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Functional characteristics of starches from the root of *Cynanchum auriculatum* Royle ex Wight grown in China

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ABSTRACT

Starches isolated from two types of *Cynanchum auriculatum* Royle ex Wight roots grown in China (BW 201001 and HSW 6-18) were investigated and compared to commercial potato starch. The protein, lipid, ash and amylose content of BW 201001 and HSW 6-18 starches were 0.12%, 0.15%, 0.16%, 17.48%, and 0.15%, 0.13%, 0.11%, 21.10%, respectively. The starch granules were irregular with a round or polygonal shape and a size ranging between 5 and 15 μm. Compared to HSW 6-18 starch, BW 201001 starch had a higher solubility, water binding capacity, average chain length, and proportion of B1 chains, and a lower swelling power, syneresis, and proportion of A chains. Both starches showed the characteristic B type X-ray pattern. The degree of crystallinity and R(1047/1022) of BW 201001 and HSW 6-18 were 43.03%, 0.50, and 38.78%, 0.42, respectively. Gelatinisation temperatures followed the order of BW 201001 > HSW 6-18. The peak viscosity, setback, and final viscosity of BW 201001 were lower than those of HSW 6-18 starch. Both starches exhibited lower RDS and higher RS, which was related to their crystal and molecular structures.

1. Introduction

Starch is the most important polysaccharide reserve in higher plants, taking the form of birefringent, semi-crystalline granules. Thus, starch deserves detailed research to understand its biochemical and functional characteristics more fully and to outline its varieties. Because of their ready availability and their extensive utilisation for several centuries in food and non-food commercial applications (BeMiller & Whistler, 2009; Srichuwong & Jane, 2007), starches separated from a variety of plant sources, such as cereal grains (corn, wheat, and rice), roots (cassava and sweet potato), and tubers (potato), have been studied extensively. Their properties and functionalities are significantly different from each other, which is attributable to the diversity of the starch sources (Hoover, 2001; Miao, Zhang, & Jiang, 2009; Miao, Zhang, Mu, & Jiang, 2011; Srichuwong & Jane, 2007).

The root of *Cynanchum auriculatum* Royle ex Wight, a member of the Asclepiadaceae family, is commonly known as Baishouwu or Erye niupixiao. It is a very famous traditional Chinese herbal medicine and is widely distributed in China, Korea, Japan, and India (Lu, Teng, Yang, & Mei, 2011; Shan et al., 2005; Sun, Liu, Wang, Xiang, & Zhu, 2009). It has been used in the clinic as a beneficial and tonic agent for more than 1000 years. Its clinical uses include the treatment of geriatric diseases, the enhancing of immunity and the prolongation of life (Lu et al., 2011; Shan et al., 2005). As reported in the literature, there are a variety of bioactive components contained in *C. auriculatum*, including steroidal glycosides, acetophenones, flavonoids, phospholipids, polysaccharides, etc. (Lu et al., 2011; Qi et al., 2009; Sun et al., 2009). In the rhizomes of *C. auriculatum* cultivars, starch was the main component, comprising up to 40–70% of the total biomass (Shan et al., 2005). A survey of the literature reveals very little investigation of starch separated from *C. auriculatum*. Therefore, we considered it worthwhile to determine the composition and physicochemical properties of starches from two different roots of *C. auriculatum* Royle ex Wight cultivars grown in China. The properties of these starches were compared with those of potato starch.

2. Materials and methods

2.1. Materials

Two different roots of *C. auriculatum* Royle ex Wight cultivars (BW 201001 and HSW 6-18) were collected from Binhai Binhuai Farm (Yancheng, Jiangsu Province, China). Potato starch was a generous gift from Wuxi Thaihua Starch Co. Ltd., China. Pancreatin

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from porcine pancreas (Cat. No. P-1625, activity 3 × USP/g) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Amyloglucosidase (EC 3.2.1.3, 3300 U/ml) and glucose oxidase-peroxidase assay kits (Cat. No. K-GLUC) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All chemicals used in the experiment were reagent grade and were obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

2.2. Starch isolation

Washed *C. auriculatum* Royle ex Wight roots were disintegrated in a laboratory blender with enough deionised water to form a slurry, which was filtered through 100-mesh sieves and then centrifuged at 3000 × *g* for 10 min. The sediment was washed thoroughly with distilled water. This step was repeated three times. The starch was then collected and dried in a mechanical convection oven at 40 °C for 12 h. The dried starch cake was ground and passed through an 80-mesh screen. Finally, the starch samples were stored in a closed container until characterisation studies were carried out, within a few months of the samples' collection.

2.3. Chemical composition

Quantitative estimation of the sample moisture, ash, lipid, and protein contents was executed according to the AACC standard methods (44-19.01, 08-17.01, 58-15.01 and 46-09.01, respectively) (AACC International, 2010). Total amylose content was determined using the iodometric method reported in an earlier publication (Miao, Zhang, et al., 2009).

2.4. Granule morphology

Micrographs of the starch samples were obtained using a scanning electron microscope (Quanta-200, FEI, Eindhoven, The Netherlands). Dried, finely ground samples were mounted on an aluminum stub using double-sided stick tape and coated with a thin film of gold (10 nm), then examined at an accelerating voltage of 5.0 kV.

The birefringence patterns of the starch granules were photographed with an XP-201 microscope (Shanghai Caikon Optical Instruments Factory, Shanghai, China) attached with an exposure control. Two polaroid filters were used with the microscope for birefringence examination.

2.5. Swelling power and solubility

The swelling power and solubility of the starch granules were determined in triplicate using the procedure described by Miao, Zhang, et al. (2009).

2.6. Freeze–thaw stability

Starch suspension (6%, w/v) was heated at 90 °C for 15 min in a temperature controlled water bath, followed by rapid cooling in an ice water bath to room temperature. The starch sample was stored at −10 °C for 24 h and at 30 °C for 12 h followed by centrifugation at 3000 × *g* for 10 min. Syneresis was measured (in triplicate) as percent of water separated after five cycles of freezing and thawing.

2.7. Water binding properties

The water binding capacity of the starch was determined using the method modified by Miao, Zhang, et al. (2009).

2.8. Branch chain length distribution

The chain length distribution of the starch was determined by high-pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Starch samples (10 mg) were dissolved with 2 ml of $NaNO_3$ solution (pH 4.0, 0.1 M) and heated in a boiling water bath for 10 min. Isoamylase (0.5 U) was added to each dispersion, and the mixtures were incubated at 40 °C with shaking for 24 h. Then, the solution was heated in a boiling water bath for 10 min to deactivate the enzyme. The debranched sample solutions were filtered through a 0.45-μm membrane filter and then injected into the HPAEC-PAD system (50 μl sample loop). The HPAEC-PAD system consisted of a Dionex DX 600 equipped with an ED 50 electrochemical detector with a gold working electrode, GP 50 gradient pump, LC 30 chromatography oven, and AS 40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: T1 = 0.40 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = −0.15 V. Data were collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA, USA). Eluents were prepared in distilled deionized water with helium sparging; eluent A was 150 mM NaOH, and eluent B was 50 mM sodium acetate in 150 mM NaOH. Linear components were separated on a Dionex CarboPac™ PA1 column with gradient elution (40% of eluent B at 0 min, 50% at 2 min, 60% at 10 min, and 80% at 40 min) at 30 °C and a flow rate of 1 ml/min.

2.9. Fourier transform infrared spectroscopy

All infrared spectra were obtained with a Nicolet Nexus 470 spectrometer (Thermo Electron Corporation, Waltham, MA, USA) equipped with a deuterated triglycine sulfate (DTGS) detector using the Digilab attenuated total reflectance (ATR) accessory at 4 cm^{-1} resolution by 64 scans. An ATR cell with a Ge crystal was used. The ATR cell was allowed to equilibrate at room temperature before each measurement. Each spectrum recorded against an empty cell as background and was subtracted from the spectrum of air. Spectra were baseline-corrected and deconvoluted by drawing a straight line at 1200 and 800 cm^{-1} (using Omnic version 6.2 software). A half-width of 26 cm^{-1} and a resolution enhancement factor of 2.4 were employed. The ratio of absorbance height at 1047 cm^{-1} to the height at 1022 cm^{-1} was obtained on the deconvoluted spectra.

2.10. X-ray diffraction

X-ray diffraction analysis was performed with an X'Pert PRO X-ray powder diffractometer (PANalytical, Almelo, The Netherlands) operating at 40 kV and 40 mA with Cu Kα radiation (λ = 1.5406 Å). The starch powders scanned at a rate of 2°/min from 2θ 5° to 35° at room temperature. The degree of crystallinity was calculated according to the equation below: $X_c = A_c/(A_a + A_c)$, where X_c = degree of crystallinity, A_c = crystalline area and A_a = amorphous area on the X-ray diffractogram.

2.11. Thermal properties

The gelatinisation and retrogradation parameters of each starch sample were examined using a differential scanning calorimeter (Pyris-1, Perkin Elmer Inc., Norwalk, CT, USA) equipped with a refrigerated cooling system. Approximately 3 mg anhydrous starch samples were mixed with 6 mg deionized water and hermetically sealed in an aluminum pan. Samples were allowed to equilibrate for 12 h at room temperature, then scanned at a heating rate of 10 °C/min from 10 to 150 °C. The differential scanning calorimetry analyzer was calibrated using indium as a standard and an

empty aluminum pan was used as the reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH) were calculated automatically.

2.12. Pasting properties

Starch samples (3 g, on a 14% moisture basis) were mixed with water (25 ml) in a canister and heated in an RVA (Newport Scientific Pvt. Ltd., Warriewood, Australia) at a rate of 5 °C/min to 95 °C, maintained at 95 °C for 7 min, and cooled at a rate of 6 °C/min to 50 °C. Changes in viscosity during heating, cooking, and cooling were recorded, and the gelatinisation temperature and peak, final, breakdown, and setback viscosities were noted.

2.13. In vitro digestion

The digestibility of each starch was analyzed according to the procedure of Englyst, Kingman, and Cummings (1992) with a slight modification. To prepare Enzyme Solution I, amyloglucosidase solution (0.14 ml) was diluted to 6.0 ml with deionised water. Enzyme Solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 ml) with magnetic stirring for 10 min the centrifuging the mixture for 10 min at 1500 × *g*. Finally, a portion (54.0 ml) of the supernatant was transferred into a beaker. Enzyme Solution III was prepared immediately before use by mixing water (4.0 ml), Enzyme Solution I (6.0 ml), and Enzyme Solution II (54.0 ml).

A starch sample (200 mg) was dissolved in 15 ml of phosphate buffer (0.2 M, pH 5.2) by vortexing. After the solution was equilibrated at 37 °C for 5 min, seven glass balls (10 mm diameter) and Enzyme Solution III (5.0 ml) were added. Then, the samples were shaken in a in a 37 °C water bath at 150 rpm. Aliquots of hydrolyzed solution (0.5 ml) were taken at different time intervals and mixed with 4 ml of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolysates was determined using glucose oxidase/peroxidase (GOPOD) assay kits (Wicklow, Ireland). The percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analyzed in triplicate.

The values of different starch fractions (RDS, SDS, and RS) were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) using the following formulas:

$$\%\mathrm{RDS} = (\mathrm{G120} - \mathrm{FG}) \times 0.9 \times 100$$

$$\%\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9 \times 100$$

$$\%\mathrm{RS} = (\mathrm{TG} - \mathrm{FG}) \times 0.9 \times 100 - (\mathrm{RDS} + \mathrm{SDS})$$

3. Results and discussion

3.1. Chemical compositions

Data concerning the composition of starches found in *C. auriculatum* Royle ex Wight are given in Table 1. The purity of the starches is judged on the basis of composition (low protein, low lipid or low ash content) and through microscopic examination. There was no significant difference between BW 201001 and HSW 6-18 starches with respect to their lipid (0.12–0.15%), protein (0.13–0.15%) and ash (0.11–0.16%) contents (Table 1). These values indicated that our procedure for isolating starch was suitable and that the starch was relatively pure. HSW 6-18 starch had a much higher apparent amylose content (21.10%) than did BW 201001 starch (17.48%). The protein content of *C. auriculatum* Royle ex Wight was almost the same as that of potato, while the lipid and ash content were almost twice that of potato. The apparent amylose content of potato was 23.16%, approximately 5% higher than that of starch from *C. auriculatum* Royle ex Wight. According to a recent literature review by Hoover (2001), the variation in the chemical compositions of *C. auriculatum* Royle ex Wight starches isolated from different cultivars could be due to inherent genetic differences. These variations were within the range reported for tuber and root starches.

3.2. Granule morphology

Scanning electron microscopy images and polarised light micrographs of *C. auriculatum* Royle ex Wight starches are presented in Fig. 1. The granule size of *C. auriculatum* Royle ex Wight starches ranged from 5 to 15 μm, with the diameters of BW 201001 granules being larger than those of HSW 6-18. These results are consistent with the granule size of root starches, which range from 1 to 110 μm, depending on the starch source (Hoover, 2001). Yu and Wang (2008) also report that the granule size of other starches in traditional Chinese medicine ranges from 1 to 40 μm. As they suggest, the variation in starch granule size and shape may be due to the biological origin of the starch. As shown in Fig. 1A, the starch granules were mainly irregular and round or polygonal in shape, whereas the shapes of potato starch granules were smooth ovals and spheres with a diameter of 10–50 μm. *C. auriculatum* Royle ex Wight starch had a larger quotient of small granules than did potato starch. Multiple Maltese crosses on birefringence were observed under polarised light microscopy for both starches (Fig. 1B). The center of the cross is at the hilum. Intact granules exhibit a well-defined birefringence pattern with a dark cross.

3.3. Physicochemical properties

The swelling power, solubility, syneresis and water binding capacity of starches separated from different *C. auriculatum* Royle ex Wight cultivars are given in Table 1. When starch molecules are heated in excess water, their crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes an increase in granule swelling and solubility. The swelling power and solubility of BW 201001 starch and HSW 6-18 starch at 85 °C were 14.10%, 13.36%, and 15.39%, 12.70%, respectively. In contrast, potato starch showed the highest swelling power (56.42%) and the lowest solubility (0.85%). The ability of starch to swell in excess water has been reported to be related to its mealy characteristics, as has its solubility. The differences between swelling power and solubility may also be due to the differences in the granule size of starches isolated from different plants (Miao, Zhang, et al., 2009; Yu and Wang, 2008). Kaur, Singh, Ezekiel, and Sodhi (2009) reported a higher swelling power and lower solubility for potato starches having large and irregular or cuboidal granules. This conclusion is in agreement with our results, in which starches with low swelling power and high solubility showed higher crystallinity while starches with high swelling power and low solubility showed lower crystallinity. Low swelling power may be attributed to the presence of a large number of crystallites formed by the association between long amylopectin chains (Table 2). Crystallite formation increases granular stability, thereby reducing the capacity for granular swelling. The freeze–thaw stability of starch gels, determined by gravimetric measurement of the water separated from gelatinised starch pastes after five cycles of freezing and thawing, is a function of the reassociation of linear starch molecules (retrogradation). BW 201001 starch showed a lower syneresis (10.51%) than HSW 6-18 starch (11.18%). The syneresis value of potato starch was observed to be 4.72%. Hoover (2001) and Miao, Jiang, and Zhang (2009) reported that the amount of the water excluded in the

Table 1
Chemical composition and physicochemical properties of *Cynanchum auriculatum* Royle ex Wight starches.[a]

Sample	Moisture content (%)	Protein content (%)	Lipid content (%)	Ash content (%)	Apparent amylose content (%)	Swelling power (g/g)	Solubility (%)	Syneresis (%)	Water binding capacity (%)
BW201001	9.2 ± 0.2a	0.12 ± 0.01b	0.15 ± 0.04bc	0.16 ± 0.03c	17.48 ± 0.40c	14.10 ± 0.07c	13.36 ± 0.10ab	10.51 ± 0.01bc	105.87 ± 0.02b
HSW 6-18	8.7 ± 0.1d	0.15 ± 0.02ab	0.13 ± 0.01b	0.11 ± 0.04c	21.10 ± 0.55bc	15.39 ± 0.14bc	12.70 ± 0.03ab	11.18 ± 0.00a	102.24 ± 0.11a
Potato	10.1 ± 0.3c	0.07 ± 0.01c	0.20 ± 0.03a	0.24 ± 0.03bc	23.16 ± 0.29a	56.42 ± 0.02a	0.85 ± 0.12b	4.72 ± 0.24c	97.05 ± 0.09b

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).

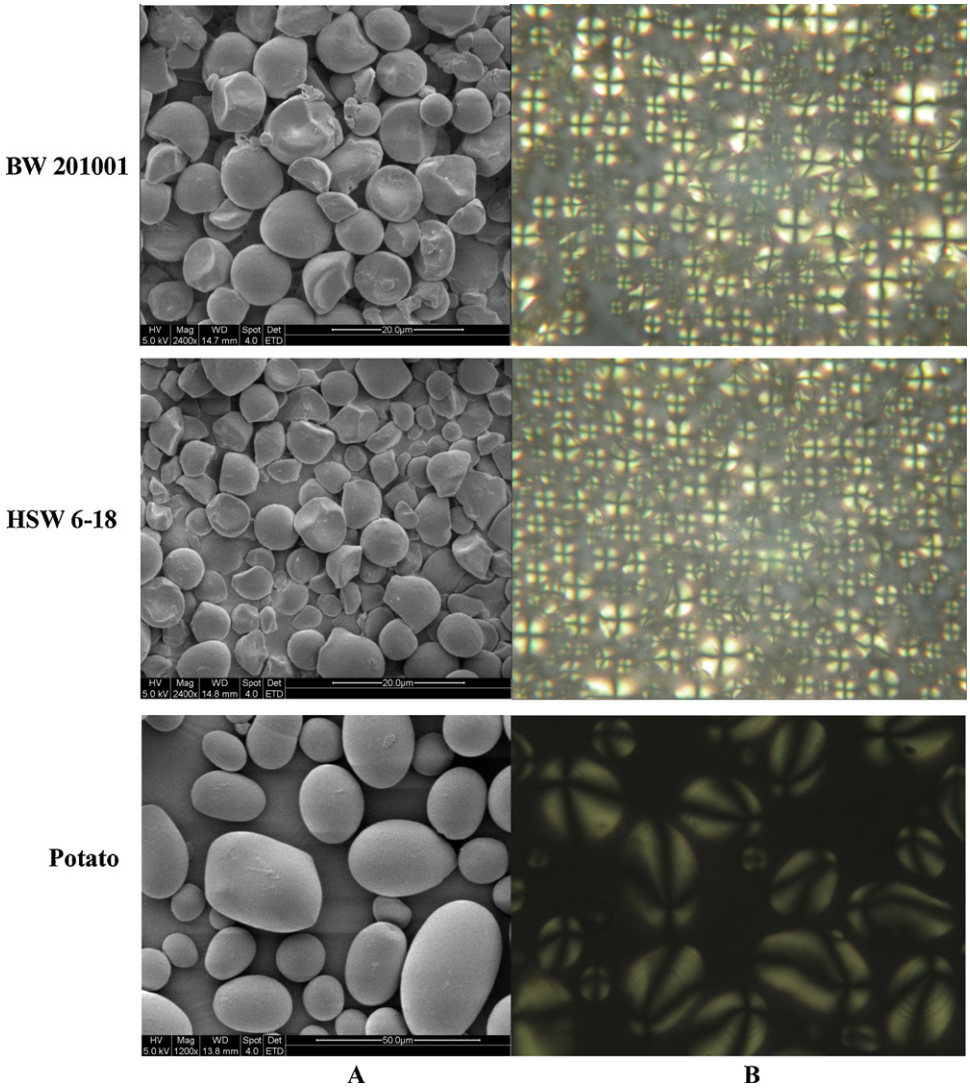

Fig. 1. Scanning electron microscopy (2400×, A) and polarised light microscopy (400×, B) of *Cynanchum auriculatum* Royle ex Wight starches.

freeze–thaw phase would be the result of increased intramolecular and intermolecular hydrogen bonding due to interaction between starch chains (amylose–amylose, amylose–amylopectin, and amylopectin–amylopectin) during frozen storage. Thus, the differences in starch concentration, centrifugal forces or structure (degree of polymerization of amylose, amylopectin length, proportion of short chains) may be the causative factors in this study. Water binding capacity (WBC) of the starches also differed significantly. BW 201001 starch showed the highest WBC (103.57%) and potato starch showed the lowest (99.85%). The difference in WBC

Table 2
Branch chain length distribution and average chain length of *Cynanchum auriculatum* Royle ex Wight starches.[a]

Sample	Average chain length	Percent distribution (%)			
		DP 6-12 (A chains)	DP13-24 (B1 chains)	DP 25-36 (B2 chains)	DP ≥37 (B3+ chains)
BW201001	18.4 ± 0.2a	22.0 ± 1.1b	51.1 ± 0.3b	14.1 ± 0.6a	12.8 ± 0.9ab
HSW 6-18	17.8 ± 0.0a	28.4 ± 1.5c	47.6 ± 0.7ab	12.3 ± 1.1ab	12.1 ± 0.2c
Potato	22.3 ± 0.1ab	21.7 ± 1.2ac	47.2 ± 0.0a	17.4 ± 0.4b	13.7 ± 0.2b

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).

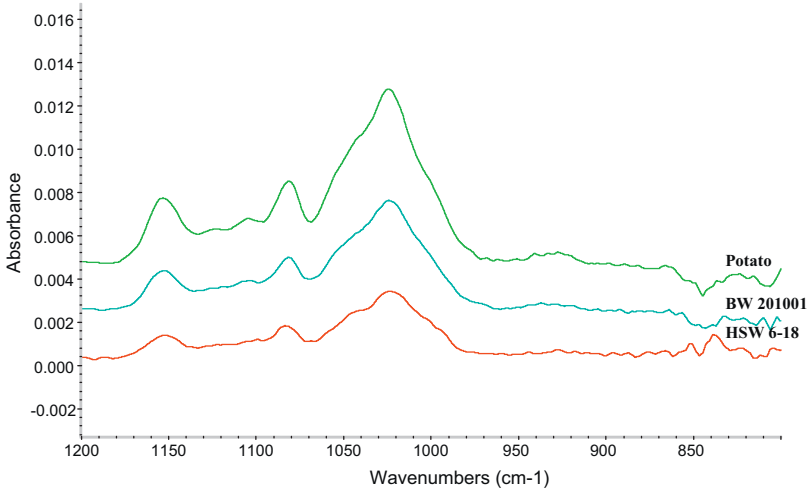

Fig. 2. Deconvoluted FT-IR spectrum of *Cynanchum auriculatum* Royle ex Wight starches.

between starches separated from different *C. auriculatum* Royle ex Wight plants may be attributed to a variation in granular fine structure. Loose association of amylose and amylopectin molecules in the native starch granules has been reported to be responsible for high WBC (Miao, Zhang, et al., 2009; Srichuwong & Jane, 2007). The engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains lowers WBC. In addition, differences among the starches in degrees of availability of water-binding sites may also have contributed to the variation in WBC.

3.4. Chain length distribution

The branch chain length distribution and average chain length of *C. auriculatum* Royle ex Wight starches are given in Table 2. The A, B1, B2, and B3+ amylopectin branch chains correspond to the chains of DP 6-12, 13-24, 25-36 and ≥37, respectively, according to Hanashiro, Abe, and Hizukuri (1996). Different cultivars of the root exhibited differences in chain length distribution. Of the *C. auriculatum* Royle ex Wight starches, BW 201001 showed higher proportions of B chains (B1 chains 51.1%, B2 chains 14.1%, and B3+ chains 12.8%, respectively) and lower proportions of A chains (22.0%), whereas HSW 6-18 showed higher proportions of A chains (28.4%) and lower proportions of B chains (B1 chains 47.6%, B2 chains 12.3%, and B3+ chains 12.1%, respectively). Compared to *C. auriculatum* Royle ex Wight starches, potato starches had fewer short A chains (21.7%) and more B chains (B1 chains 47.2%, B2 chains 17.4%, and B3+ chains 13.7%). A comparison of the average branch chain length of the three types of starches showed potato (22.3) > BW 201001 (18.4) > HSW 6-18 (17.8). The different percent distribution of chain lengths in amylopectin could be associated with the varying size and amount of the double helix in the starch and with the crystal fine structure of starch (Srichuwong & Jane, 2007).

3.5. Crystalline structure

The FT-IR spectrum of starch is sensitive to changes in structure on a molecular level (short-range order). The absorbance bands at 1022 cm^{-1} and 1047 cm^{-1} are characteristic of amorphous and crystalline structures, respectively, in starch. R(1047/1022) from a deconvoluted FT-IR spectrum is used to express the ratio of ordered crystalline domains to amorphous domains in starches (Capron, Robert, Colonna, Brogly, & Planchot, 2007; Miao et al., 2011). The infrared ratios of the starch sample absorbance bands at 1047 and 1022 cm^{-1} (Fig. 2) were 0.50, 0.42, and 0.44 for BW 201001, HSW 6-18, and potato, respectively (Table 3). HSW 6-18 starch had a much lower R(1047/1022) than did BW 201001 or potato starch. HSW 6-18 starch had a higher proportion of short A chains and lower proportions of B1 and B2 chains than did BW 201001 starch or potato starch (Table 2). Jane, Wong, and McPherso (1997) postulated that starches with a higher proportion of B chains could form longer crystallites, resulting in a more perfect crystalline structure. Therefore, we speculate that a lower R(1047/1022) in HSW 6-18 starch, which reflects a smaller amount of ordered domains, could correspond to an imperfect crystalline structure produced by a lower proportion of B chains.

Table 3
Pasting properties of *Cynanchum auriculatum* Royle ex Wight starches.[a,b]

Sample	R(1047/1022)	X_c (%)
BW201001	0.50 ± 0.12a	43.03 ± 0.84bc
HSW 6-18	0.42 ± 0.07b	38.78 ± 1.23a
Potato	0.44 ± 0.15b	38.25 ± 1.78b

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).
[b] R(1047/1022) indicates infrared ratios of the absorbance of the bands at 1047 and 1022 cm^{-1}; DP indicates degree of polymerization; X_c indicates degree of crystallinity.

The X-ray diffractograms of *C. auriculatum* Royle ex Wight starches are shown in Fig. 3. *C. auriculatum* starch showed the

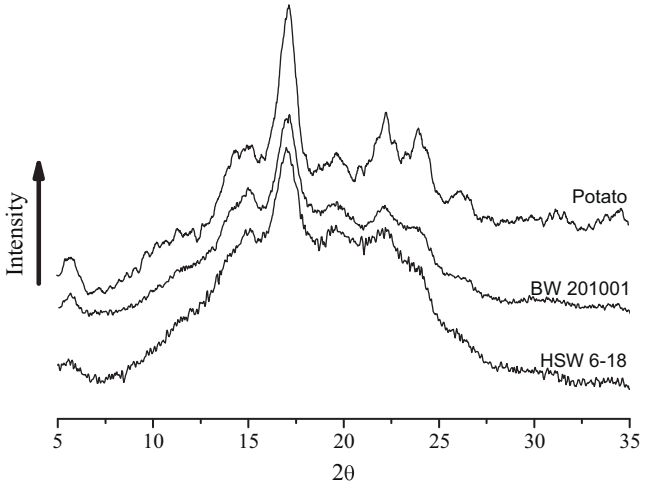

Fig. 3. X-ray diffraction pattern of *Cynanchum auriculatum* Royle ex Wight starches.

characteristic B type pattern of root starch, with very strong peaks at 17° 2θ, medium peaks at 15°, 18°, and 22–23° 2θ, and weak peaks at 5.5° 2θ. There was no significant difference in peak positions among the three starches. Ma, Chang, Zheng, Yu, and Ma (2010) reported that the starches of some traditional Chinese medicines, for example *Polygonum multiflorum*, showed highly similar X-ray diffraction patterns: the typical B-type, which has been observed by Yu and Wang (2008). The X-ray diffraction pattern of starch depended on its origin and environmental growth conditions (Miao, Zhang, et al., 2009; Miao et al., 2011). The degree of crystallinity (X_c), measured according to the diffraction intensity, was slightly higher in BW 201001 (43.03%) than in HSW 6-18 (38.78%) or potato starch (38.25%), which may be attributed to the differences in amylopectin content, as HSW 6-18 starch and potato starch have a higher amylose content and the double-helical content decreases with increasing amylose content. Similar X_c values ranging between 24% and 45% for B-type root starch has been reported (Hoover, 2001).

3.6. Thermal properties

Table 4 shows the result of DSC analysis of starches separated from different *C. auriculatum* Royle ex Wight cultivars. The transition temperatures (T_o, T_p and T_c), gelatinisation temperature range ($T_c - T_o$) and enthalpies of gelatinisation (ΔH) differed significantly. The ranges of T_o, T_p, and T_c of three starch samples were 53.42–59.35 °C, 58.20–64.54 °C, and 64.35–71.75 °C, respectively, which was similar to the study of Ma et al. (2010). The gelatinisation temperatures were ranked as follows: HSW 6-18 < potato < BW 201001. The gelatinisation parameters may be controlled in part by the molecular structure of amylopectin (perfection and ordering of amylopectin crystallites, length of the external 'A' chains of amylopectin, extent of branching, molecular weight and polydispersity), the composition of starch (amylose/amylopectin ratio, lipid complexed amylose chains), the granules' architecture (crystalline to amorphous ratio), or a combination thereof (Miao, Jiang, et al., 2009; Noda et al., 1998). The higher melting temperature for BW 201001 might be the result of (1) a rigid crystalline structure that is evidenced by its higher X_c and (2) a more ordered crystalline domain as measured by FT-IR. The range of gelatinisation (T_c - T_o) and ΔH of three starches were 12.40 °C, 10.93 °C, 16.54 °C, and 6.61 J/g, 7.70 J/g, 14.72 J/g, respectively. Differences in $T_c - T_o$ may be due to the presence of crystallites, which has varying stability within the crystalline domains, whereas differences in ΔH is due mainly to the disruption of the double helices (Cooke & Gidley, 1992).

The retrograded *C. auriculatum* Royle ex Wight starches were analyzed by DSC after 2 weeks of storage at 4 °C. The T_o, T_p, T_c, $T_c - T_o$ and ΔH values were 41.35–42.08 °C, 52.39–56.00 °C, 57.06–61.94 °C, 15.71–19.35 °C and 4.37–6.84 J/g, respectively. The T_p and T_c of retrograded HSW 6-18 starch were much lower than those of BW 201001 starch and potato starch. Retrogradation of starch was inversely correlated with the proportion of short chains of DP 6-9 (Miao, Jiang, et al., 2009). Thus, it is likely that a higher proportion of short A chains (DP 6-12) in the HSW 6-18 starch may have contributed to the low T_p and T_c obtained on heating the retrograded starch.

3.7. Pasting properties

Pasting properties of the *C. auriculatum* Royle ex Wight starches are summarised in Table 5. BW 201001 showed a higher pasting temperature (65.2 °C) than did HSW 6-18 (61.0 °C) or potato starch (60.3 °C), which was in accordance with the gelatinisation temperatures obtained with DSC (Table 4), but lower than that reported in studies of other starches used in traditional Chinese medicine

Table 4
Thermal properties of *Cynanchum auriculatum* Royle ex Wight starches.[a,b]

Sample	Gelatinisation					Retrogradation				
	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
BW201001	59.35 ± 0.12a	64.54 ± 0.23b	71.75 ± 0.35a	12.40 ± 0.18b	6.61 ± 0.15b	42.08 ± 0.25a	56.00 ± 0.11a	61.43 ± 0.09ac	19.35 ± 0.29b	4.37 ± 0.21c
HSW 6-18	53.42 ± 0.50ac	58.20 ± 0.18b	64.35 ± 0.22ab	10.93 ± 0.21c	7.70 ± 0.08b	41.35 ± 0.18a	52.39 ± 0.26ab	57.06 ± 0.26ab	15.71 ± 0.35a	4.52 ± 0.12ab
Potato	55.08 ± 0.13a	63.31 ± 0.09a	71.62 ± 0.27bc	16.54 ± 0.14ab	14.72 ± 0.05ac	41.73 ± 0.34a	55.40 ± 0.07a	61.94 ± 0.13b	19.21 ± 0.16bc	6.84 ± 0.27ac

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).
[b] T_o, T_p, T_c, indicate the temperature of the onset, peak, conclusion of gelatinisation, respectively; ΔH indicates enthalpy of gelatinisation.

Table 5
Pasting properties of *Cynanchum auriculatum* Royle ex Wight starches.[a]

Sample	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Final viscosity (cP)
BW201001	65.2 ± 0.0a	4924 ± 19c	3224 ± 15ab	1166 ± 33b	2866 ± 3b
HSW 6-18	61.0 ± 0.1b	5010 ± 26ab	2287 ± 13a	1336 ± 28b	4059 ± 25ac
Potato	60.3 ± 0.3a	3107 ± 8b	1711 ± 22c	409 ± 11a	1805 ± 14c

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).

(Ma et al., 2010). The high pasting temperature of BW 201001 starch indicates that this starch has a higher resistance to swelling and rupture. Peak and final viscosity of starches from HSW 6-18, BW 201001 and potato were 4924 cP and 2866 cP, 5010 cP and 4059 cP, and 3107 cP and 1805 cP, respectively: highest for HSW 6-18 starch and lowest for BW 201001 starch. Changes in viscosity during a cooking period (breakdown) give indications of paste stability while changes occurring during cooling (setback) might show the consistency of gel and involve retrogradation of the starch molecule. HSW 6-18 had higher values for setback and BW 201001 had higher values for breakdown. Hoover (2001) and Srichuwong and Jane (2007) reported that starch pasting properties were influenced by granule swelling, amylose leaching, starch crystallinity, and branch chain length distribution of amylopectin. BW 201001 starch has a lower swelling power (Table 1), a stronger crystalline structure as evidenced by a larger proportion of B1 chains (Table 2), and a higher X_c and larger amount of ordered crystalline domains (Table 3) that could have contributed to lower peak viscosity. BW 201001 starch also has a smaller proportion of DP 6-12 (Table 2) and a lower amylose content (Table 1) that could lead to a low finial viscosity and setback as suggested by Miao et al. (2011).

3.8. In vitro digestibility

The starch nutritional fractions of *C. auriculatum* Royle ex Wight starches are presented in Table 6. Based on the Englyst assay, RDS, SDS, and RS are the three consecutive digestion fractions divided by reaction time and represent three starch materials found in starches and processed foods (Englyst et al., 1992). The RDS, SDS, and RS were 7.5%, 15.8%, and 76.7%, 8.6%, 17.4%, and 74.0%, 8.3%, 16.9%, and 74.8% for BW 201001, HSW 6-18, and potato starches, respectively. Starch digestibility is greatly influenced by the interplay of many factors, including starch sources, granule size, amylose/amylopectin ratio, amount of amylose–lipid complexes, amylose content, specific area and granule porosity, unit cell structure, amylopectin chain length distribution, extent of molecular association between starch components, degree of crystallinity and type of crystalline polymorphic forms (Hoover, 2001; Miao, Jiang, et al., 2009; Miao et al., 2011). BW 201001 starch has a lower RDS content and higher SDS and RS content that could be influenced by its higher X_c (Table 3). Residual crystallites affect enzymatic digestibility by imposing physical limits on the accessibility of the enzymes during the early stage of degradation. This suggests that the higher X_c of BW 201001 starch induced slow digestion at the beginning of hydrolysis (related to lower RDS), but induced a larger amount of starch hydrolysis in the intermediate and late stages of digestion (related to higher SDS and RS). Based on the report of Srichuwong and Jane (2007), B-type starch granules have long and stable double helices of amylopectin chains and their homogeneous internal structure without voids make them highly resistant to enzyme hydrolysis. BW 201001 also had a higher proportion of B1 chains than did the other two starches, which could produce a perfect crystalline structure and a higher resistance to amylolytic enzymes.

Table 6
Starch nutritional fractions of *Cynanchum auriculatum* Royle ex Wight starches.[a,b]

Sample	RDS (%)	SDS (%)	RS (%)
BW201001	7.5 ± 0.6c	15.8 ± 0.3a	76.7 ± 0.7c
HSW 6-18	8.6 ± 1.1b	17.4 ± 0.5bc	74.0 ± 1.8a
Potato	8.3 ± 0.2a	16.9 ± 1.1b	74.8 ± 0.9a

[a] Values followed by a different letter in each column are significantly different ($p < 0.05$).
[b] RDS, SDS, RS, indicate the nutritional fractions of rapidly digestible starch, slowly digestible starch, resistant starch, respectively.

4. Conclusions

The *C. auriculatum* Royle ex Wight starches isolated from the two cultivars differed in properties. These differences were more marked in BW 201001 starches than in HSW 6-18, due to its higher crystallinity and larger amount of ordered domains. *C. auriculatum* Royle ex Wight starch crystal and molecular structures are the causative factors in their low digestibility. In general, *C. auriculatum* starches had a lower lipid content, swelling power and apparent amylose content, a lower average chain length, proportion of A chains (DP 6-12) and enthalpy of gelatinisation, and higher crystallinity, gelatinisation temperature, breakdown, and setback than did potato starches.

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