possible to detect minor contamination of non-polar material in the polar fraction. The FID detection limit of polar material will be determined by studying the response factors as a function of the quantity of sample spotted on the rods.

Ten used frying oil samples were studied using the standardized column chromatographic method and Sep-Pak cartridges. The non-polar fraction was eluted from the Sep-Pak cartridges with 20 ml of a mixture of PE/Et_2O (92:8). These analytical conditions were selected considering the results reported in Table 1. The total recovery ranged from 93.2 to 97.8% for the column chromatographic method and 89.3 to 99.9% for the Sep-Pak cartridges (Table 2). The plot of the amount of the polar fraction obtained with Sep-Pak cartridges (y) versus the amount obtained by column chromatography (x) gave a linear regression, $y = 0.86x + 1.35$ with a correlation coefficient of 0.996. This indicates that the results obtained using the Sep-Pak cartridges are slightly lower than those from column chromatography.

There is a good correlation between the standardized DGF, IUPAC, AOAC method and fractionation using Sep-Pak cartridges. This method, which does not require either large quantities of solvent or time to prepare the column, could be a powerful tool for fast determination of the state of degradation of a commercial frying fat.

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Supplementary Consideration of the Triglyceride Matrix Model on Reverse Phase High Performance Liquid Chromatography

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The matrix model that has been expressed as linear relationship between the logarithm of the relative retention time of a molecular species of a triglyceride versus total acyl carbon number or total double bonds when only one acyl group differs in carbon number or number of double bonds was reviewed. A similar linear relationship was observed when the fatty acid residues were substituted in the triglyceride molecule. This relationship was demonstrated by introducing the theory of partition chromatography presented by A.J.P. Martin.

The empirically determined correlation graph (Fig. 1), the matrix model of triglyceride (TG) on high performance liquid chromatography (HPLC) presented previously (1,2), was reviewed because of the following reasons: Though (18:1, 18:1, 18:1)[1] has the same equivalent carbon number (ECN) as (16:0, 18:1, 18:1), the former elutes earlier than the latter, as can be observed in the chromatograms of Kuksis et al. (3) and of Pauls (4). Or, though (16:0, 18:1, 18:1) has the same ECN as (16:0, 16:0, 18:1), the former elutes earlier than the latter, as can be observed in the chromatograms of many others (5-12). This phenomenon may be attributed to the differences in chemical potential between 16:0 and 18:1 residues in the TG molecule. If the difference in chemical potential between these two fatty acid residues is expressed as $\Delta\mu_x$, $\Delta\mu_x$ is considered to be added every time the 16:0 residue substitutes for 18:1 in the TG molecule. Therefore, the linear relationship shown in Figure 2 should hold. $\Delta\mu_x$ is proportional to the logarithm of the relative retention time (RRT) because the ratio of the partition coefficients (α) of the two homologous series exactly denote RRT, and according to the theory of Martin (13), $\log \alpha = \Delta\mu_x / R \cdot T$ should hold where R is the gas constant; T is the absolute temperature ($1/R \cdot T$ can be considered constant in most chromatographic conditions). So, a linear relationship should also hold between the increase in log (RRT) and the number of substitutions of the 16:0 residue

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[1](18:1, 18:1, 18:1) means the same as (18:1 | 18:1 | 18:1, stacked vertically), but the binding position of the acyl group is not discerned in this study.

substituted into 18:1 in the TG molecule. This correlation was ascertained by reproducing the experiment presented in the previous paper (1,2) using Figure 1.

EXPERIMENTAL

Molecular species of TG were prepared from the sources shown in Table 1 by collecting the corresponding peaks of the molecular species using HPLC. Though the molecular species of the collected peaks could be predicted by referring to the previous data (1,2), portions of the fractions were monitored by inference of results obtained by gas chromatography in order to ascertain the collected molecular species.

The operating conditions of HPLC were the same as previously reported (1,2) and are shown in Table 2.

The collected molecular species were combined and

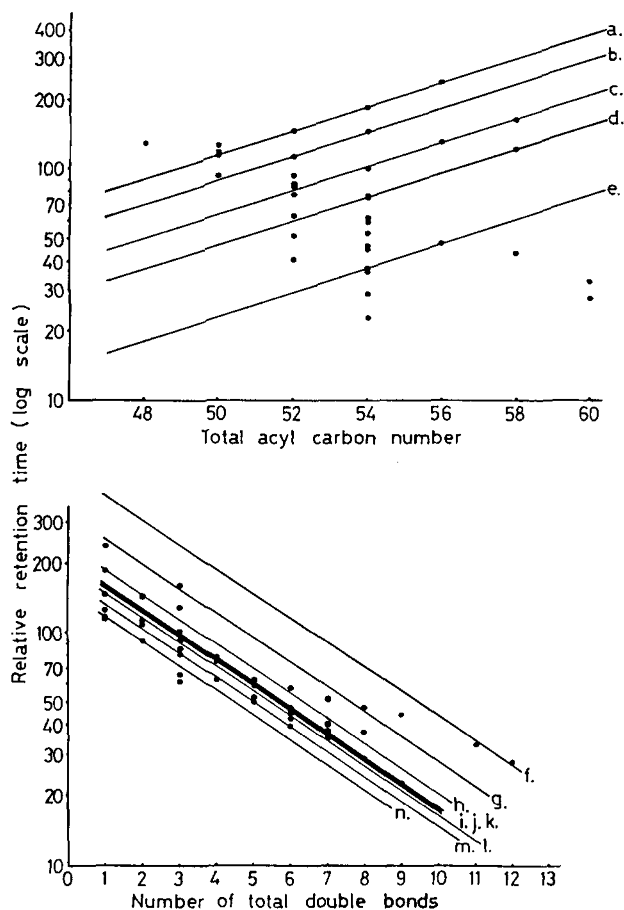
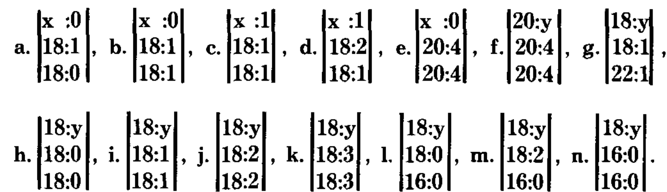


FIG. 1. Relationship between relative retention time of a molecular species of a triglyceride versus total acyl carbon number or number of total double bonds on high performance liquid chromatography. This figure was presented in *J. Amer. Oil Chem. Soc. 61*:1226 (1984).



(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. This matrix model makes it possible to distinguish the binding position of the acyl group, though at present the positional isomers are not discerned because of the difficulty of the separation of these isomers on high performance liquid chromatography.)

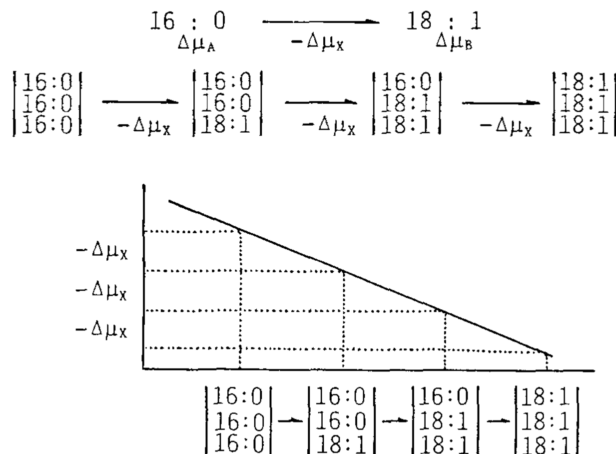


FIG. 2. Correlation between the increase (decrease) in chemical potential and the fatty acid residual substitution.

TABLE 1

Sources of the Molecular Species of Triglyceride Examined

Molecular species[a]	Sources
|16:0 16:0 16:0|, |18:1 18:1 18:1|, |18:2 18:2 18:2|, |18:3 18:3 18:3|	Standard (Gasukuro Kogyo Inc., Tokyo)
|18:2 16:0 18:1|, |16:0 16:0 18:2|, |16:0 18:2 18:2|	Palm oil (Tsukishima Food Industry Co., Ltd., Tokyo)
|16:0 18:1 18:1|, |18:2 18:1 18:1|, |18:1 18:2 18:2|	Rapeseed oil (Commercial source, Hakodate, Japan)
|16:0 16:0 18:1|, |18:0 16:0 18:1|	Cacao butter (Yunokawa Seiyaku Co., Ltd., Hakodate, Japan)
|16:0 18:3 18:3|	Linseed oil (Wako Pure Chemical Industries, Osaka, Japan)
|18:0 18:1 18:1|	Lard (Commercial source, Hakodate, Japan)

[a]Binding position of the fatty acid residue is not discerned here.

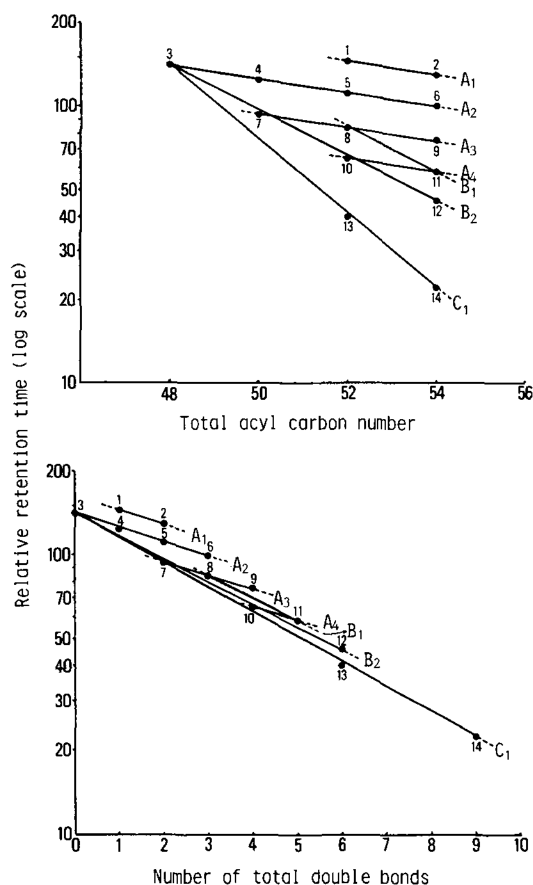
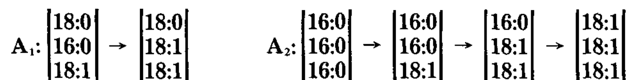
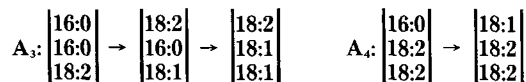
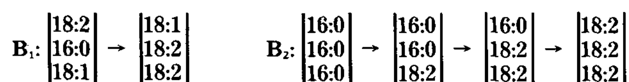
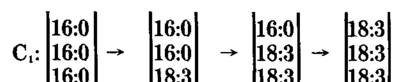


FIG. 3. Relationship between relative retention time of a molecular species of a triglyceride versus total acyl carbon number or number of total double bonds when the fatty acid residue is substituted. Lines from A_1 to A_4 demonstrate the lines of 16:0 → 18:1 substitution. Lines B_1 and B_2 demonstrate the lines of 16:0 → 18:2 substitution. Line C_1 demonstrates the line of 16:0 → 18:3 substitution. Lines are:

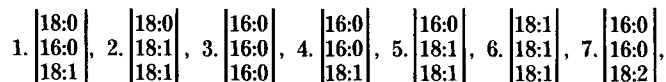


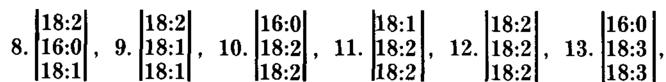


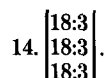




Points are:







reinjected into HPLC under the same conditions. RRTs of the peaks which appeared (identified molecular species) were calculated by dividing the retention time of each peak by that of triolein. The semilogarithmic plot of RRT of each molecular species versus total acyl carbon number and versus total double bonds was made.

RESULTS AND DISCUSSION

The RRTs of the molecular species examined are tabulated in Table 2. The semilogarithmic plots of these RRTs were used in order to generate lines that connect the fatty acid residual substituted TG molecular species as shown in Figure 3. As illustrated, four parallel lines (A_1, A_2, A_3 and A_4) demonstrate the substitution of 16:0 fatty acid residue into 18:1; two parallel lines (B_1 and B_2) demonstrate the substitution of 16:0 fatty acid residue into 18:2; and one line (C_1) demonstrates the substitution of 16:0 fatty acid residue into 18:3 in the TG molecule. The distance between (18:1, 18:1, 18:1) and (18:2, 18:2, 18:2) and the distance between (18:2, 18:2, 18:2) and (18:3, 18:3, 18:3) were the same in the upper figure in Figure 3.

Interestingly, the pattern of the lines was the same between the carbon number versus RRT plot (upper, Fig. 3) and the double bonds versus RRT plot (lower, Fig. 3), though the slopes of the lines were different between these two figures. These correlations held despite variations in the analytical condition used by reviewing

TABLE 2

Relative Retention Time of the Molecular Species Examined[a]

Molecular species[b]	RRT[c]	Molecular species[b]	RRT[c]	Molecular species[b]	RRT[c]
18:3, 18:3, 18:3	22.4	18:2, 18:1, 18:1	76.3	16:0, 16:0, 18:1	125.0
16:0, 18:3, 18:3	40.1	18:2, 16:0, 18:1	84.1	18:0, 18:1, 18:1	129.9
18:2, 18:2, 18:2	46.0	16:0, 16:0, 18:2	94.0	16:0, 16:0, 16:0	141.9
18:1, 18:2, 18:2	58.3	18:1, 18:1, 18:1	100.0	18:0, 16:0, 18:1	146.8
16:0, 18:2, 18:2	65.0	16:0, 18:1, 18:1	112.4		

[a]HPLC equipment, Hitachi 638-50; Shodex RI monitor; column, LiChrosorb RP-18 (250 × 8 mm; tandem); solvent, acetone/acetonitrile (3:1, v/v); flow, 1.5 ml/min; column temperature, ambient.
[b]The binding position of the fatty acid residue is not discerned here.
[c]Relative retention time when triolein is used as the reference peak.

TABLE 3

Quotation from other Workers' HPLC Chromatograms

Column	Solvent	Monitor	Sample	References
Supelcosil LC-18 (250 × 4.6 mm)	acetone/acetonitrile (63.6:36.4, v/v), 1.0 ml/min	RI	TG standard Palm oil Olive oil Corn oil Soybean oil Human plasma	5, 7
μBondapak C_{18} (300 × 7.8 mm)	acetonitrile/tetrahydrofuran (4:1, v/v), 2.0 ml/min	RI	Peanut oil Olive oil Ham	6
Spherisorb S30DS2 (150 × 4.5 mm, tandem)	acetonitrile/tetrahydrofuran (73:27, v/v), 1.0 ml/min	UV	Cacao butter	8
Perkin-Elmer HS-3 (100 × 4.6 mm)	acetone/acetonitrile (7:3, v/v), 2.5 ml/min	RI	Standard Olive oil Palm oil Corn oil Peanut oil Sunflower oil	10
Zorbax C_{18} ODS (250 × 4.6 mm, tandem)	acetonitrile/methylene chloride (7:3 → 2:3, v/v, 120 min linear gradient)	FID	Randomized standard Cacao butter Soybean oil Olive oil	11

other workers' chromatograms (Table 3), and therefore may be considered to be invariant rules for the reverse phase high performance liquid chromatographic analysis.

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