Characterization of starch nanoparticles prepared by nanoprecipitation: Influence of amylose content and starch type

Yang Qin, Chengzhen Liu, Suisui Jiang, Liu Xiong, Qingjie Sun*

School of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong Province, 266109, China

ABSTRACT

Starch nanoparticles (SNPs) were prepared by nanoprecipitation using seven native starches, including waxy corn, normal corn, high amylose corn, potato, tapioca, sweet potato, and pea starches (amylose content 0.8%–69.0%). The structural, morphological and physicochemical properties of SNPs were systematically investigated using dynamic light scattering, Fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry and electron microscopy. Compared to micro-scale native starch (mean size of 15–49 μm), the mean particle sizes of SNPs were mainly in the range of 30–75 nm, which is much smaller than those reported in previous published literatures. Interestingly, the smaller the starch granules were, the smaller the corresponding SNPs were obtained. All SNPs exhibited a typical V-type crystalline structure, which were independent of the crystal type of the native starch. Moreover, the relative crystallinity of SNPs increased with the increasing amylose content in native starch, and a high correlation ($R^2 = 0.95$) was observed. Compared with native starch, the gelatinization enthalpy of corresponding SNPs decreased, with the exception of high amylose corn starch.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Nano-sized particles have attracted much more attention due to their unique properties, which are significantly different from their bulk materials. Nano-technology not only decreases raw materials in particle sizes, it also improves their functional properties. Nanoparticles prepared from natural polysaccharides and protein with the advantages of nontoxicity, biocompatibility, biodegradability, being renewable and abundantly available in nature—such as in cellulose, chitin and whey protein—have attracted wide attention (Moon et al., 2011; Wu et al., 2015). As a typical biodegradable natural polysaccharide, starch is a renewable substance produced by many plants as a source of stored energy. Starch nanoparticles (SNPs) produced via nano-technological processes have drawn more attention in recent years because they provide greater opportunity for mass production and are non-hazardous to human health (Joye and McClements, 2013). These SNPs have been widely used in food packaging (Dai et al., 2015), plastic fillers (Shi et al., 2013), diagnosis and the treatment of cardiovascular diseases, and drug delivery (Muthukrishnan et al., 2015; Dar et al., 2013) due to their mechanical properties and renewable nature (Simi and Emilia-Abraham, 2007).

SNPs have been prepared using a variety of methods, including acid hydrolysis (Kim et al., 2012), extrusion (Song et al., 2011), high pressure homogenization and emulsification (Shi et al., 2011), and nanoprecipitation (Kim et al., 2015). The anti-solvent nanoprecipitation is a simple, fast and reproducible method to fabricate synthetic and natural polymer nanoparticles (Campardelli et al., 2012). It typically allows the preparation of very fine particles with an improved control over particle properties, such as size, morphology and physical state, and is suitable for producing nanoparticles from a range of different food ingredients. Chin et al. (2011) prepared SNPs by anti-solvent precipitation from sago starch with the diameters in the range of 300–400 nm. Tan et al. (2009) also obtained nanosphere using acetylated waxy maize starch by a nanoprecipitation process, and found the mean diameter increased from 249 to 720 nm as the concentration of the starch in the acetone raised from 1 to 20 mg/mL. Hebeish et al. (2014) produced SNPs by nanoprecipitation from the starch solution, and found the diameters raised from 132 to 220 nm with the concentrations of maize starch increased from 2.5% to 10%. Additionally, Juna et al. (2014) studied the influence of temperature (75, 100, and 130 °C) on the shapes and sizes of waxy corn starch nanoparticles via nanoprecipitation, and observed the sizes of SNPs decreased from 600 to 238 nm with increasing temperature.

* Correspondence author at: School of Food Science and Engineering, Qingdao Agricultural University, 700 Changcheng Road, Chengyang District, Qingdao, 266109, China.
E-mail address: phdsun@163.com (Q. Sun).

Although SNPs prepared from different volume ratios of starch aqueous solution to anti-solvent, reaction temperature, and concentration of starch have been investigated in previous literatures (Ma et al., 2008; Chin et al., 2011), no detailed information has been reported about the effects of different amylose contents and starch types on the formations and characterizations of size-controlled SNPs via nanoprecipitation. To fill this knowledge gap, we aimed to systematically investigate the differences in size, morphology, structure, and physicochemical properties among the nanoparticles fabricated by nanoprecipitation from native starches with different amylose contents and starch types. Furthermore, we would further evaluate if there is any correlation between the structural and morphological characteristics of SNPs and the different native starch (waxy corn, normal corn, high amylose corn, potato, tapioca, sweet potato, and pea starches).

2. Materials and methods

2.1. Materials

Normal corn starch (CS, 26.5% amylose content) was obtained from Ingredion China Ltd. (Guangdong, China). Potato starch (PS, 28.0% amylose content) was supplied by Tianjin Tingfung Starch Development Co., Ltd. (Tianjin, China). Sweet potato starch (SPS, 20.6% amylose content) and tapioca starch (18.9% amylose content) were obtained from Shandong Zhucheng Xingmao Starch Company, China. (Shandong, China). Waxy corn starch (WX, 0.8% amylose content), pea starch (PES, 40.0% amylose content) and high amylose corn starch (HAS, 69.0% amylose content) were purchased from the National Starch Co. (Shanghai, China). All other reagents were of analytical grade, and deionized water was used throughout.

2.2. Preparation of starch nanoparticles

The SNPs were prepared by reference to a previously reported procedure (Hebeish et al., 2014) and modified especially for the concentrations and the conditions of gelatinized starch solution, as well as the ratios of starch solution to absolute ethanol. Briefly, with the exception of HAS, starch suspension (1%, w/v) was prepared by mixing 0.1 g of starch powder in 10 ml of deionized water. The mixture was incubated at 100 °C for 30 min for the complete gelatinization of starch with constant stirring. This way, a homogeneous starch solution was obtained. Because HAS could not gelatinize at 100 °C, it was incubated at 150 °C for 30 min in an oil bath. The starch solution was quickly cooled to room temperature, and then 100 ml of ethanol was added dropwise to the gelatinized starch solution, which was continually stirred at a constant stirring rate. Whereafter, the solution was kept for another 8 h at room temperature under continuous mechanical stirring. The resulting suspension was then centrifuged (2230 g for 15 min), and the supernatants were removed to obtain the regenerated SNPs, which were rinsed three times with absolute ethanol to remove excessive water. Subsequently, the nanoparticles were dried by lyophilization for 48 h. The dry high amylose corn starch nanoparticles (HASNP), pea starch nanoparticles (PESNP), potato starch nanoparticles (PSNP), corn starch nanoparticles (CSNP), tapioca starch nanoparticles (TSNP), sweet potato starch nanoparticles (SPSNP) and waxy corn starch nanoparticles (WXNP) obtained from the lyophilization process were kept in a plastic bag and stored in the refrigerator (4–5 °C) until further use.

2.3. Transmission electron microscopy (TEM)

The SNPs sample was ultrasonically dispersed in deionized water to form a 0.01% (w/v) SNPs suspension. Then, a small droplet of the diluted SNPs suspension was deposited on a 300 mesh copper grid coated with holey carbon film. The excess liquid was removed by filter paper. The as-obtained specimen was subsequently dried under the vacuum condition. The TEM analysis was performed using an H-7650 TEM (Hitachi, Tokyo, Japan) at 80 kV.

2.4. Scanning electron microscopy (SEM)

The microstructure of native starch was observed using SEM (JSM840, Jeol, Japan). The starch granules were attached to an SEM stub using double-sided cellophane tape. Then, the stub and sample were coated with gold-palladium, and the morphologies were photographed. Average dimensions were determined using digital image analyses. Starch granules were assimilated to spherical particles. Between 150 and 200 measurements were performed depending on the source used to determine the average diameter.

2.5. Particle size analysis

The average size and size distributions of SNPs were estimated by dynamic light scattering (DLS) using a Malvern Zetasizer Nano (Malvern Instruments Ltd., UK) equipped with a He-Ne laser (0.4 mW; 633 nm) and a temperature-controlled cell holder. The intensity of the scattered light was detected at 90° to the incident beam. The measurements were performed in samples diluted in deionized water and analyzed at 25 °C (Pignatello et al., 2006).

2.6. X-ray diffraction (XRD)

The crystalline structures of the native starch and SNPs were studied using an X-ray diffractometer (AXS D8 ADVANCE; Bruker, Karlsruhe, Germany). The samples were stored in a sealed container in a saturated solution (1000 ml) of NaCl to standardize moisture content with Cu Kα radiation (λ = 1.543). Reflection angle signals of 2θ from 4° to 40° were recorded. Relative crystallinity of samples were determined by plotting the peak's baseline on the diffractogram and calculating the area using the software spectrum viewer, according to the method described by Jivan et al. (2013). The area above and under the curve corresponded to crystalline domains and amorphous regions, respectively. The ratio of upper area to total area was taken as the relative crystallinity: Relative crystallinity (%) = Area under the peaks/Total curve area × 100.

2.7. Fourier transform infrared spectroscopy analysis (FTIR)

The infrared spectra of the SNP samples were recorded on an FTIR spectrophotometer (NEXUS-870; Thermo Nicolet Corporation, Madison, WI, USA), as described by Kunal et al. (2008). Each of the samples was mixed with KBr and pressed into pellets, which were then subjected to attenuated total reflectance spectroscopy in a 4000–500 cm^{-1} range. Intensity measurements were performed on the spectra by recording the height of the transmittance bands from the baseline.

2.8. Differential scanning calorimeter (DSC)

Gelatinization parameters of the SNP samples were measured using a differential scanning calorimeter (DSC1; Mettler Toledo, Schwerzenbach, Switzerland) equipped with a thermal analysis data station and data recording software (STAR@ SW 9.20), as described by Chanvrier et al. (2007). Each sample (approx. 3–8 mg) was placed in an aluminum pan, where the same amount of moisture was added (3–8 μl). The pan was hermetically sealed before DSC analysis. The scanning temperature range and the heating rates were 25–120 °C and 10 °C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample