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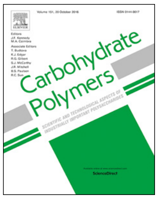

Multi-scale structures of cassava and potato starch fractions varying in granule size

Liang Zhang[a], Yan Zhao[a], Wei Hu[a], Jian-Ya Qian[a,*], Xiang-Li Ding[a], Cheng-Ran Guan[a,b], Yu-Qi Lu[a], Yan Cao[a]

[a] *School of Food Science and Engineering, Yangzhou University, Huayang Xilu 196, Yangzhou, Jiangsu 225127, People's Republic of China*
[b] *Jiangsu Key Laboratory of Dairy Biotechnology and Safety Control, Yangzhou University, Huayang Xilu 196, Yangzhou, Jiangsu, 225127, People's Republic of China*

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ABSTRACT

Large-, medium-, and small-sized granules were separated from cassava and potato starches. Fourier transform infrared spectroscopy, wide angle X-ray diffraction, small angle X-ray scattering, nuclear magnetic resonance and scanning electron microscope were used to investigate the supramolecular structures of cassava and potato starch fractions. The crystallinity of small-sized potato starch (SPS) was lower than that of its counterparts, while crystallinities of all cassava fractions were similar. The contents of lamellar structure of small-sized granules were the smallest, while those of their counterparts were similar. The lamellar repeat distance was similar for the starch fractions. Self-similar structure of SPS showed mass fractal with the lowest compactness, while that of small-sized corn starch showed surface fractal with the largest compactness. The NMR test revealed that SPS had the highest total double helix content, while its counterparts showed similar values. Moreover, a new ^{13}C peak at 64 ppm was observed for SPS.

1. Introduction

Starches from the same variety are always existing with varying sizes (1–100 μm) and shapes (spherical, lenticular, polyhedral, and irregular) (Ao & Jane, 2007). Wheat, barley, and triticale starches have a bimodal granule size distribution. These large and small fractioned granules from bimodal starches significantly differ in their molecular, supramolecular structures, functional properties and end uses (Takeda, Takeda, Mizukami, & Hanashiro, 1999; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001; Tang, Watanabe, & Mitsunaga, 2002; Zhang, Li, Liu, Xie, & Chen, 2013). Some unimodal starches which covered a wide range of granule sizes, such as potato and maize starches, were also divided into large-, medium-, and small-sized fractions (Cai et al., 2014; Dhital, Shrestha, Hasjim, & Gidley, 2011; Kaur, Singh, McCarthy, & Singh, 2007; Lin et al., 2016; Noda et al., 2005; Singh & Kaur, 2004; Utrilla-Coello, Agama-Acevedo, de la Rosa, Rodríguez-Ambriz, & Bello-Pérez, 2010). These fractions from native maize starch showed similar multi-structures and functional properties; however, fractions from high-amylose maize starch had significantly different multi-structures, thus resulting in their different functional properties (water solubility, gelatinization enthalpy, and hydrolysis and digestion degrees) (Cai et al., 2014; Lin et al., 2016). For potato starch, it is already known that larger granules have higher peak, trough, and final pasting viscosities and show lower gelatinization temperature and digestibility than smaller granules. And these functional properties (swelling, gelatinization property and digestibility) are closely connected to their compositions and multiscale structures (molecular, short-range ordered, crystalline, fractal, lamellar structure and so on) (Dhital et al., 2011; Kaur et al., 2007; Noda et al., 2005; Singh & Kaur, 2004). The composition and molecular structures (branch-chain length distribution of amylopectin) of these fractioned potato starches were studied comprehensively. The results showed that lower amylose content and higher phosphorus content were observed for smaller granules and the amylopectin of all the fractions showed similar branch-chain length distribution. However, little was known about the supramolecular structures of these potato starch fractions.

Moreover, to our knowledge, cassava starch, another kind of unimodal starches which covered a relative narrow range of granule sizes, was rarely studied. This may be because that it is hard to separate the starch fractions of a relative narrow particle distribution range. Many ways were used to separate starch fractions, such as microsieving (Kaur et al., 2007), extreme sedimentation (Ao & Jane, 2007), differential sedimentation (Takeda et al., 1999), differential supernatant (Dhital, Shrestha, & Gidley, 2010) and glycerol centrifugation (Peng, Gao,

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[*] Corresponding author.
E-mail address: jyqian@yzu.edu.cn (J.-Y. Qian).

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Abdel-Aal, Hucl, & Chibbar, 1999). Microsieving and differential sedimentation could not completely separate large-, medium-, and small-sized fractions (Peng et al., 1999). For extreme sedimentation and differential supernatant separation, it takes very long to obtain enough small-sized granules, what's more, it is also hard to separate starches of a relative narrow distribution range due to the smaller difference among sedimentation rates of different-sized granules in water. However, in glycerol solution, the difference among sedimentation rates of heterogeneous granules is large enough to separate the different-sized granules of a smaller distribution range. Moreover, glycerol-assisted sedimentation has an extensive application range compared with others since it is easier to separate different kinds of starches by adjusting the glycerol/water proportion.

In this study, glycerol-assisted sedimentation method is adopted to extract cassava and potato starches. Different concentrations of glycerol were chosen to obtain the best separation effect of these two kind of starches. X-ray powder diffraction (XRD), attenuated total reflectance Fourier transform infrared (ATR-FTIR) and solid-state ^{13}C cross-polarization magic-angle spinning nuclear magnetic resonance (13C CP/MAS NMR) were used to study the crystalline, short ranged ordered structure, single helix and double helix of starch fractions. Small-angel X-ray diffraction scattering (SAXS) is used to study the lamellar and fractal structure of these fractions. The objective of this study was to investigate the structure of starch fractions from two kinds of unimodal starches with different crystalline types, which could enrich our understanding of multiscale structure of cassava and potato starch fractions. Furthermore, it offers explanations for the different functional properties of these starch fractions.

2. Materials and methods

2.1. Materials

Potato starch was bought from Shanghai Rongya Trading Co., Ltd; Cassava starch was bought from Chaodee trading Co., Ltd. Glycerol was bought from Sinopharm Chemical Reagent Co., Ltd.

2.2. Starch fractionation according to granule size

Glycerol-assisted sedimentation has an extensive application range since it is easier to separate different kinds of starches through adjusting the glycerol concentration. Some pre-experiments were carried out to choose the proper glycerol solutions to fractionate potato and cassava starches. It was found that for potato starch, glycerol solutions of 90% and 75% concentration showed relative good separation effect, while for cassava starch, glycerol solutions of 85% and 60% concentration was much more proper.

Separation steps:

1) Potato starch: The starch was fractionated according to Peng et al. (1999) with some modifications. 40 mL of starch suspension (2.5% in 90% glycerol, w/w) was centrifuged at 100*g* for 5 min. The supernatant was removed to a beaker. The pellet was resuspended with 40 mL of 90% glycerol and centrifuged for five times to obtain starch precipitate that constituted the large-sized fraction. The supernatants were pooled and centrifuged at 5000*g* for 15 min. The resulting starch pellet was suspended with 40 mL of 75% glycerol and centrifuged at 100*g* for 5 min. The supernatant was removed to a beaker. The pellet was suspended with 40 mL of 75% glycerol and centrifuged for five times to obtain starch precipitate that constituted the medium-sized fraction. The supernatants were pooled and centrifuged at 5000*g* for 15 min. The resulting starch pellet comprised the small-sized fraction. Finally, the starch fractions were washed and dried at 40 °C for 2 days, ground into powders in a mortar with pestle, and passed through a 100 mesh sieve.
2) Cassava starch: The fraction method is similar with the way to separate potato starch, except that another two kinds of glycerol solutions (85% and 60%) were used to sedimentate the large-sized fraction and medium-sized granules, respectively.

2.3. Scanning electron microscopy (SEM)

Specimens were glued on specimen stubs using silver conducting tape and coated with gold-palladium using a sputter coater (BAL-TEC SCD 500, Liechtenstein). The cross section morphology of the cast films was observed using an environmental scanning electron microscope (XL 30 ESEM, Philips, Holland) with the parameters HJ: 20 kV, resolution ratio: 3.4 nm, and spot: 4.

2.4. Granule size analysis

Granule size of these starch fractions was measured by a laser diffraction particle size analyzer (S3500, Microtrac, USA). The starches were suspended in distilled water and stirred. The obscuration in all measurements was > 10%. Particle size distribution curve was obtained and particle size was defined in terms of the 10th percentile [d(0.1)], median [d(0.5)], 90th percentile [d(0.9)].

2.5. Attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy

Each sample was analyzed in an ATR FT-IR spectrometer (Cary 610/670, Varian, USA). The film was placed in a horizontal ATR accessory with a zinc selenide prism for the analysis. All spectra were obtained through 52 scans at a resolution of 4 cm^{-1} at ambient temperature.

2.6. Solid-state ^{13}C CP/MAS NMR analysis

The solid-state NMR experiment was performed on a Bruker Avance III 400WB spectrometer (Bruker Biospin GmbH, Germany) operating at a ^{13}C frequency of 100.63 MHz with a double resonance H/X CP-MAS 7 mm probe as described previously by Cai et al. (2014). The proton decoupled ^{31}P NMR spectra were also acquired on this machine.

2.7. X-ray diffractionmeasurement (XRD)

XRD pattern of these film samples were identified by using a Bruker AXS D8 Advance X-ray diffractometer (Bruker Inc., Germany) with Ni-filtered Cu Kα radiation at a voltage of 40 kV and a current of 40 mA ($\lambda = 0.154$ nm). The scanning scope of 2θ was 5–60° at a scanning rate of 3°/min.

The relative crystallinity of starch was calculated as the percentage ratio of diffraction peak area (crystalline area: a_c) to total diffraction area (a_t) according to Nara and Komiya (1983). The diffraction peak was separated with the smooth curve connecting each point of the peak valley in the range of 30° and 4°, and the area between the diffraction curve and the smooth curve was regarded as diffraction peak area (crystalline area: a_c). The area between the diffraction curve and the straight line joining the two points of intensity at 30° and 4° was regarded as the total area (a_t).

2.8. Small angle X-ray scattering measurement (SAXS)

SAXS measurement of starch was performed according to Cai et al. (2014). by using a Bruker NanoStar SAXS instrument equipped with Vantec 2000 detector and pinhole collimation for point focus geometry. The X-ray source was a copper rotating anode (0.1 mm filament) operating at 50 kV and 30 W, fitted with cross-coupled Göbel mirrors, resulting in Cu Kα radiation wavelength of 1.5418 Å. The optics and sample chamber were under vacuum to minimize air scattering. During X-ray exposure, the starch slurries were kept in sealed cells to prevent dehydration. SAXS data sets were analyzed using DIFFRAC plus