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Synthesis of bacterial cellulose and bacterial cellulose nanocrystals for their applications in the stabilization of olive oil pickering emulsion

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ABSTRACT:

Although bacterial cellulose (BC) and bacterial cellulose nanocrystals (BCNs) have considered to be edible, non-toxic, biocompatible and biodegradable, there is still the dearth of reports of BC and BCNs as the emulsifiers of Pickering emulsions with respect to the applications in foods, cosmetics and medicines. In this work, BC with ultrafine network architecture and 8–40 nm crystalline microfibrils in diameter was produced by *Acetobacter xylinum*, and then it was hydrolyzed by sulfuric acid followed by the oxidation of hydrogen peroxide to prepare BCNs. The physicochemical properties of BC and BCNs were contrastively evaluated by FT-IR, XRD, TGA and dynamic light scattering (DLS). The emulsifying performances of BC and BCNs in the stabilization of olive oil Pickering emulsion were further evaluated by optical microscope (OM) and fluorescent microscope (FM). Experimental results showed that the sulfuric acid hydrolysis of BC induced the removal of the amorphous components and the cleavage of the crystalline microfibrils, making BCNs possess the initiating decomposition temperature of 350 °C, the crystallinity index (CrI) of 89.6%, average size of 259.6 nm with PDI of 0.26 and zeta potential at about −34.8 mV, which exhibited high thermal stability and good emulsifying performance in comparison with BC. Furthermore, the effects of pH and ionic strength on emulsion stability were also investigated. In comparison to BC, BCNs were more sensitive in response to the change of pH and ionic strength.

1. Introduction

Cellulose, derived from animals, plants and bacteria, as the most abundant renewable natural biopolymer is a linear homopolysaccharide composed of D-glucopyranose units linked by β-1,4-glycosidic bonds (Abdul Khalil et al., 2014). Despite the same chemical structure, the inherent property and polymerization degree depend on the source of cellulose (Abdul Khalil, Bhat, & Ireana Yusra, 2012). In comparison with tunicin and plant cellulose, bacterial cellulose (BC) possesses outstanding merits, such as high crystallinity and purity with the absence of lignin or hemicelluloses, low density, high modulus and tensile strength, excellent water holding capacity and biocompatibility (Paximada, Tsouko, Kopsahelis, Koutinas, & Mandala, 2016). Furthermore, it is worth noting that the shape and some performances of BC can be adjusted during its biosynthesis process. On account of these advantages, BC can be utilized in biomedicine (Fu, Zhang, & Yang, 2013), cosmetic industry, paper industry (Chawla, Bajaj, Survase, & Singhal, 2009), food processing and packaging (Pereda, Dufresne, Aranguren, & Marcovich, 2014), and tissue engineering (Kirdponpattara, Khamkeaw, Sanchavanakit, Pavasant, & Phisalaphong, 2015).

In virtue of the three hydroxyl groups at the positions C2, C3 (secondary hydroxyl groups) and C6 (primary hydroxyl groups) of the monomer, intra- and intermolecular hydrogen bonds exist in cellulose fibers, leading to the crystalline packing for amorphous and crystalline regions (Abdul Khalil et al., 2012; Maya Jacob & Sabu, 2008). These cellulose fibers can be dealt with strong acid hydrolysis to remove amorphous regions to obtain rod-like crystalline forms of cellulose, referred to as cellulose nanocrystals which have unique surface, optical and mechanical properties (Habibi, Lucia, & Rojas, 2010; Zoppe, Venditti, & Rojas, 2012). In

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particular, cellulose nanocrystals prepared by sulfuric acid hydrolysis can be endowed with anionic sulfate half-ester groups on their surface, which could be further oxidized by hydrogen peroxide, thereby resulting in the uniform aqueous dispersions (Hu, Ballinger, Pelton, & Cranston, 2015; Zhong, Fu, Peng, Zhan, & Sun, 2012). Generally, BC and bacterial cellulose nanocrystals (BCNs) are considered to be hydrophilic because of the presence of high density of hydroxyl groups on the surface (Dankovich & Gray, 2011), while the hydrophobic interactions come from the crystalline organization along with extensive hydrogen bonding of polymer chains, making BC and BCNs be amphiphilic overall (Lindman, Karlström, & Stigsson, 2010). Therefore, their amphiphilic capacities can be applied to stabilize surfactant-free emulsions, in the so-called Pickering emulsions.

Pickering emulsions involve the irreversible adsorption of solid colloidal particles at the oil-water interface and stabilizing the emulsion droplets against coalescence by forming a mechanically robust monolayer (Hu et al., 2015). It is the irreversible adsorption that requires a much higher energy to remove the colloidal particles from the oil–water interface, making Pickering emulsions more stable to resist coalescence than conventional emulsions stabilized by surfactants. In contrast to conventional emulsions stabilized by surfactants, Pickering emulsions have a number of merits, such as more robust formulations, reduced foaming problems and lower toxicity (Duan, Chen, Zhou, & Wu, 2009), thus being widely used in biomedical, health and cosmetic fields where the use of surfactants is undesirable. By replacing conventional synthetic surfactants, they can reduce the use of hazardous surfactants and their environmental consequences (Kalashnikova, Bizot, Cathala, & Capron, 2011b).

As BCNs are easier to be hydrated and well-dispersed in aqueous medium than other types of cellulose nanocrystals, they are capable of emulsifying kerosene (Ougiya, Watanabe, Morinaga, & Yoshinaga, 1997), soybean oil (Blaker, Lee, Li, Menner, & Bismarck, 2009), hexadecane (Kalashnikova et al., 2011b) and liquid paraffin (Jia et al., 2016) to obtain stable Pickering emulsions. However, much less is available in the literature regarding the use of BCNs as a stabilizer for food-grade oil emulsion application (Wang et al., 2016), and little research has been done on the stabilizing properties of BC (Paximada, Tsouko, et al., 2016), though the emulsions stabilized by BC and BCNs have been considered to be environmentally friendly, nontoxic, edible, degradable and biocompatible (Habibi et al., 2010). Notably, olive oil, obtained from the olive of the Mediterranean Basin, is commonly used in cooking, cosmetics and pharmaceuticals. In the Mediterranean basin, olive oil is an important constituent of the diet which is considered a major factor in preserving a healthy and relatively disease-free population (Owen, Giacosa, Hull, Haubner, & Würtele, 2000). Therefore, the emulsification of olive oil by BC and BCNs to prepare serviceable Pickering emulsions is necessary to widen their applications in food emulsions, cosmetic emulsions and medical emulsions.

Paximada, Tsouko, Kopsahelis, Koutinas, and Mandala (2016) found that BC could exhibit better emulsifying capability compared to commercial celluloses, such as HPMC and CMC, and its emulsions were not affected by changes in pH, temperature or ionic strength. Despite the emulsification of olive oil droplets by BC was achieved in this report, the systematic investigation of physicochemical properties and emulsifying performance of BCNs for olive oil is still scarce. In this study, BC and BCNs, synthesized by *Acetobacter xylinum* and sulfuric acid hydrolysis, were assessed by scanning electron microscope (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), thermal gravimetric analysis (TGA) and dynamic light scattering (DLS). The olive oil Pickering emulsions prepared by ultrasonic dispersion were further evaluated by optical microscope (OM) and fluorescent microscope (FM). On account of the colloidal properties of BC and BCNs may be affected by pH and salt concentration (Binks, Duncumb, & Murakami, 2007; Zhang et al., 2012), the effects of pH and ionic strength on the stability of Pickering emulsions were also investigated. This study contrastively estimated the emulsifying performance of BC and BCNs for olive oil, aimed at expanding their applications in food emulsions, cosmetic emulsions and medical emulsions. To the best of our knowledge, the contrastive evaluation of emulsifying performance of BC and BCNs for olive oil is rarely reported so far.

2. Experimental

2.1. Materials

Acetobacter xylinum (CGMCC5173) obtained from China General Microbiological Culture Collection Center was applied to synthesize BC. Olive oil and agar obtained from Sigma-Aldrich were respectively of medical grade and biological grade. Other reagents such as sucrose, Na_2HPO_4, $(NH_4)_2SO_4$ and $MgSO_4$ were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). They were of analytical grade and used without further purification. All aqueous solutions were prepared with deionized water.

2.2. Synthesis of BC and BCNs

The cultivation of *Acetobacter xylinum* was achieved using naturally fermented coconut-water as the incubation system. 100 mL of fermented coconut-water as well as 2.0 g of sucrose, 1.5 g of agar, 0.2 g of Na_2HPO_4, 0.3 g of $(NH_4)_2SO_4$ and 0.05 g of $MgSO_4$ was well mixed to form uniform medium. The pH of the medium was adjusted to 4.5 by acetic acid, followed by high temperature sterilization for 30 min. Thereafter, *Acetobacter xylinum* was activated at 30 °C for 48 h, and then 5 mL of the suspension was inoculated in the same medium with the volume of 100 mL. The cultivation of *Acetobacter xylinum* was conducted at 30 °C for 12 days. Finally, the resultant BC gelatinous membranes were removed from cultures and washed with deionized water. Next it was treated by 0.1 M NaOH at 80 °C to eliminate remaining medium and cells, and then washed repeatedly until a neutral pH was obtained. The purified BC gelatinous membranes were crushed into small pieces with a disintegrator (TY10LB20ES, Waring, America), and their suspensions were further homogenized with a high shear homogenizer (PB100-SP10ZX21, Prima, England) to obtain uniform BC aqueous suspension, as shown in Scheme 1b.

BCNs were prepared by sulfuric acid hydrolysis of BC aqueous suspension according to previous method with some modifications (Mo et al., 2015; Zhong et al., 2012). About 5.0 g of BC was dispersed in 80 mL of 50 wt% sulfuric acid under vigorous mechanical stirring. The hydrolysis was performed at 45 °C for 3 h, and the mixture was diluted five-fold to quench the hydrolysis reaction. Then 8 mL of 30 wt% hydrogen peroxide was added to bleach and oxidize BCNs. Afterwards, the resultant suspension was centrifuged at 9000 rpm for 15 min to separate the crystals, which were washed and treated ultrasonically for 15 min to eliminate excess acid. At last, the precipitate was further dialyzed against deionized water for 8 d using a dialyzing membrane with a molecular weight cutoff of 3500 to remove residual sulfuric acid as well as other low-molecular weight impurities. The resultant BCNs suspension was stored in a refrigerator for further use. Simultaneously, a specified amount of BC and BCNs aqueous suspensions was lyophilized to calculate BC and BCNs content in the suspensions and for further characterization. Based on the weight of BC, the yield of BCNs was 61.6%.