Lipase-Catalyzed Interesterification Reaction Between Menhaden Oil and the Ethyl Ester of CLA: Uniresponse Kinetics

Carlos F. Torres, Betty Lin, Louis P. Lessard, and Charles G. Hill, Jr.*

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

ABSTRACT: The kinetics of the interesterification reaction between menhaden oil and the ethyl ester of CLA (CLAEE) in the presence of an immobilized lipase from *Rhizomucor miehei* was investigated. A 2^3 factorial design with a central point was used to define the experimental region. The factors considered were the molar ratio of reactants, the enzyme loading, and the temperature. Optimal results were obtained when the reaction was carried out at 55°C with an enzyme loading of 0.65 g per 12 g mixture and a mole ratio of CLAEE to menhaden oil equal to 0.13. A uniresponse kinetic model of the Michaelis–Menten type was developed to characterize the rate of consumption of CLAEE and the rate of release of FA ethyl esters from menhaden oil. The best fit of the data with a uniresponse model was obtained using a form based on reversible reactions and inhibition by the ethyl esters of the FA residues released by the reaction.

Paper no. J10525 in *JAOCS 80,* 873–880 (September 2003).

KEY WORDS: CLA, fish oil, interesterification, n-3 FA, *Rhizomucor miehei* lipase.

CLA is a FA that has attracted the attention of nutritional experts and the food industry because of its potential for incorporation into foods marketed as nutraceuticals. Nutraceuticals are foods (or parts of foods) that provide therapeutic or preventative medicinal values as well as nutritional benefits. The term CLA refers to a mixture of geometrical and positional isomers of linoleic acid (18:2) containing conjugated double bonds. The richest natural sources of CLA are animal fats, especially milk fat and meat from ruminant animals (1). Commercially available CLA is prepared by chemical isomerization, and analysis of these preparations by silver ion HPLC (2) indicates that there are at least 12 different peaks associated with various isomers of CLA. Anticarcinogenic effects (3), decreased risk of atherosclerosis (4), and many other health benefits (5) have been attributed to consumption of CLA. The *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of CLA are the isomers that are believed to be primarily responsible for the beneficial physiological effects of this substance. The n-3 PUFA (sometimes referred to as omega-3 FA) found in fish oils also provide health benefits relative to prevention of cardiovascular disease and certain cancers (6).

*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

One logical approach to facilitating ingestion of both CLA and n-3 FA is to produce fats and oils enriched in these substances *via* enzymatic processes. These fats and oils can be used to fortify foods to be sold as nutraceuticals, e.g., dairy spreads, frozen desserts, and salad dressings. Incorporation of CLA into fish oils by enzymatic reactions that selectively replace saturated FA and monounsaturated FA residues while leaving the n-3 FA residues relatively untouched permits one to produce TAG offering the benefits of both CLA and n-3 FA.

Several reports of enzymatic interesterification of fish oil are available in the literature (7–9). Acidolysis of fish oil with CLA has been studied (10,11). In these acidolysis reactions, the percentage of CLA esterified depended on the molar excess of CLA used, the selectivity of the lipase used toward the different FA residues present in the fish oil, and the balance reached between the hydrolysis and esterification reactions. To extend the results for acidolysis of fish oil, we investigated lipase-mediated interesterification of fish oil with CLA in ethyl ester form (CLAEE) to ascertain the rate of this process and the corresponding potential yield relative to those associated with the acidolysis reaction. A uniresponse kinetic model was used to characterize the kinetics of the reaction in terms of both the rate of incorporation of CLA and the rate of release of FA ethyl esters (FAEE) from the precursor fish oil.

MATERIALS AND METHODS

Materials. CLAEE was kindly provided by Natural ASA (Hovdebygda, Norway). As reported by the vendor, the area percentages from a GC analysis of the different isomers present in the CLA were: 40% *c*9,*t*11, 39.8% *t*10,*c*12, 2.2% other isomers, 2.5% stearic acid, 12.9% oleic acid. The balance of the CLA consisted of other FA. Menhaden oil was purchased from Sigma (St. Louis, MO). The immobilized lipase L9 (lipase from *Rhizomucor miehei*) was obtained from Biocatalytics (Pasadena, CA). According to the specifications of the vendor, the specific activity of lipase L9 is 8,000 U/g. All solvents used were HPLC grade from Fisher (Chicago, IL).

Interesterification reaction. Twelve grams of a mixture of menhaden oil and CLAEE (the relative proportions of these reactants differed for each molar ratio studied) was added to a 50-mL flask containing 1 g hexadecane (used as an internal standard) and mixed by swirling. An appropriate amount of the immobilized lipase L9 (between 3 and 6% by weight of the reactants) was then added. The flasks were stoppered and placed

in an orbital shaker (300 rpm) at different temperatures. Samples (200 µL) were withdrawn periodically for HPLC and GC analyses, and the flask was resealed after each sampling. All trials were allowed to proceed for 24 h.

Analysis of reaction products—GC. Samples from the interesterification reaction (200 µL) were mixed with 1800 µL of chloroform and immediately filtered with a 0.45 µm Whatman nylon syringe filter (Clifton, NJ). Aliquots (400 µL) were evaporated under nitrogen, redissolved in *n*-hexane, and dried with sodium sulfate.

Samples of the final transparent solution (400 µL) were ethylated by addition of 1 mL of a solution of sulfuric acid in ethanol (5%, vol/vol). This mixture was allowed to stand overnight at 50°C. After addition of 200 µL water, the resulting mixture was extracted with two 1-mL portions of *n*-hexane, and the final extract was then dried with sodium sulfate.

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 (Supelco, Bellefonte, PA; 0.32 mm i.d.). Injector and detector temperatures were set at 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. Identification of the various FA was based on a menhaden oil standard (#4-7085) obtained from Supelco. Identification of CLA and the associated retention time was accomplished by direct injection of the CLAEE.

Analysis of reaction products—HPLC. An Econosil-Silica 5U column (250 × 4.6 mm; Alltech, Deerfield, IL) with detection *via* ELSD (Alltech) was used for the HPLC analyses of reaction mixtures. Two mobile phases were used: Phase A consisted of *n*-hexane, 2-propanol, ethyl acetate, and formic acid (80:10:10:0.1, by vol), whereas phase B was *n*-hexane. The flow rate of the mobile phase was 1.5 mL/min. A splitter valve was used after the column, and only 50% of the mobile phase was directed through the detector.

The protocol used for the mobile phase involved linear elution gradients of 1% (vol/vol) A, increasing to 98% (vol/vol) A in 20 min. The 98:2 (vol/vol) mixture of A and B was used for 3 min. The system was next restored to its initial conditions by passing the 1:99 (vol/vol) A/B mixture through the column for 10 min. To conduct the analysis, 10 µL of sample (2.5 mg/mL in chloroform) was injected. The retention times for the TAG, FA, 1,3-DAG, 1,2-DAG, 1(3)-MAG, and 2-MAG were 8.5, 10.3, 11.3, 12.8, 21, and 22.3 min, respectively. Standards for these analyses were obtained from Sigma.

Mathematical modeling. To quantify the rate expression for the interesterification reaction, we used a modified version of the generalized forms of the Michaelis–Menten rate expressions proposed by Torres and Hill (12). The proposed reaction mechanism is shown in Figure 1. For the purpose of modeling the reaction kinetics, the following two assumptions were made: (i) The step involving rupture of the ester bond is the rate-determining step in the interesterification reaction, and (ii) the concentrations of ethanol ([Q]) and the intermediate lower glyceride ([IG]) are assumed to be essentially constant throughout the reaction. The total rate of release of FA residues is equal to the rate of appearance of FAEE (r_B). This rate and the rate of disappearance of CLAEE ($-r_L$) can be written for the rate-limiting steps (see Fig. 1) as:

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

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

The concentrations of the different enzymatic complexes ([F-CLA-IG], [E-A_2], [E-A_1], and [F-B-IG]) can be expressed in terms of the concentration of the free enzyme using pseudo-equilibrium relationships. Combination of these relationships with Equations 1 and 2, followed by partial normalization of the intermediate equation to facilitate the nonlinear regression analysis, leads to rate expressions of the following forms:

$$-r_L = \frac{\Psi_1[\text{CLAEE}] - \Omega_1[\text{A}]}{1 + K_1[\text{CLAEE}] + K_2[\text{B}]} \quad [3]$$

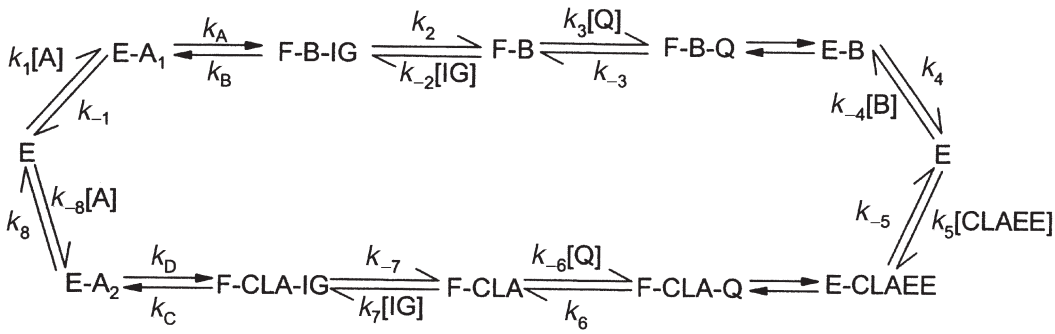

FIG. 1. Schematic representation of the interesterification reaction. E, uncomplexed nonacylated enzyme; F, acylated form of the enzyme; B, ethyl ester of FA residue liberated from the original menhaden oil; IG, lower glyceride intermediate; Q, ethanol; CLAEE, CLA ethyl ester to be incorporated, E-X, complexed form of the nonacylated enzyme with species X; F-X, complexed acylated form of the enzyme with species X.

$$r_B = \frac{\Omega_2[\mathrm{A}] - \Psi_2[\mathrm{B}]}{1 + K_1[\mathrm{CLAEE}] + K_2[\mathrm{B}]} \quad [4]$$

where $(-r_L)$ is the rate of disappearance of the CLAEE and (r_B) represents the net rate of release of the native FA residues. [CLAEE], [A], and [B] represent the concentrations of the incoming replacement FAEE, total ester bonds, and the FAEE released from the precursor menhaden oil, 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, K_1, and K_2) in Equations 3 and 4 are related to the rate constants in Figure 1 as indicated elsewhere (12).

Statistical analysis. Proposed rate expressions were fitted to the data using GREG, a general nonlinear regression package (13). This program uses the residual sum of squares as the objective function.

An extra sum of squares test (14) 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 (15). The numerical integration was accomplished using a fourth-order Runge–Kutta–Felburg method (16). The SE was estimated using a first-order approximation of the mathematical model.

RESULTS AND DISCUSSION

Factorial design. To explore a wide variety of experimental conditions for the interesterification reaction between CLAEE and menhaden oil, a 2^3 factorial design with a central point was utilized. Factor (parameter) settings are shown in Table 1. The nine different experimental trials involved determinations of product compositions vs. time over a 24-h period. Analysis of this type of factorial design using orthogonal polynomials has been utilized previously (11). However, in the present study this methodology was not appropriate for use because different aliquots were taken from the same batch reactor, a situation in which the results are not independent of one another. Instead, a kinetic model for the interesterification reaction was developed to fit the experimental data using a nonlinear regression analysis.

TABLE 1
Parameter Settings for Experimental Design[a]

	Factor settings		
Data set	MR	Temperature	Enzyme loading
A	0.13	55	0.65
B	2.21	55	0.65
C	0.13	55	0.35
D	2.21	55	0.35
E	0.13	35	0.65
F	2.21	35	0.65
G	0.13	35	0.35
H	2.21	35	0.35
K	1.17	45	0.50

[a]Factors: MR = molar ratio of the ethyl ester of CLA (CLAEE) to FA equivalents in menhaden oil; temperature (°C); and enzyme loading (g). Twelve-gram reaction mixture.

Level of hydrolysis. Interesterification reactions involve the simultaneous occurrence of both hydrolysis and re-esterification reactions. To facilitate the nonlinear regression analysis of the data for this set of experiments, the level of hydrolysis was assumed to be essentially constant throughout the course of the reaction. Inspection of Figure 2 reveals how the concentrations of the different acylglycerols present in the reaction mixture during the trial corresponding to the central point of the experimental design evolve as the reaction progresses. Because of difficulties in quantifying the FAEE by HPLC coupled with light-scattering detection, the percentage of acylglycerols in Figure 2 refers to the total FA present in the mixture in either free or residue form. For the central point of the experimental design, the concentrations of lower glycerides (i.e., 1,2- and 1,3-DAG) increased by *ca.* 6% from the initial value. No MAG were detected after 24 h of reaction. The increase in the concentration of lower glycerides indicates that there is some net hydrolysis in addition to the interesterification reaction. Because the increase in the concentration of the DAG never exceeded 10% of the initial concentration of these species, the assumption that the concentrations of DAG were substantially constant was validated.

Selection of the most appropriate model. Five increasingly complex models of the general form of Equations 3 and 4 were used to fit the nine different data sets. Model 1 included only the parameters for the forward reaction ($\Psi_{1,A-K}$ and $\Omega_{2,A-K}$). It did not account for either inhibition effects or the reversibility of the reaction. Model 2 contained two additional parameters ($\Omega_{1,A-K}$ and $\Psi_{2,A-K}$) related to the reversibility of the reaction, but omitted inhibition effects. To incorporate inhibition effects, three different approaches were considered: (i) Model 3 included individual terms for inhibition by both the replacement CLAEE and the FAEE released by hydrolysis ($K_{1,A-K}$ and $K_{2,A-K}$); (ii) Model 4 employed two common inhibition constants for both the replacement CLAEE and the FAEE released by hydrolysis reactions (K_1 and K_2); and (iii) Model 5 incorporated a common inhibition constant for inhibition by the FAEE released by hydrolysis reactions (K_2). Models 3, 4, and 5 all included kinetic parameters for the forward and reverse reactions and were analyzed for their ability to fit the composite data set for all nine trials.

Extra determinant analyses of the abilities of the different models to fit the data for all nine trials are presented in Table 2. Inspection of the different tabular entries for increasingly more complex models leads to the conclusion that of the models considered thus far, Model 5 is the most appropriate model. Hence, use of a common inhibition constant for the various FAEE is sufficient to quantify inhibition by these species. Moreover, the improvement in the fit associated with incorporation of the parameter K_1 for inhibition by the ethyl ester of CLA does not compensate the reduction in the degrees of freedom. Hence, inhibition by CLAEE can be considered negligible.

Parameter estimates for Model 5 are shown in Table 3. Scrutiny of the different tabular entries indicates that the variable that has the most significant influence on Ψ_1 and Ψ_2 is the ratio of reactants and that at 35°C the effect of the enzyme loading is very small.

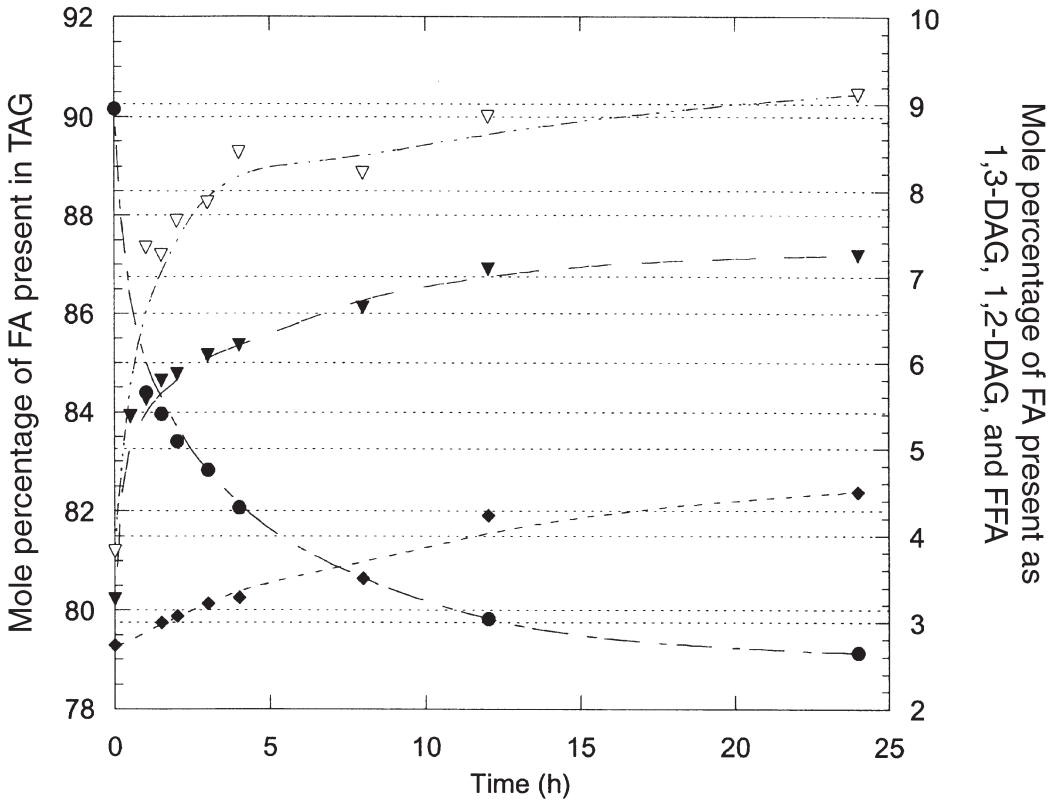

FIG. 2. Distribution of acylglycerols in the interesterification reaction between menhaden oil and CLAEE. Conditions: 5.7 g menhaden oil (935 mM), 6.5 g CLAEE (1125 mM), 0.5 g lipase Chirazyme L9 (Biocatalytics, Pasadena, CA), 1 g hexadecane, 45°C, 300 rpm. —●— TAG, —◆— 1,3-DAG, —▼— 1,2-DAG, ---▽--- FFA. CLAEE, ethyl ester of CLA.

TABLE 2
Extra Determinant Analyses of Model 1 vs. Model 2, Model 2 vs. Model 3, Model 2 vs. Model 4, and Model 2 vs. Model 5 for the Interesterification of Menhaden Oil with CLAEE

Source	SS[a]	df[b]	MSS[c]	*F* ratio[d]	*P*-value[e]
Extra	779020	18	43278.89	59.15	<0.00001
Model 2	39510	54	731.67		
Model 1	818530	72			
Extra	11102	18	616.75	0.78	0.7062
Model 3	28409	36	789.13		
Model 2	39510	54			
Extra	10103	2	5051.30	8.93	0.0005
Model 4	29407	52	565.53		
Model 2	39510	54			
Extra	9940	1	9939.50	17.81	0.0001
Model 5	29571	53	557.93		
Model 2	39510	54			

[a]Sum of squares of residuals.
[b]Degrees of freedom.
[c]Mean sum of squares of residuals.
[d]Extra mean sum of squares/mean sum of squares of full model.
[e]*P*-values below 0.05 indicate that at the 95% confidence level the model with fewer df provides a better fit of the data than the model with more df. CLAEE, ethyl ester of CLA.

TABLE 3
Parameter Estimates for Model 5 Obtained from Regression Analysis of the Nine Data Sets for the Interesterification of Menhaden Oil with CLAEE[a]

Data set	$\Psi_1 \pm$ CI[a] (h^{-1})	$\Psi_2 \pm$ CI (h^{-1})	$\Omega_1 \pm$ CI (h^{-1})	$\Omega_2 \pm$ CI (h^{-1})	$K_2 \pm$ CI (mM^{-1})
A	2.87 ± 1.09	3.14 ± 0.97	0.64 ± 0.42	0.29 ± 0.08	0.02 ± 0.01
B	0.27 ± 0.09	1.50 ± 0.40	1.23 ± 0.49	0.56 ± 0.15	0.02 ± 0.01
C	1.27 ± 0.39	1.37 ± 0.36	0.31 ± 0.20	0.12 ± 0.03	0.02 ± 0.01
E	0.15 ± 0.04	0.51 ± 0.12	0.47 ± 0.16	0.15 ± 0.03	0.02 ± 0.01
E	0.70 ± 0.19	0.81 ± 0.20	0.15 ± 0.11	0.07 ± 0.02	0.02 ± 0.01
F	0.02 ± 0.01	0.20 ± 0.06	0.02 ± 0.15	0.08 ± 0.02	0.02 ± 0.01
G	0.62 ± 0.19	0.80 ± 0.20	0.14 ± 0.13	0.07 ± 0.02	0.02 ± 0.01
H	0.04 ± 0.01	0.11 ± 0.05	0.07 ± 0.11	0.05 ± 0.01	0.02 ± 0.01
K	0.29 ± 0.08	0.74 ± 0.18	0.50 ± 0.17	0.25 ± 0.06	0.02 ± 0.01

[a]CI = 95% confidence interval. CLAEE, ethyl ester of CLA.

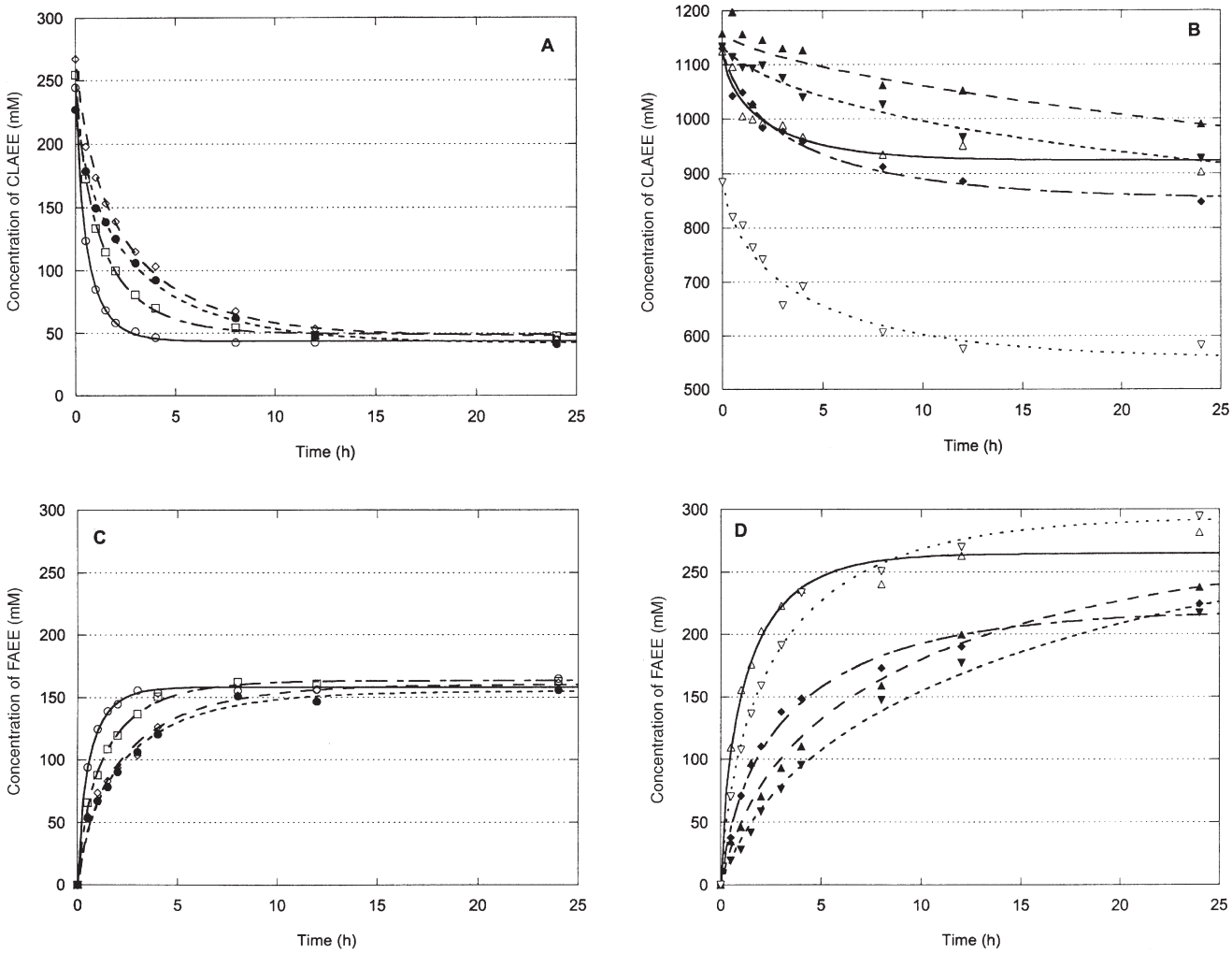

FIG. 3. Comparison of values predicted using Model 5 with experimental data for the interesterification reaction. Conditions for panels A and C: Initial concentrations of the reagents: 1940 mM menhaden oil (expressed in terms of equivalents of FA residues), 248 mM CLAEE; trial A: experimental (○), predicted (—); trial C: experimental (□), predicted (-—-); trial E: experimental (◇), predicted (– –); trial G: experimental (●), predicted (------). Conditions for panels B and D: Initial concentrations of the reagents (except for trial K): 658 mM menhaden oil, 1457 mM CLAEE; trial B: experimental (△), predicted (—); trial D: experimental (◆), predicted (- —); trial F: experimental (▲), predicted (– –); trial H: experimental (▼), predicted (-----); trial K: initial concentrations of the reagents: 935 mM menhaden oil, 1125 mM CLAEE; experimental (▽), predicted (-----). FAEE, FA ethyl esters; CLAEE, ethyl ester of CLA.

TABLE 4
Extra Determinant Analysis of Model 5 vs. Model 6 for the Interesterification of Menhaden Oil with CLAEE

Source	SS[a]	df[b]	MSS[c]	*F* ratio[d]	*P*-value[e]
Extra	3348	8	418.55	0.75	0.65
Model 5	29571	53	557.93		
Model 6	32919	61			

[a]Sum of squares of residuals.
[b]Degrees of freedom.
[c]Mean sum of squares of residuals.
[d]Extra mean sum of squares/mean sum of squares of full model.
[e]*P*-values below 0.05 indicate that at the 95% confidence level the model with fewer df provides a better fit of the data than the model with more df. CLAEE, ethyl ester of CLA.

Similar values of Ω_2 were obtained at 35°C regardless of the molar ratio and enzyme loading utilized. The effect of the molar ratio on Ω_2 and Ω_1 seems to be smaller than on Ψ_1 and Ψ_2.

Curves corresponding to fits of Model 5 to the nine data sets for interesterification of menhaden oil with CLAEE are shown in Figure 3. Figures 3A and 3C depict the data sets corresponding to a molar ratio of CLAEE to menhaden oil of 0.13 and show the consumption of CLAEE and release of the ethyl esters of the original FA residues, respectively. Figures 3B and 3D correspond to the data sets with a molar ratio of CLAEE to menhaden oil of 2.21 as well as to the central point. These panels portray the consumption of CLAEE and the release of the ethyl esters of the original FA residues, respectively. Inspection of the different plots in Figure 3 indicates that faster rates of reaction were obtained for those trials sets involving a molar ratio of CLAEE to menhaden oil of 0.13. Results of a previous study (11) also indicated that the higher the molar ratio of CLA to fish oil, the greater was the extent of incorporation of CLA in TAG, although use of such ratios involved a concomitant lower level of conversion of CLA to residue form.

Effect of the molar ratio on the rate constants. The molar ratio of the CLAEE to menhaden oil should have no effect on the rate constants but does affect the overall rate of reaction. Hence, in Model 6 the different data sets were grouped on the basis of the enzyme loading used and the temperature. The common parameters Ω_2 and Ω_1 were utilized for those data sets that had the same enzyme loading and the same temperature but different molar ratios. That is, the same values of Ω_2 and Ω_1 were used for data sets A and B, data sets C and D, data sets E and F, data sets G and H, and data set K. This strategy could not be extended to Ψ_1 and Ψ_2 because these two lumped parameters involve the concentration of lower glycerides that was assumed to be constant during a given interesterification reaction. This factor may differ from one data set to another.

As was the case for Model 5, a common inhibition constant (K_2) was employed in Model 6 for all data sets to characterize inhibition by the ethyl esters of the original FA residues released by reaction. An extra determinant analysis was conducted to characterize the abilities of Models 5 and 6 to fit the data (see Table 4). This analysis indicates that common values of Ω_2 and Ω_1 can be used for those data sets with similar enzyme loadings and temperatures.

The parameter estimates of Model 6 are shown in Table 5. Inspection of the different tabular entries indicates that for Ψ_1, Ψ_2, Ω_1, and Ω_2 the variable that has the most significant effect is temperature. The effect of enzyme loading is noticeable only at the higher temperatures.

The observed effect of the molar ratio on Ψ_1 and Ψ_2 can be explained by the fact that these lumped parameters incorporate the concentrations of lower glycerides. Although this concentration was considered constant for a given trial, it could differ from one trial to another.

Curves corresponding to fits of Model 6 to the nine data sets for interesterification of menhaden oil with CLAEE are shown in Figure 4. Figures 4A and 4C correspond to the trials with a molar ratio of CLAEE to menhaden oil of 0.13 and show the consumption of CLAEE and the ethyl esters of the original FA residues released, respectively. Figures 4B and 4D correspond to the trials with a molar ratio of CLAEE to menhaden oil of 2.21 and the central point. These panels depict the consumption of CLAEE and the release of the ethyl esters of the original FA residues, respectively. Trends similar to those depicted in Figure 3 were observed in Figure 4. Conversions of the CLAEE of *ca.* 80% were obtained in those trials involving a molar ratio of CLAEE to menhaden oil of 0.13, whereas for those trials involving a molar ratio of CLAEE to menhaden oil of 2.21 the conversion never exceeded *ca.* 25%. These conversions of CLAEE correspond to product acylglycerols containing

TABLE 5
Parameter Estimates for Model 6 Obtained from Regression Analysis of the Nine Data Sets for the Interesterification of Menhaden Oil with CLAEE

Data set	$\Psi_1 \pm$ CI[a] (h^{-1})	$\Psi_2 \pm$ CI (h^{-1})	$\Omega_1 \pm$ CI (h^{-1})	$\Omega_2 \pm$ CI (h^{-1})	$K_2 \pm$ CI (mM^{-1})
A	3.92 ± 1.46	5.89 ± 1.79	1.07 ± 0.43	0.51 ± 0.15	0.02 ± 0.01
B	0.24 ± 0.08	1.36 ± 0.41	1.07 ± 0.43	0.51 ± 0.15	0.02 ± 0.01
C	1.43 ± 0.42	1.58 ± 0.41	0.42 ± 0.14	0.14 ± 0.03	0.02 ± 0.01
E	0.14 ± 0.04	0.47 ± 0.12	0.42 ± 0.14	0.14 ± 0.03	0.02 ± 0.01
E	0.68 ± 0.19	0.89 ± 0.21	0.12 ± 0.09	0.08 ± 0.02	0.02 ± 0.01
F	0.03 ± 0.01	0.18 ± 0.05	0.12 ± 0.09	0.08 ± 0.02	0.02 ± 0.01
G	0.57 ± 0.17	0.64 ± 0.15	0.11 ± 0.08	0.06 ± 0.01	0.02 ± 0.01
H	0.04 ± 0.01	0.16 ± 0.05	0.11 ± 0.08	0.06 ± 0.01	0.02 ± 0.01
K	0.29 ± 0.09	0.74 ± 0.22	0.50 ± 0.18	0.25 ± 0.07	0.02 ± 0.01

[a]CL = 95% confidence interval. CLAEE, ethyl ester of CLA.

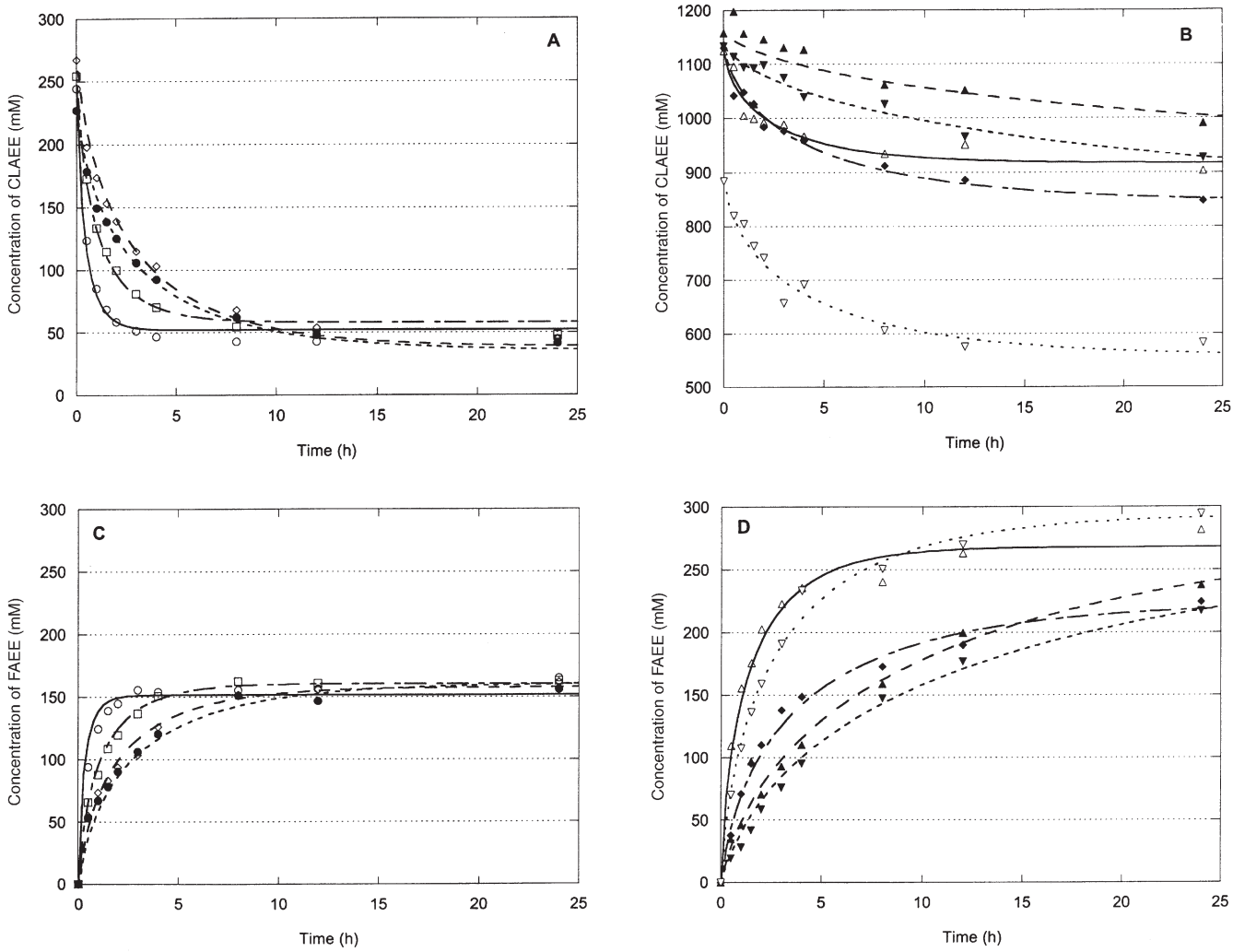

FIG. 4. Comparison of values predicted using Model 6 with experimental data for the interesterification reaction. Symbols are the same as in Figure 3.

ca. 13 and 40% CLA residues, respectively. These results are consistent with the results obtained for acidolysis of menhaden oil with free CLA (12).

Although a uniresponse model permits one to work with a small number of parameter estimates, it does not provide information about the individual species of FA residues present in the product menhaden oil. Hence, to obtain information regarding the selectivity of the lipase toward individual FA residues present in the menhaden oil, a multiresponse model is necessary.

ACKNOWLEDGMENTS

This work was supported by the University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin (Federal grant number NA 46 RG 0481, project number R/AQ-34). Additional support was provided by National Science Foundation Grant BES-0077524 and a U.S. Department of Agriculture Competitive Research Grant (Award Number 2001-35503-09912). A postdoctoral fellowship for Carlos F. Torres and a sabbatical grant for Charles G. Hill Jr. were provided by the Ministerio de Educacion y Cultura (Spain).

REFERENCES

1. 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 Compos. Anal. 5*:185–197 (1992).
2. 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).
3. 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).
4. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994).
5. 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).
6. Kinsella, J.E., Food Lipids and FA: Importance of Food Quality, Nutrition and Health, *Food Technol. 42*:124–145 (1988).
7. Garcia, H.S., J.A. Arcos, D.J. Ward, and C.G. Hill, Synthesis of Glycerides Containing n-3 FA and Conjugated Linoleic Acid by Solvent Free Acidolysis of Fish Oil, *Biotechnol. Bioeng. 70*:587–591 (2000).
8. Jennings, B., and C.C. Akoh, Enzymatic Modification of Triacylglycerols of High Eicosapentaenoic and Docosahexaenoic Acid Content to Produce Structured Lipids, *J. Am. Oil Chem. Soc.76*:1133–1137 (1999).
9. Xu, X., L.B. Fomuso, and C.C. Akoh, Modification of Menhaden Oil by Enzymatic Acidolysis to Produce Structured Lipids: Optimization by Response Surface Design in a Packed Bed Reactor, *Ibid. 77*:171–176 (2000).
10. Garcia, H.S., J.A. Arcos, D.J. Ward, and C.G. Hill, Synthesis of Glycerides Containing n-3 FA and Conjugated Linoleic Acid by Solvent-Free Acidolysis of Fish Oil, *Biotechnol. Bioeng. 70*: 587–591 (2000).
11. Torres, C.F., E. Barrios, and C.G. Hill, Jr., Lipase Catalyzed Acidolysis of Menhaden Oil with CLA: Optimization by Factorial Design, *J. Am. Oil Chem. Soc. 79*:457–466 (2002).
12. Torres, C.F., and C.G. Hill, Lipase-Catalyzed Acidolysis of Menhaden Oil with Conjugated Linoleic Acid: Effect of Water Content, *Biotechnol. Bioeng. 78*: 509–516 (2002).
13. Stewart, W.E., M. Caracotsios, and J.P. Sorensen, GREG User's Manual, Department of Chemical Engineering, University of Wisconsin–Madison, Madison, WI, 1992.
14. Bates, D.M., and D.G. Watts, Practical Considerations in Nonlinear Regression, in *Nonlinear Regression Analysis and its Applications*, John Wiley & Sons, New York, 2001, pp. 81–153.
15. 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–St. Paul, 1989.
16. 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 p.

[Received December 16, 2002; accepted May 21, 2003]