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Compositional, morphological, structural and physicochemical properties of starches from seven naked barley cultivars grown in China

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ABSTRACT

Starches from seven naked barley cultivars in China were isolated and investigated for their compositional, structural and physicochemical properties. The range of starch purity, lipid, protein, amylose and amylopectin contents and the amylose/amylopectin ratio for different varieties were 90.58% to 97.95%, 0.01% to 0.02%, 0.31% to 0.45%, 22.72% to 27.49%, 65.89% to 72.64% and 0.31 to 0.39, respectively. The starches from all naked barley cultivars exhibited A-type crystalline packing arrangements and similar granule shapes. The rapid visco analyzer (RVA) results showed the peak viscosity, trough viscosity, breakdown, final viscosity, setback, peak time and pasting temperature of starches ranged from 2977 to 3641 cP, 2253 to 2844 cP, 474 to 876 cP, 3094 to 4320 cP, and 729 to 1476 cP, 5.53 to 6.80 min and 50.25 to 84.35 °C, respectively. The transition temperatures (T_o, T_p and T_c), gelatinization temperature range (ΔT_r) and enthalpies of gelatinization (ΔH) measured using a differential scanning calorimeter analyzer (DSC) ranged from 54.07 to 58.47 °C, 57.51 to 61.54 °C, 65.49 to 72.74 °C, and 10.46 to 16.58 °C and 7.74 to 9.82 J/g, respectively. The various structural and physicochemical properties demonstrated by naked barley starch suggest their broader potential applications of this novel cereal.

1. Introduction

Barley (*Hordeum vulgare* L.) is the world's fourth largest cereal grain crop after wheat, rice and corn, which could be classified into hulled barley and hull-less barley according to the presence or absence of the husk on the grain (Mayer, 2012; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001; Wang, Pan, Nima, Tang, Cai, Liang, et al., 2011). Naked barley or hull-less barley (*H. vulgare* ssp. vulgare) is a widely adaptable crop and is more tolerant of soil salinity than wheat (Cozzolino, Roumeliotis, & Eglinton, 2013; Mayer, 2012). In temperate areas, it is grown as a summer crop, while in tropical areas, it is sown as a winter crop. Naked barley has been grown extensively for thousands of years in the Qinghai–Tibet Plateau of China (Wang et al., 2011), and has been a staple food in Tibet since the fifth century AD. Naked barley possesses a combination of the nutritive characteristics of wheat and hulled barley, it is high in true metabolizable energy and fat, and is high in protein and low in fiber (Rezaei, Dehghan, & Ayatollahy, 2008). In the most past, naked barley was used as an animal feed and as an important staple food of people that lived in plateau area and low rainfall regions (Wang et al., 2011). However, naked barley has recently attracted more and more interest among food scientists and technologists because of its high soluble dietary, β-glucans and arabinoxylan content, and its high malt quality and ease of processing (Wang et al., 2011; Zheng, Li, & Wang, 2012).

As a comprehensive nutritive cereal, naked barley contains about 80% complex carbohydrates, 3.7–7.7% β-glucans, 11.5–14.2% proteins, 4.7–6.8% lipids and 1.8–2.4% ash, respectively (Li, Vasanthan, Rossnagel & Hoover, 2001). Among all the components of naked barley grain, starch is the largest single component, constitutes about to 56–75% of kernel dry weight, and the amylose content in naked barley starches varies from 0 to 40% depending on the varieties (Bhatty, 1997; Li et al., 2001; Zheng, Han, & Bhatty, 1998). As a result, the naked barley presents an important starch source for food and non-food industrial applications. In general, the physicochemical properties and functional characteristics of starch depend on the amylose and amylopectin starch content, the amylose/amylopectin ratio and the inner structure of starch granules. The preliminary researches on naked barley starches (Bhatty, 1997; Li, Vasanthan, Hoover, & Rossnagel, 2004; Li et al., 2001; Suh, Verhoeven, Denyer, & Jane, 2004; Zheng et al., 1998) have revealed that the native naked barley starch, which displays a typical bimodal granule-size distribution, has larger molecules of amylose and amylopectin, and the amylopectin contained a larger proportion of long chains. However, the reports on naked barley starch are comparatively little, and the related naked barley varieties are also relatively few. Therefore, the more information about the naked barley starch need to be investigated and explored further.

Up to now, there is no specific report on the physicochemical and structural properties of the naked barley starches isolated from Chinese

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cultivars. In this study, morphological, pasting and thermal characteristics of seven naked barley starches isolated from Chinese cultivars were studied. Thus, this study was aimed at investigating the physicochemical, structural and morphological characteristics of starches isolated from some naked barley cultivars popularly used in major production regions of China. Thereby, scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and X-ray powder diffraction (XRD) have been used to investigate the granule characteristics. These values were combined with pasting properties analyzed by rapid visco analyzer (RVA) and thermal characteristics measured by differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

Whole grain naked barley seeds (Zangqing 8, Zangqing 148, Beiqing 6, Zangqing 25, Kunlun 12, Zangqing 320 and Xila19) were collected from the main producing areas in China by Agricultural Research Institute of Tibet Academy of Agricultural and Animal Husbandry Sciences in 2012, cultivated in the same experimental field in 2012 in Lhasa and harvested for the test. All chemicals and reagents used in the trials were of analytical grade (Chemical Company, Beijing, China).

2.2. Isolation of starch

Naked barley starch was isolated according to the method of Bello-Pérez, Agama-Acevedo, Zamudio-Flores, Mendez-Montealvo, and Rodriguez-Ambriz (2010) with modifications. Naked barley grains were washed with distilled water to remove dust and other impurity substance. The grains were soaked in an anhydrous sodium sulphite solution at a concentration about 0.1%. The ratio between soaking solution and grains was 5:1. The mixture was kept at room temperature (25 °C) for 48 h, and was stirred occasionally for 24 h. After that, the soaking liquid was drained off, the softened grains washed thoroughly with distilled water and then homogenized with a pulping machine. The slurry was then successively passed through 100 mesh nylon, and the residue was washed with distilled water until no more starch was released. Thereafter, the material retained on the cloth was discarded. The suspension was centrifuged at 3500 rpm for 15 min, and the supernatant containing proteins and fat was discarded. The upper pigmented layer of the precipitate was carefully removed. The bottom white starch fraction was repeatedly purified by resuspension in distilled water. Finally, the isolated starch was dried with an electric oven at 45 °C for 24 h.

2.3. Proximate chemical composition analysis of naked barley starch

The total starch content and ratio of amylose/amylopectin (AM/AP) were determined by using the commercially available Total Starch Assay Kit (K-TSTA, Megazyme International Ireland Ltd.) and AM/AP assay kit (K-AMYL 07/11, Megazyme International Ireland Ltd.), respectively. While the protein and lipid contents were measured according to the Chinese national standard methods GB/T 5511-2008 and GB/T 5512-2008 (Chinese national standard, 2008), respectively, and the results were reported on a dry basis.

2.4. Microscopy analysis

2.4.1. Scanning electron microscopy observation

A starch sample was mounted on a scanning electron microscope (SEM) stub with a double-sided adhesive tape, coated with gold and placed in the SEM chamber. Scanning electron micrographs were taken using an SEM (JSM-6360LV, JEOL, Japan).

2.4.2. Polarized light observation

The birefringence pattern of starch granule observation was performed using a light microscope (DMBA400, Motic China Group Co., Ltd., Guangzhou, China) with polarized light. Starch sample was suspended in a drop of a mixture of water:glycerol (1:1), and the dispersed granules were gently covered with a cover slip. The micrographs were recorded at 400× magnification.

2.4.3. Confocal laser scanning microscopy (CLSM) observation

The confocal laser scanning microscopy observation of each starch sample was conducted according to the procedure of Chen, Yu, Simon, Liu, Dean and Chen (2011) with a slight modification. Starch granules (10 mg) were dispersed in 15 μl of freshly made 8-amino-1, 3, 6-pyrenetrisulfonic acid (APTS, Sigma Chemicals, St. Louis, USA) solution (10 mM APTS dissolved in 15% acetic acid) and 15 μl of 1 M sodium cyanoborohydride (Sigma Chemicals, St. Louis, USA) solution was added. The reaction mixture was incubated at 30 °C for 15–18 h, with the granules washed 5 times with 1 ml of distilled water and suspended in 20 μl of glycerol/water mixture (1:1, v/v). After dyeing the sample with APTS the starch suspension was sealed between two microscope glass slides using silicon adhesive. Optical sections of starch granules treated with fluorescent dyes were visualized using a Digital Eclipse C1plus CLSM system equipped with He/Ne and Ar lasers (Nikon Co., Ltd., Tokyo Japan), and a stand for fixed fluorescent cell samples was used for the detection of the fluorescence signal from dye-stained starch granules. The details of the Leica objective lens used were: 60 × plan apo/1.40 oil UV. The excitation wavelength was 488 nm with 52 capacities, and the images were acquired in 512 × 512 pixel resolution.

2.5. X-ray diffraction analysis

X-ray powder diffraction (XRD) measurements were performed using an X-ray diffractometer (Rigaku D/max-2551/PC, Rigaku Corporation, Tokyo, Japan) under the following conditions: radiation source, CuKα; angle of diffraction scanned from 5 to 60°; step size, 0.02; step time, 2 s. To avoid the influence of relative humidity on relative crystallinity, the starch samples were equilibrated in a 100% relative humidity chamber for 48 h at room temperature.

Table 1
The composition characteristics of starches isolated from different naked barley varieties.

Vatieties	Starch (%)	Lipid (%)	Protein (%)	Amylose (%)	Amylopectin(%)	A/P
Zangqing 8	95.14 ± 2.20b	0.02 ± 0.01a	0.42 ± 0.07a	23.85 ± 2.05bc	71.30 ± 2.05a	0.34 ± 0.04ab
Zangqing 148	90.58 ± 2.59c	0.02 ± 0.00ab	0.44 ± 0.02a	24.75 ± 1.16abc	65.89 ± 1.63b	0.38 ± 0.03ab
Beiqing 6	97.95 ± 1.34a	0.01 ± 0.01b	0.31 ± 0.05a	27.49 ± 0.55a	70.46 ± 0.55a	0.39 ± 0.01a
Zangqing 25	97.18 ± 0.80ab	0.01 ± 0.00ab	0.33 ± 0.04a	27.12 ± 0.33ab	70.06 ± 0.33a	0.39 ± 0.00ab
Kunlun 12	95.57 ± 0.56ab	0.01 ± 0.01ab	0.45 ± 0.03a	24.97 ± 0.17abc	70.60 ± 0.17a	0.35 ± 0.00ab
Zangqing 320	96.96 ± 0.84ab	0.01 ± 0.00ab	0.39 ± 0.05a	26.23 ± 2.96abc	70.73 ± 2.96a	0.37 ± 0.06ab
Xila19	95.36 ± 0.18ab	0.01 ± 0.00ab	0.32 ± 0.09a	22.72 ± 0.85c	72.64 ± 0.85a	0.31 ± 0.02b

All values are the means of triplicate determinations ± SD. The means within columns with different letters are significantly different ($p < 0.05$).