Effect of Heat on Triglycerides of Corn Oil[1]

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Abstract

Results of a study on the effect of heating corn oil in air to a 200C temp are reported. Heated oil was separated on a silicic acid column into 8 fractions. The first four fractions, constituting about 62% original oil, were found to be unchanged triglycerides. The remaining 4 fractions constituted polymeric and degraded products of high molecular wt. Percentage losses from the respective positions in the oleo- and linoleo-glyceride fractions suggest that fatty acids in primary positions are slightly more susceptible to heat than those in the 2-position. Assuming a 1,3-random 2-random distribution, triglyceride fraction in the heated oil contained 6.7% trilinolein as compared to 17.7% in fresh oil. Evidence is presented which shows presence of branching in short chain unsaturated acids and of hydroxy acids in the saponified polymeric fractions.

Introduction

INFORMATION ON THE mechanism of thermal degradation and polymerization of vegetable oils is important. During processing, as well as in frying and baking, the oils may reach temp of 200C and above. At these elevated temp, it is common knowledge that degradation and polymerization take place.

In recent years two valuable publications have appeared in this Journal. Crossley et al. (5) and Endres et al. (7) reported their studies on the effect of heat on tri-palmitin and di-palmito-oleins and postulated the pathways of degradation. However, very little information is available on triglycerides containing predominantly the C_{18} unsaturated acids. Scholfield et al. (15), using countercurrent distribution technique, has shown that the glyceride composition of corn oil follows a random distribution pattern. Major component glycerides of corn oil are trilinolein, dilinoleo-oleins and dioleo-linoleins.

It is the purpose of this paper to report results of a study on the fractionation of corn oil before and after heating by silicic acid chromatography. TLC, GLC and IR spectroscopy were applied to analyze the heated oil.

Materials and Methods

A commercial brand of corn oil was used. One hundred g fresh corn oil contained in a 200-ml conical flask was heated 48 hr in an air oven at 200C without any added air. At the end of this period the oil was flushed with nitrogen and stored in a freezer at −20C. The peroxide value of the oil after heating was 8.8 meq./kg. Iodine values of the oil before and after heating were 127.7 and 115.0, respectively.

Fractionation. Twenty g each of fresh and heated oil was fractionated in triplicate into glyceride types by silicic acid partition chromatography. The triplicate elutions are described as series I,II and III. Chromatographic conditions were the same as described in an earlier publication from this laboratory (13). The flask assembly, used in the stepwise elution, was the same as described by Sahasrabudhe (14). In this method the sample of fat is eluted with n-hexane containing increasing proportions (0–25%) of ethyl ether. All triglycerides are eluted when the 15% level of ethyl ether in n-hexane is reached (13). In the present study, elution was carried through to 100% ethyl ether to include the degradation products. Solvents used in the stepwise elution were as follows: 400 ml n-hexane; 250 ml each of 2.5%,5%,10%, 15% and 25% ethyl ether in n-hexane; 100 ml 50% ether in hexane followed by 300 ml ethyl ether.

Twenty-five ml fractions were collected on an automatic fraction collector. Care was taken not to heat the samples above 40C and nitrogen was used wherever possible. Each individual tube fraction was evaporated on a water bath and the residues weighed. The fraction wt were plotted against tube fraction numbers (Fig. 1). In the 2nd series, tube fractions under each peak were pooled and designated letters A to F as shown in Figures 1 and 3. In the third series, all triglyceride fractions (21–60) and polymeric fractions (61–90) were pooled. These are referred to as triglycerides and polymers fractions from heated oil respectively. All fractions were flushed with nitrogen and stored at −20C in glass stoppered flasks.

Analysis of Fractions. Individual tube fractions from series I were analysed by GLC for total fatty acids as methyl esters (Fig. 2).

Methyl esters were prepared by the conventional methods of transesterification using anhydrous HCl-methanol (16). In series II, the pooled fractions were analysed for total fatty acids and also for fatty acids in the 2-position by the pancreatic lipase technique described by Mattson and Volpenhein (9), followed by the separation of 2-monoglyceride as described by Quinlin and Weiser (12), except that 16 g Mallinckrodt silicic acid as prepared in our laboratory (13) was used with 150 ml of each solvent.

Free fatty acids in the heated oil were isolated by adsorption on Amberlite IRA 400 resin and methylated by anhydrous HCl-methanol as described by Hornstein et al. (8).

Methyl esters of fatty acids were analysed by gas chromatography on a Burrell Kromo-Tog K_2 equipped with a thermal conductivity cell. Two columns were used. Most of the analysis was carried out in a 6-in. glass column packed with 20% Reoplex-400 on Chromosorb W. The column was preconditioned at 255C for 4 hr, followed with 24 hr at 225C. After a considerable bleeding of the stationary phase this column gave good resolution of the fatty acid methyl esters. Chromatographic conditions were as follows: column temp 217C; detector cell temp 250C; and helium flow 100 ml/min. A second column packed with 20% Apiezon-L on Chromosorb W was used at 216C wherever necessary. Percentage distribution of the fatty acids was calculated by areas under the peaks as suggested by Bartlet and Smith (2). As the components resolved in symmetrical peaks, correction factors were not required.

IR spectral analysis was carried out on a Perkin-Elmer instrument Model 221 with a 5X scale expansion. The sample was applied as a thin film between salt plates, or in carbon tetrachloride solutions.

[1] Presented in part at the AOCS Meeting, New Orleans, 1962.

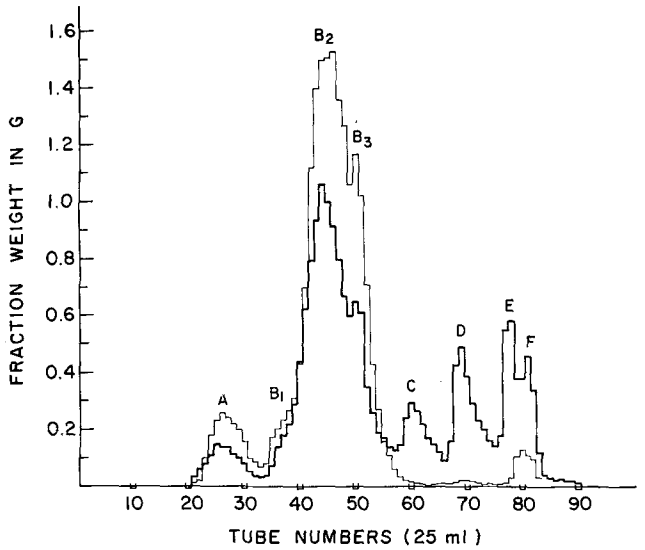

FIG. 1. Fraction wt distribution in fresh corn oil (lighter line) and heated oil (heavier line).

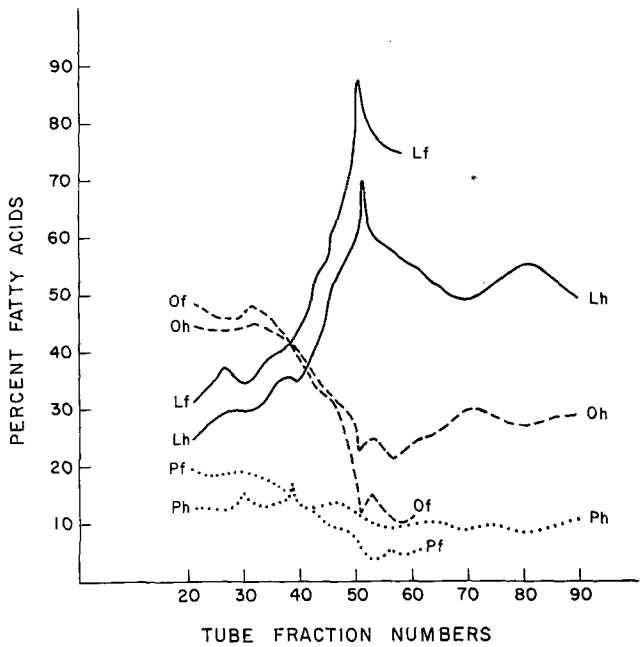

FIG. 2. Percent distribution of $C_{16:0}$ (P), $C_{18:1}$ (O) and $C_{18:2}$ (L) acids in fresh (f) and heated (h) oils.

TLC (1,11) was applied to the fractions with known standards of triglycerides.

Mol wt were determined by the Rast method with camphor as solvent (10).

Results and Discussion

The general trend in elution of triglycerides by silicic acid partition chromatography is governed by a) chain length and b) unsaturation of the constituent fatty acids. Glycerides containing shorter chain acids are more strongly adsorbed than those containing longer chain acids. Among the glycerides containing fatty acids of the same chain length, the unsaturated ones are more strongly adsorbed in direct relation to unsaturation (13).

Corn oil was chosen because the glyceride structure of corn oil has been studied by other workers (6,15). Scholfield et al. (15) has demonstrated that the glyceride composition of corn oil follows a random distribution pattern, and that corn oil glycerides constitute 22% trilinolein.

Glyceride structure of corn oil by silicic acid chromatography has been worked out in our laboratory and found to account for 20% trilinolein. Details of this study will be published elsewhere. In Figure 1, the wt distribution patterns obtained with the oil before and after heating are presented. Reproducibility was found to be very satisfactory between the three separate elutions of heated oil. The fraction wt varied within 1.5%. Recovery of the fresh oil was 99.5% while that of heated oil was 98%. Fractions A,B_1,B_2 and B_3 are essentially triglycerides. The four fractions C,D,E and F obtained in the heated oil represent 37% of the original oil. Percentage losses in fractions A–B_3 on heating the oil under these conditions are 42.0, 13.5,34.6 and 54.0, respectively. Under the conditions of column operation, fraction B_3 should include all the trilinolein present in the corn oil. This assumption is valid on the basis of separations obtained with synthetic glyceride mixtures under similar conditions (13). Assuming a random distribution (17), 70% of fraction B_3 is trilinolein. Percentage fatty acid distribution in individual tube fractions 21–60 in fresh and heated oil is shown in Figure 2 and percentage distribution of fatty acids in the pooled triglyceride fractions is listed in Table I. The first column of figures gives the percentage distribution of the fatty acids in fresh corn oil. Figures in parenthesis represent the percentage of the fatty acids in the 2-positions. For example, corn oil contains 28.5% octadecenoic (oleic) acid of which 8.7% is in the 2-position, leaving 19.8% in the primary positions. Assuming that the primary positions 1 and 3 are identical, this represents 9.9% of the fatty acid in each of the primary positions. Similarly 23.3% of the $C_{18:2}$ acid (linoleic) is in the 2-position, leaving 33% in the primary positions. The second column (Table I) gives the values for the pooled triglyceride fractions A–B_3 from the heated oil. Next in order are the values for fractions A,B_1,B_2 and B_3. For easier comparison, results of fresh and heated oils are presented side by side. Going through the values for the C_{18} monoenoic acid, differences between the fresh and

TABLE I

Fatty Acid Distribution in Triglyceride Fractions

	Fresh oil total	Heated oil A+$B_{1,2,3}$	A		B_1		B_2		B_3	
%	100	62.5	Fresh 9.8	Heated 5.7	Fresh 8.2	Heated 7.0	Fresh 52.0	Heated 34.0	Fresh 25.5	Heated 14.3
$C_{16:0}$	11.1[a] (0.6)[b]	13.5 (0.9)	13.9 (0.7)	18.6 (1.0)	14.6 (0.6)	16.2 (1.1)	12.9 (0.9)	13.6 (0.9)	4.8 (0.3)	9.0 (0.9)
$C_{18:0}$	2.1 (—)	1.9 (—)	2.5 (—)	4.4 (—)	2.4 (—)	3.2 (—)	2.3 (0.5)	1.9 (0.2)	0.5 (—)	1.3 (0.1)
$C_{18:1}$	28.5 (8.7)	31.9 (10.1)	47.0 (15.0)	44.6 (16.2)	41.2 (12.4)	43.6 (14.6)	32.0 (10.3)	32.9 (10.3)	12.7 (3.9)	23.7 (6.8)
$C_{18:2}$	56.3 (23.3)	49.0 (22.3)	35.0 (17.0)	31.4 (16.0)	41.3 (19.8)	36.8 (17.5)	52.5 (21.8)	51.4 (21.8)	80.2 (28.3)	61.8 (25.4)
$C_{18:3}$	1.5 (0.5)	0.7 (0.2)	0.4 (0.3)				0.1 (+)	0.1 (—)	2.0 (0.8)	0.9 (0.1)
Unknown I		1.5 (—)		++						
Unknown II		1.1 (—)	1.1 (++)	++		.3 (—)		0.5 (++)		

[a] Fresh oil also contained 0.5% palmitoleic acid.
[b] Figures in parenthesis indicate the percentage distribution of the particular fatty acid in 2-position.

TABLE II

Percentage Losses of Fatty Acids from 1-,3- and 2-positions in the Triglyceride Fractions

Fatty acids	Fractions							
	A		B_1		B_2		B_3	
	(1,3)	(2)	(1,3)	(2)	(1,3)	(2)	(1,3)	(2)
$C_{16:0}$	17.8	28.5	6.9	+	30.9	38.2	+	+
$C_{18:1}$	48.3	40.0	12.5	—	31.2	34.6	+	—
$C_{18:2}$	50.0	43.6	22.2	23.2	36.8	34.7	60.7	49.6

+ Indicates increase.
— Indicates no change.

heated oils are not significant with the exception of fraction B_3. In fact the B_2 fractions show a remarkable similar fatty acid composition. There is an apparent increase in the palmitic and stearic acid compensated by the reduced values of linoleic acid. These values do not take into account the loss in the fraction. The wt of the pooled fractions as a percentage of total are shown on the top of each column. For a better representation, actual values (in g) of the three major fatty acids in the above positions, were calculated. All acids, including $C_{16:0}$, are in part lost from the triglycerides. The percentage losses from the respective positions are shown in Table II.

The total loss of individual acids calculated as the difference between fresh and heated oil triglyceride fractions was as follows: 71% linolenic, 45% linoleic, 30% oleic and 24% palmitic acid. In fraction A, losses of palmitic acid from the 2-position are slightly higher than those from the primary positions. However, the amount of palmitic acid in 2-position is so small that it is insignificant. Linoleic acid on the other hand shows a greater susceptibility to heat in the primary position than in the 2-position, particularly in fractions A and B_3. Most of the oleic acid loss in fraction B_1 is from the primary position. Fraction B_2, which includes 52% of the original oil, does not show any differences in the per cent losses of fatty acids in the respective positions. It therefore appears that the whole glycerides as such were affected by heat. Fractions B_1 and B_3 show slight increases in the amount of $C_{16:0}$ and $C_{18:1}$ acids. It should be emphasized here that the identification of these acids is essentially based on retention times in gas chromatography. IR spectrophotometric analysis was carried out on some GLC fractions; however, no definite conclusions can be drawn as to the purity of the components designated as above. The identification by GLC alone becomes doubtful, particularly in the polymeric and degraded fractions. These are discussed later in the paper. Free fatty acids in the heated oil accounted for 0.2% of the original oil.

Glyceride composition. From the percentage distribution of the fatty acids between 1-,3- and 2-positions, the triglyceride composition of the whole fat was calculated. The composition of fatty acids

TABLE III

Calculation of Glyceride Structure (17)

	Oleic	Linoleic
(a) % triglyceride	28.5	56.3
(b) % 2-monoglyceride	26.2	70.0
(c) % 1,3 acids $(\frac{3a-b}{2})$	29.6	49.5

LLL = (49.5) (70.0) (49.5)/10,000 = 17.1
LOL = (49.5) (26.2) (49.5)/10,000 = 6.4
OLL = (29.6) (70.0) (49.5) (2)*/10,000 = 20.4
OOO = (29.6) (26.2) (29.6)/10,000 = 2.3
OOL = (29.6) (26.2) (49.5) (2)/10,000 = 7.6
OLO = (29.6) (70.0) (29.6)/10,000 = 6.1

* Factor 2 was used in cases of unsymmetrical glycerides to account for the isomers.

TABLE IV

Glyceride Composition

	Triglyceride	% Fresh oil	% Heated oil*	% Loss
GU_3	LLL	17.1	6.7	61.4
	LOL	6.4	2.9	54.7
	OLL	20.4	10.1	50.5
	OOO	2.3	2.0	13.0
	OOL	7.8	4.9	37.2
	OLO	6.1	4.5	26.2
	LeLL	1.0	0.2	80.0
	LLeL	0.7	0.1	85.0
	LeOO, OLeO, LeOL, LeLO	1.4	0.4	71.4
GSU_2	PLL	10.8	6.3	41.6
	PLO	6.4	5.2	18.7
	POL	4.0	2.8	30.0
	POO	2.4	2.3	4.2
	PLLe, PLeL, POLe, PLeO	0.8	0.2	75.0
	LPL, OPO, LPO	0.5	1.1	120**
	SLL	2.0	0.9	55.0
	SOL	0.6	0.4	33.3
	SLO	1.2	0.7	41.6
	SOO	0.4	0.4	.....
	SLeO, SOLe, SLLe, SLeL	0.1	++	.....

* Corrected for 62.5%.
** 120% apparent gain.

occupying the terminal positions was calculated from the formula:

$$\% \text{ composition 1:3 acids} = \frac{3a - b}{2}$$

where *a* is the % fatty acid in the triglyceride and *b* the % distribution of the fatty acid in the 2-monoglyceride (4). From these sets of values the triglyceride composition was calculated by the procedure described by VanderWal (17). As an example, the calculations for oleo-linoleo glycerides are shown in Table III. Results obtained this way for the fresh corn oil and the pooled triglyceride fraction from heated oil are shown in Table IV.

Polymeric and degraded fractions. About 37% of the heated oil was eluted into 4 fractions of polymeric and degraded products. The mol wt of the fractions C,D and E range between 2000–4800 (Fig. 3). These obviously are dimeric and polymeric fractions. Fraction F, with a mol wt of 1340, appears to be a mixture of polymeric and other degraded products. GLC analysis of methyl ester of these fractions is presented in Table V. A number of fatty acids not originally found in corn oil were observed. Some of these were tentatively identified by internal and external standards. Some of the fractions also were collected as eluates from the gas chromatograph and analysed by IR spectroscopy. Spectrophotometric analysis of

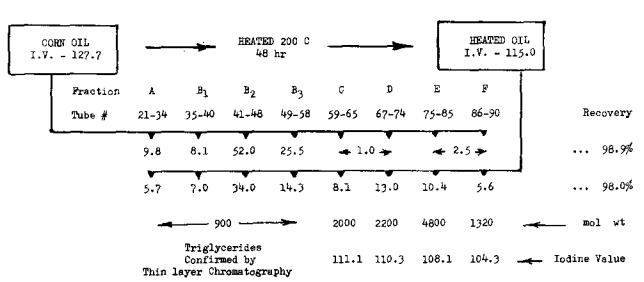

FIG. 3.

TABLE V
Analysis of Methyl Ester of Polymeric and Degraded Fractions

Fatty acid	Fractions			
	C (8.1)[1]	D (13.0)	E (10.4)	F (5.6)
A*	+	0.64	0.53	++
B*	0.42	0.36	0.44	1.2
C*	0.34	0.45	0.30	0.40
D*	....	.....		++
E*	....	0.32	+	....
F*	....		+	....
$C_{15:0}$	....	1.2	....	0.9
Azelaic†	....	++	....	1.4
$C_{16:0}$	10.8	10.0	9.9	10.7
$C_{16:1}$	5.3	4.2	3.1	1.0
Sebacic†	2.9	2.1	0.6	2.1
$C_{18:0}$	1.9	1.4	1.7	1.8
$C_{18:1}$	25.7	29.1	28.2	28.7
$C_{18:2}$	52.6	50.1	55.1	51.7

* Unidentified.
† Tentatively identified by retention time and internal standards.
[1] Figures in parenthesis indicate values in per cent of the fraction.
+ Indicates trace.
++ Indicates less than 0.1.

compounds marked A,B and E in Table V suggests the presence of short chain, unsaturated, branched acids (910 cm^{-1}). Compound B also shows an OH group. Dibasic acids tentatively identified by internal standards could not be obtained for further chemical analysis. $C_{16:0}$ and $C_{18:1}$ acids in these fractions gave remarkably broad peaks. This in itself was an indication that the compounds were not pure. Appearance of palmitoleic acid seems anomalous and no explanation is available at this time.

Fractionation into urea adduct forming fatty acids and non-adduct forming material. Fraction C,D, E and F were subjected to fractionation with urea. The procedure used was essentially the same as described by Bhalerao and Mahon (3) except that 1–2 g samples were saponified for 2 hr to ensure complete saponification. Under the conditions of urea fractionation used, all $C_{16:0}$ (palmitic) acid forms an adduct. Eight to 13% of the compound reported as $C_{16:0}$ by GLC in fractions C,D,E and F did not form an adduct. These compounds have the same relative retention time as $C_{16:0}$ under the conditions of GLC used in this study. Work on these fractions is being continued and will be reported later.

ACKNOWLEDGMENT

Interpretation of IR spectroscopic data by J. C. Bartlet; helpful criticism and encouragement from D. G. Chapman, Head of Food Section, and R. A. Chapman, Asst. Dir., Scientific Services, Food and Drug Directorate.

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[Received July 26, 1962—Accepted November 27, 1963]

The Preparation of a Series of Molecularly Homogeneous Para-*t*-Octylphenoxypoly(ethoxy)ethanols

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Abstract

Multi-gram quantities of the first ten members of a series of para-(1,1,3,3-tetramethylbutyl)-phenoxypoly(ethoxy)ethanols (p-*t*-OPE_n) have been prepared. Analytical and physical data indicate that each of these materials is composed of greater than 95% of a single molecular species.

Introduction

THE BASE-CATALYZED addition of ethylene oxide to alkylphenols is the most common method of preparation of a group of nonionic surface active materials, the alkylphenoxypoly(ethoxy)ethanols. This type of reaction leads to products having a Poisson distribution (1–5) of molecular species. The popular terms "E number" or "E_n" refer to the average number of ethylene oxide units attached to the alkylphenol in the product mixture. Thus an oxyethylated p-*t*-octylphenol having an average of five mole of ethylene oxide and a Poisson distribution of molecular weights can be called *p-t*-octylphenol-E_5 (p-*t*-OPE_5). An interesting subject is the comparison of the physical and surface active properties of such materials with those of materials having the same E_n, but consisting of a single molecular species. Such a comparison has been made possible in these laboratories by the synthesis of multi-gram quantities of the first 10 members of a series of essentially molecularly homogeneous p-*t*-OPE_n's and has been reported by Crook and co-workers (6). The synthesis and purification of these materials represent, respectively, extensions of the work of Gingras and Bayley (7), who have described the synthesis of molecularly homogeneous p-*t*-OPE_7 by the reaction of hexaoxyethylene glycol with p-*t*-octylphenyl-β-chloroethyl ether and of Kelly and Greenwald (5), who obtained very small quantities of a number of substantially homogeneous p-*t*-OPE_n's by tedious chromatographic separation of the components of normal p-*t*-$OPE_{9.7}$, prepared by the addition of ethylene oxide to recrystallized p-*t*-octylphenol.

Experimental

A. *Synthetic*

1. *Normal p-t-OPE_n.* The following preparation of p-*t*-$OPE_{8.94}$ is typical.

A mixture of 50.0 g (0.243 m) of p-*t*-octylphenol which had been recrystallized four times from heptane (mp 85–86C; congealing pt. 84.8C) and 0.3 g (0.013 g atom) sodium metal in a tared ethylene oxide reaction flask was