Surface modification of nanostarch using nano silver: a potential antibacterial for food package coating

Mina Amirsoleimani¹ · Mohammad A. Khalilzadeh¹ · Fatemeh Sadeghifar² · Hasan Sadeghifar^{3,4}

Revised: 18 May 2017 / Accepted: 18 December 2017 / Published online: 10 February 2018
© Association of Food Scientists & Technologists (India) 2018

Abstract Starch nanocrystal was prepared using weak sulfuric acid hydrolysis at 40 °C. Transmission electron micrographs of dilute suspensions of starch nanocrystals showed round particles with a diameter ranging from 20 to 40 nm. SEM of freeze dried samples showed separated particles between 40 and 100 nm and confirmed production of starch nano particles. XRD patterns obtained for the prepared nanostarch and raw starch sample showed no special pattern of crystallinity for starch sample. Extracted nanostarch showed pattern of crystallinity with the peaks at Bragg angles (2 θ) at about 15° and 23°, and a doublet at 17° and 18°. The crystalline structure of prepared sample was A-type. FTIR spectra confirmed the particles oxidation. Nano silver particle was precipitated on the starch nano particle. UV spectra confirmed the presence of silver particle on the starch particles. Inhibitions tests of nanostarch bearing nano silver on three types of bacteria was investigated. The inhibition test results were 25 μ g/mL for *S. aureus*, and *S. typhi*, and 12.5 μ g/mL for *E. coli*.

Keywords Nanostarch · Nano silver · Antibacterial · Nanoparticles · Surface modification

Introduction

Starch is one of the most abundant biomass material in the nature. Plant roots, stalks, crop seeds, and staple crops such as rice, corn, wheat, tapioca, and potato are the main sources of starch (Bule et al. 1998). It consists of mainly two macromolecules including amylose and amylopectin. In most common types of starch, amylose contains between 72 and 82 wt%, and amylopectin is the rest (Sandhu and Singh 2007). Amylose is defined as a linear molecule of glucose units linked by (1-4) α -D-glycoside bonds, slightly branched by (1-6) α -linkages. Amylopectin is a highly branched polymer consisting of relatively short branches of α -D-(1-4) glycopyranose which are interlinked by α -D-(1-6)-glycosidic linkages approximately every 22 glucose units.

Physical structure of starch is in granules shape. The granule particle size depends strongly on its botanic origin. Starch granules have concentric alternating amorphous and semi-crystalline growth rings. X-ray diffraction study showed its semi-crystalline polymer structure. The size of the crystals reported to be 120–400 nm (French 1984).

Many research has published on production of nanoparticles from natural and renewable biopolymers during last decade. Starch become important as a source of bio-based nanoparticles (LeCorre et al. 2010). Different methods were proposed for nanostarch preparation including acid treatment (Dufresne 2014), high power ultrasonication (Haaja et al. 2013), using enzymes and recrystallization (Sun et al. 2014), reactive extrusion (Song et al. 2011), nano precipitation (Chin et al. 2011), self-

✉ Hasan Sadeghifar
hsadegh@ncsu.edu

¹ Department of Phytochemistry, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

² Department of Biology, North Carolina State University, Raleigh, NC, USA

³ Department of Wood and Paper Science, Sari Branch, Islamic Azad University, Sari, Iran

⁴ Department of Forest Biomaterials, North Carolina State University, Raleigh, NC, USA

assembly (Liu et al. 2016), high pressure homogenization (Shia et al. 2011) and alkali freezing treatment (Zhang et al. 2013).

Nano starch with high specific area and surface hydroxyl groups has good potential for surface chemical modification to introduce any desired surface functionality. The accessible hydroxyl group content on the surface of starch nanocrystals reported to be around 14% of the total hydroxyl group in polymer structure which is around $0.0025 \text{ mol g}^{-1}$ of starch nanocrystal (Angellier et al. 2005; Dufresne 2014). The main reported surface modification is grafting different reagents on the surface of the particles (Dufresne 2014; LeCorre et al. 2010) to prepare new material for different application especially in blend with polymers.

In this work, nanostarch was prepared using acid hydrolysis method and its properties were characterized. Then we oxidized surface of nanostarch particles using sodium periodate and then precipitation of nano Silver on its surface through reduction chemistry. Potential of the nanostarch bearing nano silver as an antibacterial substrate was examined.

Materials and methods

Materials

Wheat starch from market, sulphuric acid (H_2SO_4 , 95%) and silver nitrate (AgNO_3) and sodium periodate (NaIO_4 , 99%) were purchased from Aldrich. Dialysis membrane (6–8000 Da) purchased from Spectrum Laboratories, Inc. *Escherichia coli* S2025, *Staphylococcus aureus* S2375 and *Salmonella typhi* PTCC: 1609 were used for antibacterial power assay of AgNPs.

Preparation of starch nanocrystals

Starch (18 g) were mixed in a 150 mL of 3.16 M H_2SO_4 solution at 40 °C and was stirred during 5 days. After hydrolysis, the suspensions were washed by successive centrifugations in distilled water until neutrality and then dialyzed using cellulose membrane against water. The cleaned product was stored at 4 °C with few drops of chloroform. The yield of nano starch was 7.5%.

Periodate oxidation of starch nanocrystals

4 mL of aqueous 0.5 M NaIO_4 was added to 25 mL of a nanocrystal suspension (1 mg/mL). After stirring for 6 h at room temperature, 1 mL of ethylene glycol was added in order to destroy the excess of sodium periodate and then

the solution was dialyzed for 48 h against water. Quantity of the aldehyde functional group was measured using Pommerening method (Pommerening et al. 1992). An aliquot of 10 mL of nanocrystal suspension was heated for 15 min with 5 mL of 0.01 M NaOH; after cooling, excess NaOH was titrated by 0.005 M HCl. Equivalents of NaOH consumed used for calculation of aldehyde functions generated by periodate oxidation.

AgNPs formation

5 mL of starch nanocrystals were added into 5 mL of $5 \times 10^{-3} \text{ M}$ AgNO_3 and 500 μL of 37% NH_4OH . The reaction was maintained at 50 °C during 30 min. The resulting brown suspension was first dialyzed against water for purification and then stored in the dark at 4 °C.

FTIR

The FTIR analysis was performed using a FTIR Bruker-Tensor 270 spectrometer. The freeze dried starch nanocrystals were mixed with analytical grade KBr at a weight ratio of 1/100 mg.

XRD

The crystallinity of the dried starch and nanostarch sample was determined using a Smart lab X-ray diffractometer. The diffractometer was equipped with Be-filtered Cu-K α radiation with a wavelength of 1.54 Å generated at 35 kV and 25 mA. The samples were scanned from a 2 θ range from 5° to 70° at an increment of 0.05°.

Scanning electron microscopy

Scanning electron micrograph was obtained with a LEO 440i scanning electron microscope under vacuum at an operating voltage of 10 kV. Dried starch sample was gold coated by sputtering for 15 s before sample scanning.

Transmission electron microscopy

Transmission Electron Microscopy (TEM) observations were performed using a LEO 906 microscope with an 80 kV voltage, and micrographs were recorded on Kodak film.

Antimicrobial activity

AgNPs (Ag^0 /nano starch) were assayed for their growth inhibition properties against *S. aureus* PTCC: 1431-(ATCC: 25923), *E. coli* PTCC: 1399-(ATCC: 25922) and *S. typhi* PTCC: 1609 using the standard dilution

method which determines the minimum inhibitory concentration (MIC). Tryptic Soy broth was inoculated at 105 colony forming units (CFU)/mL with each microorganism, and nanostarch containing Ag NPs were added at final concentration in silver of 2.5–50 mg/mL and let to grow at 37 °C under gentle shaking at 250 rpm. Absorbance at 600 nm was monitored at approximately 30 min intervals during 10 h of culture. Assays with just nanostarch (without Ag NPs) was used as negative controls.

Results and discussion

Starch nanocrystal was prepared using weak sulfuric acid hydrolysis for 5 days at 40 °C. Then the product was washed and centrifuged several times with distilled water. The final product dialyzed to neutralize using water and remove impurities. A part of purified nanostarch in solution was used for next step reactions (without drying) and the another part was dried using freeze dryer for other analysis.

FTIR absorption bands are shown in Fig. 1. 1430 cm^{-1} band assigned to a symmetric CH_2 bending vibration increased after converting starch into nanostarch. This band known as the “crystallinity band” indicates an increase in its intensity reflects in the degree of crystallinity of the samples (Zhang et al. 2015). Also, the FTIR band at 898 cm^{-1} , assigned to C–O–C stretching, is known as an “amorphous” absorption band. In the starch sample, intensity of the absorption at 897 cm^{-1} is higher than in nanostarch sample which shows starch is more amorphous than prepared nanostarch. These changes is due to differences between the crystalline and amorphous structure (VanderHart and Atalla 1984).

To evaluate the size and shape of starch nano particles and Ag NPs on its surface, TEM and SEM analysis were carried out. Figure 2a shows the transmission electron micrographs (TEM) of diluted suspensions of starch nano crystals prepared using H_2SO_4 hydrolysis. The image

Fig. 1 FTIR spectra of starch and nanostarch samples

Fig. 2 TEM (a) and SEM (b) images of starch nano particles and deposition of Ag NPS on the starch nano particle (c)

shows rounded shape particles with a diameter ranging from 20 to 40 nm. The particles seem to have a degree of aggregation. SEM of freeze dried sample (Fig. 2b) shows separated particles between 40 and 100 nm size and confirm production of starch nanoparticles. Also the Ag NPs are visible on the surface of starch nano particle (Fig. 2c).

Fig. 3 XRD diffraction of raw starch and its nano particles

Starch nanocrystals structure is known to display different X-ray diffraction (XRD) patterns based on their sources (Haaja et al. 2013; Zobel 1964). Figure 3 shows the XRD patterns obtained for the prepared nanostarch and raw starch samples. Raw starch has no special pattern of crystallinity. However, extracted nanostarch showed pattern of crystallinity with the peaks at Bragg angles (2θ) at about 15° and 23° , and a doublet at 17° and 18° . The crystalline structure of prepared nanostarch is A-type which is in agreement with reported results for wheat starch (Corre and Angellier 2014).

The main technique for determining starch crystallinity is comparing of the experimental diagrams to several discrete crystalline diffraction peaks (100% crystallinity diagrams) and an amorphous halo (100% amorphous diagram) (Buléon et al. 1998; Zobel 1988). The prepared sample crystallinity calculated to be around 43%. The degree of crystallinity remains low even after acid hydrolysis shows that an important amount of non-organized materials remains in the prepared nano particles.

Scheme 1 Chemical modification of starch nano-particle

Fig. 4 Nano starch (a) and nanostarch-Ag NPs (b) solution and their UV absorption (c) spectra

Fig. 5 diffusion capacity of the solution into nutrient agar against *S. aureus*, *S. typhi* and *E. coli*

Oxidation of starch nanocrystals and Ag nanoparticle (Ag NPs) deposition

Another part of our study was to obtain Ag nanoparticle (Ag-NPs) deposited directly onto starch nanocrystals and its antibacterial ability. First, we create reductive sites on the starch nano crystals using periodate oxidation (Scheme 1a). Oxidation was carried out at room temperature for 6 h, using NaIO_4 . The solution was then dialyzed for 48 h against distilled water to remove the salts and other impurities. The amount of aldehyde groups per anhydroglucose unit was determined by titration to be 0.34. It is likely that these units are located on the surface of the nanocrystals. At the next step, the aldehyde functions used to reduce Ag^+ into Ag^0 which will aggregate into nanoparticles directly on the surface of nanostarch (Scheme 1b).

Formation of AgNPs carried out by addition of 5×10^{-3} M AgNO_3 at 50 °C for 30 min. The resulting red brown solution was characterized by UV–Vis spectroscopy (Fig. 4). The suspension shows a new absorbance peak at 425 nm in comparison to oxidized nanostarch. This absorbance is due to the resonance of the AgNPs

Fig. 6 Effect of AgNPs against *E. coli*, *S. typhi* and *S. aureus*

(Maneering et al. 2008). Nanostarch showed no absorption in this area.

Antibacterial assays

Diffusion capacity of the starch nanocrystals bearing Ag-NPs into an agar medium investigated using well plate method for three types of bacteria (Fig. 5). The inhibition halo for 10 µg/mL concentration of AgNPs on the three strains was around 11 mm wide. With increasing of concentration to 25 µg/mL, the halo wide increased to around 15 mm for *S. aureus* and *E. coli* and around 12 mm for *S. typhi*. with increasing concentration up to 50 µg/mL, the

halo wide only increased to 17 mm wide. It seems the 25 µg/mL concentration is the optimum concentration as an antibacterial. In general, *E. coli* is more sensitive than two other bacteria.

Figure 6 shows the inhibitions test for all bacteria in different concentration of AgNPs during incubation time from 1 to 10 h. The results showed that the maximum effectiveness of all bacteria was happened around 9 h and levelled off after that.

The results generally showed that modified starch nanocrystals can be a potential for antimicrobial substrate. These nanoparticles can use as antiseptic solutions wound-healing gels and different medical application.

Conclusion

Starch nano particles were prepared using acid hydrolysis of wheat starch. TEM, SEM and XRD results showed starch nano-particles with around 40 nm in size and with around 45% crystallinity. Nano starch bearing nano silver particles were prepared using particle oxidation and precipitation of silver on its surface through reduction chemistry. The nano-starch bearing nanosilver showed good antibacterial potential for three different bacteria including *S. aureus*, *S. typhi*, and *E. coli* which is good potential for application as a coating materials for food packaging and other application.

References

- Angellier H, Molina BS, Belgacem M, Dufresne A (2005) Surface chemical modification of waxy maize starch nanocrystals. *Langmuir* 21:2425–2433
- Bule A, Colonna P, Planchot V, Ball S (1998) Starch granules: structure and biosynthesis. *Int J Biol Macromol* 23:85–112
- Buléon A, Colonna P, Planchot V, Ball SS (1998) Starch granules: structure and biosynthesis. *Int J Biol Macromol* 23:85–112
- Chin SF, Pang SC, Tay SH (2011) Size controlled synthesis of starch nanoparticles by a simple nanoprecipitation method. *Carbohydr Polym* 86:1817–1819
- Corre DL, Angellier CH (2014) Preparation and application of starch nanoparticles for nanocomposites: a review. *React Funct Polym* 85:97–120
- Dufresne A (2014) Crystalline starch based nanoparticles. *Curr Opin Colloid Interface Sci* 19:397–408
- French D (1984) Organization of starch granules. In: BeMiller J, Whistler R (eds) *Starch chemistry and technology*. Academic Press, New York
- Haaja SB, Albert M, Pétrier C, Boufia S (2013) Starch nanoparticles formation via high power ultrasonication. *Carbohydr Polym* 92:1625–1632
- LeCorre D, Bras J, Dufresne A (2010) Starch nanoparticles: a review. *Biomacromolecules* 11:1139–1153
- Liu C, Qin Y, Sun Q, Xiong L, Liu Z (2016) Preparation and characterization of starch nanoparticles via self-assembly at moderate temperature. *Int J Biol Macromol* 84:354–360
- Maneerung T, Tokura S, Rujiravanit R (2008) Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydr Polym* 72:43–51
- Pommerening K, Rein H, Bertram D, Muller R (1992) Estimation of dialdehyde groups in 2,3-dialdehyde bead-cellulose. *Carbohydr Res* 233:219–223
- Sandhu KS, Singh N (2007) Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chem* 101:1499–1507
- Shia A-m, Li D, Wangb L, Li B, Adhikaric B (2011) Preparation of starch-based nanoparticles through high-pressure homogenization and miniemulsion cross-linking: influence of various process parameters on particle size and stability. *Carbohydr Polym* 83:1604–1610
- Song D, Thio YS, Deng Y (2011) Starch nanoparticle formation via reactive extrusion and related mechanism study. *Carbohydr Polym* 85:2008–2014
- Sun Q, Li G, Dai L, Ji N, Xiong L (2014) Grean preparation and characterization of waxy maize starch nanopaticles through enzymolysis and recrystallisation. *Food Chem* 162:223–228
- VanderHart DL, Atalla RH (1984) Studies of microstructure in native celluloses using solid-state carbon-13 NMR. *Macromolecules* 17:1465–1472
- Zhang Z, Shan H, Sun J, Weng Y, Wang X et al (2013) Facile preparation of corn starch nanoparticles by alkalifreezing treatment. *RSC Adv* 3:13406–13411
- Zhang B-C, Azuma J-I, Uyama H (2015) Preparation and characterization of a transparent amorphous cellulose film. *RSC Adv* 5:2900–2907
- Zobel HF (1964) X-ray analysis of starch granules. In: Whistler RL, Smith RJ, BeMiller JN (eds) *Methods in carbohydrate chemistry*, vol 4. Academic Press, New York
- Zobel HF (1988) Starch crystal transformations and their industrial importance. *Starch* 40:1–7