Concentration of Eicosapentaenoic Acid by Selective Esterification Using Lipases

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ABSTRACT: The aim of this work was to increase the content of EPA in FFA extracts from a commercial oil (43.1% EPA) and from *Phaeodactylum tricornutum* oil, a single-cell oil, by selective enzymatic esterification. Initially, the FFA extract was esterified with lauryl alcohol using nine lipases. All the lipases concentrated EPA in the unesterified FFA fraction. The criterion used to choose the best lipase was maximization of the dimensionless effectiveness factor (F_{AE}). This factor grouped the concentration factor (ratio between the EPA concentrations in the FFA fractions before and after esterification) with EPA recovery in the final FFA fraction. Experiments were carried out to correlate F_{AE} and the degree of esterification (ED, percentage of initial FA converted to lauryl esters). Lipase AK from *Pseudomonas fluorescens* was the most effective for concentrating EPA. Studies of the optimal temperature, substrate molar ratio, solvent/substrate ratio, and treatment intensity (product of the lipase mass and the reaction time) were also carried out using the lipase. The maximum F_{AE} was obtained when the ED was 60%: EPA concentration was 72%, and recovery was 73%. Finally, this lipase was used to concentrate EPA from a FFA extract from *P. tricornutum* (23% EPA). The content of EPA in the unesterified FFA fraction increased to 71% at 78% ED (recovery of EPA, 75.5%). Comparison of the results obtained with the two FFA extracts seemed to indicate that the selectivity of Lipase AK for EPA depended on the content of EPA, with higher contents of EPA in the initial FFA mixture reducing the selectivity for EPA.

Paper no. J11217 in *JAOCS 83*, 215–221 (March 2006).

KEY WORDS: Concentration, eicosapentaenoic acid (EPA), esterification, lipase.

EPA, an n-3 PUFA, has attracted increasing attention because of its important role in human health. EPA has potential uses for the prevention or treatment of heart and circulatory diseases (1) and inflammation (2). In Japan, EPA ethyl ester has been used for the treatment of arteriosclerosis and hyperlipemia since 1991 (3). However, pharmaceutical and clinical applications require a high PUFA concentration (4). We have previously developed a three-step method to obtain highly pure PUFA from fish and microalgal oils, which involved isolation by preparative HPLC (5,6). However, industrial implementation of these methods is difficult because of the high cost of the chromatographic step.

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The aim of this work was to examine the possibility of using lipases as catalysts to produce EPA concentrates from FFA extracts originating from fish and microalgae. Lipases are well known to have little activity on PUFA (7,8). These FA have been enriched by selective hydrolysis (9), by selective esterification (10), and by selective alcoholysis (11,12). Many investigators have concentrated DHA and GLA using enzymatic reactions, and these FA were industrially produced by selective hydrolysis (3). However, concentrating EPA is difficult. Miller *et al*. (13) showed that the activity of Lipozyme RMIM, from *Rhizomucor miehei*, on a FA diminished in proportion to the proximity of a double bond on the FA to the carboxylic group. This factor explains why DHA (22:6n-3) is concentrated more easily than EPA (20:5n-3): EPA has a double bond at the carbon atom in the 5 position and DHA has a double bond at the carbon atom in the 4 position. Thus, for example, Wanasundara and Shahidi (14) concentrated DHA and EPA from seal blubber and menhaden oils by hydrolysis using several lipases. The most effective lipases were *Candida rugosa* and *Rhizopus oryzae*. The former increased the concentration of DHA from seal blubber oil from 7.6 to 24% (3.2-fold increase) and the concentration of EPA from 6.4 to 9.8% (1.5-fold increase); these results were obtained after 35 h of hydrolysis, but at 80 h the EPA content decreased to 8.5%. *Rhizopus oryzae* lipase was also effective for the enrichment of total n-3 PUFA and DHA in both oils. However, this lipase selectively hydrolyzed EPA from TAG of both oils; consequently, the contents of EPA in the final products decreased (14). However, the esterification reaction was more effective than hydrolysis in concentrating PUFA (3). Lipase AK from *Pseudomonas* sp. moderately hydrolyzed DHA from TAG compared with other constituent FA. Hence, when an oil containing DHA was hydrolyzed to a high degree with Lipase AK, the content of DHA in the FFA fraction was the same as that in the original oil (15). Esterification of the resulting FFA using *R. oryzae* lipase increased the enrichment of DHA in the FFA fraction from 23 to 73% (15,16).

This work deals with the concentration of EPA from EPA-rich FFA mixtures originating from a commercial fish oil and from a single-cell oil from *Phaeodactylum tricornutum*.

MATERIALS AND METHODS

FFA from microalgal cells and oil. Lyophilized or wet cells of the marine microalga *P. tricornutum* (UTEX 640) were used as the starting material for preparing an EPA-rich FFA mixture.

TABLE 1
FA Composition of FFA Mixtures Obtained from *Phaeodactylum tricornutum* Cells and EPAX4510TGl

FA	FFA from *P. tricornutum* (wt%)	FFA from EPAX4510TG (wt%)
14:0	6.9	—[a]
16:0	20.1	4.1
16:1n-7	19.1	1.7
16:2n-4	4.0	—
16:3n-4	5.6	—
16:4n-1	0.7	0.5
18:0	1.0	4.6
18:1n-9	2.2	7.8
18:1n-7	1.6	5.6
18:2n-6	3.0	1.0
18:3n-6	—	0.6
18:3n-3	0.5	0.7
18:4n-3	0.6	3.3
20:0	—	0.6
20:1n-9	—	4.0
20:2n-6	—	0.9
20:4n-6	2.4	2.7
20:4n-3	0.9	1.7
20:5n-3 (EPA)	**23.0**	**43.1**
22:1n-9	—	4.1
21:5n-3	—	1.4
22:5n-3	3.8	2.4
22:6n-3	2.5	7.9
24:0	0.3	—
Others	1.8	1.4

[a]A dash (—) indicates that the corresponding FA does not appear in the oils.

Cells were grown in an outdoor tubular photobioreactor, harvested by centrifugation at 1800 × *g*, and then stored at −8°C until use. A FFA mixture was obtained from *P. tricornutum* cells by a three-step process: (i) direct saponification of the microalgal cells, (ii) extraction of unsaponifiables, and (iii) extraction of FFA. This method was carried out as described in Ibáñez González *et al*. (6). The composition of FA in the extract, determined in triplicate by GC, is shown in Table 1.

EPAX4510TG (Pronova Biocare, Lysaker, Norway) is a commercial marine oil rich in EPA. Table 1 shows the composition of FA in the FFA extract obtained by saponification of the oil and extraction of the FFA (5).

Lipases. The lipases used in esterifying the FFA were (Table 2): Lipase D from *R. oryzae,* Lipase M from *Mucor javanicus*, Lipase AK from *Pseudomonas fluorescens* (Amano Pharmaceutical Co., Nagoya, Japan), Lipozyme RMIM from *R. miehei*, Novozyme 435 from *Candida antarctica* (Novo Nordisk, A/S, Bagsvaerd, Denmark), Lipase EU-093 from *Rhizopus oryzae*, Lipase EU-034 from *Pseudomonas stutzeri* (Europe Bioproducts, Cambridge, United Kingdom), Lipase QLMex from *Alcaligines* sp., and Lipase OFex from *Candida rugosa* (Meito Sangyo, Nagoya, Japan). The lipases D, M, AK, EU-093, EU-034, QLMex, and OFex were provided as powders, with a minimum water content of 10%. In some experiments these lipases were immobilized on Celite 545 AW (Fluka Chemie, Buchs, Switzerland). This immobilization was performed in our laboratory according to Soumanou *et al*. (17). The ratios of Celite/lipase were 2.5:1 and 4:1 (w/w).

Esterification. Esterifications of FFA with lauryl alcohol (1-dodecanol; Panreac, Barcelona, Spain) were conducted as follows: FFA (170–1780 mg), lauryl alcohol (222–4440 mg; molar ratio of FFA extract/lauryl alcohol, 1:1 to 1:4), *n*-hexane (0–25 mL/g substrates), and lipase (1.25–200 mg lipase/g substrates)

TABLE 2
Esterification of a FFA Mixture from EPAX4510TG with Lauryl Alcohol by Different Lipases: Degree of Esterification (ED), EPA Recovery (F_R), and EPA Concentration (F_C) in the FFA Fraction[a]

	FFA (nonesterified FA)			
Lipase	T (°C)	ED (%)	F_R (%)	F_C
M	40	37.8	73.0	1.07
D	40	53.0	66.7	1.27
EU-034	40	58.1	52.9	1.18
AK	40	17.0	90.9	1.14
EU-093	40	72.3	38.8	1.30
OFex	40	39.2	70.3	1.14
QLMex	40	90.3	16.5	1.60
M	55	53.2	52.4	0.94
D	55	25.8	83.0	1.11
EU-034	55	74.8	34.3	1.17
AK	55	32.4	84.0	1.32
EU-093	55	77.5	38.2	1.47
OFex	55	23.4	83.0	1.07
QLMex	55	37.5	68.2	1.04
Lipozyme RMIM[b]	55	87.9	11.9	1.0
Novozym 435[c]	40	54.8	47.0	0.9

[a]Operation conditions: 178 mg of FFA, 222 mg of alcohol (molar ratio 1:2), 40 mg of lipase, 4 mL of hexane, and 24 h.
[b]2.5 mg and 24 h.
[c]5 mg and 2 h.

were introduced into 50-mL Erlenmeyer flasks with silicone-capped stoppers under an argon atmosphere (to avoid oxidation of the EPA). The mixture was incubated at 30, 40, or 55°C with shaking at 400 rpm (Shaking Incubator SWB 20; Haake Mess-Technik GmbH u. Co., Karlsruhe, Germany) for different times. The reaction was stopped by addition of 6–8 mL of hexane. The mixture was then filtered (glass plate of porosity 4) and the aqueous phase was separated in a decantation funnel. The volume of the hexane phase was adjusted to 25 mL with the addition of hexane. This final mixture was stored under an argon atmosphere at –20°C until analysis.

In a previous paper (18) we verified that the amount of lipase (m_z) and the reaction time (t) had the same effect on the enzymatic reaction conversion. Therefore, it is possible to combine both variables into a single variable by defining the intensity of treatment (TI) as the product of m_z and t.

Analysis of reaction products. The final esterification mixture contained FFA, lauryl esters (LE), lauryl alcohol, hexane, and small amounts of water. The degree of esterification (ED) was expressed as the molar ratio of LE to the initial amount of FFA. The ED was determined by two procedures: acid–base titration and TLC followed by GC.

The analysis of the reaction products by TLC–GC determined the composition of FA in the two separated fractions (FFA and LE) and the total amount of FA in each fraction. ED by this procedure was determined as follows:

$$\text{ED} = \frac{\text{total amount of fatty acids obtained from the LE chromatograms}}{\text{total amount of fatty acids in the LE and FFA chromatograms}} * 100 \quad [1]$$

These TLC–GC analyses have been described elsewhere (19). The ED used in this work was the average value of the ED obtained by acid–base titration and by TLC–GC.

To quantify the degree of concentration and the recovery of EPA in the FFA fraction, the concentration factor (F_C) and the recovery factor (F_R) were used. F_C is the ratio of the content of EPA (C_{EA}) in the FFA fraction to the content of EPA (C_{EAo}) in the initial FFA extract:

$$F_C = \frac{C_{EA}}{C_{EAo}} \quad [2]$$

The recovery (F_R) was calculated as follows:

$$F_R = \frac{C_{EA}X_A}{C_{EA}X_A + C_{LE}X_L} \quad [3]$$

where C_{EA} is the concentration of EPA in the FFA fraction, C_{LE} is the concentration of EPA in the LE fraction, X_A is the weight of the FFA fraction, and X_L is the weight of the LE.

Separation of esters and FFA after esterification. FFA and LE fractions were separated by the addition of a KOH solution to neutralize the FFA. LE was recovered in the hexane phase, and FFA (as potassium salts) was recovered in the aqueous phase. The aqueous phase was acidified with HCl and the FFA were extracted with *n*-hexane.

RESULTS AND DISCUSSION

Esterification of FFA originating from EPAX4510TG. The aim of this work was to concentrate EPA in an FFA fraction by the esterification of a mixture of FFA containing EPA with lipases. First, this concentration was conducted using FFA originating from the commercial oil EPAX4510TG, which contained 43.1% EPA (Table 1), as starting material. The lipases used were selected because they displayed either high esterification activity or a certain specificity for PUFA. Esterification of FFA was carried out with lauryl alcohol because our preliminary experiments using Lipases D and M showed that this alcohol achieved higher ED than did ethanol. These preliminary results concur with those obtained by Shimada *et al.* (16), who assayed 11 alcohols and chose lauryl alcohol to concentrate DHA from a FFA mixture from tuna oil.

Table 2 shows that most concentration factors (F_C) were greater than one, indicating that EPA was concentrated in the unesterified FFA fraction. High F_C were obtained by esterification with Lipase QLM (F_C 1.60 at 40°C), Lipase D (F_C 1.27 at 40°C), Lipase EU-093 (F_C 1.30 and 1.47 at 40 and 55°C, respectively), and Lipase AK (F_C 1.32 at 55°C). The recovery of EPA (F_R) in the FFA fraction is important to effectively concentrate EPA. Among the lipases that showed high F_C, those that provided higher EPA yields were Lipase AK (84% at 55°C) and Lipase D (66.7% at 40°C). EPA yields in the reaction with the other lipases were below 50%.

Considering both the F_C and F_R, we selected Lipase AK, Lipase D, and Lipase EU-093.

Most suitable lipase for the enrichment of EPA. The FA specificity of a lipase is affected by its molecular properties, the structure of the substrate, and factors affecting the binding of the enzyme to FA (20). Factors such as substrate concentration, temperature, and amount or immobilization of lipase do not necessarily affect the lipase specificity for EPA. However, all these factors may affect the lipase activity. All the lipases used catalyzed the esterification of EPA to some degree (Table 2). Lipase selectivity for EPA will increase as the difference between the esterification rates of EPA and other FA increases. This rate is proportional to the EPA concentration, which increases as the esterification progresses. Therefore, the esterification rate of EPA increases and the selectivity of the lipase for EPA decreases. Lipase selectivity will be affected by the composition of the FFA mixture at any given reaction time; consequently, it will be related to the ED. To determine the selectivity of a lipase, it is advisable to carry out several experiments in which the variation of F_C and F_R against ED is studied.

Three selected lipases (Lipase AK, Lipase D, and Lipase EU-093) were compared using five parameters: ED, F_C, F_R, and two additional ones, the dimensionless concentration factor (F_{AC}) and the effectiveness factor (F_{AE}). To assign the same importance to the two factors affecting the selectivity of a lipase for EPA (concentration and recovery), F_C can be substituted by a parameter that, like F_R, includes values between 0 and 1. For this purpose a dimensionless concentration factor

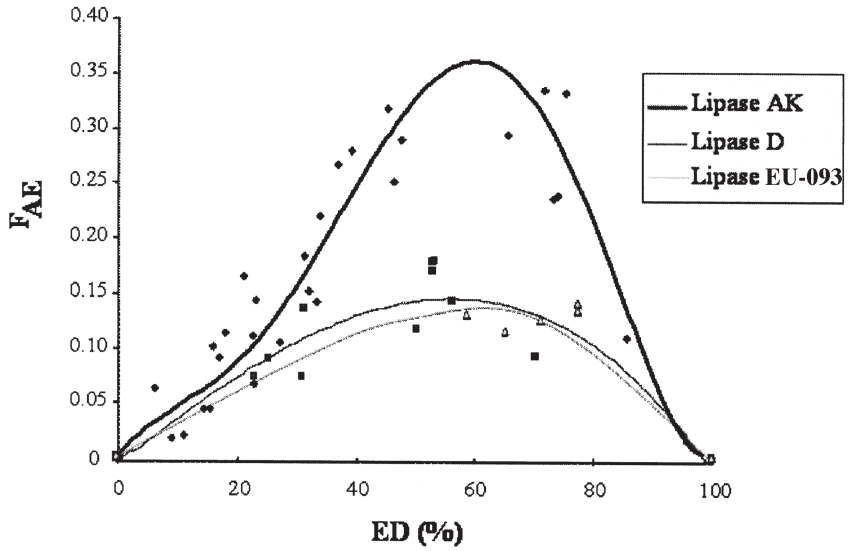

FIG. 1. Esterification of a FFA mixture from EPAX4510TG with lauryl alcohol by Lipase EU-093, Lipase D, and Lipase AK under different conditions: Influence of the degree of esterification (ED) on the effectiveness factor (F_{AE}). Operation conditions with Lipase EU-093 and Lipase D: 178 mg of FFA, 222 mg of alcohol (molar ratio 1:2), 4 mL of hexane, 24 h; immobilized and native lipases used at 40 and 55°C using 40–400 mg lipase. Operation conditions with Lipase AK: 178–1780 mg of FFA; 222–4440 mg of alcohol; FFA/alcohol molar ratios of 1:1, 1:2, 1:3, and 1:4; 40 and 55°C; 0, 4, and 10 mL of hexane; 24 and 48 h; and 4–320 mg of native or immobilized lipase.

(F_{AC}), also including values between 0 and 1, was defined to quantify the EPA concentration:

$$F_{AC} = \frac{C_{EA} - C_{EAo}}{100 - C_{EAo}} \quad [4]$$

where C_{EA} is the concentration of EPA in the unesterified FFA fraction and C_{EAo} is the concentration of EPA in the substrate FFA mixture. The value of F_{AC} is between 0 and 1, because when EPA is esterified at the same rate as the other FA, $C_{EA} = C_{EAo}$ and $F_{AC} = 0$; on the other hand, when EPA is not esterified but all other FA are esterified completely, $C_{EA} = 100$ and $F_{AC} = 1$. In this way, the concentration and recovery factors are defined by parameters (F_{AC} and F_R, respectively) that vary between 0 and 1 and can be unified in a single factor, allowing effective comparison of the results obtained with the three lipases. This parameter is termed the effectiveness factor (F_{AE}) and can be defined as

$$F_{AE} = F_{AC} \times F_R = \frac{C_{EA} - C_{EAo}}{100 - C_{EAo}} \times F_R \quad [5]$$

This parameter was used to quantify the effectiveness of the lipases in concentrating and recovering EPA (lipase selectivity for EPA) at any time.

Esterification of the FFA was performed using the three selected lipases, and the amount of lipase and other experimental conditions were changed to obtain different ED. Figure 1 shows the variation of the F_{AE} with the ED. Lipases D and EU-093 showed similar behavior, and the maximum values of F_{AE} did not exceed 0.2. However, Lipase AK gave higher F_{AE}: the maximum F_{AE} was >0.35. Therefore, among the three enzymes this lipase was the most suitable for concentrating EPA.

Optimization of the esterification conditions with Lipase AK. Immobilized Lipase AK had higher F_R but did not achieve higher ED than the lipase used without being immobilized (native lipase). Immobilization led to a significant loss of activity when Lipase AK was reused once or twice (results not shown). Hence, native Lipase AK was used in this study.

Figure 2 shows the ED obtained with Lipase AK at different FFA/lauryl alcohol molar ratios and temperatures. The highest ED was obtained with a molar ratio of 1:2. This result agreed with those obtained by other authors (21). The ED decreased when the alcohol content increased, which may be attributed to an increase in the mass transfer resistance, since the lauryl alcohol increased the viscosity of the reaction mixture and obstructed the transfer of substrates and products to the lipase.

Figure 2 also shows that the experiments carried out at 55°C gave higher ED than did those at 40°C. However, the higher temperatures increased the risk of PUFA oxidation and hexane evaporation.

Operating without solvent is of interest for the food, nutraceutical, and health care industries. There were no appreciable differences between the ED obtained with 4 and 10 mL of hexane (which corresponded to hexane/substrate ratios of 10 and 25 mL/g, respectively), indicating that this variable did not influence the esterification kinetics at this concentration range (Fig. 3). The reaction velocity with solvent was slightly faster than without solvent, which may be due to a decrease in the

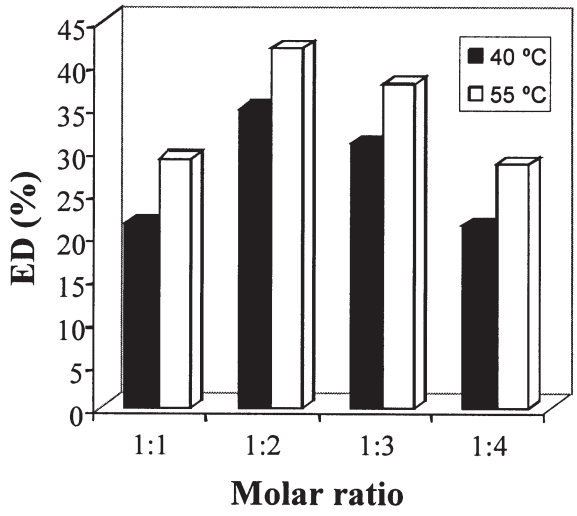

FIG. 2. Esterification of FFA from EPAX4510TG with lauryl alcohol by Lipase AK: Influence of the FFA/lauryl alcohol molar ratio on ED at different temperatures. Operation conditions: 178 mg of FFA, 4 mL of hexane, 40 mg of native Lipase AK, and 24 h. For abbreviation see Figure 1.

mass transfer rate by the increase in viscosity of the reaction mixture (22).

Optimal ED. The maximum F_C was reached at 75% ED (Fig. 4), and EPA recovery decreased as the ED increased (Fig. 5). When the ED was 75% (corresponding to a maximum F_C value), only 45% of the EPA was recovered in the FFA fraction. These F_C (or F_{AC}) and F_R values were used to calculate the F_{AE} values shown in Figure 1. The optimal ED (giving the maximum F_{AE} value) was approximately 60%.

When the ED reached 60%, F_C and F_R had values of 1.7 and 72%, respectively (Figs. 4, 5). This F_C corresponds to an EPA concentration in the FFA fraction of 73%. This ED was obtained with TI per unit of substrate mass of approximately 2000 mg lipase × h/g substrates (Fig. 3) under the following experimental conditions: 178 mg of EPAX4510TG FFA, 222 mg of lauryl alcohol (molar ratio FFA/alcohol 1:2), 4 mL of hexane, 40°C, and a TI of 800 mg of Lipase AK × h [800 mg lipase × h/(178 + 222) mg of substrates = 2000 mg × h/g substrates]. This experiment was carried out four times; the ED, F_C, F_R, and F_{AE} are shown in Table 3. These results correspond to a final FFA mixture with an EPA concentration of 71–72%, where 72–74% of the initial EPA was recovered (Table 3).

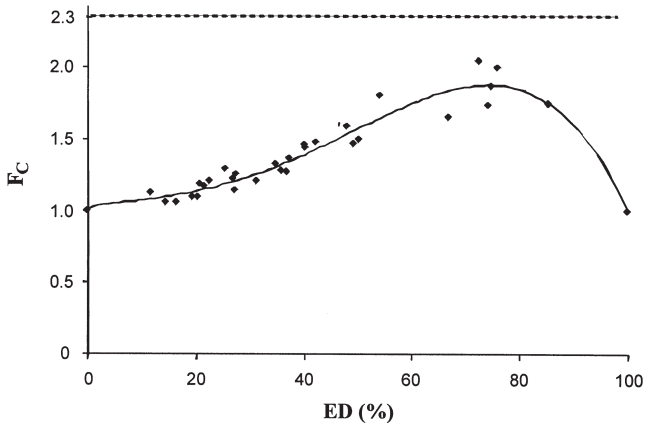

FIG. 4. Esterification of FFA from EPAX4510TG with lauryl alcohol by Lipase AK: Influence of the ED on the EPA concentration (F_C) in the unesterified FFA fraction. For other abbreviation see Figure 1.

Selective esterification of FFA originating from P. tricornutum cells. The optimized experimental conditions used in the esterification of a FFA mixture from EPAX4501TG oil with Lipase AK were used in the enzymatic esterification of FFA extracts from *P. tricornutum*. Different TI were tested by combining different lipase amounts and times. Table 4 shows the ED and percentages of EPA with respect to the total FA (C_{EA}) of the different experiments carried out. From these results, the concentration (F_C), recovery (F_R), and effectiveness (F_{AE}) factors were determined.

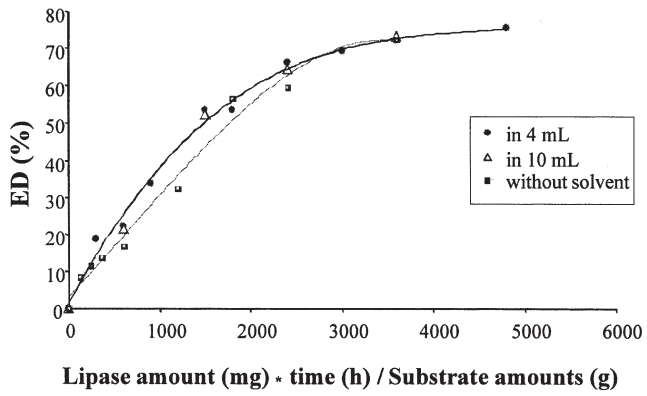

FIG. 3. Esterification of FFA from EPAX4510TG with lauryl alcohol catalyzed by Lipase AK: Influence of the treatment intensity (TI) per 1 g of substrate on the ED with different hexane volumes. Operation conditions: 178 mg of FFA, 222 mg of lauryl alcohol (molar ratio 1:2), 40°C. For other abbreviation see Figure 1.

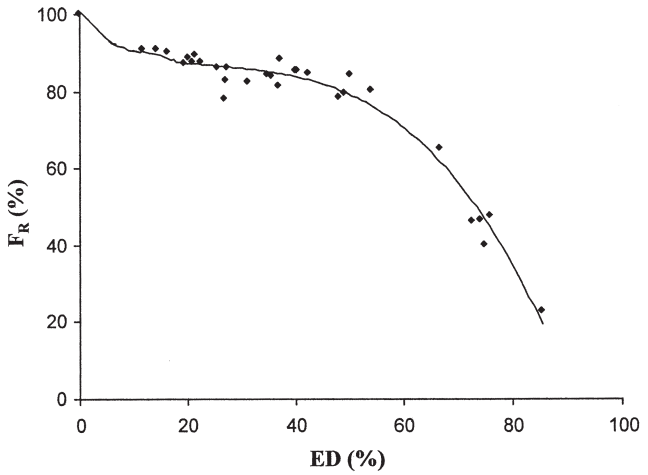

FIG. 5. Esterification of FFA from EPAX4510TG with lauryl alcohol by Lipase AK: Influence of the ED on the EPA recovery (F_R) in the unesterified FFA fraction. For other abbreviation see Figure 1.

TABLE 3
Selective Esterification of FFA Mixtures from EPAX4510TG and *P. tricornutum* Cells with Lauryl Alcohol Catalyzed by Lipase AK: Initial EPA Content of These FFA Extracts, with Treatment Intensity (TI)[a] Leading to the Optimal ED[b] or to the Maximum Effectiveness Factor (F_{AE})[c], Concentration (F_C)[d], and Recovery (F_R)[e]

	EPAX4510TG	*P. tricornutum*
Initial EPA content (%)	43.1	23.0
Treatment intensity (mg·h)[a]	816	2400
Optimal ED[b]	59.3–60.7	77.3–79.1
F_C[d]	1.66–1.68	3.0–3.2
F_R (%)[e]	72–74	74–77
Maximum F_{AE}[c]	0.36	0.46–0.49
Final EPA content (%)[f]	71.5–72.4	69.0–73.6

[a]TI that leads to the optimal ED: TI = 816 mg·h (34 mg of lipase and 24 h); TI = 2400 mg·h (100 mg of lipase and 24 h). For other abbreviation see Table 2.
[b]ED that gives the maximum effectiveness factor (F_{AE}).
[c]Maximum $F_{AE} = F_{AC} \times F_R$ (Eqs. 3, 4, and 5).
[d]Concentration of EPA corresponding to the maximum F_{AE} (Eq. 2).
[e]EPA recovery corresponding to the maximum F_{AE} (Eq. 3).
[f]Content of EPA in the final FFA mixture.

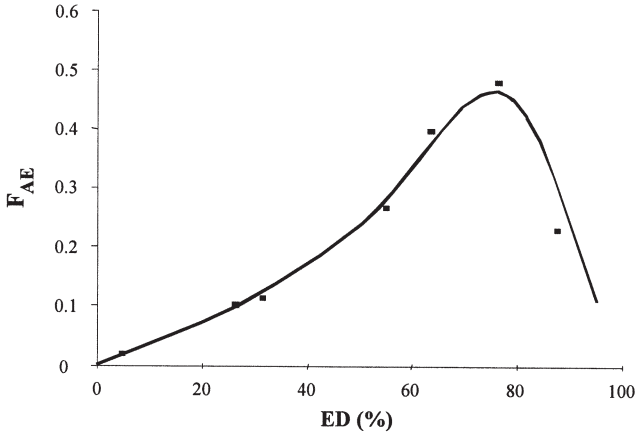

FIG. 6. Esterification of FFA from *Phaeodactylum tricornutum* cells with lauryl alcohol by Lipase AK: Influence of the ED on the effectiveness factor (F_{AE}). For operation conditions, see Table 4. For other abbreviation see Figure 1.

TABLE 4
Esterification of a FFA Mixture from *P. tricornutum* Cells with Lauryl Alcohol Catalyzed by Lipase AK: Influence of the TI (time × lipase amount) on the ED, EPA Content (wt%) in the FFA Fraction (C_{EA}), and F_C, F_R, and F_{AE}[a]

Time (h)	Lipase (mg)	TI (mg·h)	ED (%)	C_{EA} (%)	F_C	F_R (%)	F_{AE} (0–1)
24	20	480	5.2	24.9	1.04	98.4	0.01
23	34	782	26.8	33.5	1.33	97.6	0.10
24	34	816	32.1	31.9	1.39	95.1	0.11
24	51	1224	55.4	49.5	1.95	92.1	0.26
48	34	1632	63.9	59.2	2.47	89.3	0.39
48	51	2448	76.8	75.9	3.17	73.4	0.48
48	68	3264	88.1	73.4	3.06	36.5	0.23

[a]Operation conditions: 178 mg FFA, 222 mg lauryl alcohol (molar ratio 1:2), 4 mL hexane, and 40°C. For other abbreviations see Tables 2 and 3.

Figure 6 shows the variation of F_{AE} with ED. F_{AE} reached a maximum value at 78% ED. To obtain an optimal ED (78%), a TI of approximately 2400 mg of lipase × h was necessary (Table 4); the values of F_C and F_R were 3.1 and 76%, respectively. This F_C value corresponds to an EPA purity of 71% in the final FFA fraction.

Based on these results, four experiments were carried out under experimental conditions that allowed the maximum F_{AE} to be attained (178 mg of FFA extract, 222 mg of lauryl alcohol, 4 mL of hexane, 40°C, and a TI of 2400 mg of lipase AK × h). EPA was enriched to 69–74% with 74–77% recovery (Table 3).

Comparison between the results obtained with the commercial oil FFA and algal oil FFA. Although the initial concentrations of EPA from the two oils were different (Table 1), the recovery yields (F_R) were similar (Table 3). The concentration factors (F_C) were different, but the EPA contents in the final FFA fractions obtained from the two substrate FFA mixtures were similar (Eq. 2, Table 3). That is, when the content of EPA in the initial FFA mixture was low, the F_C and ED required to achieve a high content of EPA were high. Therefore, the final concentration and recovery of EPA did not depend on the EPA concentration in the initial FFA mixture.

ACKNOWLEDGMENTS

This research was supported by grants from the Ministerio de Educación y Cultura and Ministerio de Ciencia y Tecnología (Spain), Projects PPQ2000-1220 and AGL2003-03335, and Plan Andaluz de Investigación (Junta de Andalucía), CVI 0173.

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[Received August 29, 2005; accepted December 9, 2005]