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Physicochemical properties of rhizome starch from a traditional Chinese medicinal plant of *Anemone altaica*

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ABSTRACT

This study investigated the physicochemical properties of rhizome starch of *A. altaica* for the first time. The results were compared to those obtained from two common starches (potato and rice). The rhizome had a starch content of 49.8%. Isolated starch granules were mostly oval in shape with a central Maltese cross and an average long axis of 6.25 μm. The starch contained 35.5% amylose and had lower gelatinization and pasting temperatures than rice and potato starches and a swelling power comparable to potato. Altaica starch had high breakdown and setback viscosities. X-ray diffraction revealed B-type starch with relative degree of crystallinity of 17.5%. Starch possessed a high susceptibility to hydrolysis by acid, porcine pancreatic α-amylase and *Aspergillus niger* amyloglucosidase when compared with potato and rice starches.

1. Introduction

Anemone altaica Fisch. ex C. A. Mey, belonging to the family Ranunculaceae, is widespread throughout the north of Asia and Europe (The Editorial Board of Flora of China, 1980). The rhizomes of *A. altaica* are believed to have anti-inflammatory and analgesic properties and have been used in the treatment of epilepsia, neurasthenia, and arthritis in traditional Chinese medicine for a long time (Wu, Zhou, & Xiao, 1988). Previous studies on the chemical constituents of *A. altaica* lead to the isolation, purification and structure identification of some small-molecule active ingredients, such as anemonin, palmitic acid, succinic acid, isoferulic acid, cirsiumaldehyde, and carboxymethyl isoferulate (Zou & Yang, 2008; Zou, Dong, & Yang, 2005). However, the macromolecules such as starch contained in the rhizome of *A. altaica* and other medicinal plants have hardly been studied and are therefore not utilized (Wang, Gao, Chen, & Xiao, 2006). In order to make good use of traditional Chinese medicinal plant of *A. altaica* and widen its application, starch was isolated from the rhizome of *A. altaica*. The physicochemical properties of *A. altaica* starch were investigated for the first time.

2. Materials and methods

2.1. Plant material

The rhizome of *A. altaica* was collected from a valley (N34°18.209′, E106°33.507′, alt. about 1400 m) of Xiaolongshan Mountain in Tianshui, Gansu Province, in May, 2011. The tuber of potato (*Solanum tuberosum* L.) was purchased from a local natural food market (Yangzhou City, China). Mature grain of rice (*Oryza sativa* L.) cv. Teqing was obtained from Agricultural College of Yangzhou University and grown in the university experimental field, Yangzhou, China, during the growing season.

2.2. Starch content

Quantitative determination of starch contents in dry *A. altaica* rhizome and rice grain was carried out according to the colorimetric method of anthrone-H_2SO_4 (McCready, Guggolz, Silviera, & Owens, 1949). The experiments were performed in triplicate.

2.3. Isolation of native starches

Native starch granules were isolated following a method described by Wei, Qin, Zhu, et al. (2010) with a slight modification.

Abbreviations: AAG, *Aspergillus niger* amyloglucosidase; ATR-FTIR, attenuated total reflectance-Fourier transform infrared; DSC, differential scanning calorimetry; PPA, porcine pancreatic α-amylase; RVA, rapid visco analyzer; XRD, X-ray powder diffraction.

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Briefly, the rhizome of *A. altaica* and the tuber of potato were sliced into small pieces. The rice grains were steeped in double-distilled water at 4 °C for 16 h. The rhizome and tuber pieces and softened grains were homogenized with ice-cold water in a home blender. The homogenate was squeezed through four layers of cheesecloth by hand. The fibrous residue was homogenized and squeezed twice more with ice-cold water to facilitate the release of starch granules from the fibers. The combined extract was filtered with 100, 200, 300 and 400 mesh sieves and centrifuged at $3000 \times g$ for 10 min. The yellow gel-like layer on top of the packed white starch granule pellet was carefully scraped off and discarded. The process of centrifugation separation was repeated several times until no dirty material existed. The white starch sediment was further washed with anhydrous ethanol, dried at 40 °C, ground into powders, and passed through a 100-mesh sieve.

2.4. Morphology observation of starches

A starch suspension (1%, w/v) was prepared with 50% glycerol. A small drop of starch suspension was placed on the microscope slide and covered with a coverslip. The starch granule shape and Maltese cross were viewed with an Olympus BX53 polarized light microscope equipped with a CCD camera.

2.5. Particle size analysis of starches

The sizes of starch granules were analyzed. Images of starch granules were analyzed using JEDA-801D morphological image analysis system (Jiangsu JEDA Science-Technology Development Co., Ltd., Nanjing, China). More than 1 000 starch granules were analyzed per sample. Starch granules were grouped according to the long axis length, and the number of starch granules in each group was counted. Plotting the relative number of starch granules against the long axis length produced a starch granule size-distribution curve.

2.6. Amylose content determination

Amylose content of starches was determined using the iodine adsorption method of Konik-Rose et al. (2007) with some modifications. About 10 mg of starch was weighed (accurate to 0.1 mg) into a 10 ml screwcapped tube, then dissolved in 5 ml of urea dimethyl sulfoxide (UDMSO) solution. Dissolution was obtained by incubating the mixture at 95 °C for 1 h with intermittent vortexing. A 1 ml aliquot of the starch-UDMSO solution was treated with 1 ml of iodine solution (0.2% I_2 and 2% KI, w/v) and made up to 50 ml with water. The solution was immediately mixed and placed in darkness for 20 min. Apparent amylose content was evaluated from absorbance at 620 nm. The recorded values were converted to percent of amylose by reference to a standard curve prepared with amylose from potato (Sigma–Aldrich A-0512) and amylopectin from corn (Sigma–Aldrich 10120). The experiments were performed in triplicate.

2.7. Thermal property of starches

Thermal property of starches was measured using a differential scanning calorimetry (DSC) (200-F3, NETZSCH, Germany) as described previously by Wei et al. (2011). Starch (~5 mg, dry starch basis) was precisely weighed and mixed with 3 times (by weight) deionized-distilled water (~15 μl). The mixture was sealed in an aluminum pan overnight at 4 °C. After equilibrating for 1 h at room temperature, the starch sample was then heated from 25 to 110 °C at a rate of 10 °C/min. The experiments were performed in duplicate.

2.8. Swelling power determination of starches

Swelling power of starches was determined by heating starch-water slurries in a water bath at temperatures ranging from 40 to 95 °C in 5 °C intervals according to the procedures of Wei et al. (2011). The swelling power determination was performed in triplicate.

2.9. Pasting property of starches

The pasting property of starches (8% solids) was evaluated in triplicate with a Rapid Visco Analyzer (RVA-3D, Newport Scientific, Narrabeen, Australia). A programmed heating and cooling cycle was used, where the samples were held at 50 °C for 1 min, heated to 95 °C at a rate of 12 °C/min, maintained at 95 °C for 2.5 min, cooled to 50 °C at a rate of 12 °C/min, and then held at 50 °C for 1.4 min. Parameters recorded were pasting temperature, peak viscosity, hot viscosity, final viscosity, breakdown viscosity (peak-hot viscosity) and setback viscosity (final-hot viscosity).

2.10. Crystal structure analysis of starches

Crystal structure of starches was analyzed on an X-ray powder diffraction (XRD) (D8, Bruker, Germany) spectroscope. The XRD analysis and determination of the relative degree of crystallinity (%) of the starches were carried out following the method described by Wei, Qin, Zhou, et al. (2010). Before measurements, all the specimens were stored in a dessicator where a saturated solution of NaCl maintained a constant humidity atmosphere (relative humidity = 75%) for 1 week.

2.11. Structural order of starch external region

Ordered structure of starch external region was analyzed on a Varian 7 000 Fourier transform infrared (FTIR) spectrometer with a DTGS detector equipped with an attenuated total reflectance (ATR) single reflectance cell containing a germanium crystal (45° incidence-angle) (PIKE Technologies, USA) as previously described by Wei, Xu, et al. (2010). Original spectra were corrected by subtraction of the baseline in the region from 1200 to 800 cm^{-1} before deconvolution was applied using Resolutions Pro. 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, 1022, and 995 cm^{-1} were performed on the deconvoluted spectra by recording the height of the absorbance bands from the baseline using Adobe Photoshop 7.0 image software.

2.12. Hydrolysis of starch

Starches were hydrolyzed by porcine pancreatic α-amylase (EC 3.2.1.1) (PPA) (Sigma–Aldrich A-3176), *Aspergillus niger* amyloglucosidase (EC 3.2.1.3) (AAG) (Sigma–Aldrich A-7095) and HCl. The hydrolysis rates of starches by PPA and AAG were determined using the method of Li, Vasanthan, Hoover, & Rossnagel (2004) with some modifications. For PPA hydrolysis, isolated native starch (10 mg) was suspended in 2 ml of enzyme solution (0.1 M phosphate sodium buffer, pH 6.9, 25 mM NaCl, 5 mM $CaCl_2$, 0.02% NaN_3, 50 U PPA). For AAG hydrolysis, starch (10 mg) was suspended in 2 ml of enzyme solution (0.05 M acetate buffer, pH 4.5, 5 U AAG). The hydrolyses of PPA and AAG were conducted in a constant temperature shaking water bath with continuous shaking (100 rpm) at 37 and 55 °C, respectively, for 1, 2, 4, 8, 12, 24, 48, and 72 h.