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# Enzymatically-synthesized xylo-oligosaccharides laurate esters as surfactants of interest

D. Gérard [a,b], T. Méline [a], M. Muzard [b,1], M. Deleu [c,1], R. Plantier-Royon [b,1], C. Rémond [a,*,1]

[a] Université de Reims Champagne Ardenne, INRAE, FARE, UMR A 614, Chaire AFERE, 51686, Reims, France
[b] Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, Université de Reims Champagne-Ardenne, 51687, Reims Cedex, France
[c] Université de Liège, Gembloux Agro-Bio Tech, Laboratoire de Biophysique Moléculaire Aux Interfaces, 2 Passage des Déportés, B-5030, Gembloux, Belgium

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ABSTRACT

Lipase-catalyzed synthesis of xylo-oligosaccharides esters from pure xylobiose, xylotriose and xylotetraose in the presence of vinyl laurate was investigated. The influence of different experimental parameters such as the loading of lipase, the reaction duration or the use of a co-solvent was studied and the reaction conditions were optimized with xylobiose. Under the best conditions, a regioselective esterification occurred to yield a monoester with the acyl chain at the OH-4 of the xylose unit at the non-reducing end. Surface-active properties of these pure xylo-oligosaccharides fatty esters have been evaluated. They display interesting surfactant activities that differ according to the degree of polymerization (DP) of the glycone moiety.

## 1. Introduction

Hemicelluloses are the second major abundant biopolymer in plant biomass after cellulose, accounting up to 25–35% of the dry matter, especially in woody tissues. However, hemicelluloses are much less valued than cellulose in industrial fields. The most abundant hemicellulosic polysaccharides are xylans, heteropolysaccharides mainly constituted of xylose units linked by β-(1,4) glycosidic bonds [1,2]. Xylo-oligosaccharides (XOs), can be produced from xylans by auto-hydrolysis [3–5], acid hydrolysis [6–9], direct enzymatic hydrolysis [10–13] or a combination of chemical and enzymatic treatments [14,15]. These oligosaccharides have exhibited interesting properties for applications as prebiotics and in anti-obesity diets [16–18]. Indeed, xylo-oligosaccharides represent a promising oligosaccharide class that are metabolized by probiotic bacteria in the gut microbiota and also offers anti-oxidant, anti-allergy or anti-inflammatory activities [19].

Sugar-based surfactants are non-ionic amphiphilic compounds intensively studied due to their widespread applications [20]. Among them, fatty acid sugar esters represent a class of non-toxic and biodegradable surfactants with a wide range of applications in many fields such as food [21], cosmetics and detergents [22] or pharmaceuticals [23,24]. Chemical syntheses of sugar esters generally require the use of alkaline catalysts, high temperatures, hazardous solvents and produce complex mixtures of esters [25,26]. Enzymatic synthesis of sugar esters catalyzed by lipases or esterases has been studied in the recent years as a milder and greener alternative to the classical chemical routes [27,28]. The use of lipases in organic solvents is a one-pot process, with a high degree of regioselectivity and the enzymatic reaction generally leads to the production of monoesters. However, few examples concern the enzymatic acylation of oligosaccharides with a DP superior to 2. During esterification reaction in presence of fructo-oligosaccharides (DP 2–8) and lauric acid, the lipase N435 mainly catalyzed the synthesis of monoesters whereas traces of di- and tri-esters were also detected [29]. No information is available concerning the structures of the esterified fructo-oligosaccharides. Synthesis of maltodextrin esters [30–32] or XOs esters [33–36] was recently reported by enzymatic esterification in the presence of various fatty acids using the lipase from *Thermomyces lanuginosus* and studied for their properties in the stabilization of oil-in-water emulsions. However, no purification of the maltodextrin or XOs esters was performed and no data about their structures were described.

In this study, the purpose was to investigate the experimental conditions for the lipase-catalyzed synthesis of XOs esters from pure DP2 xylobiose to DP4 xylotetraose and to determine the chemical structures of the esterified products. As amphiphilic XOs are known to act as surfactants, we were also interested in the determination of their surface

* Corresponding author.
*E-mail address:* caroline.remond@univ-reims.fr (C. Rémond).
[1] These authors contributed equally to this work.

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active properties.

## 2. Results and discussion

### 2.1. Synthesis of xylo-oligosaccharide esters

We first set out to determine the best experimental conditions for the enzymatic transesterification using previously prepared pure xylobiose (see Experimental section). In a first approach, we used the optimized reaction conditions defined in our previous study reporting the synthesis of monosaccharides esters using 1% polymer-supported commercially available lipase N435 (Novozymes) from D-xylose and L-arabinose: xylobiose 10 mM, vinyl laurate 300 mM in 2-methylbutan-2-ol (2M2B), 10% 4 Å molecular sieves, 50 °C, 72h [37]. A large excess of vinyl laurate was used because of the partial enzymatic hydrolysis of the acyl donor in the less reactive lauric acid due to the presence of residual water into the reaction medium [38]. Interestingly, monitoring of the lipase-catalyzed esterification reaction by HPLC clearly indicated the production of a single product. After purification and careful studies by NMR and mass spectrometry analyses, the structure of the compound obtained was assigned to a xylobiose monoester: 4′-*O*-lauryl-xylobiose **1** (Scheme 1). No other xylobiose monoester has been detected. However, a trace of xylobiose diester could be observed by MS.

We then focused on improving the experimental conditions by playing on several parameters such as enzyme loading and reaction duration.

In a first set of experiments, different lipase N435 loadings were tested in order to catalyze the transesterification reaction in the presence of xylobiose (10 mM) during 72h. With 2% loading of lipase N435, the concentration of xylobiose ester **1** reached 4.02 g/L (8.6 mM), 24% more than the 1% lipase catalyzed reaction (3.05 mM). Sequential addition of lipase (1% at $t_0$ and 1% at 40h), tested to evaluate the impact of fresh enzyme addition during the reaction, did not increase the ester concentration. As the concentration in ester **1** was rather high (8.6 mM from 10 mM xylobiose), the following experiments were then conducted with 2% N435 lipase.

Then, a kinetic study of this enzymatic transesterification reaction was undertaken. Kinetic analyses indicated that maximal concentration of xylobiose ester **1** was reached from 48h (Fig. 1).

Finally, under the best reaction conditions: N435 lipase 2%, xylobiose 10 mM, vinyl laurate 300 mM in 2M2B, 10% 4 Å molecular sieves, 50 °C, 72h, the yield in 4′-*O*-laurylxylobiose **1** reached 86%.

Therefore, in the presence of X2 and vinyl laurate, lipase N435 catalyzed a regioselective monoacylation at the hydroxyl group OH-4 of the D-xylose residue from the non-reducing end. Previous studies concerning the acylation of disaccharides mainly concern maltose, lactose and sucrose. In the case of sucrose, in presence of vinyl laurate, lipase N435 mainly catalyzed the formation of the 6,6′-*O*-diester [39]. The *O*-6 and *O*-6′ monoesters were also produced to a lower extent. The authors concluded that the two primary alcohols from sucrose exhibit a similar reactivity. In other studies, the acylation of lactose or maltose with vinyl laurate catalyzed with different lipases, notably CALB from *Candida antarctica*, led to the synthesis of 6′-*O*-monoester indicating a preferential acylation of the primary hydroxyl group from the non-reducing moieties of the disaccharides [39,40]. This selective production of disaccharide monoesters by lipase N435 could be explained by the selectivity of the lipase due to its active site which consists of a 9.5 × 4.5 Å tunnel [41]. Therefore, a steric hindrance can occur when molecules with high molecular weight such as oligosaccharide monoesters enter the active site and thus these monoesters cannot be used as acyl acceptor for further acylation to produce diesters [42,43]. In the present study, as no primary hydroxyl groups is present on X2, lipase N435 catalyzed the acylation onto a secondary hydroxyl group. Interestingly, a total regioselectivity was obtained for this enzymatic transesterification on the 4′-OH position of X2. Such regioselectivity for an enzymatic esterification or transesterification using a polymer-supported lipase at the 4-OH group was previously described from β-xylopyranosides, especially in polar solvents [44–47]. This OH-4 group does not have the highest chemical reactivity [48,49], but studies have suggested a better availability for a lipase-catalyzed reaction due to steric effects [46]. A selective acylation at OH-4 of a xylopyranose moiety in a complex branched pentasaccharide (digitonin) was also observed [50].

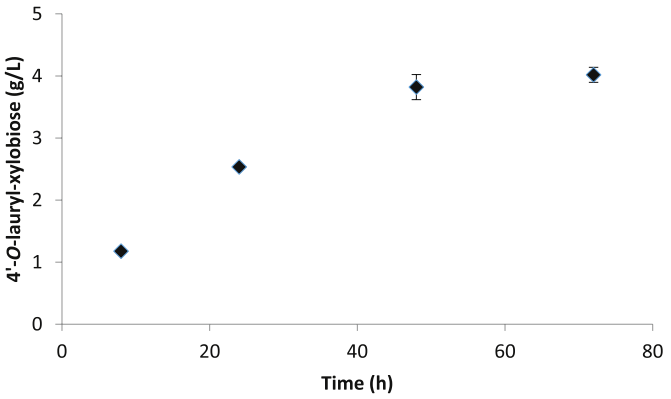
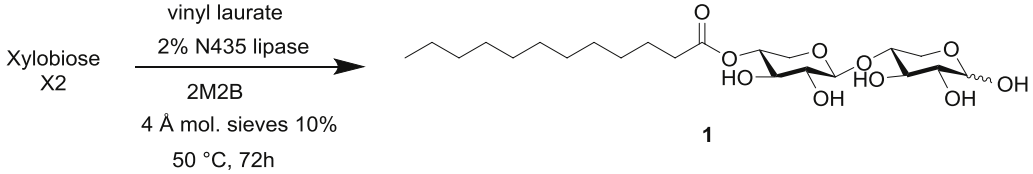



**Fig. 1.** Kinetic study for the synthesis of 4′-*O*-lauryl-xylobiose **1**. 4′-*O*-Laurylxylobiose was quantified by HPLC 10 mM X2, 300 mM VL in 2M2B, lipase N435 2%, 4 Å molecular sieves 10%, magnetic stirring (400 rpm) at 50 °C.

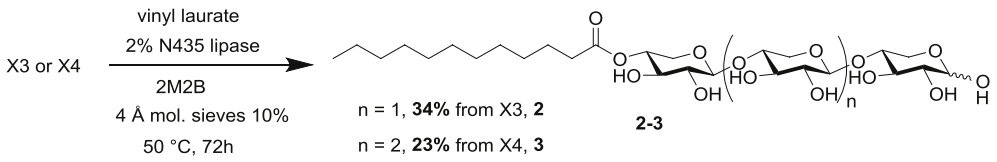
The synthesis of xylotriose-based laurate ester was then studied in the same conditions as described for xylobiose. In this case, 4″-*O*-laurylxylotriose **2** was produced with the lipase N435 as confirmed by mass spectrometry and NMR analyses. After 72h with lipase N435 at 2%, 2.04 g/L of 4″-*O*-lauryl-xylotriose **2** were produced (Table 1). Under the best reaction conditions, the yield in 4″-*O*-laurylxylotriose **2** reached 34% (Scheme 2). In the presence of xylotetraose and vinyl laurate, lipase N435 synthesized 4‴-*O*-laurylxylotetraose **3** as confirmed by mass spectrometry and NMR analyses. The concentration obtained after 72h reached 1.70 g/L (Table 1). Under the best reaction conditions, the yield in 4‴-*O*-laurylxylotetraose **3** reached 23% (Scheme 2).

**Table 1**
Concentration of XOs laurate esters produced with lipase N435 during 72h. 10 mM of XOs, 300 mM vinyl laurate in 2M2B, lipase N435 2%, 10% molecular sieves, under magnetic stirring 400 rpm at 50 °C during 72 h. 4-*O*-lauryl-XOs were quantified by HPLC. Mean values of triplicates reactions.

| | XOs loading | | 4-*O*-lauryl XOs produced | | |
|---|---|---|---|---|---|
| | mM | g/L | mM | g/L | Yield (%) |
| **X2** | 10 | 2.82 | 8.64 ± 0.25 | 4.02 ± 0.12 | 86 |
| **X3** | 10 | 4.14 | 3.42 ± 0.42 | 2.04 ± 0.25 | 34 |
| **X4** | 10 | 5.46 | 2.32 ± 0.12 | 1.70 ± 0.09 | 23 |




**Scheme 1.** Synthesis of 4′-*O*-laurylxylobiose **1**.




**Scheme 2.** Synthesis of 4″-*O*-laurylxylotriose **2** and 4‴-*O*-laurylxylotetraose **3**.

A decrease in reactivity for the acylation reaction with vinyl laurate occurred from X2 to X4. This result could be attributed to the decrease in solubility into 2M2B when the DP of XOs increased from 2 to 4. DMSO was reported as an interesting co-solvent for acylation with lipases in 2M2B or *tert*-butanol as it can improve the solubility of oligosaccharides into an organic solvent [51,52]. DMSO was generally used at concentration lower than 20% in order to not generate the denaturation of lipases. In our study, to improve the solubility of XOs at 10 mM in 2M2B, transesterification reactions were tested in presence of DMSO as a co-solvent at 10% (v/v). The solubility of XOs at 10 mM was investigated (Table 2). Results confirmed the poor solubility of XOs into pure 2M2B. Concentration of dissolved XOs in a 90:10 2M2B/DMSO mixture was highly improved.

Whereas the presence of DMSO greatly improved the XOs solubility in the reaction medium, when catalyzing the acylation into the 2M2B/DMSO mixture (90:10), no improvement of the synthesis of lauryl XOs occurred and the concentrations of 4-*O*-lauryl XOs were quite similar than those obtained into pure 2M2B. This result could be due either by a possible addition of water in the reaction mixture due to the high hydrophilicity of DMSO, used as received without treatment to remove water, leading to a competing hydrolysis reaction or to a decreasing affinity of the lipase for XOs as acyl acceptors when DP increased.

### 2.2. Surface-active properties of lauryl XOs

The surface-active properties of the three purified XOs laurate esters **1–3** were evaluated by measuring the surface tension at an air/water interface as a function of their bulk concentration (Fig. 2) and compared to the curve obtained for 5-*O*-lauryl-D-xylofuranose, the main ester produced from transesterification reaction with D-xylose and vinyl laurate in our previous study [37].

For the four molecules, a decrease of surface tension with increasing concentration is observed up to a define concentration after which the surface tension is almost constant. At this concentration, called the critical aggregation concentration (CAC) and higher, the interface is saturated and aggregates are formed in the aqueous phase. Critical aggregation concentrations (CAC) and surface pressure at CAC ($\Pi_{CAC}$) for the four XOs esters are presented in Table 3. The maximum surface excess concentration ($\Gamma_{max}$) and the interfacial area of the molecule (A) were also determined from the Gibbs adsorption equation (see experimental section).

An increasing number of xylose residues in the XOs laurate esters tends to increase the CAC and decreases the corresponding surface pressure ($\Pi_{CAC}$). It also decreases the maximum surface excess concentration (Γmax) and increases the area occupied by the xylose ester at the interface.

**Table 2**
Solubility of XOs into reaction media containing 2M2B 100% or 2M2B/DMSO 90:10. XOs were incubated under magnetic stirring 400 rpm at 50 °C during 72 h. Solubility of XOs was quantified by HPAEC-PAD.

| | XOs loading | | Dissolved XOs in 2M2B | | Dissolved XOs in 2M2B/DMSO 90:10 | |
|---|---|---|---|---|---|---|
| | mM | g/L | mM | g/L | mM | g/L |
| **X2** | 10 | 2.82 | 0.54 | 0.15 | 6.94 | 1.95 |
| **X3** | 10 | 4.14 | 0.35 | 0.14 | 4.69 | 1.94 |
| **X4** | 10 | 5.46 | 0.11 | 0.06 | 1.14 | 0.62 |

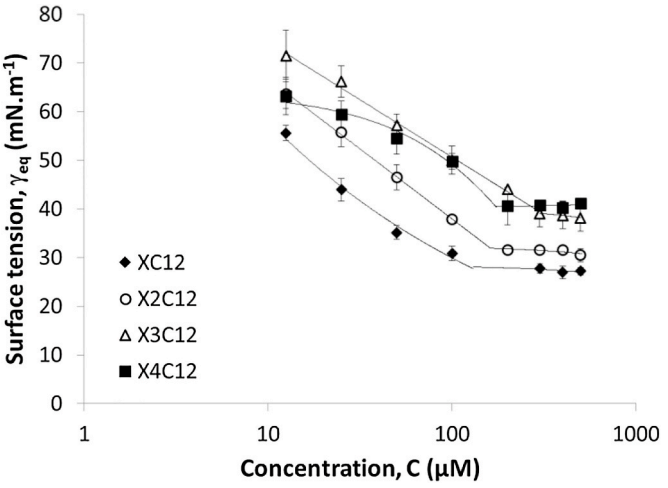



**Fig. 2.** Equilibrium surface tension ($\gamma_{eq}$) at an air/water interface as a function of the bulk concentration (C) for 5-*O*-lauryl-D-xylofuranose (XC12), 4′-*O*-lauryl-xylobiose (X2C12), 4″-*O*-lauryl-xylotriose (X3C12), 4‴-*O*-lauryl-xylotetraose (X4C12) determined at 22 °C.

**Table 3**
Interfacial parameters (critical aggregation concentration (CAC), surface pressure at the CAC ($\Pi_{CAC}$), maximum surface excess concentration ($\Gamma_{max}$) and interfacial molecular area (A)) for the purified XOs laurate esters.

| Pentose laurate esters | CAC (mg. $L^{-1}$) (µM) | $\Pi_{CAC}$ (mN. $m^{-1}$) | Maximum surface excess concentration $\Gamma_{max}$ (molecule/Å$^2$) | Interfacial area A (Å$^2$/ molecule) |
|---|---|---|---|---|
| 4′-*O*-lauryl-xylobiose **1** | 84.1 ± 0.7 (181.3 ± 1.5) | 41.9 ± 0.7 | 0.026 ± 0.003 | 38 ± 4 |
| 4″-*O*-lauryl-xylotriose **2** | 284.4 ± 3.8 (477.2 ± 6.4) | 35.9 ± 2.7 | 0.024 ± 0.001 | 42 ± 2 |
| 4‴-*O*-lauryl-xylotetraose **3** | 210.2 ± 35.8 (288.7 ± 49.1) | 31.7 ± 0.9 | 0.020 ± 0.001 | 50 ± 3 |
| 5-*O*-lauryl-D-xylofuranose | 29.4 ± 1.5 (88.6 ± 4.5) | 40.8 ± 2.3 | 0.05 ± 0.03 | 20 ± 12 |

The XOs laurate esters studied in this work reduce the surface tension of water between 30.1 and 40.3 mN $m^{-1}$. Comparatively with glucose esters with the same alkyl chain, xylose esters are a little bit more efficient even if we consider the most efficient β-anomer of glucose esters ($\gamma_{CAC}$ C12-β-D-glucoside = 33.1 mN $m^{-1}$ *vs* $\gamma_{CAC}$ 5-*O*-lauryl-D-xylofuranose = 31.2 mN $m^{-1}$ and $\gamma_{CAC}$ C12-β-D-maltoside = 33.1 mN $m^{-1}$ *vs* $\gamma_{CAC}$ 4′-*O*-lauryl-xylobiose = 30.1 mN $m^{-1}$) [53].

The formation of supramolecular aggregates like micelles depends on the equilibrium between the dispersion force and the cohesion force of the monomers in solution. The increase of xylose residues in the XOs laurate esters lessens the average hydrophobicity of the molecule. It negatively impacts the cohesion force between the monomers, and hence increases the CAC value. This relationship between the average hydrophobicity and CAC value was already observed for other oligosaccharides like oligofructose [54].

$\Pi_{CAC}$ corresponds to the ability of a surfactant to reduce the surface tension. Higher $\Pi_{CAC}$ is, higher the effectiveness of the surfactant is. From our results, we observed that a lower global hydrophobicity of the XOs laurate esters decreases their ability to reduce the surface tension. This could seem in contradiction with other studies on other sugar esters, which claimed that a higher hydrophobicity is in disfavour of surface tension reduction [55–57]. However, in those studies, sugar esters share the same sugar headgroup but vary in the chain length of the hydrophobic part. As $\Pi_{CAC}$ depends on the compactness of the molecule at the interface [54], the size of the sugar part can also influence this parameter. According to our results, we can indeed observe that a higher xylose residue number increases the interfacial molecular area and consequently reduces the concentration of the molecule at the interface ($\Gamma_{max}$ decreases), and so the effect on the surface tension.

## 3. Conclusions

In conclusion, we have developed the lipase-catalyzed synthesis of lauric esters of pure XOs from X2 to X4. This enzymatic transesterification reaction was found to be regioselective and led to the exclusive formation of a monoester featuring the acyl chain at the OH-4 position of the xylose unit at the non-reducing end. However, a decrease in reaction yields was clearly observed with the increase of the degree of polymerization of the XOs. According to their surface-active properties, XOs laurate esters studied in this work can be considered as efficient surfactants. However, an increasing number of xylose residues in the XOs laurate esters is in disfavor of the surface tension reduction and their aggregation behavior.

## 4. Experimental section

### 4.1. General information

Methylbutan-2-ol (2M2B, 99%), methanol, methylene chloride, vinyl laurate (VL, > 99%), orcinol, dimethyl sulfoxide (DMSO), activated charcoal, molecular sieves (4 Å, beads, 8–12 mesh), magnesium sulfate ($MgSO_4$), anhydrous sodium acetate ($CH_3COONa$), and the enzyme Novozym 435 (immobilized lipase on acrylic resin from *Candida antarctica*) were purchased from Sigma-Aldrich Corp. (St. Louis, USA). Acetic acid (AcOH, > 99%), ethylacetate (EA, > 99.8%), petroleum ether (PE, > 99.9%) and *n*-butanol (BuOH, > 99%) were purchased from Roth (Karlsruhe, Germany). Sulfuric acid ($H_2SO_4$, 95%) was purchased from VWR (Radnor, USA). Acetonitrile (>99.9%) was purchased from Carlo Erba Reagents (Dasit Group S.p.A, Cornaredo, Italy). Mixture of XOs DP2-DP7 was purchased from C.A.R.B. (Corvallis, Oregon, USA). Silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic acidic solution of *para*-anisaldehyde, followed by heating. Flash column chromatography was performed over silica gel Macherey-Nagel (40–63 μm) Kieselgel 60 M. NMR spectra were recorded on AC 500 (500 MHz for $^1$H, 125 MHz for $^{13}$C) spectrometer. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet.

ESI-HRMS spectra were obtained on an hybrid tandem quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass, Manchester, UK) operated in positive mode. The electrospray potential was set to 3 kV in positive ion mode (flow of injection 5 mL min$^{-1}$) and the extraction cone voltage was usually varied between 30 and 90 V.

### 4.2. Purification of xylobiose, xylotriose and xylotetraose from a mixture of XOs

The protocol was adapted from previously described procedures [58–60]. A commercially available mixture of XOs (DP 2 to 7, 20% X2, 25% X3, 22% X4, 6 g) and 5 g of sodium acetate in 50 mL of acetic acid anhydride were heated at 100 °C during 2 h. Water (300 mL) was added and peracetylated xylo-oligosaccharides were extracted with dichloromethane. The organic phase was dried over $MgSO_4$ and dichloromethane was evaporated under reduced pressure. Peracetylated xylobiose (X2), xylotriose (X3) and xylotetraose (X4) were purified using silica gel chromatography eluted using PE/EA (1:1 v/v) as eluent. Each peracetylated xylo-oligosaccharide was then deprotected under Zemplén conditions (20 mL of anhydrous MeOH/g of peracetylated XOs and catalytic amount of sodium methoxide) [61]. After neutralization with Amberlite (IR-120, $H^+$ form), MeOH was evaporated under reduced pressure. Finally, a decoloration step was carried out using 25 g/L of activated charcoal mixed in distilled water during 1 h. After centrifugation, samples were filtered (0.2 μm) and lyophilized.

In these conditions, 560 mg of X2, 456 mg of X3 et 350 mg of X4 were obtained.

### 4.3. Enzymatic transesterification of xylo-oligosaccharides

Analytical transesterification reactions (1 mL) were conducted from pure xylobiose X2, xylotriose X3 and xylotetraose X4 in screwed glass bottles with magnetic stirrer, 400 rpm, at 50 °C. Each XOs (10 mM corresponding to X2 2.82 g/L, X3 4.14 g/L, X4 5.46 g/L) was incubated with 300 mM vinyl laurate in 2M2B and 4 Å molecular sieves (5% w/v). Reaction started when the immobilized enzyme (lipase Novozym 435) was added to the mixture at 2% w/v. After 72h, reactions were centrifuged at 5500 rpm for 2 min in order to pellet molecular sieves and lipase. Supernatants were used to monitor sugar fatty esters production by TLC and HPLC. These reactions were performed in triplicates.

The effect of lipase loading was studied using pure X2 in the same reaction conditions. The influence of the solubility of XOs was studied using DMSO as a co-solvent. In this case, reactions were conducted into a 2M2B/DMSO mixture (90:10).

Higher volume syntheses (100 mL) were performed in order to produce sufficient quantities of XOs laurate esters for purification, characterization and determination of their physico-chemical properties.

### 4.4. Purification of XOs laurate esters

After removal of lipase N435, molecular sieves and insoluble XOs by centrifugation, the organic phase was washed with an equivalent volume of water to remove residual soluble XOs. Then, 2M2B was evaporated under reduced pressure and XOs laurate esters were collected simultaneously to residual vinyl laurate. XOs laurate esters were recovered by precipitation using one reaction volume of petroleum ether. The purification of xylobiose, xylotriose and xylotetraose laurate esters **1–3** respectively was performed by silica gel chromatography (9385 Merck Kieselgel 60, 230–400 mesh, 40–63 μm) using EA/methanol 9:1 as eluent.

### 4.5. 4′-O-laurylxylobiose (1)

$^1$H NMR (500 MHz, $CD_3OD$): mixture of anomers (α/β = 1/1.1), δ 5.03 (d, 0.5H, $J^{II}_{1,2}$ 3.5 Hz, H-1$^I$α), 4.72 (td,1H, $J^{III}_{4,3}$ 9.4 Hz, $J^{III}_{4,5}$ 5.3 Hz, H–4$^{II}$), 4.41 (d, 0.5H, $J^{I}_{1,2}$ 7.6 Hz, H-1$^I$β), 4.37 and 4.36 (d, 0.5H, $J^{III}_{1,2}$ 7.6 Hz, H–1$^{II}$ of each anomer), 3.96–4.02 (m, 1.5H, H–5$^{II}$ and H-5$^I$α), 3.79 (t, 0.5H, $J^{II}_{5,5} = J^{II}_{5,4}$ 10.7 Hz, H-5$^I$β), 3.74 (t, 0.5H, $J^{II}_{3,2} = J^{I}_{3,4}$ 8.9 Hz, H-3$^I$α), 3.57–3.70 (m, 2H, H-5$^I$α, H-5$^I$β, H-4$^I$α, H-4$^I$β), 3.56 (t, 1H $J^{IIII}_{3,2} = J^{IIII}_{3,4}$ 9.4 Hz, H–3$^{II}$), 3.44 (t,0.5H, $J^{II}_{3,4}$ 9.1 Hz, H-3$^I$β), 3.38 (dd, 0.5H, $J^{II}_{2,3}$ 9.1 Hz, $J^{II}_{2,1}$ 3.5 Hz, H-2α), 3.25–3.33 (m, 2.5H, H-5$^I$α, H–5$^{II}$, H–2$^{II}$), 3.16 (dd, 0.5H, $J^{II}_{2,3}$ 9.1 Hz, $J^{II}_{2,1}$ 7.6 Hz, H-2$^I$β), 2.32–2.38 (m, 2H, $CH_2$), 1.61 (qu, 2H, *J* 7.3 Hz, $CH_2$), 1.27–1.35 (m, 16H, 8 $CH_2$), 0.90 (t, 3H, *J* 7.0 Hz, $CH_3$).

$^{13}$C NMR (125 MHz, $CD_3OD$): 174.8 (C═O), 103.7 (C–1$^{II}$), 98.7 (C-1$^I$β), 93.8 (C-1$^I$α), 78.4 (C-4$^I$α), 78,1 (C-4$^I$β), 76.0 (C-3$^I$β), 75.9 (C-2$^I$β),

74.7 (C–$3^{II}$), 74.4 (C–$2^{II}$), 73.6 (C-$2^{I}\alpha$), 72.9 (C-$3^{I}\alpha$), 72.7 (C–$4^{II}$), 64.6 (C-$5^{I}\alpha$), 63.7 (C–$5^{II}$), 60.3 (C-$5^{I}\beta$), 34.9 ($CH_2$), 33.0 ($CH_2$), 30.7 (2 $CH_2$), 30.6 ($CH_2$), 30.4 ($CH_2$), 30.3 ($CH_2$), 30.1 ($CH_2$), 25.9 ($CH_2$), 23.7 ($CH_2$), 14.4 ($CH_3$). 2D experiment (HMBC): correlation between C═O and H–$4^{II}$.

ESIHRMS *m/z* 487.2516 $[M + Na]^+$ (calcd for $C_{22}H_{40}O_{10}Na$, 487.2519).

### 4.6. 4″-O-laurylxylotriose (2)

$^1$H NMR (500 MHz, $CD_3OD$): mixture of anomers (α/β = 1/1.4), δ 5.02 (d, 0.4H, $J^{I\,I}_{1,2}$ 3.5 Hz, H-$1^{I}\alpha$), 4.72 (td, 1H, *J* 9.5 Hz, *J* 5.4 Hz, H–$4^{II}$), 4.41 (d, 0.6H, $J^{I\,I}_{1,2}$ 7.6 Hz, H-$1^{I}\beta$), 4,37 (d, 1H, $J^{IIIII}_{1\,,2}$ 7.6 Hz, H–$1^{III}$), 4.33 and 4.34 (d, $J^{IIII}_{1,2}$ 7.5 Hz, H–$1^{II}$ of each anomer), 4.05 (dd, 1H, *J* 11.7 Hz, *J* 5.2 Hz, H–$5^{II}$), 3.95–4.02 (m, 1.4H, H–$5^{III}$ and H-$5^{I}\alpha$), 3.79 (t, 0.6H, *J* 10.7 Hz, H-$5^{I}\beta$), 3.10–3.75 (m, 11H, H–$4^{I}$, H–$4^{II}$, H-$5^{I}\beta$, H-$5^{I}\alpha$, H–$5^{II}$, H–$5^{III}$, H-$3^{I}\beta$, H-$3^{I}\alpha$, H–$3^{II}$, H–$3^{III}$, H-$2^{I}\alpha$, H-$2^{I}\beta$, H–$2^{II}$ and H–$2^{III}$), 2.30–2.41 (m, 2H, $CH_2$), 1.61 (qu, 2H, *J* 7.2 Hz, $CH_2$), 1.27–1.35 (m, 16H, 8 $CH_2$), 0.90 (t, 3H, *J* 7.0 Hz, $CH_3$).$^{13}$C NMR (125 MHz, $CD_3OD$): 174.8 (C═O), 103.7 (C–$1^{II}$ and C–$1^{III}$), 98.7 (C-$1^{I}\beta$), 93.8 (C-$1^{I}\alpha$), 78.6 (C-$4^{I}\alpha$), 78,2 (C-$4^{I}\alpha$), 77, 8 (C–$4^{II}$), 76.0, 75.9, 75.6, 74.7, 74.4, 74.1, 73.6, 72.9, 72.7 (C–$4^{III}$), 64.6 (C-$5^{I}\alpha$ and C–$5^{II}$), 63.7 (C–$5^{III}$), 60.3 (C-$5^{I}\beta$), 34.9 ($CH_2$), 33.0 ($CH_2$), 30.7 (2 $CH_2$), 30.6 ($CH_2$), 30.5 ($CH_2$), 30.4 ($CH_2$), 30.1 ($CH_2$), 25.9 ($CH_2$), 23.7 ($CH_2$), 14.4 ($CH_3$). 2D experiment (HMBC): correlation between C═O and H–$4^{III}$.

ESIHRMS *m/z* 619.2950 $[M + Na]^+$ (calcd for $C_{27}H_{48}O_{14}Na$, 619.2942).

### 4.7. 4‴-O-laurylxylotetraose (3)

According to the complexity of NMR data, only characteristic signals are reported.

$^1$H NMR (500 MHz, $CD_3OD$): mixture of anomers (α/β = 1/1.5), δ 5.02 (d, 0.4H, $J^{I\,I}_{1,2}$ 3.5 Hz, H-$1^{I}\alpha$), 4.72 (td, 1H, *J* 9.6 Hz, *J* 5.4 Hz, H–$4^{IV}$), 4.41 (d, 0.6H, $J^{I\,I}_{1,2}$ 7.5 Hz, H-$1^{I}\beta$), 4,30-4.38 (m, 3H, H–$1^{II}$ H–$1^{III}$, H–$1^{IV}$). $^{13}$C NMR (125 MHz, $CD_3OD$): 174.9 (C═O), 103.7 (C–$1^{II}$, C–$1^{III}$ and C–$1^{IV}$), 98.8 (C-$1^{I}\beta$), 93.8 (C-$1^{I}\alpha$).). 2D experiment (HMBC): correlation between C═O and H–$4^{IV}$.

ESIHRMS *m/z* 751.3354 $[M + Na]^+$ (calcd for $C_{32}H_{56}O_{18}Na$, 751.3364).

### 4.8. XOs laurate esters quantification by HPLC

The quantification of XOs laurate esters was performed by HPLC (Prominence, Shimadzu) using a NUCLEOSHELL® RP 18plus 5 μm, 250 × 4.6 mm (Macherey Nagel) column at 40 °C. Purified products were used as standards. XOs laurate esters were eluted at 0.8 mL $min^{-1}$, at 40 °C and with an 80% acetonitrile/20% water mobile phase. The detection was performed with a dynamic light scattering detector ELSDLTII at 40 °C under 350 kPa azote pressure. Retention times were determined for each product: XOs: 3.3 min, X2 laurate ester **1**: 4.4 min, X3 laurate ester **2**: 4.0 min, X4 laurate ester **3**: 3.9 min and lauric acid, resulting from the hydrolysis of vinyl laurate into the reaction mixture: 7.2 min.

### 4.9. Quantification of XOs solubility by HPAEC-PAD

In order to quantify the solubility of the XOs in the organic media, X2, X3 or X4 were incubated at 10 mM in 2M2B 100% or in 2M2B/DMSO 90:10 during 72 h at 50 °C under magnetic stirring. The solubilized XOs were quantified by High Performance Anion Exchange Chromatography coupled with Pulsed Amperometry Detection (HPAEC-PAD, ICS 5000, ThermoFischer, Courtaboeuf, France) after injection onto PA1 column according to a previously described method [62].

### 4.10. Determination of XOs laurate esters surface active properties

Adsorption to an air-aqueous medium interface was analyzed at room temperature with a Kibron tensiometer (trough of 1.8 cm of diameter) equipped with a Wilhelmy wire. Purified XOs esters were solubilized in 1.2 mL of Tris 10 mM pH 7.4 at different concentrations (C) between 10 μM and 1000 μM. The surface tension ($\gamma_{eq}$) was measured after 15 min when it is stable. CAC was determined from the plot $\gamma_{eq} = f(C)$ at the intersection between the regression of the ascendant and plateau parts.

The maximum surface excess concentration ($\Gamma_{max}$) and the interfacial area of the molecule (A) was determined from the Gibbs adsorption equation:

$$\Gamma_{max} = 1/RT.\ d\gamma_{eq}/d\ln C \text{ and } A = 1/(N_A\Gamma_{max})$$

with R the universal gas constant, T the absolute temperature, $\gamma_{eq}$ the equilibrium surface tension, C the bulk concentration and $N_A$ the Avogadro number.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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