Carbohydrate Polymers 97 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Nano-structure of heat–moisture treated waxy and normal starches

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ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form
23 November 2012
Accepted 12 April 2013
Available online 24 April 2013

Keywords:
Heat–moisture treatment
Atomic force microscopy
Nano-structure
Normal starch
Waxy starch

ABSTRACT

Surface regions of untreated and heat–moisture treated (HMT) normal rice, waxy rice, normal corn, waxy corn, normal potato, and waxy potato starch granules were examined by atomic force microscopy (AFM). AFM images revealed surface roughness of untreated starch granules and protrusions with a diameter of approximately 15–90 nm. After treatment, the smooth surface region on starch granules was observed, especially in normal rice, waxy rice, and normal corn starches. A significant reduction in the size of protrusions on the surface of HMT potato starch granules was also detected. The newly formed structures may act as barriers and retard water penetration into starch granules. The blocklet model of starch granule architecture was also confirmed by the AFM images.

1. Introduction

Heat–moisture treatment (HMT) refers to a physical modification of starch through incubation of starch granules at low moisture levels, below 35%, and heating at high temperature, above the glass transition temperature but below the gelatinization temperature, during a certain period of time (Jacobs & Delcour, 1998). Numerous studies have demonstrated the effects of such treatments on the structure and physicochemical properties of cereal, tuber, and legume starches, including significant changes in X-ray diffraction pattern, crystallinity, granule swelling, amylose leaching, viscosity, gelatinization parameters, retrogradation, and acid/enzyme susceptibility (Chung, Hoover, & Liu, 2009; Hoover & Manuel, 1996; Hormdok & Noomhorm, 2007; Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2011; Juansang, Puttanlek, Rungsardthong, Puncha-Arnon, & Uttapap, 2012; Shih, King, Daigle, An, & Ali, 2007; Singh, Bawa, Riar, & Saxena, 2009; Varatharajan, Hoover, Liu, & Seetharaman, 2010; Vieira & Sarmento, 2008; Watcharatewinkul, Puttanlek, Rungsardthong, & Uttapap, 2009; Watcharatewinkul, Uttapap, Puttanlek, & Rungsardthong, 2010; Zavareze, Storck, Castro, Schirmer, & Dias, 2010). Possible explanations for the observed effects of HMT on starch properties were gathered by Jacobs and Delcour (1998). These included: (1) changes with respect to crystallinity – either a change in the packing arrangement of double helices in starch crystallites from B- to A-type crystallinity, or development of new crystallites in the amorphous regions (formation of amylose crystallites or formation of crystalline amylose–lipid complexes); (2) changes with respect to the amorphous fraction (increase in order, without increase in crystallinity) such as increased interactions between amylose chains or between amylose and amylopectin, extra formation of amylose–lipid complexes, or transformation of amorphous amylose into a helix; and (3) alterations of the interactions between crystallites and the amorphous parts. Besides these, other changes at the molecular, nano- or microstructural level, which have not yet been identified, might also be involved in the physicochemical changes of starch after HMT.

Our previous reports have described the microstructure, physicochemical properties and molecular structure of waxy and normal rice, corn, and potato starches modified by HMT at 100 °C for 16 h with starch moisture content of 25%. Scanning electron micrographs showed that HMT did not change the size, shape or surface characteristics of corn and potato starch granules, while

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0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.04.044

surface change/partial gelatinization was found on granules of rice starches. The crystallinity of starch samples, except waxy potato starch, decreased significantly with HMT. The treated starches (except waxy potato starch) had higher pasting temperature and lower viscosity. Shifts of the gelatinization temperature to higher values and gelatinization enthalpy to lower values, as well as biphasic endotherms, were found in treated starches (Jiranuntakul et al., 2011). The analysis of starch chain distribution and unit chain distribution of amylopectin revealed that HMT did not alter the molecular structure of starch and amylopectin (Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012). The significant decrease in paste viscosity and marked increase in gelatinization temperature, in spite of the decrease in crystallinity and gelatinization enthalpy of HMT starches, are still not well understood. Molecular changes as mentioned above might take part in the observed effects. However, starch granule structure is complex and consists of several levels of organization, i.e. granules, growth rings, blocklets, amorphous and crystalline lamellae, and amylopectin and amylose chains (Pérez & Bertoft, 2010; Vandeputte & Delcour, 2004); therefore, changes in other structural levels could also take place during HMT. Regarding the results obtained, we have speculated that a new superficial layer was formed on the surface of starch granules during HMT, and this layer restricted water penetration into the granules and retarded granule swelling. However, the proposed structure on the surface region of starch granules was not observed by scanning electron microscopy (SEM). This might be due to the fact that resolution of this technique was not high enough to examine the nano-structure of the starch granules. Gunning, Kirby, Morris, Wells, and Brooker (1995) reported that preparation of samples for imaging by coating with metals provided thin surface coats of around 10 nm thickness. Such coats are particulate, leading to the possible misinterpretation of results at very high resolution due to the presence and size of the metal grains. To overcome this problem, a new technique should be employed to explore the nanostructure of the starch granules.

Atomic force microscopy (AFM) is a powerful technique for investigating biological surfaces, such as food materials. This technique has a high magnification with high resolution for observing nanometer-scale structures, and a high capacity for imaging samples in either atmospheric or aqueous environments without any dyeing, metal coating, or fluorescence of samples; this allows the observation of the sample structure in its nearly native condition (Yang et al., 2007). This technique has been applied by several researchers to study the external and internal structures of starch granules (Ayoub, Ohtani, & Sugiyama, 2006; Baldwin, Adler, Davies, & Melia, 1998; Juszczak, Fortuna, & Krok, 2003a,b; Ohtani, Yoshino, Hagiwara, & Maekawa, 2000; Ridout, Parker, Hedley, Bogracheva, & Morris, 2004). Depressions and protrusions on the surface of potato, tapioca, barley, oat, maize, waxy maize, wheat, rye, and rice starch granules were also viewed by AFM operated in non-contact mode (Juszczak et al., 2003a,b). Ohtani et al. (2000) observed protrusions approximately 30 nm in diameter on the outermost surface of rice, corn, wheat, potato, and sweet potato starch granules, physically destroyed by a glass homogenizer. A significant change in the surface structure of rice starch granules, which was disordered using plasticizing combined with a lyophilization process, was detected by AFM. The average size of protrusions on the granule surface of rice starch decreased from 100 to 35 and 25 nm after the second and the third disorder processes, respectively (Ayoub et al., 2006).

In this study, AFM, operated in intermittent contact mode, was conducted to investigate changes of the surface structure of waxy and normal rice, corn, and potato starch granules after HMT at 100 °C for 16 h with a moisture content of 25%. AFM images of starch granules prior to and following HMT were examined and analyzed for the size of protrusions and the width and depth of existing pores or pits. Alterations of these surface characteristics were traced and compared among the starch samples. Associations of the surface changes with the crystalline structure and gelatinization properties of HMT starches were also discussed.

2. Materials and methods

2.1. Materials

Normal rice (Pathum Thani 60) and waxy rice (RD 6) were provided by the Pathum Thani Rice Research Center, Thanyaburi, Pathum Thani, Thailand. Normal corn (sweet type), waxy corn, and normal potatoes were purchased at a local market in Bangkok, Thailand. Waxy potato starch (3.92% amylose content) was provided by National Starch Food Innovation.

2.2. Starch isolation

Normal rice, waxy rice, normal corn, waxy corn, and normal potato starches, with amylose contents of 21.72%, 1.64%, 25.19%, 2.06%, and 28.97%, respectively, were isolated from their sources by the methods described by Jiranuntakul et al. (2011).

2.3. Heat–moisture treatment

Native starch was brought to the desired moisture content (25%) by soaking 200 g of starch in 600 mL of water overnight at 4 °C. Excess water in the equilibrated slurry was drawn out by vacuum suction to obtain a starch cake. The cake obtained was rendered into small lumps by pressing the cake through a sieve (mesh size 2.36 mm) onto a tray. Water in the starch lumps was then evaporated by open air-drying until the moisture content dropped to 25%. Starch samples with adjusted moisture content were placed in 200 mL screw-capped bottles and heated in a hot-air oven at 100 °C for 16 h. After HMT, the bottles were opened and the starches were dried at 40 °C until the moisture content reached 10%.

2.4. Sample preparation for AFM imaging

Immobilization of starch granules was performed before AFM imaging by the following procedure. Untreated or HMT starch granules (20 mg) were mixed with 1 mL of MilliQ water (Millipore, Billerica MA). Then 50 μL samples of starch suspensions were taken with a pipette and dropped onto a glass slide. Excess water was removed from the sample by heating at 40 °C for 30 min using a temperature controllable hot plate.

2.5. Atomic force microscopy

AFM images of untreated or HMT starches were obtained using an AFM (Nanowizard; JPK Instruments AG, Berlin, Germany) and a standard silicon tip cantilever (OMCL-AC160TS-C2; Olympus, Japan) with typical resonant frequency and spring constant of 300 kHz and 42 N/m, respectively. The measurements were performed in air at ambient temperature (25 °C) and humidity using intermittent contact mode (also called tapping mode). Scan areas varied between 500 nm × 500 nm and 40 μm × 40 μm, with scan frequency in the range of 0.1–1.0 Hz. For each starch sample, topographic and error signal mode images of approximately 40–50 starch granules were collected, and a typical representative sample was selected as an illustrative result. Bright regions in the AFM topographic images correspond to peak features in height. The error signal mode was used to enhance the edge contrast of the sample (Ohtani et al., 2000). JPK Image Processing software (JPK Instruments) was used for analysis of the AFM images.