Ultra high pressure (UHP)-assisted acetylation of corn starch

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ABSTRACT

Potential roles of ultra high pressure (UHP) in starch granule reactivity and properties of acetylated starch were investigated. Corn starch was substituted with acetic anhydride at pressure range of 0.1–400 MPa for 15 min; also, conventional reaction (30 °C, 60 min) was conducted as reaction control. Native and acetylated corn starches were assessed with respect to degree of substitution (DS), X-ray diffraction pattern/relative crystallinity, starch solubility/swelling power, gelatinization, and pasting behavior. For the UHP-assisted acetylated starches, DS values increased along with increasing pressure levels from 200 to 400 MPa, and reaction at 400 MPa exhibited maximum reactivity (though lower than the DS value of the reaction control). Both UHP-assisted and conventional acetylation of starch likely occurred predominantly at amorphous regions within granules. Gelatinization and pasting properties of the UHP-assisted acetylated starches may be less influenced by UHP treatment in acetylation reaction, though restricted starch solubility/swelling were observed.

1. Introduction

Starch is comprised primarily of two distinct homopolymers of α-D-glucose: linear amylose and highly-branched amylopectin, both of which further assemble together to form semi-crystalline aggregates, referred to as a granule (Tester, Karkalas, & Qi, 2004). Granular starch has been used in food and non-food applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water retention agent (Singh, Kaur, & McCarthy, 2007). Nevertheless, native starch granules possess limitations of poor solubility, low heat and shear resistance, uncontrolled paste consistency, high tendency toward retrogradation and gelling, and low freeze–thaw stability of pastes (BeMiller, 1997). To overcome these undesirable defects, the majority of native granular starch utilized as food ingredients is chemically modified to enhance and improve its inherent properties in accordance with the intended end-use (Alexander, 1992).

One of the most common starch chemical modification methods, acetylation is achieved by esterification of native starch (while yet in the granular state) with either acetic anhydride or vinyl acetate in the presence of alkaline catalysts (e.g., sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium carbonate) (Huang, Schols, Jin, Sulman, & Voragen, 2007; Wang & Wang, 2002). Granular starch acetate intended for food applications possesses low degree of substitution (DS < 0.1), and relative to native (unmodified) starch granules, commonly exhibited improved properties such as increased solubility and swelling capacity, stabilized paste consistency, enhanced paste clarity, retarded retrogradation, and improved freeze–thaw stability (Huang et al., 2007; Liu, Arntfield, Holley, & Aime, 1997; Liu, Ramsden, & Corke, 1997; Saartrat, Puttanlek, Rungsardthong, & Uttapap, 2005; Singh, Chawla, & Singh, 2004; Sodhi & Singh, 2005; Wang & Wang, 2002). Despite these beneficial properties of acetylated starch, it may not meet all the diversity of physical and textural properties required by many existing and/or emerging food systems (within the current regulatory practices). Thus, it is needed to expand properties and functionalities of acetylated starch.

There has been great attention to ultra high pressure (UHP), or high hydrostatic pressure (HHP), technology for physical modification of starch (Stute, Klingler, Boguslawski, Eshtiaghi, & Knorr, 1996), though annealing and/or heat-moisture treatment are conventionally conducted to alter granule structures via starch molecule interaction and starch crystallite disruption/reorientation within the amorphous and crystalline regions, respectively (Gunaratne & Corke, 2007a). UHP treatment of the starch–water suspension reversibly hydrates amorphous regions within granules, leading to the granule swelling and crystalline region distortion, and thus, the crystalline regions become more accessible to water (Buckow, Heinz, & Knorr, 2007; Douzals, Marechal, Coquille, & Gervais, 1996; Douzals, Perrier-Cornet, Gervais, & Coquille, 1998; Oh, Pinder, Hemar, Anema, & Wong, 2008a). Finally, starch granules are non-thermally gelatinized over a critical pressure level which varied depending on botanical sources and crystal packing arrangements of starch (Blaszczak, Fornal, Valverde, & Garrido, 2005; Buckow et al., 2007; Oh et al., 2008a; Stolt, Oinonen, & Autio,

2001). Further, UHP-treated starch that is little or partially gelatinized maintains its granular structure (Blaszczak et al., 2005; Stolt et al., 2001; Stute et al., 1996), and restricts amylose leaching from granules and granule swelling capacity (Douzals et al., 1998; Katopo, Song, & Jane, 2002; Stolt et al., 2001; Stute et al., 1996). Moreover, UHP treatment has been shown to transit A-type crystallite pattern to B-type crystallite pattern (Hibi, Matsumoto, & Hagiwara, 1993; Katopo et al., 2002). These noted physical and structural characteristics of UHP-treated starch granules are anticipated to impact its reactivity in starch chemical modification and modified starch property. However, there is no study to associate chemical modification of starch with UHP treatment to develop modified starch products with novel properties for food and non-food industrial applications.

The objective of this study was to investigate the influence of UHP on reactivity of corn starch granules in acetylation reaction with acetic anhydride, and to further characterize acetylated starch properties (starch solubility, swelling power, gelatinization, and pasting behavior) so as to facilitate an understanding of the UHP effects on acetylated starch.

2. Materials and methods

2.1. Materials

Corn starch, total amylose and moisture contents of which are 26.5% (s.b.; dry starch weight basis) and 10.5% (d.b.; dry weight basis), respectively, was kindly donated from Daesang Co. (Ichon, Korea). Acetic anhydride was purchased from Sigma Co. (St. Louis, MO, USA). Other reagents and chemicals used in this study were at least of analytical grades.

2.2. Preparation of acetylated corn starches

Either UHP-assisted or conventional acetylation reaction was conducted with a reaction mixture which was prepared similar to a scheme described by Wang and Wang (2002). Corn starch (20 g, d.b.) was suspended in distilled water (80 ml) with rapid stirring, after which pH of the starch slurry was adjusted to 8.0–8.5 using 1.0 M NaOH. Acetic anhydride (12%, s.b.) was added dropwise to the starch slurry, during which period the starch slurry was maintained at pH 8.0–8.5 by periodic addition of 1.0 M NaOH.

For UHP-assisted reaction, approximately 100 g (about 86 ml) of the reaction mixture was immediately transferred into a retortable pouch and hermetically sealed using a heat sealer. It was pressurized in UHP unit (2 L capacity, Autoclave Engineers, Erie, PA, USA) at a rate of 20 MPa/s using distilled water as a pressure medium. The temperature of the UHP unit increased from 25 to 27 °C by pressurization up to 400 MPa, and returned to 25 °C in a few seconds. The UHP unit was maintained at 25 °C during pressure holding time (15 min). The UHP treatments were achieved at differing pressure levels (100–400 MPa) for 15 min to investigate UHP impacts in acetylation reaction of corn starch with acetic anhydride. While only minimal pH drop (less than 0.1) was observed in the final reaction mixtures after UHP treatments, the final pH level ranged from 8.0 to 8.5. For conventional acetylation reaction (referred to as a reaction control), the same reaction mixture was further incubated for 60 min at 30 °C with continuous stirring. Reaction system parameters for corn starch acetylation were depicted in Table 1. Furthermore, the reaction mixtures in absence of acetic anhydride were subjected to the same UHP treatments (designated as a UHP control) so as to investigate effects of UHP on gelatinization and pasting properties of the UHP-assisted acetylated starch derivatives.

Upon completion of either UHP-assisted or conventional acetylation reaction, the reaction mixtures were neutralized to pH 5.5 with 1 M HCl. The resultant acetylated starch was recovered by centrifugation (1500g, 20 min), washed at least three times with distilled water, and dried at 45 °C for 24 h. The dried starch pellets were ground with a pestle and mortar, and passed through an 80 mesh (pore size: 180 μm) sieve for further analysis.

2.3. Determination of degree of substitution (DS)

Acetyl group contents (acetyl %) within acetylated starch were titrimetrically determined similar to the method of Gunaratne and Corke (2007b). Acetylated starch (5.0 g, d.b.) was combined with distilled water (50 ml) in a 250 ml flask, and stirred for 30 min at 25 °C. The diluted NaOH (0.45 M, 25 ml) was added to the starch suspension, after which excess alkali was back-titrated with 0.2 M HCl using phenolphthalein as an indicator. Native starch was also used as a blank, and acetyl group contents were calculated as following:

$$\text{Acetyl} \% = \frac{(\text{Blank} - \text{Sample}) \times f \times 0.0086 \times 100}{\text{Sample weight (g, d.b.)}}$$

where blank and sample are titration volume (ml) of 0.2 M HCl, and f is a factor value of 0.2 M HCl. Degree of substitution (DS) was also calculated as following:

$$\text{DS} = \frac{162 \times \text{Acetyl} \%}{4300 - (42 \times \text{Acetyl} \%)}$$

2.4. X-ray diffraction

X-ray diffraction (XRD) patterns of native and acetylated starches were obtained using an X-ray diffractometer (M18XHF-SRA, Mac Science Co., Yokohama, Japan) operated in a transmission mode with a Ni filter Cu Kα radiation at 40 kV and 300 mA. The X-ray source has a wavelength of 1.54056 Å, and XRD data were collected at scanning angle 2θ from 5° to 40°. Relative crystallinity was calculated as the percent (%) ratio of sum of crystalline peak areas to that of the total diffractogram (including crystalline and amorphous peak areas) (Lee et al., 2006).

Table 1
Reaction system parameters and mean[a] degree of substitution (DS) values for acetylation of corn starch granules with acetic anhydride.

Treatment	Reagent (%, s.b.[b])	pH	Pressure (MPa)	Temperature (°C)	Time (min)	DS
Reaction control	12.0	8.0–8.5	–	30	60	0.089 ± 0.002[A]
UHP1	12.0	8.0–8.5	0.1	25	15	0.047 ± 0.000[D]
UHP2	12.0	8.0–8.5	100	25	15	0.046 ± 0.002[D]
UHP3	12.0	8.0–8.5	200	25	15	0.048 ± 0.001[D]
UHP4	12.0	8.0–8.5	300	25	15	0.055 ± 0.000[C]
UHP5	12.0	8.0–8.5	400	25	15	0.067 ± 0.002[B]

[a] Mean value of two reaction replicates; values within a column sharing a upper-case letter are not significantly different ($p < 0.05$).
[b] s.b., dry starch weight basis.

2.5. Solubility and swelling power

Solubility and swelling power of native and acetylated starches were determined using the method outlined by Koo et al. (2005). Starch sample (0.5 g, d.b.) was combined with distilled water (30 ml) in the 50-ml centrifuge tubes. Tubes were incubated in a shaking water bath at 90 °C for 30 min, after which the tubes were centrifuged for 30 min at 1500g. While the precipitates were immediately weighed, the supernatants were further dried for 16 h at 105 °C and weighed. The solubility and swelling power were calculated as following:

$$\text{Solubilty}\,(\%) = \frac{\text{Soluble material weight (g, d.b.) in supernatant}}{\text{Starch sample weight (g, d.b.)}} \times 100$$

$$\text{Swelling power} = \frac{\text{Precipitate weight (g, wet basis)} \times 100}{\text{Starch sample weight (g, d.b.)} \times (100 - \text{solubility})}$$

2.6. Differential Scanning Calorimeter (DSC) analysis

Gelatinization properties of native and acetylated starches were analyzed using a Differential Scanning Calorimeter (DSC-650, Sinco Co., Seoul, Korea). Starch sample (0.55 g, d.b.) was suspended in distilled water (0.45 ml) with vigorous stirring. The starch suspension (15 mg) was pipetted into a stainless steel sample pan, and hermetically sealed. The sample pans were scanned at a heating rate of 5 °C/min over the temperature range of 20–120 °C. A sealed, empty sample pan was used as a reference. Starch gelatinization onset (T_o), peak (T_p), and completion (T_c) temperatures and gelatinization enthalpies (ΔH) were determined from the DSC thermograms (Lee et al., 2006).

2.7. Starch pasting behavior

Pasting properties of native and acetylated starches were determined using the Rapid Visco Analyzer (RVA) (Newport Scientific, NSW, Australia). Starch sample (3.0 g, d.b.) was directly weighed into an RVA canister, followed by the addition of distilled water (25 ml). Starch suspensions were analyzed under continued shear (160 rpm) beginning with an initial hold at 50 °C (1 min), linear heating to 95 °C at a heating rate of 12 °C/min, an intermediate hold at 95 °C (2.5 min), linear cooling to 50 °C at a cooling rate of 12 °C/min, and a final hold at 50 °C (2 min).

2.8. Statistical analysis

The UHP-assisted and conventional acetylation reactions were replicated twice for each treatment. DS values and all measured starch properties were determined at least twice for each experimental replicate. Experimental data also were analyzed using Analysis of Variance (ANOVA), and expressed as mean value ± standard deviation. A Duncan's multiple range test was conducted to assess significant differences among experimental mean values ($\alpha < 0.05$). All statistical computations and analyses were conducted using SAS version 8.02 for Windows (SAS Institute, Inc., Cary, NC).

3. Results and discussion

3.1. Acetylation of corn starch with acetic anhydride under ultra high pressure (UHP)

UHP treatments, ranging from 500 to 650 MPa, are commonly known to partially and/or completely gelatinize normal corn starch

granules at temperature range of 20–30 °C (Blaszczak et al., 2005; Buckow et al., 2007; Hibi et al., 1993; Katopo et al., 2002; Oh et al., 2008a). Thus, in the present study, UHP-assisted acetylation reaction was conducted at relatively low pressure levels (up to 400 MPa) to minimize gelatinization (or rupture) of corn starch granules in the reaction medium during pressurization. For the reaction control, conventional acetylation reaction was also conducted to assess influence of the UHP-assisted acetylation reaction on starch granule reactivities and acetylated starch properties. Degree of substitution (DS) values for acetylated corn starches subjected to either conventional or UHP-assisted reaction were depicted in Table 1.

DS value (0.089) of the reaction control (Table 1), generated from conventional acetylation reaction, was much lower than those (0.133–0.150) reported by Singh et al. (2004), who derivatized normal corn starch with various levels (2–12%, s.b.) of acetic anhydride under very similar reaction conditions to those of the present study, except they used a higher initial concentration (30% vs. 20% in the present study) of the starch slurry. Pal, Singhal, and Kulkarni (2000) suggested that excess water in hydroxypropylation reaction system appeared to reduce reaction efficiency due to decreased accessibility of diluted reagent (by abundant water) to starch granules. Moreover, the highly-reacting acetic anhydride more likely undergoes the side reaction with a large portion of water (relative to starch granules) within a reaction mixture, leading to a decrease in reaction efficiency (Huang et al., 2007). Thus, the lower DS value observed in the present study may be due to the use of the relatively low concentration of the starch slurry.

For the UHP-assisted acetylated starches, DS values did not significantly differ over the pressure range of 0.1–200 MPa (Table 1). However, a significant increase in DS values was first observed at 300 MPa, and elevation of the pressure levels up to 400 MPa further enhanced reaction efficiency (Table 1). This phenomenon may be explained by the report of Douzals et al. (1996). They observed that during pressurization below 300 MPa, bulk volume of the wheat starch–water suspension was less reduced than that of pure water, implying preservation of initial starch granule volume within the suspension. Over 300 MPa, however, bulk volume reduction was greater for the starch suspension relative to pure water, which more likely resulted from starch granule hydration inducing granule swelling. Based on the observation of Douzals et al. (1996), in the present study, corn starch granules may reversibly hydrate and in turn swell, along with increasing pressure levels from 200 to 400 MPa. The pressure-induced granule hydration and/or swelling appeared to facilitate infiltration of acetic anhydride, the slow-diffusing (relative to fast-reacting) nature of which likely confines the most reaction to the granule outer regions (Chen, Schols, & Voragen, 2004; Steeneken & Woortman, 2008), into the granule interior, likely enhancing reaction efficiency. Thus, UHP may be a key factor to impact starch granule reactivity by promoting accessibility of reagent to starch molecules within the granules.

Relative to the reaction control DS value, moreover, UHP-assisted acetylated starches exhibited lower DS values across overall pressure levels (Table 1). UHP5 (reacted even at 400 MPa), representing the highest DS value among the UHP-assisted acetylated starches, generated the 25% lower DS value than the reaction control (Table 1). These differential reactivities were likely attributable to differences in reaction time between conventional and UHP (400 MPa)-assisted reactions (60 and 15 min, respectively) as demonstrated by Xu, Miladinov, and Hanna (2004), who exhibited an increase (in non-linear fashion) of acetylated starch DS values along with increasing reaction time from 30 to 240 min. Further, despite 75% shorter reaction time for the UHP-assisted (relative to conventional) reaction, UHP5 achieved a 75% level of reactivity of the reaction control, suggesting that acetylation reaction might

be accelerated by UHP treatment over a critical pressure level (400 MPa). However, UHP-assisted reaction time (pressure holding time) effects on starch granule reactivity will need to be more carefully considered under the time range preventing starch gelatinization in further study. Buckow et al. (2007) demonstrated that starch granule swelling and gelatinization under UHP appeared to be not spontaneous but time-dependent process.

To track structural changes of starch granules subjected to either conventional or UHP-assisted acetylation reaction, both X-ray diffraction pattern and relative crystallinity of acetylated starches were investigated relative to those of native starch (Fig. 1 and Table 2, respectively). Native starch exhibited major peaks at 15.1°, 17.2°, 17.9°, and 23.0° (2θ) (Fig. 1), indicating a typical A-type crystalline packing arrangement within granules. No significant changes or shifts in X-ray diffraction patterns were observed for all acetylated (relative to native) starches (Fig. 1). Relative crystallinities also did not significantly differ for native and acetylated starches (Table 2). Huang et al. (2007) and Wang and Wang (2002) reported no differences in X-ray diffraction patterns and relative crystallinities between native and acetylated (DS < 0.1) starches of normal corn, yellow pea, cowpea, and chickpea. Regarding UHP treatment of starch granules, Hibi et al. (1993) exhibited that a significant decrease in relative crystallinity of normal corn starch granules in the aqueous suspension began to be observed over 500 MPa, and an increase of pressure holding time from 20 to 60 min at 500 MPa led to transition from A-type toward B-type X-ray diffraction patterns. Thus, the results in the present study suggested that UHP-assisted (up to 400 MPa for 15 min) acetylation reaction did not appear to impact crystalline structures of normal corn starch granules. It is further implied that acetylation reaction under UHP may occur primarily at amorphous regions within granules as suggested for conventional acetylation reaction by Huang et al. (2007) and Wang and Wang (2002).

3.2. Solubility and swelling power

Starch solubility and granule swelling of native and acetylated starches were determined at 90 °C, and depicted in Table 2. All acetylated starches, generated from both conventional and UHP-assisted reactions, exhibited the enhanced starch solubility and swelling capacity compared to those of native starch (Table 2). As commonly observed, incorporation of acetyl groups in starch molecules prevents inter-chain association among acetylated starch molecules, facilitating hydration and thus, swelling of the granules; further, increased hydration and swelling of the acetylated starch granules enhanced the mobility of acetylated starch molecules, leading to an increase in starch solubility (Gunaratne & Corke, 2007b; Liu, Ramsden et al., 1997; Singh et al., 2004; Sodhi & Singh, 2005). For acetylated starches, both starch solubility and swelling power of the reaction control (generated from conven-
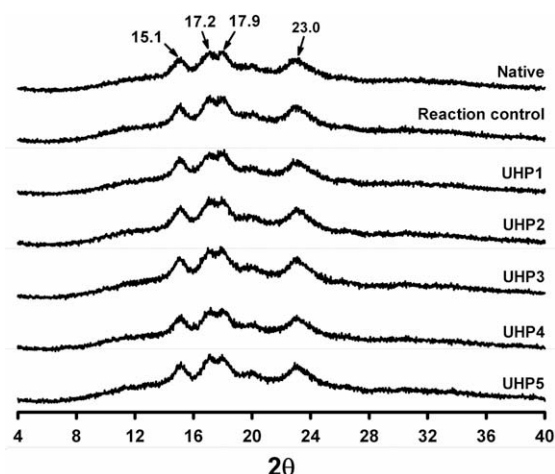

Fig. 1. X-ray diffraction patterns of native and acetylated corn starches.

tional reaction) were greater than those of the UHP-assisted acetylated starch derivatives (UHP1–UHP5) (Table 2). These results appeared to be due to higher DS for the reaction control across the UHP-assisted acetylated starch derivatives as observed by Singh et al. (2004), who exhibited increasing trends in starch solubility and swelling power along with increasing DS values of the acetylated starches.

However, subtle differences (though not likely of practical importance) in starch solubility and swelling power were observed across the UHP-assisted acetylated starches (UHP1–UHP5) (Table 2). In comparison of acetylated starch derivatives (UHP1–UHP3) representing comparable DS values, UHP1 (acetylated at 0.1 MPa), resembling the modified starch derivative subjected to 15 min conventional acetylation reaction, exhibited significantly higher starch solubility and swelling power than both UHP2 and UHP3 (acetylated at 100 and 200 MPa, respectively) (Table 2). Further, the UHP1 starch solubility and swelling power values were significantly overrode only after acetylation reaction was conducted at 400 MPa (UHP5) (Table 2). These results suggested that UHP treatment in acetylation reaction might restrict granule swelling of and/or starch leaching from acetylated starch derivatives as often observed in the UHP-treated native starch granules (e.g., normal corn, barley, wheat, normal rice) (Douzals et al., 1998; Oh, Hemar, Anema, Wong, & Pinder, 2008b; Stolt et al., 2001; Stute et al., 1996). Though the noted suggestion is not yet fully understood even in UHP-treated native starch granules, possible explanation might be due to formation of amylose–lipid complex, which inhibits granule swelling and amylose leaching from granules (Tester & Morrison, 1990), within granules during UHP treatment (Oh et al., 2008b). Katopo et al. (2002) identified the V-pattern in X-ray diffraction patterns of normal corn and rice starches subjected to UHP treatment in water, suggesting the presence of amylose–lipid complex. Moreover, Liu, Arntfield et al. (1997) revealed that in acetylated starches, amylose molecules reacted with lipids, forming amylose–lipid complex. Thus, in the present study, the UHP-assisted (relative to conventional) acetylation reaction might facilitate formation of amylose–lipid complex, likely resulting in restricted granule swelling and starch leaching. However, this proposed issue also will need to further investigate the presence and influence of amylose–lipid complex within the UHP-assisted acetylated starches.

3.3. Starch gelatinization

Gelatinization properties of native, reaction control, and UHP-assisted acetylated starches and corresponding UHP controls were

Table 2
Mean[a] values for relative crystallinity (RC), starch solubility (SS), and swelling power (SP) of native and acetylated corn starch granules.

Sample	RC (%)[b]	SS$_{90}$ (%)[c]	SP$_{90}$ (g/g)[c]
Native	7.7 ± 0.2[A]	15.5 ± 0.7[F]	18.0 ± 0.4[F]
Reaction control	7.6 ± 0.2[A]	37.7 ± 0.1[A]	26.2 ± 0.9[A]
UHP1	7.7 ± 0.1[A]	26.7 ± 0.5[C]	21.5 ± 0.5[C]
UHP2	7.6 ± 0.5[A]	23.7 ± 1.3[E]	20.1 ± 0.7[E]
UHP3	7.7 ± 0.2[A]	24.8 ± 1.0[D]	20.9 ± 0.1[D]
UHP4	7.8 ± 0.1[A]	27.4 ± 0.7[C]	21.4 ± 0.3[C]
UHP5	7.8 ± 0.0[A]	31.1 ± 1.3[B]	22.3 ± 0.3[B]

[a] Mean value of two measurements; values within a column sharing a upper-case letter are not significantly different (p < 0.05).
[b] Defined as the percent ratio (%) of total area of the crystalline peak regions to that of total diffractogram (crystalline and amorphous regions).
[c] Starch solubility (SS) and swelling power (SP) were determined at 90 °C.

investigated using DSC, and depicted in Table 3. Native starch exhibited higher gelatinization temperatures (e.g., onset, peak, completion) and enthalpies than all acetylated starches (subjected to either conventional or UHP-assisted reaction) (Table 3), compatible with previous reports of others (Gunaratne & Corke, 2007b; Huang et al., 2007; Liu, Ramsden et al., 1997; Singh et al., 2004; Sodhi & Singh, 2005; Wang & Wang, 2002). Substituted acetyl groups destabilize starch granule structures by inhibiting interchain association amongst starch molecules and facilitating granule hydration. Destabilized starch granules need less energy to gelatinize, resulting in reduced gelatinization temperatures and enthalpies (Gunaratne & Corke, 2007b; Singh et al., 2004; Sodhi & Singh, 2005).

Aside from acetylation effects on starch gelatinization, UHP has been known to gelatinize native starch granules, which alters gelatinization temperatures or reduces gelatinization enthalpies (Douzals et al., 1998; Katopo et al., 2002; Stolt et al., 2001; Stute et al., 1996). To investigate whether UHP contributes to lower gelatinization temperatures and enthalpies observed across the UHP-assisted acetylated starches, gelatinization properties of the UHP controls (subjected to the same acetylation reaction in absence of acetic anhydride at pressure ranges of 100–400 MPa) were assessed (Table 3; values in parentheses). The UHP controls, regardless of pressure levels, exhibited very similar gelatinization temperatures (e.g., onset, peak, completion) and enthalpies to those of native starch (Table 3). These results suggested that UHP did not appear to impact gelatinization properties of the UHP-assisted acetylated starches. Further, it was more likely due to relatively lower pressure levels employed in the present study compared to those (commonly more than 500 MPa, though varied depending on botanical sources of starch) in pressure-induced gelatinization (Blaszczak et al., 2005; Buckow et al., 2007; Douzals et al., 1998; Katopo et al., 2002; Oh et al., 2008a; Stolt et al., 2001; Stute et al., 1996). Thus, reduced trends in gelatinization properties of the UHP-assisted acetylated (relative to native) starches appeared to be attributable to their respective degree of derivatization.

Furthermore, the reaction control subjected to conventional acetylation reaction exhibited lower gelatinization onset and peak temperatures and similar gelatinization completion temperature compared to those of the UHP-assisted acetylated starches (except for UHP5, which acetylated at 400 MPa) (Table 3). These results were in partial agreement with common trends observed by Singh et al. (2004), who exhibited a decrease in gelatinization temperatures (e.g., onset, peak, completion) of acetylated normal corn

starches, along with increasing reagent levels. Within the UHP-assisted acetylated starches, while the highest DS value of UHP5 (Table 1) possessed the lowest gelatinization temperatures, no consistent trends were observed for other acetylated starch derivatives (UHP1–UHP4) (Table 3). As earlier suggested, these results appeared that gelatinization of the UHP-assisted acetylated starches was the function of degree of derivatization.

Despite variations in gelatinization temperatures, gelatinization enthalpies did not significantly differ across all modified starch derivatives subjected to conventional and UHP-assisted acetylation reactions. The noted phenomenon also suggested that in the present study, either acetylation reactions generating low DS levels (<0.1) or UHP treatments over the pressure range of 0.1–400 MPa did not appear to impact starch crystalline structures within granules. It was further supported by X-ray diffraction patterns and relative crystallinities (coincided with changes in gelatinization enthalpy) which revealed intact starch crystalline structures of acetylated starches subjected to both conventional and UHP-assisted acetylation reactions (Section 3.1; Table 2 and Fig. 1).

3.4. Pasting behavior

Pasting properties were investigated for native, reaction control, and UHP-assisted acetylated starches and corresponding UHP controls (Table 4 and Fig. 2). Pasting viscosities were higher at all points of the pasting profiles prior to final holding time (2 min) at 50 °C for native starch relative to the reaction control and UHP-assisted acetylated starches (UHP1–UHP5) within which any consistent trends in pasting viscosity profiles were not observed (Fig. 2). During the final holding periods, pasting viscosities of all acetylated starches (except for UHP2) transited and increased over those of native starch (Fig. 2). For pasting characteristics (Table 4), the reaction control exhibited lower peak and trough viscosities than native starch, though opposite trends were observed for final, breakdown, and setback viscosities. While the UHP-assisted acetylated starches (UHP1–UHP5) exhibited lower peak and breakdown viscosities than the native starch and reaction control, final and setback viscosities were observed in the viscosity ranges of native starch to reaction control. Within the UHP-assisted acetylated starches, no consistent trends in pasting characteristics were observed. As commonly observed in the majority of studies which investigated pasting properties of acetylated starch (Gunaratne & Corke, 2007b; Liu & Corke, 1999; Liu, Ramsden et al., 1997; Sodhi & Singh, 2005; Wang & Wang, 2002), acetylation generates rapid viscosity development and enhanced pasting viscosity relative to those of native starch, resulting from hydrophilic acetyl groups substituted to starch molecules which prevent starch chain reassociation and promote granule hydration/swelling. However, these common trends and explanations did not likely account for differences in pasting properties between native and acetylated starches, or among the UHP-assisted acetylated starches, generated in the present study. On the other hand, decreased trends in pasting properties of all acetylated (relative to native) starches generated in the present study likely resembled to those observed by Huang et al. (2007) and Saartrat et al. (2005), who reported lower pasting viscosities at all points of the pasting profiles for acetylated (relative to native) starches with the DS range from 0.05 to 0.1. Saartrat et al. (2005) suggested that reduced pasting viscosities of acetylated (relative to native) starch may result from hydrophobic behavior of acetyl groups within granules. However, their suggestion also did not likely provide reasonable explanation for the present study, which generated low-substituted acetylated starches (DS < 0.1). Xu et al. (2004) demonstrated that hydrophobic characteristic of acetylated starch increased with increasing DS values from 0.5 to 2.5. Thus, further research with acetylated starches modified at multiple reagent levels will be needed to bet-

Table 3
Mean[a] gelatinization properties of native and acetylated corn starch granules.

| Reaction	T_o (°C)[b]	T_p (°C)[b]	T_c (°C)[b]	ΔH (J/g)[b]
Native	64.5 ± 0.5[A]	70.7 ± 0.1[A]	87.5 ± 0.6[A]	7.6 ± 0.2[A]
Reaction control	56.5 ± 0.7[E]	65.0 ± 0.7[F]	85.3 ± 0.2[D]	5.3 ± 0.4[B]
UHP1	61.2 ± 0.9[B]	67.2 ± 0.5[B]	85.9 ± 0.1[B]	5.3 ± 0.0[B]
UHP2	58.8 ± 1.0[D]	66.5 ± 0.0[D]	85.3 ± 0.3[CD]	5.1 ± 0.0[BC]
	(63.9 ± 0.5)[c]	(69.6 ± 0.2)	(87.8 ± 0.9)	(6.6 ± 0.8)
UHP3	59.7 ± 1.0[C]	67.0 ± 0.4[BC]	85.7 ± 0.9[BCD]	5.0 ± 1.1[BC]
	(62.5 ± 1.2)	(69.6 ± 0.2)	(87.5 ± 0.2)	(7.1 ± 0.2)
UHP4	58.9 ± 0.5[D]	66.8 ± 0.5[C]	85.7 ± 0.8[BC]	4.8 ± 0.9[C]
	(62.3 ± 0.7)	(69.2 ± 0.1)	(87.7 ± 1.1)	(6.8 ± 0.3)
UHP5	56.6 ± 0.8[E]	66.2 ± 0.0[E]	82.2 ± 0.9[E]	4.9 ± 0.1[BC]
	(63.1 ± 0.9)	(68.9 ± 0.6)	(86.0 ± 1.5)	(7.6 ± 0.3)

[a] Mean value of three measurements; values within a column sharing a uppercase letter are not significantly different ($p < 0.05$).
[b] Gelatnization onset, peak, and completion temperatures are designated as T_o, T_p, and T_c, respectivtly; gelatnization enthalpy is denoted as ΔH.
[c] Values in the parentheses are gelatinization properties of the UHP controls subjected to the same UHP-assisted acetylation reaction in absence of acetic anhydride.

Table 4
Mean[a] pasting viscosity characteristics of native and acetylated corn starch granules.

Reaction	Peak (RVU)	Trough (RVU)	Final (RVU)	Breakdown (RVU)	Setback (RVU)
Native	262.3 ± 2.1^A	180.7 ± 4.0^A	274.0 ± 4.0^F	81.7 ± 5.7^B	93.3 ± 7.8^G
Reaction control	259.7 ± 3.8^B	128.3 ± 1.5^F	313.3 ± 3.8^A	131.3 ± 2.3^A	185.0 ± 2.6^A
UHP1	208.3 ± 2.3^D	132.3 ± 3.8^D	289.0 ± 3.6^C	76.0 ± 3.0^D	156.7 ± 0.6^C
UHP2	185.3 ± 1.5^F	120.7 ± 1.5^G	257.0 ± 1.0^G	64.7 ± 1.5^F	136.3 ± 0.6^F
	$(262.7 \pm 3.2)^b$	(177.3 ± 4.7)	(276.7 ± 2.9)	(85.3 ± 7.5)	(99.3 ± 4.7)
UHP3	221.0 ± 3.0^C	142.0 ± 3.5^B	311.0 ± 3.6^B	79.0 ± 1.7^C	169.0 ± 2.6^B
	(268.0 ± 6.1)	(181.7 ± 9.0)	(285.3 ± 6.1)	(86.3 ± 3.1)	(103.7 ± 4.0)
UHP4	207.7 ± 3.2^D	135.7 ± 1.5^C	285.3 ± 1.5^D	72.0 ± 1.7^E	149.7 ± 2.3^E
	(277.7 ± 4.5)	(200.0 ± 17.8)	(284.7 ± 2.3)	(77.7 ± 13.7)	(84.7 ± 20.0)
UHP5	200.7 ± 1.5^E	130.0 ± 2.6^E	282.3 ± 2.5^E	70.7 ± 2.1^E	152.3 ± 0.6^D
	(282.3 ± 3.2)	(185.7 ± 5.5)	(292.0 ± 2.0)	(96.7 ± 2.3)	(106.3 ± 3.8)

[a] Mean value of three measurements; values within a column sharing a upper-case letter are not significantly different ($p < 0.05$).
[b] Values in the parentheses are pasting characteristics of the UHP controls subjected to the same UHP-assisted acetylation reaction in absence of acetic anhydride.
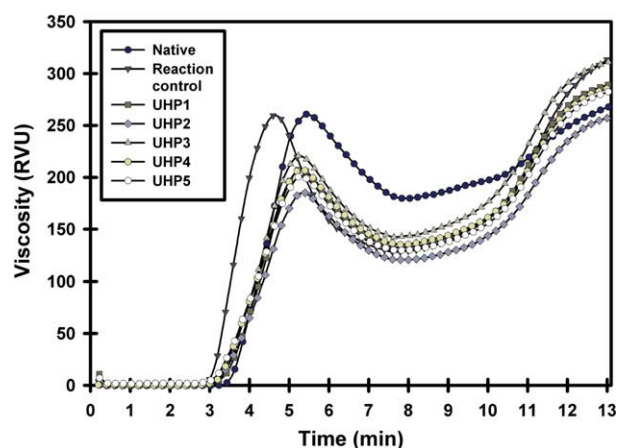
Fig. 2. Pasting profiles of native and acetylated corn starches.

ter understand the effects of acetylation on pasting properties of acetylated starch derivatives.

To assess whether UHP treatments in acetylation reaction impact pasting properties of the UHP-assisted acetylated starches, UHP controls subjected to the UHP-assisted acetylation reaction in absence of acetic anhydride were tested, and depicted in Table 4 (values in parentheses). The UHP controls (relative to native starch) exhibited the increased peak and final viscosities along with increasing pressure levels from 100 to 400 MPa, though there was no consistent variation in trough, breakdown, and setback viscosities (Table 4). Little information is available for enhanced peak and final viscosities of UHP-treated starches at relatively low pressure levels (from 0.1 to 400 MPa). Further, the UHP controls possessed higher peak, trough, and breakdown viscosities and lower setback viscosities compared to corresponding acetylated starches (Table 4). These findings suggested decreased pasting viscosities of the UHP-assisted acetylated starches were likely attributable to their respective degree of derivatization in acetylation reaction.

4. Conclusions

This study investigated reactivity and properties of corn starch in the UHP-assisted acetylation reaction with acetic anhydride. UHP treatment (up to 400 MPa for 15 min) in acetylation reaction appeared to enhance starch granule reactivity by facilitating penetration of acetic anhydride into the granule interior and/or matrix, while not induced significant changes in granule structures (e.g., granule identity, crystalline structure). The UHP-assisted (relative to conventional) acetylation reaction also appeared to reduce reac-

tion time at the maximum pressure level (400 MPa), though there was a 25% decrease in reaction efficiency. These advantageous processes would be more useful and valid for other substitution reactions such as, hydroxypropylation and/or cationization reactions which require extended reaction time and relatively higher reaction temperature. The reaction volume might be the determining factor for an acceleration or deceleration of chemical reaction under pressure with isothermal conditions. However, more researches are needed to prove this. This research demonstrated reaction system appeared to be the first successful association of acetylation reaction with UHP technology. Moreover, the UHP-assisted acetylated starches exhibited limited starch solubility and swelling capacity and reduced pasting properties compared to those of conventional starch acetate. The noted differential properties might infer differences in granular reaction patterns for UHP-assisted and conventional reactions. Overall physical properties observed for UHP-treated starch acetate may further expand applications of acetylated starch in food and non-food systems under strict regulatory practice.

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