A New Concept for Determining Triglyceride Composition of Fats and Oils by Liquid Chromatography

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ABSTRACT

The matrix concept was used to characterize the chromatographic rules in the elution of molecular species of triglyceride. To prove the hypothesis experimentally, cacao butter, palm oil, linseed oil, olive oil, rapeseed oil and triglyceride of "Ogonori" (*Gracilaria verrucosa*) were examined. A matrix model was suggested to help determine the individual molecular species of naturally occurring triglycerides.

INTRODUCTION

Prior research has concentrated on the analysis of molecular species of glycerides. Direct analysis based on chromatographic separation by gas chromatography or liquid chromatography has not yet been achieved, although partial separation and analysis by either method is readily accomplished (1,2).

Many workers have reported (3-9) that the elution of glyceride molecular species on reversed-phase chromatography is controlled by a partition number (PN) that is defined as PN=C-2D where C is the total acyl carbon number and D is the total double bonds in the molecule. This PN is restricted only under certain defined analytical conditions. Practically, during this study, many cases were observed where the relative retention time (t_R) of PN deviates from expected values, e.g., during high performance liquid chromatography (HPLC) analysis when LiChrosorb RP-18 was used as column packing. The latest work done by Dong et al. (10) gives an exceedingly good separation on triglyceride analysis. This might also suggest that a more detailed concept might be introduced in determining the chromatographic rules for glyceride analysis.

In the previous paper (11), we discussed the 2 rules in the elution of triglyceride on HPLC:

$$C = p_1 \cdot \log(t_R) + q_1 \qquad C = \begin{vmatrix} x & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix} \qquad [1]$$

$$D = p_2 \cdot \log(t_R) + q_2 \qquad D = \begin{vmatrix} c_1 & y \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix} \qquad [2]$$

where c_1, c_2, c_3 and d_1, d_2, d_3 are acyl carbon numbers and the number of double bonds in each acyl group line up in a similar way. X and y are variables of acyl carbon numbers and number of double bonds, respectively; p_1 and p_2 are the slopes and q_1 and q_2 are the intercepts on the ordinates; t_R is relative retention time.

In the present paper, availability of rules 1 and 2 are confirmed by analyzing several kinds of triglycerides (TG).

EXPERIMENTAL

Preparation of Triglyceride

Linseed oil and olive oil were purchased from Wako Pure Chemical Industries, Ltd., Osaka. Rapeseed oil was obtained from a commercial source. Cacao butter was supplied by Yunokawa Seiyaku Co. Ltd., Hakodate, Hokkaido, Japan and palm oil was kindly supplied by Tsukishima Food Industry Co., Ltd., Tokyo, Japan. Ogonori (*Gracilaria verrucosa)* was collected at the shore of Taisei-chō, Hokkaido, Japan. Total lipids were extracted from Ogonori using chloroform/methanol (1:2, v/v) with the use of an ultra-turrax for comminution.

Triglycerides from these oils were purified by preparative thin layer chromatography (TLC) using n-hexane/diethyl ether (4:1, v/v) as the developing solvent.

HPLC Fractionation of the Molecular Species of Triglyceride

The purified triglycerides were filtered through a 0.45 μ type FP-45 Fluoropore filter (Sumitomo Electric Industry, Ltd., Osaka, Japan) and subjected to HPLC. Separation of triglycerides by HPLC has been achieved on LiChrosorb RP-18 (Merck, West Germany) twin 8 x 250 mm columns. These columns were connected in series. The instruments used consisted of a Hitachi 638-50 Liquid Chromatograph (Hitachi Ltd., Tokyo) equipped with a Shodex SE-11 RI detector (Showa Denko Ltd., Tokyo, Japan). The eluting solvent used was acetone/acetonitrile (3:1, v/v). Triglycerides were solubilized in chloroform at 5 μg/25 μL and applied to the column under room temperature (20-22 C). The flow rate was 1.5 mL/min.

Identification of Molecular Species of Each Peak on HPLC

Peaks on HPLC chromatograms were numbered in sequence of elution. The fatty acid composition and total acyl carbon number of each collected predominant peak was analyzed by gas chromatography (GC) as previously reported (12).

RESULTS

Figure 1-3 show chromatograms of triglyceride on HPLC. The acyl combination of each collected predominant peak was determined by fatty acid analysis and total acyl carbon number analysis as shown in Table I. This table shows the results on linseed oil in Figure 1 as an example. Peak number 1 in Figure 1 is triglyceride composed of 18:3 alone, i.e., (18:3, 18:3, 18:3) because over 98% of this fraction was 18:3; in addition, the total acyl carbon number of this peak was mostly 54. Peak number 2 is considered to be the combination of 1 mol of 18:2 and two mol of 18:3, (18:2, 18:3, 18:3). This was supported by the data of total acyl carbon number of this fraction. Peak number 3 is mainly (18:3, 18:2, 18:2) for the same reasons as in peak number 2. Peak number 4 is (18:1, 18:3, 18:3) with 4-10% contaminants because 9.7% of 18:2 was detected as overlaps from the former peak. Peak number 5 has at least 2 contaminants, i.e., 18:1 and 18:2. This peak is concluded to be (16:0, 18:3, 18:3) from the data of total acyl carbon number. All other peaks were identified in the same manner.

The relative retention times of all peaks were determined by dividing the retention time of each peak by the retention time of triolein. When the amount of triolein was very small and the peaks overlapped considerably, purified triolein was added as an internal standard. Predominant or reliable peaks were selected to plot the t_R of individual triglycerides on semilogarithmic graph paper. Results are shown in Figure 4. As is clear from this figure, the sequence in elution might be controlled by a fixed correlation, that is, a matrix relation. If we express this by empirical equation, the 2 equations described previously can be obtained (equations 1 and 2).

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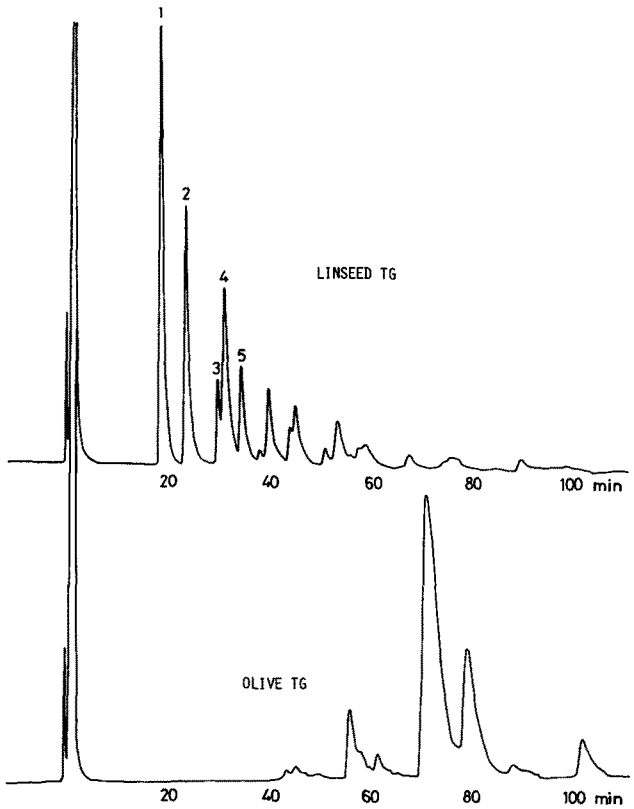

FIG. 1. HPLC chromatograms of linseed oil and olive oil triglycerides.

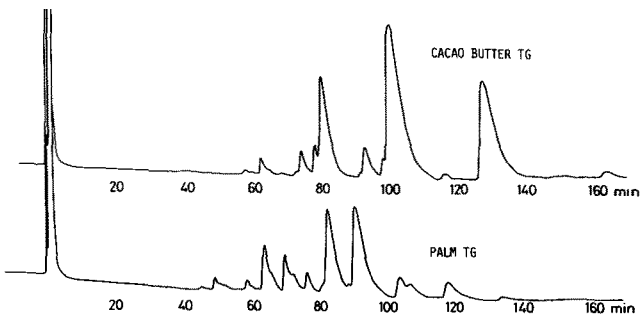

FIG. 2. HPLC chromatograms of cacao butter and palm oil triglycerides.

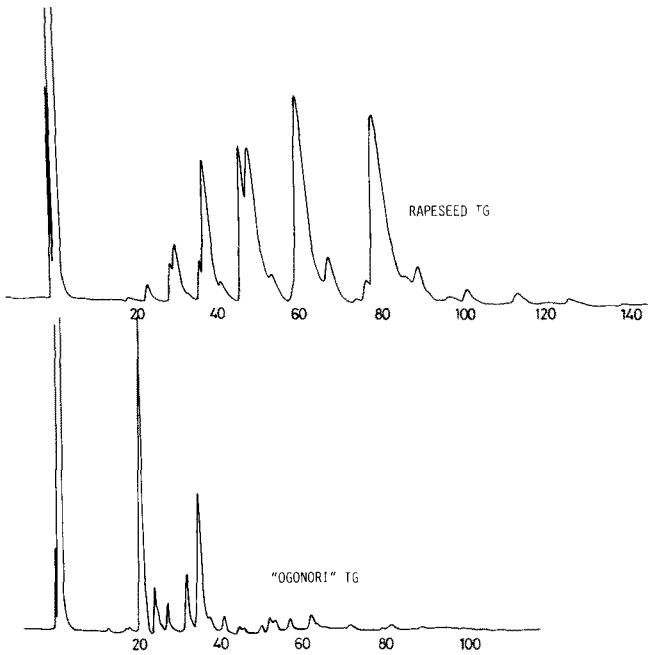

FIG. 3. HPLC chromatograms of rapeseed oil and Ogonori triglycerides.

DISCUSSION

If we take notice of the variables in equation 1 or 2, each molecular species that has the same acyl group combination in the other 2 positions would lie on an exact oblique straight line on the semilogarithmic graph. These lines would become a set defined by the third row, second column matrix (see Fig. 4). The theory of Martin (13) is closely related to this hypothesis. His theory was as follows. If we consider the partition coefficients α_A and α_B of 2 substances, A and B, which differ because B contains, in addition to those groups contained in A, a group X, we have,

$$\ln \alpha_A = \Delta\mu_A/RT,\ \ln \alpha_B = (\Delta\mu_A/RT) + (\Delta\mu_X/RT),$$

$$\ln (\alpha_B/\alpha_A) = \Delta\mu_X/RT = \ln \alpha_B - \ln \alpha_A$$

(μ: Chemical potential, that is, $dG/dn = \mu \propto t_R$; G: free energy; n: mol of the substance; R: gas constant; T: absolute temperature).

TABLE I

Determination of Triglyceride Molecular Species[a] (Percentage Per Peak)

	Peak number				
	1	2	3	4	5
Fatty acid					
16:0					29.6
18:1				28.9	3.7
18:2		33.4	63.2	9.7	1.1
18:3	98.0<	66.6	36.8	61.4	65.5
Carbon number[b]					
52					98.0<[c]
54	98.4[c]	98.3[c]	96.6[c]	96.8[c]	
Molecular species	18:3 18:3 18:3	18:3 18:3 18:2	18:3 18:2 18:2	18:3 18:3 18:1	16:0 18:3 18:3

[a]Example of linseed oil.
[b]Total acyl carbon number.
[c]Percentage by weight.

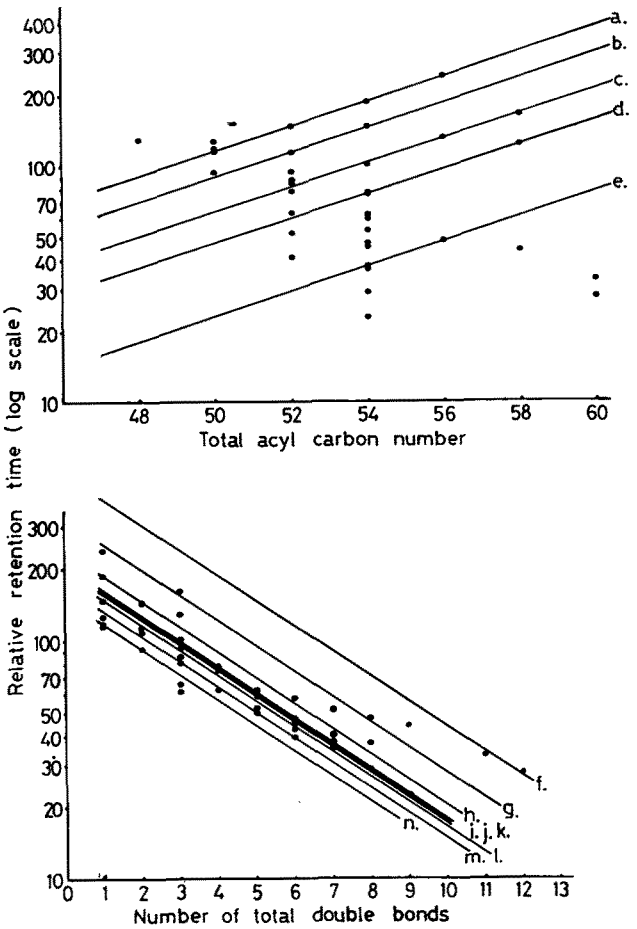

FIG. 4. Relationship between relative retention time and total acyl carbon number and relation between relative retention time and total double bonds on HPLC of triglycerides from natural sources.

a. $\begin{vmatrix}18 & 0\\18 & 1\\x & 0\end{vmatrix}$, b. $\begin{vmatrix}18 & 1\\18 & 1\\x & 0\end{vmatrix}$, c. $\begin{vmatrix}18 & 1\\18 & 1\\x & 1\end{vmatrix}$, d. $\begin{vmatrix}18 & 1\\18 & 2\\x & 1\end{vmatrix}$, e. $\begin{vmatrix}x & 0\\20 & 4\\20 & 4\end{vmatrix}$,

f. $\begin{vmatrix}20 & y\\20 & 4\\20 & 4\end{vmatrix}$, g. $\begin{vmatrix}18 & y\\18 & 1\\22 & 1\end{vmatrix}$, h. $\begin{vmatrix}18 & 0\\18 & 0\\18 & y\end{vmatrix}$, i. $\begin{vmatrix}18 & 1\\18 & 1\\18 & y\end{vmatrix}$, j. $\begin{vmatrix}18 & 2\\18 & 2\\18 & y\end{vmatrix}$,

k. $\begin{vmatrix}18 & 3\\18 & 3\\18 & y\end{vmatrix}$, l. $\begin{vmatrix}16 & 0\\18 & 0\\18 & y\end{vmatrix}$, m. $\begin{vmatrix}16 & 0\\18 & 2\\18 & y\end{vmatrix}$, n. $\begin{vmatrix}16 & 0\\16 & 0\\18 & y\end{vmatrix}$.

(X and y are variables of acyl carbon number and number of double bonds, respectively. For example, x can take 16, 18, 20, 22. . . . and y can take 1, 2, 3, 4, 5. . . . . ., etc.)

Thus, the addition of a group X, changes the partition coefficient by a given factor depending on the nature of the group, and on the pair of phases employed, but not on the rest of the molecule. In our hypothesis, we can substitute substance A as $\begin{vmatrix}c_1 & d_1\\c_2 & d_2\\c_3 & d_3\end{vmatrix}$, and substance B as $\begin{vmatrix}c_1+X & d_1\\c_2 & d_2\\c_3 & d_3\end{vmatrix}$ in equation 1. Then X will become a so-called functional group and this X is actually proportional to the increase in chemical potential by the increase of CH_2 group (or units) in the molecule. Or we can also substitute substance A as $\begin{vmatrix}c_1 & d_1\\c_2 & d_2\\c_3 & d_3\end{vmatrix}$, and substance B as $\begin{vmatrix}c_1 & d_1+Y\\c_2 & d_2\\c_3 & d_3\end{vmatrix}$ in equation 2. Then Y will also become a functional group and Y is actually proportional to the decrease in chemical potential by the decrease of 2H units in the molecule because μ is proportional to t_R at the same time. Although a limitation

TABLE II

Relationship Between Relative Retention Time and Molecular Species of Triglyceride from Natural Sources

Rapeseed oil		Linseed oil		Cacao butter		Palm oil		Ogonori		Olive oil	
RRT[a]	Molecular species	RRT[a]	Molecular species	RRT[a]	Molecular species	RRT[a]	Molecular species	RRT[a]	Molecular species	RRT[a]	Molecular species
28.6	(18:3) × 2(18:2)	22.5	(18:3) × 3	91.2	(16:0) × 2(18:2)	62.3	(18:2) × 2(16:0)	27.8	(20:4) × 3	125.4	(16:0) × 2(18:1)
35.9	(18:2) × 2(18:3)	28.6	(18:3) × 2(18:2)	117.5	(16:0) × 2(18:1)	74.7	(18:1) × 2(18:2)	32.8	(20:4) × 2(20:3)	144.8	(18:1) × 2(18:0)
37.5	(18:3) × 2(18:1)	35.5	(18:2) × 2(18:3)	136.2	(18:1) × 2(18:0)	83.6	(16:0) (18:1) (18:2)	37.0	(20:4) × 2(14:0)		
45.0	(18:2) × 3	36.8	(18:3) × 2(18:1)	143.7	(18:0) × 2(18:2)	92.1	(16:0) × 2(18:2)	43.8	(20:4) × 2(18:1)		
46.8	(18:1) (18:2) (18:3)	40.1	(18:3) × 2(16:0)	146.8	(16:0) (18:0) (18:1)	128.6	(16:0) × 3	47.8	(20:4) × 2(16:0)		
58.4	(18:2) × 2(18:1)	44.4	(18:2) × 3	184.8	(18:0) × 2(18:1)						
61.0	(18:1) × 2(18:3)	46.5	(18:1) (18:2) (18:3)	238.5	(18:0) (18:1) (20:0)						
76.3	(18:1) × 2(18:2)	51.0	(16:0) (18:2) (18:3)								
85.5	(16:0) (18:1) (18:2)	58.4	(18:2) × 2(18:1)								
100.0	(18:1) × 3	61.0	(18:1) × 2(18:3)								
112.4	(18:1) × 2(16:0)	76.6	(16:0) (18:1) (18:3)								
122.4	(18:1) (18:2) (22:1)	92.6	(16:0) (18:0) (18:3)								
128.3	(18:1) × 2(20:1)	100.0	(18:1) × 3								
143.9	(18:1) × 2(18:0)										
160.0	(18:1) × 2(22:1)										
162.8	(18:1) × (isomer)										

[a]RRT: relative retention time when (18:1) × 3 is used as the reference peak.

presently exists in the separation of individual molecular species, these chromatographic rules might be invariant until a complete separation of the individual molecular species can be accomplished. Then, a matrix model would be the most convenient way to distinguish the positional isomers.

ACKNOWLEDGMENT

M. Hatano and K. Takama provided considerable assistance. H. Ebina and M. Egi contributed experimental skills to the program.

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[Received October 26, 1983]

Adiabatic Reactor for Simulating Storage-Damaged Soybeans

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ABSTRACT

An adiabatic reactor was constructed to simulate conditions that lead to storage-damaged soybeans. The heart of the system is an electronic temperature controller that causes the reactor temperature to closely follow intrinsic heating of the soybeans. Using moisture concentrations between 17% and 20%, we observed intrinsic heating ranging from 47 C to 52 C after 3-13 weeks. Quality of the extracted oil was determined by analyses for triglyceride (TG) composition, total phosphorus (P), peroxide value and free fatty acid (FFA). Higher FFA and lower P and linolenic acid concentrations in the damaged beans compared with undamaged beans agree with the literature and indicate reliability of the adiabatic reactor for further studies, e.g., the effect of enzymatic activity on the quality of resulting soybean oil.

INTRODUCTION

Oilseeds and cereals are subject to damage by pests, weather, harvesting, handling and storing (1-7). Especially after extreme wet harvest and storage conditions, grain can be affected adversely (8). Breakdown of monitoring and control equipment also can cause grain to be damaged, even during ideal storage conditions. Damage to soybeans has a deleterious effect on the extracted oil (9-11), which affects its processing requirements (12,13). The primary purpose for designing an adiabatic reactor was to better understand storage damage and its effects on grain and extracted oil. Normally, the site of maximum storage damage is the center of large volumes of grain. Adiabatic conditions prevail because the central location is the most thermally insulated. An adiabatic system loses very little, if any, heat generated, and an exothermic reaction, even though extremely slow, can cause a positive thermal feedback resulting in accelerated spoilage or even fire. Spoilage can be simulated in an adiabatic reactor by placing moist grain in an environment that favors the activity of microorganisms with accompanying increase in temperature (14-16). This paper presents a detailed description of the apparatus and data demonstrating its satisfactory operation.

[1]Presented at the Great Lakes Regional ACS Meeting, Normal, IL, June 1982.

APPARATUS AND PROCEDURE

The adiabatic reactor, Figure 1, was designed to incorporate a 500 mL Dewar flask, which holds ca. 165 g of soybeans. Holes were drilled in the flask skirt-base to permit air circulation. Aluminum foil and a large rubber stopper were used for a seal. A squirrel-cage type fan blower circulated the temperature-controlled air within the box containing the Dewar. The motor for driving the fan was mounted ca. 1 ft from the top of the box to minimize the effect of motor heat. A flexible electric heating cord, 140 watts, 6 ft long for 115 volts AC was used as the heating element (Cole-Parmer Instr. Co., Chicago, IL). The complete box, except for the top panel, was placed in a large plastic sack for additional heat and humidity retention. Other details of the reactor are shown in the diagram.

The heart of the system is an in-house constructed temperature controller, which is shown schematically in Figure 2. Most components are mounted on a 3″ × 5″ printed circuit board, which is positioned directly on top of the commercial power supply (Polytron Devics Inc., Paterson, NJ). The sensors used are 2 type J thermocouples wired

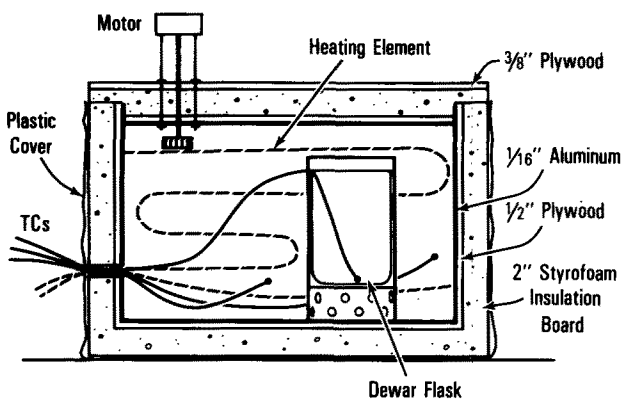

FIG. 1. Diagram of adiabatic reactor. TC—thermocouples and thermistors. Outside dimensions—18 in (length) × 12 in. (width) × 18 in. (height). Grain placed in Dewar flask. See text for details.