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Variation in the rate and extent of starch digestion is not determined by the starch structural features of cooked whole pulses

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ABSTRACT

Cooked whole pulses are widely accepted as a low glycemic food with potential health benefits of reducing the risk of obesity and type 2 diabetes. However, the structural basis for the variation in digestion rate and extent of different pulses is still unclear. In this study, intact cotyledon cells of four commercial pulses (i.e., pinto bean, garbanzo bean, green-split pea, and black-eyed pea) were isolated under controlled cooking and isolation conditions, as a model for whole foods. We investigated the macrostructure of intact cells and structural features of entrapped starch granules, such as crystallinity and thermal parameters, and diffusion of an amylase-sized FITC dextran probe into intact cells. In vitro digestion kinetic profiles of pulse cells were monitored by a reducing sugar assay with a fixed α-amylase activity, and were fitted into a first order model to obtain the apparent rate coefficient and digestion extent at 180 min. Pearson's correlation analysis suggested that there is no significant correlation between the kinetic parameters and structural features of entrapped starch granules nor the size of the cell particles. It is concluded that the starch digestibility of isolated pulses is controlled by the accessibility of digestive enzymes, which is limited by the rigid cell wall and/or pulse protein matrix, rather than starch structure.

1. Introduction

Pulses belong to the legume family, among which peas, beans, lentils and chickpeas are the most common varieties. Evidence from many epidemiological studies suggests that whole grains or pulses contribute to a low glycemic response compared with refined grain-based foods, and are associated with a reduced risk of obesity and type 2 diabetes (Marsh, Barclay, Colagiuri, & Brand-Miller, 2011; Priebe, van Binsbergen, De, & Vonk, 2008; Venn & Mann, 2004). According to the origin-based classification of resistant starch, whole grains and pulses belong to type 1, i.e., physically inaccessible starch (Englyst, Kingman, & Cummings, 1992). In order to eliminate complex intrinsic host factors and individual diversity, starch digestion has been commonly measured by in vitro methods, and is now better expressed as a kinetic phenomenon (i.e., rate and extent) rather than a thermodynamically defined entity (Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Dhital, Warren, Butterworth, Ellis, & Gidley, 2017). On the basis of the factors that influence the digestion kinetics, at least two hypotheses for the underlying structural basis for the enzyme resistance of whole pulses are proposed (Dhital et al., 2017; Zhang, Dhital, & Gidley, 2015). The first hypothesis is that accessibility of digestive enzymes is limited by physical barriers of the intact plant cell walls and/or protein matrix. Bhattarai, Dhital, Wu, Chen, and Gidley (2017) reported that the pulse cells maintained integrity with negligible starch digested (ca. 2%–3%) after simulated stomach and duodenum digestion. An ileostomy study also confirmed the recovery of intact cells of white beans at the distal ileum following up to 3 h of intestinal residence (Noah et al., 1998). Frost et al. (2016) further suggested that variation in cell wall composition and properties of potato varieties result in varied starch digestibility. In addition, water-insoluble protein can act as a binding matrix for enzymes, leading to reduced starch hydrolysis (Chen et al., 2018; Yu et al., 2018). The second hypothesis is that crystalline

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structure is retained inside the cellular structure of whole pulses, resulting from the limited swelling and gelatinization of the entrapped starch granules. Dhital, Bhattarai, Gorham, and Gidley (2016) found that pulses retained both cellular integrity and most of its ordered structure of entrapped starch granules after the cooking process, either of which could slow down the digestion rate of starch.

Although pulse starches have been widely reported to show lower digestibility compared to cereal starches (Maaran, Hoover, Donner, & Liu, 2014; Sandhu & Lim, 2008), information on the factors that control the starch digestion in whole pulses is still limited. In the current study, we aimed to understand the structural characteristics varying from nm to mm length scale for variation in the digestion rate and extent of whole pulses using isolated intact cotyledon cells as a model. Intact cells were isolated from four commercially available pulses under controlled cooking conditions, and structural analyses were conducted, including macrostructural characteristics of cells, crystalline structure and thermal properties of the entrapped starch. We also evaluated the apparent digestion rate coefficient and extent through fitting the kinetic data into a first order kinetic model, and proposed the structural factors controlling the variation of digestion kinetic values.

2. Materials and methods

2.1. Materials

Pinto bean (PB, *Phaseolus vulgaris*), garbanzo bean (GB, *Cicer arietinum*), green-split pea (GSP, *Pisumsativum*), and black-eyed pea (BEP, *Vigna unguiculata*) were purchased from Target Co., Ltd (Minneapolis, MN, USA). Porcine pancreatic alpha-amylase (A3176, 10 units/mg) and fluorescein isothiocyanate conjugate tagged dextran (FITC-dextran, molar mass 20 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Total starch assay kit was purchased from Megazyme (K-TSTA, Megazyme, Co. Wicklow, Ireland). Other chemicals used in this study were of reagent grade.

2.2. Isolation of intact cells and starch granules

Intact pulse cells were isolated following the method described by Dhital et al. (2016) with slight modifications. Pulse seeds were immersed in excess water at 4 °C overnight to fully swell, and the outer hulls were removed manually. The dehulled pulses were heated at 95 °C for 1 h in excess water with magnetic stirring. The cooked pulses were mashed manually, and the intact pulse cells were separated with sieves with openings of 150 μm and 50 μm and then freeze-dried for further use. In order to compare the digestibility of cooked entrapped starch in cell walls and starch granules, pinto bean starch (PBS) was isolated from cotyledons using a wet-milling method reported by Wang, Hasjim, Wu, Henry, and Gilbert (2014). The starch granules were cooked at 95 °C in a water bath for 30 min and then freeze-dried for X-ray diffraction analysis and enzymic hydrolysis.

2.3. Determination of total starch

To determine the total starch content of intact cooked pulse cells, the intact cell wall structure was first broken by a blender (A11, IKA, Staufen, Germany), which was confirmed by light microscopy. This was done to make sure that the entrapped starch inside the cells were available for enzyme action (Dhital et al., 2016). The total starch content was measured by absorbance at a wavelength of 510 nm using the Megazyme total starch assay kit (K-TSTA, Megazyme, Co. Wicklow, Ireland).

2.4. Microscopic observations

A light microscope (BX-51, Olympus, Japan) equipped with a cross polarizer was used to capture the bright field and polarized pictures. Freeze-dried samples were suspended in distilled water, and placed on a microscope slide before covering with a cover slip, and images were taken at 200 × magnification.

For scanning electron microscopy (SEM), the sample was sprayed onto a circular metal stub covered with double-sided adhesive carbon tape, then coated with platinum by a sputter coater (Cressington, UK). The images were acquired using a ZEISS EVO18 scanning electron microscope (Zeiss, Germany) at an accelerating voltage of 10 kV at 100 × and 500 × magnifications.

Confocal laser scanning microscopy was done following the method described by Zhang et al. (2014) with slight modifications. Pulse cell samples (~1 mg) were dispersed in 1 mL of FITC-dextran solution (2 mg/mL) in a microcentrifuge tube at 37 °C for 3 h, and then the mixture was spread onto a glass slide and observed using a confocal laser scanning microscope (TCS-SP5, Leica, Germany) at 40 × 1.25 oil magnification. The excitation wavelength of the argon-ion laser was set at 488 nm and was operating at 30% of capacity power, and the emission light was detected from 510 to 600 nm. The optical section images of intact pulse cells were recorded with the LAS AF software (Leica, Germany).

2.5. Particle size distribution

Particle size analysis was carried out using a Malvern Mastersizer Hydro MS2000 (Malvern Instruments Ltd., Malvern, UK). The sample was suspended in distilled water and mixed at 2000 rpm for 1 min to disperse the agglomerate before measurement. The obscuration was kept at the range of 13–15%, and 1.33 was used as the refractive index for size measurement.

2.6. X-ray diffractometry

Samples were analyzed with an X-ray diffractometer (D8 Advance, Bruker, Germany) operating at 40 KV and 30 mA with Cu Kα radiation (λ) at 0.154 nm (Shi, Fu, Tan, Huang, & Zhang, 2017). The scanning region was set from 4° to 30° of the diffraction angle 2θ, which covers all of the significant diffraction peaks of starch crystallites. A step interval of 0.02° and a scan rate of 0.5°/min at room temperature were employed. The relative crystallinity was calculated as the ratio of the crystalline peak area to the total diffraction using the PeakFit software (Version 4.0, Systat Software Inc., San Jose, CA, USA).

2.7. Differential scanning calorimetry

A differential scanning calorimeter (DSC8000, Perkin Elmer, Norwalk, CT, USA) with an intra-cooler was used to examine the thermal properties. Intact pulse cell samples (~3 mg, dry starch basis) were mixed with deionized water (moisture level 70%), and hermetically sealed in high-pressure stainless-steel pans with a gold-plated copper seal. After equilibrating for 24 h at room temperature, samples were scanned at a heating rate of 5/°C min from 30 to 150 °C. The enthalpy change (ΔH), onset (T_o), peak (T_p) and conclusion (T_c) temperatures were calculated using a Pyris software (Perkin Elmer, Norwalk, CT, USA).

2.8. In vitro starch digestion kinetics

In vitro starch digestion of isolated pulse cells was carried out using porcine pancreatic α-amylase with the method described by Zhang, Dhital, Flanagan, Luckman, Halley & Gidley (2015) with slight modifications. Intact pulse cells were digested with 10 units of α-amylase per mg of starch equivalent in 15 mL of phosphate buffered saline buffer in a 37 °C water bath. Aliquots (300 μL) were removed at different intervals up to 180 min, immediately mixed with the sodium carbonate solution (0.5 M, 1.2 mL) to inactivate enzymes. The reducing sugar (maltose equivalent) concentration in the supernatant was determined