Computer Determination of All Individual Structures of Triglyceride Molecules of Fats and Oils

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ABSTRACT AND SUMMARY

A method for calculating all of the individual structures of triglyceride molecules (ISTM) of fats and oils based on a digital computer program using operational language FORTRAN is presented. Qualitative and quantitative results of gas liquid chromatographic analysis of fatty acids after their stereospecific pancreatic lipolysis from C-1,3 positions of triglyceride molecules were used as main input data. The individual structures are determined with respect to the possible mono-, di-, and tricomponent combinational types of triglyceride molecules. The resulting individual structures of triglyceride molecules are presented in concentrational expressions: weight percentages, molar values, molar percentages, and molar fractions. Moreover, some other information (graphical interpretations included) concerning triglyceride molecule structures are also obtained. Two applications of this method for triglyceride molecule individual structure determinations of maize and rapeseed oils are presented.

INTRODUCTION

Present-day knowledge indicates that the chemical, physical, and biological properties of triglyceride fats and oils depend not only on the kind and quantity of participating fatty acids but also on the positions of these fatty acids in the triglyceride molecule.

Triglyceride fats and oils are essential for all organisms; they are not only species-specific but also specific for various tissues (1,2), and it seems also that a relationship exists between disease states and triglyceride structures.

The so-called "simple lipids" are the oldest known group of triglyceride fats and oils. Fifty years ago Hilditch et al. began to study the complicated problems connected with the structure of triglyceride molecules. Kartha began to work along these lines twenty-five years ago and has succeeded in experimentally verifying some of the more recent theoretical aspects of this problem. The publications of these authors (3-8) are of basic importance in the field of triglyceride structures.

New methods of separation and analysis of triglyceride structures have been reported by Dutton et al. (9,10), Sholfield et al. (11-15), Hammond et al. (16), Jones et al. (17), Privett et al. (18), and Christie (19).

The work of Savary et al. (20), Desnuelle (21), Matson et al. (22), Coleman (23), and Youngs (24,25) employing the stereospecificity of pancreatic lipase hydrolysis further contributed to a more exact understanding of positioning of fatty acids in triglyceride molecules. Methodology applied to triglyceride structure studies was worked out by Vander Wal (26-28) and Gunstone (29).

A detailed outline of present-day knowledge in the field of structures of triglycerides molecules has been published in the monography of Hilditch (3) and in the compilations of Coleman (30) and Vander Wal (31).

The interpretation potential of analytical and computing data on the structure of triglyceride molecules forms a specific field of research. The interpretations remain more or less on a level of so-called alignment structures of triglyceride molecules (ASTM) where with the help of two types of fatty acids—saturated (S) and unsaturated (U)—six possible molecular types of triglycerides have been expressed: i.e., SSS, SUS, UUS, SSU, USU, and UUU. Data for the computing of ASTM are based on the presumption of 1,3 and 2 random distribution in Vander Wal's mathematical formulae (27). Statistical distributional formulations were applied in this field by Litchfield (32), and Blank et al. (33) have evaluated mathematical methods for this purpose.

The application of computerized calculation of ASTM using the constructed program in the operating language FORTRAN as well as the comparison of results from the point of view of various distribution theories have been published by Perkins (34). The problem of determination and also of interpretations of triglyceride structures in which all individual fatty acids should occur is more complicated since all their qualitative, quantitative, and positionally differentiated aspects have to be taken into account. Such of the structures of triglyceride molecules which would be completed exactly with both qualitative-quantitative and positional parameters of all individual fatty acids determined in some fatty oil sample we can term "the individual structures of triglyceride molecule" (ISTM). Pursuing that problem Hayakawa has worked out a great number of his own equations calculating the molar fraction values of all ISTM of maize oil (35).

The foundations of the computer program for establishing ISTM using the operational language ALGOL were derived from paper (36); its application for the computing of ISTM as well as studies of intra- and interesterification of peanut oil in the process of catalytic hydrogenation have been published in another paper (37).

The aim of the present study was to work out a program algorithm for a digital computer in the operational language FORTRAN which would yield all possible information on triglyceride structure of fats and oils based on input data of qualitative representation of fatty acids on positions C-1,3 and C-2.

EXPERIMENTAL PROCEDURES

Samples Used

Freshly extracted rapeseed oil with an acid value of 0.27 mg KOH/g was used. Triglycerides were isolated after separation on thin layers using silica-gel as an adsorbant and hexane-ethylacetate (9:1 v/v) as solvent. The combined triglyceride fractions from 25 thin layer plates were used for specific positional hydrolysis with pancreatic lipase. Stereospecified fatty acids of maize oil published by Vander Wal (31) and used for calculations of triglyceride structures by Hayakawa (35) were also compared.

Stereospecific Hydrolysis of Triglycerides by Pancreatic Lipase

Pancreatic lipase (Reanal Finomvegyszegyár, Budapest, Hungary) was used for hydrolysis of fatty acids from C-1,3 positions of triglyceride molecules isolated in bulk from rapeseed oil. The method described by Luddy (38) was applied using 50 mg of sample at 40 C with addition of

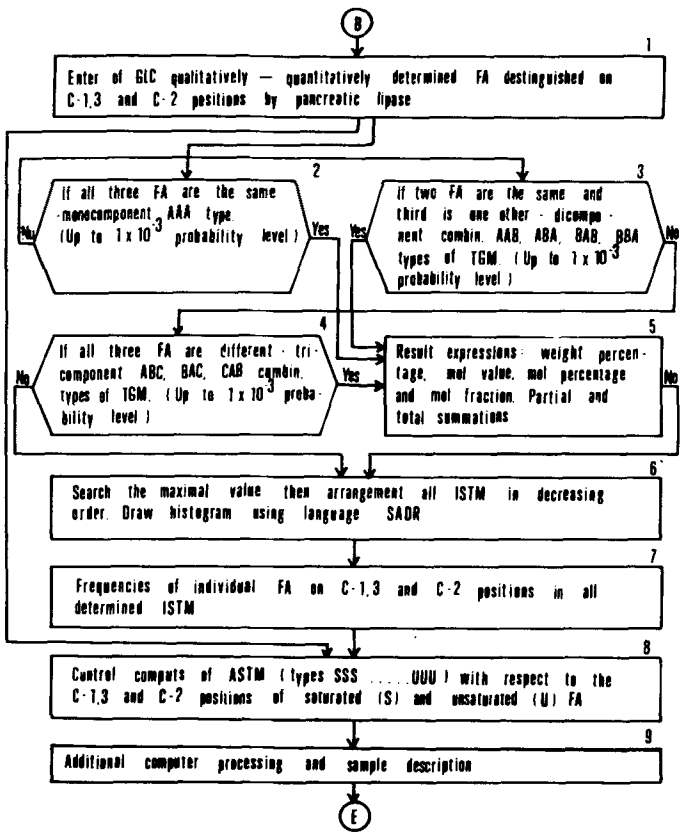

FIG. 1. Simplified form of the flowchart of program algorithm for computer determination of individual structures of triglyceride molecules of fats and oils. S = saturated; U = unsaturated; FA = fatty acids; TGM = triglyceride molecules; ISTM = individual structures of triglyceride molecules with exactly determined FA on each position of TGM; AAA. . . .ABC = general combinational types of TGM assuming mono-, di-, and tricomponentness of individual FA; SSS. . . .UUU = alignment (simple) of structural types of TGM assuming presence of S and U fatty acids only.

9 mg of pancreatic lipase, 0.1 ml of 22% solution of $CaCl_2$, and 0.25 ml of a 0.1% solution of the sodium salt of cholic acid.

Conditions for Isolation of Fatty Acids from C-1,3 and C-2 Positions of Triglyceride Molecules

The mixture, which after lipolysis consisted of free fatty acids from C-1,3 positions, of 2-monoglycerides, and of unreacted triglycerides, was again separated by thin layer chromatography (TLC) using conditions identical to those described above. After partition, the spots of free fatty acids and 2-monoglycerides were scraped off the chromatograms. The fraction of free fatty acids was extracted and converted to methyl ester according to Peisker (39) and was then subjected to gas liquid chromatographic (GLC) analysis. The isolated fraction of 2-monoglycerides was also converted to methyl esters by direct reesterification (39), and the methyl esters of fatty acids were again analyzed by GLC to obtain qualitative-quantitative data on fatty acids in the C-2 position of triglyceride molecules of rapeseed oil.

Conditions for Gas Liquid Chromatography

The chromatographic analysis of methyl esters of fatty acids from C-1,3 and C-2 positions of triglyceride molecules of rapeseed oil was carried out with a model 7620 Hewlett-Packard Gas Chromatograph with a flame ionization detector at a temperature of 250 C, injection port temperature of 250 C, an isothermal temperature of the column oven at 180 C.

A stainless steel column with active length of 200 cm, ID 2 mm, and Celite AW DMCS as the support of 100-120 mesh size coated with 10% DEGS was used (36).

One μl volumes of a 20% solution of the methyl esters of fatty acids in cyclohexane were injected. Nitrogen was used as the carrier gas with a calculated optimal linear flow speed of 4.75 cm/sec (46.7 ml/min). Qualitative (retention times) and quantitative results on the individual fatty acids belonging to both the C-1,3 and C-2 positions of the triglyceride molecules of rapeseed oil were read out from an HP model 3370 A digital integrator.

Conditions for the Algorithm, Program Construction, and Computer Processing

Vander Wal's mathematic notations for 1,3 random and 2 random distribution were first used (31). Hence it followed that from fatty acid composition aspects, the triglyceride molecules can be grouped in several combination types. So, the triglyceride molecules possessing the same fatty acids on all three positions we consider as "monocomponent combinational type" with the general interpretation form AAA. The triglyceride molecules with two of the same and one other fatty acid form "dicomponent combinational types": AAB, ABA, BBA, BAB. Triglyceride molecules containing all three different fatty acids in C-1,2,3 positions we consider as "tricomponent combinational types" of forms: ABC, CAB, and BCA. The symbols in these formulas are substituted with all of the individual fatty acids which were experimentally, qualitatively, and quantitatively determined in C-1,3 and C-2 positions. We consider the order of particular symbols in presented combinational types as corresponding to the C-1,2,3 positions in the triglyceride molecules.

The qualitative data of individual fatty acids for computer input, operational processes, and output were also coded by digits, for example, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$.

The main sequences of the constructed program algorithm for computer determination of all ISTM as well as other information concerning the triglyceride molecule structures of fats and oils are listed in the flowchart interpreted by Figure 1. For computer processing of the given program algorithm the operational language FORTRAN IV was used. For graphical interpretations of computed results the language SADR was used.

For program realization of ISTM determination we applied a Minsk 22 M digital computer with 8 K words of primary memory, 1600 K words of secondary memory, operation speed 6000/sec, operating FEL system. The transfer of entering data was done by a punched paper tape and a wide line printer was employed for the output of alphanumerical as well as graphical results.

The whole computer program constructed by algorithm sequences as shown in Figure 1 consists of 14 pages of recording from a wide line printer. Generally, it can be used for N experimentally determined fatty acids where $3 \leqslant N \leqslant 40$. The translation of programming takes about 90 min and the actual computing process about 15 min using this model of digital computer.

For control possibilities a computer ISTM determination of maize oil with model input data of fatty acid composition from the works Vander Wal (27) and Hayakawa (35) was made. After comparison of these results a computer ISTM determination of rapeseed oil used for entering our own experimental results of fatty acid composition was made.

RESULTS AND DISCUSSION

Combining analytical procedures as described in the experimental part and the computer program whose algorithm was presented in Figure 1, we can determine all individual structures of triglyceride molecules. That means

TABLE I

Shortened Form of Computer Output Part Containing Determined Individual Structures in Particular Combinational Types of Maize Oil Triglyceride Molecules

Number	Positions of individual fatty acids C-1	C-2	C-3	Weight percentages	Molar value	Molar percentages	Molar fraction	Molar fraction[a]
	A	A	A					
1	18:2	18:2	18:2	17.013	0.193	17.532	0.17532	0.1660
2	18:1	18:1	18:1	2.139	0.024	2.188	0.02188	0.0235
2	Sums			19.152	0.217	19.720	0.19720	0.1895
	A	A	B					
1	18:1	18:1	18:2	7.898	0.089	8.100	0.08100	0.0811
2	18:2	18:2	18:3	0.621	0.007	0.642	0.00642	0.0062
3	18:1	18:1	18:3	0.144	0.002	0.138	0.00138	0.0015
3	Sums			8.663	0.0981	8.880	0.08880	0.0888
	Continue through all ISTM of combinational types ABA, BBA, BAB, ABC, ACB until the last:							
	C	A	B					
1	18:3	18:1	18:2	0.266	0.003	0.274	0.00274	0.0026
2	16:0	18:2	18:3	0.227	0.003	0.241	0.00241	0.0025
2	Sums			0.493	0.006	0.515	0.00515	0.0051
26	Sums	Total		96.273	1.122	99.990	0.99990	0.9203

[a]See (35).

TABLE II

Fatty Acid Composition of Rapeseed Oil After Pancreatic Stereospecific Lipolysis and Gas Liquid Chromatographic Analysis

Fatty acids	Percent on C-1 position	Percent on C-2 position	Percent on C-3 position
Caproic ($C_{6:0}$)	1.40	1.16	1.40
Caprylic ($C_{8:0}$)	0.83	1.12	0.83
Capric ($C_{10:0}$)	0.83	1.06	0.83
Lauric ($C_{12:0}$)	2.09	1.67	2.09
Lauroleic ($C_{12:1}$)	1.77	1.91	1.77
Myristic ($C_{14:0}$)	1.68	1.92	1.68
Myristoleic ($C_{14:1}$)	2.16	1.12	2.16
Palmitic ($C_{16:0}$)	9.16	7.70	9.16
Palmitoleic ($C_{16:1}$)	2.46	1.10	2.46
Heptadecanoic ($C_{17:0}$)	-	1.50	-
Stearic ($C_{18:0}$)	4.36	3.34	4.36
Oleic ($C_{18:1}$)	13.80	9.55	13.80
Linoleic ($C_{18:2}$)	1.78	3.40	1.78
Linolenic ($C_{18:3}$)	2.19	2.04	2.19
Eicosenoic ($C_{20:1}$)	7.82	6.44	7.82
Eicosadienoic ($C_{20:2}$)	1.84	3.40	1.84
Behenic ($C_{22:0}$)	1.68	2.80	1.68
Erucic ($C_{22:1}$)	38.90	34.55	38.90
Docosadienoic ($C_{22:2}$)	3.52	3.75	3.52
Lignoceric ($C_{24:0}$)	1.49	1.67	1.49
Tetracosanoic ($C_{24:1}$)	0.24	1.60	0.24
Tetracosadienoic ($C_{24:2}$)	-	4.56	-
Cerotic ($C_{26:0}$)	-	2.64	-

all triglyceride structures possible from the given number of exactly quantitatively and quantitatively and positionally defined fatty acids in fatty oil samples with respect to their mono-, di-, and tricomponent AAA. . . .ABC types of triglyceride molecules.

A control application of the constructed program algorithm was performed for determining all ISTM of maize oil using as a model input data on fatty acid composition published in previous papers (27) and/or (35).

An example of the computer-resulting ISTM of some of the combinational types of triglyceride molecules of maize oil is interpreted in Table I. Format and the results of determined ISTM of maize oil presented in this table are like a copy of the alphanumerical data from the digital computer output record. However, the number of determined maize oil ISTM on the original output part is far larger. From the results obtained in this way we can read a number value of defined individual structures which fulfilled the conditions of possible combinational triglyceride types (column on left), all their concentrational expressions, their partial sums as well as total sums. Concretely, from the model entering fatty acid composition data of maize oil, we have received, in all its combinatorial types, a total numerical value of 26 ISTM. In the last column on the right side of Table I, the molar fraction values of the maize oil ISTM determined by presented computer method are compared with the results on molar fractions of triglyceride structures which have been received by Hayakawa (35). The

TABLE III

Shortened Form of Computer Output Part Containing Determined Individual Structures in Particular Combinational Types of Rapeseed Oil Triglyceride Molecules

Number	Positions of individual fatty acids C-1	C-2	C-3	Weight percentages	Molar value	Molar percentages	Molar fraction
	A	A	A				
1	22:1	22:1	22:1	5.228	0.050	7.717	0.07717
2	18:1	18:1	18:1	0.182	0.002	0.319	0.00319
2	Sums			5.4100	0.0517	8.0364	0.080364
	A	A	B				
1	18:1	18:1	22:1	1.025	0.011	1.694	0.01694
:							
:							
9	16:0	16:0	20:1	0.110	0.011	0.199	0.00199
9	Sums			3.9370	0.0408	6.3380	0.063380
	Continue through all ISTM of combinational types ABA, BBA, BAB, ABC, ACB until the last:						
	C	A	B				
1	22:1	16:0	18:1	0.827	0.009	1.405	0.0145
:							
:							
37	22:1	16:0	22:0	0.101	0.001	0.161	0.00161
37	Sums			7.1834	0.0780	12.1232	0.121232
182	Sums	Total				96.782	0.96782

TABLE IV

Weight Percentage Values of Alignment Structures of Triglyceride Molecules of Rapeseed Oil with Positionally Defined S and/or U Fatty Acids[a]

Type of TGM structure	SSS	SUS	SSU	USU	UUS	UUU
Percent	1,4704	4,0615	9,5625	15,5471	26,4137	42,9448

[a]S = saturated, U = unsaturated.

identity of these ISTM molar fractions of model fatty acid mixture of maize oil is evident despite of the fact that the results were obtained in two completely different ways.

Linked up with the compared results on triglyceride structures of maize oil, the constructed computer program was applied to the determination of ISTM, where, as input data, our own experimental, analytical-composition data of fatty acids of rapeseed oil were used. The entering fatty acids determined by GLC on C-1,3 and C-2 positions of rapeseed oil are presented in Table II.

Under the conditions of the described computer program as well as those of the compositional and positional data of fatty acids from Table II, we obtained much valuable information on individual structures of triglyceride molecules of rapeseed oil. The resulting form of some combinational types ISTM in the case of rapeseed oil is similar to those presented by maize oil in Table I, but the number of ISTM is proportional to the number of experimentally estimated fatty acids, but higher. It follows from computerized results that 182 ISTM of rapeseed oil in all its combinational types were defined. The results of the rapeseed oil individual structures of triglyceride molecules are in a shortened form illustrated in Table III.

The remaining information on triglyceride structures of rapeseed oil computed on the basis of input data from Table II and by the use of the presented program are listed in Tables IV-VI.

Table IV compiles adjusted results of percentage values of alignment SSS. . . .UUU structural types of rapeseed oil triglyceride molecules.

In Table V the frequencies of various fatty acids in C-1,3 and C-2 positions of rapeseed oil triglyceride molecules are presented. It can be seen from this table that frequencies of the various fatty acids are proportional to their molar percent representation in the original mixture of the rapeseed oil and that none of the found fatty acids is present preferentially in position C-2 of rapeseed oil triglyceride molecules.

The weight percents and the numbers of various triglyceride forms of rapeseed oil are presented in Table VI. They were taken and adjusted from the results of the output record of the computer. These results indicate that the occurence of the monocomponent AAA type of triglyceride molecules in rapeseed oil (trierucin and triolein) is least frequent, but if we express them in weight percentage values they can be listed in third place. In no case do the monocomponent triglyceride molecules of rapeseed oil occur in such ratios as to fulfill Hilditch's postulation (3) of the presence of one third fatty acids. On the other hand, erucic acid is originally determined to be about 40% but the number of these triglyceride molecules in rapeseed oil which do not contain erucic acid is not proportional to this finding.

The whole complex problem of triglyceride structure could not be resolved by using many other analytical methods in their single form, or without the combined use of stereospecific methods, combined with chromatographic and especially computing methods. This last aspect is partially shown in this paper on the examples given for maize and rapeseed oil, one of the most complex oils of plant origin.

Some advantages of the presented computer method for determination of fatty oil ISTM are evident since, given the number of basic data, nearly all information presently possible on triglyceride structures can be obtained in this way. The program constructed for this purpose is relatively

TABLE V

Frequencies of Individual Fatty Acids on C-1,3 and C-2 Positions of Rapeseed Oil Triglyceride Molecules

Fatty acid	Frequencies on positions C-1,3	C-2	Fatty acid	Frequencies on positions C-1,3	C-2
$C_{6:0}$	5	2	$C_{18:2}$	10	5
$C_{8:0}$	3	2	$C_{18:3}$	11	4
$C_{10:0}$	3	2	$C_{20:1}$	44	13
$C_{12:0}$	11	4	$C_{20:2}$	10	5
$C_{12:1}$	9	4	$C_{22:0}$	9	4
$C_{14:0}$	9	4	$C_{22:1}$	211	57
$C_{14:1}$	9	2	$C_{22:2}$	16	6
$C_{16:0}$	58	19	$C_{24:0}$	7	4
$C_{16:1}$	9	2	$C_{24:1}$	3	3
$C_{17:0}$	3	3	$C_{24:2}$	7	7
$C_{18:0}$	19	5	$C_{26:0}$	0	4
$C_{18:1}$	76	21			

TABLE VI

Numerical and Weight Percentage Ratios of Various Combinational Types of Rapeseed Oil Triglyceride Molecules

Type of TGM[a]	AAA	BAA	ABA	BBA	BAB	ABC	ACB	CAB
Numerical ratios	2	9	9	20	18	39	48	37
Weight percentage ratios	5.41	3.83	3.43	15.61	7.89	8.30	10.45	7.18
Weight percentage of mono- to multi component TGM	1	0.71	0.63	2.90	1.46	1.55	1.94	1.33
	1		5.70				4.83	
	1				10.52			

[a]TGM = triglyceride molecule.

more complicated than the one previously described for the determination of the physicochemical values of fats and oils (40). However, the program presented in this paper can be easily adapted for the input of fatty acids with more exactly defined positional data, for instance in combination of stereospecific enzymatic hydrolysis and magnesium methylbromide method according to Yurkowski et al. and others (41-43).

Results of the ISTM determinations from experimentally defined fatty acid compositions sent in the form of Table II or a listing of the program in FORTRAN IV written for the Minsk 22 M computer system can be obtained from the authors.

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