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Enzymatic modification and characterization of xylo-oligosaccharide esters as potential emulsifiers

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Abstract

Hydrophobically modified xylo-oligosaccharides were prepared by enzymatic esterification of xylo-oligosaccharide and three fatty acids: decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16). Lipase obtained from Thermomyces lanuginosus was used as a biocatalyst in the reaction. The degree of substitution (DS) of esterified xylo-oligosaccharide, prepared at a reaction temperature of 60°C for 4 h, ranged between 0.042 and 0.066. Esterified xylo-oligosaccharides were investigated for physicochemical properties. The esterified xylo-oligosaccharides reduced the interfacial tension and exhibited emulsifying properties. Esterified samples did not completely dissolve in water, showed higher viscosity, and changed thermal properties compared with native samples. At a concentration of 25% (w/w), esterified xylo-oligosaccharides exhibited Newtonian flow behavior similar to that of native one, except for palmitate derivatives which exhibited shear-thinning behavior. Esterified xylo-oligosaccharides were then used as emulsifiers to make *n*-hexadecane O/W emulsions. The emulsions were characterized according to their oil droplet characteristics, emulsification index, and centrifugal force-induced oil separation. Xylo laurate has potential in emulsifying activity and in preserving the stability of emulsions after centrifugal acceleration.

Introduction

Oligosaccharides are widely used in industry due to their non-toxicity and low price. They are added as ingredients in food products because of their ability to modify the functional properties of food systems (Singthong *et al.*, 2009). Most oligosaccharides are strongly hydrophilic and hence are not surface-active in emulsions. Due to the absence of lipophilic groups, they are unsuitable for emulsion systems. With the development of food science and technology, attempts are being made to synthesize amphiphilic oligosaccharides. The introduction of an ester group into an oligosaccharide constitutes an important achievement because the ester group modifies the oligosaccharide's original hydrophilic nature, obtaining an amphiphilic oligosaccharide. Amphiphilic oligosaccharides have hydrophilic and hydrophobic subregions; therefore, they can act like low-molecular-weight surfactants and may exhibit good stabilizing ability, probably due to steric stabilization with respect to their macromolecular structure (Sadtler *et al*., 2002). Enzymatic processes offer an attractive alternative route for the synthesis of poly- and oligosaccharide esters, thereby avoiding polymer degradation (van den Broek and Boeriu, 2013), reducing the use of toxic reactants, and remaining within the limits of acceptability for health (Alissandratos *et al*., 2010). Xylo-oligosaccharides presents advantages compared with other oligosaccharides in terms of healthy effects as they are prebiotic and contain low-calories, allowing their utilization in anti-obesity diets (Vázquez *et al.*, 2000). A xylo-oligosaccharide has a backbone of β-(1,4)-D-xylopyranosyl residues which are substituted in the C-2 and/or C-3 position by acyl groups. Researchers are focused on producing high DS value amphiphilic polysaccharides by chemical esterification for thermoplastic film production (Aburto *et al*., 1999; Shogren and Biswas, 2010; Fundador *et al*., 2012). These modified polysaccharides cannot be used in O/W emulsions as emulsifier because of their poor water solubility. To date, there has been little research on the enzymatic esterification of xylo-oligosaccharides. Thus, the present work attempted to synthesize amphiphilic oligosaccharide by enzymatic esterification to be used as a potential emulsifier in O/W emulsion.

Coalescence and oil separation indicate the stability of an emulsion during storage. For this

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study, centrifugal agitation was used to accelerate the coalescence and oil separation of esterified xylo-oligosaccharide-stabilized emulsions. Centrifugal force induces deformation of the oil droplet interface by frictional force and by local stretching of the film between the droplets (Hartland *et al*., 1994). Centrifugal force-induced coalescence/ oil separation may be important for the stability of high concentration biopolymer-stabilized emulsions in applications. These emulsions are often stable at rest but unstable during processing, transport, and application (van Aken and Zoet, 2000).

The purposes of the present study were: (a) to study the physicochemical properties of esterified xylo-oligosaccharides; (b) to study the emulsifying activities of esterified xylo-oligosaccharides in *n*-hexadecane O/W emulsions; and (c) to investigate the centrifugation-induced oil separation of esterified xylo-oligosaccharide-stabilized *n*-hexadecane O/W emulsions.

Materials and Methods

Materials

Xylo-oligosaccharide, extracted from corn, was supplied by San-Ei Gen F.F.I. (Osaka, Japan). The range of degree polymerization (DP) of xylo-oligosaccharide is 2 to 7. Lipase from Thermomyces lanuginosus solution, containing 2% (w/v) lipase, was purchased from Sigma-Aldrich (Buchs, Switzerland). The enzyme activity was about 100,000 U/g; the 1 g of enzyme hydrolyzes tributyrin and releases 100,000 µM of titratable butyric acid per minute under an assay condition. Decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16) were purchased from Sigma-Aldrich. All other chemicals used were of analytical grade.

Esterified xylo-oligosaccharides preparation

Esterified xylo-oligosaccharides were prepared by following the optimum reaction conditions of Udomrati and Gohtani (2014). Xylo-oligosaccharide and fatty acid in a ratio of 1:0.5 (mole of monosaccharide unit/mole of fatty acid) was used. Xylo-oligosaccharide (1 g) was dissolved in unlidded flask with 2 ml dimethyl sulfoxide (DMSO), as solvent of both hydrophilic and lipophilic substrates. The fatty acid was added into flask and then mixture was stirred by magnetic stirrer for 10 min. The purchased lipase enzyme solution (350 µl) was filled into mixture. The samples were incubated by means of a water bath at 60°C for 4 h with stirring by magnetic stirrer throughout incubation. Three fatty acids were investigated: decanoic acid, lauric acid, and palmitic acid. The ester formed was precipitated by adding ethanol. The ethanol supernatant, contains lipase and unbonded fatty acid, was poured off after centrifugation at 3000 rpm for 5 min. The precipitation by ethanol was repeated three times prior to drying of the precipitate overnight in a hot-air oven at 50°C.

Proton nuclear magnetic resonance (^1H NMR) spectra

The ^{1}H NMR spectra of the esterified samples were recorded on an Alpha 600 NMR spectrometer (JEOL, Tokyo, Japan). Samples were dissolved in DMSO-d6 to obtain a solution concentration of 15% (w/w). The measurement was operated at 70°C. All chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as a reference, which is usually used as an internal standard for NMR measurements at elevated temperature.

For esterified xylo-oligosaccharides, the maximum possible DS is 2.0, corresponding to the number of OH available on the backbone of the oligosaccharide. The DS could be calculated by the following Eq. (1):

$$DS = \frac{I_{methyl}}{3I_{anomericXyl}} \quad (1)$$

where I_{methyl} is the area of methyl protons of ester chains at 1.9–2.0 ppm, and $I_{anomeric\ Xyl}$. is the area of anomeric protons of xylo-oligosaccharide at 4.5 ppm (Belmokaddem *et al*., 2011).

Interfacial tension

Interfacial tension between *n*-hexadecane and pure water containing native or esterified xylo-oligosaccharides at a concentration of 25% (w/w) was measured by means of a drop volume method employing a computer-controlled apparatus (DVS-2000; Yamashita Giken, Tokushima, Japan) at 25 ± 0.01°C. The apparatus can automatically determine the *n*-hexadecane–water interfacial tension from the maximum volume of the pendant drop detached from a glass syringe immersed in *n*-hexadecane.

Solubility

Native or esterified xylo-oligosaccharides powder (30–50 mg) was suspended in 5 ml water, stirred for 30 min and then centrifuged at 4000 rpm for 15 min. The supernatant was collected, dried in an oven at 90°C for 6 h and weighed. Solubility (%) was calculated as follows

$$Solubility\ (\%) = \frac{weight\ of\ dry\ supernatant\ (mg)\ x\ 100}{weight\ of\ dry\ sample\ (mg)} \quad (2)$$

Rheological analysis

Shear stress (τ) and viscosity of native and esterified sample solutions of 25% (w/w) concentration were measured with a cone-and-plate type rheometer (DV-III Ultra; Brookfield Engineering Laboratories, Middleboro MA, USA) using cone number CPE-40. The angle and the gap between the cone and plate were 0.8° and 13.0 μm, respectively. Samples were placed in the measurement cell of the rheometer and allowed to equilibrate at 25°C. The shear stress of samples was measured in a shear rate range of 5 to 225 s^{-1}. Experimental data was fitted to the Herschel–Bulkley model, which is represented by Eq. (3):

$$\tau = \tau_0 + k \cdot \dot{\gamma}^{n} \quad (3)$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), n is the dimensionless flow behavior index, and k is the consistency index ($Pa.s^n$).

Differential scanning calorimetry (DSC)

Transition enthalpy (*ΔH* expressed as J/g), onset temperature (T_o) and conclusion temperature (T_c) of melting were determined by DSC (PYRIS Diamond™ DSC; Perkin-Elmer, Waltham MA, USA). Samples of native or esterified xylo-oligosaccharides (8–10 mg) were sealed in aluminum pans and scanned from −20 to 105°C at 10°C/min. An empty pan was used as a reference.

Emulsion preparation

Six ml of *n*-hexadecane was added to 4 ml of native or esterified xylo-oligosaccharides solution at concentration of 25% (w/w) for mean diameter of oil droplets and microstructure measurement and concentrations of 0-35% (w/w) for centrifugation experiment, and then homogenized by a high-speed homogenizer (T 25 digital ULTRA-TURRAX®; IKA, Staufen, Germany) at 15000 rpm for 1 min.

Determination of average oil droplet size

The average diameter of oil droplets in emulsions was determined using a laser diffraction particle size analyzer (SALD-3000; Shimadzu, Kyoto, Japan). This instrument measures the angular dependence of the intensity of light scattered from a dilute emulsion under stirring. Emulsions were stirred continuously throughout the measurement to ensure a homogeneous dispersion of the emulsion droplets.

Microscopic analysis

A microscope (BX51; Olympus, Tokyo, Japan) was used to determine the microstructure of the emulsions.

Emulsifying activity

The assay for emulsifying activity was modified from the method of Freitas *et al.* (2009), using *n*-hexadecane as the test substance. Six ml of *n*-hexadecane was added to 4 ml of aqueous phase, which was constituted of varied concentrations of native or esterified xylo-oligosaccharides (0–35% (w/w)). Emulsification was performed using a high-speed homogenizer at 15000 rpm for 1 min. After 24 h, the emulsification index was determined as follows:

$$\text{Emulsification index} = (h_e / h_T) \times 100 \quad (4)$$

where h_e (mm) is the height of the creamed layer and h_T (mm) is the overall height of the mixture.

Centrifugation experiment

Fresh emulsions were prepared as described above in emulsion preparation part and centrifuged for 5 min (Himac; Hitachi Koki, Tokyo, Japan). The experimental parameter was centrifugal rotation speed (500, 1000 and 2000 rpm).

Due to centrifugal force, the emulsion drops (with lower density than the continuous phase) move toward the axis of rotation and form a stacked layer. Depending on the oligosaccharide concentration and centrifugation speed, three different structures of the system could be observed after centrifugation: (1) complete emulsion breakup, with separated oil and water layers in the tube; (2) partial emulsion breakup, with a layer of separated oil at the top, a transparent water layer at the bottom, and a layer of creamed emulsion in between; (3) no emulsion breakup, with a creamed emulsion on top of the transparent water layer (Krebs *et al.,* 2012). The separated oil phase was carefully removed from the centrifuge tube with a Pasteur pipette. The weight of the separated oil layer was measured, and from this the oil separation was calculated as follows:

$$\text{Oil separation (\%)} = \frac{\text{weight of separated oil (g)} \times 100}{\text{weight of total oil (g)}} \quad (5)$$

Results and Discussion

Influence of type of fatty acid on degree of substitution (DS)

Fatty acids with 10, 12 and 16 carbon atoms were investigated as acyl donors for enzymatic esterification at 60°C for 4 hr. The DS values of esterified xylo-oligosaccharides ranged between 0.042 and 0.066. There was no statistical difference ($P>0.05$) in the DS values of the esterified xylo-oligosaccharides, as shown in Table 1.

Table 1. DS, interfacial tension, solubility (%) in water and rheological properties of native and esterified xylo-oligosaccharide at a concentration of 25% (w/w)

Sample	DS	Interfacial tension ($mN.m^{-1}$)	Solubility (%)	Apparent viscosity (mPa.s) at 225 s^{-1}	n	k ($Pa.s^n$)	τ_0 (Pa)
native	-	$39.77^{a} \pm 0.11$	100^{a}	$4.26^{d} \pm 0.03$	$1.05^{a} \pm 0.01$	$0.0031^{c} \pm 0.0002$	$0.06^{ns} \pm 0.02$
xylo decanoate	$0.066^{ns} \pm 0.009$	$36.27^{c} \pm 0.25$	$87.00^{b} \pm 1.82$	$5.89^{c} \pm 0.05$	$1.02^{b} \pm 0.01$	$0.0051^{b} \pm 0.0003$	0.05 ± 0.01
xylo laurate	0.050 ± 0.004	$36.55^{c} \pm 0.17$	$84.82^{b} \pm 2.02$	$5.19^{b} \pm 0.01$	$1.02^{ab} \pm 0.01$	$0.0043^{bc} \pm 0.0004$	0.05 ± 0.02
xylo palmitate	0.042 ± 0.001	$38.16^{b} \pm 0.09$	$84.90^{b} \pm 1.75$	$6.83^{a} \pm 0.10$	$0.93^{c} \pm 0.01$	$0.0098^{a} \pm 0.0007$	0.05 ± 0.03

^{ns} No statistical difference (P>0.05)
^{a,b,c} Means in same column with different lowercase superscripts are significantly different (P<0.05)

Physicochemical characterization

Interfacial tension

As shown in Table 1, the interfacial tension value of native xylo-oligosaccharide was 39.77 mN/m. After esterification, the interfacial tension of esterified xylo-oligosaccharide was lowered to 36.27, 36.25 and 38.16 mN/m for decanoic acid ester of xylo-oligosaccharide (xylo decanoate), lauric acid ester of xylo-oligosaccharide (xylo laurate) and palmitic acid ester of xylo-oligosaccharide (xylo palmitate), respectively. Esterification leads to increased surface activity of oligosaccharides and indicates the potential application of esterified oligosaccharides in the preparation of O/W emulsions. Commercial emulsifiers are more effective than esterfied xylo-oligosaccharides in reducing the interfacial tension. The published research of Kothekar *et al*. (2007) reported that soybean oil-water interfacial tension value of Tween 20, Tween 60, and Tween 80 at concentration of 1% (w/w) was 2, 2.8, and 5.1, respectively. Although, surface activities of esterifed xylo-oligosaccharide were rather low, they exhibit both emulsifying and stabilizing functions in emulsion system.

Solubility

The solubility of native and esterified xylo-oligosaccharides in water is shown in Table 1. Native xylo-oligosaccharide was fully water-soluble, while all esterified xylo-oligosaccharides were slightly insoluble in water. Esterification renders oligosaccharides more hydrophobic, which lowers the possibility of a hydrogen bond formation between the hydroxyl groups of maltodextrin and water, i.e. reducing their solubility in water (Rajan *et al.*, 2008). The solubility of esterified xylo-oligosaccharides with varied fatty acid types was not significantly different (P>0.05). This result might be attributed to no differences in the DS values.

Rheological properties

The rheological characteristics of native and esterified xylo-oligosaccharides are shown in Table 1. After esterification, the apparent viscosity at a shear rate of 225 s^{-1} and the consistency index *(k)* value of esterified samples were higher than those of unmodified sample due to an increase in the resistance to flow of the larger molecules (Ibanoğlu, 2002). This result was in good agreement with the findings of Qiao *et al.* (2006). The increased viscosity of esterified sample adduct versus the native sample suggests that these materials may be used as thickeners and perhaps can be used as emulsifiers and polymeric surfactants.

The value of n is almost 1 in native xylo-oligosaccharide, which indicates Newtonian flow behavior. There was no change in the flow behavior of esterified xylo decanoate and laurate because there was no difference in the strength of the attractive forces in the systems. However, esterification induced shear-thinning flow behavior for xylo palmitate. The shear-thinning behavior tended to increase with an increase in the chain length of fatty acids. This result concurred with Nor Hayati *et al.* (2009) who found that the degree of pseudoplasticity decreases with a decrease in molecular mass. These results may be attributed to the fact that interpenetration of oligo-polysaccharide chains forms a dynamic entangled network structure at a low rate of shear; shear thinning occurs when the rate of externally imposed disruption becomes greater than the rate of formation of new entanglements (McClements, 2005). Under such conditions, the density of the formed crosslink of the network is depleted, and hence the viscosity is reduced. As the shear rate increases, the crosslink

density further decreases, resulting in a decrease in the apparent viscosity (Wu *et al.*, 2012). Inducing shear-thinning behavior of xylo palmitate also might be related to insoluble tiny particles (lumps), which were disrupted and deformed as the shear rate increased.

Differential scanning calorimetry (DSC)

After esterification, a broader melting peak was seen for all esterified samples, compared with the native one, owing to the change of crystalline structure (Figure 1).

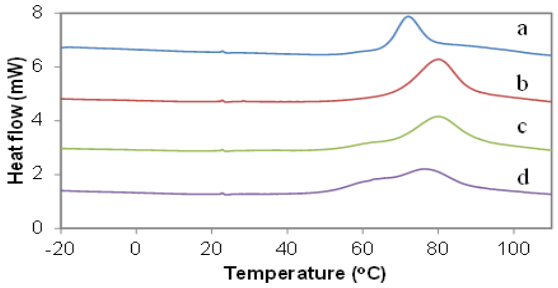

Figure 1. DSC thermograms of native xylo-oligosaccharide (a), xylo decanoate (b), xylo laurate (c), and xylo palmitate (d)

The onset temperature (T_o) of native xylo-oligosaccharide was 65.96°C. The T_o values of xylo decanoate, xylo laurate and xylo palmitate were 66.91, 65.87 and 50.33°C, respectively, as shown in Table 2, with the T_o value of xylo palmitate being clearly lower than that of the unmodified sample. The decreased T_o of xylo palmitate might be attributed to the fact that the attached long fatty acid chain could increase the free volume within the molecules due to the introduction of bulk groups, allowing more molecular mobility and also contributing to the reduction in melting temperature (Aburto *et al.*, 1999; Rajan *et al.*, 2006).

Table 2. Melting transition data of native and esterified xylo-oligosaccharides

Sample	T_o (°C)	T_c (°C)	ΔH (J/g)
native	$65.96^{a} \pm 0.25$	$79.22^{c} \pm 0.20$	$9.76^{b} \pm 0.69$
xylo_decanoate	$66.91^{a} \pm 1.81$	$88.41^{b} \pm 1.36$	$14.37^{a} \pm 0.60$
xylo_laurate	$65.87^{a} \pm 1.02$	$91.03^{a} \pm 0.61$	$13.82^{a} \pm 0.36$
xylo_palmitate	$50.33^{b} \pm 0.62$	$89.62^{ab} \pm 0.08$	$13.66^{a} \pm 0.20$

[a,b,c] Means in same column with different lowercase superscripts are significantly different (P<0.05)
T_o = Onset temperature of melting peak; T_c = Conclusion temperature of melting peak.

The DSC results showed that the ΔH of native xylo-oligosaccharide was 9.76 J/g. After esterification, the ΔH increased to 14.37, 13.82 and 13.66 J/g for xylo decanoate, xylo laurate and xylo palmitate, respectively. The ΔH of all esterified xylo-oligosaccharides was higher than that of the native one. This result may be attributed to the fact that esterification may have induced the crystalline structure of esterified oligosaccharide molecules. This result was in good agreement with the research of Udomrati and Gohtani (2014) who found that the X-ray diffraction peak of esterified maltodextrin showed a higher intensity at about $2\Theta=20^{o}$ compared with native matodextrin because of the occurrence of crystallites. The ΔH of the native sample was much lower than those of esterified samples which may have resulted from some small molecules of xylo-oligosaccharide not being precipitated by adding ethanol during the preparation process of the esterified xylo-oligosaccharides. In addition, the native sample contained small molecules and was directly used for the DSC experiment. There was no clear indication that the fatty acid chain length had an effect on the ΔH of esterified xylo-oligosaccharide.

Emulsion-forming and emulsifying behavior

Influence of esterified xylo-oligosaccharides on the emulsification index

The emulsification index of both native and esterified xylo-oligosaccharides as a function of concentration was tested for *n*-hexadecane (Figure 2).

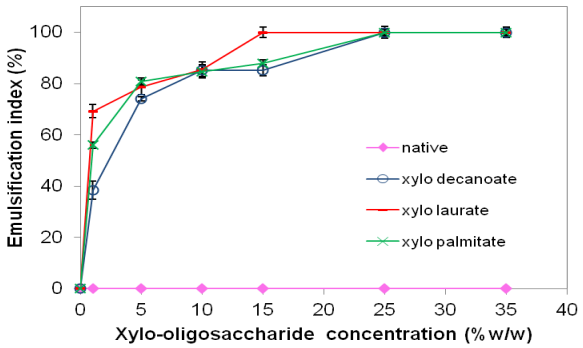

Figure 2. Emulsification index of native xylo-oligosaccharide and esterified xylo-oligosaccharides as a function of concentration for *n*-hexadecane

No emulsion-stabilizing capacity was observed for native xylo-oligosaccharide, with emulsions breaking up after only a few seconds. All esterified xylo-oligosaccharides were proved to possess emulsion-stabilization capacity, as shown by the emulsification indices being higher than those of the native sample. These data indicated that esterified xylo-oligosaccharides may be used as stabilizers for

the dispersion of hydrophobic particles in aqueous media because they have been shown to be surface-active and to have emulsifying properties. The high emulsification indices observed reflect the stability of the emulsions thus formed. The effective emulsification index of emulsions was increased by increasing the concentration, since the esterified samples were able to cover more of the emulsion droplet surface. The emulsification index was further improved with higher concentrations, reaching an index of 100% at a concentration of 15% (w/w) for xylo laurate and of more than 15% (w/w) for xylo decanoate and xylo palmitate. The hydrocarbon tails of lauric acid that had grafted along the xylo-oligosaccharide backbone may have had a stronger interaction with the oil surface, resulting in more efficient emulsifying properties, compared with the other esterified samples.

Influence of esterified xylo-oligosaccharides on oil droplet characteristics

Native xylo-oligosaccharide could not form any emulsion droplets; hence no oil droplets were observed under a microscope (Figure 3a).

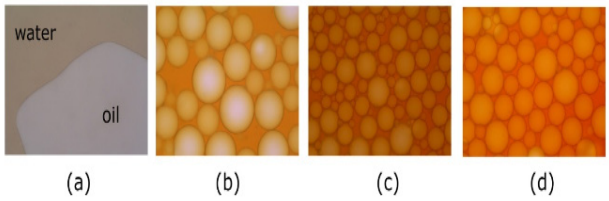

Figure 3. Micrographs of fresh *n*-hexadecane O/W emulsions stabilized by 25% (w/w) of native xylo-oligosaccharide (a), xylo decanoate (b), xylo laurate (c), and xylo palmitate (d).

On the other hand, esterified xylo-oligosaccharide formed O/W emulsions with average oil droplet sizes of 112, 75, and 80 μm, for xylo decanoate, xylo laurate, and xylo palmitate, respectively (data not shown), measured by laser diffraction particle size analyzer. Xylo laurate produced an emulsion with the smallest oil droplets, indicating a product with better emulsifying ability and also showing the highest emulsion index (Figure 2). The average oil droplet diameter of fresh emulsions was the largest when xylo decanoate was used. Esterified oligosaccharides with too many short-chain fatty acid molecules may be not sufficient for complete emulsion stabilization, which is disadvantageous for the inhibition of oil droplet coalescence and thus increases the oil droplet size.

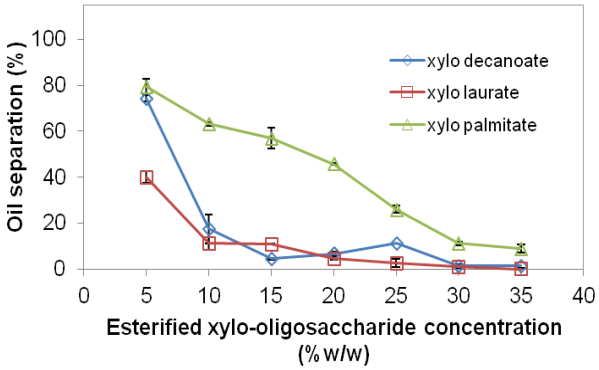

Figure 4. Oil separation of *n*-hexadecane O/W emulsions stabilized by esterified xylo-oligosaccharides as a function of type and concentration at centrifugal rotation speed of 1000 rpm.

Oil separation induced by centrifugal force

Figure 4 shows the oil separation phase (%) after centrifugation at 1000 rpm of esterified xylo-oligosaccharide-stabilzed *n*-hexadecane O/W emulsion of varied types and concentrations. All esterified xylo-oligosaccharides expressed oil separation at low concentrations. This may have occurred because the amount of esterified xylo-oligosaccharide was not sufficient to form a film to cover the oil droplet surfaces. Oil separation or coalescence may then take place if the film of the continuous phase between the droplets is thinned to some critical value (Vrij and Overbeek, 1968). The other mechanism of oil separation induced by centrifugation was probably due to film instability caused by local stretching of the film (van Aken and Zoet, 2000). For xylo laurate and decanoate, there was almost no oil separation at concentrations of 15-25% (w/w). With any further increase in concentration, the oil separation reached zero due to increasing adsorption of polymer molecules and increasing film strength and thickness surrounding the oil droplets (Walstra, 2003). It is reasonable to conclude that xylo decanoate and laurate have great potential for the stabilization of emulsions in a centrifugal field. However, xylo palmitate showed the highest oil separation value at all experimental concentrations. This result may be attributed to the tiny particles of xylo palmitate forming loose absorption layers on the oil droplet surface.

The oil separation value of xylo-laurate was the lowest compared with other types of esterified xylo-oligosaccharides. These results may explain the ability of xylo laurate to cover more of the emulsion droplet surface and/or cover the oil droplet surface with more stable films. Hence, we chose to focus on xylo laurate for studying the effect of centrifugal speed on oil separation. Figure 5 shows oil separation

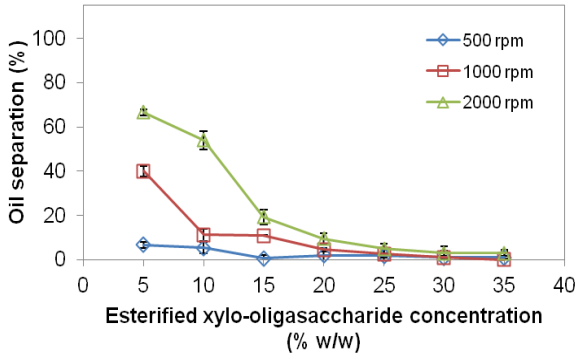

Figure 5. Oil separation of *n*-hexadecane O/W emulsions stabilized by xylo laurate as a function of centrifugal speed and concentration

as a distinct oil layer after centrifugation for 5 min for an initial series with varying centrifugal speeds and esterified xylo-oligosaccharide concentrations. Centrifugation-induced oil separation of esterified xylo-oligosaccharide-stabilized emulsions was lower at higher concentrations. Oil separation tended to increase with increasing centrifugal speed, owing to the increasing film rupture force. At low concentration, oil separation was clearly seen at high centrifugal speed. However, xylo laurate could protect the stability of emulsions, even when the centrifugal speed rose up to 2000 rpm at concentrations of 20-35% (w/w). Emulsion droplets may be stable against high collision forces because the adsorbed biopolymer creates a steric barrier against the coalescence of approaching droplets (McClements, 2005). Xylo laurate may have potential for wide use in food applications for emulsion stabilization and viscosity modification because it possesses high emulsion activity and can stabilize an emulsion in a centrifugal field.

Conclusion

Esterified xylo-oligosaccharides can be used as emulsifiers and stabilizing agents in O/W emulsion systems. The physicochemical and emulsifying properties of esterified xylo-oligosaccharide adducts depend on the type of fatty acid. Xylo laurate may have more potential in emulsifying activity and in preserving the stability of emulsions after centrifugal acceleration, compared with other esterified xylo-oligosaccharides. Hence, it may serve as a good alternative stabilizer for emulsions during processing, transport and application.

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References

Aburto, J., Alric, I., Thiebaud, S., Borredon, E., Bikiaris, D., Prinos, J. and Panayiotou, C. 1999. Synthesis, characterization, and biodegradability of fatty-acid esters of amylose and starch. Journal of Applied Polymer Science 74: 1440–1451.

Alissandratos, A., Baudendistel, N., Flitsch, S. L., Hauer, B. and Halling, P. J. 2010. Lipase-catalysed acylation of starch and determination of the degree of substitution by methanolysis and GC. BMC Biotechnology 10: 82–89.

Belmokaddem, F. Z., Pinel, C., Huber, P., Petit-Conil, M. and da Silva Perez, D. 2011. Green synthesis of xylan hemicellulose esters. Carbohydrate Polymers 346: 2896–2904.

Freitas, F., Alves, V. D., Carvalheira, M., Costa, N., Oliveira, R. and Reis, M. A. M. 2009. Emulsifying behavior and rheological properties of the extracellular polysaccharide produced by *Pseudomonas oleovorans* grown on glycerol byproduct. Carbohydrate Polymers 78: 549–559.

Fundador, N.G., Enomoto-Roger, Y., Takemura, A. and Iwata, T. 2012. Syntheses and characterization of xylan esters. Polymer 53: 3885–3893.

Hartland, S., Yang, B. and Jeelani, S. A. K. 1994. Dimple formation in the thin film beneath a drop or bubble approaching a plane surface. Chemical Engineering Science 49: 1313–1322.

Ibanoğlu, E. 2002. Rheological behaviour of whey protein stabilized emulsions in the presence of gum arabic. Journal of Food Engineering 52: 273–277.

Kothekar, S.C., Ware, A.M., Waghmare, J.T. and Momin, S.A. 2007. Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80. Journal of Dispersion Science and Technology 28:477–484.

Krebs, T., Schroën, C. G. P. H. and Boom, R. M. 2012. Separation kinetics of an oil-in-water emulsion under enhanced gravity. Chemical Engineering Science 71: 118–125.

McClements, D. J. 2005. Food emulsions: principles, practices, and techniques. 2nd edn. Boca Raton, FL: CRC Press.

Nor Hayati, I., Che Man, Y. B., Tan, C. P. and Nor Aini, I. 2009. Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides. Food Hydrocolloids 23: 233–243.

Qiao, L., Gu, Q. M. and Cheng, H. N. 2006. Enzyme-catalyzed synthesis of hydrophobically modified starch. Carbohydrate Polymers 66: 135–140.

Rajan, A., Prasad, V. S. and Emilia Abraham, T. 2006. Enzymatic esterification of starch using recovered coconut oil. International Journal of Biological Macromolecules 39: 265–272.

Rajan, A., Sudha, J. D. and Emilia Abraham, T. 2008.

Enzymatic modification of cassava starch by fungal lipase. Industrial Crops and Products 27: 50–59.

Sadtler, V. M., Imbert, P. and Dellacherie, E. 2002. Ostwald ripening of oil-in-water emulsions stabilized by phenoxy-substituted dextrans. Journal of Colloid and Interface Science 254: 355–361.

Shogren, R. L., Biswas, A. and Willett, J. L. 2010. Preparation and physical properties of maltodextrin stearates of low to high degree of substitution. Starch/ Stärke 63: 333–340.

Singthong, J., Ningsanond, S. and Cui, S. W. 2009. Extraction and physicochemical characterisation of polysaccharide gum from Yanang *(Tiliacora trianda)* leaves. Food Chemistry 114: 1301–1307.

Udomrati, S. and Gohtani, S. 2014. Enzymatic esterification of tapioca maltodextrin fatty acid ester. Carbohydrate Polymers 99: 379–384.

van Aken, G. A. and Zoet, F. D. 2000. Coalescence in highly concentrated coarse emulsions. Langmuir 16: 7131–7138.

van den Broek, L. A. M. and Boeriu, C. G. 2013. Enzymatic synthesis of oligo- and polysaccharide fatty acid esters. Carbohydrate Polymers 93: 65–72.

Vázquez, M.J., Alonso J.L., Domínguez, H. and Parajó, J.C. 2000. Xylooligosaccharides: manufacture and applications. Trends Food Science and Technology 11: 387-393.

Vrij, A. and Overbeek, J. T. G. 1968. Rupture of thin liquid films due to spontaneous fluctuations

in thickness. Journal of the American Chemical Society 90: 3074–3078.

Walstra, P. 2003. Physical chemistry of foods. New York: Marcel Dekker.

Wu, Y., Cui, S. W., Wu, J., Ai, L., Wang, Q. and Tang, J. 2012. Structure characteristics and rheological properties of acidic polysaccharide from boat-fruited sterculia seeds. Carbohydrate Polymers 88: 926–930.