International Journal of Biological Macromolecules 84 (2016) 354–360

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Preparation and characterization of starch nanoparticles via self-assembly at moderate temperature

Chengzhen Liu, Yang Qin, Xiaojing Li, Qingjie Sun*, Liu Xiong, Zhuzhu Liu

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

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 23 October 2015
Accepted 12 December 2015
Available online 17 December 2015

Keywords:
Starch nanoparticles
Self-assembly
Short glucan chains
Preparation

ABSTRACT

Starch nanoparticles (SNPs) were fabricated via short glucan chains self-assembly at 50 °C and their characteristics were evaluated by transmission electron microscopy, dynamic light scattering, molecular weight distributions, X-ray diffraction, differential scanning calorimetry, and Fourier transforms infrared spectroscopy. The results showed that SNPs exhibited spherical particles with a diameter of approximately 30–40 nm. The molecular weight of the SNPs mainly distributed at degree of polymerization (DP) 12 and DP 30. The gelatinization temperature of the SNPs increased dramatically compared to that of native waxy maize starch. The crystallinity of the samples increased as the assembling time increased and showed the same A-type in the X-ray diffraction pattern as native starch. This newly proposed SNPs approach has potential application in starch nanocomposite films due to their high gelatinization temperature.

1. Introduction

Nanoparticles have been used in a wide number of applications, including nanocomposites, drug carriers, and biosensors [1]. Due to their submicron size, nanoparticles possess a high surface-to-volume ratio, improved dispersibility, and adaptability for multiple functions. Starch, as one of the most abundant natural polymers, is biocompatible, biodegradable, non-toxic, comparatively easy to process, and is the most commonly used biopolymer in industry. Bio-based and biodegradable starch nanoparticles (SNPs) have raised a tremendous level of attention, as sustainable development policies tend to expand with the decreasing reserve of fossil fuel and the growing concern for the environment [2].

Various approaches for preparing SNPs, such as acid hydrolysis [3], high-pressure homogenization, nanoprecipitation, microemulsion, hydrolysis with pullulanase, and recrystallization [4], have been explored by researchers. There still is little research involving the preparation methods of starch nanoparticls [5]. Self-assembly is a bottom-up method to manufacture nanoparticles that are cost-effective, highly time and yield efficient, and easy to scale-up to industrial processes. Sun et al. [4] prepared short glucan chains by debranching cooked waxy maize starch and allowing it to recrystallize at 4 °C to form SNPs. However, SNPs assembled at low temperatures have limitations, such as a lower gelatinization temperature, which is major hindrance for its use in films. Although the heat moisture treatment of SNPs improved their thermal properties [6] and cross-linking increased the melting temperature of the starch nanocrystal [7], an additional modification process was needed. Therefore, the objective of this study was to develop a facile method to prepare thermostable SNPs via short glucan chains self-assembly at a moderate temperature of 50 °C and to evaluate their characteristics.

2. Materials and methods

2.1. Materials

Waxy maize starch (approximately 2% amylose and 98% amylopectin) was obtained from Tianjin Tingfung Starch Development Co., Ltd. (Tianjin, China). Disodium hydrogen phosphate (Na_2HPO_4) and Citric acid ($C_6H_8O_7$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pullulanase (E.C.3.2.1.41, 6000 ASPU/g, 1.15 g/mL, where ASPU is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 4.4 and 60 °C) was supplied by Novozymes Investment Co., Ltd. (Beijing, China). All other reagents used were analytical grade.

* Corresponding author at: 700 Changcheng Road, Chengyang District, Qingdao 266109, China.
E-mail address: phdsun@163.com (Q. Sun).

http://dx.doi.org/10.1016/j.ijbiomac.2015.12.040
0141-8130/

2.2. Preparation of starch nanoparticles (SNPs)

Starch nanoparticles were prepared using the method as described by Sun et al. [4] and with some modifications to the retrogradation procedure. Waxy maize starch (15 g) was dispersed in 100 mL of disodium hydrogen phosphate and citric acid buffer solution (pH 4.6), and the starch slurry was cooked in boiling water, with vigorous stirring for 30 min to fully gelatinize the starch. The temperature of the cooked starch was adjusted to 58 °C, and pullulanase (30 ASPU/g of dry starch) was added. After an 8 h incubation period, the reaction was stopped by heating the starch at 100 °C for 30 min to inactivate the pullulanase. Then, 400 mL of absolute ethanol were added drop wise to the gelatinized starch solution under vigorous mechanical stirring cooling to room temperature. The suspensions were washed several times with distilled water until neutrality and then freeze dried to obtain short glucan chains powder.

Twenty five grams of short glucan chains powder were placed in 75 mL aqueous solution (0.25% w/w) and then autoclaved at 121 °C for 30 min. The mixtures were then incubated in a water bath, which was preheated to 50 °C, and then kept for 1, 2, 6, 12 and 24 h. Afterwards, the mixtures were washed with distilled water several times and centrifuged with a speed of 4000 rpm for 15 min and then freeze dried to obtain SNPs.

2.3. Microscopic morphology

2.3.1. Scanning electron microscope (SEM)

A scanning electron microscope (Zeiss DSM 982 GEMINI, Germany) with a secondary electron detector was used to obtain images of the native starch. The dried starch power were evenly coated on a double-sided adhesive tabs (Leit-Tabs, 12 mm, Christine Groepel, Tulln, Austria) mounted on aluminum stubs (Stiftprobenteller, Christine Groepel, Tulln, Austria). The mounted starch precipitates were coated with gold/palladium. The voltage applied was 3.0 kV. The images and data were recorded via Carl Zeiss V2.20 windows-based software (Zeiss, Germany) [8].

2.3.2. Transmission electron microscope (TEM)

TEM observations were performed using a Hitachi (Tokyo, Japan) 7650 transmission electron microscope at an acceleration voltage of 80 kV. SNPs suspensions (0.25%, w/w) were sonicated 25 °C for 15 min. A droplet of the aqueous dispersion was placed on Formvar coated grids. Prior to complete drying, Formvar coated grid was freezed. Complete dried grid was allowed to dry for observation.

2.4. Dynamic light scattering (DLS)

The size of SNPs was determined using DLS at room temperature on a 90 Plus particle size analyzer (Brookhaven Instruments Corporation) at the 90 scattering angle. All sample solutions were filtered using disposable 0.45 μm Mill pore filters prior to analysis at a concentration of 1.0 mg/mL.

2.5. Molecular weight distributions

Molecular weight distributions of branch chains of waxy maize starch, short glucan chains powder, and SNPs were analyzed using a high-performance size-exclusion chromatograph (HPSEC) equipped with a refractive index (RI) detector. HPSEC was carried out according to Jiang et al. [9] with some modifications. The sample for branch chains length of waxy maize starch was prepared as follows: the waxy maize starch dispersed in disodium hydrogen phosphate and citric acid buffer solution (pH 4.6) was cooked in boiling water, with vigorous stirring for 30 min to fully gelatinize the starch. The temperature of the cooked starch was adjusted to 58 °C, and pullulanase (30 ASPU/g of dry starch) was added. After a 24 h incubation period to obtain completely debranched starch, the reaction was stopped by heating the starch at 100 °C for 30 min to inactivate the pullulanase, and centrifuged at 3000 × *g* for 2 min to remove the inactivated enzyme. Then, the debranched starch (short glucan chains) was analyzed for molecular weight distributions of waxy maize starch [10]. The short glucan chains powder and SNPs samples were dispersed in deionized water (0.1% w/v) and then autoclaved at 121 °C for 30 min to accomplish complete gelatinization. An aliquot (100 μL) of the above-mentioned solution was filtered through a nylon membrane (0.25 μm pore size). The filtrate was injected into a HPSEC system for molecular weight distributions analysis. The HPSEC-RI system consisted of a pump (LC-20AT), an injection valve (SIL-20A), and an RI detector. PL-aquagel-OH 30 analytical columns with a guard column were used to analyze the molecular weights of the samples. The temperature of the columns (PL-aquagel-OH 30) was maintained at 35 °C. Distilled-deionized water filtered through a membrane with a pore size of 0.25 μm was used as the eluent with a 1 mL/min flow rate. Pullulan standards (Mw 342, 1320, 6200, 10600, 21700) were used as references for the determination of molecular weights of the waxy maize starch, short glucan chains powder, and SNPs.

2.6. X-ray diffraction

X-ray diffractometer (Philips PW1710, Philips, Holland) operated at the Cu-Kα wavelength of 1.54056 Å were used. Corn starch, SNPs powders were tightly packed into the sample holder. X-ray diffraction patterns were recorded in the reflection mode in angular (2θ) range of 4–40° at ambient temperature and in 0.01° steps, 0.5°/s steps. Powders were dried at room temperature for 24 h prior to analysis.

2.7. Differential scanning calorimetry (DSC)

The thermal properties of sample, such as temperature and gelatinization enthalpy, were measured using DSC1 (METTLER TOLEDO, Switzerland). The differential scanning calorimetry (DSC) runs were operated under a nitrogen gas (30 mL/min), and an empty pan was used as the reference. The pulverized sample was further dried in a laboratory oven (105 °C) for 6 h to remove the moisture. The dried powder samples (3–5 mg) and 6–10 μL of water was added to aluminum pan. The pans were sealed and scanned at a heated rate of 10C/min with temperature ranging from 50 °C to 125 °C. The DSC thermograms were evaluated to characterize the onset (*To*), peak (*Tp*), conclusion temperature (*Tc*), and gelatinization enthalpy (ΔH).

2.8. Fourier transforms infrared spectroscopy (FTIR)

Fourier transform infrared spectra of waxy maize starch and SNPs were acquired on a FTIR TENSOR 27 spectra (Germany BRUKER) using KBr disk technique. For FTIR measurement, the samples were mixed with anhydrous KBr and then compressed into thin disk-shaped pellets. The spectra was obtained with a resolution of 2 cm^{-1} between wave number ranges of 4000–400 cm^{-1}. The assumed line shape was Lorentzian with a half-width of 19 cm^{-1} and a resolution enhancement factor of 1.9. Intensity measurements at 1047 and 1022 cm^{-1} were performed on the deconvoluted spectra by recording the height of the absorbance bands from the baseline.