Stabilizing beverage emulsions by regenerated celluloses

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ABSTRACT

Citrus-oil oil-in-water emulsions stabilized by regenerated cellulose were prepared and characterized. Ultrasonic homogenization was more effective in preparing stable emulsion than shear homogenization. Stable emulsions were prepared at cellulose concentrations from 3 to 8 g/kg using 10 mL/L oil, or at oil concentrations from 5 to 20 mL/L using 6 g/kg cellulose. Buoyancy neutral emulsion was obtained using 2 g/kg cellulose and 5 mL/L oil. All emulsions were not flocculated under optical microscope. Adsorption of cellulose on the oil droplets was evidenced by the confocal laser scanning microscopy and scanning electron microscopy. The gel nature of the emulsions was demonstrated by rheological analysis and was not influenced by changing pH from 2 to 8, ionic strength from 0 to 2 mol/L or temperature from 4 to 50 °C. The emulsion stabilization was explained by the increase of droplet density due to the adsorption of heavier cellulose layer, gel nature of continuous phase and smaller droplets produced by ultrasound. These findings offer an alternative method in stabilizing beverage emulsions, especially at relative high droplet concentrations, which may be suitable for applications as delivery carriers of bioactive compounds with health benefits.

1. Introduction

Beverages emulsions are typically oil-in-water emulsions including flavor oils or cloudy oil as the dispersed phase, water and emulsifiers as the continuous phase (McClements, 2005). Other ingredients, such as vitamins, antimicrobials, colors and nutraceuticals can be included as well and incorporated into the dispersed or continuous phase depending on their affinity to specific phase (Piorkowski & McClements, 2014). In "flavor" type beverage emulsions, the oil droplets are mainly composed of flavor oils, such as lemon, lime or orange oils, which provide taste and aroma to the finished products, whereas in "cloudy" type beverage emulsions, the oil droplets are formulated with flavorless vegetable oils, which supply a desirable cloudy appearance to the finished products by the light scattering effect (McClements, 2005). Nowadays, beverage emulsions have been considered as suitable carriers for delivering bioactive compounds with health benefits (McClements, Decker, & Weiss, 2007; Piorkowski & McClements, 2014).

Most emulsions are thermodynamically unstable systems that are susceptible to gravitational separation, flocculation, coalescence and Ostwald ripening, which eventually lead to the phase separation in emulsion and product defection (McClements, 2005). Gravitational separation is caused by the density difference between the oil phase and aqueous phase, which can be alleviated by reducing particle size, increasing continuous phase viscosity or droplet concentration, or by incorporating weighing agents including brominated vegetable oil, sucrose acetate isobutyrate, ester gum, or dammar gum into the oil phase to reduce the density contrast (Taherian, Fustier, Britten, & Ramaswamy, 2008). However, the maximum amount of weighting agents allowed for beverage emulsions is restricted by regulations. For example, in the United States the limits of brominated vegetable oil and ester gum are only 15 and 100 μg/L, respectively (Piorkowski & McClements, 2014). Addition of such a low amount of weighting agents is not enough to match the density between the oil and aqueous phase at relative high droplet concentrations. Thus, the finished products of beverage emulsions usually have a very low droplet concentration (<1 g/kg oil) (McClements, 2005). Incorporation of high doses of bioactive nutraceuticals, such as polyunsaturated fats, conjugated linoleic acid, carotenoids, phytosterols, and fat-soluble vitamins into beverage emulsions is very challenging.

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Commercially beverage emulsions are mainly stabilized by gum arabic and hydrophobically modified starch (e.g., octenyl succinate starch). Both are amphiphilic and stabilize emulsions by adsorption of their hydrophobic groups on the oil/water interface (Taherian et al., 2008). Previously, cellulose is considered as a hydrophilic polysaccharide without good emulsifying ability, and its insolubility in water is a result of the intensive inter- and intra-hydrogen bonds (McClements, 2005). Thus, the hydrophobic modification of cellulose is usually necessary for applications in emulsion stabilization (Andresen & Stenius, 2007; Xhanari, Syverud, & Stenius, 2011). Recent studies, however, show that non-modified cellulose is amphiphilic and the hydrophobic interactions play an important role in determining the crystalline structure and solubility (Cho, Gross, & Chu, 2011; Glasser et al., 2012; Medronho, Romano, Miguel, Stigsson, & Lindman, 2012). Studies of emulsion stabilization using cellulose nanocrystals show that the amphiphilic character of cellulose resides in the crystalline organization at the elementary brick level, and the emulsion stabilization is through the Pickering mechanism (Kalashnikova, Bizot, Cathala, & Capron, 2011, 2012). Another study shows regenerated celluloses prepared by dissolution of native cellulose with cold phosphoric acid and regeneration in water, which are much less crystalline than native cellulose, demonstrate good dispersibility and emulsion stabilization ability (Jia, Chen, Shi, Ye, Wang et al., 2014).

In the aforementioned studies, the emulsion stabilization ability of cellulose was tested with dodecane or hexadecane as oil phase, which might not reflect the application situation of real food emulsion system. In this paper, citrus-oil was used as an oil phase and the preparation of stable oil-in-water emulsions using regenerated cellulose as a stabilizer was studied. Since cellulose is a dense material, it is expected to make buoyancy neutral citrus-oil droplets by the weighing down effect of regenerated cellulose. The research findings may be helpful in using regenerated celluloses for beverage emulsions stabilization.

2. Material and methods

2.1. Materials

Microcrystalline cellulose powder (particle size of 20—100 μm) was purchased from Sigma Aldrich (Shanghai, China). Phosphoric acid (85% solution) and other chemicals were purchased from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). Citrus oil was purchased from a local essential oil company. Deionized water was used in all washing and dilution operations.

2.2. Preparation of regenerated celluloses

Phosphoric acid was pre-cooled to 5 °C in a refrigerator. Around 2 g of microcrystalline cellulose was mixed with 6 mL water and incubated with 100 mL cold phosphoric acid in a shaking bath at a temperature of 5 °C and a speed of 150 revolutions per minute (rpm). After 24 h, the completely dissolved particle-free cellulose solutions were diluted with 500 mL water to regenerate the celluloses. The turbid suspensions containing regenerated celluloses were centrifuged at 16,700 g (Beckman Coulter, Avanti J-30I, Palo Alto, CA, USA) for 15 min. The supernatant was discarded and the pellets were dialyzed with dialysis tubes having a molecular weight cutoff of 3500 Da to remove the phosphoric acid until a constant pH was obtained. After dialysis, the suspensions were redispersed with a disperser (IKA, Ultra-Turrax T18 digital, Staufen, Germany) at 10,000 rpm for 3 min before emulsion preparation.

2.3. Preparation of citrus-oil oil-in-water emulsions

Two homogenization methods (shear and ultrasound) were compared. Shear homogenization was conducted by the disperser (T18 digital) at 10,000 and 15,000 rpm for a duration from 2 to 8 min. Ultrasound homogenization was performed using a 20 kHz horn-type sonicator (Sonics and Materials, VC-750, Newton, CT, USA) with a 1.27 cm titanium probe in the continuous mode at two power levels: 60% and 80% pressure amplitude (PA) for a duration from 2 to 4 min. Temperature control was applied in both methods using iced bath to avoid overheating. The concentration of regenerated celluloses was in the range of 3—8 g/kg. The concentration of citrus-oil was in the range of 5—20 mL/L. The volume of emulsion in each treatment was 50 mL.

2.4. Optical microscopy, confocal laser scanning microscopy and scanning electron microscopy

After emulsion preparation, the optical photographs of emulsions were captured using a microscope equipped with a digital camera (Nikon, Eclipse 80i, Tokyo, Japan). The emulsion droplet diameters (d_{32} & d_{43}) were measured using Image J software by taking the average of at least 150 droplets and calculated according to a literature method (Timgren, Rayner, Dejmek, Marku, & Sjoo, 2013). For confocal laser scanning microscopy observation, an oil-in-water emulsion with oil concentration of 200 mL/L and cellulose concentration of 6 g/kg was prepared and stained with Nile red and Calcofluor white. Images were acquired using a confocal microscope (Zeiss, LSM710, Gottingen, Germany) using a 40X lens. To image the distribution of regenerated celluloses on the surface of emulsion droplets, styrene was mixed with radical initiator azobisisobutyronitrile at a weight ratio of 100/1 (g/g). Two mL of this mixture was added to 8.0 mL 4 g/kg cellulose suspension, dispersed by a disperser (T18 digital) at 10, 000 rpm for 3 min, and polymerized at 50 °C for 12 h to synthesize solid polystyrene particles. Scanning electron microscopy of regenerated celluloses (lyophilized from tert-butanol) and polystyrene particles was conducted using an electron microscope (Hitachi, S4800, Tokyo, Japan) at an accelerating voltage of 30 kV. The samples were gold coated in an ion sputter coater for 2 min before observation.

2.5. Rheological measurements

The rheological properties of emulsions were analyzed using a rheometer (Anton Paar, MCR 301, Graz, Austria) with a 50 mm diameter parallel plate (PP50) at 25 °C. Shear viscosity was tested with shear rate ranging from 0.1 to 10,00 s^{-1}. A pre-shear process of 100 s at 100 s^{-1} and a time-waiting process of 50 s were applied before each testing. Frequency sweep tests were conducted with a strain of 0.1% and frequencies ranging from 0.1 to 10 s^{-1}. The effects of gap size, cellulose concentrations, oil concentrations, pH values, ionic strengths and storage temperatures were studied. 0.1 mol/L HCl and 0.1 mol/L NaOH was used to adjust the pH to the targeted values at room temperature using a standard pH meter (Hanna Instruments, HI 2211, Woonsocket, RI, USA) with a plastic electrode. The ionic strength was adjusted using NaCl. Three storage temperatures 4, 25 and 50 °C were studied. All rheological tests were conducted one day after emulsion preparation.

3. Results

3.1. Morphology of regenerated celluloses and phase separation in regenerated cellulose suspension

Detail characterization of the celluloses regenerated from