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Effect of drying conditions on crystallinity of amylose nanoparticles prepared by nanoprecipitation

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ABSTRACT

In this study, amylose nanoparticles prepared by nanoprecipitation were dried at different conditions. The crystalline structure, crystallinity, re-dispersibility and morphological characteristic of the amylose nanoparticles after drying were investigated. X-ray diffraction analysis revealed that the V-type crystalline structure of the amylose nanoparticles formed in the drying process instead of the precipitation process, and drying condition significantly affects the crystallinity. The temperature cycles drying at 4 °C and 40 °C considerably increased crystallinity of the amylose nanoparticles, 24 h (4/40 °C, 12 h/12 h) drying under 11% relative humidity could give rise to a crystallinity up to 50.05%. The applied drying procedures had no obvious effect on the appearance of the amylose nanoparticles. The Z average-size (d. nm) and polydispersity index (PDI) obtained from dynamic light scattering analysis suggested that the drying processes caused some aggregates, but the dried amylose nanoparticles could be well dispersed in water.

1. Introduction

Starch nanocrystals, produced from bio-source and renewable resources, have attracted much attention in recent years because they are promising candidates as reinforcement in nanocomposites for improving mechanical, barrier and also electrical properties of polymer matrices [1–3]. The increasing scientific and industrial interest for starch nanoscrystals has led to the development of several methods to prepare starch nanocrystals for nanocomposites applications.

Acid hydrolysis approach [4], through hydrolyzing the amorphous regions in starch granules and retaining the crystalline regions, has been widely used to prepare starch nanocrystals. The main drawbacks of the acid hydrolysis are time consuming, low yield of starch nanocrystals and the tendency to aggregate [3,5], which limited the practical utilization of starch nanocrystals. For these reasons, researchers have been trying to find other procedures with physical treatments or a combination of different methods to prepare starch nanocrystals [6–13]. Crystalline starch nanoparticles were prepared with higher yield of 78% through a low temperature (4 °C) acid hydrolysis for 6 days followed by a mild ultrasonic treatment [6]. It was reported that two hours enzymatic pretreatment of waxy maize starch could effectively reduce the acid hydrolysis time from 5 days to 45 h for preparing starch nanocrystals, and the final yield was 15% [7]. Although ultrasonic treatment of starch suspensions is a physical and rapid method to prepare starch nanoparticles, and this method can disperse or break the aggregates of starch nanoparticles, X-ray diffraction analysis revealed that ultrasonication could seriously disrupt the crystalline structure of starch and led to the nanoparticles with low crystallinity or an amorphous character [8]. Crystalline starch nanoparticles were prepared by complex formation of high amylose corn starch with *n*-butanol and successive enzymatic hydrolysis, but the yield of the prepared nanoparticles was very low [9]. Recently, a green and facile method to prepare starch nanoparticles with higher crystallinity was proposed, through pullulanase debranching and recrystallization of native waxy maize starch solution, starch nanoparticles were obtained with yield above 85%, the crystallinity could up to 55.41% and the preparation time was 2 days [10]. Nanoprecipitation is a simple and fast method to prepare starch nanoparticles by dropwise addition of a dissolved starch solution to a non-solvent or inversely [11–13]. The preparation time is several hours and the yield could be over 90%, but the crystallinity of the obtained starch nanoparticles is low.

It is well known that gelatinized starch tends to re-associate in an ordered crystalline structure during storage, this process is termed retrogradation. Retrogradation behavior of starch nanoparticles prepared by recrystallization of debranched

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amylopectin-rich waxy proso millet starch was investigated, it was found that the crystallinity of the starch nanoparticles continued to increase and peaked at 49.04% at 4 °C for 240 min of retrogradation [14]. Temperature, time and moisture content/ambient relative humidity influence the retrogradation and crystallinity of starch nanoparticles. It was reported that the crystallinity of starch nanoparticles was up to 69.7% after a heat moisture treatment at 110 °C with 30% moisture content for 4 h [15]. Since preparation of starch nanoparticles via precipitation is fast, if crystallinity of the precipitated starch nanoparticles could be increased significantly through optimizing the drying condition which facilitates the association of starch molecular chains, then starch nanoparticles with higher crystallinity can be prepared effectively. As far as our literature survey could ascertain, there are no publications regarding effect of drying conditions on crystallinity of starch nanoparticles prepared by nanoprecipitation.

Amylose was selected in this study to prepare nanoparticles through nanoprecipitation because amylose has relatively simple linear molecular chains and is easily susceptible to retrogradation. The main objective of this study was to investigate effect of drying conditions on crystalline structure and crystallinity of amylose nanoparticles prepared by nanoprecipitation, hoping to find an innovative method to prepare starch nanoparticles with high crystallinity.

2. Materials and methods

2.1. Materials

Amylose with a purity of 99.5% was obtained from Shanxi Tianwei Biological Production Co. Ltd. (Xi'an, China). The amylose is isolated from corn starch and its molar mass ranges $2.4 \times 10^6 - 4.5 \times 10^8$ g/mol. Absolute ethanol and silica gel were purchased from Beijing Chemical Works (Beijing, China). Lithium chloride anhydrous and magnesium chloride hexahydrate were purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). All the materials and reagents were used as received

2.2. Preparation of amylose nanoparticles

First, 2.5 g amylose was mixed with 50 mL deionized water, and then the mixture was heated at 100 °C for 1 h with continuous stirring to obtain gelatinized amylose solution. When the temperature of the amylose solution reached 70 °C, ethanol (three times of the amylose solution in volume) was added dropwise at a rate of 10 mL/min to the amylose solution which was continuously agitated with a magnetic stirrer at a constant rate. Next, the obtained suspension was cooled to room temperature and centrifuged at 4000 rpm for 5 min. The supernatant was removed to obtain regenerated amylose nanoparticles which were then rinsed twice by centrifugation with absolute ethanol.

The prepared amylose nanoparticles were dried by putting them in desiccators at different temperatures (4 °C, 20 °C and 40 °C) and controlled relative humidities (0%, 11%, 21% and 33%) which were maintained by using silica gel and saturated salt aqueous solutions for a period of time. Freeze-dried amylose nanoparticles were also prepared at −75 °C and under vacuum for 6 h with a freeze-dryer and used as a control. After the drying, the samples were sealed in plastic bags for next test.

Moisture content of the amylose nanoparticles without drying and those dried at different conditions was determined by measuring weight loss of the samples upon drying in a vacuum oven at 105 ± 1 °C for 4 h. Moisture content was calculated according to the following equation:

$$\text{Moisture content}\,(\%) = \frac{M_w - M_a}{M_w} \times 100 \quad (1)$$

where M_w is the weight of the samples after a drying process and M_d is the weight of the fully dried samples.

2.3. Particle size analysis

Z average-size (d. nm) and polydispersity index (PDI) of the prepared amylose nanoparticles were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano-ZS90 (Malvern Instruments Ltd., UK) at 25 °C. Two types of samples were characterized: the amylose nanoparticles without drying and the ones dried under different conditions. The samples were prepared by dispersing the amylose nanoparticles in deionized water at a concentration of about 0.1%. The measurement was carried out six times for each sample and mean values were reported.

2.4. X-ray diffraction (XRD) analysis

Crystalline structures of the amylose nanoparticles were characterized using a Rigaku D/max-2500 X-ray diffractometer (Rigaku Corporation, Japan) with Cu-Ka radiation (λ = 1.542 Å) at voltage of 40 kV and electric current 200 mA. The XRD pattern of the amylose nanoparticles without drying was recorded over the 2θ range of 4–40° at a speed of 10°/min to avoid drying, while the XRD patterns of the dried amylose nanoparticles were recorded at a speed of 2°/min. Relative crystallinity was determined from the ratio of the areas of the diffraction peaks to the area of the whole diffraction pattern subtracted amorphous background patterns according to the method described by Nara and Komiya [16].

2.5. Scanning electron microscopy (SEM)

Morphology of the dried amylose nanoparticles was observed by a Zeiss EVO 18 scanning electron microscopy (Zeiss, MERLIN Compact, Germany). The samples were mounted on specimen stubs with carbon black tape and sputter-coated with gold for observation.

2.6. Differential scanning calorimeter (DSC)

The gelatinization properties of the amylose granules and dried amylose nanoparticles were investigated using a differential scanning calorimeter (DSC 7, Perkin-Elmer, USA) as described by Qin et al. [17]. Approximately 5 mg of each sample was placed in an aluminum pan, and then the same amount of distilled water was added to the DSC pan. The pan was sealed and equilibrated at room temperature for 24 h before the measurement. The scanning temperature range was 20–120 °C at a heating rate of 10 °C/min. The gelatinization onset temperature, peak temperature, conclusion temperature, and the gelatinization enthalpy (ΔH) were determined from the recorded DSC thermograms.

3. Results and discussion

To investigate the effect of drying conditions on crystalline structure and crystallinity of the amylose nanoparticles, X-ray diffraction patterns were used for comparison and determination of crystallinity. Generally, the X-ray diffraction patterns for all the dried amylose nanoparticles obtained in this study were similar regardless of the drying conditions, suggesting that the crystalline structure in the amylose nanoparticles was the same. However, the