Bull. Fac. Fish. Hokkaido Univ.
37(2), 144-147. 1986.

Differences in Relative Retention Value of Triglyceride α, β Isomers on Reverse Phase High Performance Liquid Chromatography

Koretarō TAKAHASHI*

Abstract

Relative retention values (relative retention times (RRTs)) of α, β discerned individual molecular species of triglyceride from soybean oil were calculated on a reverse phase high performance liquid chromatography (HPLC).

By applying multiple regression analysis to the retention results, the regression expression obtained indicated that the retention effect of position α is larger than that of position β on HPLC when the molecular species has the same acyl group combination.

In the previous paper,[1,2] the formula that predicts the RRT (criterion variable) of individual molecular species of triglyceride has been demonstrated, as follows:

$$\log(\mathrm{RRT}) = P_1 \cdot C_1 + P_2 \cdot C_2 + P_3 \cdot C_3 + P'_1 \cdot D_1 + P'_2 \cdot D_2 + P'_3 \cdot D_3 + Q \qquad [1]$$

where C_1, C_2, C_3 are the acyl carbon numbers (predictor variables) and D_1, D_2, D_3 are the numbers of double bonds (predictor variables) in each acyl group. P_1, P_2, P_3, P'_1, P'_2, P'_3 are the coefficients of each term. This formula [1] can be considered as the first order combination of C_1, C_2, C_3, D_1, D_2, D_3 against log (RRT). So, with the application of multiple regression analysis to the retention results from α, β discerned individual triglycerides, it is possible to perceive all inclusive correlations between the criterion variable, i.e. log (RRT) and the predictor variables.

In this study, partial regression expression coefficients of this multiple regression expression are compared in order to predict the extent of the effect of each predictor variable on RRT.

Method

The HPLC chromatogram of Park et al.[3] was used in this study, since they were able to distinguish the binding positions of the acyl groups in the triglyceride molecule. RRT of each peak on their chromatogram was calculated by dividing the retention time of each peak by that of triolein. The computer program used for multiple regression analysis was the modified program of "Personal Computer Library, Vol. 3".[4] The original program was written for the NEC PC-8001 personal

* *Laboratory of Food Chemistry I, Faculty of Fisheries, Hokkaido University* (北海道大学水産学部食品化学第一講座)

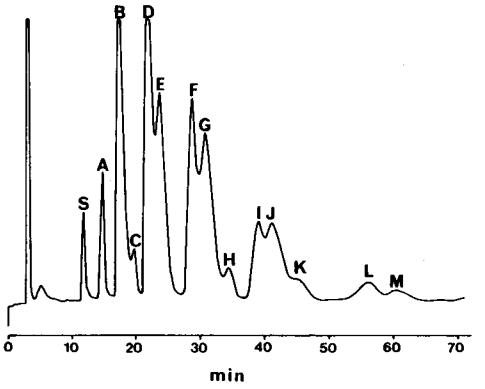

Fig. 1. HPLC chromatogram of soybean oil triglyceride analyzed by Park et al. (1983). *Agric. Biol. Chem.*, **47**(10), 2243-2249.
RRTs of the peaks are shown in Table 1.
Operating conditions ; colume (250 mm×4.5 mm) packed with Zorbax ODS ; eluting solvent, acetone and acetonitrile (64 : 36, v/v) ; flow rate, 1 ml/min.

Table 1. RRT's on HPLC of the α, β discerned molecular species of triglyceride from soybean*

Peak symbol	Molecular species	RRT**	Peak symbol	Molecular species	RRT**
S	12 : 0 12 : 0 12 : 0	24.9	I	18 : 1 18 : 1 18 : 1	100.0
A	18 : 3 18 : 3 18 : 3	32.9	J	18 : 1 18 : 2 18 : 0	105.8
B	18 : 2 18 : 2 18 : 2	39.5		18 : 1 18 : 1 16 : 0	
C	16 : 0 18 : 2 18 : 3	46.5	K	16 : 0 18 : 2 18 : 0	117.1
D	18 : 2 18 : 2 18 : 1	51.9		16 : 0 18 : 1 16 : 0	
E	18 : 2 18 : 2 16 : 0	57.6	L	18 : 1 18 : 1 18 : 0	146.9
F	18 : 1 18 : 1 18 : 2	71.4		18 : 0 18 : 3 18 : 0	
G	18 : 1 18 : 2 16 : 0	77.2	M	18 : 0 18 : 1 16 : 0	158.9
	18 : 2 18 : 2 18 : 0			18 : 0 18 : 2 18 : 0	
H	16 : 0 18 : 2 16 : 0	87.2			

* HPLC chromatogram is shown in Fig. 1.
** Triolein, is used as the reference peak.

computer but it was modified for the NEC PC-8801 mkII personal computer; for instance, the arrangement of the program was changed into N-88 BASIC.

Results and Discussion

Figure 1 shows the chromatogram of Park et al.[3], and the RRTs of the molecular species which appeared in this chromatogram are tabulated in Table 1. Results from calculation of the regression expression were:

$$\begin{aligned} 100 \cdot \log(\mathrm{RRT}) = & 4.78348 \cdot C_1 + 3.28154 \cdot C_2 + 7.01845 \cdot C_3 \\ & - 11.05191 \cdot D_1 - 5.36245 \cdot D_2 - 14.67863 \cdot D_3 \\ & - 41.38170 \end{aligned} \quad [2]$$

Since antipodes such as α, α' isomers are considered to have the same chemical potential, the coefficients of C_1 and C_3 as well as D_1 and D_3 in [2] should be equivalent. Therefore:

$$\begin{aligned} 100 \cdot \log(\mathrm{RRT}) = & \frac{4.78348 + 7.01845}{2} \cdot (C_1 + C_3) + 3.28154 \cdot C_2 \\ & - \frac{11.05191 + 14.67863}{2} \cdot (D_1 + D_3) - 5.36245 \cdot D_2 \\ & - 41.38170 \\ \therefore \quad 100 \cdot \log(\mathrm{RRT}) = & 5.900965 \cdot (C_1 + C_3) + 3.28154 \cdot C_2 \\ & - 12.86527 \cdot (D_1 + D_3) - 5.36245 \cdot D_2 \\ & - 41.38170 \end{aligned} \quad [3]$$

The multiple correlation coefficient was 0.97194.

The standard regression expression that had been calculated from the standardized predictor variables was:

$$\begin{aligned} 100 \cdot \log(\mathrm{RRT})' = & 0.37844 \cdot (C_1 + C_3) + 0.18921 \cdot C_2 \\ & - 0.53448 \cdot (D_1 + D_3) - 0.16830 \cdot D_2 \end{aligned} \quad [4]$$

For both expressions [3] and [4], the partial regression expression of position α (the coefficients of $C_1 + C_3$ as well as $D_1 + D_3$) is larger than that of position β (the coefficients of C_2 as well as D_2). This indicates that the retention effect of position α is larger than that of position β on HPLC; i.e. when the triglyceride molecular species have the same acyl group combinations, the RRTs of these molecular species are considered to be slightly shifted by the retention effect of acyl group bound in the α position.

Acknowledgments

K. Zama, T. Takagi and M. Hatano provided helpful advices. Thanks to K. Takama, T. Hirano, M. Egi and M. Watanabe for their encouragement.

References

1) Takahashi, K., Hirano, T., Egi, M. and Zama, K. (1985). A mathematical model for the

prediction of triglyceride molecular species by high performance liquid chromatography. *J. Am. Oil Chem. Soc.*, **62**, 1489-1492.

2) Takahashi, K. (1985). A novel approach for the identification of lipid molecular species. *Mem. Fac. Fish. Hokkaido Univ.*, **32**, 245-330.

3) Park, D.K., Terao, J. and Matsushita, S. (1983). Influence of triglyceride molecular species on autoxidation. *Agric. Biol. Chem.*, **47**, 2243-2249.

4) Watari, M. and Kishi, M. (1982). *Personal computer library* **3**, 9-1-9-12 pp. Kōgaku Tosho, Tokyo. (In Japanese).