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Effect of Low Level of Starch Acetylation on Physicochemical Properties of Potato Starch

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Abstract In order to find out the effect of low level of starch acetylation on physicochemical properties of potato starch, amylose content, digestibility of raw and gelatinized starch, thermal properties, pasting properties, and the swelling power of native and acetylated potato starches were measured. The amylose content was significantly lower in acetylated starch than in their counterpart native starches. Though a tendency in the decrease in digestibility of raw starch was observed with starch acetylation, acetylation did not alter the proportion of readily digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) of both raw and gelatinized potato starches. No clear increase in the swelling power was observed, however, the peak and onset gelatinization temperatures and the enthalpy required for starch gelatinization decreased with starch acetylation. Peak and breakdown viscosities were reduced due to acetylation of potato starch while final viscosity and set back were increased.

Keywords: starch acetylation, acetylated potato starch, physicochemical property

Introduction

Starch acetylation is one of the common methods of modifying starch properties by introducing acetate (CH_3CO) groups to starch granules at low temperatures. Acetylated starches are produced by treating starch granules with acetate group donors such as acetic anhydride in the presence of an alkaline agent. During acetylation 3 free hydroxyl groups on C_2, C_3, and C_6 of the glucose molecules can be substituted with acetyl groups (1). It is said that acetyl groups disrupt interactions among outer chains of amylopectin, the branched form of starch, and among amylose, chains of the linear starch polymer thus, alters a wide range of functional characteristics of native starches such as conferring higher peak viscosity, paste clarity, increasing freeze-thawed stability (2-4), and suitable for many applications in both food and non-food industries (5,6). However, the physicochemical properties of acetylated starch depend on their chemical structures, degree of substitution (DS), and acetyl group distribution.

Corn and cassava starches are been widely used for the production of acetylated starches (2,4). Though the available information is limited, potato, yams, and other starches such as starches from rice and legumes are also been studied recently for such applications (5,7-11). Potato starch in its nature has large starch granules, very high peak viscosity and swelling power than most of other starches but it forms less stable gels after cooking (12). Thus it would be modified to suit industrial applications with very low level of acetate group donors. Starch acetate with DS 0.01-0.2 is approved by the Food and Drug Administration for food use (13). In previous report, the characteristics of acetylated potato starches with relatively higher DS (0.180-0.238) were analyzed (7). Thus very low levels of acetylation of potato starch would help to utilize safe starch products in food industries and to minimize the cost of production of modified starch.

Application of starches in different industries is primarily governed by gelatinization, pasting, solubility, swelling and digestibility properties of them (14-16). Therefore, the present study was carried out to examine how physicochemical properties of native potato starch are altered by low levels of acetylation. Furthermore, the acetylated potato starches were compared with commercially available acetylated cassava starch.

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Materials and Methods

Starch samples Two potato starch samples, which were produced at the starch factory, Shihoro Agricultural Cooperative Association, Shihoro, Hokkaido, Japan, in year 2006 and 2007, were used in this study. The acetylation of starch in year 2006 was carried out as follows. Two-hundred-fifty g of starch were dispersed in 280 mL of 0.5 M NaCl. The pH of the slurry was adjusted to 9.0 using 3% NaOH, and then was kept at 25°C for 30 min. Acetic anhydride (2.0, 3.5, and 5.0%/starch, d.w.b.) was added over a period of 40 min, while maintaining a pH range of 8.5±0.5. The reaction was allowed to proceed at 25°C for 1 hr, constantly monitoring the pH between 8.0 and 9.0, after the addition of acetic anhydride was completed. The pH was then adjusted to 6.5 with 5% HCl and the slurry was kept for 1 hr. The suspension was washed thoroughly with water and was air-dried. In year 2007 sample, starch acetylation was performed using 250

kg starch (1,000-fold scale of the sample in the year 2006) with 2 levels (3.5 and 5.0% of acetic anhydride). Starch acetylated with 2.0, 3.5, and 5.0% of acetic anhydride (d.w.b.) around pH 8.5 in year 2006 and 2007 together with their native potato counterparts (potato06 and potato07, respectively) were used for the analysis of physicochemical properties. Acetated potato starches treated with 2.0, 3.5, and 5.0% acetic anhydride in 2006 were labeled as AP06-2.0, AP06-3.5, and AP06-5.0, respectively, while those treated with 3.5 and 5.0% acetic anhydride in 2007 were defined as AP07-3.5 and AP07-5.0. Furthermore, acetated cassava starch imported from Thailand was included.

Degree of substitution (DS) of acetylated starch The acetyl content in acetylated starch was measured using the following method to calculate the degree of substitution of acetylated starch. Acetylated starches (0.2-1.0 g) were weighted and added into 25 mL of 3.75% $NH_2OH \cdot HCl$ solution. After the addition of 25 mL of 9.4% NaOH solution to the starch-containing solution, the mixture was stirred for at least 10 min to solublilize the starch. For assay, 2 mL of the starch-containing mixture, 5 mL of distilled water, 5 mL of 92.96% methanol solution containing 4.928% $HClO_4$ and 13 mL of a 0.3036% $Fe(ClO_4)_2$ solution containing 1.162% $HClO_4$ and 86.34% methanol were mixed, kept for 5 min, and then filtered. After 20 min, the absorbance at 510 nm in the filterate was measured. The acetyl content (%) was calculated using 1,2,3,4,6-*penta-O*-acetyl-β-D-glucopyranose as a reference sample. DS was determined according to the following equation.

$$DS=[162\times\text{acetyl content (\%)}]/[43-42\times\text{acetyl content (\%)}]$$

Blue value and amylose content The amylose content was estimated by blue value method as described by Noda *et al.* (17) without defatting starch. Two % starch suspension was prepared by dissolving starch in dimethyl sulfoxide (DMSO) at 70°C for 3 hr and that was diluted to 0.1% starch suspension by using distilled water. The absorbance at 680 nm was recorded for a mixture (5 mL) containing 0.2 mg of starch, 0.4 mg of iodine, and 4 mg of potassium iodide (KI) 30 min after the color development. The analysis was repeated 3 times.

The apparent amylose content was calculated by using blue values of samples. For the calculations, blue values of purified amylose and amylopectin of potato (18) and cassava (19) were used.

Digestibility of raw starch by glucoamylase One mL of 2% raw starch suspension was digested with 4.15 units of crystalline glucoamylase from *Rhizopus* sp. (Oriental Yeast Co., Ltd., Tokyo, Japan) for 4 hr at 40°C according to the modified method of Noda *et al.* (17). The amount of glucose released during enzyme digestion was estimated by the phenol-sulfuric method (20), and enzyme digestibility was calculated as the % of glucose released during incubation with the enzyme to the total amount of sugar in the starch on a weight basis. The analysis was repeated 2 times.

Nutritionally important starch fractions Digestion of both raw and gelatinized starch by enzyme solution composed of mainly pancreatin was carried out according to Noda *et al.* (21). For starch gelatinization, the starch slurry was heated at 80°C for 20 min followed by in the boiling water for 15 min with frequent mixing. Measurement of nutritionally important starch fractions; readily digestible starch (RDS, starch digested during first 20 min with enzymes incubation), slowly digestible starch (SDS, starch digested between 20-120 min of enzymes incubation), and resistant starch (RS, undigested starch remained after 120 min enzymes incubation) were calculated. The analysis was repeated 2 times.

Thermal properties using differential scanning calorimetry (DSC) DSC analysis was conducted using a DSC 6100 (Seiko Instruments, Tokyo, Japan), according to Noda *et al.* (22). Ten mg of a sample (d.w.b.) was weighed in a silver pan and distilled water was then added to make a suspension of 30%(w/w, d.w.b.). A sealed pan with distilled water was used as a reference. Scans were run at a heating rate of 2°C/min from 25 to 130°C. The peak gelatinization temperature, onset gelatinization temperature, and the enthalpy for starch gelatinization were recorded. The analysis repeated twice.

Swelling power Starch slurry (20 mg of starch in dry weight basis in 5 mL of distilled water) was gelatinized at both 70 and 80°C for 20 min with frequent mixing to avoid forming of starch clots. Gels were cooled at 20°C for 5 min and then centrifuged at 9,000×g for 15 min at 10°C. The swelling power was calculated and it was expressed as the weight of swelled starch residue/1 g of starch (d.w.). The analysis was repeated 2 times.

Pasting properties The pasting properties of starches by rapid visco analyzer (RVA-4) (Newport Scientific Pty., Ltd., Warriewood, NSW, Australia) were determined with 2 replicates according to Noda *et al.* (22). Each sample of potato starch was added to 25 mL of distilled water to prepare 4 and 6%(w/w, d.w.b.) suspensions. The suspension was kept at 50°C for 1 min and then heated up to 95°C at 12.2°C/min and held for 2.5 min at 95°C. It was then cooled at 50°C (cooling rate of 11.8°C/min) and kept for 2 min. Peak viscosity, breakdown (difference between the peak and holding viscosity), final viscosity, setback (difference between final and holding viscosity), and peak time were measured from the pasting curve using Thermocline for Windows software (Newport Scientific Pty., Ltd.). The viscosity parameters were recorded in rapid visco units (RVU) and peak time was given in min. The analysis was repeated 2 times.

Statistical analysis The analysis of variance for each parameter measured were performed using Microsoft Excel 2003 and then means were compared by using least significant difference (LSD) for each property using the SAS system 9.1.for Windows.

Results and Discussion

Acetyl content and DS As shown in Table 1, the acetyl starches examined ranged between 0.66 and 1.96%, which corresponded to 0.025 and 0.075 DS, respectively. Higher

Table 1. Acetyl content, degree of substitution (DS), blue value, amylose content, and raw starch digestibility of native and acetylated potato starches

Starch type	Acetyl content (%)	DS	Blue value	Amylose content (%)	Raw starch digestibility (%)
Potato07	-	-	0.478[a1)]	20.51[a]	2.03[bc]
AP07-3.5	1.27[c]	0.048[c]	0.401[c]	13.81[d]	1.87[c]
AP07-5.0	1.78[b]	0.068[b]	0.330[f]	7.58[g]	1.59[d]
Potato06	-	-	0.481[a]	20.71[a]	1.92[c]
AP06-2.0	0.66[d]	0.025[d]	0.424[b]	15.75[c]	2.14[b]
AP06-3.5	1.30[c]	0.050[c]	0.380[d]	11.96[e]	1.29[e]
AP06-5.0	1.96[a]	0.075[a]	0.349[e]	9.29[f]	1.96[bc]
Acetylated cassava	1.88[ab]	0.072[ab]	0.293[g]	18.76[b]	3.60[a]

[1)]Values followed by the different letters in the same column are significantly different at $p<0.05$ level.

level of acetylation resulted in definitely higher DS. DS of commercially available acetylated cassava was similar to those of potato starches acetylated with 5.0% acetic anhydride.

Blue value and amylose content Acetylated cassava gave the least blue value and the highest was shown by the native potatoes (Table 1). The blue value and the amylose content of native potato starches were significantly reduced by the level of acetylation. Furthermore, significant differences were observed in blue values and amylose contents of starches treated with same levels of acetic anhydride in 2 different years showing higher blue values in starches taken from 2006 than those of 2007. According to Suzuki *et al.* (18) and Thitipraphunkul *et al.* (19), the blue values of pure amylose of potato and cassava starches were almost same (1.39 and 1.30, respectively). However, the blue values of pure amylopectin of potato and cassava starches were different (0.24 and 0.06, respectively) and thus the apparent amylose content calculated for cassava became higher than any acetylated potato starch tested. The blue values of isolated amylose or amylopectin are different from starches purified from different botanical sources and species.

According to Singh *et al.* (7), acetylated starches from corn and potato showed slightly higher amylose than their counterpart native starch. Though they observed such an increase in amylose content due to acetylation, it was very slight up to 4% of acetic anhydride used for acetylation. The amylose content was clearly increased with increased level of acetic anhydride used. On the other hand, Lawal (23) demonstrated that acetylation with 10.2% acetic anhydride reduced the amylose content of new cocoyam starch significantly from its native starch. In this experiment, acetic anhydride was used in the range of 2 to 5%, but amylose content trend is different from that of the report of Singh *et al.* (7).

The presence of acetyl groups has been reported to affect the absorption of iodine of amylose and amylopectin (8). It is also found that more substitution of acetyl groups is occurred in amylose than in amylopectin as amorphous phase is more susceptible for chemical reactions than the crystalline phase (10,24). Since acetylation reduces intermolecular association in the starch granules, it would be possible to reduce the iodine binding capacity with acetylation of starch as acetate groups disturb the helical structure of amylose. However, reasons for the drastic reduction of the blue values in acetylated starch treated with 2.0-5.0% acetic anhydride would be studied further.

Digestibility of raw starch by glucoamylase The highest digestibility of raw starch by *Rhizopus* glucoamylase was observed for acetylated cassava and all potatoes showed significantly lower digestibility of their raw starch than cassava (Table 1). Though potato starches, which were produced in 2006 and were acetylated with 2.0 and 5.0% acetic anhydride, showed relatively higher digestibility of raw starch, a tendency in the reduction in the digestibility of raw starch was observed with acetylation. Previous studies too observed similar results and concluded that the introduced acetyl groups act as barriers to enzyme attack (24,25).

Nutritionally important starch fractions A significant difference was observed in RDS, SDS, and RS of raw starch among tested samples but such differences were not observed when starch was gelatinized (Fig. 1, results of mean separation was not given in the figure).

RDS of raw starch of acetylated cassava was 7.1% while

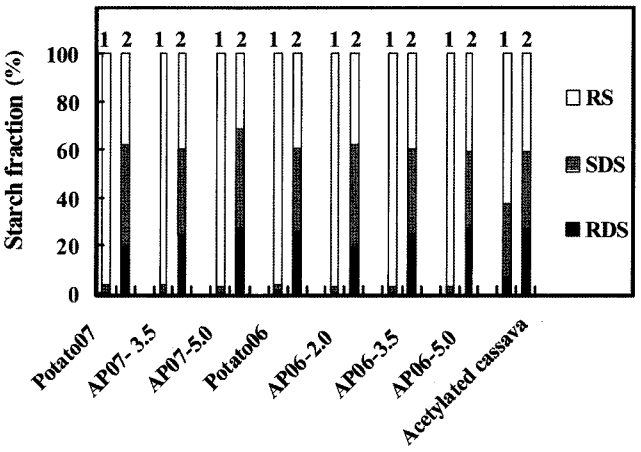

Fig. 1. Nutritionally important starch fractions based on rate of digestibility of native and acetylated potato starch. 1, Raw starch; 2, gelatinized starch; RS, resistant starch; SDS, slowly digestible starch; RDS, readily digestible starch.

Table 2. Thermal properties of native and acetylated potato starches

Starch type	Peak gelatinization temperature (°C)	Onset gelatinization temperature (°C)	Enthalpy for starch gelatinization (J/g)
Potato07	65.4[b1)]	63.2[a]	19.5[ab]
AP07-3.5	62.4[e]	59.2[bc]	18.6[b]
AP07-5.0	60.9[f]	56.1[e]	17.4[c]
Potato06	66.4[a]	62.1[a]	20.0[a]
AP06-2.0	64.9[c]	60.5[b]	19.7[a]
AP06-3.5	63.4[d]	58.4[cd]	19.5[ab]
AP06-5.0	62.4[e]	57.3[de]	18.6[b]
Acetylated cassava	65.4[b]	56.6[e]	14.1[d]

[1)]Values followed by the different letters in the same column are significantly different at $p<0.05$ level.

all potato starches gave less than 1.06% of RDS. Thus significant difference was observed in RDS of starches tested but no clear pattern of variation in RDS due to level of acetylation was shown. Furthermore, SDS and RS of raw starch only grouped cassava and all potato starches into 2 groups without showing any significant difference in potato starches due to acetylation. Thus the significant differences observed in RDS, SDS, and RS of raw starch was mainly due to the basic difference among the botanical types of starch not the chemical modification. Even such difference could not be seen after gelatinized starch. Han and BeMiller (26) prepared different types of modified starches and analyzed the variation of RDS, SDS, and RS fractions using the similar methods we used. They found that SDS and RS content could be altered with such modifications though clear conclusion could not be seen to examine the effect of acetylation itself. However, cross-linking followed by acetylation (9.0% acetic anhydride) produced more SDS and RS than untreated starch.

Though we could not find any previous information on changes in nutritionally important starch fractions due to acetylation itself, it would be possible that the acetylation up to 5.0% of acetic anhydride had no significant effect on the enzyme digestibility of both raw and gelatinized starch and thereby resulted in no changes in RDS, SDS, or RS content of acetylated starch.

Thermal properties using DSC Peak and onset gelatinization temperatures were significantly decreased with increased level of acetylation (Table 2). Furthermore, the enthalpy for starch gelatinization too decreased with increasing level of acetylation. However, the reduction was not very clear as those shown by peak and onset gelatinization temperatures. Our observations were comparable with many previous studies for many different starch types including potato (7,27). Thus, it is clear that acetylation resulted in destabilization of starch polymer structures and thereby reduction in gelatinization temperatures and enthalpy required for starch gelatinization. Liu *et al.* (2) also stated that the prime action of substituted acetyl groups is the reduction of inter-chain association.

Acetylated cassava gave peak and onset gelatinization temperatures within the range of them for acetylated potatoes. But its enthalpy for starch gelatinization is the lowest and it was at least 3.3 J/g lower than all types of potato starches.

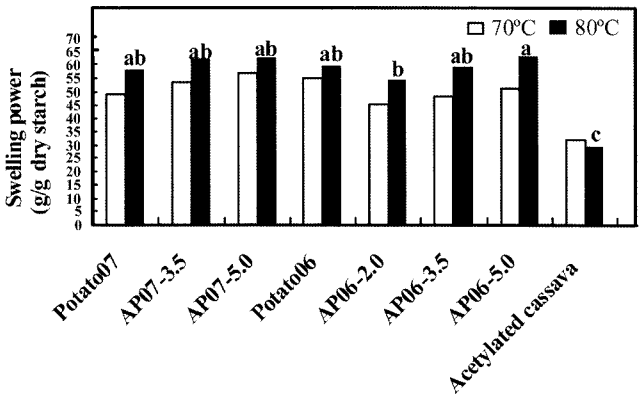

Fig. 2. Swelling power of native and acetylated potato starches at 70 and 80°C. Values labeled by the same letters in the bars representing the swelling power at 80°C are not significantly different at $p<0.05$ level. Mean separation for the swelling power at 70°C was not performed as it was not significantly different among samples.

Swelling power No significant difference was observed in the swelling power at 70°C of tested starches. However, the swelling power at 70°C clearly differentiated the all the potato starches from acetylated cassava starch (Fig. 2). Though slight differences were seen among the swelling power of potato starches, most of them were not significant. Therefore, acetylation of potato starches with 2.0-5.0% acetic anhydride did not change the swelling power of potato starch significantly. The introduction of the acetyl groups reduces the bond strength between starch molecules, alters hydrophilicity and thereby increases the hydration capacity of starch and the swelling power (7,27). Native potato starch itself gives higher swelling power due to its large granule size and/or high phosphorus content (28). Furthermore the level of acetate group donor used is limited up to 5.0% in our study. Thus both would be possible reason for observing little increase in swelling power of our acetylated potato starches. High level of acetic anhydride was used in both previous studies (7,27).

Pasting properties by RVA All pasting properties, except peak time of 6% starch suspension, measured at both starch concentrations were significantly different among the starch samples used (Table 3). When the starch concentration increased from 4 to 6% all viscosity

Table 3. Pasting properties of native and acetylated potato starch by rapid visco analyzer (RVA)

Starch type	Starch concentration	RVA properties (RVU)				
		Peak viscosity	Breakdown viscosity	Final viscosity	Setback	Peak time (min)
	4%					
Potato07		267.0$^{b1)}$	161.0^{b}	127.3^{ef}	22.2^{d}	3.4^{d}
AP07-3.5		117.6^{e}	69.6^{d}	133.0^{de}	24.9^{d}	4.4^{a}
AP07-5.0		184.1^{d}	75.2^{d}	137.1^{cd}	28.2^{c}	4.2^{b}
Potato06		295.3^{a}	187.8^{a}	125.7^{f}	18.3^{e}	3.3^{d}
AP06-2.0		181.8^{d}	72.8^{d}	140.9^{bc}	31.8^{b}	4.4^{a}
AP06-3.5		181.0^{d}	72.3^{d}	143.2^{b}	34.5^{ab}	4.2^{b}
AP06-5.0		211.8^{c}	95.4^{c}	153.6^{a}	37.2^{a}	4.0^{c}
Acetylated cassava		42.1^{f}	15.4^{e}	35.2^{g}	8.5^{f}	4.0^{c}
	6%					
Potato07		487.2^{b}	331.0^{b}	193.8^{de}	37.5^{bc}	3.0*
AP07-3.5		410.8^{e}	246.2^{f}	186.9^{e}	22.3^{d}	3.2
AP07-5.0		408.0^{e}	238.8^{g}	205.0^{c}	35.8^{c}	3.2
Potato06		536.7^{a}	374.9^{a}	197.5^{d}	35.6^{c}	3.3
AP06-2.0		451.6^{c}	273.7^{c}	222.7^{b}	44.8^{b}	3.2
AP06-3.5		437.3^{d}	256.3^{e}	218.6^{b}	37.6^{bc}	3.1
AP06-5.0		453.2^{c}	267.1^{d}	241.5^{a}	55.4^{a}	3.1
Acetylated cassava		123.8^{f}	56.5^{h}	100.3^{f}	33.0^{e}	3.7

[1)]Values followed by the different letters in the same column are significantly different at $p<0.05$ level; *Not significantly different.

properties of each starch tested increased. The lowest peak viscosity, breakdown viscosity, and final viscosity were given by acetylated cassava at both starch concentrations. Though the least set back was again given by acetylated cassava starch at 4% starch suspension, it was not largely different from native or acetylated potato with 2.0-5.0% acetic anhydride.

In potato starches, acetylation decreased peak and breakdown viscosities but increased both final viscosity and setback. But when the level of acetic anhydride increased all viscosity properties (peak, breakdown, final viscosities, and setback) tended to be also increased.

Changes in the viscosity profiles due to acetylation were observed previously (2,11,29). Although usually acetylation increases peak viscosity, such increase was depended on botanical type (29), amylose content in maize (2), and variety of rice (11). Furthermore, level of acetylation greatly influenced the swelling power of starch which is usually highly correlated to the viscosity of the starch slurry (7). Previous information explained the possible influence of acetylation on starch paste viscosity by 3 mechanisms (2). Acetate groups may prevent close association of glucose chains, alter the hydrophilicity, and improve hydrogen bonding with other starch chains thus, finally improve the swelling capacity and viscosity of starch. These changes were clear in acetated starch with high level of acetic anhydride (ca. 10%) in previous study (2,11). But, in our study, low levels of acetic anhydride (less than 5.0%) did not change in the swelling behavior so much. Therefore by combining our results with previous observations, it would be suggested that very low levels of acetylation (DS<0.08) may decrease peak viscosity of potato starch but it may be increased with increasing levels (DS 0.087-0.165) of acetylation (2,11).

Usually higher breakdown in starch was shown due to many chemical modifications (27). It is said that following modifications, the modified starches become partially degraded (30) and that cause less resistant to shear and inability to maintain the integrity of the starch granules.

However in most of above studies, increase in cold paste viscosity/final viscosity and set back was observed with acetylation of many different starch types. Therefore it would be conclude that acetate groups in starch would facilitate the reassociation of amylose during cooling.

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