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Heat–moisture treatment under mildly acidic conditions alters potato starch physicochemical properties and digestibility

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ABSTRACT

Potato starch was subjected to heat–moisture treatment (HMT; 120 °C, 3 h) under mildly acidic conditions (pH 5, 6, or 6.5 [control]) at moisture levels of 15, 20 or 25%. HMT starches exhibited significantly delayed pasting times and reduced overall paste viscosities, amylose leaching, and granular swelling characteristics relative to native starch, as well as enhanced levels of thermo-stable resistant starch (≈24%). HMT appeared to alter/enhance short-range chain associations (FT-IR) within amorphous and/or crystalline regions of starch granules. However, the extent of physicochemical change and RS enhancement during HMT was most facilitated by a mildly acidic condition (pH 6) at higher treatment moisture levels (20 or 25%). These conditions promoted limited hydrolysis of amylopectin molecules, primarily at α-(1 → 6) branch points, likely enhancing mobility and interaction of starch chains during HMT. Thus, a slightly acidic pH might reduce conditions and/or timeframe needed to impart physicochemical changes and reduced digestibility to potato starch.

1. Introduction

Heat–moisture treatment (HMT) involves heating of starch granules at low moisture levels (<35% w/w) at temperatures (84–140 °C) above that of glass transition, but below that of gelatinization, for a defined period of time (15 min–16 h) (Hoover, 2010; Jacobs & Delcour, 1998). HMT promotes the physical modification of starch chains, while in the rubbery state, by inducing structural molecular rearrangements within amorphous and/or crystalline regions of granules, retaining starch in the granular state (Hoover, 2010; Hormdok & Noomhorm, 2007; Jacobs & Delcour, 1998; Varatharajan, Hoover, Liu, & Seetharaman, 2010). Structural changes within granules resulting from HMT induce changes to granular swelling behavior, crystallinity, amylose leaching, thermal transition properties, thermal stability, and pasting behavior (Hoover, 2010; Jacobs & Delcour, 1998; Varatharajan et al., 2010). These structural and physicochemical changes incurred during HMT may also affect the susceptibility of starch granules to digestive enzymes (Hoover, 2010). From a nutritional perspective, starch may be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS), depending on the rate and extent of glucose release and absorption during passage through the upper GI tract (Englyst, Kingman, & Cummings, 1992). SDS has been linked to potential health benefits such as diabetes management and satiety (Lehmann & Robin, 2007), while RS, due primarily to its fermentation within the colon, serves as a prebiotic substrate for growth of probiotic microorganisms, reduces risk of disease associated with the large bowel (including colon cancer), contributes an antiglycemic effect, and can favorably alter blood lipid profiles (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Sajilata, Singhal, & Kulkarni, 2006). HMT represents a means for enhancing thermo-stable SDS and RS contents, which likely result from altered starch chain associations in crystalline and/or amorphous regions of granules (Chung, Hoover, & Liu, 2009; Chung, Liu, & Hoover, 2009; Shin, Kim, Ha, Lee, & Moon, 2005).

The extent of structural and physicochemical change generated during HMT is influenced by the botanical source of starch as a function of the nature, composition and organization of amylose and amylopectin chains within native granules (Hoover, 2010). For example, tuber (e.g., potato) starches have been shown to be more impacted by HMT than legume or cereal starches (Hoover & Vasanthan, 1994; Jacobs & Delcour, 1998), with conditions such as temperature, moisture content, and treatment length affecting the degree of change to starch characteristics and properties (Hoover, 2010; Shin et al., 2005). Vermeylen, Goderis, and Delcour (2006) reported that an increasing treatment temperature up to 120 °C reduced the overall long-range crystallinity of potato starch, while new crystalline structures were formed at slightly higher

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temperatures (i.e., 130 °C). At the higher temperature, thermal depolymerization of starch chains was suggested to render starch double helices sufficiently mobile to form more organized crystalline structures (Vermeylen et al., 2006).

Acid hydrolysis has been used to modify the structure and properties of granular starch via scission of glycosidic linkages (Jayakody & Hoover, 2002), with chains in amorphous regions of granules more susceptible to hydrolysis than those comprising the crystalline structure (Hoover, 2000). Consequently, pre-hydrolysis of granular starch with acid was reported to decrease the degree of polymerization of chains, and enhance the mobility and realignment of resulting chains during HMT, increasing the thermo-stable RS content (Brumovsky & Thompson, 2001; Lin, Singh, Wen, & Chang, 2011).

It was hypothesized that slightly acidic conditions during HMT might alter the molecular characteristics of starch chains within amorphous regions of granules to accelerate molecular changes during treatment without loss of granular form or excessive degradation of starch chains. The primary objective of this research was to investigate the effect of a mildly acidic condition (pH 5 or 6) during HMT on potato starch physicochemical properties, granular/molecular structural characteristics, and digestibility.

2. Materials and methods

2.1. Starch and chemical sources

Potato starch was obtained from AVEBE (Veendam, The Netherlands). Hydrochloric acid, amyloglucosidase (EC 3.2.1.3; 300 U/mL), pancreatin (Catalog No. 7545; activity 8× USP/g) and invertase (EC 3.2.1.26; 300 U/mg) were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). A glucose assay kit (Catalog No. K-GLUC) and isoamylase (EC 3.2.1.68; 1000 U/mL) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other utilized chemicals were at minimum of analytical grade.

2.2. Starch HMT under mildly acidic conditions

Potato starch (10 g, dry basis [db]) was dispersed in distilled water (50 mL), and the resulting suspension was adjusted to either pH 5.0 or 6.0 with 1.0 N HCl; the pH of the treatment control was not adjusted (≈pH 6.5). Following pH adjustment, a given starch suspension was stirred (1 h) at ambient temperature, after which the treated starch was recovered by vacuum filtration (filter paper pore size = 1 μm, VWR, Radnor, PA, USA) and allowed to air-dry to a starch moisture content below 10%. Recovered starches were equilibrated in desiccators over saturated solutions (1000 mL) of NaCl, KNO_3, or K_2SO_4 at an ambient temperature until desired starch moisture contents (15, 20, or 25%, respectively) were achieved (Brett, Figueroa, Sandoval, Barreiro, & Müller, 2009; Saibene & Seetharaman, 2006). Equilibrated starches (10 g, db) were transferred to glass containers, tightly sealed (to inhibit moisture loss during the treatment), heated at 120 °C for 3 h, and subsequently cooled to ambient temperature. Treated starches were suspended in deionized water (40 mL), neutralized with 0.1 N NaOH, washed with deionized water (40 mL × 2 times), and washed/solvent-dried with absolute ethanol (40 mL). All HMT starches were dried at ambient temperature, and ground to pass through a No. 120 sieve.

2.3. RVA pasting properties

Pasting properties of native and HMT treated potato starches were determined via the Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia) using a 7.0% (w/w) aqueous starch dispersion (2.1 g starch [db] comprising a total 30 g dispersion) under continuous shear (160 rpm). Starch suspensions were initially heated at 50 °C (1 min), heated to 95 °C (12.2 °C/min), maintained at 95 °C (2.5 min), cooled to 50 °C (11.8 °C/min), and held at 50 °C (2 min).

2.4. Light microscopy

Native and HMT starches were prepared for light microscopy by suspending granules in an excess of water on a glass slide, while starch suspensions following RVA pasting were diluted (10-fold) with deionized water, stained with 0.2% I_2/KI solution (3 mL) and transferred to a glass slide. Slides were overlaid with a cover slip, and observed on a Nikon Eclipse E600 microscope (Melville, NY, USA) equipped with a digital camera (MicroPublisher 3.3, QIMAGING, Surrey, BC, Canada).

2.5. Swelling factor determination

Swelling factors of native and HMT starches were measured at 75 °C in excess water (50 mg starch [db] in 5 mL deionized water) according to the blue dextran exclusion method of Tester and Morrison (1990), and reported as the ratio of the swollen starch granule volume to that of the dry granules.

2.6. Amylose leaching

Native or HMT starch (20 mg) was weighed into a screw cap tube (50 mL) to which deionized water (10 mL) was added, after which the capped tube was incubated in a shaking water bath (Model 406015, American Optical, Buffalo, NY, USA) at 75 °C for 30 min (160 strokes/min). Following incubation, the tube was cooled to ambient temperature and centrifuged (2000 × *g*, 10 min). The amount of liquid in the supernatant was measured and the amylose content of the supernatant was determined (Morrison & Laignelet, 1983). Amylose leaching was reported as the percentage of amylose leached per 100 g of dry starch.

2.7. X-ray diffraction (XRD)

Native or HMT potato starch (2.0 g, db) was equilibrated at ambient temperature for two weeks in a desiccator over a saturated solution (1000 mL) of NaCl to standardize moisture content. XRD patterns of moisture-equilibrated starches were obtained using a powder X-ray-diffractometer (Siemens D5000, Bruker, Madison, WI, USA) as described by Cheetham and Tao (1998). The relative crystallinity of starches was quantitatively calculated as the percent ratio of the sum of the total crystalline peak area to that of the total diffractogram (amorphous + crystalline peak area) as reported by Nara and Komiya (1983).

2.8. Fourier transform infrared spectroscopy (FT-IR)

Absorbance spectra of native and HMT starches were recorded on a Thermo Nicolet Avatar 370 spectrometer (Thermo Scientific, Waltham, MA, USA) in attenuated total reflectance (ATR) mode using a zinc selenide crystal as described by van Soest, Tournois, de Wit, and Vliegenthart (1995). After pressing starch powder onto the crystal, samples were analyzed at a resolution of 4 cm^{-1} over 64 scans. Measured spectra were baseline-corrected between 1200 and 800 cm^{-1}, while intensity measurements were performed by recording the peak heights of the absorbance bands from the baseline.

2.9. Differential scanning calorimetry (DSC)

Thermal transition properties of native and HMT starches were assessed via differential scanning calorimetry (DSC) (2920 Modulated DSC, TA instruments, New Castle, DE, USA). Starch (10.0 mg, db) and distilled water (20 μL) were transferred to a stainless steel pan, after which pans were equilibrated at ambient temperature for 24 h, and scanned from 20 to 180 °C at a heating rate of 10 °C/min using an empty pan as a reference.

2.10. Starch molecular characteristics

2.10.1. Debranched starch chain profiles

Native or HMT starch (50 mg, db) was dispersed in dimethyl sulfoxide (DMSO), vortexed mildly, and transferred to a 90 mL Teflon PFA (perfluoroalkoxy Teflon) jar (Savillex, Minnetonka, MN, USA), after which the jar was irradiated for 35 s in a 2450 MHz microwave oven (Model AR732, Emerson Radio Co., North Bergen, NJ, USA) (Kim & Huber, 2010). After cooling the dispersion to ambient temperature, starch was precipitated by addition of absolute ethanol (25 mL). Precipitated starch was collected by centrifugation (3000 × *g*, 20 min), washed with 85% (v/v) aqueous ethanol (25 mL), recovered by centrifugation (3000 × *g*, 20 min), and allowed to air dry (24 h).

Precipitated starch was re-dispersed in boiling water (9 mL) and heated for 30 min in a boiling water bath with light stirring. The starch dispersion was cooled to ambient temperature, after which 40 mM sodium acetate buffer (1 mL, pH 4.0, containing 0.2% sodium azide) was added. For debranching, isoamylase solution (12.5 μL, 2.5 μL/10 mg starch) was added to the starch dispersion followed by incubation at 40 °C for 24 h. Debranched starch solution was then heated in a boiling water bath for 15 min to inactivate the enzyme, and treated with an ion-exchange resin (1 g, IONAC NM-60 H+/OH– form, J. T. Baker Phillipsburg, NJ, USA) for 1 min to remove impurities (e.g., inactivated enzyme, salts, etc.). The purified debranched starch solution was filtered through a porous syringe filter (5.0 μm, National Scientific Co., Pittsburgh, PA, USA), and injected onto an intermediate-pressure size-exclusion chromatography (IPSEC) system (Waters Corp., Milford, MA, USA), consisting of a 1525 binary HPLC pump, a Rheodyne 7725i manual sample injector with a 200 μL loop, and a 2410 refractive index (RI) detector (operating at an internal temperature of 30 °C). Debranched starch chains were eluted on two Tricorn 10/300 columns packed with Superdex 75 and 30 prep grade gels (GE Healthcare Life Sciences, Piscataway, NJ, USA), respectively, at ambient temperature using a deionized water mobile phase (containing 0.02% sodium azide) regulated at 0.4 mL/min.

Pullulan standards (M_w 47,300, 22,800, 11,800, and 5900; Shodex Standard P82, JM Science, Inc, NY, USA) were used to estimate the molecular weight values of fractionated starch chains. A semi-logarithmic plot of molecular weight versus K_{av} at the maximum RI index values for pullulan standards was used for molecular weight calculations.

2.10.2. Starch intact chain profiles

Native or HMT starch was solubilized as previously noted, except that starch (36 mg, db) was dispersed in a mixture of aqueous urea (0.5 mL, 6 M) and KOH (10 mL, 1 M) in a Teflon PFA jar prior to microwave treatment (Kim, Huber, & Higley, 2006). After cooling to ambient temperature, starch solution was neutralized with 4 M HCl, and treated with an ion-exchange resin (1 g, IONAC NM-60 H+/OH– form) for 1 min. The resulting starch mixture was passed through a 5 μm nylon syringe filter and subjected to IPSEC analysis. Starch chains were eluted at ambient temperature on HR 16/50 and Tricorn 10/300 columns operated in series, and each packed with Sephacryl S-500 HR gel (70 cm gel height, exclusion range M_r 4×10^4–2×10^7, GE Healthcare Life Sciences, Piscataway, NJ, USA). The mobile phase consisted of deionized water (containing 0.02% sodium azide) operated at flow rate of 0.8 mL/min. Pullulan standards (M_w 788,000, 404,000, 212,000, and 47,300; Shodex Standard P82, JM Science, Inc, NY, USA) were used to estimate the molecular weights of fractionated starch chains.

2.11. In vitro digestibility

In vitro digestibility of native and HMT starches was determined on both a "cooked" and "raw" basis according to the method of Englyst et al. (1992) with minor modification. Starch (600 mg, db) was suspended in 0.1 M sodium acetate buffer (pH 5.2, 20 mL) containing 4.0 mmol of calcium chloride and 5.9 mmol of benzoic acid. For "cooked" analyses, starch suspensions were first heated in a boiling water bath (30 min), and cooled to ambient temperature prior to analysis. "Raw" starch suspensions were analyzed directly without the "cooking" step. All prepared starch suspensions were digested in the presence of pancreatin and amyloglucosidase, after which rapidly digestible starch (RDS, digested within 20 min), slowly digestible starch (SDS, digested between 20 and 120 min) and resistant starch (RS, undigested after 120 min) amounts were determined as otherwise described.

2.12. Experimental design and statistical analysis

The HMT experiment consisted of two full treatment replications, with individual analyses for each replicate conducted at minimum in duplicate (duplicate values were averaged for each replicate). Experimental data were analyzed using Analysis of Variance (ANOVA, $\alpha = 0.05$), while a Duncan's test was used to determine differences among experimental mean values ($p < 0.05$). Pearson's correlation analysis was used to examine relationships among experimental factors. All statistical analyses were conducted using SAS version 9.1 for Windows (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. Physicochemical properties of HMT starches

All HMT starches, regardless of treatment conditions, exhibited delayed pasting times and significant alterations to their pasting profiles relative to native potato starch (Fig. 1). While overall pasting viscosities and pasting temperatures of all HMT starches were greatly influenced by treatment moisture content, HMT starches generated in the presence of 20 or 25% moisture exhibited greatly reduced viscosities over the course of pasting and no longer exhibited traditional pasting profiles, lacking a true peak viscosity, as well as a typical breakdown (Fig. 1). Watcharatewinkul, Puttanlek, Rungsardthong, and Uttapap (2009) and Olayinka, Adebowale, and Olu-Owolabi (2008) reported that pasting property effects became more pronounced when moisture content during HMT was increased over the range of 15–27%. A combination of high temperature heating (between the glass transition and melting temperatures) and a limited amount of plasticizing water (10–30%) during HMT control the extent of molecular mobility (Saibene & Seetharaman, 2006), promoting partial disruption of organized chain structures (without inducing full gelatinization of starch granules) followed by rearrangement of disrupted chains within granules (Zavareze & Dias, 2011). Thus, a relatively higher moisture content likely enhanced molecular mobility during HMT, inducing a greater degree of structural change and impact on starch pasting behaviors in our experiments. However, given equal moisture contents, a much greater inhibition of starch pasting viscosity

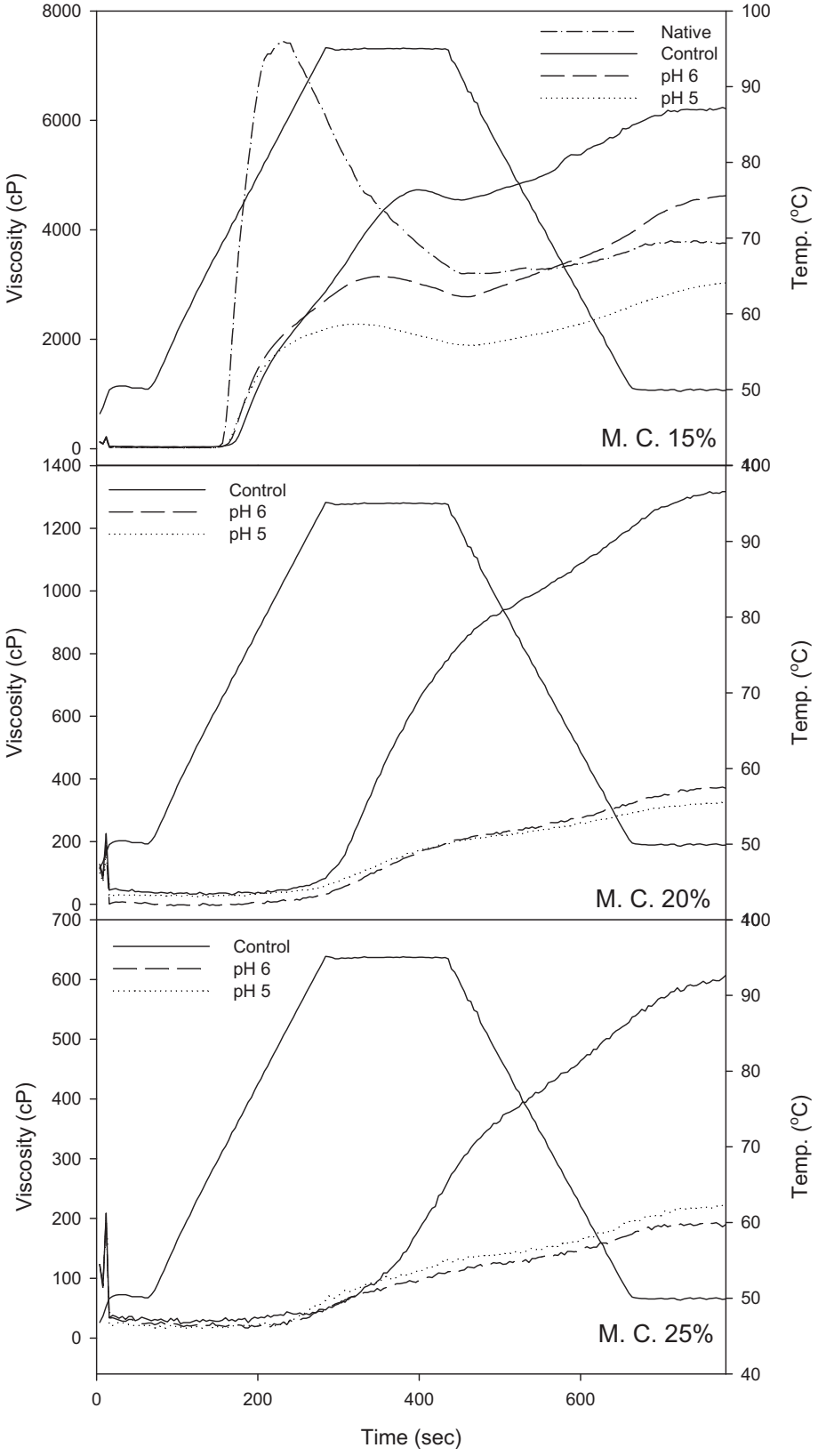

Fig. 1. Pasting profiles of native and heat–moisture treated (120 °C, 3 h) potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or 6.5 [Control]) conditions.

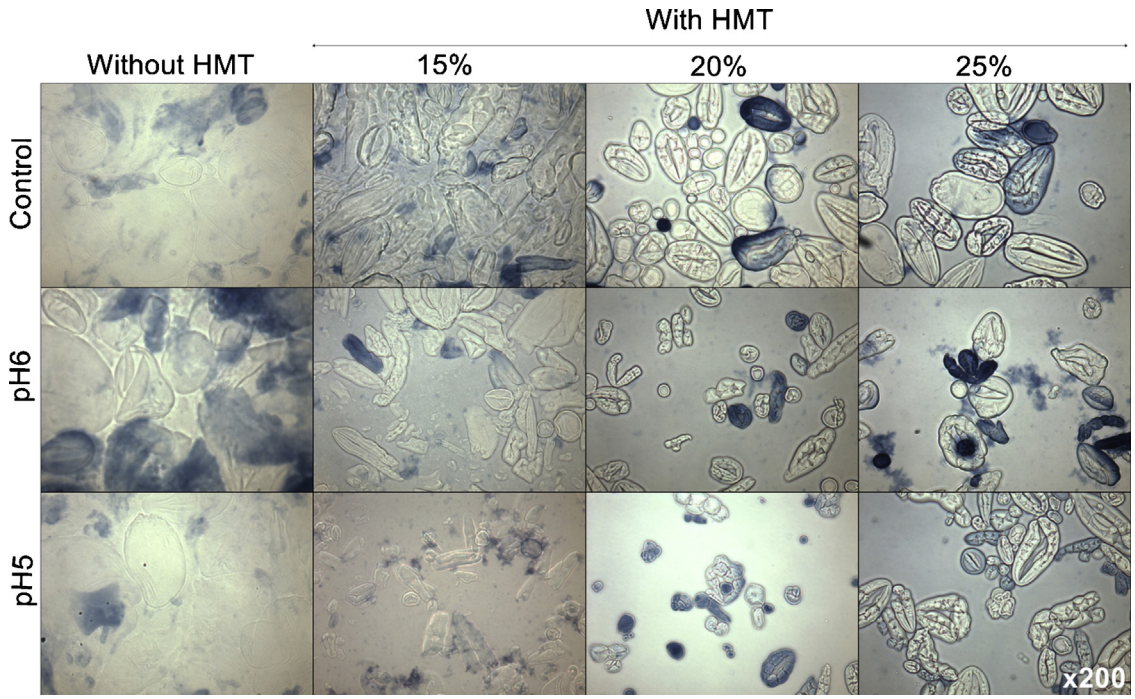

Fig. 2. Paste morphologies (after Rapid Visco Analyzer analysis) of native and heat–moisture treated (120 °C, 3 h) potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or ~6.5 [Control]) conditions.

(Fig. 1) was apparent when HMT was conducted under mildly acidic conditions (pH 5 or 6), most likely due to a reduced extent of starch granule swelling over the course of pasting.

Viscosity development during heating of aqueous starch dispersions in the presence of shear is influenced by granular rigidity, extent of granular swelling, resistance between swollen granules, extent of leaching (especially amylose) from granules, and interactions between leached macromolecules (Hoover, 2010; Jacobs, Eerlingen, Clauwaert, & Delcour, 1995; Lan et al., 2008). Fig. 2 illustrates the morphology of native and HMT starch pastes following RVA analysis. While native (without HMT) potato starch pastes or gels were comprised of swollen granule remnants or ghosts (Fig. 2), granular shapes of HMT starches were largely retained due to the more inhibited swelling of starch granules during RVA analysis. For HMT starches generated under mildly acidic conditions (most notably pH 5), some degree of granule fragmentation and/or the presence of granule fissures was observed in pastes following RVA analysis, though even fragments consisted of discrete granule particles (no fragmentation or external damage was observed in HMT starch granules before RVA analysis). However, prior to pasting, granules subjected to HMT in the presence of acid (most notably those of the 25% moisture level, but also for HMT starches generated at pH 5 for 15 and 20% moisture levels) exhibited a hollowing of granule centers as has been previously reported (Kawabata et al., 1994; Vermeylen et al., 2006). For the 25% moisture level, the extent of granule hollowing was enhanced as treatment pH decreased (Fig. 3). This phenomenon could have contributed to the fracture of the granule structure during pasting (Fig. 2), possibly associated with the degradation and/or rearrangement of starch chains within internal regions of granules. In short, HMT appeared to limit granule swelling and stabilize the stability of the starch granule structure (including fragmented granule particles) in the presence of heat and shear.

Consistent with the limited swelling of granules, amylose leaching from granules (heated to 75 °C) was not only decreased for HMT starches compared to that of native potato starch, but also declined as HMT moisture levels increased (Fig. 4a), in harmony with the report of Olayinka et al. (2008). The impact of HMT pH on amylose leaching did vary with treatment moisture content, with the tendency for a reduction in amylose leaching due to a lower pH condition (pH 5 or 6) during HMT generally declining with increasing treatment moisture levels (most notably the 25% moisture level). However, of the two pH conditions, the pH 6 (relative to the pH 5) treatment resulted in a greater reduction in amylose leaching only at the 20% treatment moisture level.

Swelling factors (Fig. 4b) determined for native and HMT potato starches coincided with pasting behavior trends previously observed for RVA measurements (Figs. 1 and 2). A higher treatment moisture level generally resulted in a greater reduction in starch granule swelling, while a mildly acidic condition during HMT further reduced starch swelling factors over and above treatment moisture level alone. In particular, the pH 6 treatment condition consistently exhibited lower swelling factors than the HMT control across all treatment moisture levels. Swelling factors of starch granules subjected to HMT at 20 and 25% moisture levels under reduced pH conditions (pH 5 or 6) were relatively similar (Fig. 4b).

In short, HMT enhanced granular stability to heat and shear by inhibiting the swelling of starch granules during heating, which effect was enhanced by mildly acidic conditions during HMT. The reduction in granular swelling and amylose leaching for HMT starches has been attributed to changes in crystalline properties and enhanced associations between starch chains (Hoover, 2010).

3.2. Crystalline properties of HMT starches

Fig. 5 illustrates starch long-range crystalline characteristics determined via XRD, in which both native and HMT potato starches possessed typical B crystalline packing arrangements, exhibiting 2θ reflection intensities at 5.5°, 17.1° and 22–24°. Multiple researchers have reported that HMT may transform the crystalline arrangement of potato starch from a B to an A or C pattern (Donovan, Lorenz, & Kulp, 1983; Gunaratne & Hoover, 2002; Hoover & Vasanthan, 1994; Varatharajan et al., 2010), though this transition was not

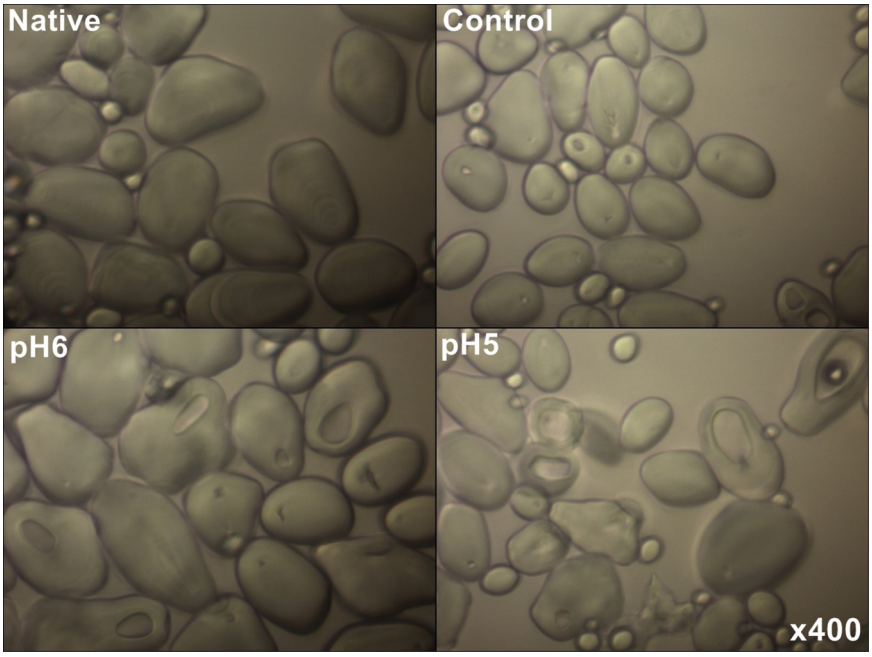

Fig. 3. Light microscopy of native and heat–moisture treated (120 °C, 3 h) potato starch granules generated at 25% moisture and various pH (5, 6, or 6.5 [Control]) conditions.

observed for HMT starches of this study. However, not all HMT conditions induce such a shift in crystalline structure (Jacobs & Delcour, 1998), as relatively high thermal energies and moisture contents are required to drive this change (Vermeylen et al., 2006). The relatively short length of HMT (3 h) in this study did not likely bring about sufficient dehydration to alter the crystalline packing arrangement.

For all HMT starches generated at 15% moisture, few if any changes in XRD intensity or relative crystallinity were observed relative to native potato starch (Fig. 5). However, more significant changes in XRD peak intensity and relative crystallinity were observed for HMT starches generated at the 20 or 25% moisture level. At the 20% moisture level, a mildly acidic treatment condition caused significant decreases in both the crystalline peak intensities and the area of the amorphous region relative to the HMT control (Fig. 5a). The sum of these changes explained the lack of an observed decrease in relative crystallinity for the pH 6 HMT starch (relative to that of native potato starch) (Fig. 5b), since relative crystallinty was estimated by the ratio of the total crystalline peak area to the sum total of the crystalline and amorphous peak areas. At the 25% moisture level, a similar phenomenon was observed, except that the HMT control also experienced extensive structural changes to amorphous regions similar to the HMT starches treated under mildly acidic conditions. Nevertheless, relative crystallinity values for the pH 6 HMT starch consistently exceeded those other HMT starches (20 and 25% treatment moisture levels), and did not significantly differ from that of native potato starch. Mildly acidic conditions during HMT (20–25% treatment moisture levels) did not in reality enhance long-range crystallinity, but rather reduced native long-range chain associations within crystalline regions and altered chain arrangements within amorphous regions of potato starch granules. It should be noted that a mildly acidic condition (most notably pH 6) during HMT (20 and 25% treatment moisture contents) resulted in the greatest restriction in granule swelling (Fig. 4b), as well the most significant alteration of granule amorphous regions (Fig. 5b – indirectly reflected by the high relative crystallinity due to the decrease in overall XRD peak area for amorphous regions).

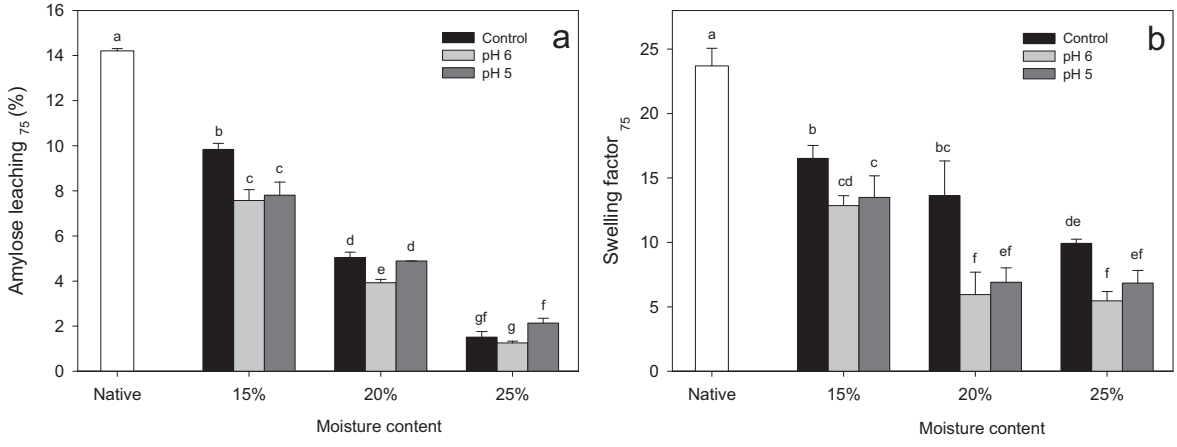

Fig. 4. Amylose leaching (a) and swelling factors [determined at 75 °C] (b) for native and heat–moisture treated potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or 6.5 [Control]) conditions. (bars along the x-axis denoted a common letter are not significantly different, $p < 0.05$).

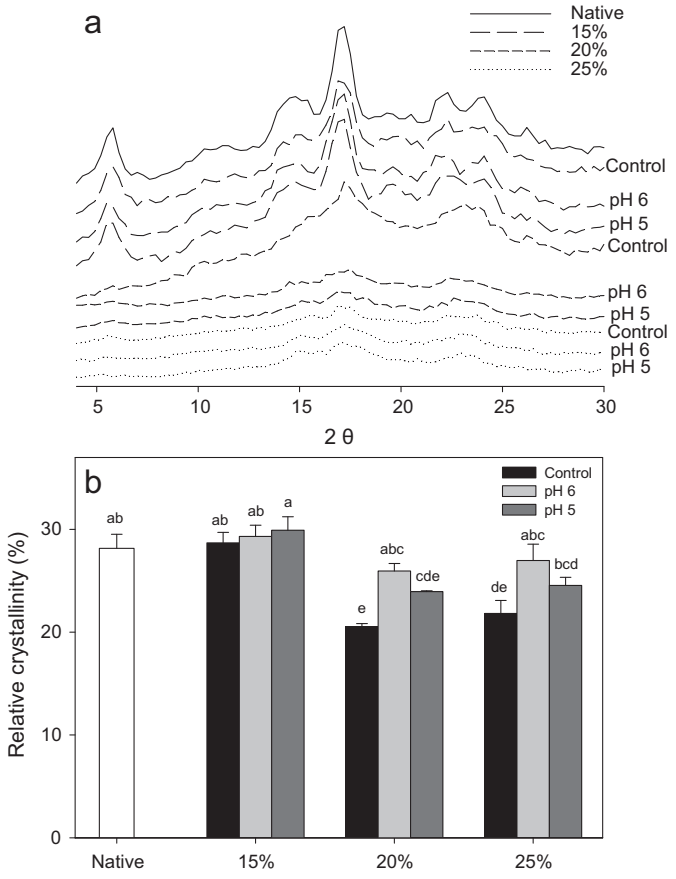

Fig. 5. X-ray diffraction patterns (a) and corresponding relative crystallinities [bars along the *x*-axis denoted by a common letter are not significantly different, $p < 0.05$] (b) for native and heat–moisture treated potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or 6.5 [Control]) conditions.

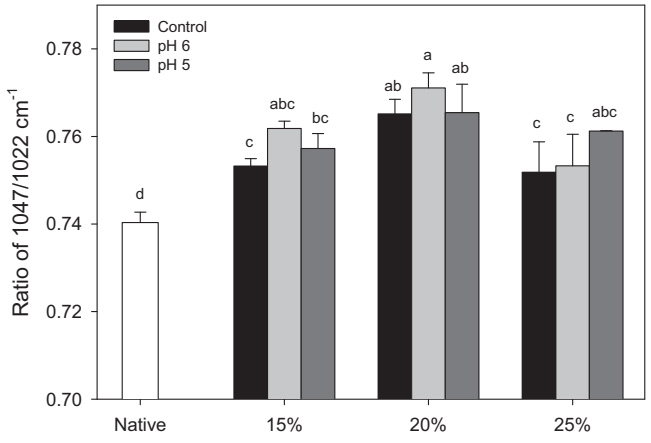

Fig. 6. Fourier transform infrared spectroscopy peak height ratios (1047 to 1022 cm^{-1}) for native and heat–moisture treated (120 °C, 3 h) potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or 6.5 [Control]) conditions (bars along the x-axis denoted a common letter are not significantly different, $p < 0.05$).

Short-range, ordered, structures of starch polymers, such as chain conformation and crystallinity, have been detected by Fourier transform infrared spectroscopy (FT-IR) (Chung et al., 2009a, 2009b; van Soest, de Wit, Tournois, & Vliegenthart, 1994; van Soest, de Wit, Tournois, & Vliegenthart, 1995). The ratio of FT-IR band intensities 1047–1022 cm^{-1} has been proposed to provide a relative measure of the proportion of crystalline to amorphous domains in starch granules (Capron, Robert, Colonna, Brogly, & Planchot, 2007; Chung et al., 2009a, 2009b), as these two IR bands (1047 and 1022 cm^{-1}) are sensitive to ordered and amorphous structures in starch granules, respectively (van Soest et al., 1995). Compared to native potato starch, all HMT starches exhibited higher 1047/1022 cm^{-1} band ratios, suggesting that short-range, ordered crystallinity of potato starch granules was enhanced by HMT (Fig. 6). Statistical differences amongst HMT starches were few, though observed increases in the ratio of 1047/1022 cm^{-1} was most obvious for HMT starches generated at the 20% moisture level (HMT control [20%] > HMT control [15 or 25%]; HMT pH 6 [20%] > HMT pH 6 [25%]) irrespective of the treatment pH condition (Fig. 6). In short, no discernible statistical differences in FT-IR band ratios were detected amongst HMT starches in response to the pH condition.

Thermal transition properties of native and HMT starch granules are presented in Table 1. After HMT, overall endothermic transitions of HMT starches shifted to higher temperatures (20 and 25% treatment moisture levels only), while melting enthalpies for all HMT starches decreased relative to native potato starch. The relative extent of change to thermal properties was enhanced by increasing the moisture content during HMT, corroborating previous reports (Donovan et al., 1983; Gunaratne & Hoover, 2002; Zavareze & Dias, 2011). For 15 and 20% treatment moisture contents, a mildly acidic condition during HMT did not induce meaningful changes in starch thermal transition properties over and above those experienced by HMT controls. As starch melting enthalpy and transition temperature reflect the extent and perfection of double helical order, respectively (Cooke & Gidley, 1992; Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003), DSC findings suggest that while the relative extent of double helices decreased, crystalline perfection of double helices was enhanced, by HMT. Gunaratne and Hoover (2002) suggested that the partial disruption of relatively unstable double helices in crystalline and amorphous regions of starch granules during HMT accounts for the decreased melting enthalpies of these starches (Gunaratne & Hoover, 2002; Hormdok & Noomhorm, 2007). During starch gelatinization, increased hydration, swelling, and mobility of chains within amorphous regions accelerate the melting of crystallites by imparting strain on chains comprising crystalline regions (Donovan, 1979). Conversely, the decrease in starch swelling due to HMT alters interactions between starch chains to reduce chain mobility within amorphous regions, consequently requiring a higher temperature to invoke sufficient swelling for disruption of crystallites within starch granules (Chung et al., 2009a, 2009b; Hoover, 2010; Zavareze & Dias, 2011).

3.3. Molecular structure of HMT potato starches

Chromatograms of native and HMT potato starches are provided in Fig. 7, illustrating the molecular distributions for amylopectin and amylose. The distribution of the amylopectin fraction of HMT starches was shifted to a slightly lower molecular weight relative to that of native potato starch, resulting also in an increase in the proportion of the amylose fraction of HMT starches. In addition, the molecular distribution of the amylose fraction was also shifted to a lower molecular weight under these same conditions. These effects became more pronounced as either the treatment moisture content increased or the HMT pH condition decreased, suggesting that starch molecules became increasingly depolymerized during HMT treatment under slightly acidic conditions and/or increased starch hydration. Chung et al. (2009a, 2009b) reported that amylopectin long chains are thermally degraded during HMT, though the mild acidic treatment conditions utilized in our investigations could also have promoted some degree of starch hydrolysis. Molecular degradation due to acid treatment would most likely

Table 1
Thermal transition characteristics[a] of heat–moisture treated starches relative to native potato starch.

Sample		Thermal transition temperature[b] (°C)			Melting enthalpy ΔH (J/g)
		T_o	T_p	T_c	
Native potato		64.12 ± 0.16cd	69.93 ± 0.30d	83.56 ± 0.11c	14.87 ± 0.13a
M.C.[c] 15%	Control	64.40 ± 0.44cd	70.46 ± 0.01cd	81.47 ± 0.84c	11.59 ± 0.30bc
	pH 6	64.08 ± 0.03cd	70.15 ± 0.01d	82.78 ± 0.17c	12.26 ± 1.19b
	pH 5	64.33 ± 0.15cd	70.57 ± 0.12cd	83.43 ± 1.63c	12.37 ± 1.50b
M.C. 20%	Control	64.72 ± 0.01cd	73.49 ± 0.03b	91.60 ± 0.35b	10.31 ± 0.14cd
	pH 6	65.08 ± 0.27c	74.65 ± 0.21b	91.89 ± 0.06b	10.15 ± 0.64cd
	pH 5	63.35 ± 0.16d	71.83 ± 0.65c	91.35 ± 0.28b	10.55 ± 0.48c
M.C. 25%	Control	63.78 ± 0.18cd	74.92 ± 0.08b	101.75 ± 0.52a	8.82 ± 0.14ed
	pH 6	69.45 ± 1.47a	85.15 ± 1.22a	101.54 ± 0.18a	7.33 ± 0.00e
	pH 5	67.95 ± 1.04b	85.20 ± 1.58a	102.73 ± 0.45a	7.49 ± 0.05e

[a] Mean values of two replicate measures; values within the same column sharing a common letter are not significantly different ($p < 0.05$).
[b] T_o, T_p and T_c are the onset, peak and conclusion gelatinization temperatures, respectively.
[c] M.C., moisture content.

favor starch chains located within granule amorphous as opposed to crystalline regions (Hoover, 2000).

Debranched chain profiles for native and HMT starches were also analyzed to assess molecular degradation patterns on individual starch chains (Table 2). Though a slight increase in the proportion of amylopectin short [SC] chains was observed for pH 5 and 6 HMT starches (20 or 25% moisture), few significant differences in weight average chain-length profiles of HMT starches were observed compared to native potato starch. Nevertheless, the detection of significant molecular degradation of amylopectin and amylose native chains (Fig. 7) and the minimal significant changes to starch debranched chain-length profiles (Table 2), together imply that starch molecular degradation during HMT occurred primarily at α-(1 → 6) branch points. Cleavage of α-(1 → 6) glycosidic linkages, which are clustered in granule amorphous regions, are preferentially cleaved over α-(1 → 4) linkages during acid hydrolysis of granular starch (Jane, Wong, & McPherson, 1997). Additionally, similar observations were observed for HMT control starches (relative to native potato starch), further suggesting that starch molecular degradation associated with HMT conditions investigated of this study favored cleavage of α-(1 → 6) linkages (but to a lesser extent than pH 5 or 6 HMT starches), irrespective of the pH condition.

A light starch hydrolysis phenomenon would be consistent with enhanced chain flexibility, allowing for new structures to be formed and/or existing structures to be perfected within starch granules during HMT. While it is likely that a mildly acidic condition during HMT primarily promoted hydrolysis of amylopectin branch points within granule amorphous regions, hydrolysis of starch chains comprising granule crystalline regions cannot be ruled out. Evidence in support of this possibility is based on the prior observation that starch pastes of HMT starches generated under mildly acidic conditions (particularly pH 5 at 15 or 20% treatment moisture levels) following RVA analysis experienced some degree of granule fragmentation during pasting (Fig. 2).

Aside from strictly a hydrolytic mechanism, potato starch amylopectin chains possess some charged monostarch phosphate moieties that contribute to intermolecular repulsion and hinder hydrogen bonding between starch chains (Craig, Maningat, Seib, & Hoseney, 1989). A slightly depressed pH (i.e., 5 or 6) could decrease the electronic charge of substituted starch chains, since the pK_a2 value for phosphate groups is approximately 6.11 (Lim & Seib, 1993). Consequently, a mildly acidic condition could further promote starch chain associations not only due to reduced electrostatic repulsion, but also, enhanced hydrogen bonding between starch chains.

3.4. *In vitro digestibility of potato starches*

RDS, SDS, and RS values for raw (uncooked) and cooked (boiled 30 min) native and HMT potato starches are presented in Table 3. Native potato starch exhibited a relatively high RS value (93.08%), which falls in the range of the reported literature values (Champ, Martin, Noah, & Gratas, 1999; Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2011; Noda et al., 2008; Zhang, Ao, & Hamaker, 2006). In the case of raw starch analyses, SDS values generally increased, while RS values decreased as moisture content increased during HMT, inducing progressively greater changes in RDS, SDS and RS values. RS values for raw HMT potato starch exhibited significant positive correlation with melting enthalpy (Table 4), while the opposite (negative correlation) was observed for RDS and SDS values. Thus, RS content of ungelatinized HMT starches was directly tied to the amount of native double helical order within granules, while alteration of or damage to

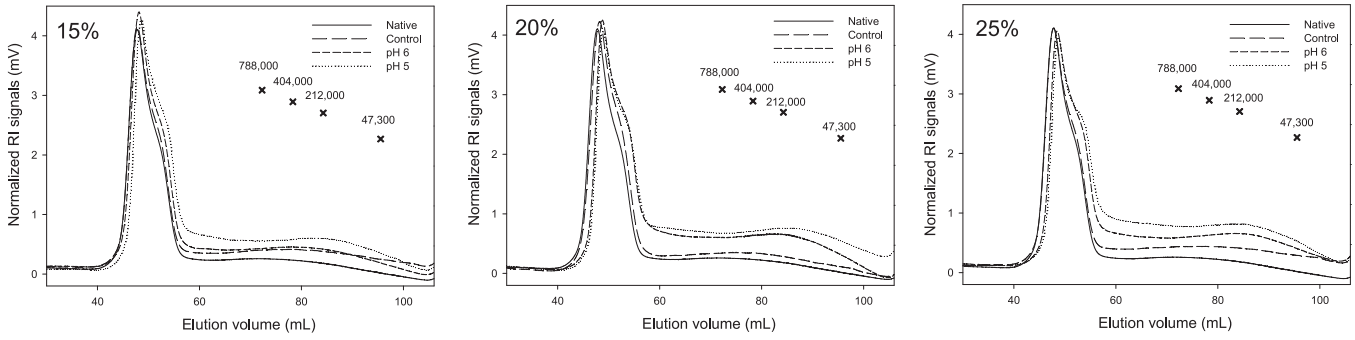

Fig. 7. Size exclusion chromatograms of native and heat–moisture treated (120 °C, 3 h) potato starches generated under varied moisture (15, 20, or 25%) and pH (5, 6, or 6.5 [Control]) conditions (number above each symbol [×] indicates the weight average molecular weight of the specific pullulan standard).

Table 2
Debranched chain profiles of heat–moisture treated starches relative to native potato starch.

Sample		Fraction of starch chains[a,b]				
		AM	LC	IM	SC	
Native potato		CL_w[d]	295.90 ± 3.30a	82.32 ± 0.19a	47.71 ± 1.10a	9.19 ± 0.39a
		%	24.79 ± 0.49A	6.62 ± 0.41A	24.37 ± 0.02A	44.23 ± 0.93AB
M.C.[c] 15%	Control	CL_w[d]	292.65 ± 8.19a	81.52 ± 1.13a	45.19 ± 1.81b	9.50 ± 0.46a
		%	24.61 ± 0.13A	6.56 ± 0.49A	24.71 ± 0.09A	44.14 ± 0.45AB
	pH6	CL_w	296.06 ± 2.30a	80.03 ± 2.29a	45.46 ± 0.47ab	9.23 ± 0.27a
		%	24.68 ± 1.44A	7.48 ± 0.78A	23.75 ± 0.44A	44.10 ± 1.10AB
	pH5	CL_w	286.95 ± 5.53a	80.63 ± 0.28a	45.76 ± 0.37ab	8.94 ± 0.14a
		%	25.72 ± 0.19A	6.69 ± 0.25A	24.29 ± 0.29A	43.32 ± 0.15AB
M.C. 20%	Control	CL_w[d]	289.85 ± 5.53a	82.80 ± 0.86a	45.95 ± 0.85ab	9.12 ± 0.44a
		%	24.89 ± 1.44A	6.96 ± 0.38A	25.24 ± 0.64A	42.92 ± 1.70B
	pH6	CL_w	294.45 ± 0.02a	80.66 ± 1.21a	45.73 ± 1.16ab	9.08 ± 0.31a
		%	25.75 ± 0.65A	7.15 ± 0.36A	23.90 ± 0.44A	43.21 ± 0.18AB
	pH5	CL_w	291.69 ± 1.64a	80.59 ± 0.00a	45.51 ± 0.72ab	9.54 ± 0.17a
		%	24.67 ± 0.53A	6.37 ± 0.42A	23.86 ± 0.00A	45.11 ± 0.11A
M.C. 25%	Control	CL_w[d]	300.71 ± 6.84a	81.52 ± 0.56a	45.68 ± 0.16ab	9.33 ± 0.09a
		%	24.62 ± 1.93A	7.51 ± 0.66A	24.33 ± 1.10A	43.55 ± 0.17AB
	pH6	CL_w	291.87 ± 4.18a	80.54 ± 0.82a	46.25 ± 1.28ab	8.95 ± 0.34a
		%	23.62 ± 1.10A	7.40 ± 0.18A	24.63 ± 0.48A	44.36 ± 0.45AB
	pH5	CL_w	291.87 ± 0.93a	79.89 ± 2.31a	46.10 ± 0.96ab	9.04 ± 0.12a
		%	24.15 ± 0.77A	6.85 ± 0.38A	23.96 ± 1.43A	45.05 ± 1.04A

[a] Mean values of two replicate measures; values within the same column sharing a common upper or lower letter are not significantly different ($p < 0.05$).
[b] Fractions are defined as follows: AM, amylose; LC, amylopectin long chains; IC, amylopectin intermediate chains; SC, amylopectin short chains.
[c] M.C., moisture content.
[d] CL_w, weight average chain length.

Table 3
RDS, SDS, and RS values for raw ("as is") and cooked heat–moisture treated potato starches subjected to heat–moisture treatment relative to native potato starch.

Sample		RDS (%)[a,b]	SDS (%)[a,b]	RS (%)[a,b]
Raw starch	Native potato	5.18 ± 0.09ef	1.95 ± 0.42e	93.08 ± 0.33a
	Control	5.40 ± 0.69ef	11.21 ± 0.40d	83.39 ± 0.30b
M.C.[c] 15%	pH6	4.10 ± 0.84f	10.37 ± 0.32d	85.53 ± 0.53b
	pH5	4.25 ± 0.21f	9.92 ± 1.16d	85.83 ± 1.37b
	Control	8.08 ± 1.31d	21.77 ± 1.20bc	70.16 ± 2.50c
M.C. 20%	pH6	6.61 ± 1.09de	18.06 ± 1.09c	75.34 ± 2.19c
	pH5	5.46 ± 0.87ef	22.38 ± 2.28bc	72.16 ± 3.15c
	Control	22.85 ± 0.99b	23.20 ± 5.85b	53.96 ± 4.86d
M.C. 25%	pH6	28.53 ± 0.10a	26.14 ± 3.58b	45.33 ± 3.48e
	pH5	20.39 ± 1.29c	31.82 ± 0.99a	47.79 ± 0.30e
Cooked[d] starch	Native potato	92.51 ± 1.24a	1.62 ± 0.09d	5.88 ± 1.24c
	Control	81.57 ± 1.59b	2.94 ± 0.40d	15.49 ± 1.98b
M.C.[c] 15%	pH6	76.69 ± 3.48c	5.82 ± 0.21bc	17.49 ± 3.69b
	pH5	80.72 ± 2.00bc	2.46 ± 0.95d	16.82 ± 2.95b
	Control	81.99 ± 1.20b	2.54 ± 0.76d	15.47 ± 0.44b
M.C. 20%	pH6	72.24 ± 1.69d	5.34 ± 1.99bc	22.42 ± 1.49a
	pH5	80.07 ± 0.22bc	3.69 ± 1.52cd	16.24 ± 1.31b
	Control	76.73 ± 0.10c	6.38 ± 0.89b	16.89 ± 0.99b
M.C. 25%	pH6	70.76 ± 3.38d	5.48 ± 1.99bc	23.76 ± 1.39a
	pH5	77.16 ± 0.30c	8.76 ± 1.29a	14.09 ± 0.99b

[a] Mean values of two replicate measures; for a specfic analysis condition (raw or cooked), values within the same column sharing a common letter are not significantly different ($p < 0.05$).
[b] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.
[c] M.C., moisture content.
[d] Cooked starches were boiled 30 min in an excess if deionized water.

Table 4
Correlation coefficient values[a,b] between in vitro digestion measures (RDS, SDS, or RS)[c] and physicochemical/crystalline properties of heat–moisture treated potato starches.

		Swelling factor	Amylose leaching	Crystallinity via XRD[d]	Crystallinity via FT-IR[d]	Melting enthalpy (ΔH)	Thermal transition temperature (T_p)
Raw starch	RDS	−0.522	-0.730^{*}	−0.362	−0.191	-0.825^{**}	0.862^{**}
	SDS	-0.831^{**}	-0.920^{***}	−0.660	0.453	-0.957^{***}	0.810^{**}
	RS	0.731^{*}	0.981^{**}	0.551	−0.143	0.962^{***}	-0.902^{***}
Cooked starch	RDS	0.891^{**}	0.835^{**}	0.121	−0.567	0.774^{**}	−0.578
	SDS	-0.688^{*}	-0.727^{*}	−0.230	0.300	-0.746^{*}	0.708^{*}
	RS	-0.800^{**}	-0.712^{*}	−0.045	0.573	−0.627	0.399

[a] Statistical significance denoted as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.
[b] $n = 10$.
[c] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.
[d] XRD, X-ray diffraction; FT-IR, Fourier transform infrared spectroscopy.

crystalline regions by HMT resulted in ungelatinized starch granules being more readily hydrolyzed by digestive enzymes (i.e., enhanced levels of RDS and SDS). In contrast, a negative correlation was observed between RS content and starch thermal transition temperature (Table 4), as HMT simultaneously decreased melting enthalpy (decreased native structure) and increased thermal transition temperature (due to enhancement of amorphous and/or crystalline structures) as a function of these competing phenomena. Similar opposing correlations for RS and RDS/SDS were observed in their respective relationships to granule swelling and amylose leaching. In short, RS was more linked to native ordered structure (melting enthalpy), while SDS levels were more affected by altered structures (increased transition temperatures) resulting from HMT. For 15 and 20% treatment moisture contents, mildly acidic conditions during HMT caused virtually no significant changes in raw starch digestibility compared to their respective HMT controls, largely because no significant differences in melting enthalpy (Table 1) were induced by pH treatment (enthalpy was positively correlated with RS). On the other hand, mildly acidic conditions during HMT at the 25% moisture level decreased RS content, but increased SDS content (altered thermal transition temperatures, Table 1) compared to the HMT control. In short, a greater extent of damage to the native granule crystalline arrangement adversely impacted the RS content of raw HMT potato starch.

Relative to raw starch values, RDS contents within cooked HMT starch were significantly increased, while SDS and RS contents were significantly reduced after heating (Table 4). RS, SDS, and RDS values for cooked HMT starches exhibited significant correlations with thermal properties (melting enthalpy [RDS, SDS only], transition temperature [SDS only]) and physicochemical properties (swelling factor, amylose leaching), but not with long-range crystalline properties (XRD) (Table 4). During heating, native crystalline structures inherent to raw HMT starches were destroyed as granules became gelatinized, making the native granule structure less of a factor in cooked starch digestibility. Swelling factors and amylose leaching tendency, both which were greatly impacted by HMT (Fig. 4), exhibited significant positive correlations with RDS, and negative correlations with SDS and RS levels (Table 4). Thus, granular stability to heating was increased during HMT, and the RS and SDS levels remaining after cooking (Table 4) were indicative of enhanced, heat-stabile structures formed during HMT (Table 1). In particular, a pH 6 treatment condition (20 or 25% treatment moisture level) during HMT generated the highest levels of heat-stabile RS of all treatment conditions evaluated in this study (Table 3). Consequently, these same HMT conditions generated starches with the most restricted swelling factors (Fig. 4b). Nevertheless, a pH 5 treatment condition (regardless of treatment moisture content) did not elevate RS content as previously noted for the aforementioned pH 6 condition, possibly due to more extensive molecular degradation of starch chains (Fig. 7) and/or potential for granule fragmentation during heating (Fig. 2). On a practical level, swelling factor values, which were negatively correlated with thermo-stabile RS/SDS contents (Table 4), would appear to be potentially useful indicators or predictors of RS/SDS content in HMT starches.

4. Conclusions

HMT significantly decreased the swelling of potato starch and leaching of amylose from granules during pasting, which physicochemical phenomena were associated with increased levels of thermo-stable RS. The increased granular stability may have resulted from enhancement of short range crystalline structure due to interaction of starch chains in amorphous and/or crystalline regions of granules during HMT. Noted changes in physicochemical phenomena and digestibility during HMT (particularly at 20 or 25% moisture levels) were most effected by a mildly acidic condition (pH 6), which promoted limited hydrolysis of amylopectin molecules, primarily at α-(1 → 6) branch points and facilitated enhanced rearrangement of starch chains. However, a pH 5 (relative to a pH 6) condition during HMT appeared to promote lower levels of RS, most likely due to an excessive degree granular damage and/or molecular hydrolysis. In short, mildly acidic conditions during HMT could potentially reduce the conditions and/or timeframe (3 h) needed to achieve a desired level of physical modification.

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