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Stabilizing oil-in-water emulsions with regenerated chitin nanofibers

Ying Zhang [a], Zhigang Chen [a], Wenyang Bian [b], Li Feng [b], Zongwei Wu [c], Peng Wang [b], Xiaoxiong Zeng [b], Tao Wu [a,*]

[a] Glycomics and Glycan Bioengineering Research Center (GGBRC), College of Food Science and Technology, Nanjing Agricultural University, Weigang 1, Nanjing 210095, People's Republic of China
[b] College of Food Science and Technology, Nanjing Agricultural University, Weigang 1, Nanjing 210095, People's Republic of China
[c] College of Life Science and Technology, Henan Institute of Science and Technology, Xinxiang 453003, People's Republic of China

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ABSTRACT

Natural chitin is a highly crystalline biopolymer with poor aqueous solubility. Thus direct application of chitin is rather limited unless chemical modifications are made to improve its solubility in aqueous media. Through a simple dissolution and regeneration process, we have successfully prepared chitin nanofibers with diameters around 50 nm, which form a stable suspension at concentrations higher than 0.50% and a self-supporting gel at concentrations higher than 1.00%. Additionally, these nanofibers can stabilize oil-in-water emulsions with oil fraction more than 0.50 at chitin usage level of 0.01 g/g oil. The droplet sizes of the resulting emulsions decrease with increasing chitin concentrations and decreasing oil fraction. Confocal laser scanning micrographs demonstrate the adsorption of chitin nanofibers on the emulsion droplet surface, which indicates the emulsion stabilization is through a Pickering mechanism. Our findings allow the direct application of chitin in the food industry without chemical modifications.

1. Introduction

Chitin is a linear polysaccharide consisting of acetyl-glucosamine mainly and glucosamine partially via β (1–4) linkage (Kumirska et al., 2010). It is the second most abundant biopolymer on earth that mainly exists as the structure component in crustacean shell, fungal cell wall and insect cuticle (Cauchie, 2002; Guo, He, Feng, & Chen, 2008; Wu, Zivanovic, Draughon, & Sams, 2004). Natural chitin is however a highly crystalline biopolymer with poor solubility in water and common organic solvents, and thus its direct application is rather limited (Kumirska et al., 2010). Usually, chitin has to be converted into its acid soluble derivative – chitosan by a deacetylation reaction using concentrated alkali at nearly boiling temperatures (Shahidi, Arachchi, & Jeon, 1999). Due to their biodegradability, biocompatibility and non-toxicity, chitin and chitosan have been extensively explored for applications in the biomedical and food industries (Jayakumar, Prabaharan, Nair, & Tamura, 2010; Shahidi et al., 1999).

Although chitin is not soluble in water, chitin particles and chitin nanocrystals demonstrate good emulsion stabilization abilities (Magdassi & Neiroukh, 1990; Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). A recent study shows high internal phase emulsions with an internal phase of up to 96% can be stabilized with chitin nanocrystals at concentration as low as 0.30% w/v (Perrin, Bizot, Cathala, & Capron, 2014). The chitin nanocrystals are rod-like particles, with widths in the range of 10–50 nm and lengths varying from 150 to 500 nm (Perrin et al., 2014). They are usually prepared by acid hydrolysis of chitin with hydrochloric acid (Goodrich & Winter, 2007). This preparation method relies on the different hydrolytic kinetics between the amorphous and crystalline regions of chitin: the swelling and hydrolysis of amorphous regions are much faster than these of crystalline regions (Zeng, He, Li, & Wang, 2012). Thus the amorphous regions are preferentially attacked by the acid, leaving the highly crystalline portion to be harvested as chitin nanocrystals. However, this process may result in significant loss of chitin biomass due to the degradation of amorphous or less crystalline domains (Fan, Saito, & Isogai, 2008). Although data regarding the yield of chitin nanocrystals is not commonly reported, the yield can be estimated based on data from cellulose nanocrystal production. The yield of cellulose nanocrystals prepared by acid hydrolysis is as low as 30%, even after optimization of the reaction condition (Bondeson, Mathew, & Oksman, 2006).

Chitin nanofibers can be prepared by mechanical disassembly of native chitin. Chitin nanofibers can be produced from a dried chitin

* Corresponding author. Tel./fax: +86 (25)84395671.
E-mail address: twu@njau.edu.cn (T. Wu).

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under acid condition, or from an undried chitin under acidic or neutral conditions by grinding (Ifuku et al., 2009, 2011, 2010). Recently chitin nanofibers with good reinforcement effect were prepared by a microfluidizer (Yuan et al., 2014). Although mechanical disassembly results in higher yields, the energy consumption can be very high (Klemm et al., 2011). Compared to chitin nanocrystals, the emulsion stabilization ability of these chitin nanofibers has not been studied.

We have successfully prepared chitin nanofibers by dissolving chitin in phosphoric acid and then regenerating it in water. Dissolution of chitin in concentrated phosphoric acid has been proceeded with the formation of an intermediate – glucofuranosyl oxazolinium ion from the acetyl-glucosamine unit. Regeneration of dissolved chitin in water leads to hydrolysis of this intermediate ion and liberates chitin with almost the same structure (Vincendon, 1997). Compared to preparing chitin nanocrystals, the recovery yield of the regenerated chitin was more than 90%. Detailed physicochemical characterization of the regenerated chitin, such as X-ray diffraction, fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy analyses were reported in one of our earlier manuscripts. In this report, the oil-in-water emulsions stabilized with regenerated chitin were characterized and the underlying stabilization mechanism was discussed.

2. Materials and methods

2.1. Materials

Chitin powder (from shrimp shells), calcofluor white, nile red and dodecane (98%) were purchased from Sigma Aldrich (Shanghai, China). Dodecane was purified by extraction with water three times, which involves mixing equal volume of water and dodecane in a separation funnel by shaking and then discharging the water after phase separation. Phosphoric acid 85% and other common chemicals were purchased from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). Deionized water was used throughout the experiments unless specified.

2.2. Purification of chitin

Chitin powder (20.00 g) was incubated in 200 ml 1 M NaOH in a shaking bath at 45 °C and speed at 150 rpm. After 3 h, the chitin suspensions were filtered and the pellet was re-suspended into 200 ml 1 M NaOH. This alkali-washing process was repeated twice more to remove the residual protein and the resulting pellets were washed with water extensively until a constant pH was reached. Then, 1 M HCl was used to wash the chitin pellet following the same protocol described above. The final pellets were washed with water repeated to reach a constant pH value of approximately 4.2. The purified chitin was then freeze-dried and kept in a desiccator before further experimentation.

2.3. Preparation of regenerated chitin

The phosphoric acid and deionized water were equilibrated to 4 °C in a refrigerator before use (Haier, China). Purified chitin (3.00 g) was pre-wetted with 9 ml deionized water and then mixed with 150 ml 85% phosphoric acid to reach a homogenous state. The chitin suspensions obtained were incubated in a shaking bath at 5 °C and speed at 150 rpm for 12 h to obtain a clear solution. Deionized water (750 ml) was used to dilute the chitin solution to obtain a milky dispersion, followed by centrifugation at 16,700*g* (Beckman, J-30I, USA) for 15 min. The supernatant was discarded and the pellet was dialyzed with water to reach a constant pH value. The concentration of chitin in the final dispersion was determined gravimetrically to be 1.000% w/v. A transmission electron microscopy (TEM) analysis of regenerated chitin was performed using a CM100 TEM (Philips, Holland) at 80 kV. Drops of diluted chitin suspensions were placed on a carbon-coated copper grid (400 mesh), dried at room temperature and negatively stained by uranyl acetate (2%, w/v) for 1 min.

2.4. Preparation of oil-in-water emulsion stabilized with regenerated chitin

Regenerated chitin (1.000% w/v) dispersion was diluted with deionized water to obtain chitin dispersions at a series of concentrations, i.e. 0.007%, 0.014%, 0.021%, 0.035%, 0.056%, 0.070%, 0.084%, 0.105%, 0.140%, 0.210%, 0.350% and 0.700% w/v. Purified dodecane (15 ml) was added into the glass tube containing 35 ml chitin dispersions followed by 1 min vortex. The oil water mixture was then emulsified with a disperser (IKA T18 homogenizer, Germany) at 10,000 rpm for 2 min, followed by sonication (Sonics and Materials VC-750, USA, 0.5 in. probe) at 60% pressure amplitude for 2 min in a cold water bath. The emulsions with varied dodecane fractions were prepared in the same way by changing the volumes of dodecane added.

2.5. Visual inspection of emulsion stability and accelerated stability test by centrifugation

Immediately after preparation, 6 ml emulsion was transferred into a plastic centrifuge tube and centrifuged at 4000*g* for 5 min to accelerate the phase separation. A layer of floated oil, a concentrated layer of droplets (designated as creamed emulsions) and a clear bottom phase were usually observed and their volumes were calculated from their thickness, which were measured with a digital caliper. These volumes were used to calculate the cream fraction (volume of creamed emulsion/total volume of emulsion), the oil fraction in cream (volume of dodecane/volume of creamed emulsion) and the usage level of chitin (g/g oil) (Jia et al., 2015). All experiments were repeated in triplicate and data are represented as mean ± standard derivation.

2.6. Optical and confocal laser scanning microscopy (CLSM)

The optical photographs of emulsions were captured by an Eclipse microscope equipped with a digital camera (Nikon 80i, Japan). The diameters of the emulsion droplets were measured in software Image J by taking average of at least 300 droplets. For CLSM observation, a 20% oil-in-water emulsion was prepared and double stained with nile red and calcofluor white. Images were acquired using a Zeiss LSM710 confocal microscope (Zeiss, Gottingen, Germany) using a 40× lens.

3. Results and discussions

3.1. Phase separation of the regenerated chitin suspensions as affected by their concentrations and morphology of regenerated chitin analyzed by TEM

As shown in Fig. 1a, untreated chitin was not soluble in water and precipitation was observed immediately after mixing the water and chitin. Regenerated chitin was also insoluble in water. At concentrations of 0.05%, 0.10% and 0.30%, precipitations of regenerated chitin were observed, which indicated that the regenerated chitin was denser than water. However, the regenerated chitin occupied a much greater volume when it was dispersed in water compared to untreated chitin. The volume of 0.10%