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Evaluation of regioselectivity of lipases based on synthesis reaction conducted with propyl alcohol, isopropyl alcohol and propylene glycol

Pierrick Fevrier [a], Philippe Guégan [a], Florent Yvergnaux [a], Jean Pierre Callegari [a], Laurent Dufossé [b], Adrien Binet [b,*,1]

[a] *PROFIL, Av. du Gal Leclerc, Campus de Beaulieu, 35700 Rennes, France*
[b] *LUMAQ, EA 2651, Université de Bretagne Occidentale, 29000 Quimper, France*

Abstract

Preliminary investigations on the regioselectiviy of various lipases were performed. Ten commercial lipases from different origins, including three immobilized lipases, were tested by esterification reaction between caprylic acid and propyl or isopropyl alcohol in *n*-hexane. Reaction products were analyzed with a gas chromatograph. Best yields were obtained with immobilized lipase IM60 from *Rhizomucor miehei*. Therefore, this enzyme was chosen as biocatalyst for a second step of regioselectiviy study with propylene glycol which bears primary and secondary alcohol groups. It was shown, by using several solvents, that polarity could influence the product profile in situations in which multiple products of various polarities can be formed. Furthermore, the major role of silica gel in reaction mixture was established.

Keywords: Lipase; Regioselectivity; Esterification; Solvent polarity

1. Introduction

The lipases (EC 3.1.1.3) constitute a group of enzymes which is becoming increasingly attractive in the oil industry. Stability and especially selectivity are the properties that are responsible for their success in biotransformations. Apart from the selectivity, regioselectivity is the most widely used in modification of natural fats and oils, or for the production of structured triacylglycerols. These contain mixtures of either short-chain or medium-chain fatty acids or both, and long chain fatty acids, preferably esterified on the same glycerol molecule. Structural lipids are of potential advantage in human nutrition because they can be tailor-made to target specific diseases and metabolic conditions [1]. The interest of the structured lipids lies in the composition and position of different fatty acids on the glycerol backbone. Therefore, with the aim of an industrial application of lipases in synthesis of structured lipids, a detailed understanding and control of regioselectivity is essential.

* Corresponding author.
E-mail address: binet@iutquimp.univ-brest.fr (A. Binet).
[1] Fax: +33-2-98-90-85-44.

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With these substrates, regioselectivity is defined as the ability of lipases to distinguish the two external positions (primary ester bond) and the internal position (secondary ester bond) of glycerol. In the present paper, we describe a preliminary approach to determine the regioselective activity of lipases in synthesis reactions. In a first step, the behaviour of nine lipases and one esterase towards primary and secondary alcohol (propyl and isopropyl alcohol, respectively) was studied by esterification reaction with caprylic acid. In a second step, a study with propylene glycol was achieved. In this case, silica gel was added in reaction media to improve yields and the influence of solvent polarity was compared with respect to their efficiency in improving the enzymatic activity. These results represent a preliminary approach on regioselectivity study, a further step using glycerol as substrate is in progress.

2. Experimental

2.1. Material and analytical methods

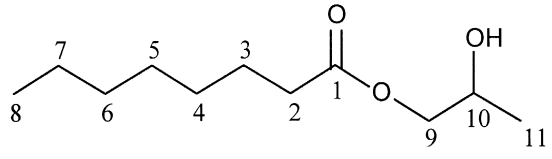
Ten enzymes were obtained from available sources. Lipase from *Aspergillus niger* ("A6"), *Mucor* sp ("M10") and *Pseudomonas* sp ("PSL") were from Amano Pharmaceutical (Nagoya, Japan). Immobilized lipase IM60 from *Mucor miehei* and SP435 from *Candida antarctica* B were supplied by Novo Nordisk. Immobilized lipase Chirazyme from *C. antarctica* A (CAA) was from Boehringer Mannheim. Porcine pancreatic lipase (PPL) was from Aldrich Chemical. Lipase from *C. cylindracea* (CCL) was from Fluka Biochemica. Lipase from *Rhizopus arhizus* (F30) was from MMP. Esterase (EST) was purchased from Gist Brocades.

Acetone, Acetonitrile, 2-methyl-2-propanol, isopropyl alcohol, propylene glycol diacetate and molecular sieve (3 Å) were obtained from Acros. Isopropyl ether, *tert*-butylmethyl ether and propyl alcohol were purchased from Fluka Chemica. Propylene glycol was purchased from Sigma. Caprylic acid and methyl laurate were purchased from Aldrich. *n*-hexane and silica gel were purchased from Merck.

Over the time course of the reaction, samples of 50 μl were removed periodically and diluted with 100 μl internal standard solution. Samples were analyzed by gas chromatography (GC). Analysis was performed in a Chrompack Gas Chromatograph CP 9001 equipped with a flame-ionization detector and a fused-silica capillary column (BPX70, 25 m × 0.22 mm i.d.; SGE) and operated in a split mode. The carrier gas was helium, and the total gas flow rate was 27 cm/s. The injector and detector temperatures were 270°C and 300°C, respectively. For esterification reaction between caprylic acid and propyl alcohol or isopropyl alcohol, the column was held at 50°C for 1 min and heated to 200°C for 2 min at the rate of 15°C/min. The esters were quantified with methyl laurate as an internal standard. For esterification reaction between caprylic acid and propylene glycol, oven temperature was programmed from 80°C for 4 min to 190°C at the rate of 8°C/min, increased to 250°C at the rate of 30°C/min and held 3 min. The esters were quantified with propylene glycol diacetate as an internal standard.

Different compounds were analyzed by NMR. Proton spectra were recorded at 400 MHz in $CDCl_3$. Carbon spectra were recorded at 100 MHz in $CDCl_3$. Chemical shifts were referenced to internal TMS.

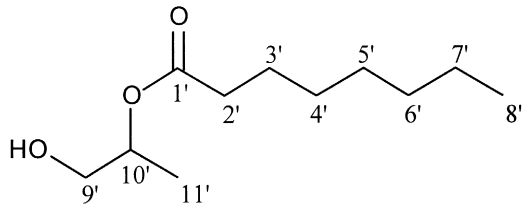
Primary monoester: 2-hydroxy-1-propyl octanoate



RMN ^1H (400.13 MHz, $CDCl_3$, δ): 4.10 (dd, $^2J_{H9a-H9b} = 10.9$ Hz, $^3J_{H9a-H10} = 3.1$ Hz, 1H, H_{9a}); 4.03 (m, 1H, H_{10}); 3.94 (dd, $^2J_{H9b-H9a} = 10.9$ Hz, $^3J_{H9b-H10} = 7.1$ Hz, 1H, H_{9b}); 2.35 (t, $^3J_{H2-H3} = 7.6$ Hz, 2H, H_2); 1.64 (m, 2H, H_3); 1.28 (m, 8H, H_4–H_7); 1.21 (d, $^3J_{H11-H10} = 6.3$ Hz, 3H, H_{11}); 0.88 (*t*, $^3J_{H8-H7} = 6.8$ Hz, 3H, H_8).

RMN ^{13}C (100.61 MHz, $CDCl_3$, δ): 174.1 (C_1); 69.5 (C_9); 66.1 (C_{10}); 34.2 (C_2); 31.7 (C_6); 29.1 (C_4); 28.9 (C_5); 25.0 (C_3); 22.6 (C_7); 19.2 (C_{11}); 14.1 (C_8).

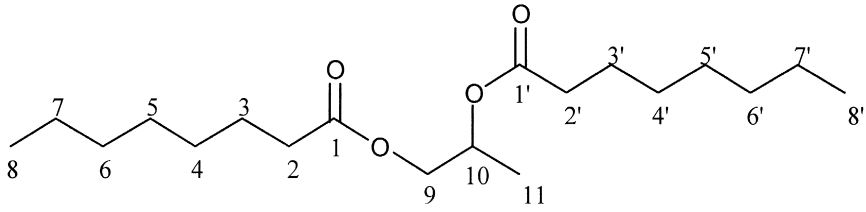
Secondary monoester: 1-hydroxy-2-propyl octanoate



RMN ^{1}H (400.13 MHz, $CDCl_3$, δ): 5.14 (m, $^{3}J_{H10-H11} = 6.5$ Hz, $^{3}J_{H10-H9b} = 6.7$ Hz, $^{3}J_{H10-H9a} =$ 3.6 Hz, 1H, H_{10}); 4.17 (dd, $^{2}J_{H9a-H9b} = 11.7$ Hz, $^{3}J_{H9a-H10} = 3.6$ Hz, 1H, H_{9a}); 4.05 (dd, $^{2}J_{H9b-H9a} = 11.7$ Hz, $^{3}J_{H9b-H10} = 6.7$ Hz, 1H, H_{9b}); 2.3 (fq, $^{3}J_{H2.2'-H3.3'} = 7.6$ Hz, 4H, H_2, $H_{2'}$); 1.61 (m, 4H, H_3, $H_{3'}$); 1.29 (m, 16H, H_4–H_7, $H_{4'}$–$H_{7'}$); 1.24 (d, $^{3}J_{H11-H10} = 6.5$ Hz, 3H, H_{11}); 0.88 (t, $^{3}J_{H8.8'-H7.7'} =$ 6.8 Hz, 6H, H_8, $H_{8'}$).

RMN ^{13}C (100.61 MHz, $CDCl_3$, δ): 173.6, 173.3 (C_1, $C_{1'}$); 68.0 (C_{10}); 65.9 (C_9); 34.5 ($C_{2'}$); 34.2 (C2); 31.7 ($C_{6'}$); 31.7 (C_6); 29.1 ($C_{4'}$); 29.1 (C_4); 29.0 ($C_{5'}$); 29.0 (C_5); 25.0 ($C_{3'}$); 25.0 (C_3); 22.7 ($C_{7'}$, C_7); 16.6 (C_{11}); 14.1 ($C_{8'}$, C_8).

Diester: 1,2 propyl dioctanoate



RMN ^{1}H (400.13 MHz, $CDCl_3$, δ): 4.99 (m, $^{3}J_{H10'-H11'} = 6.5$ Hz, $^{3}J_{H10'-H9'b} = 6.5$ Hz, $^{3}J_{H10-H9'a} = 3.7$ Hz, 1H, $H_{10'}$); 3.67 (dd, $^{2}J_{H9'a-H9'b} = 11.1$ Hz, $^{3}J_{H9'a-H10'} = 3.5$ Hz, 1H, $H_{9'a}$); 3.60 (dd, $^{2}J_{H9'b-H9'a} = 11.1$ Hz, $^{3}J_{H9'b-H10'} = 6.5$ Hz, 1H, $H_{9'b}$); 2.32 (t, $^{3}J_{H2'-H3'} = 7.6$ Hz, 2H, $H_{2'}$); 1.64 (m, 2H, $H_{3'}$); 1.28 (m, 8H, $H_{4'}$–$H_{7'}$); 1.23 (d, $^{3}J_{H11'-H10'} = 6.5$ Hz, 3H, $H_{11'}$); 0.88 (t, $^{3}J_{H8'-H7'} = 6.8$ Hz, 3H, $H_{8'}$).

RMN ^{13}C (100.61 MHz, $CDCl_3$, δ): 174.1 ($C_{1'}$); 71.8 ($C_{10'}$); 65.9 ($C_{9'}$); 34.6 (C2′); 31.7 ($C_{6'}$); 29.1 ($C_{4'}$); 28.9 ($C_{5'}$); 25.0 ($C_{3'}$); 22.6 ($C_{7'}$); 16.2 ($C_{11'}$); 14.1 ($C_{8'}$).

2.2. Esterification reaction with propyl alcohol and isopropyl alcohol

The reaction was initiated by adding 15 or 150 mg of lipase (15 mg for IM60 and SP435, 150 mg for the other enzymes) to 2 ml of *n*-hexane, which contained 288 mg of caprylic acid, 120 mg of propyl alcohol or isopropyl alcohol and 400 mg of molecular sieve. The temperature was kept at 40°C, except for SP435 which was 55°C, and the mixture was stirred magnetically at 250 rpm. Lipases were added on weight basis taking into account previous results obtained in esterification reaction in our laboratory. The objective is to compare the behaviour of the same lipase towards primary and secondary alcohols.

2.3. Esterification reaction with propylene glycol

Substrates were adsorbed as described by Berger et al. [2]: 152 mg of propylene glycol and 152 mg of silica gel were carefully mixed until a homogeneous powder was obtained. The reaction was initiated by adding 15 mg of IM60 to 2 ml of *n*-hexane, which contained 288 mg of caprylic acid, silica gel-adsorbed substrate and 400 mg of molecular sieve. The temperature was kept at 40°C and the mixture was stirred magnetically at 250 rpm. In the case of non-adsorbed substrate, 152 mg of propylene glycol was used.

3. Results and discussion

3.1. Esterification reaction with propyl and isopropyl alcohol

In order to undertake a further synthesis of structured lipids for nutritional applications from glycerol,

the behaviour of 10 enzymes towards a primary and a secondary alcohol, respectively, propyl and isopropyl alcohol, was tested by esterification reaction with caprylic acid. The solvent, hexane, was chosen according to the results of Valivety et al. [3] who studied the reaction between the dodecanol and decanoic acid in various solvents and concluded that esterification is preferred in nonpolar solvents. Ratio of alcohol/fatty acid was 1:1. In order to shift esterification equilibrium, water was removed by addition of molecular sieve at the beginning of the reaction.

For simplicity purpose, the same amount of enzyme was used except for IM60 and SP435 because of their high catalytic activity. Fig. 1 shows that the behaviour of these two lipases is in accordance with supplier data which reported IM60 as 1,3 selective and SP435 as nonselective lipases. Indeed for IM60, at $t = 24$ h yield with propyl alcohol is 100% and 30% with isopropyl alcohol, while for SP435 yields are about equal with the two alcohols. Time course of esterification reaction is illustrated in Fig. 2.

The eight other enzymes can be grouped into three classes. Group I (PSL, A6 and F30) displays a poor activity in the described conditions. It is interesting to note that PSL, which was the best enzyme for the synthesis of propylene glycol esters of C_{12}–C_{18} fatty acids [4], apparently showed a weak activity for the synthesis of propyl or isopropyl ester of C_8. This could be due to substrate specificity of the enzyme. Group II is represented by M10, PPL, EST and CCL. M10 and PPL are known as 1,3 selective lipases in hydrolysis reaction [5]. They gave a similar profile in synthesizing propyl or isopropyl ester in organic solvents. On the other hand, CCL which is known as a nonselective lipase in hydrolysis reaction [5,6], displays in this case a preference for primary alcohol. This behaviour can be explained by the fact that lipases properties in hydrolysis can be different when placed in an organic media [7]. As far EST, it displays a high activity with propyl alcohol since at $t = 2$ h yield is 100% (Fig. 2). Activity with isopropyl alcohol is weaker and yield at $t = 24$ h is only 46%. Finally, group III, which is represented by CAA, is the most conspicuous case. Whereas initial reactivities seem to be similar, this lipase prefers secondary alcohol on a long period. Indeed, at $t = 24$ h, yield with isopropyl alcohol is 97% whereas with propyl alcohol is 30%. This case is surprising and singular. However, Rogalska et al. [8] reported, for the first time, that CAA hydrolyzes preferentially the *sn*-2 position of triglyceride backbone.

1,3 positional selectivity towards glycerol is the most useful lipase property in modification of natural fats and oils. The above described data allow to estimate the ability of enzymes to catalyse esterification reaction and to value each lipase activity towards primary or secondary alcohol. Among the 10 lipases tested, five could be 1,3 selective: IM60, M10, PPL, EST and CCL. For industrial application

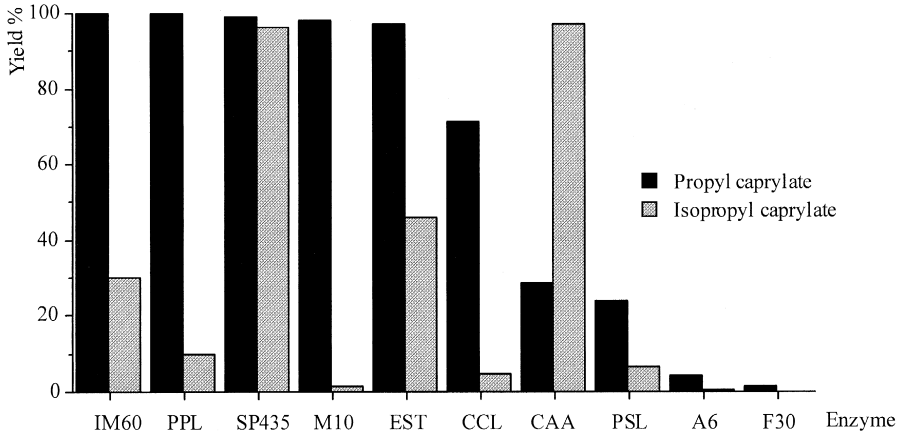


Fig. 1. Yield at $t = 24$ h in propyl and isopropyl caprylate. Esterification conditions: 2 ml *n*-hexane, 120 mg propyl or isopropyl alcohol, 288 mg caprylic acid, 400 mg molecular sieve, 15 or 150 mg of lipase (15 mg for IM60 and SP435, 150 mg for the other enzymes), 40°C, except for SP435 which was 55°C and 250 rpm.

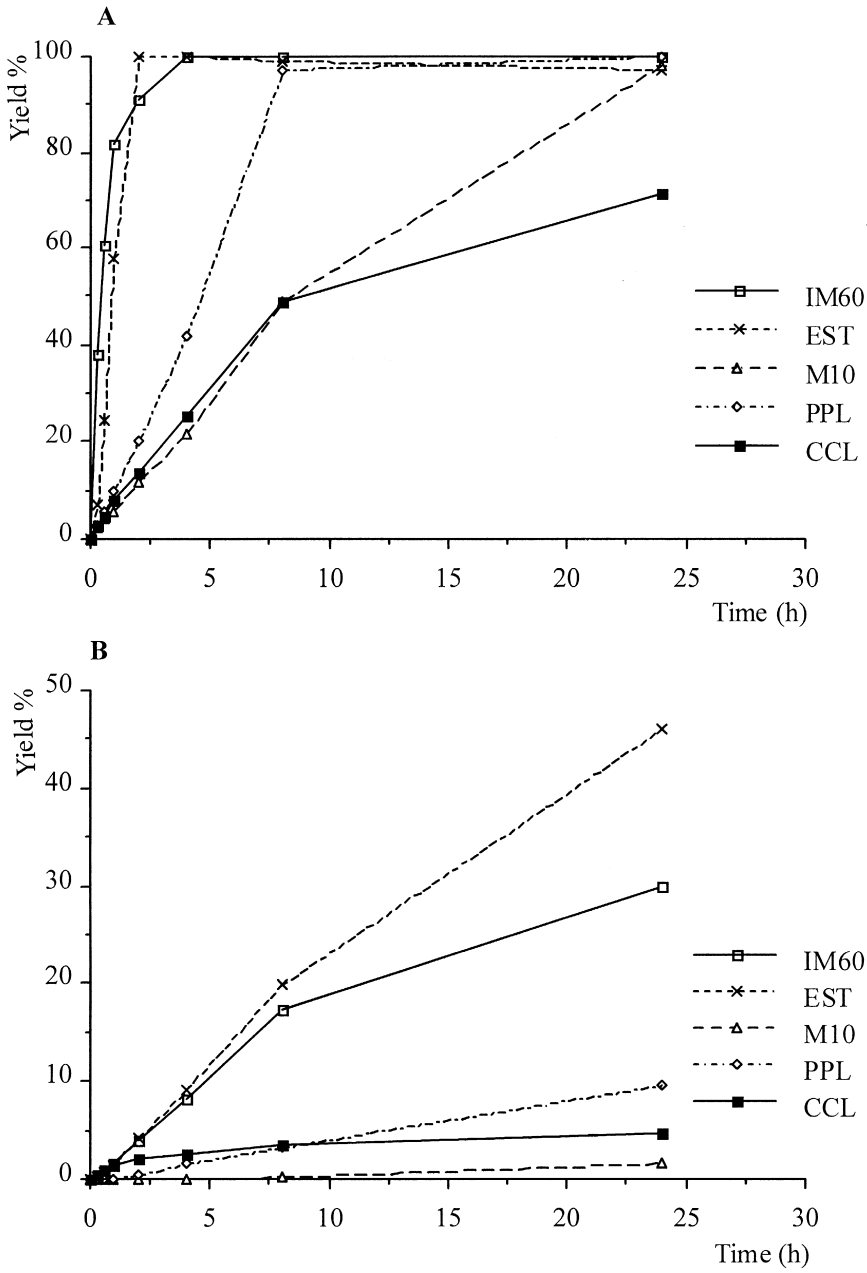


Fig. 2. Time course of esterification reaction of propyl and isopropyl alcohol with caprylic acid catalyzed by IM60, EST, M10, PPL, CCL. Samples of 50 μl were removed and diluted with 100 μl internal standard solution. Esterification conditions: 2 ml *n*-hexane, 120 mg propyl or isopropyl alcohol, 288 mg caprylic acid, 400 mg molecular sieve, 15 or 150 mg of lipase (15 mg for IM60, 150 mg for the other enzymes), 40°C. (A) Time course of esterification reaction with propyl alcohol. (B) Time course of esterification reaction with isopropyl alcohol.

in synthesis of structured lipids, IM60 appears the most adapted compared to M10 and EST (Fig. 2). Indeed, it displays high activity with primary alcohol and after 2 h reaction with *n*-propanol is almost

complete whereas yield is only 4% with isopropanol. Furthermore, IM60 is an immobilized lipase that provides improved stability and makes enzyme recovery and reuse easier. This is a great advantage compared to M10 and EST, which are powder lipases. Therefore, in the second part of this investigation, IM60 was chosen to catalyse esterification reaction between caprylic acid and propylene glycol which bears one primary and one secondary alcohol.

3.2. Esterification with propylene glycol and IM60

Propylene glycol is a polar molecule, which is immiscible in hydrophobic organic solvents such as *n*-hexane. Therefore, esterification reaction with propylene glycol in this media is bound to fail. This problem can be overcome by adsorbing propylene glycol onto a solid support. In 1992, Berger et al. [2] adsorbed solvent-immiscible substrates, such as ethylene glycol and glycerol, onto solid support of silica gel. This lipase-catalyzed esterification was found to be very efficient in producing mono- and diacylglycerols using *n*-hexane, diethyl ether or *tert*-butylmethyl ether as solvents. In 1995, Charlemagne and Legoy [9] reported the enzymatic synthesis of polyglycerol-fatty acid esters in a solvent-free system using silica gel as support for different polar polyglycerols. In 1997, Castillo et al. [10] studied the role of silica gel and different supports in lipase-catalyzed esterification reactions of high-polar substrates, such as 1,3-propanediol and glycerol. They presented the reaction conditions under which the use of silica gel was advantageous.

Fig. 3 presents the synthesis of primary monoester (ME1), secondary monoester (ME2) and diester (DE) with and without silica gel in reaction media. It is obvious that the yield of different esters is improved when reaction is carried out using silica gel as a support for propylene glycol. In this last case, consumption of caprylic acid starts immediately after addition of enzyme, being initially incorporated into primary monoester. This one is accumulated for 2 h up to a maximum of 51% and then decreases to reach 33% at $t = 24$ h. Secondary monoester synthesis is weak. At $t = 2$ h, yield is 5% and does not exceed significantly this value. Diester, which is formed more slowly than primary monoester, goes up to 38% at $t = 24$ h. This production profile of

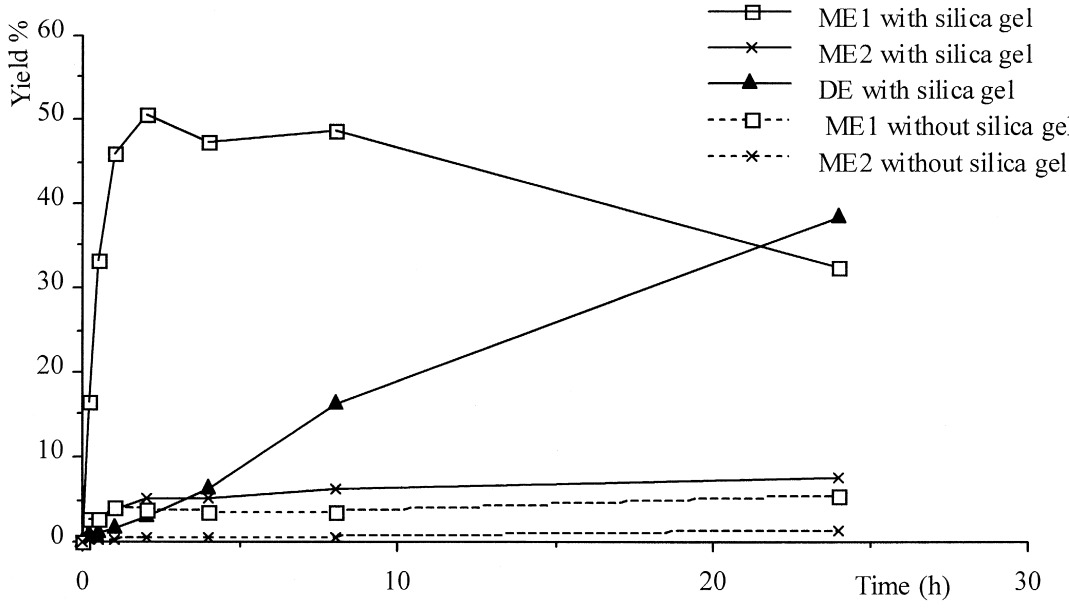


Fig. 3. Effect of silica gel on the esterification of propylene glycol with caprylic acid, catalyzed by IM60. Esterification conditions: 2 ml *n*-hexane, 152 mg propylene glycol, 288 mg caprylic acid, 400 mg molecular sieve, 15 mg of lipase, 40°C, 250 rpm. In the reaction with silica gel, 152 mg of silica gel is used to adsorb propylene glycol (see Materials and methods).

dicaprylin is in accordance with the results published by Castillo et al. [10]. They achieved esterification reaction of oleic acid and silica gel adsorbed glycerol with Lipozyme from Novo Nordisk Industry. They suggested that the low rate of disappearance of 1-(3)-monoolein, 1,3-diolein and oleic acid observed at the end of their reaction might be directly associated with the increase in triolein formation and a function of isomerization rate 1,3-products, i.e. spontaneous acyl migration. On the basis of their previous paper and results of Ergan et al. [11] and Lortie et al. [12], they claimed that, in lipase-catalyzed esterification reaction of glycerol in different reaction media, the use of silica gel significantly improved the reaction rate and conversion yields, without significantly changing the profile of synthesized products. Furthermore, to confirm this conclusion, we followed by gas chromatography the evolution of the ratio ME1/ME2 during 24 h in the presence of silica gel and hexane (at $t = 0$, ME1/ME2 = 2.21 and at $t =$ 24 h, ME1/ME2 = 2.33). Amount of silica-gel, solvent volume and temperature are the same as that in esterification reaction. During this time, the ratio ME1/ME2 did not change significantly. Hence, from these results and from the data reported by Castillo et al. [10], silica gel would not catalyze acyl migration.

Polarity of the reaction medium can influence the product profile or "reaction selectivity" in situations in which multiple products of differing polarities can be formed [13]. Polarity is expressed by log P value (P is the partitioning coefficient between 1-octanol and water). To select the best solvent for propylene glycol esterification reaction, six solvents, including *n*-hexane, with different log P, were tested (Table 1). In the solvents with $1.4 \leq \log P \leq 3.5$, a rapid production of primary monoester was observed. In the three solvents, yield of primary monoester reached a maximum at $t = 2$ h then decreased. The highest maximum, 65%, was obtained in *tert*-butylmethyl ether. Yield of diester, at $t = 24$ h, was greatly higher in *n*-hexane, 38%, than in isopropyl ether and *tert*-butylmethyl ether, 11% and 16%, respectively. These production profiles of esters agree with the results published by Janssen et al. [14]. These authors postulated that for the synthesis of polar products, a polar solvent is favourable, while for the synthesis of nonpolar products, it is better to choose a nonpolar solvent. In fact, primary monoester is a relatively polar product, whose accumulation would not be easily accommodated by too apolar solvents because of limited solvatation capac-

Table 1
Yields of primary, secondary monoesters and diester produced by IM60 in the presence of propylene glycol and caprylic acid in various solvents

Solvent	log P [15]	Reaction time (h)	Yield%		
			Primary monoester	Secondary monoester	Diester
n-Hexane	3.5	0.25	16	1	1
		0.5	33	2	1
		1	46	4	2
		2	51	5	3
		4	47	5	6
		8	49	6	16
		24	33	7	38
Isopropyl ether	1.9	0.25	14	1	0
		0.5	24	3	0
		1	37	3	1
		2	57	4	2
		4	53	6	3
		8	48	6	5
		24	36	8	11
tert-Butyl-methyl ether	1.4	0.25	15	1	1
		0.5	28	2	1
		1	46	3	1
		2	65	5	2
		4	60	6	3
		8	61	7	7
		24	49	11	16
2-Methyl-2-propanol	0.79	0.25	2	0.2	0
		0.5	3	0.3	0
		1	5	0.4	0
		2	10	1	0
		4	13	1	1
		8	26	2	1
		24	37	3	1
Acetone	−0.23	0.25	1	0.2	0
		0.5	2	0.4	0
		1	3	0.5	0
		2	4	0.6	0
		4	7	0.8	0
		8	11	1	1
		24	22	3	1
Acetonitrile	−0.33	0.25	1	0.1	0
		0.5	2	0.2	0
		1	3	0.3	0
		2	5	0.5	0
		4	7	0.7	0
		8	12	1	1
		24	23	2	1

ity [13]. However, when solvents with log $P \leq 0.79$ are used, catalytic activity decreases and primary monoester is formed more slowly than in the three previous solvents. Indeed, in 2-methyl-2-propanol, yield is 10% at $t = 2$ h compared to 65% in *tert*-butylmethyl ether. The attenuation of reactivity in the three most polar solvents can be explained by the fact that the stability of enzymes in these solvents is not always good. Nevertheless, Fig. 4 shows that the more polarity solvent increases, the less role of silica

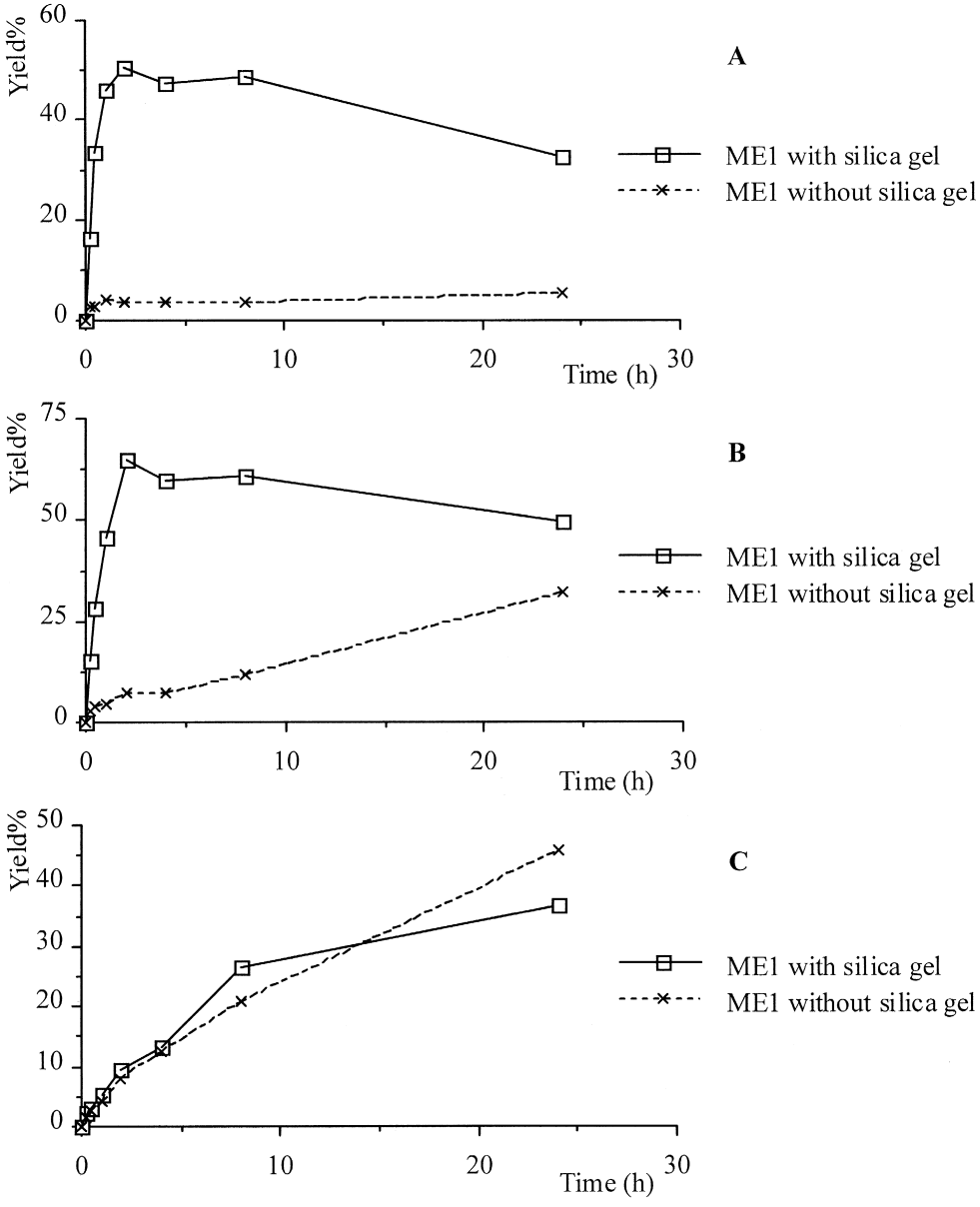


Fig. 4. Influence of silica gel according to the solvent polarity in esterification of propylene glycol with caprylic acid catalyzed by IM60. Esterification conditions: 2 ml solvent, 152 mg propylene glycol, 152 mg of silica gel, 288 mg caprylic acid, 400 mg molecular sieve, 15 mg of lipase, 40°C, 250 rpm. (A) *n*-Hexane (log $P = 3.5$); (B) *tert*-Butylmethyl ether (log $P = 1.4$); (C) 2-Methyl-2-propanol (log $P = 0.79$).

gel is important. In 2-methyl-2-propanol, yield of primary monoester profile is similar with or without silica gel. One interpretation of this trend is that the solubilization of propylene glycol increases with solvent polarity.

4. Conclusion

The first step described in this paper is an approach, which allows, at once, to estimate the ability of enzymes to catalyse esterification reaction and to value lipases activity towards primary and secondary alcohols. IM60 was chosen for the second step because this enzyme displays the best compromise between catalysis activity and regioselectivity. Results obtained with propylene glycol confirm the trend observed in the previous step and in the literature for this lipase. It is shown that the addition of silica gel to the reaction mixture is extremely important in order to facilitate the reaction without significantly changing the relative ratio of the different products [10]. The choice of the organic solvent is crucial. Indeed, polarity of the solvent can influence the product profile. Thus, production of primary monoester is increased in a relatively polar solvent such as *tert*-butylmethyl ether ($\log P = 1.4$). On the other hand, a more polar solvent ($\log P < 1.4$) can deteriorate the stability of enzymes and decrease the catalytic activity of the lipase used. The transposition of this preliminary study on the regioselectivity of lipases to the synthesis of structured lipids is in progress in our laboratory.

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References

[1] L.B. Fomuso, C.C. Akoh, J. Am. Oil Chem. Soc. 74 (1997) 269.
[2] M. Berger, K. Laumen, M.P. Schneider, J. Am. Oil Chem. Soc. 69 (1992) 955.
[3] R.H. Valivety, G.A. Johnston, C.J. Suckling, P.J. Halling, Biotechnol. Bioeng. 38 (1991) 1137.
[4] J.F. Shaw, S. Lo, J. Am. Oil Chem. Soc. 71 (1994) 715.
[5] T. Godfrey, Lipid Technol. 7 (1995) 58.
[6] R.G. Jensen, F.A. Dejong, R.M. Clark, Lipids 18 (1983) 239.
[7] P. Villeneuve, M. Pina, J. Graille, Ol., Corps Gras, Lipides 3 (1996) 459.
[8] E. Rogalska, C. Cudrey, F. Ferrato, R. Verger, Chirality 5 (1993) 24.
[9] D. Charlemagne, M.D. Legoy, J. Am. Oil Chem. Soc. 72 (1995) 61.
[10] E. Castillo, V. Dossat, A. Marty, J.S. Condoret, D. Combes, J. Am. Oil Chem. Soc. 74 (1997) 77.
[11] F. Ergan, M. Trani, G. André, Biotechnol. Bioeng. 35 (1990) 195.
[12] R. Lortie, M. Trani, F. Ergan, Biotechnol. Bioeng. 41 (1993) 1021.
[13] S.J. Kuo, K.L. Parkin, J. Am. Oil Chem. Soc. 73 (1996) 1427.
[14] A.E.M. Janssen, M. Hadini, N. Wessels Boer, R. Walinga, A. Van der Padt, H.M. Van Sonsbeek, K. Van't Riet, in: J. Tramper (Ed.), Biocatalysis in Non-Conventional Media vol. 8, Elsevier, Amsterdam, 1992, 155, Session 5.
[15] A.E.M. Janssen, A. Van der Padt, H.M Van Sonsbeek, K. Van't Riet et al., Biotechnol. Bioeng. 41 (1993) 95.