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Starch chain interactions within the amorphous and crystalline domains of pulse starches during heat-moisture treatment at different temperatures and their impact on physicochemical properties

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ABSTRACT

Pulse (faba bean [FB], black bean [BB] and pinto bean [PB]) starches were heat-moisture treated (HMT) at 80, 100 and 120 °C for 12 h at a moisture content of ~23%. Structural changes on HMT were monitored by microscopy, HPAEC-PAD, ATR-FTIR, WAXS, DSC and susceptibility towards acid and enzyme hydrolysis. Amylopectin chain length distribution remained unchanged in all starches on HMT. In all starches, HMT increased crystallinity and gelatinisation temperatures. The gelatinization enthalpy remained unchanged in some starches, whereas it decreased slightly in other starches on HMT. Slowly digestible starch content decreased at all temperatures of HMT, whereas resistant starch content increased at HMT80 and HMT100 (HMT80 > HMT100), but decreased at HMT120. Birefringence, B-type crystallites and acid hydrolysis decreased on HMT. The extent of the above changes varied amongst starch sources and genotypes. HMT altered the X-ray pattern from A + B → A. The results of this study showed that structural reorganisation of starch chains during HMT temperature was influenced by starch chain flexibility, starch chain interactions and crystalline stability of the native granules.

1. Introduction

Native pulse starches have poor functional properties such as low shear and acid resistance, low thermal stability and high retrogradation tendency (Hoover, 2010). Therefore, modification of pulse starches is needed to meet industrial demands. In this respect, chemical modifications are commonly used to produce pulse starches with desirable properties. However, at the present time, there is a great interest in the use of physical modification techniques, such as heat-moisture treatment (HMT) which changes the physicochemical properties of starches by facilitating starch chain interactions within the amorphous and crystalline domains (Hoover, 2010). HMT involves treatment of starch granules at low moisture levels (<35% moisture w/w) during a certain time period (15 min-16 h) and at temperatures (80–130 °C) above the glass transition temperature (T_g) but below the gelatinization temperature (Hoover, 2010). Several studies have shown that HMT influences granule morphology, X-ray diffraction pattern, gelatinization parameters, crystallinity, granule swelling, amylose leaching, viscosity, retrogradation and susceptibility towards acid and α-amylase hydrolysis (Chung, Liu, & Hoover, 2009; Gunaratne & Hoover, 2002; Varatharajan, Hoover, Liu, & Seetharaman, 2010). The type and extent of change has been shown to vary with botanical origin, starch composition and HMT conditions (Hoover, 2010).

Published data on HMT starches have involved studies on a single starch source or comparative studies on starches differing widely in amylopectin chain length distribution (APCLD) and amylose content (Hoover, 2010). Consequently, it is difficult to ascertain the extent to which structural changes on HMT are influenced by APCLD and amylose (AM) content. The influence on HMT on the structure and properties of cereal and tuber starches that contain pure A- and B-type crystallites, respectively, is well documented. However, similar studies on C-type pulse starches that contain varying proportions of both A- and B-type crystallites are fragmentary. Furthermore, the impact of HMT on AM content, starch nutritional fractions (slowly digestible starch[SDS] and resistant starch[RS] contents), acid hydrolysis, crystallinity, granule morphology and short range molecular order in pulse starches is not well documented. The objectives of this study were therefore, to unravel the structural changes (at molecular and supramolecular levels) and morphological changes during HMT of faba bean[FB], black bean[BB] and pinto bean[PB] starches at different temperatures, and their impact on physicochemical properties. The above starches were shown in our earlier study (Ambigaipalan et al., 2011) to have similar APCLD, marginal differences in AM content and trace quantities of bound lipids. Therefore, it is hypothesized, that structural changes on HMT may reflect the interplay

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between the following factors: (1) packing density (loose or compact) of AM and AP chains within native starch granules and (2) differences in polymorphic composition.

2. Materials and methods

2.1. Materials

Faba bean (*Fatima*, FB9-4) and Black bean (*Expresso*, BRT1519-10) were obtained from the Crop Development Centre, University of Saskatchewan, Canada. Pinto bean (*AC Pintoba*, *Pecos*) was obtained from the Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food, Canada. Pancreatin from porcine pancreas (cat.No. P-1625, activity3 × USP/g) was purchased from Sigma Chemical (St. Louis, MO, USA). Amyloglucosidase (EC 3.2.1.3.,3,300 U/mL) and glucose oxidase–peroxidase assay kit (cat. No. K-GLUC) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All chemicals and solvents were of ACS certified grade. Starches were isolated according to the procedure described in our earlier publication (Ambigaipalan et al., 2011).

2.2. Heat-moisture treatment (HMT)

Starch samples were equilibrated at room temperature in a desiccator using a saturated salt solution of K_2SO_4 (a_w0.97). Following equilibration, the moisture content (~23%) of the samples was determined by the standard AACC (2000) method. The starch samples were sealed and then heated at 80, 100 and 120 °C for 12 h in a forced air oven. The HMT starches were subsequently air dried to uniform moisture content (~10%).

2.3. Polarised light microscopy

Native and HMT starch suspensions (water-glycerol, 50:50,v/v) were observed under crossed polarised light (magnification 400×) using a binocular microscope (Nikon Microscope, Eclipse 80i, NY, USA) equipped with a real time viewing (Q-capture Pro™, BC, Canada).

2.4. Scanning electron microscopy (SEM)

The granule morphologies of native and HMT pulse starches before and after amylolysis were analysed using a Hitachi (S570, Nissei Sangyo Inc., Rexdale, ON, Canada) SEM at an accelerating potential of 5 kV. The dry starch samples were brushed onto the surface of double-sided carbon adhesive tape mounted on an aluminum stub and then coated with a thin film (20 nm) of gold in an argon atmosphere.

2.5. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The amylopectin branch chain length distribution of HMT starches was determined by isoamylase debranching of whole starch accompanied by HPAEC-PAD (Liu, Gu, Donner, Tetlow, & Emes, 2007).

2.6. Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of native and HMT starches were recorded on a Digilab FTS 7000 spectrophotometer (Digilab USA, Randolph, MA, USA). The intensity measurements were performed on the deconvoluted spectra by recording the peak height of the absorbance bands from the base line (Chung et al., 2009).

2.7. Wide angle X-ray diffraction (WAXS)

Native and HMT starches were kept in a desiccator over saturated solutions of K_2SO_4 (a_w0.97), at room temperature for 14 days prior to X-ray diffraction measurements. The IGOR pro 6.1 software (WaveMetrics Inc. Portland, OR, USA) was used to calculate the relative crystallinity described by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008). A Gaussian function was used for curve fitting. Polymorphic composition (proportion of A-and B-type granules) of the starches was calculated using the procedure described in our earlier publication (Ambigaipalan et al., 2011).

2.8. Differential scanning calorimetry (DSC)

The gelatinization parameters of native and HMT starches were measured using a Mettler Toledo DSC (DSC1/700/630/GC200, Mettler-Toledo LLC, OH, USA) equipped with a thermal analysis data station and data recording software (STAR@SW 9.20). Water (11 μl) was added to starch (3.0 mg, db) in the DSC pans, and equilibrated overnight at room temperature. The scanning temperature range and the heating rates were 30–110 °C and 10 °C/min, respectively.

2.9. Swelling factor (SF) and amylose leaching (AML)

SF and AML were determined at 80 °C according to the method of Hoover and Ratnayake (2004).

2.10. Acid hydrolysis

Native and HMT starches were hydrolysed with 2.2 M HCl (1 g, db, starch/40 mL) at 35 °C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for periods ranging from 0 to 15 days. The amount of total reducing sugar in the supernatant was determined as outlined in an earlier publication (Varatharajan et al., 2010).

2.11. In vitro starch digestibility and expected glycemic index (eGI)

The *in vitro* digestibility of native and HMT starches was determined by the AACC approved method (AACC International, 2000). Pulse starch (100 mg) was incubated with pancreatin (10 mg) and amyloglucosidase (12 U) in 4 mL of 0.1 M sodium maleate buffer (pH 6.0) at 37 °C with continuous shaking (200 strokes/min) for 0.5–16 h. After incubation, 4 mL of ethanol (95%) were added to inactivate the enzyme and the sample was centrifuged at 1500*g* for 10 min. The glucose content of the supernatant was measured by a glucose oxidase–peroxidase assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Starch classification based on the rate of hydrolysis was: rapidly digestible (digested within 0.5 h) starch (RDS), slowly digestible (digested between 0.5 and 16 h) starch (SDS) and resistant (undigested after 16 h) starch (RS). The expected glycemic index (eGI) of the starches was calculated in accordance with the procedure established by Goni, Garcia-Alonso, and Saura-Calixto (1997). The hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve of each starch sample by the corresponding area obtained from a standard material (white bread). The eGI was calculated using the equation described by Grandfeldt, Björck, Drews, and Tovar (1992), which is shown below:

$$eGI = 8.198 + 0.862HI.$$