Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification

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A B S T R A C T

This article describes the synthesis of a series of oligofructose monoesters with fatty acids of different chain length (C8, C12, C16 and C18) to obtain food-grade surfactants with a range of amphiphility. Reactions were performed in a mixture of DMSO/Bu’OH (10/90 v/v) at 60 °C and catalysed by immobilised Candida antarctica lipase B. MALDI-TOF-MS analysis showed that the crude reaction products were mixtures of unmodified oligofructose and mostly mono-esters. The conversion into mono-esters increased with the length of the fatty acid chain, reflecting the specificity of the lipase towards more lipophilic substrates. Reverse phase solid phase extraction was used to factionate the products, which lead to sufficient purity (>93%) of the fatty acid esters for functional testing. It was shown that derivatives of longer (C16 and C18) fatty acids were more efficient in lowering surface tension and gave a much higher dilatational modulus than derivatives of the shorter (C8 and C12) fatty acids.

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1. Introduction

Sugar esters are food-grade non-ionic surfactants (Cao, Bornscheuer, & Schmid, 1999; Coulon, Ismail, Girardin, & Ghoul, 1998) and have many attractive properties: they are produced from renewable and largely available feedstock (Coulon, Girardin, & Ghoul, 1999) and are biodegradable, non-toxic, odourless and tasteless (Tarahomjoo & Alemzadeh, 2003). They are synthesized by esterification of a sugar with a fatty acid (Cao et al., 1999; Tarahomjoo & Alemzadeh, 2003). The synthesis of fatty acid esters of mono- and disaccharides has been thoroughly investigated (Habulin, Šabeder, & Knez, 2008; Piao, Takase, & Adachi, 2007; Šabeder, Habulin, & Knez, 2006) and lauric, palmitic and stearic esters of sucrose are manufactured and applied industrially. To the best of our knowledge oligosaccharides have been used rarely (Degn, Larsen, Duus, Petersen, & Zimmermann, 2000; Ferreira, Gil, Carvalho, Geraldes, Kim, 2002; Pérez-Victoria & Morales, 2006; Riva, Nonini, Ottolina, & Danieli, 1998; van den Broek & Boeriu, 2012). Therefore, this study will focus on oligofructose, which is derived from inulin, a readily available, water soluble, prebiotic fibre extracted from chicory roots (Boscher, Van Loo, & Franck, 2006). It is a linear polydisperse carbohydrate, which mainly consists of β-(1,2,1) fructose units and usually is terminated by an α-(2,1) glucopyranose unit. By partial hydrolysis, oligofructose is obtained (Robe rfroid, 2007).

By esterification of oligofructose with a fatty acid an amphiphilic molecule is obtained that can be used in food products. Esterification of sugars with fatty acids can be performed chemically or enzymatically (Coulon et al., 1999; Tarahomjoo & Alemzadeh, 2003). Chemical synthesis usually takes place in the presence of an alkaline catalyst at high temperatures, accompanied by discoloration and degradation of the carbohydrate moiety (Cao et al., 1999; Sarney & Vulfson, 1995). One important limitation of chemical esterification is the low regioselectivity which, due to the multiple hydroxyl groups of the substrate, leads to the formation of a complex mixture of sugar esters with different degrees of esterification at different locations on the monomer unit (i.e. C-2, C-3, C-6) and on the oligosaccharide chain (Coulon et al., 1998; Degn, Pedersen, Duus, & Zimmermann, 1999). This has an impact on the functional properties of the product. Alternatively, esterification can be performed enzymatically (Coulon, Ismail, Girardin, Rovel, & Ghoul, 1996; Sarney & Vulfson, 1995). Enzymatic catalysis has the advantage of the high regio- and enantioselectivity, which leads to mono-esters as predominant reaction products (Coulon et al., 1996, 1998). In addition, enzymatic reactions can be performed at moderate temperatures, usually below 80 °C (Soultani, Engasser, & Ghoul, 2001; Tarahomjoo & Alemzadeh, 2003).
2. Experimental

2.1. Materials

Oligofructose (Orafti P95) was obtained from Beneo-Orafti (Tienen, Belgium) and had a DP that ranged between 2 and 8. It was used without further modification. Immobilised lipase from C. antarctica (Novozym 435) was received from Novozymes (Bagsvard, Denmark). The enzyme activity, determined in a synthetic reaction between butyric acid and BuOH, was 0.0679U (expressed as µmol butyl butyrate formed per milligramme of enzyme per minute).

Dimethylsulfoxide (>99%) (DMSO), butyl butyrate, and 2,5-dihydroxybenzoic acid (>99.5%) (DHB) were obtained from Fluka (Buchs, Switzerland). Deuterium oxide (D2O), deuterated chloroform (CDCl3) and caprylic acid were obtained from Acros (Geel, Belgium). Lauric acid, palmitic acid, stearic acid, acetone, toluene, n-hexadecane and n-hexane were obtained from Merck (Darmstadt, Germany). Molecular sieves (4Å, 8–12 mesh), 1-butanol, butyric acid and BuOH were obtained from Sigma (Zwijndrecht, The Netherlands). Methanol and acetonitrile were obtained from Biosolve ( Valkenswaard, the Netherlands). D2O–DMSO was obtained from Cambridge Isotope Laboratories (Andover, USA). Water was purified using a Purelab Ultra system (Elga Labwater, Edé, The Netherlands).

2.2. Methods

2.2.1. Lipase activity

Esterification activity: The esterification activity was determined for the reaction between butyric acid and butanol in 5 mL of a mixture of toluene and BuOH (70/30 v/v) containing 0.73 mmol butyric acid, 1.54 mmol butanol, 0.034 mmol n-hexadecane as internal standard and 0.5 g of molecular sieves (Habeych, Juhl, Pleiss, Vanegas, & Eggink, 2011). The reaction was initiated by the addition of 5 mg of lipase and the reaction mixture was incubated for 30 min at 40 °C while stirring at 200 rpm. The reaction was terminated by separating the enzyme and molecular sieves by filtration through a Whatman Spartan 13/0.45SR filter. The amount of butyl butyrate formed was determined using gas chromatography (Focus GC with AS3000 autosampler, Interscience, Breda, Netherlands). The system was calibrated using butyl butyrate. The gas chromatograph was equipped with a Flame ionisation Detector and a Restek Rxi-5 ms column with dimensions of 30 m × 0.25 mm × 0.25 µm. The carrier gas was helium and the make-up gas was nitrogen. Conditions: injection volume was 1 µL; oven temperature was 50 °C for 2 min and was then raised to 300 °C at a rate of 20 °C/minute and stayed at 300 °C for 2 min; software was Chrom-Card v2.4.1.

Hydrolytic activity: The hydrolytic activity was determined using a pH-stat device (Metrohm) with triolein as substrate at 40 °C and pH 7.5, according to the method of Hoppe and Theimer (Hoppe & Theimer, 1996). Liberated fatty acids were titrated automatically with 0.1 M NaOH to maintain a constant pH. One unit (U) of lipase activity was defined as the amount of lipase that liberates 1 µmol fatty acids per minute.

Enzyme stability: 50 mg of Novozym 435 was suspended in 5 mL of a mixture of DMSO/BuOH (10/90, v/v) and kept at 60 °C with magnetic stirring at 200 rpm. After 1, 2, 3, 4, 5, 6, 16, 24, 40, 48 and 70 h hours the enzyme was removed from the solvent by filtration and analysed to determine the residual activity.

2.2.2. Synthesis of caprylic acid, lauric acid, palmitic acid and stearic acid esters of oligofructose by esterification

Oligofructose was dried in a vacuum oven (Gallenkamp, Loughborough, UK) at 50 °C for 48–72 h until the water content was reduced to less than 1%. The water content of the oligofructose was determined with the Mettler Toledo DL-39 Karl Fischer Coulometer linked to a Mettler Toledo Stromboli sample oven and sample changer (Columbus, USA). The determination was performed at a temperature of 125 °C. Oligofructose (final concentration 1% (w/v)) was dissolved in dimethylsulfoxide (DMSO). Caprylic acid, lauric acid, vinyl laurate, palmitic acid or stearic acid (molar ratio fatty acid:oligofructose 3:1, calculated based on the average molecular weight of the oligofructose) was dissolved in warm (~50 °C) BuOH. The solutions were slowly mixed until all material was dissolved while stirring at 200 rpm at a temperature of 60 °C. The final composition was DMSO/BuOH (10/90 v/v). Molecular sieves (3% (w/v)) and lipase (68 U/g oligofructose, 1% (w/v)) were added to start the reaction. The reaction mixture was incubated for 69 h at 60 °C while stirring at 200 rpm using overhead mechanical stirring. Samples were taken after 0.5, 1, 2, 3, 19, 27, 43 and 69 h. After 19 and 43 h another portion of lipase was added (2 × 68 U/g oligofructose). After 69 h the reaction was stopped by decanting to separate the molecular sieves and the lipase from the solvent. The solvents were evaporated using rotary vacuum evaporation. The product was washed twice with 50 mL of acetone and one time with 50 mL of n-hexane.

2.2.3. Purification

For reverse phase solid phase extraction Vac 35 cc C18 10 g cartridges (Waters, Milford, USA) with a loading volume of 16 mL were used. The columns were activated by washing them with 45 mL of methanol followed by 45 mL of water. 16 mL of a suspension of esters in water (14 mg/mL) was loaded onto the column. Water/methanol mixtures were used to elute the components of interest, starting with 100% water and ending with 100% methanol, using 10% increments. The last step (100% methanol) was per-
formed twice. 45 mL was used for each step. The fractions were eluted with the help of a vacuum pump. After purification, the fractions were analysed using MALDI-TOF MS, after which similar fractions were added together. Finally, the solvent was evaporated using rotary vacuum evaporation.

2.2.4. MALDI-TOF MS

Mass spectrometry of the products was performed according to a previously described protocol (Ter Haar et al., 2010) on an Ultraflex MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany), using DHB as a matrix.

From the spectra the relative abundance of the different components was calculated. To that end, the intensities of all identifiable peaks of both oligofructose and ester products (sodium and potassium adducts) were added and set to 100%. The relative contribution of each component was determined from the sum of the sodium and potassium adducts. A similar procedure has been used before for the same type of molecules (Ter Haar et al., 2010).

2.2.5. Nuclear magnetic resonance (NMR) analysis

For 1H NMR analysis caprylic acid mono-esters and lauric acid mono-esters were dissolved in D2O, palmitic acid mono-esters were dissolved in D6-DMSO and stearic acid mono-esters were dissolved in CDCl3. 100 mg of sample was dissolved in 1 mL of solvent.

1H NMR spectra were recorded on a Bruker Avance III 400 spectrometer operating at 400.17 MHz.

C8 mono-ester (D2O, δ): 0.8 (t, 3H, CH3), 1.3 (m, 8H, (CH2)4), 1.6 (m, 2H, CH2CH2CO), 2.4 (t, 2H, CH2CO), 3.3–5.4 (m, CH, CH2 and OH in oligofructose).
C12 mono-ester (D2O, δ): 0.9 (t, 3H, CH3), 1.3 (m, 16H, (CH2)14), 1.6 (m, 2H, CH2CH2CO), 2.4 (t, 2H, CH2CO), 3.5–5.4 (m, CH, CH2 and OH in oligofructose).
C16 mono-ester (DMSO-d6, δ): 0.8 (t, 3H, CH3), 1.2 (m, 24H, (CH2)12), 1.5 (m, 2H, CH2CH2CO), 2.5 (t, 2H, -CH2CO), 3.2–5.2 (m, CH, CH2 and OH in oligofructose).
C18 mono-ester (CDCl3, δ): 0.9 (t, 3H, CH3), 1.3 (m, 28H, (CH2)14), 1.6 (m, 2H, CH2CH2CO), 2.3 (t, 2H, CH2CO), 3.6–5.4 (m, CH, CH2 and OH in oligofructose).

For the 2D NMR analysis, 40 mg of lauric acid mono-esters was dissolved in 0.5 mL D6-DMSO. NMR spectra were recorded at a probe temperature of 297 K on a Bruker Avance-III-500 spectrometer located at the Wageningen NMR Centre. The 1H signal at 2.5 ppm and 13C signal at 39.52 ppm of D6-DMSO were used as internal references. The spectra were acquired using standard pulse sequences delivered by Bruker. For the 1H, 13C)-HMBC 800 spectra of 32 scans were recorded, resulting in a measuring time of 12.5 h. For the 1H, 13C)-HMQC 512 spectra of 16 scans were recorded, resulting in a measuring time of 4 h.

2.2.6. Determination of surface tension and surface dilatational modulus

Purified mono-ester fractions of caprylic, lauric, palmitic and stearic acid were dissolved in water at a concentration of 0.01% (w/v). The surface tension and surface dilatational modulus were monitored as a function of time using an automated drop tensiometer (ADT, ITCONCEPT, Longessaigne, France). Cycles of sinusoidal moduli were alternated with five blank cycles. The drop area was monitored as a function of time using an automated drop tensiometer (ADT, ITCONCEPT, Longessaigne, France). Cycles of sinusoidal moduli were alternated with five blank cycles. The drop area was monitored as a function of time using an automated drop tensiometer (ADT, ITCONCEPT, Longessaigne, France). Cycles of sinusoidal moduli were alternated with five blank cycles. The drop area was monitored as a function of time using an automated drop tensiometer (ADT, ITCONCEPT, Longessaigne, France).

The temperature was 25 °C. Measurements were performed at least in duplicate. If reproducibility was insufficient, measurements were performed in triplicate. Reported values for surface tension and surface dilatational modulus are equilibrium values.

3. Results and discussion

3.1. Establishment of reaction conditions

3.1.1. Esterification vs. transesterification

Elaborating on the work of Sagis et al. (2008), a fatty acid was linked to oligofructose by an ester bond to create an amphiphilic molecule that can be used as a food-grade surfactant. Because of the increased specificity and the milder reaction conditions, enzymatic procedures were preferred over chemical procedures. Therefore, lipase-catalysed esterification and transesterification reactions were used to obtain the oligofructose fatty acid ester. Both reactions are favoured at low water content to prevent the hydrolysis of the newly formed esters. During esterification, water is formed as the second product in addition to the ester. Therefore, molecular sieves could be added to this system to control the water content. Since the oligofructose and all solvents are dried before the reaction, the addition of molecular sieves should not be necessary during the transesterification reaction. They could, however, remove some of the water that remained after drying or absorb water that enters the system through the atmosphere. Therefore, three different reaction procedures were tested; transesterification of oligofructose with vinyl laurate (both in the absence and in the presence of molecular sieves) as well as an esterification reaction of oligofructose with lauric acid (in the presence of molecular sieves).

Table 1 shows the relative abundance of mono-esters formed by time interval, as estimated based on the intensity of the peaks in the MALDI-TOF mass spectrum. The relative abundance of monooesters (defined as one fatty acid esterified with one oligofructose molecule) at the end of the reaction time was about 30–40% for all procedures (Table 1). These results are comparable to previously reported data on the transesterification reaction between oligofructose and vinyl laurate (Ter Haar et al., 2010) despite the difference in DMSO concentration between the two studies (20% DMSO compared to 10% DMSO in this research). Although a decrease in the DMSO level reduces the solubility of the oligofructose, it will increase the enzyme activity. Apparently the combination of the two effects leads to a similar yield.

There were no major differences between the three reaction procedures with regard to the amount of product formed. Generally, transesterification reactions with, for instance, vinyl esters of fatty acids as acyl donors are used for enzymatic ester synthesis since they lead to higher conversions (Ter Haar et al., 2010). However, in this study esterification was considered as well for a few reasons. During transesterification with vinyl esters of fatty acids, acetaldehyde is formed which can inactivate the enzyme. Furthermore, vinyl esters of fatty acids are not commercially available (at least not for all fatty acid chain lengths) while free fatty acids are commercially available. Finally, in a food-grade reaction procedure it is more desirable to use the more natural fatty acids instead of vinyl esters. Therefore, it was decided that further reactions would be esterification reactions instead of transesterification reactions.

Table 1

<table>
<thead>
<tr>
<th>Reaction procedure</th>
<th>Molecular sieves present?</th>
<th>Relative abundance of mono-ester (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Esterification</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Transesterification</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Transesterification</td>
<td>No</td>
<td>0</td>
</tr>
</tbody>
</table>
3.1.2. Enzyme activity

To investigate whether the catalytic activity and stability of the immobilised lipase is affected under the conditions of the reaction, the enzyme was incubated at 60°C in DMSO/BuOH (10/90; v/v) for 72 h, with magnetic stirring. At different time intervals the residual enzyme activity was determined in (a) aqueous conditions, to compensate for reversible inactivation due to dehydration, if any, and (b) in an almost dry solvent mixture. The enzyme activity as a function of time is available in Supplementary data 1. The activity of the rehydrated enzyme (i.e., hydrolytic activity, in emulsion) decreased to about 50% of the initial activity at incubation times longer than 24 h in DMSO/BuOH (10/90; v/v). Since the enzyme was rehydrated in a medium of very high water activity, reversible inactivation due to dehydration is not an issue and the irreversible inactivation observed can be assigned to effects of temperature and abrasion due to mechanical shear. When the residual esterification activity was measured in a medium of very high water activity, reversible inactivation due to dehydration is not an issue and the irreversible inactivation observed can be assigned to effects of temperature and abrasion due to mechanical shear. When the residual esterification activity was measured in an anhydrous organic medium without rehydration the enzyme retained only 17% of its initial activity after 24 h incubation, with a half-life time of about 13 h. This additional inactivation can be ascribed to the reversible dehydration by polar organic solvents which can strip the essential water molecules from the enzyme (Zaks & Klibanov, 1988). The inactivation of immobilised lipase B from Candida antarctica by dehydration in polar organic solvents and by mechanical stirring has been reported earlier and many studies address solutions to diminishing these effects (Chen, Hu, Miller, Xie, & Cai, 2008; Li, Zong, Wu, & Lou, 2006; Ter Haar et al., 2010; Widjaja, Yeh, & Ju, 2008). Ter Haar et al. (2010) have shown the inactivating effect of DMSO on Novozym 435 in both the esterification and trans-esterification reaction and succeeded in minimizing the loss of activity by minimizing the concentration of DMSO to 20% (v/v) in a binary mixture with tert-butanol as cosolvent. Other approaches were to replace the polar reaction medium with a hydrophobic solvent, such as hexane or toluene (Widjaja et al., 2008), or with a two-phase solvent system consisting of a polar solvent, i.e., DMSO, dimethylformamide, or pyridine, and a hydrophobic solvent, such as hexane and diisopropyl ether (Li et al., 2006). Generally, reducing the speed of agitation was used to prevent mechanical degradation and leakage of the enzyme adsorbed on the solid support due to shear.

In this study, to compensate for the loss of activity of the biocatalyst and to enhance the product yield, in further batch experiments the addition of enzyme after 19 h and 43 h was incorporated into the synthesis protocol. This approach was suitable for the production of preparative amounts of oligofructose monoesters at a laboratory scale, but is less appropriate for industrial application due to the impact of the enzyme costs on process economics. This limitation could be overcome by developing a continuous process for the enzymatic esterification of oligofructose using a fixed-bed reactor with immobilised enzyme and a rehydration step between the cycles (personal communication).
3.2. Synthesis of caprylic acid, lauric acid, palmitic acid and stearic acid esters of oligofructose by esterification

To produce oligofructose fatty acid esters with differences in hydrophobicity, the length of the fatty acid was varied. Caprylic, lauric, palmitic and stearic acid were esterified with oligofructose to obtain amphiphilic molecules.

The oligofructose that was used as substrate is a mixture of oligomers with degrees of polymerization (DP) between 2 and 8, and an average DP of 4.4 (Fig. 1A). The esterification with fatty acids yielded a mixture of unmodified oligofructose and predominantly mono-esters (illustrated for caprylic acid esters in Fig. 1B).

The formation of products as a function of time was studied and the relative abundance of mono-esters was estimated based on the intensity of the peaks in the mass spectrum (Fig. 2).

After 69 h, the relative abundance of unreacted oligofructose was 87% for caprylic acid, 64% for lauric acid, 48% for palmitic acid and 67% for stearic acid. The reaction product consisted of mono- and di-esters, although mono-esters were the predominant reaction products. The amount of di-esters was below the detection limit for caprylic acid esters, 2.1% for lauric acid esters, 3.8% for palmitic acid esters and 1.2% for stearic acid esters (data not shown). The specificity of lipase is responsible for the fact that mono-esters were the predominant reaction product (Woudenberg-Van Oosterom, Van Rantwijk, & Sheldon, 1996). A significant increase in oligofructose conversion with the increase of fatty acid chain length was observed, with the highest conversion being for the C16 acid. This is consistent with earlier reports of C. antarctica lipase specificity towards more lipophilic substrates (Cao, Fischer, Bornscheuer, & Schmid, 1997; Soultani et al., 2001).

The results in Fig. 3 clearly show that both the length of the alkyl chain of the fatty acid and the degree of polymerization (DP) of the individual oligomers in the oligofructose substrate are relevant factors influencing the type of products formed (Fig. 3B). For all oligomers, increased fatty acid chain length led to an increase in mono-ester formation. Furthermore, when comparing the distribution of the different oligomers in the unmodified oligofructose (Fig. 3A) to that in the mono-esters (Fig. 3B), the oligomers with DP 3 and DP 4 seemed to be better substrates for the enzyme than the oligomers with a higher DP, although for smaller fatty acids DP5 also seemed to be a good substrate. Finally, di-esters were only formed for oligomers with a lower DP (3 and 4, results not shown). These effects could be related to the anatomy of the catalytic cleft of the lipase. It is an elliptical, steep funnel with dimensions of 9.5 × 4.5 Å (Pleiss, Fischer, & Schmid, 1998). When larger molecules attempt to access the catalytic cleft, they may be hindered due to steric effects. This could explain why mono-esters with a higher DP of the oligofructose moiety are formed for shorter fatty acids and why di-esters are only formed for oligofructose moieties with a lower DP (Cramer, Dueholm, Nielsen, Pedersen, & Wimmer, 2007; Pedersen, Wimmer, Emmersen, Degn, & Pedersen, 2002).

3.3. Isolation and purification of mono- and di-esters of oligofructose

After removing molecular sieves and lipase, evaporation of the solvents and washing with acetone and hexane, the products were fractionated using reverse phase solid phase extraction (RP-SPE). RP-SPE is an effective purification method that: (1) generates large amounts of materials in a relatively short time, (2) gives a sufficient purity for functionality experiments, and (3) requires only minor modifications when switching between products derived from fatty acids with different chain lengths.

Unmodified oligofructose was eluted from the column during the first two steps: 100% water and 10% methanol (Fig. 4). Mono-esters of caprylic acid eluted with 50% and 60% methanol, mono-esters of lauric acid with 70% and 80% methanol, mono-esters of palmitic acid with 90% methanol and mono-esters of stearic acid with 90% and 100% methanol. Di- and tri-esters of caprylic acid eluted with 80% and 90% methanol, di- and tri-esters of lauric acid with 90% methanol, di-esters of palmitic acid with 100% methanol and di-esters of stearic acid only during the second washing step with 100% methanol. The difference in hydrophobicity between the different fractions is reflected in the polarity of the eluting solvent.

Purifications have also been performed using flash chromatography which fractionates the material using the same column material in a larger column and a linear gradient of water and methanol (details about methods are available in supporting information 2). Similar results were obtained and are therefore not shown.
3.4. Characterization of purified fractions

Fractionation of the products of each reaction by SPE allowed the separation of two purified fractions; one enriched in monoester (entries 1–4 in Table 2) and one containing di- and tri-esters (not shown). Di-esters of palmitic acid could not be isolated in sufficient quantities.

MALDI-TOF-MS analysis showed that the products obtained have sufficient purity for functionality testing (Table 2). The mono-ester fractions (especially caprylic and lauric acid derivatives) were highly pure. The mono-esters were mixtures of DP2 to DP8 oligomers, with predominately DP3, DP4 and DP5. Surprisingly, low amounts of di- and tri-esters could be isolated, although they were not identified in all crude reaction mixtures using MALDI-TOF-MS analysis, probably because their concentration was below the detection limit.

$^1$H NMR spectra of the monoesters of caprylic acid, lauric acid, palmitic acid and stearic acid with oligofructose confirmed the presence of the fatty acid ester group, as illustrated for the oligofructose C12-monoester, ($\delta$ ppm, D$_2$O): 0.8–0.9 (br t, 3H, CH$_3$; $\delta$1.1–1.3 (m, 16H, (CH$_2$)$_8$); $\delta$1.4–1.6 (m, 2H, CH$_2$CH$_2$CO); $\delta$2.2–2.4 (br t, 2H, CH$_3$CO); $\delta$3.3–5.5 (multiple coupling, CH, CH$_2$ and OH of the oligofructose unit).

2D NMR analysis was performed for lauric acid mono-esters to confirm further the structure of the molecule. In the HMBC spectrum, 3-bond couplings between the C=O of the fatty acid and protons of the oligofructose could be identified, indicating the positions where fatty acids are attached to the oligofructose chain. This attachment appeared to be heterogeneous, since multiple overlapping signals for sugar protons coupled with C=O were found. In the HMQC spectrum, the single-bond couplings between these proton signals and their adjacent carbon atoms could be identified. From the chemical shifts below 67 ppm of these carbon atoms, it is clear that the fatty acids are mainly attached at fructosyl C-1 and/or C-6 positions of fructose and glucose, showing the preference of the enzyme for primary hydroxyl groups. All NMR spectra are available in Supplementary data 3.

Apart from the C-1 hydroxyl group of the terminal fructosyl residue, all other C-1 hydroxyls are involved in the glycosidic links. Thus, the position of the esterified fatty acid is expected to be restricted to the C-6 position and the terminal fructosyl C-1.

Although 2D NMR analysis could prove the location of the esterified fatty acid on a single monosaccharide unit, it could not conclusively identify the position of the fatty acid on the oligofructose chain. However, the location of the fatty acids is most probably at the primary OH groups of either terminal glucose or terminal

### Table 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Fraction</th>
<th>Composition (%)</th>
<th>Unmodified oligofructose</th>
<th>Mono-ester</th>
<th>Di-ester</th>
<th>Tri-ester</th>
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<tbody>
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<td>1</td>
<td>Caprylic acid mono-esters</td>
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<td>99.6</td>
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<tr>
<td>2</td>
<td>Lauric acid mono-esters</td>
<td>2.7</td>
<td>97.3</td>
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<td>3</td>
<td>Palmitic acid mono-esters</td>
<td>6.1</td>
<td>93.9</td>
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<td>nd</td>
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<tr>
<td>4</td>
<td>Stearic acid mono-esters</td>
<td>6.1</td>
<td>93.9</td>
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</tr>
</tbody>
</table>
fructose moieties of the oligosaccharides due to geometrical constraints in the catalytic cleft of the lipase. Therefore, the reaction can be summarized according to the reaction scheme as presented in Scheme 1.

3.5. Functionality

As a first step in the functional characterization, the surface tension and surface dilatational modulus of air/water interfaces stabilized by purified mono-esters were determined (Table 3). There is a clear dependence of these properties on the length of the fatty acid that is esterified with the oligofructose. In general, when the fatty acid chain length increases, the surface tension decreases. The efficiency of a surfactant is inversely proportional to the amount of surfactant that is necessary to achieve a certain surface tension reduction. Therefore, derivatives with a longer fatty acid chain are more efficient in reducing surface tension. Since the critical micelle concentrations of the derivatives are not yet known, no statements about the effectiveness (the maximum surface tension reduction) can yet be made.

The palmitic and stearic acid derivatives have a significantly higher dilatational modulus compared to that of the shorter chain derivatives. The reproducibility of the measurements, as reflected in the standard deviations of the modulus, was poor. The reason for the poor reproducibility is that the measurements are outside of the linear regime, where the stress is no longer proportional to the strain. This means that the structure at the interface, which is responsible for the high modulus, is affected by the deformation that is imposed. Unfortunately, with the currently available equipment, it is not possible to take measurements within the linear regime. This topic will be further explored in a forthcoming paper. However, despite the large variation in the reported values, a strong dependence of the modulus on the length of the fatty acid chain was found. Air/water interfaces stabilized by proteins generally have a surface tension of 47–57 mN/m and a surface dilatational modulus ranging between 20–80 mN/m, while interfaces stabilized by LMW surfactants generally have a surface tension of 22–42 mN/m and a negligible surface dilatational modulus (Bos & Van Vliet, 2001). For example, an air/water interface stabilized by sucrose monolaurate had a surface tension of 38 mN/m and a surface dilatational modulus of 1 mN/m (Sagis et al., 2008). This shows that because of the low surface tension combined with a high dilatational modulus, especially oligofructose esters of palmitic and stearic acid perform well compared to commonly used food surfactants. A high dilatational modulus is often linked to high foam stability (Sagis et al., 2008). Commercially available sucrose esters of longer fatty acids are also capable of lowering the surface tension considerably and of creating an interface with a high dilatational modulus. However, they are often produced by chemical methods and therefore are usually mixtures of esters with a wide range of degrees of substitution and even different fatty acid chain lengths. In contrast, as a result of enzymatic synthesis, the oligofructose fatty acid esters that were created in this study are mono-esters with a single fatty acid chain length. Furthermore, because of the larger hydrophilic head, the solubility in water is increased.

These results imply that oligofructose fatty acid esters with longer fatty acid chains could be efficient surfactants for use in foods. Experimental work is in progress at the moment focusing on elucidating structure–function relations and on the emulsifying and foaming capabilities of the different reaction products.
4. Conclusion

Enzymatic esterification of oligofructose with fatty acids of different chain lengths yielded a mixture of unmodified oligofructose, mostly mono-esters and small amounts of di- and tri-esters. Fractionation using reverse phase solid phase extraction led to a sufficient degree of purification (>93%) of most products. By combining MALDI-TOF, 1H NMR and 2D NMR techniques, the products were identified as monosubstituted oligofructose fatty acid esters. These results show that using relatively simple methods with only minor modifications when changing between the different fatty acids, it is possible to synthesize esters of oligofructose and a range of fatty acids. Initial functional experiments showed that the derivatives of longer (palmitic and stearic) fatty acids had a low surface tension and a high surface dilatational modulus, which makes them promising novel food-grade surfactants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.09.133.

References