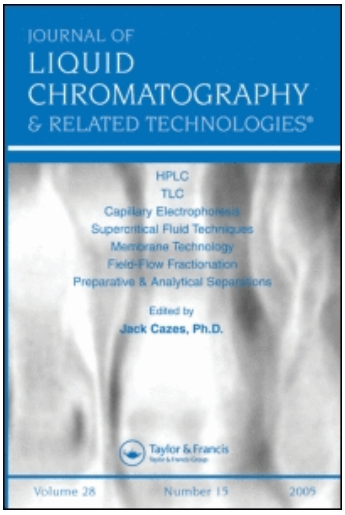

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Molecular Prediction of Rat Liver Triglycerides by High Performance Liquid Chromatography

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MOLECULAR PREDICTION OF RAT LIVER TRIGLYCERIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Triglyceride composition of rat liver was analyzed by HPLC with a non-linear elution gradient of acetone in acetonitrile using a light-scattering detector. Triglyceride molecular species were predicted by means of simple and multiple linear regression analysis between the log k' of the HPLC peaks and molecular variables, equivalent carbon number (ECN) of the possible triglycerides, and chain length (CL) and number of double bounds (DB) of each of the fatty acids in the glycerol molecule.

The triglycerides presenting high random percentages were considered the principal molecular species in each HPLC peak. The main molecular species of rat liver triglycerides were palmitoyl-oleoyl-linoleoyl-glycerol, triolein, palmitoyl-dilinoleoyl-glycerol, dipalmitoyl-oleoyl-glycerol and oleoyl-dilinoleoyl-glycerol. The systematics predicted molecular species

with arachidonic acid, such as dioleoyl-arachidonoyl-glycerol, dipalmitoyl-arachidonoyl-glycerol, and oleoyl-linoleoyl-arachidonoyl-glycerol, and with docosahexaenoic acid, including dipalmitoyl-docosahexaenoyl-glycerol, palmitoyl-oleoyl-docosahexaenoyl-glycerol, and palmitoyl-linoleoyl-docosahexaenoyl-glycerol.

INTRODUCTION

In the last years, the progress in chromatographic techniques has enormously facilitated the analysis of triglycerides. Argentation-thin layer chormatography (TLC) has been used to separate triglycerides according to their degree of unsaturation, while reversed-phase-TLC separates them according to the carbon number.[1] Through capillary column gas chromatography (GC) it is possible to separate triglycerides according to both variables.[2,3] The separation is made by the total number of carbons, and within each triglyceride class with the same number of carbons the retention is higher in growing order of unsaturation degree. However, reversed-phase high performance liquid chromatography (RP-HPLC) is the most commonly employed chromatographic technique for separating mixtures of triglycerides. The mechanism for separating triglycerides in RP-HPLC includes chain length and degree of unsaturation of their constituent fatty acids.[4] Wada et al.[5] were the first to establish a parameter termed partition number (PN; PN=CN - 2 ND, where CN is the total number of carbons and ND is the total number of double bonds in fatty acids) for characterizing triglyceride molecules. Following their lead other workers have developed other parameters analogous to PN for molecularly discriminating between triglycerides with the same PN value, mainly to improve identification of chromatogram peaks.[6-8]

Most of researchers obtained good RP-HPLC separations of triglycerides from natural fats using octadecylsylane columns with the combination of a solvent, generally acetonitrile, and an organic modifier as mobile phase.[2] Although many different substances have been used as organic modifier, acetone is the most commonly employed, due to its effect in improving the selectivity of triglyceride mixtures.[9,10] One of the most challenging research areas in RP-HPLC of natural samples of triglycerides is the identification of the molecular species.

A significant number of researches use the theoretic prediction of triglycerides in the RP-HPLC peaks as a useful tool in the triglyceride identification process. The prediction provides a number of possible

triglycerides for each chromatographic peak. The more or less accuracy in the prediction will depend on the complexity of the fat and the mathematic equations employed.[7,11-13]

A low number of works have been found in the bibliography about the triglyceride composition of the liver. One of the main analytical difficulties is the triglyceride extraction, due to the high proportion of phospholipids and water present in the liver.[14] The most significative results were obtained by RP-HPLC with light-scattering detector, using as mobile phase acetonitrile with isopropanol or cloroform as organic modifier.[15,16] In these conditions, Huang et al.[15] reported 19 triglycerides in the rat liver being the major triglyceride molecular species palmitoyl-oleoyl-linoleoyl-glycerol, palmitoyl-dioleoyl-glycerol, palmitoyl-dilinoleoyl-glycerol, and oleoyl-dilinoleoyl- glycerol.

This study has been carried out in order to determine the rat liver triglycerides, proposing for this a highly resolutory analytical method and a theoretic prediction systematics for molecular species.

MATERIALS AND METHODS

Sample Preparation

Wistar type rats (Letica) weighing 250-300 g were used. They were sacrificed by decapitation and the liver was then obtained and preserved at -80°C until used.

Total liver lipids were extracted following the method described by Folch et al.[17] Different lipid fractions were separated by thin layer chromatography (TLC) on silica gel 60 plates (Kieselgel 60 F_{254}, Merck) using an elution system of hexane/diethylether/acetic acid (80:20:1,v/v/v) (Merck), according to the method of Ruiz-Gutiérrez et al.[18]

HPLC Analysis

The triglyceride fraction, vacuum-evaporated to dryness, at temperature below 30°C, was redissolved in n-hexane and passed through a filter having a pore size of 0.2 μm (Millipore). Injections of 10 μL-volume containing 60 mg of triglycerides were used for the HPLC analysis. The chromatographic system consisted of two pumps, model 422M and 422S (Kontron), a model 7161 injector (Rheodyne), two 20 cm-long stainless-steel columns connected in series

with an internal diameter of 4.6 mm containing a bonded-phase of Spherisorb ODS-2 (Phase Separations) with a particle size of 3 μm, and a light scattering detector model Sedex 45 (Sedere) provided with an automatic air compressor model Compact 106 (Cedime). The system was controlled by a computer through MT-450 Data System (Kontron), and connected to a matrix printer model LQ 570 (Epson). The columns were sumerged in a thermostatized water bath at 30°C. The mobile phase consisted of an initial elution gradient of 20 to 80% (v/v) of acetone in acetonitrile during 100 minutes. After this time, the percentage of acetone was raised to reach the 100% at minute 105, and held to the end of the analysis. The flow rate was 1.0 mL/min.

Quintupled runs of 10 μL of n-hexane solution containing 0.5 mg/mL of pure triglycerides (Sigma Grade, 99% pure) tritridecanoin, 1,2-dipalmitoyl-oleoyl-glycerol, trimiristin, 1,2-distearoyl-3-miristoyl-glycerol, triheptadecanoin, 1,2-distearoyl-3-palmitoyl-glycerol, 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol, 1,2-dioleoyl-3-palmitoyl-glycerol, 1,2-dipalmitoyl-3-miristoyl-glycerol, 1,2-dimiristoyl-3-palmitoyl-glycerol, tripentadecanoin, 1,3-distearoyl-2-oleoyl-glycerol, 1,2-dimiristoyl-3-lauroyl-glycerol, 1-miristoyl-2-oleoyl-3-palmitoyl-glycerol, tripalmitin, triolein, and 1,3-dipalmitoyl-2-linoleoyl-glycerol were analysed in order to calculate the equations used in the prediction of the triglyceride molecular species of rat liver.

All of the solvents, used both in dissolving sample triglycerides and in the mobile phase were HPLC grade (Normasolv, Scharlau). The dead volume (v_0) was calculated as the difference in column weight when saturated in methanol and when saturated in water.

GLC Analysis

Lipids were transmethylated and the resulting fatty acid methyl esters (FAME) analyzed by GLC as described by Ruiz-Gutierrez et al.[19] using a model 5890 series II gas chromatograph (Hewlett-Packard) equipped with a flame ionization detector (FID) and a capillary silica column Omegawax 320 (Sulpelco) of 50 m length, 0.32 mm internal diameter and 0.25 mm film thickness.

Calculation of Triglyceride Composition

Triglyceride composition of the rat liver by HPLC was predicted by means of relationships between the capacity factors (k') and molecular variables of the

pure triglycerides. The equivalent carbon number (ECN) was calculated according to the following equation:

$$ECN = CN - a_1'*DB - a_2'*NUFA \quad (1)$$

where CN is the total carbon number of the three fatty acids, and DB the total number of double bonds and NUFA the number of unsaturated fatty acids of the triglyceride molecule. The values of the constants a_1' and a_2' were calculated by multiple linear regression analysis of the experimental values of the dependent variable, log k', and the independent variables CN, ND, and NUFA for the pure triglycerides (log $k' = q' + b'$ CN + c' ND + d' NUFA), where a_1' is the quotient between the coefficients c' and b', and a_2' between the coefficients d' and b'.

Firstly, a simple linear regression analysis was applied to relate ECN with log k' of the pure triglycerides. According to the methodology proposed by Takahashi et al.[20] a multiple linear regression was secondly applied to relate chain length (CL) and number of double bounds (DB) of each of the three fatty acids of the triglyceride molecule to log k' of the pure triglycerides. The stereoespecific positions in the glycerol molecule were equivalent because HPLC analysis cannot separate positional isomers.[21] Finally, the random composition was used to establish the probability for the presence of the triglycerides in each HPLC peak when more than one molecular species were predicted.

RESULTS AND DISCUSSION

Until the present, ECN has always been defined as a variable depending on CN and ND.[20,22] However, other authors have provided experimental data in which triglycerides with the same ECN were separated by RP-HPLC according to NUFA,[23] showing lower retention times the triglycerides with higher NUFA. For this reason, in this study ECN was redefined as a dependent variable of NC, ND, and NUFA (equation (1)).

The experimental results obtained for constant a_1' were close to -2.0, as established by Wada et al.[5] for the effect of the total number of double bonds (ND) on the chromatographic retention. On the other hand, the experimental value obtained for constant a_2', and checked for different types of gradients, was aproximately ten times lower to a_1'. Following Wada et al. criteria,[5] fixed values were adopted for these constants (ECN= NC - 2.0 ND - 0.2 NUFA) checking experimentally that triglycerides eluted in growing order of ECN.

The simple linear regression analysis results between log k' and ECN for the pure triglycerides are shown in the equation (2)

$$ECN = -18.6190 + 46.7656 * \log k' \qquad s.e. = 0.6593 \qquad (2)$$

Fifteen fatty acids were identified by GLC analysis of the liver triglyceride fraction. Only the fatty acids with percentages equal or higher than 0.5 were used for the prediction. These fatty acids were oleic (29.5 %), linoleic (28.7 %), palmitic (23.5 %), palmitoleic (6.0 %), arachidonic (4.0 %), vaccenic (2.5 %), stearic (2.0 %), docosahexaenoic (1.5 %), linoleic (1.1 %), and miristic acid (0.5 %) acids. Making all the possible combinations between these fatty acids taking three by three, the total number of possible triglycerides in rat liver fat was 220. The range of ECN, calculated by the equation [2] and its standard error, provided the selection of the possible molecular species for each HPLC peak. According to the methodology proposed by Takahashi et al.[20] the equations used to make a new selection of triglycerides in each HPLC peak were the following:

$$CL_1 = -12.6145 - 0.3003*CL_2 - 0.8515*CL_3 + 2.0008*DB_1 + 1.2242*DB_2 + 1.6101*DB_3 + 32.8917*\log k' \quad s.e. = 0.6059 \qquad (3)$$

$$CL_2 = -10.5601 - 0.3336*CL_1 - 0.1830*CL_3 + 0.9847*DB_1 + 1.5240*DB_2 + .5732*DB_3 + 27.3474*\log k' \quad s.e. = 0.6392 \qquad (4)$$

$$CL_3 = -10.8486 - 0.7303*CL_1 - 0.1410*CL_2 + 1.3483*DB_1 + 1.0991*DB_2 + 1.6268*DB_3 + 24.6057*\log k' \quad s.e. = 0.5611 \qquad (5)$$

$$DB_1 = 2.8837 + 0.2033*CL_1 + 0.0899*CL_2 + 0.1597*CL_3 - 0.0880*DB_2 - 0.2803*DB_3 - 7.1007*\log k' \quad s.e. = 0.1931 \qquad (6)$$

$$DB_2 = 4.2992 + 0.2341*CL_1 + 0.2621*CL_2 + 0.2453*CL_3 - 0.1658*DB_1 - 0.4573\,DB_3 - 11.3311*\log k' \quad s.e. = 0.2651 \qquad (7)$$

$$DB_3 = 5.8440 + 0.4123*CL_1 + 0.1320*CL_2 + 0.4860*CL_3 - 0.7070*DB_1 - 0.6121\,DB_2 - 15.6067*\log k' \quad s.e. = 0.3067 \qquad (8)$$

where CL_i is the chain length and DB_i the number of double bonds of each of the three fatty acids of the triglyceride molecule. The equations corresponding to DB_1 and DB_2 were not used because the percentages of explained variance were minor to 85 %.

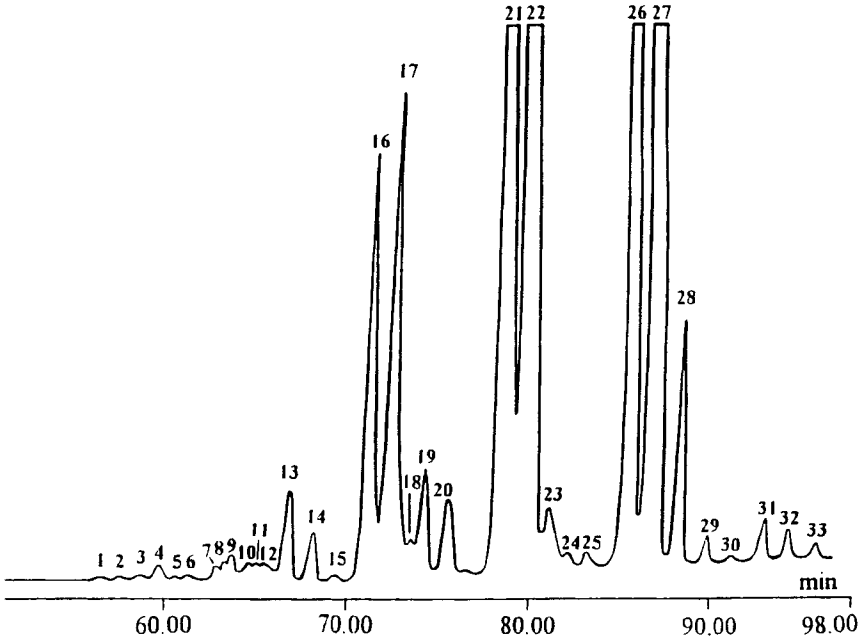

Figure 1. High-performance liquid chromatography analysis of rat liver triglycerides. Two columns (Spherisorb ODS-2 3 μm) connected in series with a non-linear elution gradient of 20-100 % to 80-100% (v/v) acetone in acetonitrile were developed at 30 °C with a flow of 1.0 mL/min. Sample volume was 10 μL containing 60 μg of triglycerides.

From the value of log k' of the HPLC peaks, the value of CL_1, CL_2, CL_3 and DB_3 was calculated for the triglycerides previously selected by equation (2); the value of the rest of the variables remaining constant (equations (3), (4), (5), and (6). This systematics allowed a new selection of triglycerides, assigning one triglyceride to one chromatographic peak when at least two of the above mentioned equations was in compliance. For the compliance of the equations an arbitrational arrangement of the fatty acids in the glycerol molecule was considered, being assigned in the positions 1, 2 and 3 in growing order of chain length and degree of unsaturation. On the other hand, the experimental values of log k' for the triglyceride standards, and the logic sequence of elution in growing order of ECN, were also taken into account for the final estimation of triglycerides to the individual chromatographic peaks.[22] The final results of the molecular species of rat liver triglycerides estimation process are shown in Table 1 which corresponds to the HPLC chromatogram depicted in Figure 1.

Table 1a

Results of the Application of the Estimation Systematics for the Rat Liver Triglycerides (TG) by HPLC

Peak	ECN	Estimated TG	% Random
1,2	38.9833, 39.0665	$C_{18:2}$-$C_{18:2}$-$C_{20:4}$	0.99
		$C_{18:1}$-$C_{18:2}$-$C_{22:6}$	0.76
		$C_{16:1}$-$C_{18:2}$-$C_{20:4}$	0.41
		$C_{18:2}$-$C_{18:2}$-$C_{18:3}$	0.27
		$C_{18:1}$-$C_{20:4}$-$C_{20:4}$	0.14
		$C_{18:1}$-$C_{18:3}$-$C_{20:4}$	0.08
		$C_{16:1}$-$C_{16:1}$-$C_{20:4}$	0.04
		$C_{16:1}$-$C_{16:1}$-$C_{18:3}$	0.01
		$C_{18:1}$-$C_{18:3}$-$C_{18:3}$	0.01
3,4	39.5124, 39.8918	$C_{16:0}$-$C_{18:2}$-$C_{22:6}$	0.61
		$C_{16:0}$-$C_{16:1}$-$C_{22:6}$	0.13
		$C_{16:0}$-$C_{20:4}$-$C_{20:4}$	0.06
		$C_{16:0}$-$C_{18:3}$-$C_{20:4}$	0.01
		$C_{16:0}$-$C_{18:3}$-$C_{18:3}$	0.01
		$C_{14:0}$-$C_{16:1}$-$C_{20:4}$	<0.01
		$C_{18:0}$-$C_{20:4}$-$C_{22:6}$	<0.01
		$C_{14:0}$-$C_{16:1}$-$C_{18:3}$	<0.01
		$C_{18:0}$-$C_{18:3}$-$C_{22:6}$	<0.01
5	40.2978	$C_{14:0}$-$C_{14:0}$-$C_{20:4}$	<0.01
		$C_{14:0}$-$C_{14:0}$-$C_{18:3}$	<0.01
6	40.4757	No TG estimated	-
7,8,9	40.9353, 41.0432,41.0969	$C_{18:2}$-$C_{18:2}$-$C_{18:2}$	2.36
		$C_{18:1}$-$C_{18:2}$-$C_{20:4}$	2.03
		$C_{16:1}$-$C_{18:2}$-$C_{18:2}$	1.48
		$C_{18:1}$-$C_{18:2}$-$C_{18:3}$	0.56
		$C_{16:1}$-$C_{18:1}$-$C_{20:4}$	0.42
		$C_{18:1}$-$C_{18:1}$-$C_{22:6}$	0.39
		$C_{16:1}$-$C_{16:1}$-$C_{18:2}$	0.31
		$C_{16:1}$-$C_{16:1}$-$C_{16:1}$	0.02

ECN= Equivalent Carbon Number. Peak Numbers come from Figure 1.

Table 1b

Results of the Application of the Estimation Systematics for the Rat Liver Triglycerides (TG) by HPLC

Peak	ECN	Estimated TG	% Random
10, 11, 12	41.4376, 41.6894, 41.8452	$C_{16:0}$-$C_{18:2}$-$C_{20:4}$	1.62
		$C_{16:0}$-$C_{18:1}$-$C_{22:6}$	0.62
		$C_{16:0}$-$C_{18:2}$-$C_{18:3}$	0.45
		$C_{16:0}$-$C_{16:1}$-$C_{20:4}$	0.34
		$C_{14:0}$-$C_{16:1}$-$C_{18:2}$	0.05
		$C_{18:0}$-$C_{18:2}$-$C_{22:6}$	0.05
		$C_{18:0}$-$C_{20:4}$-$C_{20:4}$	0.01
		$C_{14:0}$-$C_{16:1}$-$C_{16:1}$	0.01
		$C_{18:0}$-$C_{18:3}$-$C_{20:4}$	0.01
		$C_{18:0}$-$C_{18:3}$-$C_{18:3}$	<0.01
13	42.1432	$C_{16:0}$-$C_{16:0}$-$C_{22:6}$	0.25
		$C_{14:0}$-$C_{16:0}$-$C_{20:4}$	0.03
		$C_{14:0}$-$C_{14:0}$-$C_{18:2}$	<0.01
		$C_{14:0}$-$C_{14:0}$-$C_{16:1}$	<0.01
14	42.5768	$C_{14:0}$-$C_{14:0}$-$C_{14:0}$	<0.01
15	43.0015	No TG estimated	-
16	43.5321	$C_{18:1}$-$C_{18:2}$-$C_{18:2}$	7.29
		$C_{18:1}$-$C_{18:1}$-$C_{20:4}$	1.04
		$C_{16:1}$-$C_{16:1}$-$C_{18:1}$	0.32
17	43.9094	$C_{16:0}$-$C_{18:2}$-$C_{18:2}$	5.81
		$C_{16:0}$-$C_{16:1}$-$C_{18:2}$	2.43
		$C_{16:0}$-$C_{16:1}$-$C_{16:1}$	0.25
		$C_{18:0}$-$C_{18:2}$-$C_{20:4}$	0.14
		$C_{14:0}$-$C_{16:1}$-$C_{18:1}$	0.05
		$C_{18:0}$-$C_{18:2}$-$C_{18:3}$	0.04
18, 19	44.0676, 44.4535	$C_{16:0}$-$C_{16:0}$-$C_{20:4}$	0.66
		$C_{14:0}$-$C_{16:0}$-$C_{18:2}$	0.20
		$C_{16:0}$-$C_{16:0}$-$C_{18:3}$	0.18
20	44.6707	$C_{14:0}$-$C_{14:0}$-$C_{16:0}$	<0.01
21	45.6748	$C_{18:1}$-$C_{18:1}$-$C_{18:2}$	7.49
		$C_{16:1}$-$C_{18:1}$-$C_{18:1}$	1.57

ECN= Equivalent Carbon Number. Peak Numbers come from Figure 1.

Table 1c

Results of the Application of the Estimation Systematics for the Rat Liver Triglycerides (TG) by HPLC

Peak	ECN	Estimated TG	% Random
22	46.0034	$C_{16:0}$-$C_{18:1}$-$C_{18:2}$	11.94
		$C_{16:0}$-$C_{16:1}$-$C_{18:1}$	2.50
		$C_{18:0}$-$C_{18:2}$-$C_{18:2}$	0.49
		$C_{18:0}$-$C_{18:1}$-$C_{20:4}$	0.14
		$C_{18:0}$-$C_{18:1}$-$C_{18:3}$	0.04
23	46.3793	$C_{16:0}$-$C_{16:0}$-$C_{18:2}$	4.75
		$C_{14:0}$-$C_{16:0}$-$C_{18:1}$	0.21
		$C_{14:0}$-$C_{16:1}$-$C_{18:0}$	<0.01
24	46.5934	$C_{14:0}$-$C_{16:0}$-$C_{16:0}$	0.08
25	46.9344	No TG estimated	-
26	47.5140	$C_{18:1}$-$C_{18:1}$-$C_{18:1}$	2.57
27	47.8632	$C_{16:0}$-$C_{18:1}$-$C_{18:1}$	6.14
		$C_{18:0}$-$C_{18:1}$-$C_{18:2}$	1.02
28	48.1988	$C_{16:0}$-$C_{16:0}$-$C_{18:1}$	4.89
29	48.5886	$C_{16:0}$-$C_{16:0}$-$C_{16:0}$	1.30
		$C_{14:0}$-$C_{16:0}$-$C_{18:0}$	0.01
30	48.9418	$C_{18:0}$-$C_{18:1}$-$C_{18:1}$	0.52
31	49.3408	$C_{18:0}$-$C_{18:0}$-$C_{18:2}$	0.03
32	49.6494	$C_{16:0}$-$C_{18:0}$-$C_{18:1}$	0.83
33	50.0095	$C_{14:0}$-$C_{18:0}$-$C_{18:0}$	<0.01

ECN= Equivalent Carbon Number. Peak Numbers come from Figure 1.

In the cases in which the prediction systematics assigned more than one triglyceride to only one HPLC peak the random composition was used to establish the highest probability for the existence of the triglycerides in the peaks.

So, although it has been reported that significant differences do occur between experimental quantitative composition and theoretical random composition for the natural fat triglycerides,[24,25] the random composition may, from a qualitative standpoint, furnish extremely useful information to complete the triglyceride molecular prediction.

It must be also emphasized that the values for the variables CL_i and DB_i for the positional isomers and the *cis-trans* isomers are exactly the same, and according to the GLC results, the estimated triglycerides containing $C_{18:1}$ could be constituted by oleic acid (18:1, n-9) or vaccenic acid (18:1, n-7). However, if the percentages in which these fatty acids are found in the rat liver triglycerides are compared, it should be considered that the main of these would be oleic acid, in a total ratio of 11.8 times superior to vaccenic acid.

Taking into account the results of Table 1 and the quantitative significance of the HPLC peaks of the chromatogram (Figure 1) it could be asserted that the main triglycerides in the rat liver were palmitoyl-oleoyl-linoleoyl-glycerol, dioleoyl-linoleoyl-glycerol, palmitoyl-dioleoyl-glycerol, triolein, palmitoyl-dilinoleoyl-glycerol, dipalmitoyl-oleoyl-glycerol, and oleoyl-dilinoleoyl-glycerol.

These molecular species were constituted by the three major fatty acids obtained through the fatty acid analysis of the total triglycerids (oleic, linoeic, and palmitic acids).

Three main triglycerides containing arachidonic acid (20:4, n-6) were predicted: dioleoyl-arachidonoyl-glycerol,dipalmitoyl-arachidonoyl-glycerol, and oleoyl-linoleoyl-arachidonoyl-glycerol; and other three main triglycerides containing docosahexaenoic acid (22:6, n-3): dipalmitoyl-docosahexaenoyl-glycerol, palmitoyl-oleoyl-docosahexaenoyl-glycerol, and palmitoyl-linoleoyl-docosahexaenoyl-glycerol.

In both cases arachidonic and docosahexaenoic acids were combined with oleic, linoleic, or palmitic acids to form the triglyceride molecules.

Due to the scarce number of works about the triglyceride composition of rat liver, the comparison of these results with those of other authors is very difficult. Nevertheless, the triglycerides predicted in this work are in concordance with the triglyceride composition reported by other authors.[15,26-28]

Chen et al.[16] found a triglyceride containing docosahexaenoic acid, probably palmitoyl-linoleoyl-docosahexanoyl-glycerol, but they did not make a strict identification process; only the fatty acid composition of each chromatographic peak was determined.

It may be concluded that the proposed chromatographic method for the analysis and prediction of the rat liver triglyceride molecular species has turned out to be effective and useful for the knowledge of the triglycerides.

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REFERENCES

1. H. P. Kaufmann, Z. Makus, Fette Seifen Anstrichm., **63**, 125 (1961).

2. J. J. Myher, A. Kuksis, Can. J. Cell. Biol., **62**, 352-362 (1984).

3. N. Frega, F. Bocci, G. Lercker. Ital. J. Food Sci., **4**, 257-265 (1990).

4. V. Ruiz-Gutiérrez, L. J. R. Barrón, J. Chromatogr. B., **671**, 133-168 (1995).

5. S. Wada, C. Koizumi, J. Nonaka, Yukagaku, **26**, 95-99 (1977).

6. A. H. El-Hamdy, E. G. Perkins, J. Am. Oil Chem. Soc., **58**, 867-872 (1981).

7. J. P. Goiffon, C. Reminiac, D. Fenon. Rev. Fr. Corps Gras, **28**, 199-206 (1981).

8. M. T. G. Hierro, A. I. Nájera, G. Santa-María., Rev. Esp. Cienc. Tecnol. Aliment., **32**, 635-651 (1992).

9. L. J. R. Barrón, G. Santa-María, Chromatogr., **23**, 209-214 (1987).

10. M. J. Wojtusik, P. R. Brown, J. G. Turcotte, Biochromatogr., **3**, 76-83 (1988).

11. K. Takahashi, T. Hirano, K. Zama, J. Am. Oil Chem. Soc., **63**, 1543-1546 (1986).

12. L. J. R. Barrón, M. V. Celaa, G. Santa-María, N. Corzo, Chromatogr., **25**, 609-612 (1988).

13. P. Ruiz-Sala, M. T. G. Hierrro, I. Martínez-Castro, G. Santa-María, J. Am. Oil Chem. Soc., **73**, 283-293 (1996).

14. G. A. Rao, D. E. Rieley, E. C. Larkin, Lipids, **20**, 531-535 (1985).

15. Y. S. Huang, X. Lin, R. S. Smith, P. R. Redden, G. W. Ells, D. F. Horrobin, Med. Sci. Res., **20**, 691-692 (1992).

16. Z. Y. Chen, S. C. Cunnane, J. Nutr. Biochem., **4**, 421-425 (1993).

17. J. Folch, M. Less, G. H. Sloan Stanley, J. Biol. Chem., **33**, 497-509 (1957).

18. V. Ruiz-Gutiérrez, J. L. Prada, F. Pérez-Jiménez, J. Chromatogr. B, **622**, 117-124 (1993).

19. V. Ruiz-Gutiérrez, A. Cert, J. J. Ríos, J. Chromatogr. B, **575**, 1-6 (1992).

20. K. Takahashi, Hirano, M. Egi, K. Zama, J. Am. Oil Chem. Soc., **62**, 1489-1492 (1985).

21. J. L. Perrin, A. Prévot, Rev. Fr. Corps Gras, **11**, 437-445 (1986).

22. B. Herslöf, O. Pdlaha, B. Töregard, J. Am. Oil Chem. Soc., **56**, 864 (1979).

23. M. T. G. Hierro, M. C. Tomás, F. Fernández-Marín, G. Santa-María, J. Chromatogr., **607**, 329-338 (1992).

24. M. Bugaut, J. Bezard, Oleagineaux, **34**, 77-87 (1979).

25. J. Gresti, M. Bugaut, C. Maniongui, J. Bezard, J. Diary. Sci., **76(7)**, 1850-1869.

26. L. Y. Yang, A. Kuksis, J. J. Myher, G. Steiner, J. Lipid Res., **36**, 125-136 (1995).

27. Z. Y. Chen, J. Yang, C. R. Menard, S. C. Cunnane, Lipids, **27**, 21-24 (1992).

28. Z. Y. Chen, S. C. Cunnane, Am. J. Physiol., **263**, R233-R239(1992).

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