TABLE I

Correlations between Analytical Values of Rice Bran Oil and Double-Fractionated Palm Olein

Rice bran oil	Double-fractionated palm olein					
	Polar components	Dielectric constant	A_{232}	A_{268}	Refractive index	Polymer content
Polar components	–	0.981	0.928	0.827	0.953	0.877
Dielectric constant	0.984	–	0.952	0.883	0.977	0.909
A_{232}	0.904	0.964	–	0.972	0.889	0.774
A_{268}	0.971	0.988	0.947	–	0.821	0.711
Refractive index	0.995	0.993	0.927	0.976	–	0.975
Polymer content	0.995	0.975	0.882	0.974	0.990	–

polymer and DF palm olein only 9%. As shown in Figure 5, the polymerization occurred more rapidly in rice bran oil than in DF palm olein.

Among analytical methods tested, conjugated diene content, refractive index and polymer content discriminate clearly between the two oils after 10 hr heating, whereas oxidized polar components and dielectric constant do not discriminate each other even in absolute abuse level.

The correlation coefficients between every two analytical values were obtained by linear regression analysis and are given in Table I. In the case of rice bran oil, polar components, dielectric constant, refractive index and polymer content were shown to have a high correlation. Dielectric constants especially appeared to possess correlation coefficients of 0.96 or higher with all methods. In the case of DF palm olein, polar components, dielectric constant and refractive index were shown to have relatively good correlation. However, spectroscopic methods measuring the existence of double bonds appeared not to be suitable for DF palm olein, which is composed of less unsaturated fatty acids than is rice bran oil.

REFERENCES

1. Gray, J.I., JAOCS 55:539 (1978).
2. Fritsch, C.W., JAOCS 58:272 (1981).
3. Fritsch, C.W., D.C. Egberg and J.S. Magnuson, JAOCS 56:746 (1979).
4. Paradis, A.J., and W.W. Nawar, J. Food Sci. 46:449 (1981).
5. Freeman, I.P., Chem. Ind. 15:623 (1974).
6. Waltking, A.E., and H. Wessels, J. Assoc. Off. Anal. Chem. 64: 1329 (1981).
7. Waltking, A.E., W.E. Seery and G.W. Bleffert, JAOCS 52:96 (1975).
8. Peled, M., J. Sci. Food Agric. 26:1655 (1975).
9. Arya, S.S., S. Ramarujam and P.K. Vijayaraghavan, JAOCS 46:28 (1969).

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A Mathematical Model for the Prediction of Triglyceride Molecular Species by High Performance Liquid Chromatography

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ABSTRACT

The physicochemical and theoretical relationships between the traditional equivalent carbon number (ECN) and a proposed elution theory for triglyceride molecular species that has been expressed as a matrix model were demonstrated by multiple regression analysis. It was concluded that the ECN expression has two independent variables, that is, the total acyl carbon number (CN) and the total double bonds (DB), and one dependent variable , the relative retention time (RRT). In the proposed elution equation, there are six independent variables, including the carbon numbers and number of double bonds in each acyl group to be considered.

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INTRODUCTION

Equivalent carbon numbers (ECN) or partition numbers (PN) have been widely accepted as empirically derived values for determining the molecular species of triglycerides when using reverse-phase high performance liquid chromatography (HPLC) (1-7). The definition of ECN or PN is ECN(PN)= CN−2•DB where CN is the total acyl carbon number and DB is the total number of double bonds in the molecule. In addition, a matrix model for the identification of lipid molecular species has been proposed by the authors (8,9). The matrix model (For ease in expressing the constants and variables among the acyl groups in triglyceride molecules; it does not follow mathematical operations.) can be written as:

$$CN=p_1 \cdot \log(RRT)+q_1 \qquad CN=\begin{vmatrix} x & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$$

$$DB=p_2 \cdot \log(RRT)+q_2 \qquad DB=\begin{vmatrix} c_1 & y \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$$

where RRT is the relative retention time, c_1, c_2, c_3 and d_1, d_2, d_3 are the acyl carbon numbers and the number of double bonds in each acyl group, respectively. In this expression, x and y represent 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 ordinate of the semilogarithmic plots of the RRT's against CN's or DB's.

By the application of multiple regression analysis to retention results from standard triglycerides, we have been able to predict retention times of molecular species more accurately.

METHODS

The chromatograms of Perkins et al. (2,10) were used in this study. RRT's of each peak on their chromatograms were calculated by dividing the retention time of each peak by that of triolein. Multiple regression analysis was performed against the RRT using a personal computer Model PC-8001 (NEC, Tokyo).

DISCUSSION

Martin (11) formulated the equation:

$$\Delta\mu_B/R\cdot T=\Delta\mu_A/R\cdot T+\Delta\mu_X/R\cdot T,\ \ln(\alpha_B/\alpha_A)=\Delta\mu_X/R\cdot T \quad [1]$$

where A and B are members of a homologous series, differing by the functional group X; α is the partition coefficient, and $\Delta\mu_X$ is the difference in chemical potential of the group X in a polar or non-polar phase of the chromatographic system. It follows that each functional group in the solute molecule contributes more or less independently to the differences in standard free energy of the solute between the two chromatographic phases. Thus, in general, there is a linear relationship between ln α or log α and the number of functional groups in a homologous series (12). By substituting A in Martin's (11) equation with a triglyceride species $\begin{vmatrix} c_1 & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$ and B with $\begin{vmatrix} c_1+X & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$, or substituting A with $\begin{vmatrix} c_1 & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$ and B with $\begin{vmatrix} c_1 & d_1+Y \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$, then X and Y will denote functional groups. In simple triglycerides, X corresponds to the -CH_2- unit and Y corresponds to the -CH=CH- unit. The chemical potential of the triglyceride molecule is affected principally by X or Y because an elongation of the hydrocarbon chain or an increase in the number of double bonds in the acyl group affects the RRT of each molecular species. Plots of (empirically determined) log(RRT) against CN or DB on semilogarithmic paper draw ascending or descending straight lines (9). Though there is no doubt that the chemical potential of a triglyceride molecule is affected principally by the number of -CH_2- units or -CH=CH- units, more precise concepts can be introduced. These include:

(i) One hydrocarbon chain of the triglyceride molecule -CH_2- and -CH=CH- can be considered as a physicochemical functional group. Adding the differences in arrangement of these units might affect the total chemical potential of the triglyceride molecule and should be considered. (We will call this the Ω factor.).

(ii) Unless the three carbon chains are the same, we should consider differences between the positional isomers (i.e., binding position of the acyl group).

(iii) Specific fatty acids such as iso-, *trans*- or hydroxy fatty acids also should affect the total chemical potential of triglyceride molecule.

For the purpose of this discussion, we will not consider (iii), because retention data on these triglycerides are not forthcoming. The chemical potential (μ) of triglyceride $\begin{vmatrix} c_1 & d_1 \\ c_2 & d_2 \\ c_3 & d_3 \end{vmatrix}$ can then be written as follows:

$$\mu=g\left\{f(c_1, d_1, \Omega_1), f(c_2, d_2, \Omega_2), f(c_3, d_3, \Omega_3)\right\} \quad [2]$$

where Ω is the Ω factor, f is the chemical potential given by the hydrocarbon chain and g is the function of chemical potential given by the differences in positional isomers. Function [2] sums up the chemical potential of the triglyceride molecule, and therefore represents factors that control the sequence of elution on HPLC.

In order to derive an ECN from function [2], we shall neglect factor (ii), and suppose that there is no contribution from positional isomers. We can rewrite function [2] as:

$$\mu=f(c_1, d_1, \Omega_1)+f(c_2, d_2, \Omega_2)+f(c_3, d_3, \Omega_3) \quad [3]$$

In addition to this, if we neglect the Ω factor, function [3] will become:

$$\mu=f(c_1, d_1)+f(c_2, d_2)+f(c_3, d_3) \quad [4]$$

A commutative law should hold at function [4] because positional isomers (binding position of the acyl group) are not considered. Therefore:

$$\mu=f(c_1+c_2+c_3,\ d_1+d_2+d_3) \quad [5]$$

$$=f(CN, DB) \quad [6]$$

Function [6] corresponds to the function for ECN.

There is an empirically derived linear relationship between the logarithm of the RRT of a molecular species of a triglyceride versus CN or DB when only one acyl group differs in carbon number or number of double bonds (9). For ease of comprehension, a matrix mode has been used to exhibit this relationship. Mathematically, this can be written as follows:

$$\log(RRT)=P_1\cdot C_1+q_1 \quad [1]$$

$$\log(RRT)=P_2\cdot C_2+q_2 \quad [2]$$

$$\log(RRT)=P_3\cdot C_3+q_3 \quad [3]$$

$$\log(RRT)=P_1'\cdot D_1+q_1' \quad [4]$$

$$\log(RRT)=P_2'\cdot D_2+q_2' \quad [5]$$

$$\log(RRT)=P_3'\cdot D_3+q_3' \quad [6]$$

where RRT is the relative retention time, C_1, C_2, C_3 are the acyl carbon numbers (variable) in each acyl group. D_1, D_2, D_3 are the numbers of double bonds (variable) in each acyl group. P_1, P_2, P_3, P_1', P_2', P_3' are the slopes (constant) and q_1, q_2, q_3, q_1', q_2', q_3' are the intercepts (constant) from the semilogarithmic plots of log(RRT) of each molecular species of triglyceride against CN and DB.

By adding both sides of equations [1] through [6]:

$$6\cdot\log(RRT)=P_1\cdot C_1+P_2\cdot C_2+P_3\cdot C_3+P_1'\cdot D_1+P_2'\cdot D_2+P_3'\cdot D_3+q_1+q_2+q_3+q_1'+q_2'+q_3' \quad [7]$$

If we substitute Pn=1/6 Pn, P'n=1/6 P'n, Σq/6=Q, the following function can be obtained:

$$\log(RRT) = P_1 \cdot C_1 + P_2 \cdot C_2 + P_3 \cdot C_3 + P_1' \cdot D_1 + P_2' \cdot D_2 + P_3' \cdot D_3 + Q \quad [8]$$

This formula [8] can be considered as the first order combination of $C_1, C_2, C_3, D_1, D_2, D_3$ against log(RRT).

$C_1, C_2, C_3, D_1, D_2, D_3$ are independent variables (predictor variables) and log(RRT) corresponds to the dependent variable (the criterion variable). Thus, we have reached an equation that predicts the RRT of individual molecular species of triglyceride. By calculating the regression expression, we can predict the RRT's from several kinds of predictor variables that are $C_1, C_2, C_3, D_1, D_2, D_3$. Or at the same time, it is possible to see all-inclusive correlations between the criterion variable, i.e. RRT and the predictor variables.

Standard chromatograms of Perkins et al. (2,10) were introduced in order to calculate the RRT's of each molecular species of triglyceride (Table I). Results from calculation of the regression expression were:

$$\log(RRT) = 5.57391 \cdot C_1 + 4.90109 \cdot C_2 + 7.00699 \cdot C_3 - 11.63260 \cdot D_1 - 13.40241 \cdot D_2 - 15.40738 \cdot D_3 - 85.77448 \quad [9]$$

The multiple correlation coefficient was 0.99418. Unfortunately, in the standard chromatograms mentioned above, we were unable to distinguish triglyceride isomers (the binding position of the acyl groups). So, the coefficients of C_1, C_2, C_3 should be equivalent. And the coefficients of D_1, D_2, D_3 also should be equivalent. Therefore, formula [8] can be rewritten as:

$$\log(RRT) = P \cdot (C_1 + C_2 + C_3) + P' \cdot (D_1 + D_2 + D_3) + Q = P \cdot (CN) + P' \cdot (DB) + Q \quad [10]$$

Function [10] is actually the same as the definition of ECN because P'/P corresponds to the coefficient -2 in ECN. The actual calculation of P'/P from formula [9] gave -2.2, nearly but not equal to the ECN definition ratio of -2. If we use -2.2 for P'/P, it is possible to distinguish the following pairs that have the same ECN (=38):

$$\begin{vmatrix} 18 & 1 \\ 20 & 4 \\ 22 & 6 \end{vmatrix} \quad (A) \qquad \begin{vmatrix} 16 & 0 \\ 20 & 4 \\ 22 & 6 \end{vmatrix} \quad (B)$$

By using a coefficient -2.2, the ECN' of (A) becomes 60-2.2 x11=35.8 and the ECN' of (B) becomes 58-2.2x 19=36.0. The authors have performed a sequence simulation on the HPLC data of Table I using formula [10]. The results are shown in Figure 1. It is clear from Figure 1 that all the molecular species in Table I become resolvable (shown as a solid line).

Formula [10] that corresponds to the definition of ECN has two independent variables and one dependent variable, whereas formula [8] has six independent variables. This implies that with only two independent variables, it is impossible to accurately predict the RRT of an individual molecular species, and that six independent variables are needed for the prediction of RRT's of triglyceride molecular species.

However, we can conclude that formula [8] can be used for contemporary HPLC analysis.

TABLE I

Relative Retention Time Calculated from HPLC Chromatograms[a]

Molecular species	RRT[b]	Molecular species	RRT[b]
LaLaLa	21.9	M O P	101.1
tri-13:0	33.9	S O L	104.1
L L Le	34.3	S P L	108.7
M M M	42.1	P O O	113.1
L L L	44.8	S O M	117.3
L O L	58.7	P O P	120.1
L L P	63.3	P P P	132.9
L O O	76.7	S O O	144.2
tri-15:0	81.8	S S L	145.0
P L O	83.7	S O P	152.7
P P L	89.5	(S O O)	155.3
P L P	90.7	S P O	162.3
L P P	94.2	S P P	179.8
O O O	100.0	S O S	208.1

[a]Calculations made from HPLC chromatograms of E.G. Perkins et al., JAOCS 58:867 (1981) and Lipids 17:460 (1982).

[b]RRT: relative retention time when 000 is used as the reference peak.

Abbreviations: La, lauryl; L, linoleyl; Le, linolenyl; M, myristyl; O, oleyl; P, palmityl.

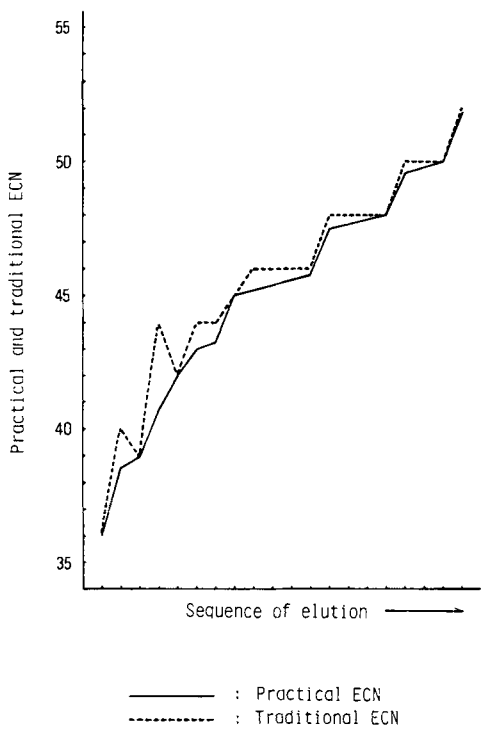

FIG. 1. Practical ECN (——) suggested in this study and the traditional ECN (- - -) in relation to the sequence of elution of triglyceride molecular species on HPLC; deduced from Table I.

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REFERENCES

1. Petersson, B., O. Podlaha and B. Töregård, JAOCS 58:1005 (1981).
2. El-Hamdy, A.H., and E.G. Perkins, JAOCS 58:867 (1981).
3. Plattner, R.D., G.F. Spencer and R. Kleiman, JAOCS 54:511 (1977).

4. Plattner, R.D., JAOCS 58:638 (1981).
5. Wada, S., C. Koizumi and J. Nonaka, Yukagaku 26:11 (1977).
6. Wada, S., C. Koizumi, A. Takiguchi and J. Nonaka, Yukagaku 27:21 (1978).
7. Wada, S., Jasco Report 18:18 (1983).
8. Takahashi, K., T. Hirano and K. Zama, Bull. Japan. Soc. Sci. Fish. 49:1301 (1983).
9. Takahashi, K., T. Hirano and K. Zama, JAOCS 61:1226 (1984).
10. Perkins, E.G., D.J. Hendren, N. Pelick and J.E. Bauer, Lipids 17:460 (1982).
11. Martin, A.J.P., Biochem. Soc. Symposia (Cambridge, England) 3:4 (1950).
12. Vereshchagin, A.G., J. Chromatog. 14:184 (1964).

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☘Effect of Caustic Refining, Solvent Refining and Steam Refining on the Deacidification and Color of Rice Bran Oil

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ABSTRACT

Degummed rice bran oil was deacidified by caustic, solvent and steam refining processes. The steam refining process was optimized through a series of experiments with varying refining times (1-5 hr), temperatures (220-280 C) and amounts of steam (4-20%), at a pressure of 4 mmHg. The most significant factors affecting the degree of deacidification were the refining temperature and amount of steam. The correlation coefficient between quadratic equation obtained and experimental results was 0.96. Acid value and color of steam refined oil were not as good as those of caustic refined oil, but steam refining showed better retention of natural antioxidants than caustic or solvent refining. Steam refining is preferred for deacidification of rice bran oil because of reduced neutral oil loss and elimination of soap production.

The important criteria in selecting a deacidification process are known to be the degree of deacidification, neutral oil loss, effect on bleaching and production of soapstock (2,8-10). In comparing caustic refining, solvent refining and steam refining, caustic refining of degummed rice bran oil resulted in satisfactory acid values and color but showed the worst result in neutral oil loss and produced large amounts of soapstock. Solvent refining was not shown to be efficient because of poor deacidification, high losses of neutral oil and darkening of color. Steam refining also was less effective than caustic refining in deacidification and bleaching. However, the degree of deacidification could be improved by development of a process to remove all the free fatty acids (8), and the color problem could be eliminated by including a preliminary bleaching step before steam distillation (10). The application of steam refining to rice bran oil will result in many advantages such as reduced neutral oil loss, no production of soap, and the production of high purity, industrial fatty acids.

INTRODUCTION

Rice bran oil has been difficult to refine because of its high content of free fatty acid, unsaponifiable materials and color (1,2). Rice bran oil has been deacidified in industry by caustic refining, but this process gives considerably greater losses of neutral oils than caustic refining of many other vegetable oils with similar free fatty acid content (3,4). The solvent extraction method (2) and progressive acetylation of hydroxylated compounds in the oil (5) also have been used to reduce the refining loss of neutral oil of rice bran oil in the deacidification process. In liquid-liquid extraction refining, furfural-naphtha (6), liquid propane (7) or alcohols (2) have been used as the fractionating solvent. Alcohol extraction in combination with alkali refining has been used to give satisfactory deacidification, reduction of refining loss and color removal.

In recent years, steam refining of crude oils with high free fatty acids has been used to avoid both excess losses of neutral oil and production of large quantities of soapstock which require expensive waste-water treatment before discharge (8,9). Furthermore, steam refining gives savings on equipment, space, time and labor in comparison to caustic refining; it also yields high quality fatty acids (9). However, with dark-colored oils having a high free fatty acid content, the steam refining process is not as successful as caustic refining in achieving a low free fatty acid refined oil which gives satisfactory oil-color during the subsequent bleaching step. However, the bleaching problem of dark crude oils has been solved by inserting the bleaching process prior to steam refining (10,11).

At present, steam refining is used mostly in the palm oil industry. It also has been applied successfully to soybean oil, coconut oil, palm kernel oil, peanut oil, sesame oil and olive oil, tallow and lard (8,9). Optimization studies of steam refining concerning time, temperature and the amount of steam for any fats and oils are very scarce (8), and information about steam refining of rice bran oil is not found anywhere. In this study, rice bran oil was deacidified by steam refining, conventional caustic refining and solvent extraction method in order to compare the neutral oil loss and characteristics of the oil after each deacidification process. In the steam refining process, the independent variables were analyzed statistically in order to identify the factors affecting free fatty acid content and color. The residual contents of tocopherol and oryzanol also were measured to observe the effects of the deacidification processes upon the removal of natural antioxidants of neutralized oil.

MATERIALS AND METHODS

Materials

Crude rice bran oil was obtained from a local refinery and was laboratory degummed with 4% oxalic acid at a level of

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