation. Factors affecting the scission reaction have been little explored, and it is not clear how the glyceride structure of a fat might affect the interaction of hydroperoxides and their breakdown, especially if hydroperoxides are always on separate molecules. Neither is it clear how a pro- or antioxidant that might be present would affect the scission reaction.

The scission products, aldehydes and alcohols, are subject to further oxidation and might accelerate the oxidation rate (42b). However, the kinetics that fit the reaction, the logarithm of hydroperoxide concentration versus time is linear, assume that the radicals are initiated by a reaction involving two hydroperoxides (57b), and if some scission products were a major source of radicals, the kinetics should change unless the scission products and hydroperoxides always occur in a fixed ratio.

SUMMARY

Natural corn oil, randomized corn oil and corn oil methyl esters were purified by passage through alumina and oxidized in 5-g lots to peroxide values of 30 to 40. The purified samples were free from tocopherols. The hydroperoxides in the oxidized samples were reduced to alcohols with iodide (41). Two major bands, bands B and C, were isolated from unoxidized triglycerides or methyl esters by thin-layer chromatography on silica gel G. The R_f of band C corresponded to the monohydroxy-triglycerides region as indicated by TLC standards. Band C in the oxidized triglycerides was converted to methyl esters with methanolic sodium methoxide. The methyl hydroesters from oxidized natural and randomized corn oil and corn oil methyl esters were silylated with trimethylchlorosilane (TMS). The silylated products were analyzed by gas-liquid chromatography using methyl heptadecanoate as an internal standard. The recovery of TMS-ether derivatives of methyl hydroxyesters accounted for 70 to 80% of the hydroperoxides generated in the early stage of autoxidation. The ratio of unoxidized to hydroxy fatty acids was about 1.5-2 to 1. A ratio of 2 to 1 would be expected for monohydroxy-triglycerides; so it could be concluded that C-band is monohydroxy-triglycerides.

Band B had a greater R_f than monohydroxy-triglycerides but less than unoxidized triglycerides or methyl esters. The

properties of band B suggested that it might contain a scission product. Data from gas-liquid chromatography, mass spectrometry and infrared spectroscopy indicated the B-band in oxidized methyl esters could include methyl azelaic semialdehyde, dimethyl azelate, nonanal and an unidentified aldehyde. In oxidized oil, band B consisted of a triglyceride containing two fatty acids and one azelaic semialdehyde or methyl azelaic group. The unidentified aldehyde also was present.

The oxidative rate studies showed that randomized corn oil oxidized three to four times faster than natural oil and corn oil methyl esters oxidized approximately as fast as randomized oil. The results suggested that the natural glyceride structure of corn oil may affect its rate of oxidation, or perhaps the difference in rates of oxidation was due to the presence of either an unknown antioxidant or prooxidant.

BIBLIOGRAPHY

1. Bading, H. T. 1959. Isolation and identification of carbonyl compounds formed by autoxidation of ammonium linoleate. J. Am. Oil Chemists' Soc. 36:648-650.

2. Bading, H. T. 1960. Principles of autoxidation process in lipids with special regard to the development of autoxidation off-flavors. Neth. Milk Dairy J. 14: 215-242.

3. Baldwin, A. R. and M. S. Sniegowski. 1967. Fatty acid composition of lipids from corn and grain sorghum kernels. J. Am. Oil Chemists' Soc. 28:24-27.

4. Bank, A., J. N. Keay and J. G. M. Smith. 1957. Structure of conjugated methyl linoleate hydroperoxide. Nature 179:1078.

5. Bergstrom, S. 1945. On the autoxidation of the methyl ester of linoleic acid. Mineral Geol. 21A, No. 14.

6. Bolland, J. L. 1949. Kinetics of olefin oxidation. Q. Rev. 3:1-21.

7. Bolland, J. L. and G. Gee. 1946. Kinetic studies in the chemistry of rubber and related materials. II. The kinetics of oxidation of conjugated olefins. Trans. Faraday Soc. 42:236-244.

8. Brockerhoff, H. J. 1967. Sterospecific analysis of triglycerides: an alternative method. J. Lipid Res. 8:167-169.

9. Buss, C. D. and G. MacKinney. 1955. Carbonyl compounds in rancid corn oil. J. Am. Oil Chemists' Soc. 32:487-489.

10. Catalino, M., M. D. Felice and V. Sciancalipore. 1975. Autoxidation of monounsaturated triglycerides. Influence of the fatty acid position. Ind. Aliment 14:89-92.

11. Collins, G. and T. P. Hilditch. 1929. Regularities in the glyceride structure of vegetable seed-fats. Biochem. J. 23:1273-1289.

12. Day, E. A. and D. A. Lillard. 1960. Autoxidation of milk lipids. I. Identification of volatile monocarbonyl compounds from autoxidized milk fat. J. Dairy Sci. 43:585-595.

13. Dugan, L. 1976. Lipids. Pages 139-203 in O. Fennema, ed. Principles of food science. Part I. Food chemistry. Marcel Dekker, Inc., New York, NY.

14. Evans, C. D. 1961. Chemical changes accompanying flavor deterioration of vegetable oils. Pages 123-148 in Campbell Soup Company, ed. In proceedings of flavor chemistry symposium. Campbell Soup Company, Camden, NY.

15. Evans, C. D., D. G. McConnell, G. R. List and C. R. Scholfield. 1969. Structure of unsaturated vegetable oil by glycerides: Direct Calculation from fatty acid composition. J. Am. Oil Chemists' Soc. 46:421-424.

16. Farmer, E. H. and D. A. Sutton. 1943. The course in autoxidation reactions in polyisoprenes and allied compounds. Part IV. The isolation and constitution of photochemically-formed methyl oleate peroxide. J. Chem. Soc. 1943:119-122.

17. Fatemi, S. H. and E. G. Hammond. 1980. Analysis of oleate, linoleate and linolenate hydroperoxides in oxidized ester mixtures. J. Am. Oil Chemists' Soc. 15:379-385.

18. Frankel, E. N. 1962. Hydroperoxides. Pages 51-78 in M. W. Schultz and E. A. Day, eds. Symposium on foods: Lipids and their oxidation. AVI Publishing Co., Inc., Westport, Conn.

19. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khamby, R. F. Grawood and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas chromatography-mass spectrometry: I. Methyl oleate. Lipids 12: 901-907.

20. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khamby, R. F. Grawood and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas-chromatography-mass spectrometry. II. Methyl linoleate. Lipids 12:908-1013.

21. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khamby, R. F. Grawood and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas-chromatography-mass spectrometry. III. Methyl linolenate. Lipids 12:1055-1061.

22. Gaddis, A. M., R. Ellis and G. T. Currie. 1961. Carbonyls in oxidized fats. V. The composition of neutral volatile monocarboxyl compounds from autoxidized oleate, linoleate, linolenate esters and fats. J. Am. Oil Chemists' Soc. 38:371-375.

23. Gunstone, F. D. 1962. The distribution of fatty acids in natural glycerides of vegetable origin. Chem. Ind. (London) 1214-1223.

24. Gunstone, F. D., R. J. Hamilton, F. B. Padley and M. I. Qureshi. 1965. Glyceride studies. V. The distribution of unsaturated acyl groups in vegetable triglycerides. J. Am. Oil Chemists' Soc. 42:965-969.

25. Gunstone, F. D. and T. P. Hilditch. 1945. The union of gaseous oxygen with methyl oleate, linoleate and linolenate. J. Chem. Soc. 1945:836-841.

26. Hall, G. E. and D. G. Roberts. 1966. A study by intrared and proton magnetic resonance spectroscopy of the monohydroperoxides of oleate, linoleate esters. J. Chem. Soc. 1966:1109-1112.

27 Hamm, D. L., E. G. Hammond, V. Parvanah and H. E. Snyder. 1965. The determination of peroxides by the Stamm method. J. Am. Oil Chemists' Soc. 42:920-922.

28. Hammond, E. G. and F. D. Hill. 1964. The oxidized-metallic and grassy flavor components of autoxidized milk fat. J. Am Oil Chemists' Soc. 41:180-183.

29. Henderson, S. K., A. Witchwost and W. W. Nawar. 1980. The autoxidation of linoleates at elevated temperatures. J. Am. Oil Chemists' Soc. 57:409-413.

30. Hoffman, G., J. Stroink and R. Polman. 1973. The oxidative stability of some diacyl triglyceride. International symposium on deterioration of lipids, Gdansk 1971. Problemowe Postepow Nauk Robniczych 136:93-98.

31a. Holman, R. T. and O. C. Elmer. 1947. The rates of oxidation of unsaturated fatty acids and esters. J. Am. Oil Chemists' Soc. 24:127-129.

31b. INCOS® Version 3-1 software tape. 1980. National Veterinary Services Laboratories, Ames, Ia.

32. Jensen, R. G., T. A. Marks, J. Sampugna, J. G. Quinn and D. L. Carpenter. 1966. Purification of triglycerides with an alumina column. Lipids 1:451-452.

33. Johnson, D. C. 1972. Factors affecting perioxide types in oxidizing fatty acid mixtures. Unpublished Ph.D. Thesis. Library, Iowa State Univ., Ames, Ia.

34. Kartha, A. R. S. 1953. The glyceride structure of natural fats. II. The rule of glyceride type distribution of natural fats. J. Am. Oil Chemists' Soc. 30:326-329.

35. Kartha, A. R. S. 1954. The glyceride structure of natural fats. III. Factors governing the content of fully saturated glycerides. J. Am. Oil Chemists' Soc. 31:85-88.

36. Khan, N. A., W. E. Tolberg, D. H. Wheeler and W. O. Lundberg. 1954. Photochemical oxidation of fatty acid esters with and without chlorophyll. J. Am. Oil Chemists' Soc. 31:460-466.

37. Keeney, M. 1962. Secondary degradation products. Pages 79-89 in H. W. Schultz and E. A. Day, eds. Symposium on foods: Lipids and their oxidation. AVI Puvlishing Co., Inc., Westport, Conn.

39. Luddy, F. E., R. A. Barford, S. F. Herb, P. Magidman and R. W. Riemenschneider. 1964. Pancreatic lipase hydrolysis triglycerides by a semimicro technique. J. Am. Oil Chemists' Soc. 41:693-695.

40. Mattil, K. F., F. A. Norris, A. J. Stirton and D. Swern. 1964. Composition and characteristics of individual fats and oils. Pages 210-211 in D. Swern, ed. Bailey's industrial oil and fat products. Interscience Publishers, Inc., New York, NY.

41. Mehlengacker, V. C., T. H. Hopper and E. M. Sallea, eds. 1968. Official and tentative methods of American Oil Chemists' Soc.: Tentative mthods cd 13-60. American Oil Chemists' Soc., Chicago, Il.

42a. Meijboom, W. and G. A. Jongenotter. 1979. A quantitative determination of tocotrienols and tocopherols in palm oil by TLC-Glc. J. Am. Oil Chemists' Soc. 56:33-35.

42b. Michalski, S. T. and E. G. Hammond. 1972. Use of labeled compounds to study the mechanism of flavor formation in oxidizing fats. J. Am. Oil Chemists' Soc. 49:563-566.

43. National Academy Of Science. 1972. Specification and criteria for biochemical compounds. 3rd ed. National Academy of Science, Washington, D.C.

44a. Norris, F. A. and K. F. Mattil. 1947. An approach to the glyceride structure of natural fats. J. Am. Oil Chemists' Soc. 24:274-275.

44b. Quinn, G., J. Lampugna and R. G. Jensen. 1967. Synthesis of 100-gram quantities of highly purified mixed acid triglycerides. J. Am. Oil Chemists' Soc. 44: 439-442.

45a. Raghuveer, K. G. 1969. Fatty acid composition and the flavor of autoxidized butteroil and the effect of glyceride structure on the rate of oxidation of fats. Unpublished M.S. Thesis. Library, Iowa State Univ., Ames, Ia.

45b. Raghuveer, K. G. and E. G. Hammond. 1967. The influence of glyceride structure of autoxidation. J. Am. Oil Chemists' Soc. 44:239-243.

46. Ross, J., A. I. Grbhart and J. F. Gerecht. 1949. The autoxidation of methyl oleate. J. Am. Chem. Soc. 71:282-286.

47. Sahasrabudhe, M. R. and I. G. Farn. 1964. Effect of heat on triglycerides of corn oil. J. Am. Oil Chemists' Soc. 41:264-267.

48. Saunders, D. H., C. Ricciuti and D. Swern. 1955. Reactions of fatty materials with oxygen. XVI. Relation of hydroperoxide and chemical peroxides to total oxygen absorbed in autoxidation of methyl oleate. J. Am. Oil Chemists' Soc. 32:79-83.

49. Scholfield, C. R., J. Nowakowska and H. J. Dutton. 1961. Glyceride structure of vegetable oils by counter current distribution. VI. Corn oil. J. Am. Oil Chemists' Soc. 38:175-177.

50. Screenivasan, B. 1978. Interesterification of fats. J. Am. Oil Chemists' Soc. 55:796-805.

51. Selke, E., F. N. Frankel and W. E. Neff. 1978. Thermal decomposition of methyl oleate hydroperoxides and identification of volatile components by gas chromatography-mass spectrometry. Lipids 13:511-513.

52. Selke, E., W. K. Rohwedder and H. J. Dutton. 1977. Volatile components from triolein heated in air. J. Am. Oil Chemists' Soc. 54:62-67.

53. Selke, E., W. K. Rohwedder and H. J. Dutton. 1980. Volatile components trilinolein heated in air. J. Am. Oil Chemists' Soc. 57:25-29.

54. Septon, H. H. and D. A. Sutton. 1956. The chemistry of polymerized oils. V. The autoxidation of methyl linoleate. J. Am. Chemists' Soc. 33:263-272.

55. Stirton, A. H., J. Turner and R. W. Reimenschneider. 1945. Oxygen absorption of methyl esters of fat acids and the effect of antioxidants. J. Am. Oil Chemists' Soc. 22:81-83.

56. Swern, D., J. E. Coleman, H. B. Knight, C. Disciuti, C. O. Willits and C. R. Eddy. 1953. Reactions of fatty materials with oxygen. XIV. Polarographic and infrared spectrophotometric investigation of peroxides from autoxidized methyl oleate. J. Am. Chemists' Soc. 75:3135-3137.

57a. Swift, C. E., F. G. Dollear, L. E. Brown and R. T. O' Conner. 1948. Decomposition of methyl hydroperoxide oleate. J. Am. Oil Chemists' Soc. 25:39-40.

57b. Uri, N. 1961. Physio-chemical aspects of autoxidation. Pages 55-104 in W. O. Lundberg, ed. Autoxidation and antioxidants. Vol. I. Interscience Publishers, Inc., New York, N.Y.

58. Vander Wal, R. J. 1960. Calculation of the distribution of the saturated and unsaturated acyl groups in fats, from pancreatic lipase hydrolysis data. J. Am. Oil Chemists' Soc. 37:18-20.

59. Wilkins, R. A. 1964. Theories of mechanisms of oxidized flavor development. Australian Commonwealth Scientific and Industrial Research Organization, Division of Dairy Research, International Report No. 4.

60. Wong, W. S. and E. G. Hammond. 1977. Analysis of oleate and linoleate hydroperoxides in oxidized ester mixtures. Lipids 12:475-479.

ACKNOWLEDGMENTS

I would like to express my appreciation to my major professor, Dr. Earl Hammond, for his patience, understanding, guidance and support throughout this research project. Above all, the philosophy of life which I have learned from him will be treasured and remembered.

Thanks are also expressed to Frank Ross for his work on the mass spectroscopy.

Finally, I wish to thank my husband, Henry Chu, for his love, care and support which made the completion of this dissertation a reality.