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# Impact of fatty acid structure on CALB-catalyzed esterification of glucose

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## Abstract:

Enzymatic synthesis of fatty acid glucose esters from different fatty acyl donors were performed *via* enzymatic catalysis in the presence of CALB, using acetonitrile as the solvent. The acyl donor nature (fatty acid or fatty acid vinyl ester) and structure were varied. Lower reaction rates and lower conversions were obtained with fatty acids in comparison to their corresponding vinyl esters. Moreover, the acyl donor with the longest chain length gave the highest conversions. The presence of unsaturation on the acyl donor chain was also shown to be detrimental to the conversion.

## 1. Introduction

Glycolipids, also known as fatty acid sugar esters (FASE), are molecules composed of a fatty alkyl chain and a saccharide moiety. These non-ionic amphiphilic molecules are generally biodegradable and biocompatible (1; 2) and are therefore molecules of great interest for markets such as detergents,

(3; 4; 5) cosmetics (6; 7) and food (3; 4; 8; 9) as emulsifiers, emollients or conservatives, respectively. Highly substituted sucrose esters have been commercialized by Procter & Gamble as substitutes for resins in paint formulations, while low-substituted sucrose esters are used as additives for food industry, known as 'E473'. Depending on the fatty chain functionalization, glycolipids properties can also be tuned to exhibit antioxidant, (10) antimicrobial, (11; 12) or antibacterial behavior (13). Some FASE have also been proved to be gelating agents. (14)

FASE synthesis is usually performed in the presence of alkaline catalysts such as potassium carbonate, potassium lactate, or metallic sodium. Besides requiring the use of toxic solvents and high temperatures, this route is generally not selective and mixtures of esters with different substitution degrees are obtained. (15) Enzymatic catalysis offers an interesting alternative for glycolipids synthesis. Indeed, esterases from group EC3.1, such as lipases and proteases, can catalyze FASE synthesis under milder and less toxic conditions than chemical catalysts. In particular, lipases are generally strictly selective toward primary alcohols, which leads to FASE of controlled structure. Hence, lipase-catalyzed FASE synthesis has encountered a large interest in the last decades. Nevertheless, while extensively studied, a lot of contradictions exist in the literature concerning the influence of the main reaction parameters. One of the most typical examples concerns the influence of the fatty acid or ester chain length on its conversion into glycolipid. Ghoul *and coll.* investigated the esterification of fructose with FAs having chain lengths from C8 to C18 in 2-methyl-2-butanol. The authors observed an increase of final conversions into FASEs while increasing the acyl donor chain length. (16) Schmid *and coll.* investigated the *Candida antarctica* Lipase B (CALB)-catalyzed glucose esterification in solid phase, composed of glucose, FA (C6 to C18), and about 100%-300% of organic solvent as adjuvant (17; 18), and came to the same conclusions. On the contrary, Pedersen *and coll.* studied the esterification of several disaccharides with FAs (C4 to C12) and observed higher initial conversion rates and higher conversions for shorter chains. (19) Lin *and coll.* studied glucose acylation by vinyl diesters of different chain lengths (C4 to C10) and also observed the highest conversions into glycolipid for shorter chains. (20) Those papers studied the influence of chain length

on either fatty acids, vinyl esters, or methyl esters as acyl donors but, to our knowledge, no existing study compares results obtained from direct esterification or trans-esterification. In our previous work, (21) we described an exhaustive study of the influence of the lipase, the solvent and the temperature on the synthesis of 6-O-glucose palmitate. It was shown that a full acylation of glucose was possible within 40 hours in anhydrous acetonitrile at 45°C, in the presence of only 5 wt-% of CALB. In this paper, we investigate the effect of the acyl donor chain length on FASE synthesis catalyzed by CALB. Based on our previous results, reactions were performed in anhydrous acetonitrile at 45°C. Both saturated fatty acids (FA, C8 to C18) and fatty acid vinyl esters (FAVE, C4 to C18) were used as acyl donors and their influence on reaction kinetics and final conversion into FASE was evaluated. The influence of the presence of unsaturations and their configuration was also investigated.

## **2. Material and methods**

### 2.1 Material

Vinyl butyrate, vinyl hexanoate, vinyl decanoate and vinyl palmitate were purchased from TCI Europe. Vinyl oleate was provided by Iteq (Pessac, France). Octanoic acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and lipase B from *Candida antarctica* immobilized on acrylic beads were purchased from Sigma Aldrich. Elaidic acid, linoleic acid, and linoleidic acid were purchased from Nu-Chek Prep. Anhydrous glucose was purchased from Fluka. Acetonitrile (Fluka, HPLC grade) was dried over alumina column. All the other compounds were used as received.

### 2.2 Characterization methods

NMR experiments were performed at 298K on a Bruker Avance 400 spectrometer operating at 400MHz. Deuterated DMSO was used as solvent.

HPLC analysis was performed on a HPLC apparatus with an evaporating light scattering detector (ELSD, Varian 380-LC) and a Prevail carbohydrate ES 5 $\mu$  column. The evaporator and nebulizer temperatures were set at 90°C and 40°C, respectively. 50  $\mu$ L of the samples were injected. The eluent

was a solution of 75/25/5 v/v/v methanol/acetonitrile/water with a flow rate of 0.5 mL.min<sup>-1</sup>.

## 2.3 Synthetic procedures

### 2.3.1 Synthesis of fatty acid vinyl esters (FAVEs)

Vinyl esters from octanoic acid, lauric acid, myristic acid, stearic acid, elaidic acid, linoleic acid and linoleaidic acid were synthesized according to the procedure described by Vilela *and coll.* (22)

Saturated vinyl esters: Yield: 50-70 %; <sup>1</sup>H NMR (DMSO, 298 K): 7.28 (1H, dd, **CH=CH<sub>2</sub>**), 5.34 (2H, **CH=CH**), 4.86 (1H, dd, **CH<sub>2</sub>=CH**), 4.56 (1H, dd, **CH<sub>2</sub>=CH**), 2.38 (2H, t, **CH<sub>2</sub>-CO**), 1.65 (2H, q, **CH<sub>2</sub>-CH<sub>2</sub>-CO**), 1.17-1.39 (m, alkyl chain CH<sub>2</sub>), 0.88 (3H, t, CH<sub>3</sub>)

Vinyl elaidate: Yield: 77 %; <sup>1</sup>H NMR (DMSO, 298 K): 7.29 (1H, dd, **CH=CH<sub>2</sub>**), 5.38 (2H, m, **CH=CH**), 4.86 (1H, dd, **CH<sub>2</sub>=CH**), 4.56 (1H, dd, **CH<sub>2</sub>=CH**), 2.38 (2H, t, **CH<sub>2</sub>-CO**), 1.96 (4H, m, **CH<sub>2</sub>-CH=CH**) 1.65 (2H, q, **CH<sub>2</sub>-CH<sub>2</sub>-CO**), 1.20-1.30 (18H, m, alkyl chain CH<sub>2</sub>), 0.88 (3H, t, CH<sub>3</sub>)

Vinyl linoleate: Yield: 70 %; <sup>1</sup>H NMR (DMSO, 298 K): 7.28 (1H, dd, **CH=CH<sub>2</sub>**), 5.36 (4H, m, **CH=CH**), 4.86 (1H, dd, **CH<sub>2</sub>=CH**), 4.56 (1H, dd, **CH<sub>2</sub>=CH**), 2,77 (2H, t, **CH=CH<sub>2</sub>=CH**), 2.38 (2H, t, **CH<sub>2</sub>-CO**), 2.04 (4H, m, **CH<sub>2</sub>-CH=CH**), 1.66 (2H, q, **CH<sub>2</sub>-CH<sub>2</sub>-CO**), 1.19-1.36 (14H, m, alkyl chain CH<sub>2</sub>), 0.89 (3H, t, CH<sub>3</sub>),

Vinyl linoleaidate: Yield: 74 %; <sup>1</sup>H NMR (DMSO, 298 K): 7.28 (1H, dd, **CH=CH<sub>2</sub>**), 5.40 (4H, m, **CH=CH**), 4.86 (1H, dd, **CH<sub>2</sub>=CH**), 4.56 (1H, dd, **CH<sub>2</sub>=CH**), 2,67 (2H, t, **CH=CH<sub>2</sub>=CH**), 2.38 (2H, t, **CH<sub>2</sub>-CO**), 1,99 (4H, m, **CH<sub>2</sub>-CH=CH**), 1.65 (2H, q, **CH<sub>2</sub>-CH<sub>2</sub>-CO**), 1.19-1.39 (14H, m, alkyl chain CH<sub>2</sub>), 0.88 (3H, t, CH<sub>3</sub>)

### 2.3.2 Synthesis of glycolipids

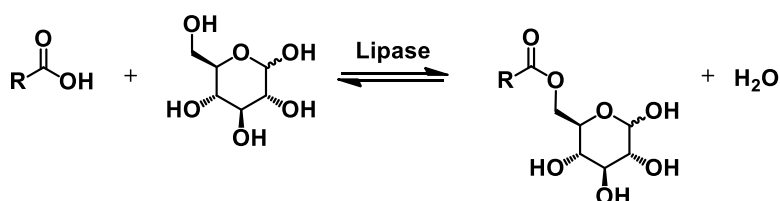
0.9 mmol of either FA or FAVE and 0.9 mmol of glucose were poured in an oven-dried Schlenk with 10 mL of anhydrous acetonitrile under argon. 20 mg of immobilized CALB were therefore added. The reaction was carried under a magnetic stirring of 250 rpm at 45°C heated by a thermostet oil bath. Reactions were followed by collecting 0.2 mL aliquots and the compounds proportions were determined by <sup>1</sup>H NMR (See Supplementary Information). In all cases, only the OH6 of glucose was esterified, secondary alcohols remaining untouched. At the end of the reaction, acetonitrile was

evaporated. THF was poured in the crude mixture and the obtained mixture was filtered under vacuum in order to remove the lipase and most of the glucose. The soluble part was evaporated. The obtained solid was dispersed into water then filtrated on a Büchner in order to remove traces of glucose. 5–10mL of acetone was then added to dissolve the unreacted fatty chains and the suspension was filtrated again. The remaining insoluble white powder was characterized by  $^1\text{H}$  NMR spectroscopy and was found to be pure 6O-glucoester. Glucoesters were isolated with yields of 50-90% depending on the chain length. Each glycolipid synthesis was performed in duplicates.

### 3. Results and discussion

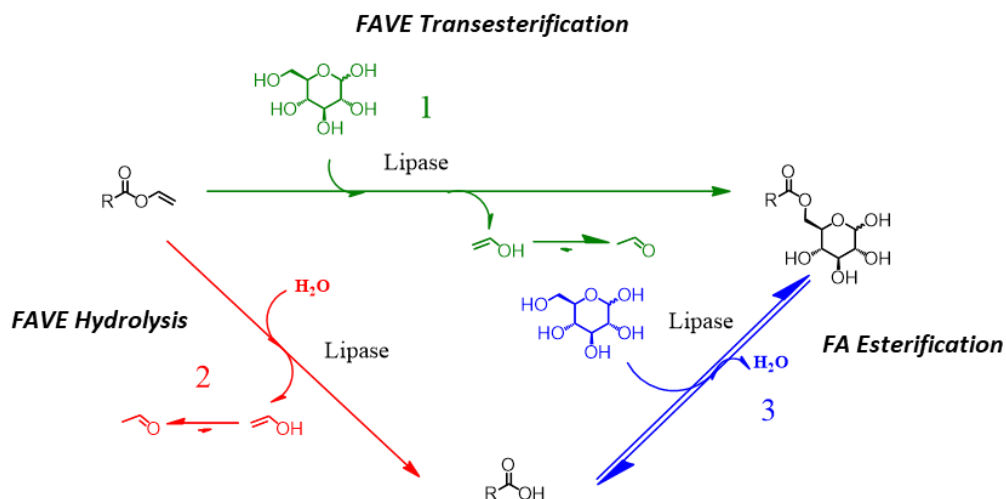
#### 3.1 Influence of acyl donor chain length

Enzyme-catalyzed esterification reactions were first carried out starting from glucose and saturated FAs with chain lengths from C8 to C18 (Scheme 1).



**Scheme 1: FASE synthesis from fatty acid and glucose**

Enzymatic acylation of glucose was then examined using FAVEs with chain lengths from C4 to C18. (Scheme 2) FAVEs are also good candidates for transesterification reactions as transesterifications are much faster than FAs esterifications and lead to higher conversion rates. (23) Indeed, on the contrary to classical esterification using FAs, there is no need to shift the equilibrium, as released vinyl alcohol is immediately converted to acetaldehyde, making the reaction irreversible (reaction 1 on Scheme 2). Therefore, because of the presence of residual water in the reaction medium, the enzyme can also catalyze FAVE hydrolysis (reaction 2 on Scheme 2), which is also irreversible. An equilibrium can then take place between the so-formed FA and the corresponding glycolipid (reaction 3 on Scheme 2).

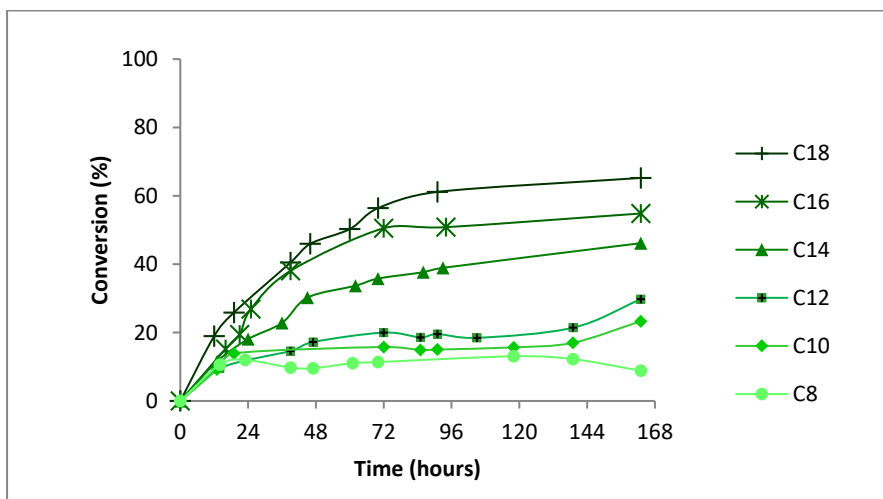


**Scheme 2: FASE synthesis from vinyl ester and glucose**

All the reactions were carried out at 45°C during 72 hours and were monitored using <sup>1</sup>H NMR. Plots of 6-O-glucose monoester conversion versus time for each FA and each FAVE are shown in Figure 1 and Figure 2, respectively. Glucose acylation initial rates have been calculated from those plots and are exposed in Table 1.

	FA		FAVE	
	Acylation after 7 days (%)	Initial FASE formation rate (μmol.min <sup>-1</sup> .g <sup>-1</sup> CALB beads)	Acylation after 3 days (%)	Initial FASE formation rate (μmol.min <sup>-1</sup> .g <sup>-1</sup> CALB beads)
C4	-	-	81	24
C6	-	-	83	44
C8	9	6	55	33
C10	17	5	42	35
C12	21	5	75	29
C14	46	6	100	29
C16	55	7	100	28
C18	65	12	100	30

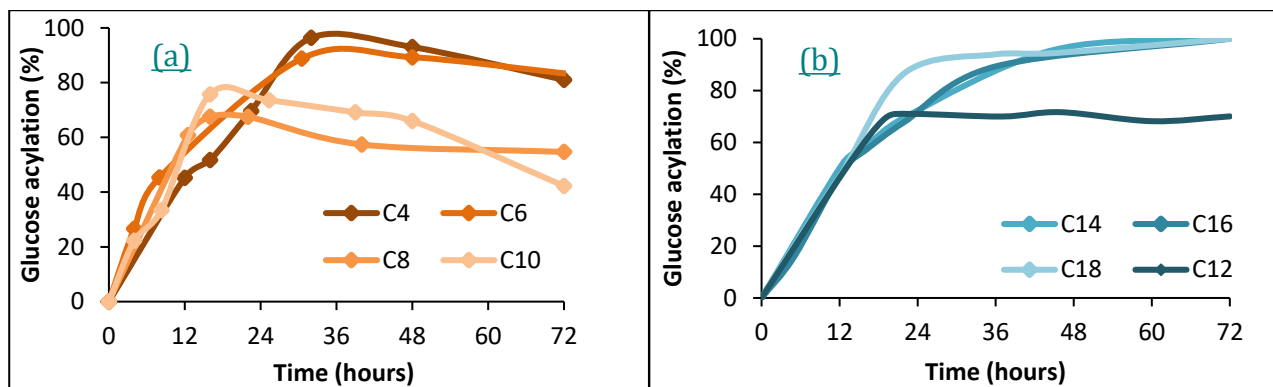
**Table 1 – Final conversions into FASEs and glucose initial conversion rates depending on FA or FAVEs chain lengths**



**Figure 1 : Effect of the fatty acid chain length on glucoester synthesis**

Whatever the FA used, glycolipid was produced although the formed water was not removed to shift the equilibrium. After 168 hours of reaction, only the C8 plot seems to have reached the equilibrium, while conversions into other glucoesters are still slightly increasing. Conversions, as well as initial rate, increased with FA chain length. This preference of CALB for longer chains, which are more hydrophobic, is not very surprising as CALB exhibits a hydrophobic elliptical, steep funnel of  $9.5 \times 4.5 \text{ \AA}$  active site. (24) Those results are consistent with those published by Ghoul *and coll.* (17) On the contrary, as already mentioned, Pedersen *and coll.* observed the opposite trend on the esterification of disaccharides. (20) In that case, the size of the disaccharides probably hinders the FA access to the active site, short chain FAs accessing more easily the active site than the long chain FAs.



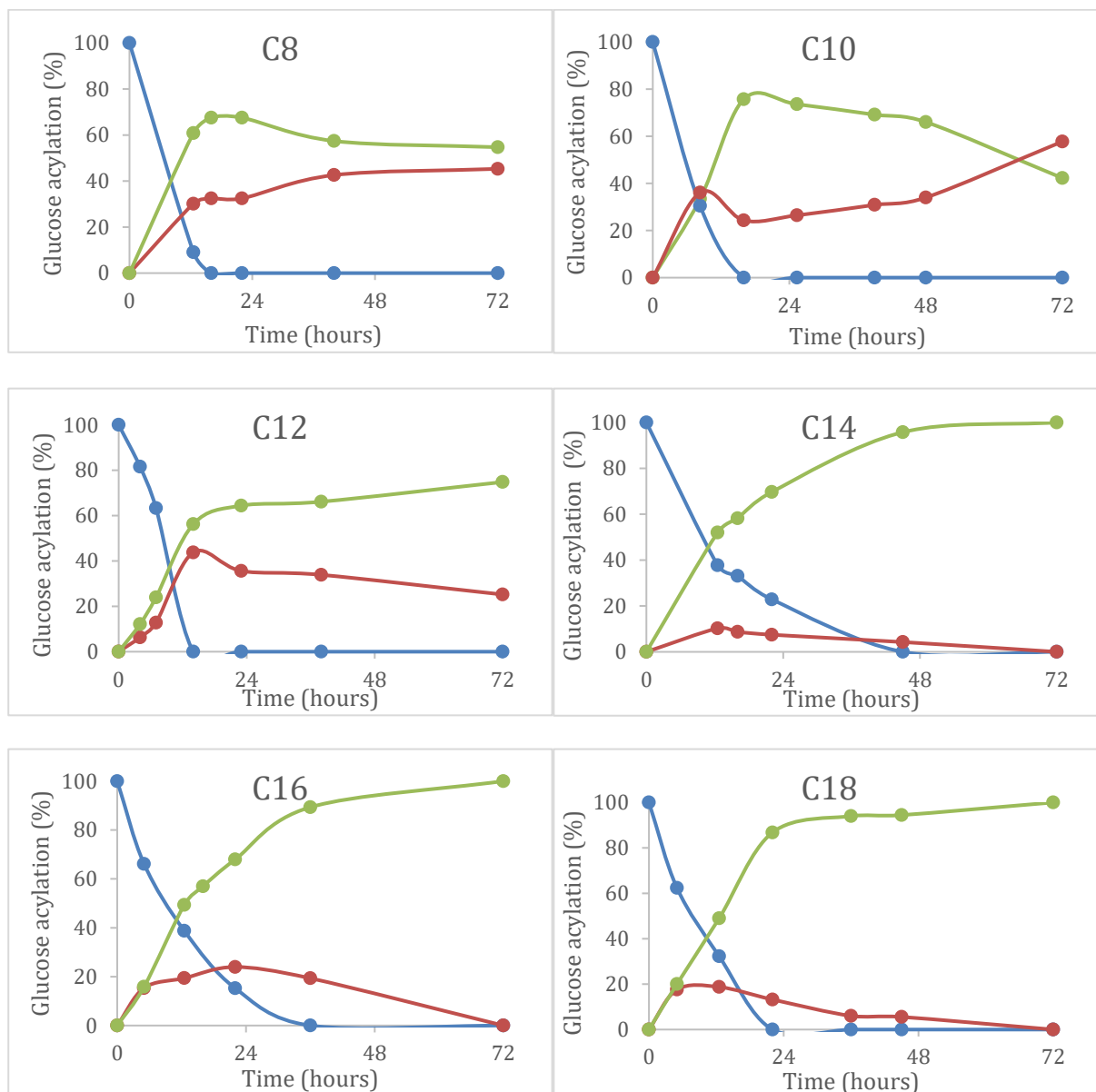


**Figure 2: Kinetics of FAVEs conversion into glucoesters determined by  $^1\text{H}$  NMR: effect of FAVEs chain length ; (a) left = C4 to C10 FAVEs; (b) right = C12 to C18 FAVEs**

As already observed with fatty acids, the best conversions were obtained for the long chain FAVEs (C14, C16 and C18). In the case of C18, the conversion into glycolipid was even total. More surprisingly, conversions up to 90-95% were reached with C4 and C6 FAVEs. Lower conversion rates were obtained for C8, C10 and C12, with a minimum conversion of 42% for C10. For FAVE with the largest chain lengths (C12 to C18), FASE concentrations increase linearly with time at a very similar conversion rate (Table 1). Whereas C12 FASE concentration reached a conversion of 75 % after 72 hours, full conversion into FASEs was observed with all other long-chain FAVEs. For the shortest FAVE chains (C4 to C10), FASE concentration reaches an optimum and then decreases slowly with time.

The variation of FAVE, FA and FASE with time for FAVEs with chain lengths from C8 to C18 is shown in Figure 3. Because of the volatility of FAVE and FA with the shortest chains, it was not possible to obtain accurate data for C4 and C6 FAVEs. For all chain lengths, FAVE conversion into both FASE and FA strongly prevailed. When all the FAVE was consumed (most of the time in 24 h), only the reversible reaction between FA and FASE remained. Nevertheless, depending on the chain length, two different behaviors could be observed. For C12, C14, C16 and C18 FAVEs, the equilibrium is shifted toward FASE formation. Total consumptions of FAs were observed for C14, C16 and C18, while C12 glucoester, was much slower and was still ongoing after 72 hours. For C8

and C10 FAVEs, the equilibrium is shifted to the FA formation and FASE is slowly hydrolyzed. In all cases, FAVE trans-esterification allows reaching much higher glucose acylation rates than FA direct esterification (See Table 1).



**Figure 3 : Variation of FAVE, FA and glucoester contents for FAVEs from C8 to C18 determined by  $^1\text{H}$  NMR. Blue = FAVE ; Red = FA ; Green = glucoester**

In order to explain those results, maximal FASE solubilities for C10 to C18 chains in acetonitrile at 25°C have been determined by HPLC (See Table 2 and supplementary information). Indeed,

Schmid *and coll.* observed that the longer the FA chain, the less soluble the corresponding glucoester. (18; 19) Saturated glucoesters show very poor solubility in acetonitrile; precipitation of long-chain glucoesters could then shift the equilibrium and lead to higher conversions than for short chain FAs.

FASE	Maximal solubility ( $\mu\text{mol/mL}$ )	Highest FASE amount measured ( $\mu\text{mol/mL}$ )	
		From FA	From FAVE
<b>C4</b>	-	-	86.7
<b>C6</b>	-	-	80.4
<b>C8</b>	-	8.0	60.8
<b>C10</b>	23.6	26.7	68.1
<b>C12</b>	6.85	26.8	67.3
<b>C14</b>	1.99	41.5	90.0
<b>C16</b>	0.60	49.3	90.0
<b>C18</b>	0.26	58.7	90.0

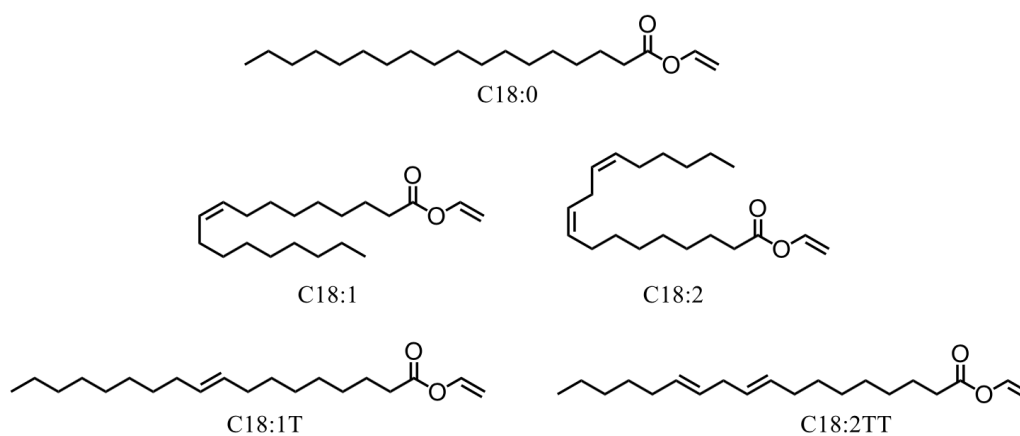
**Table 2 – Maximal FASEs solubilities in acetonitrile at 25°C compared to FASEs highest measured concentrations during esterification and trans-esterification reactions**

Solubilities decrease from 23.6 to 0.26  $\mu\text{mol/mL}$  while increasing the chain length from C10 to C18. On another hand, FASE amounts go from 8.0 to 58.7  $\mu\text{mol/mL}$  during direct esterification reactions, and from 60.8 to 90.0  $\mu\text{mol/mL}$  during FASE trans-esterification reactions. As C10 glucoester maximal solubility is higher than the others, starting from C4 to C10 FAVEs, even if a fraction of formed glucoesters could precipitate out of the reactional medium, a significant part remains in solution. As they are smaller molecules than C12 to C18 glucoesters, they are mobile enough to access the CALB active site and therefore be hydrolyzed, (See Scheme 2) causing a decrease of measured conversion. Starting from C8 and C10 FAs, their esterification rates being

significantly lower, it could be assumed that all the so-formed glucoesters remains in solution. In that case, the system converges to the thermodynamic equilibrium. On the opposite, starting for C12 FAs or FAVEs and higher, almost all the so-formed glucoesters precipitate out of the reaction medium, shifting the equilibrium into their formation. As only a tiny amount of glucoesters remains in solution, there is no hydrolysis occurring.

### 3.2 Influence of unsaturations

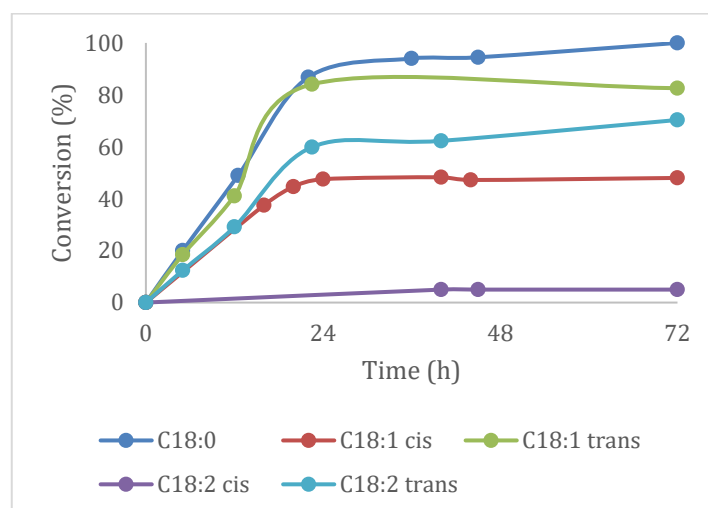
As the best results were obtained with vinyl stearate, the influence of double bonds was investigated with unsaturated C18 vinyl esters: vinyl oleate (vinyl *cis*-9-octadecenoate, C18:1), vinyl elaidate (vinyl *trans*-9-octadecenoate, C18:1T), vinyl linoleate (vinyl *cis*-9,*cis*-12-octadecadienoate, C18:2), and vinyl linoleaidate (vinyl *trans*-9,*trans*-12-Octadecadienoate, C18:2TT). Enzymatic transesterifications of those FAVEs were monitored by <sup>1</sup>H NMR. In all cases, all the FAVEs were transformed into FAs and/or FASEs, (See Scheme 3) and the reaction equilibrium between the glycolipid and the fatty acid was reached after 24 hours (See Figure 4). Both conversion into glycolipid and initial conversion rate were higher for *trans* FAVEs than for *cis* FAVEs and they both decreased with increasing number of double bonds. For vinyl linoleate, only traces of glycolipid were detected (See Table 3).



**Scheme 3 : C18 FAVEs: C18:0 = vinyl stearate ; C18:1 = vinyl oleate ; C18:2 = vinyl linoleate ; C18:1T = vinyl elaidate ; C18:2TT = vinyl linoleaidate**

FAVE	C18:0	C18:1T	C18:2TT	C18:1	C18:2
<b>Conversion after 72h</b>	100%	83 %	70 %	47 %	<5 %
<b>Initial conversion rate (<math>\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}</math> lipase)</b>	29,6	26,0	18,3	17,1	-

**Table 3 : Influence of the number and the configuration of unsaturations on FAVE conversion into glucoester. Initial conversion rate into 6-O-glucose linoleate was too low to be calculated**



**Figure 4 : Influence of the number and configuration of unsaturations on FAVE conversion into glucoester**

*Cis* configuration seems to cause steric hindrance, making the FAVE access to the enzyme active site more difficult and thus decreasing a lot the formation of FASE. The lower decrease observed for C18:1T and C18:2TT can be due to the reduction of the chain mobility because of the presence of double bonds. Again, contradictory results can be found in the literature. Indeed, in the case of flavonoids esterification with oleic, linoleic and linolenic acids, conversions of 70, 80 and 68% were respectively obtained at 50°C in acetone, showing almost no influence of the number of unsaturations.

(25) Ardhaoui *et al.* also scarcely found a difference of behavior between stearic acid and oleic acid for flavonoid esterification. (26) In both cases, a huge amount of lipase was used (0.7 to 1.2 g of CALB per mmol of flavonoid) whereas, in this study, only 22 mg of CALB per mmol of glucose was employed. It could be assumed that, in their case, the influence of unsaturations could have been smoothed by the important quantity of catalyst. On the contrary, Hollman *et al.* (27) studied the influence of a methyl substituent in valeric acid on the rate of CALB-catalyzed esterification of 1-octanol and found that it strongly decreased for 2- and 3-methyl valeric acids. Lin *et al.* found similar results comparing esterification of acetoin with linear and branched fatty acids. (28) These results show the importance of the substrate geometry to obtain high conversions into glycolipid: a saturated, linear fatty acid will easily access to the enzyme active site while hindrance caused by unsaturations will lead to lower conversions.

#### **4. Conclusion**

In this paper, the influence of the acyl donor type and geometry (fatty acid or vinyl ester with chain lengths from 4 to 18 carbons, saturated or unsaturated) on the enzymatic synthesis of glucose-based FASEs were investigated. From both types of acyl donors, CALB showed more affinity for alkyl chains longer than 12 carbons. Starting from fatty acids, an equilibrium between FA and FASE is reached within 4 days. Higher glucose acylation rates were obtained with FAVEs, as the continuous natural elimination of so-formed acetaldehyde allows the reaction system to exceed equilibrium, and maximum FASE concentrations were reached within 16 to 32 hours. The presence of unsaturation on the fatty chain was showed to hinder the access of the enzyme active site, causing lower acylation rates.

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# Effect of the nature of fatty acid on CALB-catalyzed esterification of glucose

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## Supplementary information

### 1. Calculation of conversions into FASE based on <sup>1</sup>H NMR spectra: example of 6-O-glucose palmitate:

<sup>1</sup>H NMR spectra of vinyl palmitate, palmitic acid, 6-O-glucose palmitate, glucose and crude mixture after 16 hours of reaction are plotted in Figure S1. Attributions have been checked by COSY, HSQC and HMBC NMR. All signals and their attributions are plotted in Table S1. Vinyl palmitate, palmitic acid and 6-O-glucose palmitate contents have been calculated using Equation S1.

%VP

$$= \frac{\frac{1}{2} * (0.5 I_{2.41 \text{ ppm}} + I_{7.21 \text{ ppm}})}{\frac{1}{2} * (0.5 I_{2.41 \text{ ppm}} + I_{7.21 \text{ ppm}}) + \frac{1}{2} (0.5 I_{2.17 \text{ ppm}} + I_{11.93 \text{ ppm}}) + \frac{1}{7} * (0.5 I_{2.26 \text{ ppm}} + I_{3.76 \text{ ppm}} + I_{3.99 \text{ ppm}} + I_{5.01 \text{ ppm}} + I_{5.07 \text{ ppm}} + I_{6.33 \text{ ppm}} + I_{6.64 \text{ ppm}})}$$

%PA

$$= \frac{\frac{1}{2} (0.5 I_{2.17 \text{ ppm}} + I_{11.93 \text{ ppm}})}{\frac{1}{2} * (0.5 I_{2.41 \text{ ppm}} + I_{7.21 \text{ ppm}}) + \frac{1}{2} (0.5 I_{2.17 \text{ ppm}} + I_{11.93 \text{ ppm}}) + \frac{1}{7} * (0.5 I_{2.26 \text{ ppm}} + I_{3.76 \text{ ppm}} + I_{3.99 \text{ ppm}} + I_{5.01 \text{ ppm}} + I_{5.07 \text{ ppm}} + I_{6.33 \text{ ppm}} + I_{6.64 \text{ ppm}})}$$

%GP

$$= \frac{\frac{1}{7} * (0.5 I_{2.26 \text{ ppm}} + I_{3.76 \text{ ppm}} + I_{3.99 \text{ ppm}} + I_{5.01 \text{ ppm}} + I_{5.07 \text{ ppm}} + I_{6.33 \text{ ppm}} + I_{6.64 \text{ ppm}})}{\frac{1}{2} * (0.5 I_{2.41 \text{ ppm}} + I_{7.21 \text{ ppm}}) + \frac{1}{2} (0.5 I_{2.17 \text{ ppm}} + I_{11.93 \text{ ppm}}) + \frac{1}{7} * (0.5 I_{2.26 \text{ ppm}} + I_{3.76 \text{ ppm}} + I_{3.99 \text{ ppm}} + I_{5.01 \text{ ppm}} + I_{5.07 \text{ ppm}} + I_{6.33 \text{ ppm}} + I_{6.64 \text{ ppm}})}$$

**Equation S1: Calculation of vinyl palmitate (VP), palmitic acid (PA), and 6-O-glucose palmitate (GP) percentages of a given crude sample. I represents the integral value for each signal on the corresponding NMR spectrum. Integrals were calculated by setting I<sub>0.85 ppm</sub> = 3**



(conditions: vinyl palmitate/glucose molar ratio = 1/1, concentration = 90 mM, 5% of CALB (20 mg), in 10 mL of anhydrous acetonitrile; reaction performed at 45°C)

**Table S1: List of chemical shifts on a typical raw mixture of vinyl palmitate, palmitic acid, glucose and 6-O-glucose palmitate**

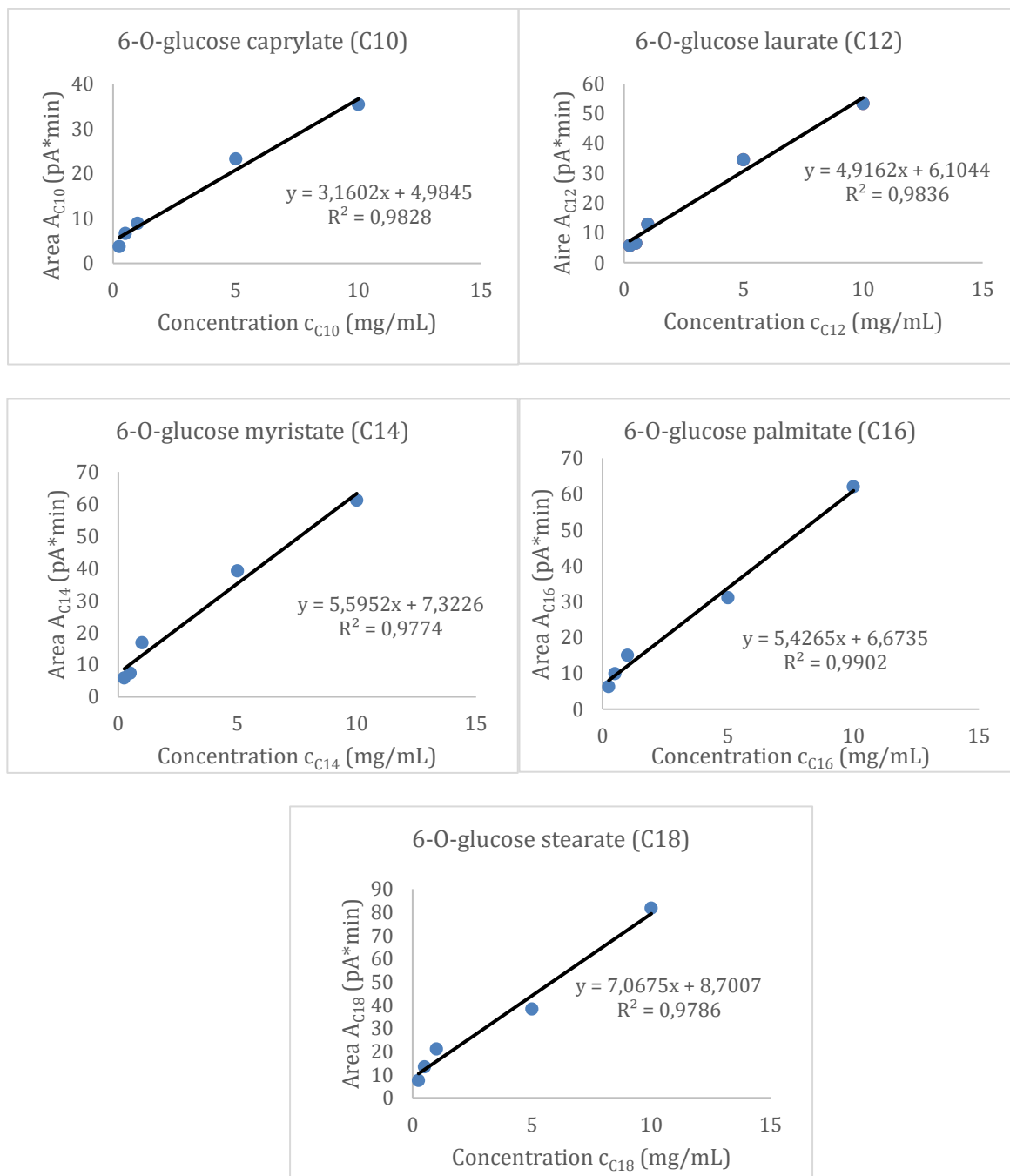
<b>Chemical shifts (ppm)</b>	<b>Vinyl palmitate</b>	<b>Palmitic acid</b>	<b>Glucose</b>	<b>6-O-glucose palmitate</b>
<b>0.85</b>	CH <sub>3</sub>	CH <sub>3</sub>		
<b>1.14-1.34</b>	CH <sub>2</sub> alkyl chain	CH <sub>2</sub> alkyl chain		CH <sub>2</sub> alkyl chain
<b>1.54</b>	CH <sub>2</sub> -CH <sub>2</sub> -CO	CH <sub>2</sub> -CH <sub>2</sub> -CO		CH <sub>2</sub> -CH <sub>2</sub> -CO
<b>2.17</b>		CH <sub>2</sub> -CO		
<b>2.26</b>				CH <sub>2</sub> -CO
<b>2.41</b>	CH <sub>2</sub> -CO			
<b>2.89</b>			H2 $\beta$	
<b>3.04</b>				H4
<b>3.10</b>				H2
<b>3.42</b>			H5 $\alpha$ , H6b $\alpha$ , H6b $\beta$	H3
<b>3.57</b>			H4 $\alpha$ , H6a $\beta$	
<b>3.66</b>			H6a $\alpha$	
<b>3.76</b>				H5
<b>3.99</b>				H6a
<b>4.26</b>			H1 $\beta$	H6b
<b>4.33</b>			H6' $\beta$	
<b>4.41</b>			H2' $\beta$	

4.45		H6'α	
4.50			
4.56	CH <sub>2</sub> =CH		
4.60		H3'β	
4.73			H3'
4.74		H4'β	
4.80		H2'α, H3'α, H4'α	
4.86	CH <sub>2</sub> =CH		
4.90		H1α	H1
5.01			H4'β
5.07			H4'α
6.18		H1'β	
6.33			H1'β
6.55		H1'α	
6.64			H1'α
7.21	CH=CH <sub>2</sub>		
11.93	COOH		

## 2. Determination of FASEs solubilities by HPLC

HPLC conditions are given in the Materials and Methods section

For each FASE, a calibration curve for glucose was determined by injecting in HPLC samples from six FASE solutions in DMSO at known concentrations. (See Figure S2) For each sample, peak areas were measured and the obtained values were plotted versus injected mass. The relation between measured areas ( $A_{Cn}$ ) and corresponding glucose mass ( $m_{Cn}$ ) was obtained by linear regression.



**Figure S2 – Calibration curves for FASEs from C10 to C18**

#### Determination of FASEs maximal solubilities in acetonitrile

An excess of each FASE was put in a capped glass vial containing a magnetic bar, in presence of 1 mL of acetonitrile. All the vials were put under agitation in a thermostated oil bath at 25°C for 72 hours. The samples were filtrated on 0.4  $\mu\text{m}$  cellulose filters to remove insoluble FASE and the

soluble parts were analyzed by HPLC. For each sample, the FASE peak area was measured and the corresponding concentration was calculated using the previously plotted calibration curves. All results are plotted in Table S2.

FASE	Injected volume ( $\mu\text{L}$ )	Area ( $\text{pA}\cdot\text{min}$ )	Concentration	
			mg/mL	$\mu\text{mol/mL}$
<b>C10</b>	50	16.720	5.29	26.7
<b>C12</b>	50	7.646	1.56	6.85
<b>C14</b>	50	2.827	0.51	1.99
<b>C16</b>	50	1.061	0.17	0.60
<b>C18</b>	50	0.564	0.08	0.26

**Table S2. C10, C12, C14, C16 and C18 FASE maximal solubilities in acetonitrile at 25°C**