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Production of structured triglycerides rich in n-3 polyunsaturated fatty acids by the acidolysis of cod liver oil and caprylic acid in a packed-bed reactor: equilibrium and kinetics

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Abstract

Structured triglycerides (ST) enriched in *n*-3 polyunsaturated fatty acids (PUFAs) (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) in position 2 of the triglyceride backbone were synthesised by acidolysis of cod liver oil (CLO) and caprylic acid (CA) catalysed by the 1,3-specific immobilised lipase Lipozyme IM. The reaction was carried out in three ways: (1) in a batch reactor (where the influence of temperature on the incorporation of CA into the CLO triglyceride was studied); (2) in an immobilised lipase packed-bed reactor (PBR) by recirculating the reaction mixture from the exit of the bed to the substrate reservoir (product recirculation) to determine the equilibrium composition; and (3) in a PBR without recirculation. A "lag" period of duration inversely proportional to the initial water amount of the lipase, was observed when new lipase was used. Apparently, during this "lag" period the hydro-enzymatic layer that surrounds the lipase surface reaches its water equilibrium content. A reaction scheme, where only the fatty acid in the positions 1 and 3 of the glycerol backbone were exchanged by CA, was proposed. The exchange equilibrium constants between CA and the native fatty acids of CLO were determined. The *n*-3 PUFAs (EPA and DHA) were the most resistant native fatty acids to exchange with exchange equilibrium constants of 1.32 and 0.28, respectively. Also, average reaction rates and kinetic constants of exchange of CA and native fatty acid of CLO were calculated. Low kinetic constants were observed for EPA, DHA and palmitic acid. For acidolysis reaction in the continuous mode PBR, the lipase amount/(flow rate × substrate concentration) ratio ($m_L/q[\mathrm{TG}]_0$) could be considered as the intensive variable of the process for use in scale up of the PBR. A simple equation was proposed for the prediction of the fatty acid composition of the ST at the exit of the PBR as a function of the intensive variable $m_L/q[\mathrm{TG}]_0$. At equilibrium, the ST produced had the following composition: CA 57%, EPA 5.1%, DHA 10.0% and palmitic acid 6.3% (only considering the major fatty acids). In addition, the proportion of EPA and DHA that esterified the position 2 of the ST was 13.5%, which represented 44% of the total fatty acids in the position 2 of the resultant ST.

Keywords: Equilibrium acidolysis; Lipase-catalysed reaction kinetics; Structured lipids; Polyunsaturated fatty acids; Packed-bed reactor; Immobilised lipase

1. Introduction

The *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) have been recognised for their important role in health. Eicosapentaenoic acid (EPA) can affect the circulatory system and can help to prevent arteriosclerosis and thrombosis (Simopoulos, 1991), and docosahexaenoic acid (DHA) is particularly important for brain development; thus, a normal adult brain contains more than 20 g of DHA (Nettleton, 1993). Methods for recovering of PUFAs from algal oils as both free fatty acids and ethyl esters (Robles Medina et al., 1995; Molina Grima, Robles Medina, Giménez Giménez, & Ibáñez González, 1996; Giménez Giménez et al., 1998; Belarbi, Molina, & Chisti, 2000) and as highly concentrate triglycerides (Esteban Cerdán, Robles Medina, Giménez Giménez, Ibáñez González, & Molina Grima, 1998; Robles Medina et al., 1999) have been previously reported.

On the other hand, for clinical nutrition purposes there has been an increasing interest in the production of structured triglycerides (ST) with medium-chain fatty acids (M) located at the positions 1 and 3 of the glycerol backbone and functional long-chain polyunsaturated fatty acids (L)

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at the position 2 (MLM). ST have been used in absorption studies (Christensen, Hoy, Becker, & Redgrave, 1995; Jandacek, Whiteside, Holcombe, Volpenheim, & Taulbee, 1987) and for clinical nutrition (Heird, Grundy, & Hubbard, 1986). The structure of the triglyceride affects the digestion and absorption of fat. Thus, for example, Christensen et al. (1995) found that MLM triglycerides with EPA and DHA in the position 2 were more readily absorbed sources of these PUFAs than other triglycerides with similar fatty acid compositions but with a random fatty acid distribution. This better absorption is because pancreatic lipase hydrolyses ester bonds at the positions 1 and 3 in triglycerides, and shows higher activity toward medium-chain fatty acids than toward long-chain fatty acids, especially PUFAs (Bottino, Vandenburg, & Reiser, 1967). The liberated medium-chain free fatty acids are directly absorbed into the portal vein and the 2-monoglycerides (with the essential long-chain fatty acid) are well absorbed via the lymphatic route (Jandacek et al., 1987).

The simplest and direct route for the synthesis of ST of the MLM type is the acidolysis between long-chain triglycerides and medium-chain free fatty acids catalysed with a 1, 3-specific lipase (Shimada et al., 1996a, b; Shimada, Suenaga, Sugihara, Nakai, & Tominaga, 1999; Akoh & Huang, 1995; Xu, Balchen, Hoy, & Adler-Nissen, 1998a). The lipases offer high catalytic efficiency, specificity and selectivity by incorporation of the required acyl group into a specific position of native triglyceride. Acyl migration is a major problem in the synthesis of ST in batch reactors and this causes a decrease of yield in the targeted ST. The high substrate/enzyme ratio demands long reaction times to reach equilibrium, and consequently results in acyl migration. In contrast, the continuous enzymatic reactor with the lipase immobilised into a column is advantageous over batch reactor in reducing the acyl-migration (Mu, Xu, & Hoy, 1998). This reactor is one of the most commonly employed for solid–fluid contacting in heterogeneous catalysis, because (i) it facilitates the contact and subsequent separation; (ii) it allows reuse of the enzyme without the need for a prior separation; (iii) it permits the use of a continuous mode of operation; and (iv) it is more cost effective than the batch operation (Mu et al., 1998; Shimada et al., 1999; Xu et al., 1998a).

In contrast to the wealth of information pertaining to the kinetics of hydrolysis reactions catalysed by lipases, little has been reported on the kinetics of lipase-catalysed interesterification between free fatty acids and heterogeneous triglycerides. Perhaps the most detailed kinetic study of this type is the work of Reyes and Hill (1994). These authors studied the kinetics of the acidolysis between free fatty acids and heterogeneous triglycerides such as olive oil and milk fat. They proposed a kinetic model that could account for the effect of the concentration of all chemical species participating in the interesterification reaction. Also, little information has been reported on the magnitude of rate constants in the esterification reactions. However, this information is essential for understanding how reaction variables affect reaction rates and for rational design and scaling up of interesterification reactors.

Table 1
Characteristics of the immobilised enzyme particles (determined by mercury porosimetry) hexane, and packed-bed reactor

Immobilised enzyme particles	
Bulk density, ρ	0.668 g/cm^3
Specific pore volume, V_v	0.679 cm^3/g
Solid density	1.221 g/cm^3
Specific surface area, S_g	104.7 m^2/g
Average particle diameter, d_p	0.4 mm[a]
Average pore radii, $R_P = 2V_v/S_g$	13.0 nm
Porosity, $\varepsilon = V_v\rho$	0.453
Particle density admitting that the pore fraction is occupied by hexane, ρ_S	0.99 g/cm^3
Hexane	
Viscosity (30°C), μ	0.288 cp
Density (30°C), ρ_L	0.655 g/cm^3
Packed-bed reactor	
Internal diameter	1.5 cm
Bed porosity	0.46
Bed density, ρ_B	0.36 g/cm^3

[a]Data provided by Novo Nordisk Bioindustrial (0.2–0.6 mm).

This work aims to study the synthesis of ST by acidolysis between a commercial heterogeneous triglyceride (cod liver oil) as source of *n*-3 PUFAs and caprylic acid, using the immobilised 1, 3-specific lipase Lipozyme®IM immobilised in a packed-bed reactor. The objective of this study was to investigate the equilibrium and kinetics of ST's synthesis and simultaneously, the effects of temperature and the nature of fatty acids. A simple model is proposed which accounts for the effects of enzyme load, flow rate through the lipase bed and substrate concentration on the composition of synthesised ST. Also aspects related to the scaled up of the immobilised lipase packed-bed reactor are discussed.

2. Materials and methods

2.1. Chemicals and materials

Lipozyme®IM was donated by Novo Nordisk A/S (Bagsvaerd, Denmark). This lipase, containing 2–3% water determined by Karl–Fischer titration (Compact titrator microKF 2026, Crimson, Alella, Spain), was immobilised on a macroporous anion exchange resin and showed a 1, 3-positional specificity. Table 1 shows the immobilised enzyme characteristics determined using a mercury porosimeter (Quantachrome Autoscan 60). Analytical-grade caprylic acid (CA) and hexane were obtained from Sigma Aldrich (St. Louis, MO). Table 2 shows the fatty acid composition of the commercial cod liver oil used (CLO,

Table 2
Composition of the original cod liver oil (CLO), average value of the equilibrium composition of structured triglycerides (ST), F_{Xe}, fatty acid composition in the position 2 at the equilibrium, F_{Li2} (statistical distribution), and exchange equilibrium constants between native CLO fatty acids and caprylic acid (CA), K_i

Fatty acid	CLO (Mole fraction)	F_{Xe}[a] (Mole fraction)	F_{Li2}[b] (Mole fraction)	K_i[c]	K_i[d]
8 : 0	0.0	0.57 ± 0.01	0.0	—	—
14 : 0	0.047	0.023 ± 0.001	0.018	1.01	1.00
16 : 0	0.124	0.063 ± 0.003	0.049	0.96	0.93
16 : 1*n*7	0.087	0.033 ± 0.002	0.021	1.59	1.58
18 : 0	0.029	0.0094 ± 0.00005	—	—	—
18 : 1*n*9	0.193	0.060 ± 0.002	0.030	2.19	2.13
18 : 1*n*7	0.051	0.016 ± 0.0005	0.008	2.11	2.11
18 : 4*n*3	0.023	0.0048 ± 0.0004	—	—	—
20 : 1*n*9	0.120	0.035 ± 0.001	0.016	2.37	2.34
20 : 5*n*3	0.092	0.051 ± 0.003	0.042	0.79	0.77
22 : 1*n*9	0.087	0.029 ± 0.001	0.016	1.94	1.92
22 : 5*n*3	0.019	0.008 ± 0.002	—	—	—
22 : 6*n*3	0.128	0.100 ± 0.006	0.093	0.28	0.27
Total	1	1	0.305		

[a] $F_{Xe} = F_{Me}$ for CA (8 : 0) and $F_{Xe} = F_{Lie}$ for CLO native fatty acids (14 : 0 to 22 : 6*n*3).
[b] Fatty acid composition of the position 2 determined by Eqs. (10) and (11) admitting a statistical distribution (i.e. $K = K_1 = \cdots = K_n = 1$).
[c] Calculated by Eqs. (10) and (11).
[d] Calculated by Eqs. (12) and (13) (simplified method).

Acofarma, Barcelona, Spain). From this composition an average molecular weight for the CLO of 910 Dalton may be calculated.

2.2. Batch reactor

The reaction mixture consisted of CLO, 100 mg (0.110 mmol); CA, 97.8 mg (0.679 mmol); hexane, 3 ml and lipase, 8.4 mg. In all the experiments these amounts were kept constant. These amounts determined a CA/CLO molar ratio, $m_0 = 6$. This reaction mixture was placed in 50-ml Erlenmeyer flasks with silicone-capped stoppers. The mixture was incubated at temperatures between 25°C and 50°C and agitated in an orbital shaking air-bath at 200 rpm during a reaction time of 24 h. These experimental conditions were found to be optimal in other work on acidolysis of triolein with CA (Camacho Páez, Robles Medina, Camacho Rubio, Esteban Cerdán, & Molina Grima, 2001). Also, in a previous paper, it was found that an increase in the agitation rate did not influence on the experimental results (Robles Medina et al., 1999). The reaction was stopped by separation of lipase by filtration and the mixture, product of the reaction, was stored at −20°C until analysis. All reactions, and their corresponding analysis, were carried out in triplicate. The standard deviations were always lower than 8%.

2.3. Packed-bed reactor (PBR)

Fig. 1 shows a scheme of the reaction system. The immobilised lipase (2.08 g or 6 g) was packed into a glass column of 1.5 i.d. × 15 cm length, covered with aluminium foil to prevent photo-induced oxidation. The column had two mobile perforated disks, which allowed to adjust the column volume to the volume of the packed lipase. When 6 and 2.08 g of Lipozyme® IM were charged into the PBR the lipase height in the column were 9.5 and 3.3 cm, respectively. Taking into account the bulk density of the catalyst (Table 1), the bed porosity in this confined volume was 0.46 and the bed density of catalyst was 0.36 g/cm^3 (Table 1). The substrate mixture was kept in a reservoir

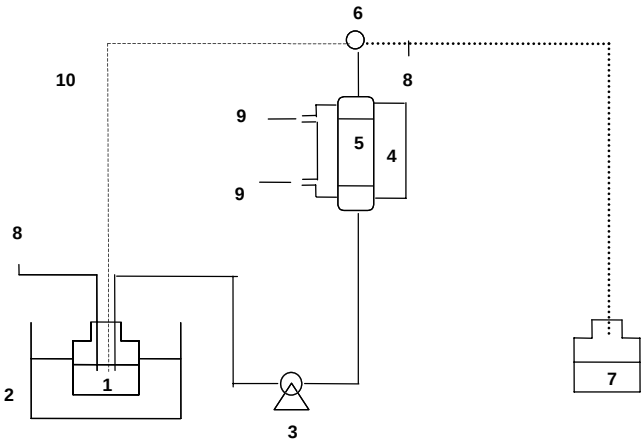

Fig. 1. Immobilised lipase packed-bed reactor (PBR). (1) Substrate reservoir, (2) reactor temperature control, (3) peristaltic pump, (4) water jacket, (5) bed of immobilised enzyme, (6) three-way valve, (7) product reservoir, (8) sampling, (9) cooling/heating water; and (10) recirculation. The dashed line describes the flow of materials for recirculating operation; the dotted line describes the flow for the continuous mode.

submerged in a thermostated water bath. The mixture consisted of CLO, 10 g (0.011 mol), caprylic acid, 9.69 g (0.067 mol) and hexane, 300 ml. These amounts were kept constant in all the experiments. This implies an initial CLO concentration $[TG]_0 = 0.0367$ M and a CA/CLO molar ratio $m_0 = 6.1$. This reaction mixture was pumped upward into the column by a peristaltic pump at flow rates between 15.8 and 630.3 ml/h, which corresponds to residence times between 0.49 and 0.012 h, respectively (residence time = void bed volume/flow rate). The column also had a jacket for controlling the reaction temperature and the column and substrate mixture temperature was maintained constant at 30°C by the circulation of water from the water bath. A three-way valve was placed at the exit of the lipase bed that permitted two modes of operation (Fig. 1): (1) by recirculating to the substrate reservoir the mixture that came out of the bed (product recirculation), or (2) directing the reaction mixture that comes out from the bed to an additional reservoir (continuous operation). When the system was operated with recirculation the reaction was followed by sampling at different times (between 0.5 and 60 h) either at the exit of the bed or in the substrate reservoir. When the PBR was operated in continuous mode, sampling occurred only at the exit of the bed. The samples were stored at −20°C until analysis. All analyses were carried out in triplicate. The standard deviation was always lower than 6%.

2.4. Identification of reaction products and estimation of the molar fraction of fatty acids in triglycerides

Hexane was removed from the product mixture in a vacuum evaporator. Glycerides (monoglycerides, diglycerides and triglycerides) were extracted by three extractions with 3 ml hexane each after adding 2 ml of 0.5 N KOH (20% ethanol solution) to 70 mg of the reaction mixture. These glycerides were identified by thin-layer chromatography (TLC) followed by quantitative gas chromatography (GC). TLC analysis has been described elsewhere (Robles Medina et al., 1999). Fractions corresponding to each glyceride type were scraped from the plates and methylated by direct transesterification with acetyl chloride/methanol (1 : 20) by following the method of Lepage and Roy (1984). These methyl esters were analysed with a Hewlett Packard 4890 gas chromatograph (Avondale, PA) connected to a capillary column of fused silica Omegawax (0.25 mm × 30 m, 0.20 μm standard film; Supelco, Bellefonte, PA), and a flame-ionisation detector. Nitrogen was the carrier gas, and the total column flow was 36 ml/min. The oven temperature program was the following: 150°C for 3 min, from 150°C to 185°C at 10°C/min, 185°C for 10 min, from 185°C to 240°C at 10°C/min, and finally 240°C for 12 min. Matreya (Pleasant Gap, PA) *n*-3 PUFAs standard (catalog number 1177) was used for the qualitative fatty acid determination. Caprylic acid was also injected to determine its retention time. Nonadecanoic acid (19 : 0) was used as an internal standard for quantitative determination of fatty acids. The signal was analysed and integrated by an on-line computer. The amounts of fatty acids were calculated by the equation

$$\text{Fatty acid (mg)} = 0.125\,\frac{f_X\,\text{area}_X}{\text{area}_{IS}}, \tag{1}$$

where 0.125 is the amount of the internal standard (19 : 0), f_X is the fatty acid response factor, area_X is the fatty acid chromatographic area and area_{IS} is the internal standard chromatographic area. The response factors were close to 1 for all the fatty acids of molecular weight close to the internal standard and therefore a $f_X = 1$ was taken for all the fatty acids, except for the caprylic acid, for which a factor equal to 1.49 was calculated.

3. Equilibrium and rate equations

3.1. Acidolysis equilibrium

The displacement of a native fatty acid, L_i, located in the position 1 or 3 of a heterogeneous triglyceride, GL_i, by an odd fatty acid, M, can be expressed by the reaction:

$$GL_i + M \overset{\text{lipase}}{\Leftrightarrow} GM + L_i. \tag{2}$$

Admitting the hypothesis that, due to the 1,3 positional specificity of lipase, only the fatty acids in the positions 1 and 3 of triglyceride are exchanged and that the change in position 1 does not depend on the nature of fatty acid in position 3 (and vice versa), at the equilibrium we can obtain (see Appendix A, Eq. (A.17)):

$$\frac{[GM]_e[L_i]_e}{[GL_i]_e[M]_e} = K_i\,\frac{(1 + 2K[M]_e/[L]_e)}{(1 + (K/K_i)[L_i]_e/[L]_e + K[M]_e/[L]_e)}, \tag{3}$$

where the subscript "*e*" refers to the equilibrium concentrations. $[GL_i]$ represents the concentration of ester bonds between the glycerol backbone and the CLO native fatty acids and [GM] is the concentration of ester bond between the glycerol backbone and the odd fatty acid; $[GL_i]$ and [GM] are expressed as native and odd equivalent fatty acid concentration, respectively. K represents the average equilibrium constant for the exchange of the native fatty acid, L, by the odd fatty acid, M, and $[L]_e$ the overall concentration of native free fatty acids (see Appendix A, Eq. (A.6)):

$$\frac{[L]_e}{K} = \sum_i \frac{[L_i]_e}{K_i}, \quad [L]_e = \sum_i [L_i]_e. \tag{4}$$

Using mass balances for both odd (M) and native fatty acid (L_i) and considering the stoichiometry of reaction (2) we can obtain the following relationships:

$$[M]_e = m_0[TG]_0 - 3[TG]_0 F_{Me}, \tag{5}$$

$$[L]_e = 3[TG]_0 F_{Me}, \tag{6}$$

$$[L_i]_e = 3[TG]_0(F_{Li0} - F_{Lie}), \tag{7}$$

$$[GM]_e = 3[TG]_0 F_{Me}, \tag{8}$$

$$[GL_i]_e = 3[TG]_0 (F_{Lie} - F_{Li2}), \tag{9}$$

where m_0 is the initial CA/CLO molar ratio, F_{Me} is the molar fraction of CA incorporated into triglycerides, and F_{Li0}, F_{Lie} and F_{Li2} are the molar fractions of the native fatty acid L_i in the original CLO, in the resulting ST at the equilibrium and esterifing the position 2 of the ST at the equilibrium, respectively. These mass balances take into account that the initial concentration of triglycerides $[TG]_0$ was equal to the concentration of triglycerides at any time, since it was experimentally observed that the formation of monoglycerides and diglycerides was negligible. The calculation of $[GL_i]_e$ by Eq. (9) takes into account that the fraction of the fatty acid L_i that esterifies the position 2 of the triglyceride does not participate in the exchange. Introducing Eqs. (5)–(9) into Eqs. (3) and (4), we obtain:

$$\frac{1}{(1+2K(m_0/3F_{Me}-1))} = K_i \frac{(F_{Lie}-F_{Li2})}{(F_{Li0}-F_{Lie})} \frac{(m_0/3F_{Me}-1)}{(1+(K/K_i)(F_{Li0}-F_{Lie})/F_{Me}+K(m_0/3F_{Me}-1))}, \tag{10}$$

$$K = \frac{F_{Me}}{\sum_i (F_{Li0}-F_{Lie})/K_i}. \tag{11}$$

Eq. (10), for each native fatty acid, and Eq. (11) allow the calculation of the exchange equilibrium constants, K_i, from m_0, F_{Me}, F_{Li0}, F_{Lie} and F_{Li2}. F_{Me}, F_{Li0} and F_{Lie} were determined by gas chromatography, while F_{Li2} cannot easily be determined.

When the odd fatty acid/native triglyceride molar ratio is sufficiently high the degree of incorporation of the odd fatty acid at the equilibrium is high and the concentration of native triglycerides must be negligible, so the equilibrium constant can be determined by the simplified equations (see Appendix A):

$$K = \frac{1.5F_{Me} - 0.5/(2-3F_{Me}) + \sqrt{(1.5F_{Me}-0.5/(2-3F_{Me}))^2 + (1.5F_{Me}/(2-3F_{Me}))}}{(m_0/3F_{Me}-1)}, \tag{12}$$

$$K_i = \frac{(F_{Li0}-F_{Lie})}{(F_{Lie}-F_{Li2})} \frac{(1+K(m_0/3F_{Me}-1))}{(1+2K(m_0/3F_{Me}-1))(m_0/3F_{Me}-1)}, \tag{13}$$

which have been obtained from Eqs. (A.20) and (A.19), respectively, taking into account Eqs. (5)–(9). In this work, the incorporation of caprilic acid attained at the equilibrium, $F_{Me} = 0.57$ (Table 2), for the used molar ratio, $m_0 = 6.1$, is only slightly higher that the one would be obtained if the exchange equilibrium was independent on the nature of the fatty acids ($K = K_1 = \cdots = K_n = 1$), $F_{Me} = 0.53$ (see Appendix A, Eq. (A.18)).

With the goal of comparing the calculation methods of the exchange equilibrium constant, F_{Li2} may be estimated by considering that it is proportional to the content of these fatty acids at equilibrium, i.e.,

$$F_{Li2} = 0.33 \frac{F_{Lie}}{\sum_i F_{Lie}} = 0.33 \frac{F_{Lie}}{1-F_{Me}}. \tag{14}$$

3.2. Kinetics of acidolysis

Bearing in mind the fatty acid profile of the CLO, it was impracticable to apply a kinetic model such as the one utilised in another work for the acidolysis of triolein with CA, in which only two fatty acid participated in the reaction (Camacho Páez et al., 2001). However, we can obtain representative values of the apparent kinetic constant, k_X, for each fatty acid, X (M or L_i) by admitting that the rate of incorporation of a fatty acid X into triglycerides by a unit amount of enzyme, r_X (mol/h g lipase), is proportional to the separation from the equilibrium (driving force) for each fatty acid in the form:

$$r_X = k_X (F_{Xe} - F_X) \tag{15}$$

r_X is positive for caprylic acid (M) and negative for the native CLO fatty acids (L_i). Initially, the acidolysis kinetics were tackled by determining the average rate of incorporation, r_{mX} (mol/(h g)), into the triglycerides of a fatty acid X by a unit amount of enzyme between the entrance and the exit of the lipase bed. r_{mX} can be obtained by means of a mass balance for any free fatty acid, X, applied to the bed, i.e.,

$$q[X]_{in} - r_{mX} m_L = q[X]_{out}, \tag{16}$$

where m_L is the lipase amount in the bed (g), q is the substrate mixture flow rate through the lipase bed (ml/h) and $[X]_{in}$ and $[X]_{out}$ are the concentrations of the free fatty acid (M or L_i), at the entrance and at the exit of the bed, respectively. The average reaction rate may be calculated as a function of the entrance and exit molar fractions of fatty acids in the ST (F_{Xin} and F_{Xout}, respectively), by substituting Eq. (5) (for caprylic acid, M) and Eq. (7) (for native fatty acid, L_i) into Eq. (16):

$$r_{mX} = \frac{3[TG]_0 (F_{Xout} - F_{Xin})}{m_L/q}. \tag{17}$$

In this case it was taken into account that the equilibrium was not reached during the residence time of the reaction mixture in the PBR. Therefore F_{Me} and F_{Lie} from Eqs. (5) and (7), respectively, must be substituted by F_{Xout} and F_{Xin} (molar fractions of CA and native fatty acids, L_i, in ST at the exit and at the entrance of the PBR, respectively).

To relate the average rate (Eq. (17)) with the kinetic equation (15), it was necessary to hypothesise about the degree of mixing in the PBR. When PBR was operated with a small lipase amount, small height/diameter ratio and at significant circulation rates, the bed would tend to fluidise if the upper perforated disc was not set properly. Therefore, the hypothesis of perfect mixing within the bed can be admitted and, in these conditions, the average incorporation rate would correspond to the conditions at the exit. In these circumstances, using Eq. (15), the kinetic constants for each fatty acid could be calculated:

$$k_X = \frac{r_{mX}}{F_{Xe} - F_{X\text{out}}}. \tag{18}$$

By substituting Eq. (17) into Eq. (18) we can obtain an equation that allows predicting the molar fractions of any native fatty acids (L_i) and CA in the ST at the exit of the PBR, $F_{X\text{out}}$. This equation is

$$\frac{F_{Xe} - F_{X\text{out}}}{F_{Xe} - F_{X\text{in}}} = \frac{1}{1 + k_X m_L / 3[\text{TG}]_0 q} = \frac{1}{1 + k_V m_L / q}. \tag{19}$$

Also, the reaction rates calculated by Eq. (17) may be used in the kinetic equation (Eq. (15)) if the change of composition in the bed was very small. In this circumstance, the bed would behave as a differential reactor and the system would allow the direct measurement of the reaction rate in the form:

$$r_X = 3[\text{TG}]_0 \frac{\text{d}F_X}{\text{d}(m_L/q)}. \tag{20}$$

This equation shows that in a PBR, the intensive variable (equivalent to the product between the reaction time and the lipase amount in a batch reactor) is the enzyme amount/flow rate.

In a well agitated batch reactor of volume V, assuming perfect mixing, a free fatty acid balance on the overall system and over a differential time (dt) leads to

$$r_X = \frac{3[\text{TG}]_0 V}{m_L} \frac{\text{d}F_X}{\text{d}t}, \tag{21}$$

where Eqs. (5) and (7) also, for no equilibrium conditions, have been taken into account. Thus, by comparing Eqs. (20) and (21) both continuous and batch reactors should give similar results wherever the following occurs:

$$\left(\frac{m_L t}{V[\text{TG}]_0}\right)_{\text{batch}} = \left(\frac{m_L}{q[\text{TG}]_0}\right)_{\text{continuous PBR}}. \tag{22}$$

This equation permits to compare both reactors. Eq. (22) is based on the hypothesis that the change of composition in a PBR is very small and the PBR behaves as a differential reactor ($r_X = r_{mX}$), i.e. the conversion attained in the PBR should be equal to the one attained in a small time in a batch reactor. Eq. (22) implies that the reaction rate (and the rate equation) should be the same in both reactors and therefore the substrate molar ratio, m_0, should be equal in both reactors and the external mass transfer resistance should not be important in the PBR. This latter condition is true in a bed where axial mixing occurs (perfect mixing hypothesis).

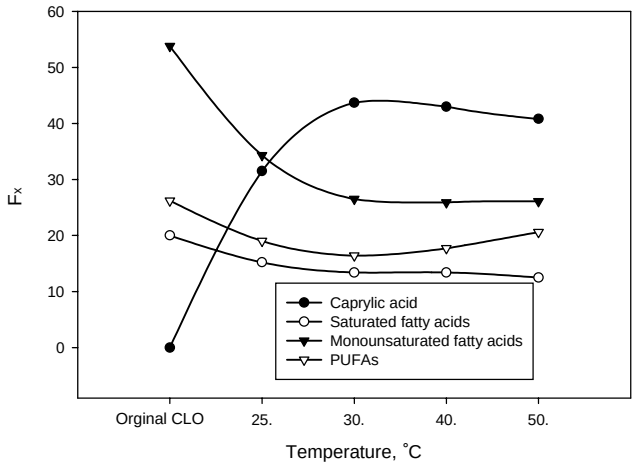

Fig. 2. Acidolysis of CLO and CA in a batch reactor. Effect of temperature on the fatty acid composition of the produced ST. Experimental conditions: initial CLO concentration, $[\text{TG}]_0 = 0.0367$ M; CA/CLO molar ratio, $m_0 = 6.1$; lipase/CLO mass ratio, 0.084; water content 3% dw of lipase; reaction time 24 h.

On the other hand, it can be shown that Eq. (22) also would be valid if the hypothesis of plug flow in the PBR is assumed.

4. Results and discussion

4.1. Acidolysis in a batch reactor: effect of temperature

The optimal temperature of enzymes depends on the source (microorganism), immobilisation process, chemical modification of enzymes and pH (Dordick, 1989). Fig. 2 shows that at 25°C a smaller incorporation of CA and a greater retention of the three groups of fatty acid (saturated, mono- and polyunsaturated) were achieved. However, at 30°C, 40°C and 50°C, the differences were small and did not increase the intensity of the transformation with the temperature in this interval. These results may indicate that the difference between the temperature of 25°C and the others is due to an increase of the reaction rate with temperature. However, small differences between the displacement of the different fatty acids were observed at temperatures between 30°C and 50°C. These differences are partly compensated and this determines an incorporation of caprylic acid that decreases slightly with the temperature. At this point it seems that the equilibrium determines the composition of the product. Effectively, in Fig. 2 is also observed that the maximum incorporation of CA occurs at 30°C, although the difference was small with respect to 40°C and 50°C. Monounsaturated fatty acids were the most displaced between 30°C and 50°C, being displaced by more than 50% (from 53.8% to 26%). Saturated fatty acids were only displaced by 35% (from 20% to 13%). PUFAs were the fraction of fatty acids less displaced by the CA in the acidolysis reaction (from 26% to 18%). PUFAs show a minimal retention at 30°C. Thus, the retention of these fatty acids at 30°C

was 67.6%, while at 50°C it was 79%. Taking into account the 1,3 positional specificity of Lipozyme IM, these results seem to indicate that PUFAs of CLO are found mainly in the 2 position of TG. A similar result with respect to the effect of temperature on the incorporation of CA in TG was reported by Mu et al. (1998) in the acidolysis of sunflower oil and CA with Lipozyme®IM (Fig. 5 of their work). These authors attained lower incorporation at the lowest temperatures (30°C and 40°C) although the experimental conditions were very different (they carried out the acidolysis with the enzyme immobilised in a column reactor and no solvent was added to the reaction mixture). As a consequence of this set of experiments we decided to carry out the subsequent experiments in the PBR at 30°C, because the reaction rate was similar in the temperature range 30–50°C. In addition, 30°C is a temperature sufficiently low to preserve PUFAs from potential oxidation, decreases the acyl-migration (Xu et al., 1998a), protects inactivation of enzyme (Dordick, 1989) and facilitates handling with a highly volatile solvent such as hexane.

4.2. Acidolysis in the PBR: product recirculation

These experiments were performed by recirculating the reaction mixture (substrates and reaction products) exiting the PBR to the substrate reservoir (dashed line in Fig. 1). With this operational mode the influence of the initial water content of the lipase and the acidolysis equilibrium were studied.

4.2.1. Influence of the initial water content of the lipase

Initially, three experiments were carried out in apparently equal conditions: 6 g of Lipozyme®IM in the bed and a flow rate of substrate mixture through the lipase bed of 15.8 ml/h. Sampling in these experiments was only at the substrate reservoir. In experiment A, (Fig. 3) fresh lipase with a water content of 3% dw was used; in experiment B, this same lipase was reused, and in experiment C, again fresh lipase with a water content of 2% dw was used. Fig. 3 shows that the incorporation of CA increased regularly with time, except for the first hours of operation in experiments A and C, in which no incorporation of CA took place. Apparently, there was a "lag" period. In experiment B, this "lag" period was not observed and the CA incorporation in ST increased uniformly with time. In experiment C, that was performed with a new enzyme lot of lower initial water content, a lag period of longer duration than in experiment A was clearly seen. Fig. 3d depicts all the experimental results shown in Fig. 3a–c. In this figure the data related to the duration of the lag period for Figs. 3a and c were eliminated and the reaction times for these two experiments were consequently delayed (2 h for experiment A and 12 h for experiment C). The results for the three experiments practically coincide, which agrees with the hypothesis of the occurrence of a lag period when new lipase was used.

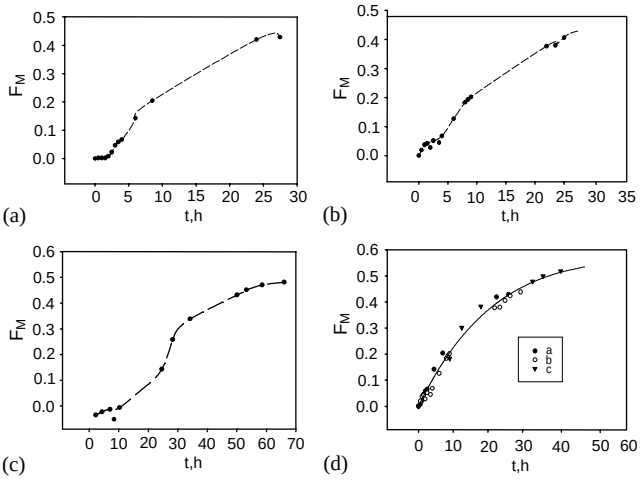

Fig. 3. Acidolysis of CLO and CA in a PBR with product recirculation to the substrate reservoir. Effect of reaction time on the fraction of CA incorporated into TG (compositions measured at the substrate reservoir): (a) fresh lipase with a water content of 3% dw; (b) reused lipase, water content 3% dw; and (c) fresh lipase with water content 2% dw; (d) pool of data from (a)–(c) delaying the times corresponding to the "lag" period (2 h for the experiments in (a) and 12 h for the experiment in (c)). Experimental conditions: $[TG]_0 = 0.0367$ M; $m_0 = 6.0$ mol/mol; substrate mixture flow rate, $q = 15.8$ ml/h; 30°C, and $m_L = 6$ g.

The lag period appears in the first experiment carried out with each new lot of enzyme and its duration is longer for the enzyme with smaller water content. Therefore, it seems logical to think that, because the water content of the lipase affects the initial reaction rate, throughout the lag period the hydro-enzymatic layer that surrounds the surface of the support-matrix reaches its equilibrium water content. This layer is formed using the water of saturation contained in the hexane phase and the water liberated by the formation of a small amount of diglycerides. Diglycerides need to be formed for the exchange reactions to occur (although diglycerides were practically not detected by TLC in the reaction products). This phenomenon has also been observed by Shimada et al. (1999). These authors carried out the acidolysis of borage oil and caprylic acid using the *Rhizopus delemar* lipase immobilised on a ceramic support. To display activity this enzyme needs to be activated at the beginning by contacting with the substrate saturated of water (1.18% dw). Subsequently, the reaction continues with appreciable performances by feeding the substrate without water.

4.2.2. Equilibrium of the acidolysis reaction

To study the acidolysis equilibrium, a new set of experiments were carried out by recirculating the reaction mixture through the packed-bed. The objective of these experiments was to determine both the fatty acid composition at equilibrium and the equilibrium constants. Lipozyme®IM (6 g) was packed into the column and a flow rate of 15.8 ml/h was used. In contrast to the earlier described recirculating experiments, sampling in these experiments occurred both at the exit of the PBR and at the substrate reservoir. The

Table 3
Kinetics of acidolysis of CA and CLO in the PBR in continuous operation mode: variation of the molar fractions of fatty acids (F_X) in the obtained triglycerides at increasing lipase amount/flow rate ratios for the fatty acids constituting over 3% of the original CLO. The last row represents the experimental values obtained at equilibrium (F_{Xe}, Table 2)

m_L/q (g h)/ml	F_X									
	8 : 0	14 : 0	16 : 0	16 : 1*n*7	18 : 1*n*9	18 : 1*n*7	20 : 1*n*9	20 : 5*n*3	22 : 1*n*9	22 : 6*n*3
Original CLO	0.000	0.047	0.124	0.087	0.193	0.051	0.120	0.092	0.087	0.128
0.00330	0.074	0.043	0.124	0.075	0.177	0.047	0.104	0.091	0.083	0.128
0.00495	0.100	0.041	0.123	0.070	0.170	0.045	0.102	0.090	0.082	0.126
0.00990	0.190	0.040	0.114	0.062	0.145	0.037	0.087	0.088	0.068	0.123
0.0198	0.217	0.038	0.114	0.059	0.133	0.033	0.087	0.088	0.068	0.122
0.0190	0.269	0.037	0.112	0.056	0.124	0.031	0.078	0.086	0.06	0.119
0.0286	0.298	0.036	0.105	0.053	0.113	0.028	0.074	0.084	0.055	0.118
0.0571	0.344	0.034	0.102	0.047	0.101	0.027	0.063	0.078	0.048	0.118
0.0952	0.409	0.033	0.094	0.045	0.084	0.020	0.052	0.073	0.043	0.118
Equilibrium	0.570	0.023	0.063	0.033	0.060	0.016	0.035	0.051	0.029	0.100

composition of the reaction mixture that left the PBR remained constant from 12.5 to 38.5 h, which demonstrated that the equilibrium was reached. The equilibrium incorporation of CA varied in the range 58.3–55.4% and was never $> 67\%$. This result and the tested 1, 3-specificity of Lipozyme®IM shows that only the positions 1 and 3 participated in the interesterification reaction. This result and the negligible formation of partial glycerides, through which acyl-migration occurs (Xu et al., 1998b), also indicated that acyl-migration practically did not occur. Table 2 shows the values at the equilibrium (F_{Xe}) for CA (57%) and the native fatty acids of the CLO calculated as the mean values of the experimental data obtained for increasing times at the exit of the bed. These results have been used to calculate the K_i values by Eqs. (10) and (11) and Eqs. (12) and (13) (simplified method) for those fatty acids with a composition in the original CLO greater than 3%. Table 2 also shows the fatty acid composition that esterifies the position 2, calculated by Eqs. (10) and (11), admitting a statistical distribution for the positions 1 and 3 ($K = K_1 = \cdots = K_n = 1$). Although the K_i values are based on Eq. (14), it can be observed that saturated fatty acids 14 : 0 and 16 : 0 have equilibrium exchange constants with caprilic acid of about 1.0, as if the nature of the fatty acid does not affect the exchange (statistical distribution). However, for the unsaturated fatty acids, the K_i values indicate that the degree of displacement depends on the nature of the fatty acid. The monounsaturated fatty acids were the most displaced; it is observed that the equilibrium exchange constants increase with the number of carbon atoms, being the 20 : 1*n*9 the most displaced. However, for the long-chain polyunsaturated fatty acid this tendency is inverted, being the PUFAs EPA (20 : 5*n*3) and DHA (22 : 6*n*3) the most resistant acids to the exchange. These results are consistent with those previously observed in Fig. 2. In Table 2, it can be seen that the fatty acids that esterifies the position 2 of glycerol in the CLO are mainly DHA (22 : 6*n*3), palmitic acid (16 : 0), EPA (20 : 5*n*3) and oleic acid (18 : 1*n*9). These results coincide with the fatty acid composition at the position 2 of refined fish oil used by Xu et al. (1998a) (24.6% C16 : 0, 12.4% EPA and 20.2% DHA, Table 1 of their work). Also the composition of tuna oil used by Shimada et al. (1996b) indicates a high proportion of these fatty acid at position 2.

4.3. Continuous operation: kinetics of acidolysis

The reaction rates and apparent kinetic constants for CA and for each one of the native CLO fatty acids were determined by experiments carried out in continuous mode, without recirculation of the reaction products leaving the reactor, and using 6 and 2.08 g of Lipozyme®IM in the bed at flow rates of 63.0, 105.0, 210.0, 315.1, 420.2 and 630.3 ml/h. Table 3 shows the fatty acids profiles of the ST obtained in the kinetic experiments at increasing values of m_L/q and Fig. 4 shows the incorporations of CA, oleic acid and EPA and the fitting of experimental results to Eq. (19) (similar figures were obtained for the other fatty acids). In continuous operation mode $F_{X\text{in}}$ is zero for CA and equal to the original CLO fatty acid composition for native fatty acids (Table 2). In Fig. 4, we see that all the experimental results fall on the same curve, irrespective of the fatty acid and the amount of enzyme used in the bed. These results clearly show that, indeed, the quotient m_L/q can be considered as the intensive process variable for continuous acidolysis when using an immobilised enzyme PBR. The fitting shown in Fig. 4 was carried out using the values of k_V (Eq. (19)) that minimised the sum of the squared residuals for the incorporation of CA and for the substitution of oleic acid and EPA. Table 4 shows the values of k_V and the values of the apparent kinetic constants, k_X, (Eq. (19)) for those fatty acids whose content in the original oil was above 3% (mol/mol) and for CA. It is seen that the fastest exchange corresponds to the monounsaturated fatty acid, with k_X values higher than 0.0050 mol/(g h), except for 22 : 1*n*9. Among them the exchange rate seems to increase inversely with

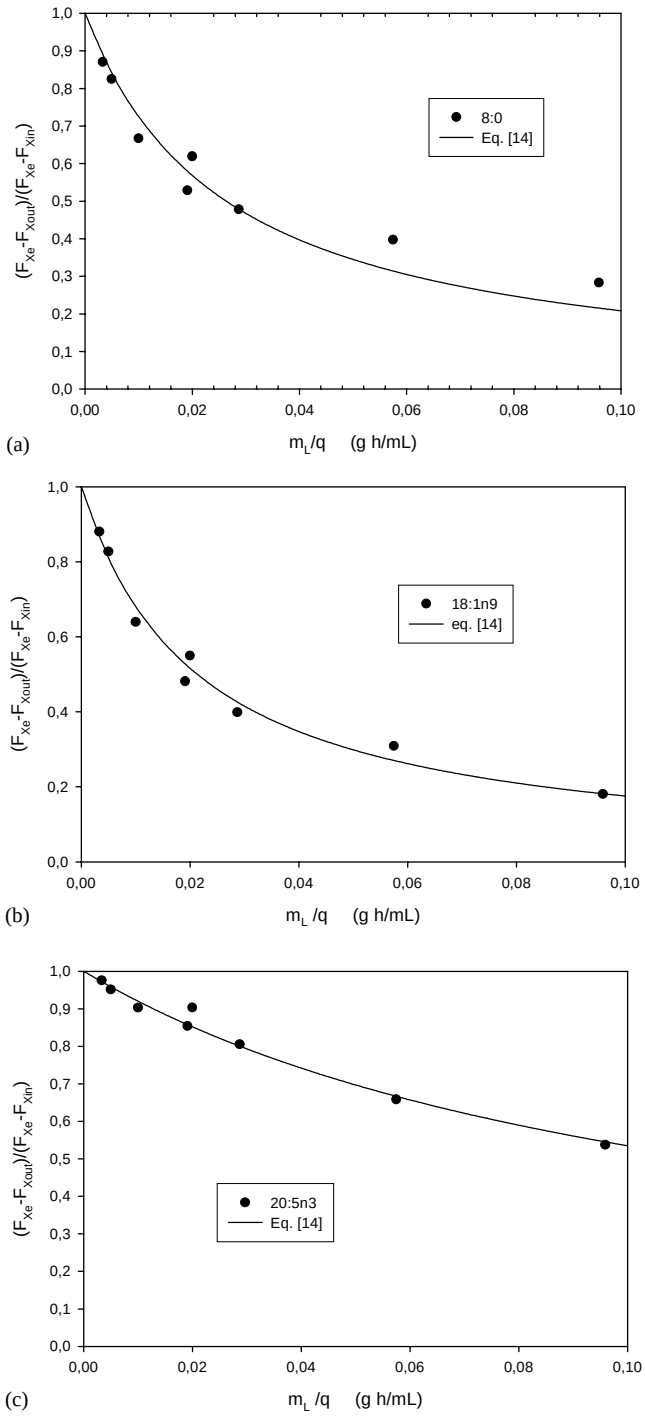

Fig. 4. Acidolysis in the PBR (continuous operation). Effect of the treatment intensity lipase amount/flow rate ratio, m_L/q, on the molar fractions ($F_{X\text{out}}$) of caprylic acid (a), oleic acid (b) and EPA (c) in TG. Continuous lines represent the values predicted by Eq. (19). Experimental conditions: $[TG]_0 = 0.0367$ M; $m_0 = 6.0$ mol/mol and 30 °C.

the molecular weight of the fatty acids. Also the exchange rate seems to be higher when the unsaturation is in position 7 than when it is in position 9, although the scatter of the experimental data do not permit a clear conclusion on this. The behaviour of the CA and 14 : 0 was similar,

Table 4
Apparent kinetic constants (k_V and k_X) for the exchange of CA and the CLO native fatty acids (Eq. (19))

Fatty acid	k_V, ml/(g lipase h)	k_X, mol/(g h)
8 : 0	38 ± 9	0.0042
14 : 0	37 ± 18	0.0041
16 : 0	10 ± 6	0.0011
16 : 1*n*7	67 ± 20	0.0074
18 : 1*n*9	47 ± 7	0.0052
18 : 1*n*7	57 ± 16	0.0063
20 : 1*n*9	48 ± 13	0.0053
20 : 5*n*3	9 ± 2	0.0010
22 : 1*n*9	34 ± 11	0.0037
22 : 6*n*3	14 ± 8	0.0015

with k_X values of the order of 0.005 mol/(g h). The lowest values were obtained for palmitic acid and the PUFAs EPA and DHA that are the fatty acids that give the lowest reaction rates. These results coincide with the conclusions obtained from the equilibrium study (these fatty acids were the less displaced by CA), although the kinetic constants and the equilibrium constants are independent. The special resistance to the exchange of the DHA has been observed previously also (Shimada et al., 1996b).

Because the continuous curves represented in Fig. 4 have been obtained with Eq. (19), this equation can be used to predict the composition of ST at the exit of the PBR at different m_L/q values. These results show that the perfect mixing hypothesis agrees acceptably well the experimental results in the range of values of m_L/q tested. These results could be explained partly by the fact that although the lipase was confined to the bed, the flow rates used were enough to cause appreciable axial mixing (partial fluidisation of the lipase particles due to the low difference between the densities of the catalyst particles and hexane) (Vainshtein, Fichman, Shapiro, Moldavsky, & Gutfinger, 1999). In effect, the minimum fluidisation rate, u_{mf}, could be calculated (Kunii & Levenspield, 1969):

$$u_{mf} = \frac{d_P^2(\rho_S - \rho_L)g}{1650\mu} \quad \text{for } Re_P \leqslant 20. \tag{23}$$

Using the variable values shown in Table 1, the u_{mf} were 0.028 and 0.11 cm/s for the lowest (0.2 mm) and the medium (0.4 mm) particle size, respectively. The superficial reaction mixture velocities used were in the range 0.025–0.099 cm/s, which indicates that, at least, the smallest particles could be fluidised at the highest flow rates tested.

4.4. Equivalence between the batch and the continuous flow reaction

The experimentally obtained incorporations of CA indicate that the PBR does not behave as a differential reactor. Only in the experiments carried out with the greater flow rate, 630 ml/h, and the lower amount of lipase, 2.08 g (the minimal treatment intensity assayed), the behaviour of the

reactor could be considered as differential, since the incorporation of CA was only 7.4%. In the rest of the experiments the incorporation was always over 10%. Eq. (22) is based on the hypothesis that the PBR behaves as a differential reactor or as an integral plug flow reactor and therefore it should be taken only as approximate.

Nevertheless, Shimada et al. (1999) achieved a complete agreement between the conversion to ST attained in a batch reactor and a continuous PBR in the acidolysis of borage oil and CA, by using Eq. (22). With respect to our results Eq. (19) and/or Fig. 4 show that, for example, an incorporation of 52% (mol/mol) of CA into ST (and also 6.2% EPA, 10.6% DHA, 7.8% palmitic acid, 6.9% oleic acid, etc.) can be attained for $m_L/q[\mathrm{TG}]_0$ values of about 8200 g lipase h/mol TG. This value of the intensive variable coincides with the value obtained for $m_L t/V[\mathrm{TG}]_0$ in another work on acidolysis between triolein and CA in a batch reactor (Camacho Páez et al., 2001). In this latter work, a 60% (mol/mol) of CA was incorporated into the ST for $m_L t/V[\mathrm{TG}]_0$ values of 8000 g lipase/h mol triolein. In addition, as can be observed in Eq. (19) the term corresponding to the continuous PBR in Eq. (22), $m_L/q[\mathrm{TG}]_0$ is the variable that determines the composition of the synthesised ST (F_X values). Thus, an important application derived from Eq. (19) is related to scale up of the PBR, which should keep constant the ratio $m_L/q[\mathrm{TG}]_0$. Also, the fluid dynamics should be arranged to avoid potential mass transfer resistance.

On the other hand, as shown in Section 2, in the PBR the residence time corresponding to flow rates between 63 and 630 ml/h are 0.12 and 0.012 h, respectively. Eq. (22) shows that similar conversions could be attained in both reactors but with significant lower times of contact between the lipase and the substrates due to the higher lipase/substrate ratio used in the continuous PBR. This implies the following additional advantages for the continuous operation mode: decreased acyl-migration (Mu et al., 1998), no oxidation and no polymerisation of PUFAs and little or negligible formation of partial glycerides (Shimada et al., 1999). In addition, the continuous operation has other advantages, e.g. no destruction of the catalyst particles by agitation and the possibility of eliminating the organic solvent, which implies that a smaller reactor can be used.

4.5. Conclusions

Both equilibrium and kinetics of the acidolysis of CLO with CA are consistent with the reaction scheme proposed in Eq. (2). The average equilibrium constant for the exchange of CA and native fatty acids may be determined by Eqs. (10) and (11) or (12) and (13). The equilibrium constants obtained for the PUFAs show that these fatty acids are the most resistant to substitution by CA. The equilibrium composition of the produced ST shows that the position 2 of the original CLO was mainly occupied by EPA, DHA, oleic acid and palmitic acid. For reactor design Eqs. (10) and (11) (or (12) and (13)) and (18) (or (19)) can be used for estimating the necessary equilibrium and kinetic constants, respectively. For scaling up purposes, Eq. (19) points out the necessity to keep constant the quotient $m_L/q[\mathrm{TG}]_0$. In the acidolysis of CLO with CA, an incorporation of CA into the resultant ST of 57% (close to the theoretical maximum value of 66.7%) was reached. Nevertheless, the results obtained in another work indicate that it is not possible to increase the conversion to ST of the MLM type by increasing the molar ratio CA/triglycerides above a specific value because the reaction rate decreases (Camacho Páez et al., 2001). Therefore, the incorporation of CA into ST when working in a continuous PBR is likely to be increased by removing the free fatty acids after an initial acidolysis in a first PBR and adding new CA (removing water by molecular sieves, if necessary) and carrying out a new acidolysis reaction in a second PBR.

Notation

area_X	chromatographic areas of a fatty acid in GC analysis
area_{IS}	chromatographic area of the internal standard in GC analysis
CA	caprylic acid
CLO	cod liver oil
d_p	particle diameter of catalyst
DHA	docosohexaenoic acid
EPA	eicosapentaenoic acid
f_X	response factor of fatty acids in GC analysis
F_M	molar fraction of the medium-chain fatty acid (caprylic acid) incorporated into ST
$F_{\mathrm{M}e}$	molar fraction of the medium-chain fatty acid (caprylic acid) incorporated into ST at the equilibrium
$F_{\mathrm{Li}e}$	molar fraction of a cod liver oil native fatty acid into ST at the equilibrium
F_{Li0}	molar fraction of a CLO native fatty acid into the original CLO triglycerides
F_{Li2}	molar fraction of a CLO native fatty acid esterifing the position 2 of the ST
F_X	molar fraction of a fatty acid (CA or a native CLO fatty acid) into the triglycerides
F_{X0}	molar fraction of a fatty acid (CA or a native CLO fatty acid) into the original CLO triglycerides
GL_i	triglyceride with one or two native fatty acid at position 1 and/or 3 changeable by an odd fatty acid molecule
GM	triglyceride with one or two odd (medium-chain) fatty acid molecules (M) incorporated at position 1 and/or 3
GC	gas chromatography
k_X	kinetic constant for acidolysis reaction, defined by Eq. (18), mol/g lipase h

k_V constant to fit the experimental results to Eq. (19), ml/g lipase h
K_i equilibrium constant for the acidolysis reaction (2) (or Eq. (A.1)), defined by Eq. (3) (or Eq. (A.2)) (see Appendix A).
L_i native long-chain free fatty acid from cod liver oil
m_0 initial CA/TG molar ratio
m_L amount of lipase, g
M medium-chain free fatty acid (caprylic acid)
MLM structured triglycerides with medium-chain fatty acids in the positions 1 and 3 and a long-chain fatty acid in the position 2 of the glycerol backbone
PBR packed-bed reactor
PUFAs polyunsaturated fatty acids
q flow rate of reaction mixture through the PBR, ml/h
r_{mX} average rate of incorporation into triglycerides of a fatty acid X between the entrance and the exit of the PBR, defined by Eq. (17), mol/g lipase h
r_X rate of incorporation into triglycerides of a fatty acid X, defined by Eq. (15), mol/g lipase h
Re_P Reynolds number of particle
ST structured triglycerides
t reaction time, h
TG triglyceride
TLC thin-layer chromatography
u_{mf} minimum fluidisation rate (Eq. (23)), cm/s
V reaction volume of a batch reactor
X free fatty acid (CA and/or a native fatty acid from CLO)
[-] concentration of ester bonds (expressed as mole of equivalent acyl group) between the glycerol backbone and the acyl group and concentration of free fatty acids, M

Greek letters

ε catalyst particle porosity
μ hexane viscosity at 30°C, cp or g/cm s
ρ_L hexane density at 30°C, g/cm^3
ρ_S catalyst particle density admitting that the pore fraction is occupied by hexane, g/cm^3

Subscripts

e equilibrium
in entrance of the PBR
out exit of the PBR
0 initial concentration or molar fraction

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Appendix A. Overall treatment of the equilibrium of acidolysis reactions catalysed by 1, 3 specific lipases

Let an oil formed by heterogeneous triglycerides, GL_iL_j, in which participate the native fatty acids L_i ($i=1\ldots n$) and L_j ($j=1\ldots n$). This oil experiments an acidolysis reaction with an odd fatty acid, M. An overall treatment of the acidolysis reactions involved can be performed admitting the following hypothesis: (i) only the fatty acids in the positions 1 and 3 are exchanged, (ii) the change in the position 1 does not depend on the nature of the fatty acid in the position 3 and vice versa, (iii) the percentage of diglycerides in the equilibrium is negligible, although they are necessary intermediates for the exchange reactions, and (iv) the acyl-migration between the position 2 and the extreme positions 1 and 3 is also negligible. This treatment will be carried out considering that the equilibrium constant depends on the nature of the native fatty acid exchanged.

Any exchange reaction corresponding to the first step of the acidolysis process can be written as

$$GL_jL_i + M \;\Leftrightarrow\; GL_jM + L_i, \tag{A.1}$$

where only the fatty acids in the positions that participate in the exchange of acyl group are indicated in the triglyceride (G represents the glycerol backbone and the acyl group in the position 2 that does not participate in the exchange). For Eq. (A.1) the equilibrium constant for the change of the native fatty acid, L_i, by the odd fatty acid, M, will be

$$K_i = \frac{[GL_jM]_e[L_i]_e}{[GL_jL_i]_e[M]_e} \tag{A.2}$$

and

$$[GL_jM]_e = K_i \frac{[M]_e}{[L_i]_e}[GL_jL_i]_e.$$

Summing all the equivalent equations for each L_j:

$$\sum_j [GL_jM]_e = K_i \frac{[M]_e}{[L_i]_e} \sum_j [GL_jL_i]_e \tag{A.3}$$

that can be written as

$$\sum_j [GL_jL_i]_e = \frac{\sum_j [GL_jM]_e}{[M]_e} \frac{[L_i]_e}{K_i}.$$

Summing now all the equivalent expressions for each L_i:

$$\sum_i \sum_j [GL_jL_i]_e = \frac{\sum_j [GL_jM]_e}{[M]_e} \sum_i \frac{[L_i]_e}{K_i}. \tag{A.4}$$

Eq. (A.4) includes all the equilibrium reactions of (A.1) type, including the two possible reactions for the same original triglyceride:

$$GL_jL_i + M \;\Leftrightarrow\; GL_jM + L_i,$$

$$GL_jL_i + M \;\Leftrightarrow\; GL_iM + L_j,$$

therefore, Eq. (A.4) can be used to define an average equilibrium constant for this first step of exchange:

$$K = \frac{[GLM]_e[L]_e}{[GL_2]_e[M]_e} \tag{A.5}$$

taking into account that,

$$[GL_2]_e = \sum_i \sum_j [GL_j L_i]_e; \quad [GLM]_e = \sum_j [GL_j M]_e;$$

$$[L]_e = \sum_i [L_i]_e$$

and comparing Eqs. (A.4) and (A.5), it can be obtained as

$$\frac{[L]_e}{K} = \sum_i \frac{[L_i]_e}{K_i}. \tag{A.6}$$

For the exchange reaction:

$$GL_i M + M \Leftrightarrow GMM + L_i \tag{A.7}$$

the equilibrium constant is

$$K_i = \frac{[GMM]_e [L_i]_e}{[GL_i M]_e [M]_e}$$

that can be written as

$$[GL_i M]_e = \frac{[GMM]_e}{[M]_e} \frac{[L_i]_e}{K_i}$$

summing all the equivalent expressions for the different native fatty acids L_i and taking into account Eq. (A.6):

$$\sum_i [GL_i M]_e = \frac{[GMM]_e}{[M]_e} \sum_i \frac{[L_i]_e}{K_i} = \frac{[GMM]_e}{[M]_e} \frac{[L]_e}{K} \tag{A.8}$$

this equation shows that the previously defined average constant is also useful to represent the second step of the exchange reaction

$$K = \frac{[GMM]_e [L]_e}{[GLM]_e [M]_e}. \tag{A.9}$$

Let $[GM]_e$ and $[GL_i]_e$ the equilibrium concentrations of ester bonds between the glycerol backbone and the odd and native fatty acids, respectively, at the positions 1 and 3 (expressed as fatty acid concentrations), it can be written as

$$[GM]_e = \sum_j [GL_j M]_e + 2[GMM]_e, \tag{A.10}$$

$$[GL_i]_e = \sum_j [GL_j L_i]_e + [GL_i L_i]_e + [GL_i M] \tag{A.11}$$

from Eq. (A.8) it can be obtained as

$$[GMM]_e = K \frac{[M]_e}{[L]_e} \sum_i [GL_i M]_e$$

and substituting into Eq. (A.10):

$$[GM]_e = \sum_j [GL_j M]_e \left(1 + 2K \frac{[M]_e}{[L]_e}\right),$$

$$\sum_j [GL_j M]_e = \frac{[GM]_e}{(1 + 2K[M]_e/[L]_e)}. \tag{A.12}$$

For the exchange reactions:

$$GL_i L_i + M \Leftrightarrow GL_i M + L_i,$$

$$GL_i L_j + M \Leftrightarrow GL_i M + L_j,$$

the equilibrium constants are

$$K_i = \frac{[GL_i M]_e [L_i]_e}{[GL_i L_i]_e [M]_e}, \qquad K_j = \frac{[GL_i M]_e [L_j]_e}{[GL_i L_j]_e [M]_e},$$

which can be written as follows:

$$[GL_i M]_e = [GL_i L_i]_e [M]_e \frac{K_i}{[L_i]_e} = [GL_i L_j]_e [M]_e \frac{K_j}{[L_j]_e} \tag{A.13}$$

and from the last equality of Eq. (A.13):

$$[GL_i L_j]_e = [GL_i L_i]_e \frac{K_i}{[L_i]_e} \frac{[L_j]_e}{K_j}$$

summing all the equivalent expressions for the different L_j and taking into account Eq. (A.6):

$$\sum_j [GL_i L_j]_e = [GL_i L_i] \frac{K_i}{[L_i]_e} \sum_j \frac{[L_j]_e}{K_j} = [GL_i L_i] \frac{K_i}{[L_i]_e} \frac{[L]_e}{K},$$

$$[GL_i L_i]_e = \frac{K}{K_i} \frac{[L_i]_e}{[L]_e} \sum_j [GL_i L_j]_e \tag{A.14}$$

and from the first equality of Eqs. (A.13) and (A.14):

$$[GL_i M]_e = [GL_i L_i]_e [M]_e \frac{K_i}{[L_i]_e} = K \frac{[M]_e}{[L]_e} \sum_j [GL_i L_j]_e. \tag{A.15}$$

Substituting Eqs. (A.14) and (A.15) into Eq. (A.11):

$$[GL_i]_e = \sum_j [GL_j L_i]_e \left(1 + \frac{K}{K_i} \frac{[L_i]_e}{[L]_e} + K \frac{[M]_e}{[L]_e}\right),$$

$$\sum_j [GL_j L_i]_e = \frac{[GL_i]_e}{(1 + (K/K_i)[L_i]_e/[L]_e + K[M]_e/[L]_e)} \tag{A.16}$$

and also substituting Eqs. (A.12) and (A.16) into Eq. (A.3):

$$\frac{[GM]_e [L_i]_e}{[GL_i]_e [M]_e} = K_i \frac{(1 + 2K[M]_e/[L]_e)}{(1 + (K/K_i)[L_i]_e/[L]_e + K[M]_e/[L]_e)}. \tag{A.17}$$

The n equations of (A.17) form for each native fatty acid in the positions 1 and 3, and Eq. (A.6), permit the calculation of the exchange equilibrium constant for each fatty acid from variables that can be determined by analysis of the reaction products and by triglyceride and fatty acid balances. If the nature of the native fatty acids does not affect their degree of exchange with the odd fatty acid, $K = K_1 = \cdots = K_n$, and a statistical distribution of the fatty acids present at positions 1 and 3 would be obtained when the equilibrium was attained, and the n equations (A.17) would permit to calculate the fatty acid composition of the position 2.

For a homogeneous triglyceride Eqs. (A.6) and (A.17) drive to

$$\frac{[GM]_e[L]_e}{[GL]_e[M]_e} = K\frac{(1+2K[M]_e/[L]_e)}{(2+K[M]_e/[L]_e)}, \quad (A.18)$$

which allows to calculate the exchange equilibrium constant between the odd and native fatty acid. In this equation, if $K = 1$, the nature of the fatty acid does not affect at the exchange equilibrium and it can be used to determine the degree of incorporation of an odd fatty acid that corresponds to a statistical distribution at the equilibrium.

In Eq. (A.17) the second term of the denominator $[L_i]_e/[L]_e$ represents the triglycerides GL_iL_j, that, for high degrees of incorporation, must be negligible compared with the others (GL_iM and GMM), and Eq. (A.17) can be written as

$$\frac{[GM]_e[L_i]_e}{[GL_i]_e[M]_e} = K_i\frac{(1+2K[M]_e/[L]_e)}{(1+K[M]_e/[L]_e)} \quad (A.19)$$

that can be reordered as

$$\frac{[L_i]_e}{K_i} = \frac{[M]_e}{[GM]_e}\frac{(1+2K[M]_e/[L]_e)}{(1+K[M]_e/[L]_e)}[GL_i]_e$$

and adding for all the native fatty acids:

$$\frac{[GM]_e[L]_e}{[GL]_e[M]_e} = K\frac{(1+2K[M]_e/[L]_e)}{(1+K[M]_e/[L]_e)}. \quad (A.20)$$

This equation permits the calculation of K and after the equilibrium constant for each native fatty acid, K_i, can be calculated by Eq. (A.19).

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