Lipase-Mediated Acidolysis of Tristearin with CLA in a Packed-Bed Reactor: A Kinetic Study

Carlos F. Torres, Farida Munir, Louis P. Lessard, and Charles G. Hill Jr.*

Department of Chemical Engineering, University of Wisconsin–Madison, Madison, Wisconsin 53706

ABSTRACT: Solvent-free acidolysis of tristearin with CLA has been carried out in a packed-bed reactor. An immobilized lipase from *Thermomyces lanuginosa* (Lipozyme TL IM) was employed as the biocatalyst. Elevated temperatures (75°C) were utilized to eliminate solid substrates. The reaction kinetics were modeled by using a rate equation of the general Michaelis–Menten form. Both the extent of incorporation of CLA and the extent to which FFA were released were investigated. Positional analysis of the purified TAG obtained after a pseudo space time of 0.6 h indicated that CLA was preferentially incorporated at the *sn*-1,3 positions of the glycerol backbone, although 10% of the *sn*-2 positions were occupied by CLA residues. At a pseudo space time of 0.6 h, 38% of the initial CLA was incorporated in acylglycerols; the associated extent of hydrolysis was 8.3%.

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KEY WORDS: Acidolysis, conjugated linoleic acid, lipase, packed bed reactor, structured lipids, tristearin.

In the past decade numerous industrial and academic research groups have investigated the use of enzymatic processes to produce modified fats and oils (structured TAG) that can be employed in the formulation of nutraceuticals. These products are foods containing constituents that confer therapeutic or preventative medicinal benefits when ingested as part of the human diet.

Semisolid fats are normally produced by hydrogenation, fractionation, or chemical interesterification (1,2). However, partial hydrogenation processes form products containing *trans* FA that may produce adverse health effects (3). Recent research efforts to address this problem have emphasized lipase-catalyzed interesterification reactions (4). Use of *sn*-1,3 specific lipases offers the possibility of preferentially substituting beneficial FA residues for undesirable residues at the *sn*-1 and *sn*-3 positions on the glycerol backbone. Hence, this approach provides positional specificity in producing modified fats in contrast to the random distribution obtained *via* chemical interesterification (5). Lipase-mediated reactions occur under mild processing conditions, and consumers may view the resulting products as "natural" products rather than chemically modified substances (6).

Bloomer *et al.* (7) reported that the level of DAG produced during lipase-catalyzed interesterification in a stirred batch reactor is about 10%. Reaction parameters such as temperature, holding time in a batch reactor, and water content of the process fluids are all in part responsible for the level of DAG in the product mixture. Another important factor to be considered is the phenomenon of acyl migration, which occurs when DAG are present in the process fluid. Factors such as temperature, water content, reaction time, and the nature of the support used for immobilization of the lipase also influence the rate and the extent of acyl migration. In this respect, a particular disadvantage of employing stirred tank reactors in either a batch or continuous flow mode is the potential for rupture of the particles supporting the immobilized lipase, thereby exposing the surface of the support for the lipase (8). Such rupture not only reduces the potential for reutilization of the immobilized lipase in a subsequent run but may also facilitate migration of acyl groups.

In the present work, we investigated the acidolysis of tristearin with CLA in a continuous-flow packed-bed reactor in an effort to better understand possible adverse effects (formation of DAG and acyl migration) associated with the use of stirred tank reactors. When this reaction is carried out in a packed-bed reactor, solid tristearin must be melted (melting point = 74°C) prior to entering the reactor. Because of the necessity of employing this relatively high temperature to melt the feedstock, it is desirable to employ reaction conditions that minimize undesired secondary reactions of the indicated types. Tubular packed-bed reactors offer a number of advantages for this application.

CLA is a FA that has attracted the attention of nutrition experts and the food industry because of its potential for incorporation into foods marketed as nutraceuticals. The term CLA refers to a mixture of geometrical and positional isomers of linoleic acid (C18:2) containing conjugated double bonds. The richest natural sources of CLA are animal fats, especially milkfat and meat from ruminant animals (9). Commercially available CLA is prepared by chemical isomerization, and analysis of these preparations by silver ion HPLC (10) indicates that there are at least 12 different peaks associated with various isomers of CLA. Anticarcinogenic effects (11), decreased risk of atherosclerosis (12), and many other health benefits (13) have been attributed to consumption of CLA. The *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers of CLA are the isomers believed to be primarily responsible for the beneficial physiological effects of this substance. CLA is a particularly intriguing substance for use in the production of modified fats and oils.

*To whom correspondence should be addressed at Department of Chemical Engineering, University of Wisconsin–Madison, 1415 Engineering Dr., Madison, WI 53706. E-mail: hill@engr.wisc.edu

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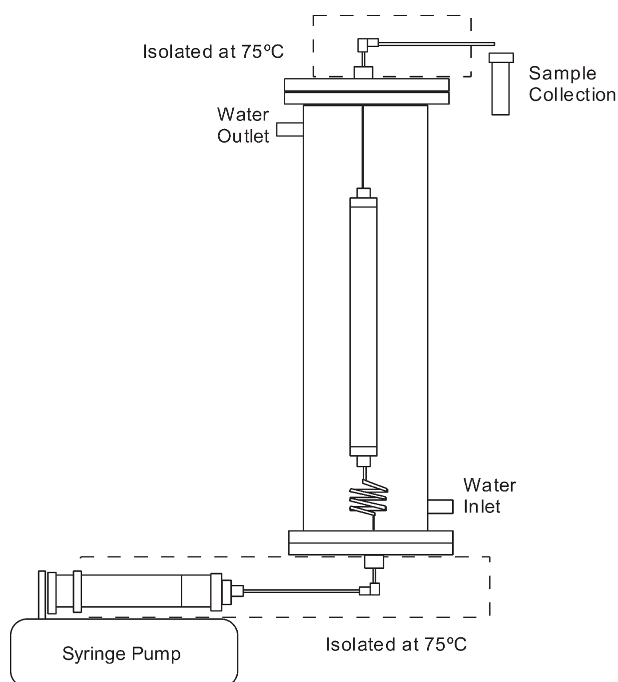
Differences in absorption and metabolism of various FA have been attributed to their position in the precursor TAG. It has been established that FA at the *sn*-2 position are more effectively absorbed in the digestive tract (14). However, it has also been reported that the digestive tracts of the rat, dog, and human have low capacities for emulsifying and digesting stearic acid from tristearin (15). Consequently, restructuring tristearin to obtain 1,3-CLA-2 stearoyl-glycerol offers an interesting route for producing a structured fat with properties different from the precursor tristearin, in particular, a TAG with enhanced nutritional and physiological benefits that could be readily ingested as a constituent of a wide variety of food products (dairy spreads, frozen desserts, salad dressings, etc.).

MATERIALS AND METHODS

Tristearin (90% pure and with a palmitic acid content of *ca.* 10%) was obtained from ICN Biomedicals (Aurora, OH) and type II porcine pancreatic lipase (L-3126) from Sigma (St. Louis, MO). CLA (C18:2) was kindly provided by Natural Asa (Hovdebygda, Norway). As reported by the vendor, the area percentages of the different isomers present in the CLA were: 0.42% *c*9,*c*12; 42.73% *c*9,*t*11; 44.24% *t*10,*c*12; 1.33% *c*9,*c*11; 1.35% *c*10,*c*12; 1.19% (*t*9,*t*11 + *t*10,*t*12). The balance of the vendor's CLA (8.74%) consisted of other FA (primarily oleic acid). Lipase TL IM was donated by Novo Nordisk (Franklinton, NC). Hexadecane (internal standard) was obtained from Aldrich (Milwaukee, WI). All solvents used were HPLC grade.

Apparatus. The packed-bed reactor (Scheme 1) consisted of 10 cm of tubing (0.64 cm i.d.) containing 1.9 g of immobilized enzyme. The tubing was packed manually with the dry immobilized enzyme. The packing was then fixed in place using a mesh connected to stainless steel plugs. This tubing was submerged in a constant temperature water bath. A syringe pump (model 220; KD Scientific, New Hope, PA) was used to supply the mixture of CLA and tristearin. To melt the reaction mixture, a 50-mL glass syringe was held at 75°C in a heater jacket. The inlet and outlet tubing were also held at 75°C with heating tape.



SCHEME 1

Operating protocol. Prior to initiating flow of CLA and tristearin to the reactor, nitrogen was passed through the packed bed for 5 min to remove air. Each experiment was initiated by quickly flushing the reactor with a total volume of the mixture of substrates equal to at least twice the void volume of the reactor. After quasi-steady state operating conditions were achieved, several samples of the effluent stream were manually collected over a time frame corresponding to at least three reactor space times. (The reactor space time is the ratio of the void volume of the reactor to the total volumetric flow rate of the two feedstocks.) For a set of experiments corresponding to a specified operating temperature, the experiments at different space times were conducted in random order.

Determination of the void volume and the space time. The void volume (1.3 cm^3/g of catalyst) was calculated using the difference between the weight of the packed-bed reactor (tubing + catalyst) when filled with a FA of known density, and the corresponding weight of the packed-bed reactor in the absence of this fluid. Corrections were made for the regions of the tubing outside the packed bed. Reactor space times were calculated as the ratio of the void volume of the reactor to the total volumetric flow rate of the two feedstocks.

Analytical methods. Samples (100 µL) of the product oil stream containing 7% (w/w) hexadecane (used as internal standard) were mixed with 1400 µL of chloroform. Aliquots of the resulting transparent solution (400 µL) were methylated by addition of 1 mL of methanolic NaOH (0.1 N). This mixture was then allowed to stand for 30 min at 60°C. Then 200 µL water was added. The resulting mixture was extracted with two 1-mL portions of *n*-hexane. The pooled extracts were dried with sodium sulfate and then centrifuged for 2 min at 5035 × *g*. One microliter of sample was injected into a Hewlett-Packard (Avondale, PA) gas chromatograph (Model 5890, series II) fitted with a 60-m HP Supelcowax 10 column (0.32 mm i.d.). Injector and detector temperatures were 220 and 230°C, respectively. The temperature program was as follows: starting at 100°C and then heating to 180°C at 20°C/min, followed by heating from 180 to 220°C at 15°C/min. The final temperature (220°C) was held for 30 min.

Purification of TAG from the reaction mixture. Separation and recovery of the TAG were accomplished *via* solid phase extraction on 1 g silica precision extraction Accubond cartridges from J&W Scientific (Folsom, CA) (16). The columns were conditioned by washing with 4 mL hexane, taking care to prevent them from becoming dry. The sample (10 mL containing TAG at a concentration of *ca.* 10 mg/mL as determined by stoichiometric calculations) was applied to a column and then eluted under vacuum (5 mm Hg) with solvent mixtures of increasing polarity: first, 8 mL hexane/diethyl ether (200:3, vol/vol); second, 40 mL hexane/diethyl ether (96:4, vol/vol).

The eluate from the second elution (containing the TAG) was collected and evaporated for subsequent analysis of the distribution of FA residues along the glycerol backbone.

Positional distribution of FA residues in TAG. Modified versions of the methods of Luddy *et al.* (17) and Williams *et al.* (18) were employed to release FA from the *sn*-1,3 positions of acylglycerols. A known weight of TAG and an appropriate (*ca.* 20–50 mg) weight of porcine pancreatic lipase were added to a 60-mL stoppered flask. Next, 0.65 mL Tris buffer (1 M sodium salt, pH 8.0), 0.35 mL sodium borate (0.19 M), 0.1 mL $CaCl_2$ (22%, w/w), and 0.25 mL bile salts (0.1%, w/w) were added. The resulting mixture (pH = 7.91) was maintained at 40°C for 1 min without shaking, then shaken at 300 rpm at 40°C for 7 min. The reaction was stopped by addition of 1 mL acetic acid (0.1 M). The mixture was extracted three times with 1 mL chloroform. The pooled organic phases were passed through a 0.45 µm syringe filter and then methylated with 0.1 M methanolic NaOH as described above. This protocol provides information concerning the distribution of FA residues at the *sn*-2 position. The distribution of FA residues at the *sn*-1,3 positions was then calculated by subtracting the amount of a FA residue at the *sn*-2 position from the total quantity of this FA present in the corresponding unhydrolyzed acylglycerols as determined by GC.

Statistical analysis. Proposed rate expressions were fit to the data using GREG, a general nonlinear regression package (Stewart, W.E., M. Caracotsios, and J.P Sorensen, GREG user's manual, Department of Chemical Engineering, University of Wisconsin–Madison, 1992). This program uses the residual sum of squares (RSS) as the objective function.

An extra sum of squares test (19) was used to discriminate between the mathematical models. Quantile values for Fisher's F distribution were determined using XLISP-STAT version 2.1, release 2, 1989 (20). The numerical integration was accomplished using a fourth-order Runge–Kutta–Felburg method (21). The SE was estimated using a first-order approximation of the mathematical model.

RESULTS AND DISCUSSION

To quantify the rate expression for the acidolysis reaction, we employed the generalized forms of the Michaelis–Menten rate expressions proposed by Torres and Hill (22). The proposed reaction mechanism is shown in Figure 1. For the purpose of modeling the reaction kinetics, two assumptions are made: (i) the step involving rupture of the ester bond is the rate-determining step in the interesterification reaction, and (ii) the water concentration is assumed to be constant throughout the reaction. The rate of release of FA residues by hydrolysis is equal to the rate of appearance of free stearic acid (r_S). This rate and the rate of disappearance of the replacement CLA ($-r_L$) can be written for the rate-limiting steps (see Fig. 1) as

$$-r_L = k_C[\text{F-CLA-IG}] - k_D[\text{E-A}_2] \quad [1]$$

$$r_S = k_A[\text{E-A}_1] - k_B[\text{F-S-IG}] \quad [2]$$

The concentrations of the different enzymatic complexes ([F-CLA-IG], [E-A_2], [E-A_1], [F-S-IG]) can be expressed in terms of the concentration of the free enzyme by means of pseudo-equilibrium relationships. Combination of these equations followed by partial normalization of the intermediate equation to facilitate the nonlinear regression analysis leads to rate expressions of the forms indicated in Equations 3 and 4 (Model I):

$$-r_L = \frac{\Psi_1[\text{IG}][\text{CLA}] - \Omega_1[\text{A}]}{1 + K_1[\text{S}]} \quad [3]$$

$$r_S = \frac{\Omega_2[\text{A}] - \Psi_2[\text{IG}][\text{S}]}{1 + K_1[\text{S}]} \quad [4]$$

where ($-r_L$) is the rate of disappearance of the replacement FA (CLA) and (r_S) represents the net rate of release of the original FA residues (stearic) by hydrolysis. IG, CLA, A, and S represent the concentrations of the lower acylglycerol species formed as an intermediate, the incoming replacement FA, total ester bonds, and the FA (stearic acid) released from the precursor tristearin, respectively. The rate expressions in Equations 3 and 4 are of the general Michaelis–Menten form. The parameters of the rate expressions [Ψ_1, Ω_1, Ω_2, Ψ_2, and K_1] in Equations 3 and 4 are related to the rate constants in Figure 1 as indicated elsewhere (22).

Model I.1 includes only parameters for the forward reaction and deactivation of the enzyme. It does not account for either inhibition effects or the reversibility of the reaction. Model I.2 contains two additional parameters related to the reversibility of the reaction but omits inhibition effects. Model I.3 incorporates the effects of both inhibition by FFA and the reversibility of the reaction. All three models incorporate the effects of deactivation of the enzyme, although Model I.1 may be characterized by a very different value of the deactivation rate constant because of its failure to account for either inhibition or reversibility of the reaction.

In order to establish the effects of the pool of FA on the apparent inhibition constant K_1, a second model (Model II, Eqs. 5 and 6) was also considered. In this case, both FA present in the reaction mixture, [S] and [CLA], were lumped together to assess their combined effect on the inhibition constant K_1:

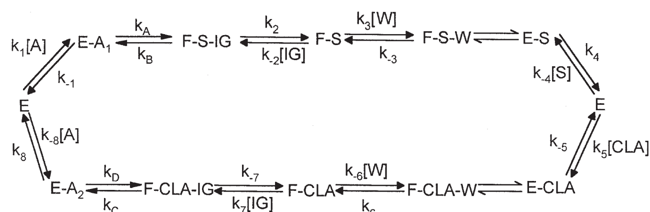


FIG. 1. Schematic representation of the acidolysis reaction. A_1 = native ester bond (reactant glyceride), S = stearic acid liberated from the original tristearin, IG = lower glyceride intermediate, W = water, CLA = new FA to be incorporated, A_2 = new ester bond formed with the new FA, F = complex between the enzyme and the species defined above, E = enzyme.

$$-r_L = \frac{\Psi_1[\mathrm{IG}][\mathrm{CLA}] - \Omega_1[\mathrm{A}]}{1 + K_1[\mathrm{S} + \mathrm{CLA}]} \quad [5]$$

$$r_S = \frac{\Omega_2[\mathrm{A}] - \Psi_2[\mathrm{IG}][\mathrm{S}]}{1 + K_1[\mathrm{S} + \mathrm{CLA}]} \quad [6]$$

It is also important to account for thermal deactivation of the enzyme in both models by incorporating a factor that quantifies this phenomenon. Deactivation was treated as if it occurs in a single step. Several models of deactivation kinetics were tested for their ability to fit the data. In Model II.1 it is presumed that the loss of enzymatic activity with time can be regarded as statistically insignificant. For Model II.2 it is assumed that the irreversible deactivation of the active enzyme (E) to an inactive form (E_d) obeys first-order kinetics,

$$E \xrightarrow{k_d} E_d \quad [7]$$

The manner in which the enzyme activity varies with time is then given by

$$a\{t\} = a_0 \exp\left[-k_d\left(t - t_0\right)\right] \quad [8]$$

where t is time; t_0 is the time at which the set of experiments is started; $a\{t\}$ is the activity of the enzyme at a particular time t; a_0 is the activity of the enzyme at time t_0; and k_d is the first-order deactivation rate constant. The parameter a_0 is arbitrarily assigned a value of 1, and subsequent activities are thus normalized with respect to this initial activity. Thus, multiplication of the rate expressions in Equations 3–6 by the factor $\exp[-k_d(t - t_0)]$ yields the corresponding activity-dependent forms of the rate expressions.

The mathematical models used in the analysis of the data thus can incorporate the effects of the rate of enzyme deactivation in the generalized Michaelis–Menten expressions for the rates of disappearance of the incoming replacement FA and the release of FFA from the precursor TAG.

In the analysis of the data for the continuous-flow packed-bed reactor, the activity-dependent forms of the rate expressions are combined with the differential form of the design equation for a plug flow reactor (23). Solution of the resulting set of differential equations then gives the composition of the effluent from the packed bed. Thus, all of the models investigated employ two independent variables to characterize two important characteristic times for these reactors. To characterize the loss of enzyme activity, the variable of importance is the time elapsed since the start of the experiments ($t - t_0$). The amount of CLA incorporated in the TAG and the amount of free stearic acid released from the precursor TAG not only depend on the reactor space time but also the enzymatic activity during the period between the time the reaction mixture enters the reactor and the time it exits the reactor. Graphical depictions of the resulting functional dependence thus require the use of a 3-D plot. However, to obtain plots that can be more readily interpreted, one may conveniently employ the concept of pseudo space time to permit representation of the data in two dimensions (24). The pseudo space time represents a mathematical combination of the time elapsed since the start of the series of experiments and the real reactor space time. The pseudo space time is defined as

$$\tau^* = \int_{t-\tau}^{t} a\{\hat{t}\} d\hat{t} \quad [9]$$

where τ^* is the pseudo space time, τ is the real space time, and $\hat{t}$ is a dummy variable. In physical terms, when the rate of deactivation of the enzyme is negligible, the pseudo space time becomes equal to the reactor space time. (In the present situation the space time is numerically equal to the mean residence time of the fluid in the reactor.) When the rate of deactivation is significant, the difference between the pseudo space time and the actual space time indicates how much longer the fluid must remain in the reactor to achieve the conversion that would have been achieved if the enzyme had not been partially deactivated. In terms of Equations 8 and 9,

$$\tau^* = \frac{a_0}{k_d}\left\{\exp\left[-k_d\left(t - t_0 - \tau\right)\right] - \exp\left[-k_d\left(t - t_0\right)\right]\right\} \quad [10]$$

Table 1 contains the extra determinant analysis of the different models tested for their ability to fit the data. The test criterion for the 95% confidence level was that if the P-value was less than 0.05, then the model with fewer degrees of freedom was selected as being more appropriate in a statistical sense. Examination of the tabular entries for progressively more complex models leads to the conclusion that Model I.3 is the most appropriate model of those considered thus far, i.e., the effects of reversibility and enzyme inhibition must be accounted for.

To assess the importance of inactivation of the enzyme in determining the observed rates of reaction, Models II.1 and II.2 were developed. In Model II.1 the loss of enzymatic activity with time was regarded as insignificant. In Model II.2 it is assumed that the deactivation of the enzyme obeys first-order kinetics. The P-values of the extra sum of squares test

TABLE 1
Extra Determinant Analyses of Model I.1 vs. Model I.2, Model I.2 vs. Model I.3, and Model II.1 vs. Model II.2 for the Acidolysis of Tristearin with CLA

Source	SS[a]	DF	MSS[b]	Observed F ratio[c]	P-value[d]
Extra	395,459	2	197,729	8.47	0.0014
Model I.1	630,120	27	23,338		
Model I.2	234,661	25			
Extra	40,951	1	40,951	4.36	0.047
Model I.2	234,661	25	9,386		
Model I.3	193,710	24			
Extra	531,105	1	531,105	18.58	0.0002
Model II.1	714,660	25	28,586		
Model II.2	183,555	24			

[a]Sum of squares of residuals.
[b]Mean sum of squares of residuals.
[c]Extra mean sum of squares/mean sum of squares of full model.
[d]P-values below 0.05 indicate that the model with fewer DF provides a better fit of the data than the model with more DF.

(Table 1) are much less than 0.05, a result that clearly indicates that deactivation of the enzyme plays an important role in the kinetic model.

Finally, to discriminate between Models I.3 and II.2, one must consider factors such as patterns in the residuals and magnitudes of the residuals. A residual is the difference between the actual response and the predicted response. In addition, the 95% confidence intervals of the parameters should also be inspected to ensure that the range is reasonable. The parameter estimates for all five models are shown in Table 2. Models I.3 and II.2 provide similar values for the residual sum of squares and similar confidence intervals for the parameter estimates. However, since use of a common value of the inhibition constant (K_1) for all FA present in the reaction mixture has previously been reported to be adequate for the kinetics of analogous hydrolysis reactions (25), we concluded that Model II.2 is more realistic than Model I.3 in which K_1 is affected only by the concentrations of the FFA released by hydrolysis.

Figures 2 and 3 permit the reader to visually assess the fits of the various models and indicate how the values of the concentrations predicted by applying Equations 3 and 4 (Models I.1–I.3), and by applying Equations 5 and 6 (Models II.1 and II.2), respectively, approximate the observed values. In examining these figures, it is important for the reader to recognize that the same set of raw data is used to test the various models. The set of dependent variables (namely, the concentrations of CLA and stearic acid) are the same in all five panels, but the pseudo space times differ because these times encompass not only the actual space time but also the effect of enzyme deactivation as reflected in the values of k_d determined in the several regression analyses. Visual assessments indicate that Models I.3 and II.2 both provide good fits of the data, but Model II.2 is preferred for the reasons stated previously. The data in Figures 2 and 3 also indicate that at long pseudo space times the consumption of CLA (in molar percentage) totals *ca.* 56%. (This value corresponds to 23 mol% of the FA residues in the original tristearin being converted

TABLE 2
Parameter Estimates for the Best Fits of the Experimental Data for the Interesterification Reaction for the Different Models Considered

Model	Parameters	Parameter value	Confidence interval
I.1	Ψ_1	7.81×10^{-4}	$\pm 4.25 \times 10^{-4}$
	Ω_2	1.03×10^{-1}	$\pm 2.87 \times 10^{-2}$
	k_d	7.50×10^{-3}	$\pm 7.04 \times 10^{-3}$
I.2	Ψ_1	1.42×10^{-2}	$\pm 3.57 \times 10^{-3}$
	Ω_1	5.74×10^{0}	$\pm 1.10 \times 10^{0}$
	Ω_2	4.36×10^{-1}	$\pm 9.98 \times 10^{-2}$
	Ψ_2	1.57×10^{-3}	$\pm 5.42 \times 10^{-4}$
	k_d	2.95×10^{-2}	$\pm 4.48 \times 10^{-3}$
I.3	Ψ_1	1.96×10^{-2}	$\pm 1.65 \times 10^{-2}$
	Ω_1	7.70×10^{0}	$\pm 7.11 \times 10^{0}$
	Ω_2	8.47×10^{-1}	$\pm 3.55 \times 10^{-1}$
	Ψ_2	2.79×10^{-3}	$\pm 1.43 \times 10^{-3}$
	K_1	2.70×10^{-3}	$\pm 1.72 \times 10^{-3}$
	k_d	3.22×10^{-2}	$\pm 6.17 \times 10^{-3}$
II.1	Ψ_1	1.57×10^{-2}	$\pm 1.14 \times 10^{-2}$
	Ω_1	1.05×10^{-3}	Not determined
	Ω_2	8.44×10^{-3}	$\pm 3.63 \times 10^{-2}$
	Ψ_2	0.00×10^{0} (lower limit)	Not determined
	K_1	3.34×10^{-4}	$\pm 4.05 \times 10^{-4}$
II.2	Ψ_1	2.66×10^{-2}	$\pm 2.67 \times 10^{-2}$
	Ω_1	1.47×10^{0}	$\pm 1.10 \times 10^{+1}$
	Ω_2	9.95×10^{0}	$\pm 9.36 \times 10^{-1}$
	Ψ_2	5.35×10^{-3}	$\pm 3.48 \times 10^{-3}$
	K_1	8.55×10^{-6}	$\pm 6.49 \times 10^{-6}$
	k_d	3.32×10^{-2}	$\pm 6.81 \times 10^{-3}$

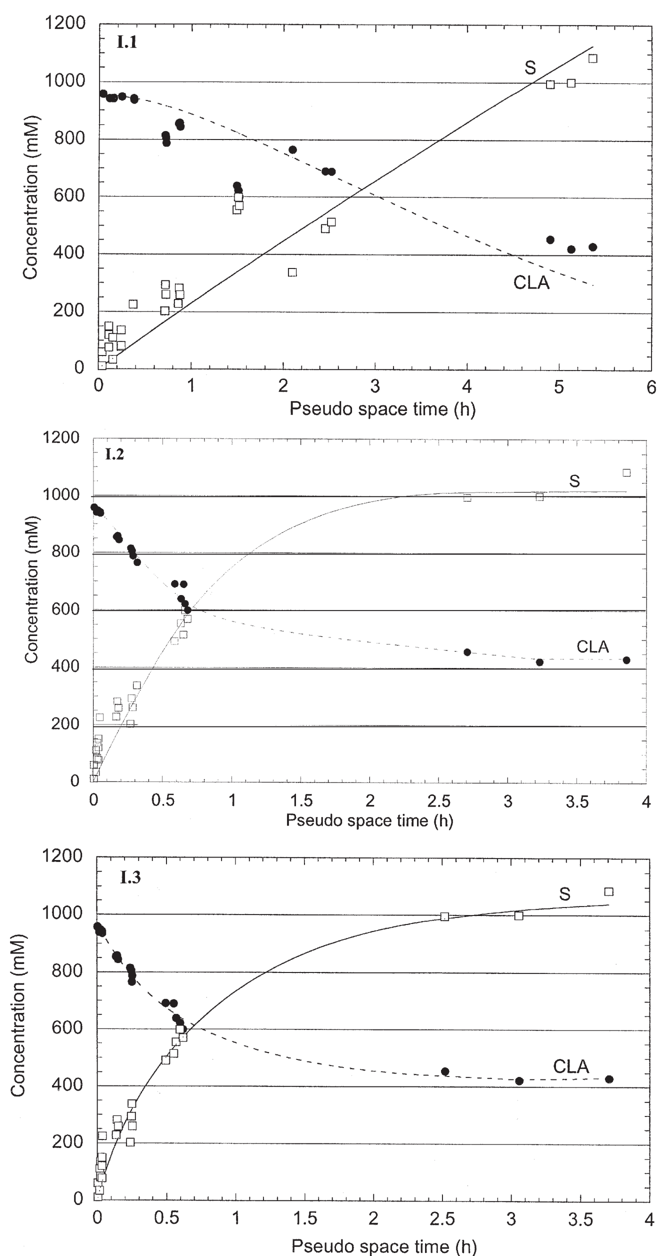


FIG. 2. Comparison of predicted values with experimental data for the acidolysis reaction. Panel labels refer to Models I.1, I.2, and I.3. Initial concentrations of the reagents: 2262 mM tristearin (expressed in terms of equivalents of FA residues), 958 mM CLA. CLA = consumption of CLA: experimental value (●), predicted value (----); S = FFA released: experimental value (□), predicted value (—).

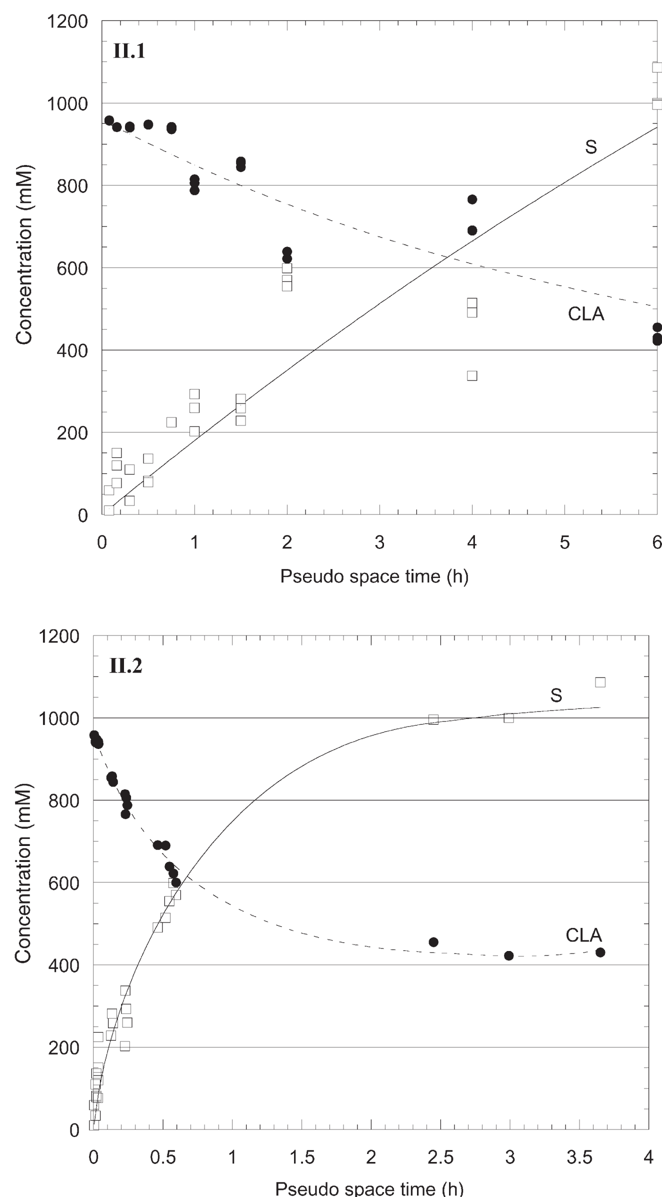


FIG. 3. Comparison of the predicted values with experimental data for the acidolysis reaction. Panel labels refer to Models II.1 and II.2. Initial concentrations of the reagents: 2262 mM tristearin (expressed in terms of equivalents of FA residues), 958 mM CLA. CLA = consumption of CLA: experimental value (●), predicted value (----); S = FFA released: experimental value (□), predicted value (—).

from stearic acid to CLA.) However, the total concentration of ester bonds (A in Fig. 1) decreased from *ca.* 2300 mM to 1850 mM at the end of the reaction. This value indicates a hydrolysis level of *ca.* 20%.

Because it was necessary to use a temperature of 75°C to ensure that all the tristearin in the packed-bed reactor employed in the present study was a liquid throughout the process, the level of hydrolysis was expected to be higher than the levels previously reported for interesterification reactions (7). This relatively high level of hydrolysis was obtained at the largest value of the pseudo space time studied. High levels of hydrolysis have previously been observed at temperatures above 60°C (26). Consequently, one needs to establish a compromise between the extent of incorporation of CLA and the quantity of FFA residues released. At a pseudo space time of 0.6 h, the conversion of CLA is 38% (mol) (16% of the residues in the acylglycerols were CLA), but the hydrolysis level was less than 8.3% (mol) of the original tristearin.

TABLE 3
Positional Distributions of FA (in mol%) in the TAG Products of the Acidolysis of Tristearin with CLA After a Pseudo Space Time of 0.69 h

	Percentage of total	Percentage of *sn*-1,3	Percentage of *sn*-2
Palmitic acid	8.21	8.47	7.71
Stearic acid	69.24	66.12	75.49
Oleic acid	1.62	1.88	1.10
CLA	15.92	18.53	10.69

The positional distribution of CLA in the final TAG product was also investigated. The results of the positional analysis of the purified TAG obtained at a pseudo space time of 0.59 h are shown in Table 3. The presence of palmitic acid and oleic acid in the interesterified products arises because these two FA are present as impurities in the tristearin and CLA, respectively. Inspection of the tabular entries indicates that 18.5% of the *sn*-1,3 positions are occupied by CLA and 10% of the *sn*-2 positions contain CLA. The presence of CLA at the *sn*-2 position of the acylglycerol is an indication that acyl migration occurs. However, for this particular reaction the presence of CLA at the *sn*-2 position has no negative implications with regard to intestinal absorption of this FA residue. By contrast, it is well known that FA at the *sn*-2 position are readily absorbed in the gastrointestinal tract, regardless of the type of FA residue present at this position (14).

The process described herein permits one to transesterify tristearin with CLA in a packed-bed reactor under solvent-free conditions. The conversion of free CLA into glyceride-bound FA residues was *ca.* 50% at the longest pseudo space time employed (3.65 h). However, one must strike a compromise between this conversion and the concomitant level of hydrolysis. A reasonable compromise is a pseudo space time of 0.59 h, at which the conversion of CLA is 38% and the level of hydrolysis is 8%. Higher molar ratios of CLA to tristearin would produce higher levels of incorporation of CLA, although lower percentage conversions of CLA would be obtained. The most appropriate mathematical model of the reaction kinetics (Model II.2) is based on the use of a combination of Equations 5, 6, and 8 so that the effects of inhibition by both CLA and stearic acid, of the reversibility of the reaction, and of deactivation of the enzyme are all accounted for.

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REFERENCES

1. List, G.R., T.L. Mounts, F. Orthoefer, and W.E. Neff, Margarine and Shortening Oils by Interesterification of Liquid and Trisaturated Triglycerides, *J. Am. Oil Chem. Soc. 72*:379–382 (1995).
2. List, G.R., R. Pelloso, F. Orthoefer, M. Chrysam, and T.L. Mounts, Preparation and Properties of Zero *Trans* Soybean Oil Margarines, *Ibid. 72*:383–384 (1995).
3. Aro, A., M. Jauhiainen, R. Partanen, I. Salminen, and M. Mutanen, Stearic Acid, *Trans* Fatty Acids, and Dairy Fats: Effect on Serum and Lipoprotein Lipids, Apolipoproteins, Lipoprotein(a), and Lipid Transfer Proteins in Healthy Subjects, *Am. J. Clin. Nutr. 65*:1419–1426 (1997).
4. Zainal, Z., and M.S.A. Yusoff, Enzymatic Interesterification of Palm Stearin and Palm Kernel Oil, *J. Am. Oil Chem. Soc. 76*:1003–1008 (1999).
5. Shimada, Y., A.K. Sugihara, H. Nakano, T. Yokota, T. Nagao, S. Komemushi, and Y. Tominaga, Production of Structured Lipids Containing Essential Fatty Acids by Immobilized *Rhizopus delemar* Lipase, *Ibid. 73*:1415–1420 (1996).
6. Rousseau, D., and A.G. Marangoni, Chemical Interesterification of Foods Lipids: Theory and Practice, in *Food Lipids: Chemistry Nutrition and Biotechnology,* edited by C.C. Akoh and D.B. Min, Marcel Dekker, New York, pp. 251–281 (1998).
7. Bloomer S., P. Adlecreutz, and B. Mattiasson, Triglyceride Interesterification by Lipases 2. Reaction Parameters for the Reduction of Trisaturated Impurities and Glycerides in Batch Reactions, *Biocatalysis 5*:145–162 (1991).
8. Xu, X, Enzymatic Production of Structured Lipids: Process Reaction and Acyl Migration, *inform 11*:1121–1131 (2000).
9. Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha, and M.W. Pariza, Dietary Sources of Conjugated Dienoic Isomers of Linoleic Acid, a Newly Recognized Class of Anticarcinogens, *J. Food Comp. Anal. 5*:185–197 (1992).
10. Sehat, N., M.P. Yurawecz, J.A.G. Roach, M. Mossoba, J.K.G. Kramer, and Y. Ku, Silver Ion High-Performance Liquid Chromatographic Separation and Identification of Conjugated Linoleic Acid Isomers, *Lipids 33*:217–221 (1998).
11. Ha, Y.L., N.K. Grimm, and M.W. Pariza, Anticarcinogens from Fried Ground Beef: Heat-Altered Derivatives of Linoleic Acid, *Carcinogenesis 8*:1881–1887 (1987).
12. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994)..
13. Chin, S.F., J.M. Storkson, K.J. Albright, M.E. Cook, and M.W. Pariza, Conjugated Linoleic Acid Is a Growth Factor for Rats as Shown by Enhanced Weight Gain and Improved Feed Efficiency, *J. Nutr. 124*:2344–2349 (1994).
14. Haumann, B.F., Structured Lipids Allow Fat Tailoring, *inform 8*:1004–1011 (1997).
15. Livesey, G., The Absorption of Stearic Acid from Triacylglycerols: An Inquiry and Analysis, *Nutr. Res. Rev. 13*:185–214 (2000).
16. Prieto, J.A., A. Ebri, and C. Collar, Optimized Separation of Nonpolar and Polar Lipid Classes from Wheat Flour by Solid Phase Extraction, *J. Am. Oil Chem. Soc. 69*:387–391 (1992).
17. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman, and R.W. Riemenschneider, Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *Ibid. 41*:693–696 (1964).
18. Williams, J.P., M.U. Khan, and D. Wong, A Simple Technique for the Analysis of Positional Distribution of Fatty Acids on Di- and Triacylglycerols Using Lipase and Phospholipase A_2, *J. Lipid Res. 36*:1407–1412 (1995).
19. Bates, D.M., and D.G. Watts, *Nonlinear Regression Analysis and Its Applications,* John Wiley & Sons, New York, 2001.
20. Tierney, L., XLISP-STAT: A Statistical Environment Based on the XLISP Language (Version 2.0), Technical Report No. 528, School of Statistics, University of Minnesota, Minneapolis, 1989.
21. Press, W.H., B.P. Flannery, S.A. Teukolsky, and W.T. Vetterling, *Numerical Recipes in FORTRAN: The Art of Scientific Computing,* Press Syndicate of the University of Cambridge, Cambridge, 1992, 702 pp.
22. Torres, C., and C.G. Hill, Lipase Catalyzed Acidolysis of Menhaden Oil with Conjugated Linoleic Acid: Efffect of Water Content, *Biotechnol. Bioeng. 78*:509–516 (2002).
23. Hill, C.G., Jr., *An Introduction to Chemical Engineering Kinetics and Reactor Design,* John Wiley & Sons, New York, 1977, p. 263.
24. Malcata, F.X., C.G. Hill, and C.H. Amundson, Hydrolysis of Butteroil by Immobilized Lipase Using a Hollow Fiber Reactor. Part II. Uniresponse Kinetic Studies, *Biotechnol. Bioeng. 39*: 984–1001 (1992).
25. Lessard, L.P., and C.G. Hill, Effect of pH on the Production of Lipolyzed Butter Oil by a Calf Pregastric Esterase Immmobilized in a Hollow-Fiber Reactor: II. Multiresponse Kinetics, *Ibid. 70*:332–341 (2000).
26. Mu, H., X. Xu, J. Adler-Nissen, and C.E. Høy, Production of Structured Lipids by Lipase-Catalyzed Interesterification in a Packed Bed Reactor: Effect of Reaction Parameters on the Level of Diacylglycerols in the Products, *Fett/Lipid 101*:158–164 (1999).

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