Random Nature of Triacylglycerols Produced by the Catalyzed Interesterification of Short- and Long-Chain Fatty Acid Triglycerides

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Sodium methoxide catalyzed interesterification of saturated long-chain fatty acid (LCFA) triglycerides and short-chain fatty acid (SCFA) triglycerides has been used to produce new triacylglycerols for food use. The triacylglycerol compositions contain both long- and short-chain fatty acids. Excess, unreacted SCFA triglycerides were removed from the reaction mixture by vacuum steam deodorization. The recovered compositions have been examined by proton nuclear magnetic resonance, supercritical fluid chromatography, high-pressure liquid chromatography, capillary high-temperature gas chromatography, and total acid analysis. The analytical data are used to quantitate the amounts of triesters containing one, two, or three long-chain fatty acids. The relative concentrations of these three triester structures are found to be in good agreement with values calculated by the statistical model for random interesterification.

INTRODUCTION

Triacylglycerols containing both 16–20-carbon long-chain fatty acids (LCFA) and 2–4-carbon short-chain fatty acids (SCFA) occur in nature (Christie and Clapperton, 1982; Kleiman et al., 1967). Available data suggest that the SCFA groups are not distributed randomly in these natural glycerol esters. For example, the 11.8 mol % of butyric acid found in cow's milk is concentrated in the *sn*-3 position (Christie and Clapperton, 1982). In the optically active seed oil of *Euonymus verrucosus*, the structural specificity is even greater, with more than 90% of the oil having the (*S*)-1,2-diacyl-3-acetin structure (Kleiman et al., 1967), where the long-chain acyl groups are predominantly palmitic, oleic, and linoleic acids.

On the other hand, triglycerides derived by catalyzed interesterification have completely random structures when the reaction occurs at temperatures that maintain phase homogeneity without enzymatic catalysis. The interesterification reaction has been studied extensively, and mathematical models have been developed that can be used to predict the distribution of LCFA groups in the products (Rozendaal, 1989; Sreenivasan, 1978). While the interesterification of SCFA and LCFA triglycerides has been reported Bauer and Lange (1949), the nature of the product mixture has not been studied in detail. It seems reasonable to question whether the significantly different size, and concomitantly reduced steric demands, of the short-chain fatty acids could give rise to a positional specificity and observable deviations from otherwise anticipated random interesterification chemistry. To probe this question, we have interesterified several combinations of SCFA and LCFA triglycerides and have compared analytical data for the resulting products to a compositional model predicted by random interesterification theory.

MATERIALS AND METHODS

Preparation of Triacylglycerol Compositions Containing SCFA and LCFA Groups. Triacetin and tripropionin were obtained from Aldrich Chemical Co., Milwaukee, WI. Tributyrin was purchased from Schweizerhall, Inc., Piscataway, NJ. Fully hydrogenated oils were obtained from the following sources: canola oil from CSP Foods, Ltd., Saskatoon, SK; cottonseed oil from Karlshamns USA, Columbus, OH; soybean oil from Van den Bergh Foods, Inc., Lisle, IL. Mixtures of SCFA and LCFA triglycerides were combined with a catalytic amount of sodium methoxide and maintained at a temperature of 100–150 °C for 5–60 min. After the reaction mixture was cooled and about 5 wt% water was added, the aqueous phase was removed and the organic phase was filtered through Tonsil Optimum 105 bleaching clay. The filtrate was subjected to vacuum steam deodorization for sufficient time to remove all of the volatile SCFA triglycerides. The resulting triacylglycerol product was characterized by a variety of spectroscopic, chromatographic, and analytical techniques.

Mono-, di-, and tristearin analytical standards employed in the SFC procedure were obtained from Nu Chek Prep, Elysian, MN.

Characterization of Triacylglycerol Compositions Containing SCFA and LCFA Groups. Compositions prepared by interesterification of SCFA and LCFA triglycerides were characterized by several analytical techniques. Supercritical fluid chromatography (SFC) was performed on dichloromethane solutions using a Suprex SFC/200A instrument operating with a 100 mm × 1 mm Deltabond Cyano 5-μm column (Keystone Scientific Co., Bellefonte, PA) and a flame ionization detector. A typical SFC chromatogram (cf. Figure 1) shows resolution of the major triacylglycerol fractions, where S and L represent a shorthand notation for the SCFA and LCFA groups, respectively. The observed retention times for the triester fraction are as follows: LSS + SLS, 12–14 min; LLS + LSL, 16–18 min; and LLL, 19–20 min. HPLC chromatograms were obtained on a Hewlett-Packard Model 1090 instrument coupled to a Varex IIA evaporative light scattering detector; HPLC data were handled with a Model 79994A Chem Station. A representative HPLC chromatogram (cf. Figure 2) shows resolution of the LSS + SLS fraction (8–15 min) and the LLS + LSL fraction (20–23 min). Capillary high-temperature gas chromatographic (CHTGC) analyses were performed on a Hewlett-Packard 5890 Series II GC equipped with a pressure programmable injector and flame ionization detector. A Quadrex aluminum-clad methyl 65% phenyl silicone analytical column (25 m × 0.25 mm i.d., 0.1-μm film thickness) from Quadrex Corp., New Haven, CT, was employed. Details of the CHTGC procedures have been pub-

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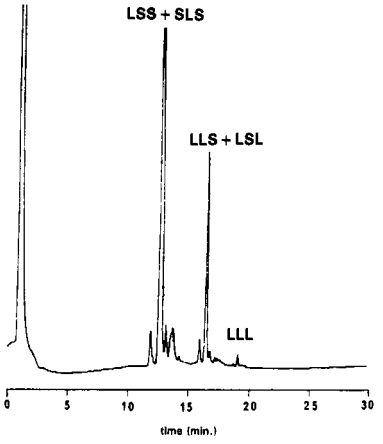

Figure 1. Supercritical fluid chromatogram of product A.

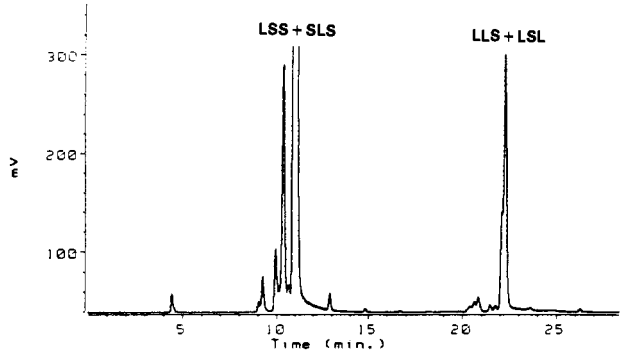

Figure 2. High-performance liquid chromatogram of product B.

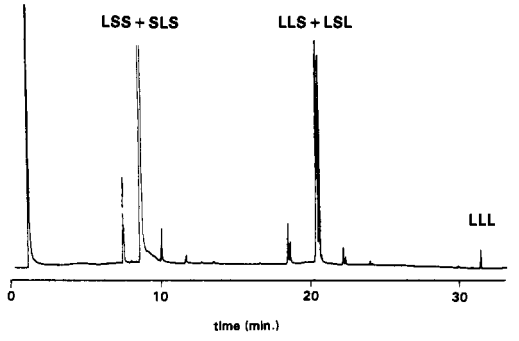

Figure 3. Capillary high-temperature gas chromatogram of product C.

lished separately (Huang et al., 1994). A typical CHTGC chromatogram is presented in Figure 3. The methodology for CHTGC peak assignments is beyond the scope of this paper and is reported separately (Huang et al., 1994). Proton NMR spectra were obtained in deuteriochloroform or $C_6D_6/DCCl_3$ solution using either a Varian VXR300 or VXR400 spectrometer (Henderson et al., 1994). The relative molar acid compositions were determined by integration of the methyl peaks for the SCFA at 2.08 (acetic), 1.14 (propionic), and 0.95 ppm (butyric) and for the LCFA at 0.88 ppm (cf. Figure 4). The SCFA and LCFA analysis was performed at Experimental Pathology Laboratories and at the Nabisco RMS Technology Center using gas chromatography.

DEVELOPMENT OF A RANDOM INTERESTERIFICATION MODEL

The interesterification of triglycerides bearing SCFA and LCFA groups could be anticipated to produce, at equilibrium, a random mixture of products as shown in eq 1. The designations S and L refer to SCFA and LCFA

$$SSS + LLL \rightleftharpoons LSS + SLS + LLS + LSL \quad (1)$$

groups in the product triacylglycerols, respectively, and their relative order reflects their respective points of attachment to glycerol. The relative concentration of any individual triester component in this mixture can be determined using published equations (Rozendaal, 1989; Sreenivasan, 1978). If we use X_S and X_L to represent the mole fractions of SCFA an LCFA, respectively, in this random mixture, then we can model the concentrations of species containing one, two, and three LCFA groups. The unnormalized probabilities of components with specific combinations of SCFA and/or LCFA groups can be expressed as follows:

$$P_{LSS/SLS} = X_S X_S X_L + X_S X_L X_S + X_L X_S X_S = 3X_S^2 X_L$$

$$P_{LLS/LSL} = X_L X_L X_S + X_L X_S X_L + X_S X_L X_L = 3X_S X_L^2$$

$$P_{LLL} = X_L^3$$

The respective unnormalized mole fractions, X, of LSS + SLS, LLS + LSL, and LLL can be represented by

$$X_{LSS+SLS} = 3X_S^2 X_L/(3X_S^2 X_L + 3X_S X_L^2 + X_L^3) \quad (2)$$

$$X_{LLS+LSL} = 3X_S X_L^2/(3X_S^2 X_L + 3X_S X_L^2 + X_L^3) \quad (3)$$

$$X_{LLL} = X_L^3/(3X_S^2 X_L + 3X_S X_L^2 + X_L^3) \quad (4)$$

By normalizing these mole fractions to unity, the theoretical mole fractions of triesters, can be obtained for any random reaction mixture that has been stripped of unreacted SCFA triglyceride (by steam deodorization, for example). By inclusion of the respective formula weights for the various fractions, the composition can be expressed in terms of the weight percent values for the three triester classes (i.e., LSS + SLS, LLS + LSL, and LLL). These calculated values can be compared directly with analytical results obtained by capillary high-temperature gas chromatography (CHTGC), supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC). Since analytical techniques such as proton nuclear magnetic resonance (NMR) and total acid analysis provide a direct measure of the relative molar amounts of SCFA and LCFA in a final product, data from these sources cannot be used with eqs 2–4 to generate the relative mole fractions of LSS + SLS, LLS + LSL, and LLL triesters. A method that can be used to convert this S/L ratio to compositional information is required.

Equations 2–4 provide the theoretical relationships that can be used to calculate a product composition from the mole fraction of SCFA and LCFA groups contained in the reactant triglycerides. Since the relative molar amounts of SCFA and LCFA groups in each component are known, the total molar SCFA and LCFA content of the product can be calculated and expressed as a ratio of SCFA/LCFA groups (S/L ratio). Additional mathematical relationships can then be derived for the S/L ratio for any composition of LSS + SLS, LLS + LSL, and LLL triesters. These relationships are presented graphically in Figure 5. The values for the S/L ratio plotted in Figure 5 have an upper limit of 2.0 and a lower limit of 0.5, since these are, respectively, the highest and lowest S/L ratios possible for triacylglycerols that contain both SCFA and LCFA groups. The calculated data in Figure 5 can be fit reasonably by simple linear approximations in the interval $1.0 < S/L < 2.0$. These expressions are provided in Table 1. Correlation coefficients for these linear equations (cf.

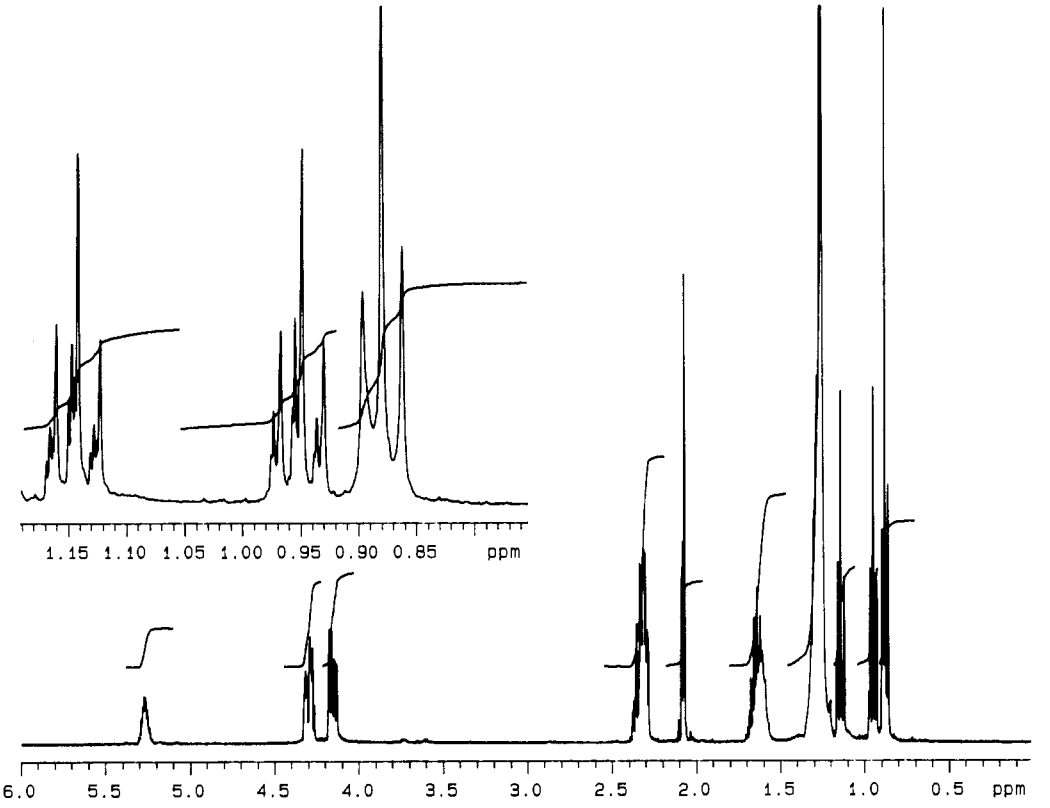

Figure 4. 400-MHz proton nuclear magnetic resonance spectrum of product D.

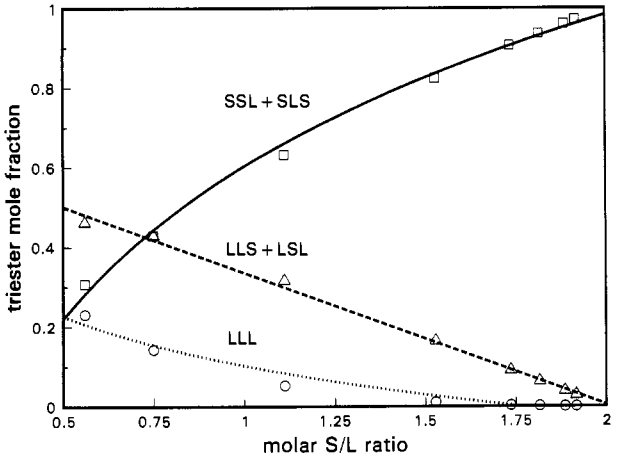

Figure 5. Short/long ratio and product composition.

Table 1. Linear Approximations for the Relationship of S/L Ratio to Triacylglycerol Component Mole Fraction in the Interval 1.0 < S/L < 2.0

triacylglyceride components	analytical expression that approximates mole fraction
LSS/SLS	$X_{LSS/SLS} = 0.4362(S/L) + 0.1440$
LLS/LSL	$X_{LLS/LSL} = -0.3560(S/L) + 0.7107$
LLL	$X_{LLL} = -0.08022(S/L) + 0.1453$

Table 1) are 0.999, −0.999, −0.957 for S/L vs [LSS + SLS], [LLS + LSL], and [LLL], respectively. Use of these relationships allows experimental data that provide a direct measure of the S/L ratio to be expressed as a specific mole fraction distribution of LSS + SLS, LLS + LSL, and LLL components.

Having developed the necessary models that permit the quantitative estimation of the three triester structural classes expected in all SCFA/LCFA containing triacylglycerol compositions, it is now posible to use these models as tools to enable comparison of theory with experiment. The calculated composition of any product is predicted by insertion of the respective mole fractions of reactant SCFA and LCFA triglycerides into eqs 2–4 and applying the appropriate molecular weights to generate weight percent values. All that is needed experimentally is a means to measure either the S/L ratio or the respective mole fractions or weight fractions of LSS + SLS, LLS + LSL, and LLL species. The latter may be used directly, while the former (S/L ratio) can be treated by the relationships provided in Table 1, along with the appropriate molecular weights, to provide individual triester weight percent values. The calculated and experimentally determined results can then be compared. If the experimental results show adequate agreement with theory, then the compositions are random.

RESULTS AND DISCUSSION

Four representative triacylglycerol compositions were prepared using the interesterification reaction and mixtures of various SCFA and LCFA triglycerides. The specific reactants and their initial mole ratios are listed in Table 2.

The results of analytical studies which employed NMR, SFC, HPLC, CHTGC, and total acid analysis to characterize triacylglycerol products A–D are given in Table 3. The data for each product are expressed as weight percent values which are compared with the respective compositions that would be predicted from the random interesterification reaction model developed earlier. As can be seen, the experimental data appear to be in reasonable agreement with the respective values calculated from the random reaction model.

Mean values obtained from the array of analytical methods surveyed for the three triester components (i.e., SSL + SLS, LLS + LSL, and LLL) in each triacylglycerol

Table 2. Reactants and Reactant Ratios Used To Produce Triacylglycerol Interesterification Products

triacylglycerol product	identity[a] (ref and lot no.)	triglyceride reactants	molar ratios of reactants
product A	SATATRIM 23CA (A8200 A014)	triacetin, tripropionin, hydrogenated canola oil	11:1:1
product B	SALATRIM 32CA (A8200 A015)	triacetin, tripropionin, hydrogenated canola oil	1:11:1
product C	SALATRIM 4CA (A7200 A006)	tributyrin, hydrogenated canola oil	2.5:1
product D	SALATRIM 234CS (A9100 A018)	triacetin, tripropionin, tributyrin, hydrogenated cottonseed oil	4:4:4:1

[a] SALATRIM (an acronym derived from short and long chain acid triglyceride molecules) is the common name for a reduced-calorie fat.

Table 3. Comparison of Compositional Data with the Random Composition Model for Triacylglycerol Products A–D

			values found, wt % unless noted, determined by					
product	components	wt % calcd	NMR	SFC	HPLC	CHTGC	acid anal.	mean (SD)
A	LSS + SLS[a]	88.5[b]	88.4[c]	83.7	89.9[d]	82.0[e]	77.4[f]	84.3 (4.5)
	LLS + LSL	11.1	11.1	15.4	10.1	17.2	19.5	14.7 (3.6)
	LLL	0.4	0.5	0.9	N/D	0.8	3.1	1.3 (1.1)
	sum of the means							100.3
B	LSS + SLS[g]	88.9[h]	88.8[i]	82.3	85.1[j]	84.5[k]	90.7[l]	86.3 (3.0)
	LLS + LSL	10.7	10.7	15.7	14.9	16.0	9.3	13.3 (2.8)
	LLL	0.4	0.5	2.0	N/D	0.6	0.0	0.8 (0.7)
	sum of the means							100.4
C	LSS + SLS[m]	60.5[n]	56.6[o]	66.7	60.3[p]	64.8[q]	56.1[r]	60.9 (4.3)
	LLS + LSL	33.7	35.5	29.6	39.7	32.2	35.8	34.6 (3.4)
	LLL	5.8	7.9	3.7	N/D	3.0	8.1	5.7 (2.3)
	sum of the means							101.2
D	LSS + SLS[s]	88.9[t]	97.2[u]	88.4	94.1[v]	86.6[w]	96.1[x]	92.5 (4.2)
	LLS + LSL	10.7	2.8	11.1	5.9	13.4	3.9	7.4 (4.1)
	LLL	0.4	0.0	0.5	N/D	N/D	0.0	0.2 (0.2)
	sum of the means							100.1

[a] Respective molecular weights for LSS + SLS, LLS + LSL, and LLL are 442, 666, and 890. [b] Calculation based on an 11:1:1 starting ratio of triacetin/tripropionin/fully hydrogenated canola oil. [c] Relative areas of C2:C3:LCFA were 51.0, 13.0, and 36.0% (S/L = 1.78 used with expressions found in Table 1). [d] Relative area % values have been normalized to 100%. [e] Relative weight % data have been normalized to 100%. [f] Mole % values of SCFA:LCFA were 61.5 and 38.5% (S/L = 1.59 was used with the expressions found in Table 1). [g] Respective molecular weights for LSS + SLS, LLS + LSL, and LLL are 470, 680, and 890. [h] Calculation based on a 1:11:1 starting ratio of triacetin/tripropionin/fully hydrogenated canola oil. [i] Relative areas of C2:C3:LCFA were 7.0, 57.0, and 36.0% (S/L = 1.78 used with expressions found in Table 1). [j] Relative area % values have been normalized to 100%. [k] Relative weight % data have been normalized to 100%. [l] Mole % values of SCFA:LCFA were 64.4 and 35.6% (S/L = 1.81 was used with the expressions found in Table 1). [m] Respective molecular weights for LSS + SLS, LLS + LSL, and LLL are 498, 694, and 890. [n] Calculation based on a 2.5:1 starting ratio of tributyrin/fully hydrogenated canola oil. [o] Relative areas of C4:LCFA were 54.0 and 46.0% (S/L = 1.17 used with expressions found in Table 1). [p] Relative area % values have been normalized to 100%. [q] Relative weight % data have been normalized to 100%. [r] Mole % values of C4:LCFA were 53.6 and 46.3% (S/L = 1.16 was used with the expressions found in Table 1). [s] Respective molecular weights for LSS + SLS, LLS + LSL, and LLL are 470, 680, and 890. [t] Calculation based on a 4:4:4:1 starting ratio of triacetin/tripropionin/tributyrin/fully hydrogenated cottonseed oil. [u] Relative areas of C2:C3:C4:LCFA were 20.0, 23.0, 23.0, and 34.0% (S/L = 1.94 used with expressions found in Table 1). [v] Relative area % values have been normalized to 100%. [w] Relative area % data have been normalized to 100%. [x] Mole % values of SCFA:LCFA were 65.7 and 34.3% (S/L = 1.92 was used with the expressions found in Table 1).

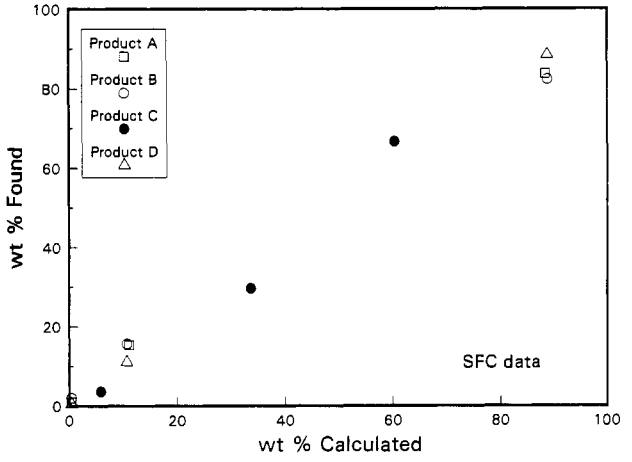

Figure 6. Comparison of calculated weight fraction composition with supercritical fluid chromatography data.

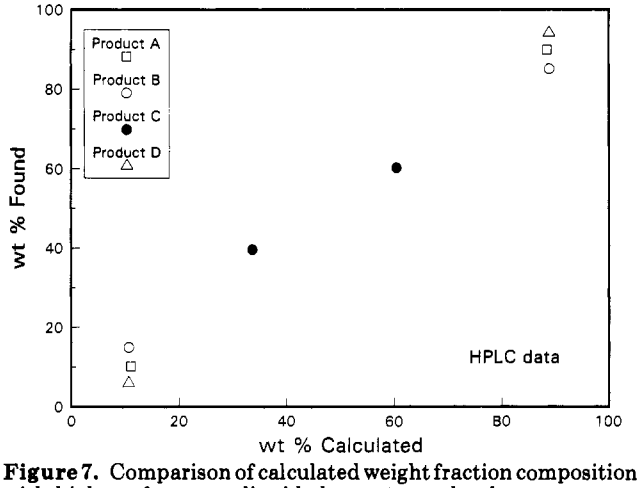

Figure 7. Comparison of calculated weight fraction composition with high-performance liquid chromatography data.

product are also provided in Table 3 and can be compared with their corresponding calculated values. Considering the concentration of the major triester component (between 60.5 and 88.9% SSL + SLS) in each product, the value found is within −11.1 to +8.3% of the respective calculated value. The mean difference between percent found and calculated values for the LSS + SLS concentration is −0.7%. The sum total of the individual triester components falls between 100.1 and 101.2% for the four products with a mean value of 100.5% (SD ± 0.4). These statistics support the position that the sodium methoxide catalyzed interesterification of SCFA and LCFA triglycerides produces a random and therefore highly predictable distribution of triester components.

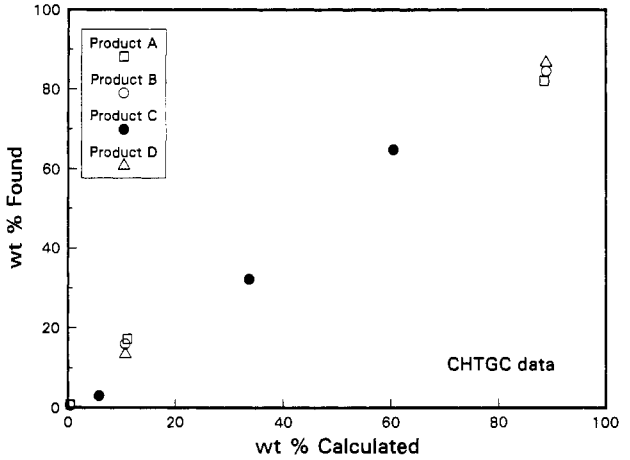

Figure 8. Comparison of calculated weight fraction composition with capillary high-temperature gas chromatography data.

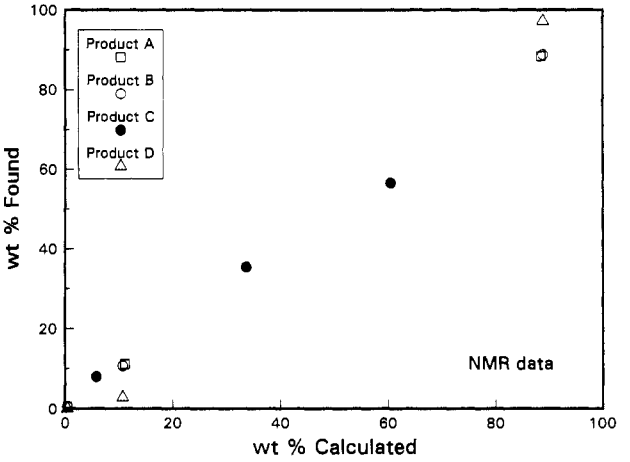

Figure 9. Comparison of calculated weight fraction composition with nuclear magnetic resonance data.

Table 4. Regression Parameters for Experimental Data against Values Calculated by the Random Model

data source	no. of data points	r^2	slope	intercept
NMR vs calcd	12	0.996	1.031	−1.017
SFC vs calcd	12	0.995	0.951	+1.604
CHTGC vs calcd	9	0.994	0.933	+2.387
HPLC vs calcd	8	0.994	1.008	+0.476

To compare the data obtained from NMR, SFC, HPLC, CHTGC, and acid analysis to the respective calculated values, linear regressions were performed. Plots of experimental data against calculated weight percent values for LSS + SLS, LLS + LSL, and LLL are provided in Figures 6–9. The regression parameters for these plots are listed in Table 4. As is evident, there is a strong linear correlation between experiment and random theory for data obtained by NMR, SFC, HPLC, and CHTGC. In all four cases examined, slopes near the expected unit values were obtained.

The apparent agreement between the calculated compositions and those determined by multiple and independent experimental probes supports a random reaction model for the interesterification of SCFA and LCFA triglycerides. Therefore, given the amounts and types of triglyceride reactants, a reasonable approximation of the composition of a triacylglycerol product can be predicted by the application of published equations (Rozendaal, 1989; Sreenivasan, 1978). It should also be pointed out that the random reaction model for the products of the interesterification of SCFA and LCFA triglycerides can also be extended to the relative amounts of isomers in respective isomeric pairs. Examination of both the NMR and CHTGC data has shown that for the LSS + SLS and LLS + LSL isomeric pairs, the nonsymmetrical isomer occurs at approximately twice the concentration of the symmetrical form, as would be expected by the random reaction model. A detailed discussion of relative isomer composition is beyond the scope of this paper. A detailed discussion of the application of ^{13}C NMR to identify and quantitate isomer content in SCFA/LCFA containing triacylglycerols is available (Henderson et al., 1994).

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