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# Effects of regenerated cellulose on oil-in-water emulsions stabilized by sodium caseinate

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ABSTRACT

This study investigated the influence of regenerated cellulose particles (RC) on the physical stability, rheological properties and microstructure of oil-in-water (o/w) emulsions stabilized by sodium caseinate (Na-SC). The stability of o/w emulsions was examined by measuring droplet size and size distribution as well as visualization of their creaming properties. Creaming stability of emulsions was enhanced with the addition of 1.0% RC which eliminated any separation. With increased addition of cellulose particles, there was an improved packing of proteins onto the droplet surface which resulted in a gradual reduction in droplet sizes. Rheological measurements and microscopy showed that RC possessed superb thickening and gelling properties. The influence of RC on emulsions stabilized by Na-SC was concentration dependent, with 1.5% RC being the critical concentration, showing only slight flocculation, a smaller droplet size and a stronger gel network. These results indicate that regenerated cellulose particles have the potential to substitute for conventional polysaccharides particularly those which require larger quantities to stabilize emulsions.



## 1. Introduction

Food emulsions are thermodynamically instable systems, and as a consequence of various phenomena, may result in creaming, sedimentation, flocculation, coalescence, Ostwald ripening and/or phase inversion (Calero, Muñoz, Cox, Heuer, & Guerrero, 2013; Palazolo, Sorgentini, & Wagner, 2004). This challenges food manufacturers to seek desirable ingredients to stabilize such food emulsion systems. Previous reports have shown that emulsions could be favorably stabilized by the use of surfactants and hydrocolloids, and most effectively by proteins, polysaccharides and nano-/micro-particles (Dickinson, 2009; Jia, Xu, et al., 2014). Proteins are widely used as emulsifiers and emulsion stabilizers in food processing (Speiciene, Guilmineau, Kulozik, & Leskauskaite, 2007; Ye, 2008). They contribute to the formation of stable emulsions and enhance the physical stability of systems by reducing of interfacial tension and by forming an adsorption layer around fat droplets which inhibits coalescence. However, proteins have limited application in food processing considering their vulnerability to changes in temperature, pH and ionic strength. Alternatively, polysaccharides, including gum Arabic, modified starches, modified celluloses and pectins, could impart desirable texture and improve stabilities of o/w emulsions by modifying the emulsion viscosity, gel-forming networks as well as providing a steric barrier through the adsorption on the surface of oil droplets.

Many researchers in the field of food science and technology have focused on producing emulsions using different types of charged polysaccharides (Chitosan, Carboxymethyl cellulose and Xanthan) in the presence of proteins (Calero et al., 2013; Chuah, Kuroiwa, Kobayashi, & Nakajima, 2014; Liang et al., 2014; Liu et al., 2012; Long et al., 2013), or polysaccharides of nano-/micro-particles (microcrystalline cellulose (MCC), nanocrystal cellulose and microfibrillated cellulose) in the absence of proteins (Winuprasith & Suphantharika, 2014; Xhanari, Syverud, & Stenius, 2011; Zoppe, Venditti, & Rojas, 2012). SC processed from milk casein is commonly used as an emulsifier imparting stability to oil-in-water emulsions as a result of the combination of it behaving as a steric barrier and by its electrostatic properties (Moschakis, Murray, & Dickinson, 2005). Cellulose consists of repeated D-glucose residues connected by β-1, 4-glycosidic bonds. MCC is insoluble in

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water, having poor surface activities due to the strong intermolecular hydrogen bonding. However, it can be used as a suspension stabilizer, emulsifier, thickener and as a dietary fiber supplement, a filling agent and a fat substitute in food products. Regenerated cellulose, derived from microcrystalline cellulose through acid dissolution and water regeneration possesses strong surface activities which are attributed to the presence of its hydrophobic and hydrophilic regions (Glasser et al., 2012; Jia, Xu, et al., 2014). The combination of proteins with polysaccharides is of great value considering their cooperative attributes which improve stabilization of o/w emulsion systems by reducing interfacial tension, as well as enhancing the thickness of the adsorption layer around the fat globules. However, stabilities of emulsions prepared with RC and Na-SC are largely unknown. The current study was aimed at investigating the mechanism when using different amounts of RC on the stability, rheological properties and microstructures of o/w emulsion when prepared with soybean oil as dispersing phase, sodium caseinate as the emulsifier and regenerated cellulose as the emulsion stabilizer.

## 2. Materials and methods

### 2.1. Materials

Food grade Na-SC containing 90% protein, 5.09% moisture, 1.42% fat and 4.5% ash, microcrystalline cellulose and phosphoric acid were purchased from Tabo Commercial Company, Qufu Tianli Medicinal Materials Company, Shandong and Jiangsu Chengxing Phosph-chemicals Company, respectively. Soybean oil was supplied by local market. Fluorescent dyes of Nile blue, Nile red and Calcofluor White were obtained from Sigma–Aldrich (Shanghai, China).

### 2.2. Preparation of regenerated cellulose

The preparation of regenerated cellulose was carried out as previously described with slight modification (Jia, Chen, et al., 2014). Briefly, 3.00 g MCC was swollen with 9 ml of deionized water and then blended twice with 150 ml of 85% phosphoric acid to form a homogeneous solution. The cellulose dispersion was mixed in an oscillator at speed of 150 rpm for 24 h at a temperature of 5 °C to acquire a transparent solution. The solution was then diluted with 750 ml of deionized water and centrifuged at 16,700 *g* (Beckman Counter, JA-10, USA) for 20 min. The centrifugation process was repeated by washing with deionized water until a constant pH was achieved. The final concentration of the cellulose was determined gravimetrically and found to be 4.57% (wt/wt).

### 2.3. Emulsion preparation

The emulsions were formulated (all in weight proportion) with 2.0% Na-SC, 30% soybean oil and various levels of RC (0%, 0.5%, 1.0%, 1.5% and 2.0%). Na-SC was dissolved in deionized water at the ratio of 1:8 using a magnetic stirring apparatus for 3 h at room temperature and then stored at 4 °C for 24 h for utilization. Soybean oil (30% wt/wt) was added later and was homogenized (Ultra TurraxT25 BASIS, Germany) at the speed of 15,000 rpm for 1.5 min in ice bath to avoid overheating of the sample. Prior to homogenization, sodium azide (0.02%) was added to control microbial growth. The remaining water and regenerated cellulose were mixed with the pre-formed emulsion at a speed of 10,000 rpm for another 1.5 min in ice bath. The final emulsion was kept at room temperature for 24 h after emulsification and then analyzed the same day and again after storage for one week. Each emulsion was prepared at least in triplicate for follow-up measurements.

### 2.4. Emulsion stability

Freshly prepared emulsions were separately transferred into individual 8 ml glass tubes and stored at ambient temperature for visual inspection of the height differences of the emulsion layers during storage. Creaming index was calculated as the serum volume fraction. Photographs were taken at 1 d and 7 d following the preparation of emulsions.

### 2.5. Particle size and distribution measurement

Particle sizes and size distribution of the prepared emulsions were monitored using a Malvern Mastersizer 3000 (Malvern Instruments Ltd., England). The average droplet size was represented by mean volume diameter $D_{4,3}$ which was more sensitive to fat droplet flocculation/coalescence than $D_{3,2}$. An individual droplet size was measured in the presence of SDS which avoided aggregate formation that usually occurred after emulsification (Kim, Renkema, & Van Vliet, 2001). Thus, two volumes of emulsion were diluted with deionized water (one volume) and SDS (1.0%, one volume) (Palazolo, Sorgentini, & Wagner, 2005; Relkin & Sourdet, 2005). The refractive and absorption indices of dispersed droplets were set as 1.436 and 0.001 respectively. Flocculation extent of emulsions was calculated using the following formula:

$$F\% = \left[\left(D_{4,3-SDS} - D_{4,3+SDS}\right)/D_{4,3+SDS}\right] \times 100\%$$

where $D_{4,3-SDS}$ and $D_{4,3+SDS}$ are the mean droplet sizes of the middle zone of emulsion measured with or without SDS.

The micrographs of the emulsions as a function of time were recorded using Zeiss Axio Scope. A1(Carl Zeiss AG, Germany). Samples diluted with deionized water (1:100, v:v) were placed on a microscope slide, covered and observed under a microscope using a 40 × objective lens.

### 2.6. Rheological measurements

The rheological behaviors of steady-shear rates and dynamic measurements for the final emulsion after storage for 1 d and 7 d were performed on a rotational Physical MCR 301 rheometer (Anton Paar, Graz, Austria). Each emulsion was taken at 2 cm from the upper part of the test tube added to a 50 mm diameter parallel plate using a fixed gap of 0.5 mm at a sustained temperature of 25 °C. Steady shear flow tests were carried out at small amplitude ranging from 0.01 to 10 1/s. Strain sweeps were conducted to ascertain the linear viscoelastic region with the strain from 0.01 to 100% at a fixed frequency 1 Hz. Frequency sweeps of the storage modulus (G′) and loss modulus (G″) were performed from 0.1 to 100 Hz with a strain of 0.1%. All rheological tests were repeated three times with the same emulsion and were conducted at 1 and 7 d after sample preparation, without applying high shear forces before the rheological measurements.

### 2.7. Confocal laser scanning microscope

The distribution of proteins and polysaccharides in emulsions were assessed by Confocal Laser Scanning Microscope (Zeiss LSM710, Germany) in the fluorescence mode with an aging time of 1 d. One-milliliter of emulsion was placed in a test tube and then 80 μL Nile blue (protein staining dye) solution and Nile red (oil staining dye) solution (both 0.01% in isopropanol) as well as Calcofluor White (polysaccharide staining dye) solution (0.1% in