Parameters affecting productivity in the lipase-catalysed synthesis of sucrose palmitate

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ABSTRACT

The industrial application of lipases for the synthesis of sucrose esters is usually limited by its low productivity, as we need a medium where a polar reagent (the sugar) and a non-polar fatty acid donor are soluble and able to react in presence of the biocatalyst. In this work, we have studied the difficulties encountered when trying to increase the volumetric productivity of sucrose esters. The synthesis of sucrose palmitate was performed in 2-methyl-2-butanol:dimethylsulfoxide mixtures by transesterification of different palmitic acid donors with sucrose, catalysed by the immobilized lipase from *Candida antarctica* B (Novozym 435). A protocol for substrate preparation different to that previously reported by our group was found to improve the reaction rate. Several parameters, such as sucrose and acyl donor loadings, the percentage of DMSO in the mixture and the nature of acyl donor, were investigated. Under the best experimental conditions (15% DMSO, 0.1 mol/l sucrose, 0.3 mol/l vinyl palmitate), a maximum of 45 g/l sucrose palmitate was obtained in 120 h. Using methyl or ethyl palmitate, the highest productivity was 7.3 g/l in 120 h using 20% DMSO with 0.2 mol/l sucrose and 0.6 mol/l acyl donor. The formation of free fatty acid, and the effect of DMSO percentage on the selectivity monoester/diester were also studied. To our knowledge, this is the first report on enzymatic synthesis of sucrose esters of long fatty acids using alkyl esters as acyl donors.

Key words: Carbohydrate esters, Sucrose esters, Lipases, Enzymatic transesterification, Alkyl esters, Vinyl fatty acid esters.

1. INTRODUCTION

Carbohydrate fatty acid esters, synthesized from renewable resources, have a vast number of applications in the food, cosmetics, oral-care, detergent and pharmaceutical industries (Watanabe, 1999). Their properties as antimicrobials useful in food storage (Marshall and Bullerman, 1994), antitumorals (Okabe *et al.*, 1999) and insecticidals (Puterka *et al.*, 2003) indicate their great versatility. We have recently demonstrated the inhibitory activity of several di- and trisaccharide fatty acid esters against microorganisms involved in caries development (Devulapalle *et al.*, 2004). Among them, sucrose esters are the most developed carbohydrate esters and are being produced at about 4000 Tm/year (Hill and Rhode, 1999).

Sugar esters can be synthesized using either chemical or biological catalysts. Although their current chemical synthesis, a base-catalysed reaction at high temperatures, is being used, the poor selectivity and the formation of coloured side-products (Polat and Linhardt, 2001) have focused the attention to the more selective enzymatic process, using lipases or proteases (Plou *et al.*, 2002).

Methodologies for enzymatic sugar acylation (also applicable to other hydrophilic compounds) need to find a medium where a polar reagent (the carbohydrate) and a nonpolar acyl donor are soluble and able to react in presence of a biocatalyst. Lipases, the most adequate biocatalysts when dealing with long-chain fatty acids, are readily inactivated by polar solvents capable of dissolving di- and trisaccharides. This occurs with most of the enzymes, and only some proteases of the subtilisin-family are able to catalyse the acylation of sugars in solvents such as dimethylformamide

(DMF) and pyridine (Plou *et al.*, 1995; Polat *et al.*, 1997).

In this context, we developed a simple process for the lipase-catalysed acylation of sucrose (Ferrer *et al.*, 1999) and other di- and trisaccharides (Ferrer *et al.*, 2000) with vinyl esters. The method was based on the pre-solubilization of sucrose in a polar solvent (dimethylsulfoxide -DMSO-) and its further mixing with a tertiary alcohol (2-methyl-2-butanol -2M2B-), being the final DMSO content close to 20% v/v. These mixtures of miscible solvents, which represented a compromise between sugar solubility and enzyme stability, have been applied by other researchers to the regioselective acylation of several polyhydroxilic compounds (Pedersen *et al.*, 2002; Castillo *et al.*, 2003; Simerska *et al.*, 2004).

Although lipases offer an exquisite regioselectivity for the synthesis of sucrose esters, the lower productivity compared with the currently used chemical process, limits their industrial exploitation. In this work, we have investigated the applicability of solvent mixtures to synthesize sucrose esters using high concentrations of reagents (sucrose and vinyl ester). In addition, non-activated acyl donors such as methyl or ethyl esters, characterized by their great availability and low price, have been tested. Using a more efficient “*solvents-mixing*” protocol to that previously reported by our group, we carried out the synthesis of sucrose monopalmitate catalysed by the immobilized lipase B from *Candida antarctica* (Novozym 435). Three parameters were studied: (a) the concentration of sucrose and acyl donor; (b) the nature of the acyl donor (methyl, ethyl and vinyl palmitate, palmitic acid); and (c) the percentage of DMSO.

2. EXPERIMENTAL

2.1. Chemicals

Immobilized lipase from *C. antarctica* B (Novozym 435) was a kind gift from Novozymes A/S. Methyl palmitate, ethyl palmitate, palmitic acid and 2-methyl-2-butanol (2M2B) were from Sigma. Sucrose and dimethylsulfoxide (DMSO) were supplied by Merck. Vinyl palmitate was from TCI (Tokyo, Japan). All other reagents were of the highest available purity. Solvents were dried over 3 Å molecular sieves (Sigma), at least for 24 h before use.

2.2. Enzymatic synthesis of sucrose monopalmitate

Original “solvents-mixing” method

Sucrose was first dissolved in one volume of DMSO, and then slowly added to 4 volumes of 2M2B equilibrated at 60°C. After that, the acyl donor was added, the mixture equilibrated for 15 min, and the biocatalyst (25 g/l Novozym 435) finally incorporated. Reactions were performed at 60°C with orbital shaking (150 rpm). Aliquots were removed at intervals, filtered using a 0.45 µm Durapore® filter coupled to an eppendorf tube and analysed by HPLC.

Modified “solvents-mixing” method

The reaction mixture consisting of sucrose and two volumes of 2M2B was stirred overnight with orbital shaking (150 rpm) at room temperature. After that, one volume of DMSO and two volumes of 2M2B were added in this order. The homogeneous sucrose suspension was heated to 60°C. Then, the acyl donor and the biocatalyst (25 g/l Novozym 435) were added. Reactions

were performed at 60°C with orbital shaking (150 rpm). Aliquots were removed at intervals, filtered using a 0.45 µm Durapore® filter coupled to an eppendorf tube and analysed by HPLC.

2.3. HPLC analysis.

Reactions aliquots were analysed by HPLC, using a 9012 pump (Varian) and a Nucleosil 100-C18 column (4.6 x 250 mm, Análisis Vínicos, Spain), maintained at 30°C. Detection was performed using an evaporative light-scattering detector DDL-31 (Eurosep) equilibrated at 60°C. Methanol:water 95:5 (v/v) containing 0.1% (v/v) acetic acid was used as mobile phase (flow rate 1.2 ml/min) for 6 min. Then, a gradient from this eluent to pure methanol was performed in 1 min, after which the flow rate was increased to 1.7 ml/min in 1 min. Methanol was held as mobile phase at 1.7 ml/min during 7 min.

3. RESULTS AND DISCUSSION

The synthesis of sucrose palmitate was performed by transesterification of different palmitic acid donors with sucrose in 2M2B:DMSO mixtures, catalysed by Novozym 435. To optimise the synthesis in terms of yield and productivity, several parameters were assessed, such as the mixing strategy, the effect of increasing concentrations of sucrose and acylating agent, and the use of cheap and available acyl donors (alkyl esters).

3.1. The problem of increasing sucrose concentration

Fig. 1(a) illustrates the original process that we developed for sugar acylation, using a reaction medium constituted by two miscible solvents (Ferrer *et al.*, 1999). The carbohydrate was dissolved in one volume of DMSO, and this was slowly added to four volumes of a tertiary alcohol (2M2B). Finally the acyl donor and the biocatalyst were incorporated into the mixture. Following this protocol sucrose is totally soluble up to approx. 10 g/l (0.03 mol/l). Sucrose solubility in such medium is notably higher than in pure 2M2B (0.42 g/l at 60°C; Tsavas *et al.*, 2002a). In addition, the inactivation of the enzyme is greatly reduced compared with pure DMSO, where the enzymatic activity decreased rapidly. As a result this methodology has proven very successful for low and moderate concentrations of reagents.

Above 10 g/l sucrose in the final mixture, the medium stops being transparent as the partial precipitation of sucrose takes place when the DMSO solution is slowly added to the 2M2B. The majority of sucrose is undissolved at the beginning of the reaction. As the reaction proceeds, the dissolved sucrose is consumed and the excess solid sugar starts to dissolve.

However, Halling's group observed in the synthesis of glucose esters that sugar dissolution rate in organic solvents was rather slow, which limited the rate of sugar esters production (Flores and Halling, 2002; Flores *et al.*, 2002).

3.2. Effect of mixing order of reagents

To improve sucrose dissolution rate, a new reaction protocol was developed for high reagents loadings, represented in Fig. 1(b). Sucrose was added to 2 volumes of 2M2B, and the obtained suspension was incubated overnight with stirring. This allowed forming a fine dispersion of sucrose crystals in 2M2B. Then, one volume of DMSO and two volumes of 2M2B were subsequently added, resulting in a more homogeneous system –at high reagents loadings– than that obtained with the original protocol 1(a), in which large agglomerates are formed by sucrose precipitation.

We compared the two methodologies using 0.2 mol/l sucrose (68 g/l) and 0.6 mol/l methyl palmitate (162 g/l). Although in our previous works on sugar acylation we commonly employed the lipase from *Thermomyces lanuginosus* (Ferrer *et al.*, 2002a), we found that this enzyme was not able to use alkyl esters as acyl donors, at least in polar or moderately polar media. For that reason, we selected the lipase from *Candida antarctica* B, whose behaviour in organic solvents differs substantially from that of *T. lanuginosus* (Salis *et al.*, 2003).

As shown in Fig. 2A, reaction is significantly faster using the modified protocol. As a consequence, substrate preparation also produces an effect on the conversion measured at 120 h: the new protocol yields 7.2 g/l sucrose

palmitate compared with 2.7 g/l using the original method. However, both reactions seem to be still far from the equilibrium position. In absence of DMSO the acylation rate is slower and sucrose palmitate yield (approx. 1 g/l) is very low.

Fig. 2B shows the dissolved sucrose during the reaction. It is noteworthy that sucrose concentration was higher at the beginning of the reaction when using the new approach (approx. 9 mM *vs.* 6 mM). This may explain the faster initial rate observed in Fig. 2A. However, in the following 3 h, sucrose concentration is basically the same in both cases. A small jump in sucrose concentration was observed between hour 3 and 5, possible due to a better solubilization of sucrose helped by the higher production of sucrose palmitate, which is a good surfactant. Then, the amount of dissolved sucrose is constant during the process (although slightly higher with the new protocol), which implies that dissolution rate must be equal to the rate of reaction. Taking into account that reaction rate is higher with the new protocol throughout the process, overnight incubation in 2M2B (protocol Fig. 1b) may produce a positive effect on medium viscosity and mass transfer, compared with pre-dissolution and precipitation strategy (protocol Fig. 1a). It is noteworthy that approx. 30 mM sucrose can stay completely in solution (supersaturation) using the original method at low sucrose loading, but if more sucrose is added, only about 6 mM sucrose is found in solution. This effect seems to be related with nucleation effects of the system (Sgualdino *et al.*, 1996), i.e. conglomeration of sucrose molecules into a new phase.

In absence of DMSO, sucrose solubility throughout the acylation process is approx. 0.5 mM, which explains the low reaction rate and yield obtained. From the above results, the rest of experiments in this work were performed following protocol 1b.

3.3. Effect of sucrose loading on conversion and yield

When sucrose concentration increases, the final productivity must also increase. The initial sucrose loading was varied in the range 0.1-0.3 mol/l using two different acylation agents: methyl palmitate and vinyl palmitate. The amount of acyl donor was adjusted to have a molar excess 3:1 with respect to sucrose.

Results are presented in Table I. As shown, the increasing concentration of acyl donor makes the medium less polar and also contributes to a decrease of sucrose solubility. In fact, although the sucrose loading increases, the dissolved sucrose diminishes from 4.8 to 2 g/l when increasing the concentration of vinyl palmitate from 0.3 to 0.9 mol/l. A similar influence on sugar solubility when rising fatty acid concentration has been reported by Tsavas *et al.* (2002a and 2002b). A similar effect occurs with methyl palmitate, although sucrose solubility is slightly lower compared with vinyl donor (Table I).

We observed that reaction rate and productivity did not increase significantly when increasing reagents loadings. With methyl palmitate as acyl donor, the maximum productivity (7.3 g/l in 120 h) was achieved using 0.2 mol/l sucrose, whereas a further increase in sucrose concentration resulted in a lower value. For vinyl palmitate, a maximum of 16.9 g/l

sucrose palmitate in 120 h was obtained at 0.3 mol/l sucrose, but productivity did not increase as much as expected when moving from 0.1 to 0.3 mol/l sucrose.

In consequence, the negative effect of fatty acid donor on sucrose solubility may explain why the yield (measured at 120 h) did not suffer a substantial increment when increasing reagents loadings. In addition, it has been reported that dead-end substrate inhibition may occur in lipase-catalysed acyl-transfer processes (Rizzi *et al.*, 1992), and cannot be ruled out to occur here.

3.4. The formation of free fatty acid as a side reaction

The hydrolysis of the acyl donor to fatty acid, also catalysed by the lipase, is an undesirable reaction in these processes. The most generally accepted mechanism for acyl-transfer reactions catalysed by lipases is the ping-pong bi-bi mechanism (Fig. 3), which involves an acyl-enzyme intermediate (Martinelle and Hult, 1995). The nucleophile alcohol R_3OH (i.e. the sugar) attacks the acyl-enzyme to give an ester molecule (product). However, water can also act as nucleophile yielding free fatty acid. This side reaction competes with sugar acylation and is responsible for the low conversion obtained in many experiments. Most of the water in the reaction mixture comes from the biocatalyst and the solvents. In fact, commercial Novozym 435 preparations contain $\geq 2\%$ H_2O (w/w) as measured by Karl-Fisher titration (Scholz, 1984), in accordance with Piyatheerawong *et al.* (2004).

Table II shows the formation of palmitic acid maintaining the initial sucrose loading and varying the concentration of acyl donor. Interestingly, when the content of vinyl palmitate is low (50 mM), approx. 96% of the initial amount is transformed into palmitic acid, which explains the low yield of sucrose palmitate obtained (0.08 g/l). Using 0.6 M vinyl palmitate, the percentage of acyl donor converted into palmitic acid is approx. 20%, resulting in the formation of 14.7 g/l sucrose palmitate. From Table II it remains clear that a careful control of the amount of water in the system –by addition of molecular sieves, drying the biocatalyst, etc.– is crucial in terms of sugar ester yield and downstream processing (the fatty acid must be removed from the final product).

3.5. Effect of DMSO content on transesterification

We analysed the effect of DMSO percentage on the acylation of sucrose with methyl palmitate. In previous works studying the transesterification of vinyl laurate with sucrose catalysed by the lipase from *T. lanuginosus*, we demonstrated that DMSO percentage in the solvent mixture substantially modified the molar ratio monoester/diester (Ferrer *et al.*, 1999; Ferrer *et al.*, 2002a). In particular, at $\leq 10\%$ DMSO the synthesis of diesters was favoured, whereas at $\geq 15\%$ DMSO the formation of monoester (6-*O*-acylsucrose) was majoritary. These results are in agreement with the fact that selectivity of acyl-transfer reactions can be modulated varying the organic solvent (Cernia *et al.*, 1998; Rendon *et al.*, 2001).

When using Novozym 435, the sucrose monopalmitate obtained is an approx. equimolar mixture of the monoesters at the 6- and 6'-hydroxyl

groups (Woudenberg-van Oosterom *et al.*, 1996). The formation of the 6,6'-diester is an important reaction to consider when using this biocatalyst. In fact, using 0.03 mol/l sucrose and 0.3 mol/l vinyl laurate in 2-methyl-2-butanol/DMSO 80:20 (v/v), the synthesis of the diester 6,6'-di-*O*-lauroylsucrose was the major process (Ferrer *et al.*, 2004).

In this work, we analysed the formation of mono- and diesters at different DMSO percentages using methyl palmitate as acyl donor (Fig. 4). As shown, and in accordance with our previous studies, an increase in solvent polarity enhanced the ratio monoester/diester. In particular, moving from 0 to 20% DMSO changed the molar ratio monopalmitate/dipalmitate from 65:35 to 99.5:0.5 (Table III).

The effect of solvent polarity on reaction selectivity seems to be also related with sucrose solubility. As indicated in Table III, the amount of dissolved sucrose is higher with increasing DMSO content, going from 0.2 g/l in pure 2M2B to 29.5 g/l in 30% DMSO. In consequence, at high DMSO contents, sucrose competes more efficiently with the formed monoester for the acyl-enzyme intermediate, which results in a major presence of monoester.

In terms of volumetric productivity, the maximum yield obtained in 120 h (4.3 g/l sucrose monopalmitate and 0.5 g/l sucrose dipalmitate) was found at 15% DMSO (Fig. 4). At higher DMSO contents the yield decreased, despite a higher concentration of sucrose in the medium. To understand why such an increase in sucrose solubility did not exert an improvement on sugar ester yield, one must consider the deleterious effect of polar organic solvents on many biocatalysts, and in particular on the activity and stability

of lipases (van Rantwijk et al., 2003). In fact, at 30% DMSO the amount of fatty acid produced was also substantially lower than at lower DMSO contents.

3.6. Effect of acyl donor

The most commonly used acyl donors for enzymatic sugar ester synthesis are the vinyl esters, due to the fast reaction rates and high yields achieved (Ferrer *et al.*, 1999). Although their availability is enormously growing, vinyl esters are still too expensive to be used at high-scale in the food and drinks industries. Among the alternatives to the vinyl esters, the use of methyl or ethyl esters, which are about 10-fold cheaper and widely available, seems to be more practical.

We analysed the behaviour of methyl, ethyl and vinyl palmitate under the optimal DMSO content (15%), using 0.1 mol/l sucrose and 0.3 mol/l acyl donor. Esterification with palmitic acid was also assayed. The yield of sucrose monopalmitate is represented in Fig. 5. From this plot it remains clear that vinyl palmitate gives rise to a productivity one order of magnitude higher than the obtained with the other acylating agents. The volumetric productivity was approx. 0.34 g sucrose palmitate/l-h (45 g/l in 120 h, plus 12 h of overnight incubation), which corresponds to a sucrose conversion of 77%. Sugar ester production in this work is notably higher than the values available in the literature, most of them using low sugar loadings (0.01-0.06 mol/l) and shorter fatty acids such as caprylic or lauric acids (Woudenberg-van Oosterom *et al.*, 1996; Pedersen *et al.*, 2002). Regarding alkyl esters as acyl donors, the highest productivity did not exceed 0.04 g sucrose

palmitate/1·h (5 g/l in 120 h, plus 12 h of overnight incubation) at 15% DMSO. Although the values obtained with alkyl esters may appear low, it is noteworthy that this is the first report of lipase-catalysed synthesis of long-chain ($\geq C_{16}$) sucrose esters using ethyl or methyl esters as acyl donors. In this context, Woudenberg-van Oosterom *et al.* (1996) studied the acylation of sucrose at low loading (0.02 mol/l) with a large excess of ethyl dodecanoate (0.97 mol/l) in refluxing tert-butanol (82°C), achieving 35% conversion after 7 days.

It is noteworthy from Fig. 5 the apparently almost identical performance of palmitic acid and its simple esters. The physico-chemical problems usually associated with esterification processes (i.e. a non-polar organic solvent is unable to accommodate the water produced during the reaction, and water forms a discrete second phase that may separate the substrates and the biocatalyst) are manifested at moderate or high conversions (Plou *et al.*, 2003). In our experiments, conversions are low and no phase separation is observed, resulting in similar reaction profiles for esterification and transesterification.

CONCLUSIONS

Enzymatic sucrose ester synthesis with high volumetric productivity is difficult to achieve as many factors are involved in these processes. The use of mixtures of tertiary alcohols with polar solvents such as DMSO enhances sugar solubility, although DMSO content is critical on enzyme activity. Sucrose dissolution rate is a key parameter and can be favoured by the pre-incubation strategy presented here. However, increasing acyl donor concentration to improve rate and yield, has a negative effect in terms of sucrose solubility and may also cause enzyme inhibition. Hydrolysis of acyl donor yielding free fatty acid is an undesirable side reaction that needs to be carefully controlled. The use of activated vinyl esters gives rise to higher rates and sugar ester production. Under our best conditions, a maximum of 45 g/l sucrose palmitate in 120 h was obtained. Bioreactor design and water control are key factors to improve volumetric productivities.

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Table I. Effect of sucrose and acyl donor loadings on sucrose palmitate synthesis. Reactions were performed at 60° C in 2M2B/DMSO 80:20 (v/v), using 25 g/l Novozym 435 and 150 rpm.

Sucrose loading		Dissolved sucrose ^a		Acyl donor	Acyl donor loading		Sucrose palmitate ^b		Conversion ^{b,c}
mmol/l	g/l	mmol/l	g/l		mmol/l	g/l	mmol/l	g/l	(%)
100	34.2	12.6 ± 0.9	4.3 ± 0.3	Methyl palmitate	300	81.1	6.6	3.8	6.6
100	34.2	13.9 ± 0.7	4.8 ± 0.2	Vinyl palmitate	300	84.7	21.5	12.5	21.5
200	68.4	7.9 ± 0.4	2.7 ± 0.1	Methyl palmitate	600	162.3	12.5	7.3	6.3
200	68.4	9.1 ± 0.5	3.1 ± 0.2	Vinyl palmitate	600	169.5	25.4	14.7	12.7
300	102.6	4.8 ± 0.3	1.6 ± 0.1	Methyl palmitate	900	243.4	5.6	3.3	1.9
300	102.6	6.2 ± 0.2	2.1 ± 0.1	Vinyl palmitate	900	254.2	29.0	16.9	9.7

^a Average value from 6 to 120 h of reaction.

^b In 120 h.

^c Referred to sucrose concentration.

Table II. Effect of molar ratio sucrose/vinyl palmitate on the transesterification/hydrolysis ratio. Reactions were performed in 2M2B/DMSO 80:20 (v/v) at 60°C.

Reagents		Products formed ^a			
Sucrose	Vinyl palmitate	Sucrose palmitate		Palmitic acid	
(mmol/l)	(mmol/l)	mmol/l	g/l	mmol/l	g/l
200	600	25.4	14.7	131.5	33.7
200	200	11.7	6.8	81.3	20.9
200	100	8.2	4.8	88.0	22.5
200	50	0.14	0.08	48.0	12.3

^a Measured at 24 h.

Table III. Effect of DMSO percentage on sucrose solubility and in the monoester/diester ratio, measured under the following experimental conditions: 0.1 mol/l sucrose, 0.3 mol/l methyl palmitate, 25 g/l Novozym 435, 60°C, 150 rpm.

Percentage of DMSO	Dissolved sucrose		Products distribution ^a		
	mmol/l	g/l	Monoesters (%)	Diester (%)	Molar ratio
0	0.5 ± 0.05	0.2 ± 0.02	65	35	1.9
10	2.9 ± 0.1	1.0 ± 0.03	81.5	18.5	4.4
15	5.0 ± 0.2	1.7 ± 0.1	92.5	7.5	12.3
20	12.6 ± 0.9	4.3 ± 0.3	99.5	0.5	199
30	86.2 ± 8.4	29.5 ± 2.9	n.r.	n.r.	n.r.

^a Molar composition calculated at 120 h.

n.r. No reaction products were detected.

Figure legends

- Fig. 1.** Scheme of the original **(a)** and modified **(b)** “solvents-mixing” protocols for the synthesis of sugar esters.
- Fig. 2.** Transesterification of methyl palmitate with sucrose in mixtures 2M2B:DMSO using different solvents-mixing protocols. Conditions: 0.2 mol/l sucrose, 0.6 mol/l methyl palmitate, 25 g/l Novozym 435, 60°C, 150 rpm. **(O)** Original protocol, 20% DMSO; **(λ)** Modified protocol, 20% DMSO; **(v)** Modified protocol, in absence of DMSO. **(A)** Formation of sucrose monopalmitate; **(B)** Dissolved sucrose during reaction.
- Fig. 3.** Ping-pong bi-bi mechanism of a transesterification process catalysed by a lipase: **(I)** ester synthesis; **(II)** hydrolysis. Ser-OH represents the catalytic-site serine residue.
- Fig. 4.** Effect of DMSO percentage on the ratio monoester:diester in the transesterification of methyl palmitate with sucrose. Conditions: 0.1 mol/l sucrose, 0.3 mol/l methyl palmitate, 25 g/l Novozym 435, 60°C, 150 rpm.
- Fig. 5** Effect of the nature of the acyl donor on transesterification. Conditions: 0.1 mol/l sucrose, 0.3 mol/l acyl donor, 2M2B:DMSO 85:15 (v/v), 25 g/l Novozym 435, 60°C, 150 rpm. Acyl donors: **(λ)**

vinyl palmitate; (O) methyl palmitate; (v) ethyl palmitate; (◊)
palmitic acid.

FIG. 1

FIG. 2

FIG. 3

FIG 4

FIG. 5