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Formulation and characterization of esterified xylo-oligosaccharides-stabilized oil-in-water emulsions using microchannel emulsification

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ABSTRACT

A series of amphiphilically esterified xylo-oligosaccharides (xylo esters) with different fatty acids residues – decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16) – were enzymatically modified at 60 °C for 4 h. These xylo esters were used as emulsifiers to formulate oil-in-water (O/W) emulsions by microchannel emulsification (MCE). Grooved and straight-through MCE was used to investigate the droplet generation and/or emulsion stability. Xylo ester-stabilized oil droplets were generated smoothly from microchannels arranged linearly or two dimensionally, while xylo ester-stabilized emulsions were less monodispersed owing to low surface activity of the xylo esters. The combined use of xylo esters (2.5% (w/w)) and Tween series (0.1% (w/w)) in the continuous phase can improve the monodispersity of the resultant oil. Successful droplet generation was achieved with the straight-through MCE using 2.5% (w/w) xylo laurate and 0.1% (w/w) Tween 20. The optimized combination of xylo laurate and Tween 20 inhibited coalescence and oiling off more efficiently than the droplets solely stabilized by Tween 20 during 30 days of storage.

1. Introduction

Amphiphilically esterified xylo-oligosaccharides (xylo esters) have both hydrophilic and hydrophobic sub-regions. Their functional properties can be fine-tuned by adjusting the ratio between the hydrophilic part (xylo-oligosaccharide) and the hydrophobic part (fatty acid), as well as the amount and chain length of the alkyl residue. Therefore, xylo esters have the ability to act as low-molecular-weight surfactants and may exhibit good stabilizing ability, probably owing to steric stabilization based on their macromolecular structure [1]. The emulsion stabilization effect of xylo esters may be attributed to the fatty acid tails combined with the xylo-oligosaccharide backbone. This newly established interaction with the oil surface and hydrophilic parts protects the emulsions against flocculation [2,3]. Enzymatic processes offer an attractive alternative route for the synthesis of xylo esters, as they are environmentally and health-friendly processes [4]. Udomrati and Gohtani [5] used xylo esters as stabilizers to improve the stability of Tween 80-stabilized Oil-in-Water (O/W) emulsions, which were produced by a high-pressure homogenizer. Their results showed that xylo esters extended the critical flocculation concentration and delayed creaming rate. Xylo esters were used as sole emulsifiers in *n*-hexadecane O/W emulsion at a concentration range of 5–35% (w/w) [6] and in soybean O/W emulsion with concentrations of 10–50% (w/w) [7]. These emulsions were also prepared by a high-speed homogenizer. These papers reported that xylo esters exhibit both emulsifying and stabilizing activities. For xylo ester (10–20% (w/w))-stabilized soybean O/W emulsion, oiling off was observed during 7 days of the storage period because of insufficient emulsion

Abbreviations: Xylo_D, Xylo-oligosaccharide decanoate (Xylo decanoate); Xylo_L, Xylo-oligosaccharide laurate (Xylo laurate); Xylo_P, Xylo-oligosaccharide palmitate (Xylo palmitate); K_c, Rate of coalescence (h^{-1}).

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stabilization in terms of coalescence. However, there was no oiling off at concentrations of 30–50% (w/w) and no creaming at a concentration of 50% (w/w), suggesting that the stability of the emulsion was remarkably improved by increasing the xylo ester concentration as a result of the increasing viscosity of the continuous phase and/or the sufficient emulsion stabilization. The emulsion systems described above were polydisperse emulsions with the coefficient of variation (CV) ranging from 28 to 50%.

Microchannel emulsification (MCE) is an advanced technique for producing monodisperse emulsions with the CV values lower than 5%. MCE involves a very mild droplet generation process that is further driven owing to interfacial tension difference at the micrometer scale [8,9]. Monodisperse emulsions have the advantage of controllable properties such as texture and stability of food and cosmetic products, and bioavailability, dispersibility, and permeability for pharmaceutical products. Several researchers have encapsulated hydrophilic/lipophilic bioactive molecules using MCE, such as β-carotene [10] and L-ascorbic acid [11–13]. The emulsion stabilization mechanism for xylo esters in O/W emulsions is assumed to be the adsorption of their hydrophobic part on the oil droplet surface, and they later act as a stabilizing agent [7]. MCE may be a suitable method to produce polysaccharide-stabilized emulsions, since MCE enables formulation of oil droplets under a very mild process, thereby avoiding emulsifier particle degradation. Udomrati and Gohtani [5–7] have reported that the surface activities of xylo esters are rather low, and hence, the combination of xylo esters and Tween-series may increase the stabilization effect. The main aim of this research is to reduce the amount of Tween-series when formulating monodisperse emulsions by MCE. The preparation and stability of monodisperse O/W emulsions stabilized by xylo esters are of particular interest, since there are no reports on this in the literature. The purpose of the present study was to investigate the effect of the concentration and type of xylo esters and Tween-series on the emulsion droplet generation by MCE, to study the effect of the combination of xylo esters and Tween-series on the emulsion droplet generation by MCE, and to evaluate the stability during the storage period.

2. Materials and methods

2.1. Materials

Xylo-oligosaccharide extracted from corn was supplied by San-Ei Gen F.F.I. (Osaka, Japan). The range of degree polymerization of the xylo-oligosaccharide was 2–7. Lipase from *Thermomyces lanuginosus* solution, containing 2% (w/v) lipase, was purchased from Sigma-Aldrich (Buchs, Switzerland). *Thermomyces lanuginosus* lipase was used, because the present work set out to complement the results of our previously published research [14] that investigated optimum reaction condition for esterified oligosaccharides synthesis catalyzed by *Thermomyces lanuginosus* lipase. The enzyme activity was about 100,000 U/g; 1 g of enzyme hydrolyzes tributyrin and releases 100,000 μM of titratable butyric acid per minute under assay conditions. Decanoic acid (C-10), Lauric acid (C-12), Palmitic acid (C-16), and ethanol 99.5% were purchased from Sigma-Aldrich. Soybean oil, analytical grade sodium azide (NaN_3), Tween 20 (polyoxyethylene (20) sorbitan monolaurate; HLB = 16.7), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate; HLB = 15.6), Tween 60 (polyoxyethylene (20) sorbitan monostearate; HLB = 14.9), Tween 80 (polyoxyethylene (20) sorbitan monooleate; HLB = 15.0), and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of analytical grade.

2.2. Esterified xylo-oligosaccharide preparation

Xylo-oligosaccharide decanoate (Xylo_D), xylo-oligosaccharide laurate (Xylo_L), and xylo-oligosaccharides palmitate (Xylo_P) were prepared by following the optimum reaction conditions of Udomrati and Gohtani [14]. Xylo-oligosaccharide and fatty acid were used in a molar ratio of 1:0.5. Xylo-oligosaccharide (1 g) was dissolved in an unlidded flask with 2 mL DMSO as a solvent for both hydrophilic and lipophilic substrates. Fatty acid was added to the flask, and the mixture was stirred by a magnetic stirrer for 10 min. The purchased lipase enzyme solution (350 μL) was added to the mixture. The samples were incubated by means of a water bath at 60 °C for 4 h with continuous stirring by a magnetic stirrer throughout the incubation period. The ester formed was precipitated by adding ethanol. The ethanol supernatant contains lipase and unbounded fatty acid, and it was poured off after centrifugation at 3000 rpm for 5 min. Precipitation by ethanol was repeated thrice prior to drying the precipitate overnight in a hot-air oven at 50 °C. Udomrati and Gohtani [6] found that the degrees of substitution (DS) of Xylo_D, Xylo_L, and Xylo_P were 0.066, 0.050, and 0.042, respectively, and their solubility (%) in water was 87.00, 84.82, and 84.90, respectively. DS value was measured by proton nuclear magnetic resonance (^{1}H NMR) with two replications.

2.3. Viscosity and density measurement

The viscosity of esterified oligosaccharide suspensions, Tween series solutions, and combination of xylo-oligosaccharide and Tween suspensions was measured using a vibrational viscometer (SV-10, A&D Company, Ltd., Tokyo, Japan) at 25 ± 1 °C. The densities of these suspensions or solutions were measured with a density meter (DA–130N, Kyoto Electronics Manufacturing, Co., Kyoto, Japan).

2.4. Interfacial tension measurement

The interfacial tension between the soybean oil and pure water containing esterified xylo-oligosaccharide, Tween-series, or combination of esterified xylo-oligosaccharide and Tween was measured by using a fully automatic interfacial tensiometer (PD-W, Kyowa Interface Science Co., Ltd., Niiza, Japan) at 25 ± 1 °C. The apparatus can automatically determine the oil-water interfacial tension from the maximum volume of a pendant drop detached from a stainless steel needle containing esterified xylo-oligosaccharide suspension or Tween solution immersed in soybean oil without droplet holding time during measurement.

2.5. O/W emulsion formulation by grooved MCE

The continuous phases used for MCE were esterified xylo-oligosaccharide dispersions (1, 2.5, and 5% (w/w)), Tween-series solutions (0.05–1% (w/w)), and combination of esterified xylo-oligosaccharide (1, 2.5, and 5% (w/w)), and Tween-series (0.1% (w/w)) dispersions. Soybean oil was used as the dispersed phase. The continuous and dispersed phases were supplied into the MCE module by syringe pumps (Fig. S1b). The flow rates of the continuous and dispersed phases were 1 and 0.05 mL/h, respectively. Cross flow microchannel (MC) array plate (model CMS 6-2, EP Tech. Co., Ltd., Hitachi, Japan) consists of 540 MCs fabricated on a 25 mm × 28 mm silicon plate (Fig. S1a) with 10 parallel arrays. Each MC array contains 54 parallel MCs with a depth of 5 μm, width of 18 μm, and length of 140 μm, and a terrace with depth of 5 μm and length of 60 μm. The dispersed phase introduced into the MCE module was forced to break through the MC arrays to generate oil droplets (Fig. S1). The droplet generation through the MC arrays was observed in real-time by using a microscopic video sys-