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ORIGINAL PAPER

Lipase-Catalyzed Synthesis of D-Psicose Fatty Acid Diesters and their Emulsification Activities

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Abstract The diesterification of D-psicose (the C-3 epimer of D-fructose) with fatty acid vinyl esters of selected acyl chain lengths (C_8, C_{10}, and C_{12}) was successfully carried out using *Candida antarctica* lipase (Novozym 435) at 45 °C for 24 h to give the 1,6-diacyl-D-psicofuranoses with a high regioselectivity in good yields (83–90%). These diesters of D-psicose have hydrophilic-lipophilic balance (HLB) values (6.5–8.2) similar to HLB values of monoglyceride compounds which constitute the largest single type of emulsifiers employed by the food industry. Ability of the D-psicose diesters to stabilize oil-in-water emulsions and the weight-averaged oil-droplet diameter in the emulsions was evaluated in this study. Emulsion stability of oil droplets stabilized by D-psicose dicaprylate (0.3%, w/v in oil phase) was comparable to D-fructose dicaprylate (0.2%, w/v). It was further confirmed that the D-psicose diesters exhibited an emulsification activity depending on the chain length of fatty acid; D-psicose dicaprate showed better emulsion stability than the other diesters.

Keywords D-psicose · Sugar fatty acid ester · *Candida antarctica* lipase · Interfacial tension · Emulsion stability

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Introduction

Recently, Ghoul et al. reported that fructose monoesters and a blend of mono- and diesters was more efficient in stabilizing oil-in-water (O/W) emulsions than commercially available sucrose esters [1]. These fructose esters are nonionic emulsifiers used as the ingredient of low-fat spreads, sauces, ice creams, or mayonnaise [2] and have many advantages such as being non-toxic and having an excellent biodegradability. However, investigations of sugar-ester type emulsifiers containing other kinds of monosaccharides have not yet been adequately performed. Matsuo et al. [3] reported that D-psicose (the C-3 epimer of D-fructose) suppressed hepatic lipogenic enzyme activities and reduced intraabdominal fat accumulation. In addition, they described that D-psicose was a sweet monosaccharide that does not provide any energy to growing rats [4], inhibits intestinal α-glucosidase activity, and suppresses the glycemic response after sucrose and maltose ingestion [5]. Further, D-psicose added as a supplement was demonstrated to decrease plasma glucose levels and reduce body fat accumulation. Therefore, D-psicose might be useful in preventing postprandial hyperglycemia in diabetic patients [6]. Taking into account these biological activities of D-psicose, many promising applications are expected for fatty acid esters of D-psicose as functional emulsifiers in food.

We have recently succeeded in carrying out regioselective esterification of another bioactive monosaccharide D-allose (the C-3 epimer of D-glucose) [7] using a similar lipase-catalyzed transesterification of ordinary monosaccharides [8–10] and demonstrated that the growth inhibiting activity of 6-*O*-capryloyl-D-allose on lettuce seedlings was about 6-fold greater than the activity of D-allose [11]. These results suggested that the hydrophilic-lipophilic balance

(HLB) of sugar derivatives might be an important factor for controlling their bioactivities. It is further expected that esterification with fatty acids could improve or at least maintain the activity of D-psicose after hydrolysis by lipases in a cell.

In this paper, we describe enzymatic synthesis of D-psicose fatty acid diesters with selected acyl chains (C_8, C_{10}, and C_{12}) via lipase-catalyzed transesterification (see Fig. 1) and the emulsification activity of the D-psicose diesters.

Experimental Procedures

Enzymes and Chemicals

Candida antarctica lipase (Novozym 435) immobilized on a macroporous acrylic resin was purchased from Novo Nordisk (Bagsvaerd, Denmark). D-Psicose was provided by the Rare Sugar Research Center at Kagawa University. The vinyl fatty acids (caprylate, caprate, and laurate) esters, acetone, acetonitrile, tetrahydrofuran (THF), and xylene (mixture of *o, m, p* isomers) were purchased from Wako Chemical Co. (Tokyo, Japan). Commercially available corn oil of Ajinomoto Co. (Tokyo, Japan) was purchased from a local supermarket. The water was purified by passing through a Barnstead D3750 system. All other reagents were purchased from the Wako Chemical Co., Tokyo, Japan.

General Procedure for Synthesis of D-Psicose Diesters

The reaction was performed by adding Novozym 435 (80 mg) to 8 ml of a solution of the acylating agent (vinyl caprylate, vinyl caprate, and vinyl laurate, 1.332 mmol) and well- mashed D-psicose (80 mg, 0.444 mmol) in an organic solvent (acetone, CH_3CN, or THF). The reaction mixture was stirred by a magnetic bar at 350 rpm in a test tube flask (personal organic synthesizer, Chemi Station PPS-2510, EYELA, Japan) at 45 °C. Time course of lipase-catalyzed reaction was monitored by thin layer chromatography (TLC) on silica gel 60 (Merck, 0.25 mm) using a solvent mixture of ethyl acetate/hexane (4:1, v/v) as the mobile phase. The TLC plates were inspected after dipping in a molybdenum solution in ethanol followed by heating. The R_f values for the D-psicose, monoacyl derivatives, diacyl derivatives, and fatty acid vinyl esters were 0.0, 0.24, 0.79, and 0.81, respectively.

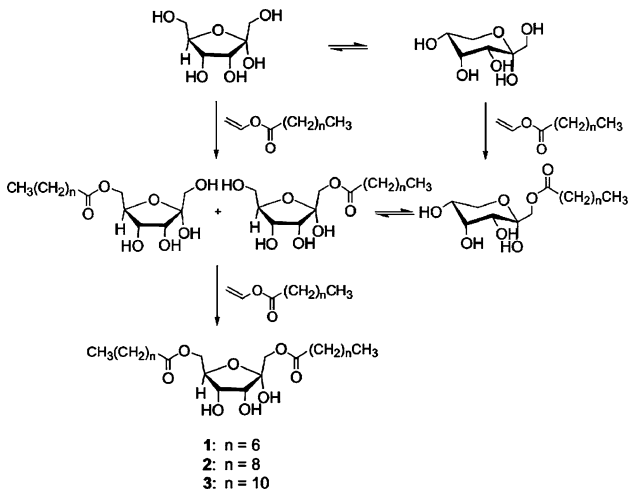

Fig. 1 Lipase-catalyzed transesterification of D-psicose with selected fatty acid vinyl esters

Purification of the Produced D-Psicose Diesters

After 24 h, the immobilized lipase was removed from the reaction mixture via filtration through a column packed with Celite. After extraction and evaporation under reduced pressure, the crude mixtures were washed several times with hexane in order to remove the excess fatty acid vinyl esters as well as triesters. Further purification of the mono- and diacyl psicoses was accomplished by column chromatography on silica gel. The diesters were eluted with a mixture of ethyl acetate and hexane (4:1, v/v) as the mobile phase and then elution of the monoesters was accomplished using a mixture of ethyl acetate and methanol (80:1, v/v).

Characterization of D-Psicose Diesters

The optical rotation values of D-psicose diesters in methanol solution were obtained using a Jasco P-1010 optical rotation polarimeter. The IR spectra were taken by a Jasco A 302 FT-IR spectrometer, using KBr disk. The mass spectra were recorded by a Jeol JMS-SX 102A mass spectrometer. The ^{1}H- and ^{13}C-NMR spectra were determined at 400 and 100 MHz, respectively, by a Jeol JNM-A400 spectrometer in CD_3OD at room temperature using tetramethylsilane (TMS) as the internal standard. The ^{13}C-NMR spectrum of the psicose monoacyl derivatives contains six sets of signals corresponding to a mixture of the α and β anomers of 1-acyl psicopyranose, 1-acyl psicofuranose, and 6-acyl psicofuranose. An additional two α anomers of 1-acyl psicopyranose and psicofuranose were observed when compared to acyl fructose [12]. The ^{13}C-NMR spectra of the psicose 1,6-diacyl derivatives contain signals corresponding to the α and β anomers in proportions of approximately 3:1, and the spectral data of the sugar region and carbonyl atom of the psicose diesters are summarized in Table 1. The carbon atoms corresponding to the acyl skeleton of the psicose dicapryloyl had the following chemical shift values at 34.9, 32.9, 30.2,

Table 1 ^{13}C-NMR chemical shift (ppm) of the three different synthesized diacyl D-psicoses

Position	α-A	β-A	α-B	β-B	α-C	β-C
C-1	66	66.9	66	66.9	66	66.9
C-2	103.2	106	103.1	106	103.1	106
C-3	72.5	76.2	72.5	76.2	72.5	76.2
C-4	72.5	73.3	72.4	73.3	72.5	73.3
C-5	81.5	81.7	81.5	81.7	81.6	81.7
C-6	65.1	65.7	65.1	65.7	65.1	65.7
C=O	175.4, 175.1	175.4, 174.7	175.1, 174.7	175.4, 174.7	175.1, 175.4	175.4, 174.7

A, 1,6-dicapryloyl-D-psicofuranose; B, 1,6-dicaproyl-D-psicofuranose; C, 1,6-dilauroyl-D-psicofuranose

Table 2 Melting points, optical rotations, MS data, and IR data for D-psicose fatty acid diesters

D-psicose fatty acid diesters	Mp (°C)	$[\alpha]_D^{25}$	EIMS (30 eV) m/z	IRν_{max} (KBr) cm^{-1}
1,6-Di-*O*-capryloyl-D-psicofuranose	58–61	+10.0	414 (M^+–H_2O)	3,421, 2,956, 1,743, 1,704, 1,197, 1,112, 964, 613
1,6-Di-*O*-caproyl-D-psicofuranose	71–74	+4.0	470 (M^+–H_2O)	3,418, 2,921, 1,745, 1,703, 1,469, 1,195, 1,114, 965, 721
1,6-Di-*O*-lauroyl-D-psicofuranose	80–82	+2.0	526 (M^+–H_2O)	3,336, 2,918, 1,725, 1,466, 1,183, 1,083, 949, 861, 720

30.1, 26.0, 23.7 ppm (−CH_2– carbon atoms), and 14.4 ppm (−CH_3 carbon atom). D-Psicose dicaprate and dilaurate had similar carbon spectra to that of dicaprylate with the exception of the carbons corresponding to the additional carbon atoms (30.4, 30.6, and 30.7 ppm). Melting points, optical rotations, MS data, and IR data for D-psicose fatty acid diesters are summarized in Table 2.

HLB of the Synthesized D-Psicose Diesters

Emulsifiers can be classified according to the HLB system which was originally devised by Griffin and can be used to predict the surface-active properties of a molecule [13]. The HLB value can be determined by the weight ratio of the hydrophilic versus lipophilic portions in the molecule using the following equation:

$$\text{HLB} = 20 \times \frac{\text{molecular mass (hydrophilic fraction)}}{\text{molecular mass (whole molecule)}}$$

The HLB values of the D-psicose diesters (caprylic, capric, and lauric) were 8.2, 7.3, and 6.5, respectively.

Interfacial Tension

Interfacial tension between pure water and xylene (or corn oil) containing the sugar diester was measured by means of drop volume method employing a computer-controlled apparatus (DVS-2000, Yamashita Giken, Tokushima, Japan) [14]. (All sugar diesters used in this study were soluble in xylene or corn oil but not in water.) The apparatus can automatically determine the oil–water interfacial tension from the maximum volume of a pendant drop detached from the glass syringe immersed in the oil.

Stability of Emulsion and Oil Droplet Size

The emulsion stability was assessed as follows. The sugar diester solution in xylene (or corn oil) was prepared and homogenized with pure water using a rotor-stator-type homogenizer (Polytron PT10-35, Kinematica, Switzerland) at room temperature for 5 min. The oil-to-water volume ratio was 3:2. To estimate the oil droplet size distribution after the homogenization, a part of the specimens was immediately diluted by water for the optical microscopy observation. The oil droplets were observed in transmitted light with a microscope (Labophot-2, Nikon, Japan) equipped with CCD camera (Moticam 2000, Shimadzu, Japan), and the microscopic images were recorded on a PC. The diameters of the droplets were measured (one by one) from the recorded images using Image J software. The weight-averaged mean diameter, d_{43} ($\Sigma n_i d_i^4/\Sigma n_i d_i^3$, where n_i is the number of particles with diameter d_i), was calculated from the size distribution histogram.

On the other hand, the emulsions without dilution was transferred to a graduated cylinder and kept in a thermostated water bath at 30 °C. The volume of separated oil was measured as a function of time and the volume percentage of the separated oil relative to the initially added oil was used as a measure of the emulsion stability. When no clear oil release was observed, the time course of light transmittance at 500 nm (T_{500}) for the turbid oil-in-water emulsion was recorded. We can use T_{500} as a

measure of the emulsion stability since T_{500} increases with time for unstable emulsions in which coalescence steadily occurs between the dispersed oil droplets to give larger droplets. A spectrophotometer (UV-1600, Shimadzu, Japan) and a 1-cm cell was employed for the transmittance measurements.

Results and Discussion

Synthesis of D-Psicose Diesters

First, we investigated the transesterification reaction of D-psicose with vinyl caprylate (3 equiv) using *Candida antarctica* lipase and an evaluation of reaction solvent revealed that tetrahydrofuran (THF) is the optimum solvent for the selective production of psicose diesters, providing the complete conversion to 1,6-di-*O*-capryl-D-psicose diester in 83% yield at 45 °C for 24 h without the monoester (Fig. 2). This solvent effect is consistent with diacetylation of D-fructose using vinyl acetate by Antona et al. [15]. This regioselective acylation at the two primary hydroxyl groups of D-psicose suggested that the produced monoesters are present in solution as a mixture of the pyranose and furanose form and the equilibrium between psicofuranose and psicopyranose monoester would be continuously shifted toward the furanose isomer until the complete conversion of D-psicose to the 1,6-diacyl psicofuranose is achieved (Fig. 1).

Furthermore, D-psicose dicaprate and dilaurate were obtained using THF at 45 °C for 48 h under similar reaction conditions in 86 and 90% yields, respectively. This increase in the yield of the psicose diesters with longer alkyl chains (C_{10} and C_{12}) is mainly due to the decreasing solubilities of the formed psicose diesters as the chain length of the acyl moiety increases.

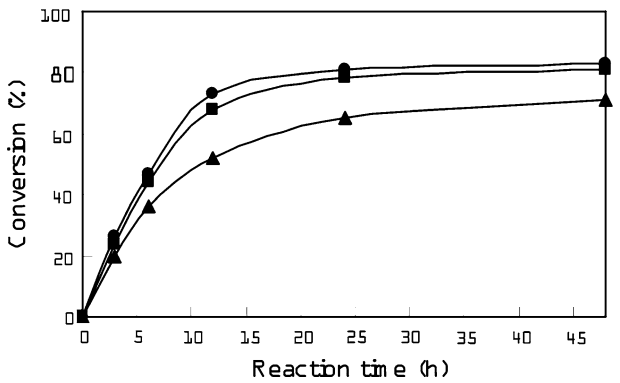

Fig. 2 Reaction progress of lipase-catalyzed transesterification of D-psicose with vinyl caprylate at 45 °C. Solvent; (*filled circles*): THF, (*filled squares*): acetonitrile, (*filled triangles*): acetone

Oil–Water Interfacial Tension

The oil–water interfacial tension for the solutions of D-fructose dicaprylate, D-psicose dicaprylate, dicaprate, and dilaurate in xylene (or corn oil) are summarized in Table 3. We can see that dissolution of sugar diesters in xylene led to a significant reduction of interfacial tension and the lowest value (8.8 mN m^{-1}) was obtained for D-fructose dicaprylate at 0.3%. No clear acyl-chain length dependence was confirmed for the interfacial tension of D-psicose diesters. When corn oil was used as the oil phase, D-fructose dicaprylate was able to reduce interfacial tension more than D-psicose dicaprylate.

Stability of the Emulsions

Oil droplet size in the emulsions prepared by mixing xylene (or corn oil) and water was measured immediately after the homogenization by optical microscopy (see Fig. 3). Figure 3a is the cumulative size distribution, *Q*, for the volume of oil particles stabilized by D-fructose dicaprylate (A) and D-psicose dicaprylate (B) at 0.3%. For both sugar diesters, smaller-sized oil droplets were obtained for corn oil than xylene. It is interesting that although the oil–water interfacial tension for D-fructose dicaprylate was lower than D-psicose dicaprylate, the former gave larger-sized droplets than the latter. These results suggest the interfacial tension did not control the droplet size for the present emulsion systems. Figure 3b shows the weight-averaged mean diameter of oil droplets stabilized by all types of the sugar diesters studied. With the increase of acyl-chain length of D-psicose diesters, the mean diameter of oil droplets became larger for both xylene and corn oil emulsions.

Table 3 Interfacial tension (γ) between water and xylene (or corn oil) dissolved D-psicose dicaprylate, dicaprate, dilaurate, or D-fructose dicaprylate at 25 °C

Oil	Emulsifier	Concentration (%, w/v)	γ (mN m^{-1})
xylene	–	0	36.9
	D-Psicose dicaprylate	0.025	21.8
		0.10	15.9
		0.30	11.0
	D-Psicose dicaprate	0.30	14.6
	D-Psicose dilaurate	0.30	12.7
	D-Fructose dicaprylate	0.025	22.2
		0.10	15.4
		0.30	8.8
corn oil	–	0	20.4
	D-Psicose dicaprylate	0.30	15.8
	D-Fructose dicaprylate	0.30	14.5

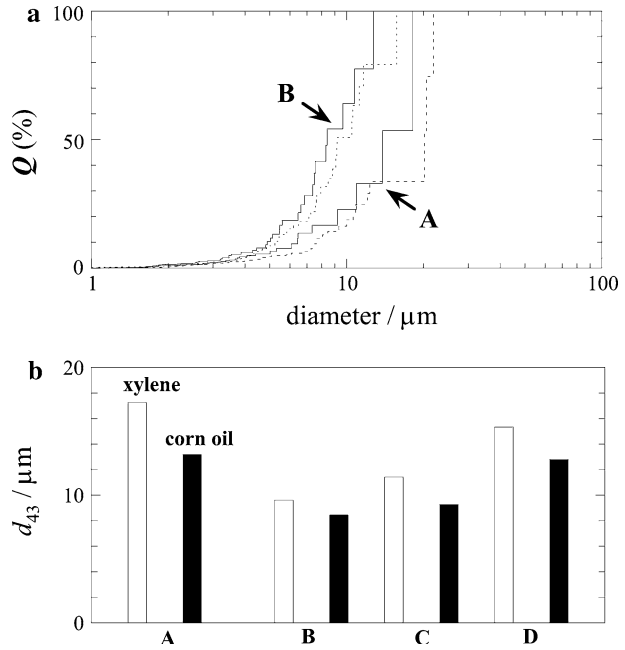

Fig. 3 Cumulative size distribution, Q, for particle volume in emulsions immediately after the homogenization. *Broken* (*solid*) *line* indicates the distribution for xylene-in-water (corn oil-in-water) emulsions stabilized by D-fructose dicaprylate and D-psicose dicaprylate. The weight-averaged mean diameter, d_{43}, for oil droplets stabilized by D-fructose dicaprylate (**a**), D-psicose dicaprylate (**b**), D-psicose dicaprate (**c**), and D-psicose laurate (**d**), respectively

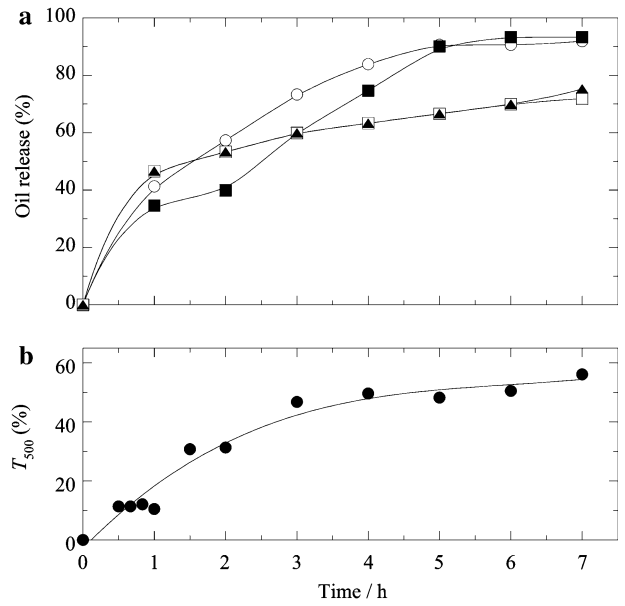

Fig. 4 Stability of xylene-in-water emulsions at 30 °C using selected concentrations of D-psicose dicaprylate. **a** Time dependence of oil release. **b** Time dependence of transmittance at 500 nm. Concentration (%, w/v); (*open circles*): 0.025, (*filled squares*): 0.05, (*open squares*): 0.1, (*filled triangles*): 0.2, (*filled circles*): 0.3

Figures 4 and 5 suggest stability of the emulsions prepared by homogenizing xylene containing D-fructose dicaprylate and D-psicose dicaprylate with pure water,

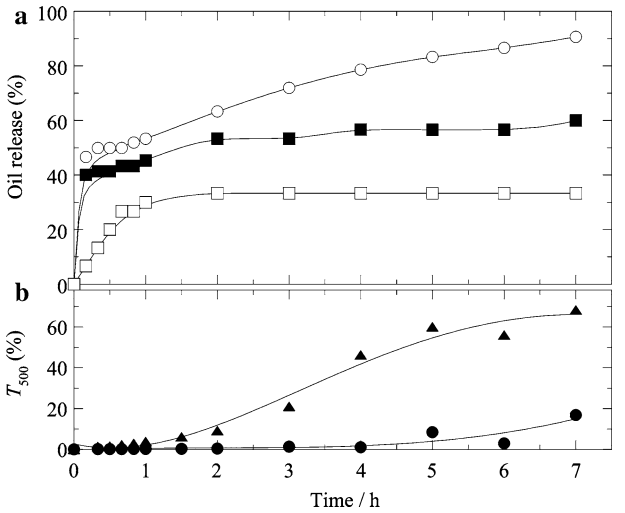

Fig. 5 Stability of xylene-in-water emulsions at 30 °C using selected concentrations of D-fructose dicaprylate. **a** Time dependence of oil release. **b** Time dependence of transmittance at 500 nm. Concentration (%, w/v); (*open circles*): 0.025, (*filled squares*): 0.05, (*open squares*): 0.1, (*filled triangles*): 0.2, (*filled circles*): 0.3

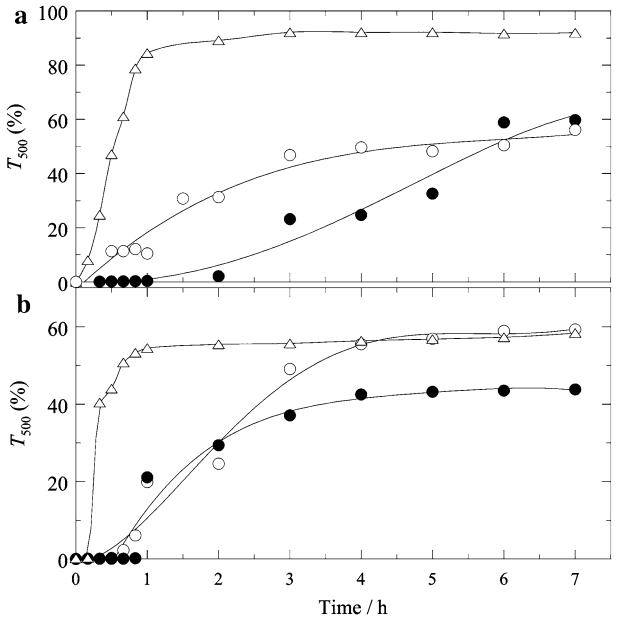

Fig. 6 Time dependence of transmittance at 500 nm for the oil-in-water emulsions stabilized by D-psicose dicaprylate (*open circles*), dicaprate (*filled circles*), and dilaurate (*open triangles*) at 30 °C. Concentration of emulsifiers was 0.3%, w/v. **a** xylene-in-water emulsion. **b** corn oil-in-water emulsion

respectively. We were able to observe clear oil release from the emulsions when the content of D-fructose dicaprylate and D-psicose dicaprylate was below 0.1 and 0.2% in xylene, respectively. By increasing the sugar diester concentration, the clear oil separation could not be detected within the measured time range and the gradual increase in T_{500} was instead measured (see Figs. 4b, 5b). Comparing

the increasing rates of T_{500} for 0.3% samples, the emulsion stability of D-fructose dicaprylate was better than D-psicose dicaprylate although the initial droplet size was confirmed to be larger (Fig. 3).

Figure 6 shows the stability of xylene (or corn oil) droplets stabilized by three different D-psicose diesters (0.3%, w/v). The most stable emulsion was the one prepared with D-psicose dicaprate for both xylene and corn oil emulsions, while D-psicose dilaurate gave a very unstable emulsion.

We established a one-step process for lipase-catalyzed diacylation of the primary hydroxyl groups (C-1 and C-6) of D-psicose without protection by using fatty acid vinyl esters with selected acyl chain lengths and the immobilized lipase from *Candida antarctica* in a highly regioselective manner with good yields (83–90%). The synthesized D-psicose dicaprate showed a good emulsification activity and, therefore, might be used as emulsifiers with biological activities in functional foods.

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