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Generating phytosterol nanoparticles in nanoporous bioaerogels via supercritical carbon dioxide impregnation: Effect of impregnation conditions

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ABSTRACT

Phytosterols are known for promoting human health and wellness; however, their water-insolubility limits their bioaccessibility and consequently bioavailability. In this study, a novel method based on supercritical carbon dioxide (SC-CO_2) impregnation of phytosterols into nanoporous starch aerogels (NSAs) was optimized to form phytosterol nanoparticles with reduced crystallinity in order to improve their bioaccessibility. Impregnation conditions, namely temperature and cooling rates, were optimized for the highest impregnation capacity, smallest particle size, and improved phytosterol distribution. The highest impregnation capacity was 99 mg phytosterol/g NSA, and the size of the impregnated phytosterols ranged between 59 and 87 nm. More isolated phytosterol nanoparticles were generated via fast cooling which drastically decreased the solubility of phytosterols in SC-CO_2. The crystallinity of the impregnated phytosterols was reduced compared to the original phytosterol. The proposed method is a novel method to generate food grade phytosterol nanoparticles with reduced crystallinity which improves the water solubility.

1. Introduction

Interest in preparing foods to improve the overall human health and wellness is growing with the trending food for health concept. One approach is to improve the nutritional value of the foods and beverages by incorporating bioactive compounds. Among bioactive compounds, phytosterols have received much attention in managing hypercholesterolemia, or abnormally high blood cholesterol, and recently inflammation (Navarro et al., 2001) and inflammatory bowel disease (IBD), a chronic inflammatory condition of the gastrointestinal tract (Cheon et al., 2006; Kang et al., 2013; Somani et al., 2015). Typical western diet is poor in terms of phytosterols and only ~5% of the dietary phytosterol is absorbed in the human body. Therefore, enriching foods with phytosterols is required, but incorporating phytosterols into foods is challenging because phytosterols are high melting point crystalline compounds that are insoluble in water and poorly soluble in fats and oils, which results in a gritty texture that affects the sensory and quality of the product negatively. Moreover, bioavailability of phytosterols is very low (1.5–5%) (Nguyen, 1999) due to poor water solubility and high crystallinity (Law, 2000).

Decreasing the size of bioactives is known to increase the bioaccessibility and in turn the bioavailability (Acosta, 2009; Kesisoglou et al., 2007). In recent years, there has been many efforts to decrease the size of phytosterols. These include emulsion formation (Chen et al., 2016; Ribeiro et al., 2016; Rozner et al., 2009), microencapsulation by spray drying (Di Battista et al., 2015), formation of inclusion complexes of phytosterols with hydroxypropyl β-cyclodextrin (Meng et al., 2012), and precipitation from organic solvents (Christiansen et al., 2003; Kawachi et al., 2006; Rossi et al., 2010). However, most of the current methods generate liquid products that are difficult to incorporate into all types of foods, have undesirable flavor profile due to the presence of surfactants, and their application is limited due to the use of organic solvents and/or non-food grade surfactants. A recent study

Abbreviations: SC-CO_2, Supercritical carbon dioxide; NSA, nanoporous starch aerogel; RESS, rapid expansion of supercritical solution into air; RESSAS, rapid expansion of supercritical solution into aqueous solution; DELOS, depressurization of an expanded liquid organic solution; SPSS, sudden precipitation of supercritical solutions; BET, Brunauer–Emmett–Teller; BJH, Barrett-Joyner-Halenda; SEM, scanning electron microscopy.

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investigated the preparation of functional foods by co-crystallization of phytosterols and triacylglycerols; however, this approach is not applicable for many low fat foods which have an increasing consumer demand (Acevedo and Franchetti, 2016).

Particle formation using SC-CO_2 is a new and exciting area that offers alternative approaches to form various micro- and nanoparticles. CO_2 is non-toxic, non-flammable, environmentally friendly, inexpensive, abundant, and has mild critical temperature (31 °C) and pressure (7.4 MPa). So far, rapid expansion of supercritical solution into aqueous solution (RESSAS) (Türk and Lietzow, 2004), rapid expansion of supercritical solution into air (RESS) (Türk et al., 2002; Türk and Lietzow, 2008), and depressurization of an expanded liquid organic solution (DELOS) (Moreno-Calvo et al., 2014) processes have been implemented for micronization of phytosterols. However, agglomeration of the particles and use of organic solvents/surfactants are the disadvantages of those SC-CO_2-based techniques.

We have recently reported a novel approach to improving water solubility of the phytosterols using SC-CO_2 and a novel NSAs impregnation method (Ubeyitogullari and Ciftci, 2016b). In that approach, NSAs with extraordinary properties (59 m^2/g surface area and 20 nm pore size) were used as a mold to generate phytosterol nanoparticles. Starch is cheap, abundant, biodegradable, biocompatible and food grade polysaccharide which can form nanoporous gel network without any chemical cross-linker. Therefore, starch is a great candidate to form NSAs for food applications (Ubeyitogullari and Ciftci, 2016a). In our previous study (Ubeyitogullari and Ciftci, 2016b) phytosterol nanoparticles were formed at a single impregnation condition (70 °C and 45 MPa), and the phytosterol impregnation capacity was relatively low (55 mg phytosterol/g NSA). It was also observed that the phytosterol nanoparticles form a very thin plate-like structure. For an improved bioaccessibility, higher amount of isolated phytosterol nanoparticles are required. Hence, there is a critical need for detailed investigation of the impregnation conditions to improve the impregnation capacity and form isolated phytosterol nanoparticles.

Therefore, the objectives of this study were to (a) form NSAs (b) impregnate NSAs with phytosterols to form phytosterol nanoparticles using a novel SC-CO_2 impregnation method (c) investigate the effects of impregnation conditions, namely, temperature, pressure, and cooling rate, for the impregnation capacity, and phytosterol particle size and distribution.

2. Materials and methods

2.1. Materials

Wheat starch was kindly supplied by Manildra Milling Corporation (IA, USA). Crude phytosterols were obtained from MP Biomedicals (OH, USA) and their composition (52.2 ± 0.7% β-sitosterol, 23.3 ± 0.6% stigmasterol, and 24.5 ± 0.1% campesterol) was determined using gas chromatography-mass spectrometry (GC-MS). Pyridine was purchased from EMD Chemicals, Inc. (NJ, USA). Sylon BFT [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA):trimethylchlorosilane (TMCS), 99:1] was obtained from Supelco Inc. (PA, USA) and 5α-cholestane (>98%) was purchased from Acros Organics (NJ, USA). Liquid CO_2 (purity> 99.99%) was supplied by Matheson Tri-Gas, Inc. (PA, USA), while ethanol (100%) was obtained from Decon Laboratories, Inc. (PA, USA).

2.2. Preparation of nanoporous starch aerogels (NSAs)

NSAs were produced from wheat starch according to the method that was optimized previously (Ubeyitogullari and Ciftci, 2016a). Briefly, starch was gelatinized in a closed high pressure reactor (4520 Bench Top Reactor, Parr Instrument Company, IL, USA) at 120 °C for 20 min with a mixing rate of 600 rpm to obtain the hydrogels, followed by a retrogradation step at 4 °C for 48 h. Subsequently, the water in the hydrogel was replaced with ethanol with a five-step solvent exchange (30, 50, 70, and 100% (v/v) ethanol for 1 h residence time then 100% ethanol for 24 h) to obtain an alcogel. Finally, the alcogels were converted to aerogels by SC-CO_2 drying of the alcogels at 40 °C and 10 MPa for 4 h with a CO_2 flow rate of 0.5 L/min (measured at ambient conditions). NSAs were stored at room temperature (21 °C) until characterized (Ubeyitogullari and Ciftci, 2016a) and impregnated. The properties of the formed NSAs are presented in Table 1.

2.3. Impregnation of the NSAs with phytosterols

Phytosterols were impregnated into the NSAs using a custom-made laboratory scale SC-CO_2 impregnation system (Fig. 1). Impregnation vessel was divided into two compartments with a sintered filter. NSAs (1 g) and phytosterols (0.5 g) were separately wrapped in a Whatman #41 filter paper (NJ, USA). Wrapped phytosterols were placed at the bottom compartments, and the NSA was placed into the top compartment. The vessel temperature (70, 90 and 120 °C) was set prior to the impregnation and the temperature of the micrometering valve was set at 80 °C to prevent freezing due to Joule Thompson effect during depressurization. The system was pressurized with CO_2 to 45 MPa using the double head high pressure syringe pump (Model 260D, Teledyne Isco Inc., NE, USA) and kept at constant set pressure and temperature. Impregnations were carried out at semi-dynamic mode for 3 h. The shut off valve was opened every 10 min for 1 min and the CO_2 flow rate was adjusted to 1 L/min (measured at ambient conditions) using the micrometering valve. At the end of 3 h, the system was cooled down to 25 °C at different cooling rates with either natural cooling or fast cooling by blowing CO_2 to the impregnation vessel from a compressed CO_2 cylinder at 6 MPa. Table 2 shows the details of the impregnation conditions. Then, the system was depressurized to atmospheric pressure at a CO_2 flow rate of 1 L/min (measured at ambient conditions). Finally, phytosterol-impregnated NSAs were collected from the vessel and stored at room temperature (21 °C) until characterized.

Phytosterols were also processed at the same impregnation conditions shown in Table 2 and collected by sudden precipitation of supercritical solutions (SPSS) without using NSAs. SPSS-phytosterols were recrystallized from phytosterol-SC-CO_2 solvato complex on the Whatman #41 filter paper (NJ, USA) in the top compartment of the vessel (Fig. 1).

2.4. Determination of the phytosterol impregnation capacity

Approximately 0.15 g of impregnated NSA was dispersed in 5 mL of chloroform in a 40-mL glass vial and heated at 50 °C for 1 h with occasional vortexing. Then, the mixture was filtered using a 0.45 μm pore-size filter. Filter cake and the vial were washed with 5 mL of chloroform three times and filtered. Finally, the chloroform in the combined filtrate was evaporated to dryness by blowing nitrogen using a Reacti-Vap evaporation unit (Model TS-18825,

Table 1
The textural properties of the NSAs.

BET surface area (m^2/g)	61.6 ± 1.4
BJH pore size (nm)	17.8 ± 0.4
Pore volume (cm^3/g)	0.27 ± 0.01
Density (g/cm^3)	0.11 ± 0.00
Porosity (%)	92.78 ± 0.00

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