

Characterization of Acetylated Corn Starch Prepared under Ultrahigh Pressure (UHP)

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To investigate the impact of ultrahigh pressure (UHP) on the physicochemical properties of the UHP-assisted starch acetate, common corn starch was subjected to either conventional (0.1 MPa, 30 °C, 60 min) or UHP-assisted (400 MPa, 25 °C, 15 min) acetylation reactions at three levels (4, 8, or 12%) of acetic anhydride. Without significant changes in starch granule crystal structure, UHP-assisted reaction exhibited lower degree of substitution values than conventional reaction across reagent addition levels. An increase in reagent addition levels exhibited common trends in starch solubility/swelling power, gelatinization, and pasting properties for the conventional and UHP-assisted starch acetates relative to native starch. Within an equivalent derivatization level, however, the UHP-assisted (relative to conventional) starch acetates revealed restricted starch solubility/swelling power, reduced gelatinization temperatures, and lower pasting viscosities. Overall, this result suggested that UHP treatment in acetylation reaction might influence the physicochemical properties of starch acetate by facilitating the formation of lipid-complexed amylose or altering granular reaction patterns to acetic anhydride.

KEYWORDS: Ultrahigh pressure (UHP); conventional acetylation; UHP-assisted acetylation; acetic anhydride; corn starch; starch acetate

INTRODUCTION

Since Hite (*1*) investigated the effect of high pressure on the shelf-life extension of milk, ultrahigh pressure (UHP), or high hydrostatic pressure (HHP), technology has been extensively applied to various food systems in accordance with the intended purposes, that is, the inactivation of microorganisms and the alteration of enzyme behaviors and/or protein functionalities (*2*). More recently, further attention to the UHP technique is paid for physical modification of starch (*3*), although annealing and/or heat–moisture treatment are conventionally conducted to modify starch granule structures (*4*). UHP treatment of the starch–water suspension reversibly hydrates amorphous regions within granules, leading to granule swelling and crystalline region distortion, and thus, the crystalline regions become more accessible to water (*5–8*). Finally, starch granules are nonthermally gelatinized over a critical pressure level, which varied depending on the botanical source and crystal packing arrangement of starch (*7–10*). Furthermore, UHP minimizes leaching of starch (including amylose) from granules and inhibits granule swelling (*3, 6, 10, 11*). UHP treatment has been shown to transit A-type crystallite pattern to B-type crystallite pattern (*11, 12*). Moreover, UHP-treated starch that is little or partially gelatinized maintains its granular structure (*3, 9, 10*). While the noted physical and structural characteristics of UHP-treated starch granules are anticipated to affect the reactivity and properties

of chemically modified starches, little information is available regarding combination of starch chemical modification with UHP and characterization of UHP-assisted modified starch products.

Several studies have been conducted to investigate UHP effects on derivatization of starch granules with acids (*13, 14*), phosphorus oxychloride (*15*), and acetic anhydride (*16*). Lee et al. (*13*) and Choi et al. (*14*) hydrolyzed common corn starch using acids (e.g., hydrochloric acid, sulfuric acid, oxalic acid) with the aid of the UHP and demonstrated that UHP (at least 600 MPa) likely facilitated starch hydrolysis by acids in the nonthermal state. Choi et al. (*14*) further suggested that action patterns of acids to starch molecules under UHP likely varied depending on pressure levels, pressure holding periods, and acid concentrations. For cross-linking reaction with phosphorus oxychloride (*15*), UHP-assisted (relative to conventional) reaction likely stabilized starch granules (as supported by restricted granule swelling and lower breakdown viscosity), although similar trends in relative crystallinity, starch solubility, and gelatinization property were observed for UHP-assisted and conventionally cross-linked starch derivatives. Choi et al. (*16*) reacted common corn starch with acetic anhydride under UHP and revealed enhanced reactivities of common corn starch in the acetylation reaction along with increasing pressure levels (up to 400 MPa). In this experiment, it was suggested that although UHP promoted acetylation reaction of common corn starch with acetic anhydride, it did not appear to influence gelatinization and pasting properties of UHP-assisted acetylated starch derivatives. To date, all studies tend to

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Table 1. Reaction System Parameters and Mean^a Degree of Substitution (DS) Values for Acetylation of Corn Starch with Acetic Anhydride

sample	reagent (% _{sb}) ^b	pH	time (min)	temperature (°C)	pressure (MPa)	DS
ACT1 ^c	4.0	8.0–8.5	60	30	0.1	0.048 ± 0.002 c
ACT2 ^c	8.0	8.0–8.5	60	30	0.1	0.067 ± 0.004 b
ACT3 ^c	12.0	8.0–8.5	60	30	0.1	0.090 ± 0.002 a
UHP1 ^d	4.0	8.0–8.5	15	25	400.0	0.033 ± 0.001 d
UHP2 ^d	8.0	8.0–8.5	15	25	400.0	0.048 ± 0.002 c
UHP3 ^d	12.0	8.0–8.5	15	25	400.0	0.067 ± 0.001 b

^a Mean value of two replicate reactions; values within a column sharing a lower case letter are significantly different ($p < 0.05$). ^b Dry starch weight basis. ^c Acetylated starch samples subjected to conventional acetylation reaction (ACT) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3). ^d Acetylated starch samples subjected to UHP-assisted acetylation reaction (UHP) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3).

focus on beneficial processing aspects (i.e., nonthermal and rapid reaction) of the UHP-assisted (relative to conventional) acid hydrolysis and chemical derivatization of starch, even though their findings may imply potential contribution of UHP treatment to properties of the UHP-assisted modified starch derivatives. There may be a lack of information on what differentiates the physicochemical properties of modified starch derivatives prepared via UHP-assisted reactions from those prepared via conventional reactions. Thus, this study investigated the effects of UHP-assisted and conventional acetylation on starch solubility, swelling power, gelatinization, and pasting behavior of common corn starch as a function of reagent addition levels and contrasted the properties of the UHP-assisted starch acetate with those of the conventional starch acetate at equivalent derivatization levels to elucidate UHP effects on acetylated starch derivatives.

MATERIALS AND METHODS

Materials. Common corn starch, possessing 26.5% (sb or dry starch weight basis) amylose and 10.5% (db or dry weight basis) moisture contents, was kindly donated from Daesang Co. (Ichon, Korea) and used for this study without further purification. Acetic anhydride was obtained from Sigma Co. (St. Louis, MO). Other reagents and chemicals used in this study were of at least analytical grade.

Preparation of Acetylated Corn Starch. Either conventional or UHP-assisted acetylation reaction was conducted with reaction mixtures that were prepared similarly to a scheme outlined by Wang and Wang (17). Common corn starch (20 g, db) was dispersed in distilled water (80 mL) with rapid stirring, after which the pH of the starch slurry was adjusted to 8.0–8.5 using 1.0 M NaOH. Acetic anhydride (4, 8, or 12%, sb) was added dropwise to the starch slurry at 25 °C under continuous stirring, during which period the pH of the starch slurry was maintained at 8.0–8.5 by periodic addition of 1.0 M NaOH. Reaction system parameters for conventional and UHP-assisted acetylation are depicted in **Table 1**.

For conventional acetylation reaction, the reaction mixture was further incubated at 30 °C for an additional 60 min with continuous stirring, and during reaction, maintained at the pH range of 8.0–8.5. The resultant reactions were designated ACT1, ACT2, and ACT3, corresponding to three reagent addition levels (4, 8, and 12%, respectively) (**Table 1**). In the case of UHP-assisted acetylation reaction, approximately 100 g (about 86 mL) of the reaction mixture was immediately poured into a retortable pouch and hermetically sealed using a heat sealer. It was pressurized in a UHP unit (2 L capacity, Autoclave Engineers, Erie, PA) 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 compression up to 400 MPa and returned to 25 °C within a few seconds. The UHP unit was maintained at 25 °C during pressure holding time (15 min). Although only a minimal pH drop (<0.1) was observed in the final reaction mixtures after UHP treatment, the final pH level ranged from 8.0 to 8.5. The resultant reactions were referred to as UHP1, UHP2, and UHP3, corresponding to three reagent addition levels (4, 8, and 12%, respectively) (**Table 1**).

At the conclusion of both conventional and UHP-assisted reaction periods (60 and 15 min, respectively), the reaction mixtures were neutralized to pH 5.5 with 1 M HCl. The acetylated starches were recovered by centrifugation (1500g, 20 min), washed 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.

Determination of Degree of Substitution (DS). DS levels of acetylated starch derivatives were assayed for acetyl group contents (acetyl %) according to a scheme described by Choi et al. (16). Acetylated starch (5.0 g, db) 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. Acetyl group contents and DS values were calculated using the equations

$$\text{acetyl\%} = [(\text{blank} - \text{sample}) \times f \times 0.0086 \times 100] / \text{sample weight (g, db)}$$

where blank and sample are titration volume (mL) of 0.2 M HCl and f is a factor value of 0.2 M HCl and

$$\text{DS} = (162 \times \text{acetyl \%}) / [4300 - (42 \times \text{acetyl \%})]$$

Native and Acetylated Starch Analyses. X-ray diffraction (XRD) patterns of native and acetylated starches were determined using an X-ray diffractometer (M18XHF-SRA, Mac Science Co., Yokohama, Japan) (13). For relative crystallinity (RC), the total areas under diffractograms and the areas of amorphous regions of the diffractograms were calculated, respectively, using SigmaPlot software (version 8.0, Jandel Scientific, San Rafael, CA). RC was calculated using the following equation:

$$\text{RC (\%)} = [(\text{total diffractogram area} - \text{amorphous region area}) \times 100] / \text{total diffractogram area}$$

Solubility and swelling power of native and acetylated starches were assessed by the scheme described by Choi et al. (16). Gelatinization properties (gelatinization temperatures and enthalpies) were analyzed using a differential scanning calorimeter (DSC-650, Sinco Co., Seoul, Korea) (14). Pasting viscosity profiles and characteristics were determined using the Rapid Visco Analyzer (RVA) (Newport Scientific, NSW, Australia) according to standard method 1 (STD1) offered by the supplier.

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

RESULTS AND DISCUSSION

Acetylation of Corn Starch with Acetic Anhydride. Our previous studies (15, 16) have realized that UHP-assisted derivatization of common corn starch with phosphorus oxychloride and acetic anhydride did not show significant gelatinization (or rupture) during reaction. In the UHP-assisted acetylation reaction where DS values were directly determined for modified starch derivatives (16), maximum reactivity of common corn starch was achieved at 400 MPa and 15 min pressure holding time, and these conditions were employed for UHP-assisted acetylation

reaction in this study. Moreover, conventional acetylation reaction was conducted to investigate how UHP may contribute to the physicochemical properties of the UHP-assisted acetylated starch derivatives. For both UHP-assisted and conventional reactions, three reagent addition levels (4, 8, and 12%) were utilized to track trends in common corn starch reactivities and to seek acetylated starch derivatives possessing equivalent derivatization levels. DS values for acetylated starch derivatives subjected to either conventional or UHP-assisted reaction are depicted in **Table 1**.

For conventional acetylation reaction, an increase in reagent addition levels enhanced DS values for acetylated starch derivatives (**Table 1**) as commonly reported by others (18–20). However, the DS values (**Table 1**) were much lower than those of the other studies (17, 18, 21) that reacted starch granules (e.g., corn, potato, canna) with various levels (2–12%) of acetic anhydride under reaction conditions comparable to those of the present study, except they used higher initial concentrations (30–35 vs 20% in the present study) of the starch slurry. Choi et al. (16) suggested that an abundant water phase within the initial starch slurry in the acetylation reaction may dilute reagent and/or induce the side reaction (i.e., conversion of acetic anhydride into acetic acid) with reagents, leading to reduced accessibility of reagent to starch granules and, in turn, decreased reaction efficiency. Thus, the lower DS values observed in the present study appear to be due to the relatively low ratio of starch to water within the initial starch slurry.

For UHP-assisted acetylation reaction, DS values of acetylated starch derivatives increased along with increasing reagent addition levels from 4 to 12% (**Table 1**). However, at equivalent reagent addition levels, UHP-assisted reaction generated lower DS values of acetylated starch derivatives compared to conventional reaction (**Table 1**). This result was in agreement with that observed by Choi et al. (16), who reported the 25% less DS value for the UHP-assisted (relative to conventional) starch acetate (reacted at 400 MPa with 12% acetic anhydride). Furthermore, they suggested that the reactivity differences likely resulted from different reaction periods for conventional and UHP-assisted reactions (60 and 15 min, respectively). Despite a probable impact of reaction time on reduction in DS values, reduction in reactivity of UHP-assisted reaction relative to conventional reaction was not likely consistent across reagent addition levels. At 4 and 8% reagent additions (**Table 1**; ACT1 vs UHP1 and ACT2 vs UHP2), approximately 32 and 28% reductions in DS values were observed for the UHP-assisted (relative to conventional) starch acetate, respectively. The further increase of reagent addition levels to 12% (ACT3 vs UHP3) reduced deviation in DS values between conventional and UHP-assisted reaction (up to 25%) (**Table 1**). These findings might imply that higher reagent addition levels enhance efficiency in UHP-assisted acetylation reaction.

XRD Pattern. To investigate whether either conventional or UHP-assisted acetylation reaction altered crystallite structures within starch granules, all acetylated starch derivatives (relative to native starch) were assessed with respect to XRD pattern and relative crystallinity (**Figure 1** and **Table 2**, respectively). Native starch possessed major diffraction peaks at 15.1°, 17.2°, 17.9°, and 23.0° (2 θ) (**Figure 1**), indicating a typical A-type crystalline packing arrangement within cereal starch granules (21). Significant peak shifts or changes in X-ray diffraction patterns were not observed for acetylated (relative to native) starches from both conventional and UHP-assisted reactions (**Figure 1**). Also, all acetylated starch derivatives exhibited relative crystallinities very similar to that of native starch (**Table 2**). Wang and Wang (17) and Huang et al. (23) reported no differences in XRD patterns and relative crystallinities between native and low-substituted

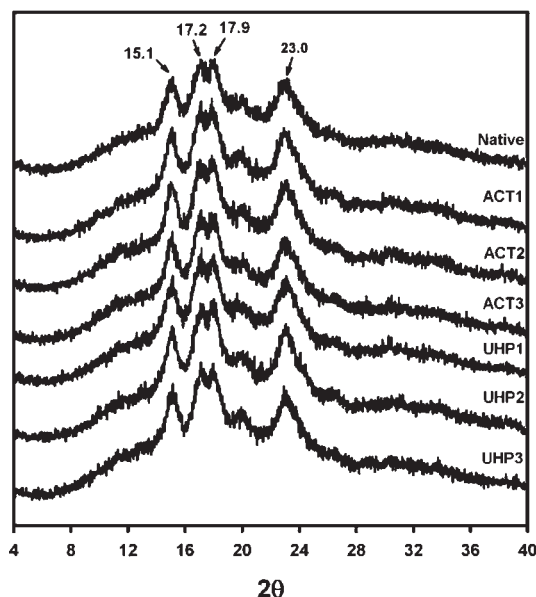


Figure 1. X-ray diffraction patterns of native and acetylated corn starches. ACT1, ACT2, and ACT3 correspond to acetylated starch samples subjected to conventional acetylation reaction with 4.0, 8.0, and 12.0% acetic anhydride (based on starch weight basis), respectively. UHP1, UHP2, and UHP3 correspond to acetylated starch samples subjected to UHP-assisted acetylation reaction with 4.0, 8.0, and 12.0% acetic anhydride (based on starch weight basis), respectively.

Table 2. Mean^a Values for Relative Crystallinity (RC), Starch Solubility (SS), and Swelling Power (SP) of Native and Acetylated Corn Starches

sample	RC ^b (%)	SS ₉₀ ^c (%)	SP ₉₀ ^c (g/g)
native	7.7 ± 0.1 a	15.5 ± 0.7 f	18.1 ± 0.5 d
ACT1 ^d	7.7 ± 0.1 a	24.4 ± 0.9 d	22.4 ± 0.4 b
ACT2 ^d	7.9 ± 0.7 a	33.4 ± 0.6 b	24.9 ± 0.5 a
ACT3 ^d	7.6 ± 0.2 a	37.7 ± 0.1 a	26.2 ± 0.9 a
UHP1 ^e	7.8 ± 0.1 a	22.8 ± 0.2 e	19.8 ± 0.1 c
UHP2 ^e	7.7 ± 0.7 a	23.4 ± 0.7 de	20.2 ± 0.8 c
UHP3 ^e	7.8 ± 0.0 a	26.7 ± 0.3 c	22.1 ± 0.0 b

^a Mean value of two measurements; values within a column sharing a lower 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 Determined at 90 °C. ^d Acetylated starch samples subjected to conventional acetylation reaction (ACT) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3). ^e Acetylated starch samples subjected to UHP-assisted acetylation reaction (UHP) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3).

(DS < 0.1) acetylated starches of normal corn, yellow pea, cowpea, and chickpea. With regard to UHP-assisted derivatization, Hwang et al. (15) and Choi et al. (16) revealed that cross-linking and acetylation reactions of common corn starch at relatively mild pressurization conditions (up to 400 MPa for 15 min) did not alter crystalline structures within starch granules. Consequently, the findings in the present study suggested that at low derivatization levels (DS < 0.1), either the conventional or UHP-assisted acetylation reaction occurs predominantly at an amorphous region within starch granules, as supported by others (15–17, 22).

Solubility and Swelling Power. For ease of interpretation and discussion, acetylated starch derivatives (prepared from conventional and UHP-assisted reaction systems) possessing equivalent derivatization levels were grouped on the basis of their DS values (i.e., DS ≈ 0.048, ACT1 vs UHP2; DS ≈ 0.067, ACT2 vs UHP3) (**Table 1**) to exclude impacts of degree of derivatization on

acetylated starch properties. It is anticipated to facilitate an understanding of functional roles of UHP in the UHP-assisted acetylated starch properties by comparing two acetylated starch derivative pairs.

Starch solubility and granule swelling of native (unmodified) and acetylated starches were determined at 90 °C and are depicted in **Table 2**. Acetylated starch derivatives, generated from either conventional or UHP-assisted reaction, exhibited enhanced starch solubility and swelling power relative to those of native starch (**Table 2**). Within a given reaction system, an increase in reagent addition levels also enhanced the solubility and swelling capacity of common corn starch granules (**Table 2**). In general, incorporation of bulky, hydrophilic acetyl groups into starch molecules restricts interchain association among acetylated starch molecules, promoting hydration and, thus, swelling of the granules. Increased granule hydration and swelling further facilitate the mobility of acetylated starch molecules within granules, enhancing starch solubility (18, 20, 24, 25).

Within an equivalent derivatization level, the UHP-assisted starch acetates exhibited lower starch solubility and swelling power than the conventional starch acetates (**Table 2**; ACT1 vs UHP2 and ACT2 vs UHP3). These results were consistent with those of Choi et al. (16), who observed restricted swelling and starch leaching for UHP-assisted (relative to conventional) starch acetates, with increasing pressure levels up to 400 MPa. Although the decreases in starch solubility and swelling capacity were often reported for UHP-treated native starch granules (e.g., normal corn, barley, wheat, normal rice) (3, 6, 10, 26), it is not yet fully understood how UHP treatment inhibits solubility and swelling of native and modified starch granules. Nevertheless, as suggested by Choi et al. (16), formation of an amylose–lipid complex within common corn starch granules during UHP-assisted acetylation reaction might provide a reasonable explanation for limited solubility and swelling capacity of the UHP-assisted (relative to conventional) starch acetates. The presence of lipid-complexed amylose in starch granules is well-known to prevent granule swelling and amylose leaching from granules (27). For UHP-treated normal corn and rice starches, Katopo et al. (11) observed the V-type XRD patterns, suggesting the presence of the amylose–lipid complex. Liu et al. (28) demonstrated that amylose molecules (which were either native or derivatized) in acetylated starch granules formed complexes with internal lipids. However, they did not detect the V-type XRD patterns, as observed in the present study (**Figure 1**), because bulky acetyl groups on starch molecules may prevent the formation of the V-type polymorphic forms among amylose–lipid complexes (28). These reports in the literature and results (**Table 2** and **Figure 1**) in the present study increase the possibility that UHP-assisted acetylation reaction might facilitate the formation of lipid-complexed amylose within granules, although no evidence is yet provided. Thus, to provide strong evidence for supporting the noted suggestion, there is a need for careful investigation of whether native and acetylated starch molecules might form complexes with lipids under which UHP treatment was applied to UHP-assisted acetylation reaction.

Thermal Properties. Thermal properties of native (unmodified) and acetylated starches were investigated using DSC and are depicted in **Table 3**. All acetylated starches, obtained from either conventional or UHP-assisted reaction, exhibited lower gelatinization temperatures (e.g., onset, peak, completion) and enthalpies than native starch (**Table 3**), consistent with previous reports of others (17, 18, 20, 23–25). As commonly known, substitution of bulky acetyl groups onto starch molecules destabilizes starch granule structures by preventing interchain association among starch molecules and facilitating granule hydration. Destabilized

Table 3. Mean^a Thermal Properties of Native and Acetylated Corn Starch Granules

reaction	T_o^b (°C)	T_p^b (°C)	T_c^b (°C)	ΔH^b (J/g)
native	64.7 ± 0.6 a	75.4 ± 0.3 a	87.9 ± 0.2 a	7.5 ± 0.1 a
ACT1 ^c	62.2 ± 0.5 b	73.3 ± 0.2 c	86.2 ± 1.1 bc	4.8 ± 0.4 b
ACT2 ^c	59.7 ± 0.6 c	71.1 ± 0.0 e	82.4 ± 0.0 d	5.0 ± 0.5 b
ACT3 ^c	56.3 ± 0.6 d	70.1 ± 0.3 f	85.2 ± 0.1 c	5.3 ± 0.4 b
UHP1 ^d	61.0 ± 1.0 bc	74.3 ± 0.1 b	85.0 ± 0.1 c	6.9 ± 1.1 a
UHP2 ^d	61.4 ± 0.6 b	72.5 ± 0.4 d	86.8 ± 0.5 ab	5.5 ± 0.8 ab
UHP3 ^d	56.7 ± 0.8 d	70.4 ± 0.1 f	82.2 ± 1.0 d	4.9 ± 0.1 b

^a Mean value of three measurements; values within a column sharing a lower case letter are not significantly different ($p < 0.05$). ^b Gelatinization onset, peak, and completion temperatures are designated T_o , T_p , and T_c , respectively; gelatinization enthalpy is denoted ΔH . ^c Acetylated starch samples subjected to conventional acetylation reaction (ACT) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3). ^d Acetylated starch samples subjected to UHP-assisted acetylation reaction (UHP) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3).

starch granules require less energy to gelatinize, leading to reduced gelatinization temperatures and enthalpies (18, 20, 25).

Upon increasing reagent addition levels from 4 to 12%, gelatinization onset and peak temperatures of acetylated starches decreased, which was in good agreement with the findings of Singh et al. (18) (**Table 3**). However, consistent trends in gelatinization completion temperatures across acetylated starches were not observed within reagent addition levels or within reaction systems (**Table 3**). Unfortunately, there is little information to explain the noted inconsistency, although it might result from erroneous measurements caused by different extents of the tailing of the DSC thermograms. Moreover, despite a decrease in gelatinization onset and peak temperatures along with an increase in derivatization levels (**Table 3**), no statistical differences in gelatinization enthalpies among acetylated starches suggested that either conventional or UHP-assisted acetylation reaction generated low-substituted starch derivatives ($DS < 0.1$) and did not appear to alter starch crystalline structures within granules, as supported by their respective XRD patterns and relative crystallinities (coincident with changes in gelatinization enthalpy) (**Table 2** and **Figure 1**).

Within an equivalent derivatization level (ACT1 vs UHP2 or ACT2 vs UHP3; **Table 3**), gelatinization onset (which was not statistically different for ACT1 and UHP2) and peak temperatures of the UHP-assisted starch acetates were lower relative to those of the conventional starch acetates, although gelatinization completion temperature and enthalpy did not statistically differ. Choi et al. (16) suggested that reduction of gelatinization properties of the UHP-assisted starch acetates was attributable to differences in degree of derivatization, regardless of UHP treatment at a pressure range from 100 to 400 MPa (which are not likely sufficient to gelatinize starch granules or alter starch gelatinization property). In the present study, the obtained results (**Table 3**) provided apparent evidence that the gelatinization property of the UHP-assisted starch acetate was influenced by degree of derivatization, as well as UHP treatment during UHP-assisted reaction. Nevertheless, degree of acetylation appeared to be much more important to gelatinization properties of the UHP-assisted starch acetates than UHP treatment. To confirm the noted suggestion, a control sample (UHP control) subjected to the same UHP-assisted acetylation reaction without acetic anhydride was prepared and compared to native and UHP-assisted starch acetates with respect to gelatinization property. The UHP control exhibited slightly lower gelatinization properties (T_o , 63.7 ± 0.1 °C; T_p , 74.9 ± 0.1 °C; T_c , 86.5 ± 1.8 °C; ΔH , 7.4 ± 0.1 J/g) than native starch (**Table 3**). However, gelatinization properties of the UHP-assisted starch acetates were much lower

Table 4. Mean^a Pasting Characteristics of Native and Acetylated Corn Starches

reaction	T_p^b (°C)	viscosity (RVU)				
		peak	trough	final	breakdown	setback
native	75.4 ± 0.4 a	261.5 ± 2.1 b	183.0 ± 0.0 a	272.0 ± 2.8 e	78.5 ± 2.1 e	89.0 ± 2.8 f
ACT1 ^c	73.3 ± 0.3 c	286.5 ± 0.7 a	146.0 ± 0.0 b	307.0 ± 4.2 ab	140.5 ± 0.7 a	161.0 ± 4.2 bc
ACT2 ^c	71.1 ± 0.0 e	257.5 ± 0.7 b	127.5 ± 2.1 c	293.5 ± 3.5 c	130.0 ± 2.8 b	166.0 ± 1.4 b
ACT3 ^c	70.1 ± 0.2 f	257.5 ± 0.7 b	127.5 ± 0.7 c	312.0 ± 4.2 a	130.0 ± 0.0 b	184.5 ± 3.5 a
UHP1 ^d	74.3 ± 0.1 b	243.5 ± 0.7 c	144.0 ± 0.0 b	283.0 ± 1.4 d	99.5 ± 0.7 c	139.0 ± 1.4 e
UHP2 ^d	72.5 ± 0.5 d	233.5 ± 3.5 d	145.5 ± 0.7 b	301.0 ± 0.0 b	88.0 ± 2.8 d	155.5 ± 0.7 dc
UHP3 ^d	70.4 ± 0.1 f	200.0 ± 1.4 e	128.5 ± 0.7 c	281.0 ± 1.4 d	71.5 ± 2.1 f	152.5 ± 0.7 d

^a Mean value of three measurements; values within a column sharing a lower case letter are not significantly different ($p < 0.05$). ^b Pasting temperature, defined as the temperature at which viscosity of the starch suspension begins to develop. ^c Acetylated starch samples subjected to conventional acetylation reaction (ACT) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3). ^d Acetylated starch samples subjected to UHP-assisted acetylation reaction (UHP) at three reagent addition levels (4.0, 8.0, and 12.0%, designated 1, 2, and 3).

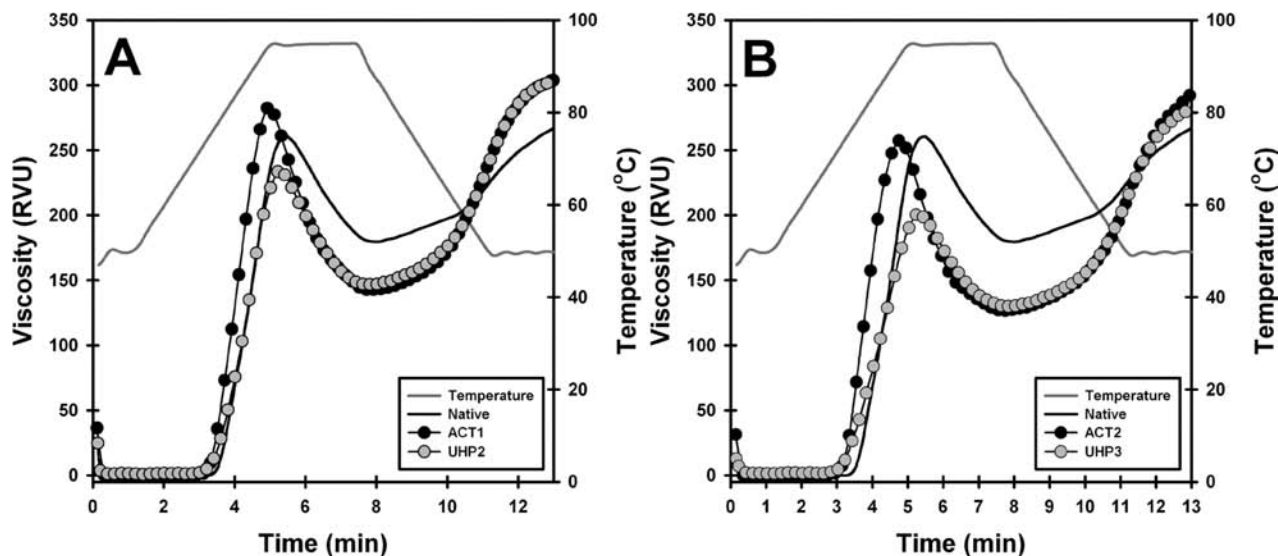


Figure 2. Pasting profiles of conventional and UHP-assisted starch acetates possessing low (**A**, DS \approx 0.048) and high (**B**, DS \approx 0.067) equivalent derivatization levels. ACT1 and ACT2 correspond to acetylated starch samples subjected to conventional acetylation reaction with 4.0 and 8.0% acetic anhydride (based on starch weight basis), respectively. UHP2 and UHP3 correspond to acetylated starch samples subjected to UHP-assisted acetylation reaction at 8.0 and 12.0% acetic anhydride (based on starch weight basis), respectively.

compared to those of native starch; the UHP-assisted starch acetates exhibited lower gelatinization onset and peak temperatures than the conventional starch acetates (**Table 3**). These findings suggested that conventional acetylation reaction combined with UHP treatment may reduce gelatinization temperatures of starch acetates to greater extents than conventional acetylation reaction alone. Thus, UHP might indirectly influence the gelatinization properties of the UHP-assisted (relative to conventional) starch acetates by altering reagent accessibility to starch molecules and/or reaction patterns within granule matrix without significant melting of starch crystallites. This suggestion was supported by similar gelatinization completion temperatures and enthalpies (**Table 3**) and intact starch crystalline structure (**Table 2** and **Figure 1**) for both conventional and UHP-assisted starch acetates (**Table 3**).

Pasting Behavior. Starch pasting characteristics were investigated for native (unmodified) and acetylated starches subjected to either conventional or UHP-assisted acetylation reaction and are depicted in **Table 4**. The pasting temperature (at when starch paste viscosity began to develop) of acetylated starches was lower than that of native starch. Acetylated starches exhibited lower peak (except for ACT1) and trough viscosities relative to native starch, while the opposite trends were observed for final, breakdown (except for UHP3), and setback viscosities. As commonly observed in the majority of studies investigating pasting

properties of low-substituted (DS $<$ 0.1) starch acetate (17,20,24,25), hydrophilic acetyl groups substituted to starch molecules facilitate granule hydration and swelling; consequently, rapid viscosity development and enhanced peak viscosity are achieved, resulting in higher breakdown viscosity. However, these general trends do not likely account for differences in peak and trough viscosities (in the present study; **Table 4**) between native and acetylated starches. Nevertheless, the lower peak and trough viscosities for acetylated (relative to native) starches may be explained by (1) destabilization of starch granules by acetylation and/or UHP treatment, which reduces resistance to shear, inducing breakdown of starch granules prior to arrival at maximum pasting viscosity (20), and (2) continued shear that leads to sustained fragmentation of the ruptured granule remnants possessing bulky acetyl groups, respectively. On the other hand, it failed to provide a reasonable explanation for higher final and setback (derived from final viscosity) viscosities of acetylated (relative to native) starches (**Table 4**). Sodhi and Singh (25) reported higher final and setback viscosities for acetylated (relative to native) rice starches (without sufficient and reasonable explanation), whereas conflicting trends also were reported (20, 24). Therefore, further research will need to better understand the effects of acetylation on pasting properties of acetylated starch derivatives.

Within an equivalent derivatization level (ACT1 vs UHP2 and ACT2 vs UHP3), the conventional starch acetates exhibited more rapid viscosity development relative to the UHP-assisted starch acetates, until the maximum viscosities were achieved (**Figure 2** and **Table 4**). Peak viscosities were higher for the conventional (relative to UHP-assisted) starch acetates (**Figure 2** and **Table 4**). These results may be explained by differences in swelling power between the conventional and UHP-assisted starch acetates. In general, the swelling property of starch granules is positively correlated to their pasting viscosities (e.g., peak, trough, final) (29). Thus, slow viscosity development and lower peak viscosities of the UHP-assisted (relative to conventional) starch acetates are possibly due to their restricted swelling capacity (see Solubility and Swelling Power; **Table 2**). This was further supported by Katopo et al. (11), who suggested that lipid-complexed amylose formed by UHP treatment restricted starch granule swelling and dispersion, generating lower peak viscosities of the UHP-treated unmodified starches (e.g., corn, rice, tapioca, potato). Furthermore, subtle variations in pasting viscosity profiles between the conventional and UHP-assisted starch acetates were observed over the course of pasting at the beginning of breakdown of the swollen granules until the final viscosities were achieved (**Figure 2** and **Table 4**). These trends were the results of equivalent derivatization degrees for both conventional and UHP-assisted acetylation reactions. Contrary to the suggestion of Choi et al. (16) (no effects of UHP on pasting properties of the UHP-assisted starch acetates), the overall findings in the present study revealed that the UHP treatment in acetylation reaction was responsible in part for alteration of pasting viscosity profiles and characteristics of the UHP-assisted starch acetates.

In conclusion, this study contrasted reactivity and physicochemical properties of conventional and UHP-assisted starch acetates as a function of three addition levels of acetic anhydride. Although UHP-assisted (relative to conventional) acetylation reaction exhibited lower reaction efficiencies, the increase in reagent addition levels supplemented lower efficiencies of UHP-assisted acetylation reaction. In UHP-assisted acetylation reaction, reagent addition levels appear to be an important factor to achieve reaction efficiency comparable to that of conventional acetylation reaction. UHP-assisted acetylation reaction may induce interaction of amylose molecules and internal granule-associated lipids, resulting in the formation of an amylose–lipid complex (further research will be needed to elucidate effects of UHP on interacting behavior of amylose and lipid). This lipid-complexed amylose may lead to inconsistencies in starch solubility, swelling power, and pasting properties between conventional and UHP-assisted starch acetates at equivalent derivatization levels. Furthermore, physicochemical properties observed for UHP-treated and conventional starch acetate possessing equivalent DS values may suggest that varied reaction processes in the preparation of the modified starch product may offer more potential to expand properties of existing starch acetates under strict practical regulatory with constraints of derivatizing reagents and reagent addition levels.

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LITERATURE CITED

- Hite, B. H. The effect of pressure in the preservation of milk. *Bull. - W. Va., Agric. Exp. Stn.* **1899**, *58*, 15–35.
- Rastogi, N. K.; Raghavarao, K. S. M. S.; Balasubramaniam, V. M.; Niranjana, K.; Knorr, D. Opportunities and challenges in high pressure processing of foods. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 69–112.
- Stute, R.; Klingler, R. W.; Boguslawski, S.; Eshtiagh, M. N.; Knorr, D. Effects of high pressures treatment on starches. *Starch/Stärke* **1996**, *48*, 399–408.
- Gunaratne, A.; Corke, H. Effect of hydroxypropylation and alkaline treatment in hydroxypropylation on some structural and physicochemical properties of heat-moisture treated wheat, potato and waxy maize starches. *Carbohydr. Polym.* **2007**, *68*, 305–313.
- Douzals, J. P.; Marechal, P. A.; Coquille, J. C.; Gervais, P. Microscopic study of starch gelatinization under high hydrostatic pressure. *J. Agric. Food Chem.* **1996**, *44*, 1403–1408.
- Douzals, J. P.; Perrier-Cornet, J. M.; Gervais, P.; Coquille, J. C. High-pressure gelatinization of wheat starch and properties of pressure-induced gels. *J. Agric. Food Chem.* **1998**, *46*, 4824–4829.
- Buckow, R.; Heinz, V.; Knorr, D. High pressure phase transition kinetics of maize starch. *J. Food Eng.* **2007**, *81*, 469–475.
- Oh, H. E.; Pinder, D. N.; Hemar, Y.; Anema, S. G.; Wong, M. Effect of high-pressure treatment on various starch-in-water suspensions. *Food Hydrocolloid* **2008**, *22*, 150–155.
- Blaszczak, W.; Fornal, J.; Valverde, S.; Garrido, L. Pressure-induced changes in the structure of corn starches with different amylose content. *Carbohydr. Polym.* **2005**, *61*, 132–140.
- Stolt, M.; Oinonen, S.; Autio, K. Effect of high pressure on the physical properties of barley starch. *Innovat. Food Sci. Emerg. Technol.* **2001**, *1*, 167–175.
- Katopo, H.; Song, Y.; Jane, J.-L. Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. *Carbohydr. Polym.* **2002**, *47*, 233–244.
- Hibi, Y.; Matsumoto, T.; Hagiwara, S. Effect of high pressure on the crystalline structure of various starch granules. *Cereal Chem.* **1993**, *70*, 671–676.
- Lee, J.-H.; Choi, H.-W.; Kim, B.-Y.; Chung, M.-S.; Kim, D.-S.; Choi, S. W.; Lee, D.-U.; Park, S.-J.; Hur, N.-Y.; Baik, M.-Y. Nonthermal starch hydrolysis using ultra high pressure: I. Effects of acids and starch concentrations. *LWT—Food Sci. Technol.* **2006**, *39*, 1125–1132.
- Choi, H.-W.; Lee, J.-H.; Ahn, S.-C.; Kim, B.-Y.; Baik, M.-Y. Effects of ultra high pressure, pressing time and HCl concentration on non-thermal starch hydrolysis using ultra high pressure. *Starch/Stärke* **2009**, *61*, 334–343.
- Hwang, D.-K.; Kim, B.-Y.; Baik, M.-Y. Physicochemical properties of non-thermally cross-linked corn starch with phosphorus oxychloride using ultra high pressure (UHP). *Starch/Stärke* **2009**, *61*, 438–447.
- Choi, H.-S.; Kim, H.-S.; Park, C.-S.; Kim, B.-Y.; Baik, M.-Y. Ultra high pressure (UHP)-assisted acetylation of corn starch. *Carbohydr. Polym.* **2009**, *78*, 862–868.
- Wang, Y.-J.; Wang, L. Characterization of acetylated waxy maize starches prepared under catalysis by different alkali and alkaline-earth hydroxides. *Starch/Stärke* **2002**, *54*, 25–30.
- Singh, N.; Chawla, D.; Singh, J. Influence of acetic anhydride on physicochemical, morphological and thermal properties of corn and potato starch. *Food Chem.* **2004**, *86*, 601–608.
- Xu, Y.; Miladinov, V.; Hanna, M. A. Synthesis and characterization of starch acetates with high substitution. *Cereal Chem.* **2004**, *81*, 735–740.
- Saartrat, S.; Puttanlek, C.; Rungsardthong, V.; Uttapap, D. Paste and gel properties of low-substituted acetylated canna starches. *Carbohydr. Polym.* **2005**, *61*, 211–221.
- Gunaratne, A.; Corke, H. Influence of prior acid treatment on acetylation of wheat potato and maize starches. *Food Chem.* **2007**, *105*, 917–925.
- Zobel, H. F. Starch crystal transformations and their industrial importance. *Starch/Stärke* **1988**, *40*, 1–7.
- Huang, J.; Schols, H. A.; Jin, Z.; Sulman, E.; Voragen, A. G. J. Characterization of differently sized granule fractions of yellow pea, cowpea and chickpea starches after modification with acetic anhydride and vinyl acetate. *Carbohydr. Polym.* **2007**, *67*, 11–20.

- (24) Liu, H.; Ramsden, L.; Corke, H. Physical properties and enzymatic digestibility of acetylated *ae*, *wx*, and normal maize starch. *Carbohydr. Polym.* **1997**, *34*, 283–289.
- (25) Sodhi, N. S.; Singh, N. Characteristics of acetylated starches prepared using starches separated from different rice cultivars. *J. Food Eng.* **2005**, *70*, 117–127.
- (26) Oh, H. E.; Hemar, Y.; Anema, S. G.; Wong, M.; Pinder, D. N. Effect of high-pressure treatment on normal rice and waxy rice starch-in-water suspensions. *Carbohydr. Polym.* **2008**, *73*, 332–343.
- (27) Tester, R. F.; Morrison, W. R. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chem.* **1990**, *67*, 551–557.
- (28) Liu, H.; Arntfield, S. D.; Holley, R. A.; Aime, D. B. Amylose–lipid complex formation in acetylated pea starch–lipid systems. *Cereal Chem.* **1997**, *74*, 159–162.
- (29) Blazek, J.; Copeland, L. Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydr. Polym.* **2008**, *71*, 380–387.

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