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The Triglyceride Composition of Linseed Oil

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Abstract

The triglyceride composition of linseed oils obtained under different ecological conditions and having different fatty acid compositions was determined by a combination of several chromatographic techniques. The triglyceride mixture was first separated in 8 fractions of different polarity by reversed-phase paper chromatography. Each glyceride fraction was then separated in a partition chromatographic system as the triglyceride coordination complexes with silver ions into individual compounds. The fatty acid compositions of the original oil, single glyceride fractions, and individual triglycerides were determined by gas-liquid chromatography. The molar ratio between the two neighboring glyceride fractions was determined by relating the fatty acid composition of each fraction to the fatty acid composition of their sum. The triglyceride composition of the total oil was then calculated from these results.

The presence of 18–19 triglycerides was ascertained in the samples studied, and the molar concentration of each glyceride was estimated. Linseed oil contains only triunsaturated and monosaturated-diunsaturated triglycerides. Within each of these types the fatty acid distribution is close to random. At the same time, the content of some triglycerides departed regularly from a random pattern.

A method for calculation of linseed oil triglyceride composition from the fatty acid composition is given.

The same general pattern of glyceride formation in linseed is followed regardless of ecological conditions; therefore, the qualitative and quantitative triglyceride composition reflects the differences in fatty acid composition of linseed oil.

Introduction

PREVIOUS CONTRIBUTIONS from our laboratory (1,2) have established the triglyceride composition of poppyseed and cottonseed oils. These oils are rather simple in their major fatty acid composition, containing only palmitic, stearic, oleic and linoleic acids. However, most natural fats are much more complex in their fatty acid composition, and the quantitative determination of the triglyceride composition of these fats involves considerable difficulty in spite of modern separation techniques.

Of all vegetable oils containing five major fatty acids, linseed oil has the greatest practical importance. Consequently, the glyceride composition of this oil has been studied by many workers. The qualitative and quantitative triglyceride composition of linseed oil has been investigated by crystallization of brominated glycerides (3), adsorption chromatography on an alumina column (4), fractional crystallization from acetone followed by ester distillation (5), countercurrent distribution (6), reversed-phase paper chromatography (7), selective hydrolysis by pancreatic lipase (8,9), and partition chromatography on a vulcanized rubber column (10,11).

As a result of these studies, some glycerides were identified and their amounts estimated (3,4,6). Also the quantities of the various triglyceride types present (triunsaturated, monosaturated-diunsaturated, etc.) and the number of various glyceride fractions of different polarity were determined (7–11). Nevertheless, the triglyceride composition of linseed oil has not yet been fully characterized.

The present work was undertaken to investigate the triglycerides of two linseed oils of different fatty acid composition by reversed-phase partition chromatography, partition chromatography in a silver nitrate-containing system, and gas-liquid chromatography (1,2,12–14). It was found that the specific distribution of fatty acids among glycerides and the individual triglyceride content of the oil is markedly influenced by the genotype and phenotype characteristics of the linseed plants.

Experimental

Material

Linseed plants of the Krupnosemyanny-3 variety were grown in 1961 on a field plot at this Institute. A part of the harvested seed was sown out again on the same plot in the spring of 1962. In 1962, the development of plants was retarded considerably by the cold and rainy summer. The weather conditions of the growing seasons and the cultivation of the experimental plants have been described in detail earlier (15). The oil was obtained by cold expression. The oil content of seeds was evaluated by refractometery (15). The iodine value of the oil was determined by the Hanus method (1).

Methods

The triglycerides were resolved into separate fractions of different polarity by reversed-phase paper partition chromatography (1,2,13) using an acetone: acetic acid (1:1)/aliphatic hydrocarbons (bp 260–310C) system. For the separation of the more polar glyceride fractions we used dodecane instead of the high-boiling hydrocarbons. The triglyceride fractions were identified by their polarity constant K_2 and relative chromatographic mobility R_2 (12). The fractions were then eluted from the chromatograms with n-hexane (1,2) and converted into fatty acid methyl esters. The fatty acid compositions of single fractions and of the total oil were determined by gas-liquid

TABLE I
Linseed Oil Content and Triglyceride Fatty Acid Composition

	1961 seeds	1962 seeds
Oil content (% of air-dry seed)	40.8	35.8
Fatty acid composition (mole %)		
Palmitic	7.4	6.8
Stearic	5.7	4.0
Oleic	23.1	14.5
Linoleic	14.9	18.6
Linolenic	48.6	55.9
Iodine value of the oil		
Calculated	177.1	.....
Found (Hanus)	177.2	.....

chromatography (2) on a Pye argon chromatograph. The Lovelock ionization detector voltage was maintained at 750 v to ensure a linear mass response for methyl linolenate (14).

The quantitative determination of the triglycerides was based on molar ratios between the two neighboring glyceride fractions. The molar ratio was estimated by relating the fatty acid composition of each fraction to the fatty acid composition of their sum. For example, let x and y be molar amounts of any two neighboring fractions I and II in the sum of these fractions I + II. Hence, $x + y = 1$. Let the fatty acid molar concentrations in fraction I be $[Le]_{I'}$ $[L]_{I'}$ $[O]_{I'}$ etc., the concentrations in fraction II be $[Le]_{II'}$ $[L]_{II'}$ $[O]_{II'}$ etc., and the concentrations in the sum be $[Le]_{I+II'}$ $[L]_{I+II'}$ $[O]_{I+II'}$ etc. Hence,

$$y = 1 - x, \text{ and}$$

$$[Le]_I \cdot x + [Le]_{II} \cdot y = [Le]_{I+II}$$

$$[L]_I \cdot x + [L]_{II} \cdot y = [L]_{I+II}$$

$$[O]_I \cdot x + [O]_{II} \cdot y = [O]_{I+II}$$

From these equations,

$$x = \frac{[Le]_{I+II} - [Le]_{II}}{[Le]_I - [Le]_{II}} = \frac{[L]_{I+II} - [L]_{II}}{[L]_I - [L]_{II}} = \frac{[O]_{I+II} - [O]_{II}}{[O]_I - [O]_{II}}$$

When the molar ratios x/y between all glyceride fractions of different polarity are known, the molar concentration of each fraction in the total oil can be calculated (2).

To determine the glyceride composition within each separate fraction, the triglycerides were then separated as their coordination complexes with silver ions in a reversed-phase system (13). The individual triglycerides thus obtained were identified by gas-chromatographic evaluation of their fatty acid composition. The relative polarity (K_3) and chromatographic mobility of complexes (R'_2) were determined as described earlier (13).

TABLE II
Quantitative Separation of Linseed Oil Triglycerides by Partition Chromatography in an Acetone: Acetic Acid (1:1)/Aliphatic Hydrocarbon System (mole %)

R_2[a]	Percent of total oil	[P][c]	[S]	[O]	[L]	[Le]	Triglyceride identification
A. 1961 Oil							
0.16	1.3	17.4	24.1	46.4	5.8	6.3	Mixture of triglycerides
0.18	4.9	15.4	14.4	55.0	7.3	7.8	Mixture of triglycerides
0.21	11.4	12.0	16.1	35.2	15.6	21.1	Mixture of triglycerides
0.25	14.4	10.4	5.3	40.0	14.6	29.7	Mixture of triglycerides
0.32	15.6	7.5	8.8	16.9	23.5	43.3	Mixture of triglycerides
0.42	24.4	8.8	.....	20.4	9.7	61.0	Mixture of triglycerides
0.63	11.7	.....	.....	.....	33.4	66.6	LLeLe
0.78	16.2	.....	.....	.....	.....	100.0	LeLeLe
B. 1962 Oil							
0.16	1.4	18.9	21.2	38.2	9.3	12.4	Mixture of triglycerides
0.18	4.3	15.8	13.8	51.3	10.3	8.7	Mixture of triglycerides
0.21	7.3	13.6	14.5	33.7	21.2	17.0	Mixture of triglycerides
0.25	10.1	12.1	6.5	34.1	18.8	28.5	Mixture of triglycerides
0.32	16.4	9.7	7.6	15.0	28.8	38.9	Mixture of triglycerides
0.42	28.0	8.9	.....	17.8	14.8	58.5	Mixture of triglycerides
0.63	14.3	.....	.....	.....	33.2	66.8	LLeLe
0.78	18.1	.....	.....	.....	....	100.0	LeLe

[a] R_2 = ratio of R_F of triglyceride to the R_F of butyl hexabromostearate.
[b] $R_2 = 0.16$ and $R_2 = 0.18$ fractions of both oils contain also nonglyceride neutral lipids not separable by adsorption chromatography on alumina (2). These lipids appear as distinct spots on the $AgNO_3$-chromatograms.
[c] P,S,O,L,Le = palmitic, stearic, oleic, linoleic, and linolenic acids respectively. The sequence of symbols in triglycerides is not significant. [ ] = mole %.

Results

The oil content and fatty acid composition of both seed samples is shown in Table I. The unfavourable weather conditions of 1962 caused a marked decrease in oil content and considerable change in fatty acid composition (15). The fairly good agreement between the experimental iodine values and those calculated from fatty acid composition demonstrates the satisfactory accuracy of the gas-chromatographic analysis. Palmitoleic acid, always present as a minor component (0.2%), was not considered in calculating the triglyceride composition.

TABLE III
Quantitative Separation of 1961 Linseed Oil Triglycerides by Partition Chromatography in an H_2O:CH_3OH:$AgNO_3$/Dodecane System (mole %)

K_2[a]	R_2	K_3	R_2'	Aqueous methanol concentration % v/v	[P]	[S]	[O]	[L]	[Le]	Triglyceride identification
47	0.16	56	0.07	98	1.6	31.5	60.4	5.2	1.3	SOO
49	0.18	54	0.17	98	29.7	3.0	60.6	3.1	4.2	POO
		57	0.29		1.6	.....	90.2	3.1	5.1	OOO
51	0.21	54	0.27	95	34.6	4.7	32.5	28.2	.....	POL
		56	0.38		1.3	31.4	32.7	2.0	32.6	SOLe
		57	0.51		4.3	.....	61.5	34.1	.....	OOL
53	0.25	54	0.58	95	31.0	.....	33.0	3.4	32.6	POLe
		56	0.71		3.0	30.3	.....	33.3	33.4	SLLe
		57	0.93		.....	.....	63.5	7.7	28.8	OOLe + OLL
55	0.32	54	0.71	80	30.6	1.5	2.9	30.2	34.7	PLLe
		56	0.79		2.1	31.3	.....	1.1	65.5	SLeLe
		57	1.02		4.9	.....	31.8	31.4	31.8	OLLe
57	0.42	56	0.74	80	32.6	1.7	4.1	0.9	60.7	PLeLe
		57	2 51		.....	.....	28.4	12.4	59.0	OLeLe + LLLe

[a] For an explanation of K_2, R_2, K_3, R_2' see "Methods."

TABLE IV
Triglyceride Composition of Linseed Oil (mole %)

Triglycerides	A. 1961 Oil			B. 1962 Oil		
	Found	Calculated		Found	Calculated	
		Random	Present method		Random	Present method
SSS	.....	0.0	0	.....	0.0	0
SSP	.....	0.0	0	.....	0.0	0
SSO	.....	0.1	0	.....	0.1	0
SPP	.....	0.1	0	.....	0.0	0
SPO	.....	0.4	0	.....	0.2	0
SSL	.....	0.1	0	.....	0.0	0
SOO	1.3	0.7	0.9	1.4	0.3	0.4
PPP	.....	0.0	0	.....	0.0	0
PPO	.....	0.4	0	.....	0.3	0
SPL	.....	0.3	0	.....	0.2	0
POO	3.2	1.1	1.4	2.8	0.7	0.9
SSLe	.....	0.3	0	.....	0.2	0
SOL	.....	0.9	1.4	.....	0.1	0.1
OOO	1.7	1.1	1.0	1.5	0.6	0.6
PPL	.....	0.2	0	.....	0.3	0
SPLe	.....	1.0	0	.....	0.8	0
POL	4.1	1.4	1.8	2.9	1.3	1.6
SOLe	5.8	3.2	4.1	3.1	2.0	2.6
SLL	.....	0.3	0.3	.....	0.3	0.3
OOL	1.3	2.1	2.0	1.4	1.7	1.6
PPLe	.....	0.8	0	.....	0.8	0
POLe	4.5	5.1	6.4	3.7	4.4	5.4
PLL	.....	0.4	0.5	.....	0.6	0.7
SLLe	2.3	2.0	2.5	2.0	1.9	2.3
OOLe	6.0	7.9	7.4	3.0	5.7	5.5
OLL	2.0	1.4	1.3	1.9	1.6	1.5
PLLe	3.5	3.2	4.0	4.8	4.0	4.9
SLeLe	4.1	3.7	4.7	3.7	3.1	3.8
OLLe	7.9	10.0	9.4	7.4	10.4	10.0
LLL	.....	0.3	0.3	0.7	0.5	0.5
PLeLe	6.4	5.9	7.5	7.5	6.6	8.1
OLeLe	14.9	18.3	17.3	14.9	16.9	16.2
LLLe	3.5	3.1	2.9	6.2	4.8	4.6
LLeLe	11.7	11.5	10.9	14.3	15.5	14.9
LeLeLe	16.2	14.0	13.2	18.1	16.8	16.1

The fatty acid composition of the separate glyceride fractions, the molar percentage of the fraction in the total triglycerides (calculated as described under "Methods"), and the tentative identification of the fractions are given in Table II. Only trilinolenin and linoleodilinolenin can be identified from these data. Other fractions seem to be mixtures of several triglycerides of the same polarity.

The triglyceride mixtures within each of these fractions were resolved in a reversed-phase system of aqueous methanol saturated with silver nitrate and dodecane/dodecane (Table III). From these results 16 more triglycerides can be identified in the 1961 seeds. The 1962 oil contains in addition, a small amount of trilinolein.

The separate chromatographic fractions of different polarity were assumed in all the following calculations to consist only of the triglycerides shown in Tables II and III, in spite of some additional fatty acids found in the $AgNo_3$ = fractions of Table III. These impurities are due to the presence of small amounts of nonglyceride neutral lipids not separable from the triglycerides by adsorption chromatography on alumina (2), or reflect the limits of the accuracy of our separation methods.

If the qualitative pattern of triglycerides and the fatty acid composition of a given fraction are known, it is then possible to calculate the content of each glyceride in this fraction. The calculation can be illustrated with the example of the $K_2 = 55$ fraction of the 1962 oil. There are PLLe, SLeLe, OLLe, and LLL in this fraction. The mole percentage of [PLLe], [SLeLe], [OLLe], and [LLL] in this fraction were calculated as follows:

$$[P] = [PLLe]/3 \quad (1)$$
$$[S] = [SLeLe]/3 \quad (2)$$
$$[O] = [OLLe]/3 \quad (3)$$
$$[L] = [LLL] + [OLLe]/3 + [PLLe]/3 \quad (4)$$
$$[Le] = [OLLe]/3 + 2[SLeLe]/3 + [PLLe]/3 \quad (5)$$

If we take [P], [S], etc., from Table II B, then

$$[PLLe] = 3 \times 9.7 = 29.1\%$$
$$[SLeLe] = 3 \times 7.6 = 22.8\%$$
$$[OLLe] = 3 \times 15.0 = 45.0\%$$

Hence from equation (4)

$$[LLL] = \frac{3\,L - OLLe - PLLe}{3} = 4.1\%$$

Hence from equation (5)

$[Le] = [OLLe]/3 + 2[SLeLe]/3 + [PLLe]/3 = 39.9\%$

The value of [Le] (Table II B) is 38.9. It will be seen that there is fair agreement between experimental and calculated values of [Le].

By multiplying these experimental values by the mole percentage of the corresponding fraction in the total triglycerides (Table II), the content of each triglyceride in the oil may be calculated. The quantitative triglyceride composition of both oil samples is shown in Table IV.

The estimated triglyceride composition given in Table IV can be used to calculate the fatty acid composition of the original oils. Table V shows the results of this computation. These results were used for the calculation of the random triglyceride composition. Comparison of the calculated fatty acid compositions in Table V with those determined experimentally in Table I shows moderately good agreement, indicating that our estimated triglyceride composition is moderately accurate.

TABLE V
Linseed Oil Fatty Acid Composition Calculated from Triglyceride Composition Data (Table IV)

Fatty acid	1961 oil	1962 oil
Palmitic	7.3	7.2
Stearic	4.6	3.4
Oleic	22.6	18.5
Linoleic	14.2	17.0
Linolenic	51.9	55.2

Discussion

The triglycerides of linseed oil contain five major fatty acids (Table I). The proportions among these acids can be considered as being typical for linseed oil (16).

In the reversed-phase paper chromatographic system the triglycerides separate into 8 fractions of different polarity (Table II). The same number of fractions has been found by column partition chromatography of linseed oil (10).

The data of Tables II and III show that we determined 18 glycerides in the 1961 seeds, and 19 glycerides in the 1962 seeds out of 35 possible triglycerides of different fatty acid composition (without taking into account positional isomers). Generally, the triglycerides having proportions calculated by random distribution, at the 0.1 to 1.0% level were not found in the oil. However, no direct relationship between the random proportion of a given compound and its occurrence in the seeds could be established in this concentration range. Thus, LLL (random proportion 0.5%) did occur in 1962 oil, whereas SPLe (random proportion 1.0% in 1961 oil) was absent.

Dutton and Cannon (6) studied the triglyceride composition of a linseed oil which was similar to our 1961 oil in its fatty acid composition. They stated that linseed oil glycerides follow essentially the random pattern of distribution. The data of Table IV generally support this conclusion. There are, how-

ever, certain regular deviations from the random distribution rule in the concentration of some glycerides. Thus, the content of LeLeLe, LLLe, POL, SOLe and some other glycerides in both oils is higher than random, whereas the content of OLeLe and OLLe is below the random level. The determination of some triglycerides by Dutton and Cannon gave following results (mole %):

	LeLeLe	LLeLe	LLLe	OLeLe
Experimental	18.2	12.3	4.1	19.5
Random	14.3	12.2	3.5	19.9

The results of the American authors are close to those of Table IV, and the same relationship between experimental and random values is observed.

The departure of linseed oil glyceride composition from random distribution theory becomes all the more evident when the experimental and random molar concentrations of each triglyceride type—trisaturated (S_3), disaturated-monounsaturated (S_2U), monosaturated-diunsaturated (SU_2), and triunsaturated (U_3)—are compared (S and U are saturated and unsaturated fatty acids, respectively). The data of this calculation are shown in Table VI. It can be seen that in both oils the concentration of U_3 is less than random, and the amount of SU_2 is above the random level.

The triglyceride composition of cottonseed oil (2) is in agreement with a positional theory of fatty acid distribution recently advanced by Gunstone (17). The calculation for our linseed oil based on Gunstone's theory (and omitted in this discussion) gives results which are close to random proportions. The same coincidence was noted in the calculations of Gunstone himself (17).

According to the random distribution principle, linseed oil must contain some quantity of S_3 and S_2U glycerides (Table VI). But the data of Table IV and the results of other workers (8) show that these glyceride types are absent in linseed oil. The same relationship has been found in poppyseed oil, but here, unlike linseed oil, the amount of U_3 glycerides is higher than random (2). Thus, the linseed oil triglycerides seem to be synthesized by a somewhat different pathway from cottonseed oil or poppyseed oil glycerides.

The saturated fatty acids of linseed oil can form only SU_2 triglycerides (Table IV). Therefore, in order to calculate the glyceride composition which would be similar to the experimental one, the saturated acids of "random" glycerides S_3 and S_2U must be "redistributed" to form SU_2 glycerides. The equations for these calculations are:

$$[SU_2] = [su_2] + 2[s_2u] + 3[s_3] \quad (6)$$

$$[U_3] = [u_3] - [s_2u] - 2[s_3] \quad (7)$$

where $[s_3]$, $[s_2u]$, $[su_2]$, and $[u_3]$ are random concentrations of corresponding glyceride types.

The calculated values of $[SU_2]$ and $[U_3]$ are shown in the last columns of Table VI A and VI B. It can be seen that these values are in agreement with experimental ones.

The computation of individual triglyceride concentrations based on the calculated $[SU_2]$ and $[U_3]$ values (Table VI) involves multiplying the random glyceride proportions (Table IV) by a correction factor F. Evidently, for $[S_3]$ and $[S_2U]$ glycerides

$$F_{s_3} = 0 \quad (8)$$

$$F_{s_2u} = 0 \quad (9)$$

TABLE VI

Found and Calculated Composition of Triglyceride Types of Linseed Oil (mole %)

Type of triglyceride[a]	A. 1961 Oil			B. 1962 Oil		
	Found	Calculated		Found	Calculated	
		Random	Present method		Random	Present method
S_3	.....	0.1	.....	.....	.....	.....
S_2U	.....	3.6	.....	.....	2.9	.....
SU_2	35.2	27.9	35.4	31.9	25.3	31.1
U_3	65.2	69.7	65.9	69.4	74.5	71.6

[a] S = acyl groups of saturated fatty acids; U = acyl groups of unsaturated fatty acids.

For the calculation of SU_2 and U_3 triglycerides the correction factors are

$$F_{su_2} = [SU_2]/[su_2] \quad (10)$$

$$F_{u_3} = [U_3]/[u_3] \quad (11)$$

The concentrations of individual triglycerides calculated from equations (8–11) are shown in the last columns of Table IV A and IV B. Some 80% of calculated values are closer to experimental results than random ones. On the other hand, our proposed distribution scheme does not account for the high values of trilinolenin, dilinoleolinolenin, and some other glycerides, but calls for an even lower value than random distribution. It is known that the iodine value of linseed oil increases markedly by the end of maturation due to the increase of linolenic and linoleic acid synthesis in this period. It is possible, therefore, that the higher values obtained reflect a preferential synthesis of LeLeLe, LLLe, etc., during the last stages of seed development. Values for simple triglycerides that are higher than random cannot be accounted for by any theory that has been proposed so far.

Thus, for the computation of triglyceride composition moderately similar to the natural linseed oil, it is necessary to calculate from its fatty acid composition the concentration of individual triglycerides and glyceride types according to the random distribution rule, and then to recalculate the corrected random values by equations (6–11).

Interestingly enough, selective lipase hydrolysis of oxidized linseed oil has shown that a significant amount of symmetrical USU glycerides (about 4% of the total triglycerides) is present (8). This is unusual, since most liquid vegetable oils contain SU_2 glycerides only in the unsymmetrical SUU form.

The results of our investigation show that linseed oil contains only triunsaturated and monosaturated-diunsaturated triglycerides. Within each of these types the fatty acyl distribution is close to random, but the content of some triglycerides may regularly depart from a random pattern.

The increase of linolenic and linoleic acid content and sharp decrease of oleic acid concentration in 1962 seeds as compared with the previous generation (Table I) produced a marked effect on the triglyceride composition (Table IV). The concentrations of all triglycerides containing only linolenic and linoleic acids increased, whereas the amount of mono-, di-, and trioleo- and monostearo-triglycerides decreased as compared with 1961 oil. The increased biosynthesis of linoleic acid in 1962 led to the appearance of trilinolein which was formerly absent in oil. Therefore, the genotype does not strictly control the qualitative triglyceride pattern. It seems that the triglyceride composition reflects the phenotypic changes in fatty acid composition by the appearance of some

glycerides and the disappearance of others. Nevertheless, a general pattern of glyceride formation is followed regardless of ecological conditions of plant growth and regardless of the composition of the fatty acid mixture which is available for triglyceride biosynthesis.

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Analysis of Triglycerides by Consecutive Chromatographic Techniques. II. Ucuhuba Kernel Fat[1,2]

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Abstract

The triglycerides from ucuhuba kernel fat (*Virola surinamensis*) were analyzed using thin-layer adsorption chromatography (TLC) followed by gas-liquid chromatography (GLC). The triglycerides were first separated into three fractions containing 0, 1, and 2 or more double bonds per molecule on silica gel TLC plates impregnated with $AgNO_3$. The total triglycerides and each individual TLC fraction were then analyzed by GLC for the molecular weights of their component triglycerides and for their fatty acid composition. Quantitation of the TLC fractions was achieved by GLC analysis of their fatty acids using an added internal standard and confirmed by solving simultaneous equations derived from GLC analysis of their triglycerides and fatty acids.

Application of these combined chromatographic techniques separated the ucuhuba kernel fat into 23 triglyceride components. Trimyristin and laurodimyristin comprised over half the total triglycerides, which was expected since the fat contained 20.0 mole % lauric and 71.3% myristic acids.

Introduction

THE INTRODUCTION of new analytical techniques for the investigation of glyceride structure has added great impetus towards a better understanding of the glyceride composition of natural fats. These methods include the widely applied silver-ion method of de Vries for separating triglycerides into fractions differing only in unsaturation (1), GLC separation of triglycerides according to molecular weight (2), and numerous other analytical techniques (3-5).

The complexity of naturally occurring fats and the lack of refinement in our present analytical methods do not, however, permit the use of a single analytical technique for determining triglyceride composition. Combinations of these techniques have likewise failed to provide comprehensive analyses, but do produce experimental data which permit detailed estimation of triglyceride compositions. Such an application of combined analytical techniques for determining triglyceride composition is illustrated in the recent work of Blank et al. (6) who applied Ag^+ TLC in conjunction with lipase hydrolysis to demonstrate the triglyceride composition of synthetic mixtures and of several natural fats. A similar integration of Ag^+ TLC, and GLC, was employed by this laboratory in characterizing the triglyceride composition of *Cuphea llavia* seed fat (7).

Ucuhuba (*Virola surinamensis*) is a tree of the nutmeg family which grows along the swampy coast of northern Brazil and the Guianas. The fruit has a spherical hull which encloses a seed 12–14 mm in diameter. Fatty acid analyses of the seed fat by several investigators (8–13) have shown that myristic and lauric acids comprise 66.6–73.4 and 5.0–20.8 wt %, respectively. Small amounts of additional fatty acids including decanoic, palmitic, oleic and linoleic were also found. Employing a systematic crystallization of the glycerides from acetone, Atherton and Meara (8) estimated that six triglycerides were present in the ucuhuba kernel fat. Trimyristin (MMM) and laurodimyristin (LaMM) were found to comprise 42.6 and 30.7 mole %, respectively, with the remaining composition (26.7%) consisting of OLaM (12.1%), LaMP (10.3%), OMM (3.1%), and LaLaM (1.2%). (See Table IV for explanation of abbreviations).

The current investigation employed the integration of Ag^+ adsorption chromatography with GLC in characterizing the triglycerides of ucuhuba (*V. surinamensis*) kernel fat. The triglycerides were separated by Ag^+ TLC according to the number of double bonds per molecule, with subsequent GLC analysis of the fractionated triglycerides.

Procedures

Materials

Ucuhuba (*V. surinamensis*) seeds were obtained through the courtesy of Mr. H. Bloom, American Consul, Belem, Para, Brazil. The fruits were gathered in 1963 from trees in the Brazilian state of Para in the lower Amazon basin. The seeds were sorted to remove any diseased or damaged ones and stored in a refrigerator for future analysis.

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