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PII: S0144-8617(97)00130-6

Carbohydrate Polymers **34** (1997) 283–289

0144-8617/97/$17.00 + .00

Physical properties and enzymatic digestibility of acetylated *ae*, *wx*, and normal maize starch

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(Received 9 February 1997; revised version received 9 June 1997; accepted 27 August 1997)

The physical properties and enzymatic digestibility of acetylated starches prepared in the laboratory from high amylose (Hi-Maize™, 66% amylose; and GELOSE 50, 47% amylose), waxy (MAZACA 3401X, 3.3% amylose), and normal (22.4% amylose) maize starches provided by Starch Australasia Limited were studied. Acetylation decreased temperature at peak viscosity, while slightly increasing peak viscosity compared to the matching unmodified starch. It increased cool paste viscosity except in the case of normal starch. All the acetylated starches had lower onset temperature (T_o), intermediate temperature (T_p), completion temperature (T_c) and endothermic energy (ΔH) than their unmodified starches, but acetylation increased swelling power and solubility. After acetylation, the hardness of all the starch gels decreased; adhesiveness decreased and springiness increased except for waxy starch where it was the reverse; cohesiveness increased in each case. Acetylation increased the clarity of all the starches, except for waxy which showed a decrease. Acetylation increased the enzymatic digestibility compared to the unmodified starches. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Starch has many diverse applications both in food production and in many other industries. For this reason, chemical modification is often made to starch to extend the range of specific physical properties available for certain uses. Chemically modified starches have markedly altered physicochemical properties compared with their parent starches (Rutenberg & Solarek, 1984). Acetylation is a widely used method for starch modification. Rutenberg & Solarek (1984) proposed a mechanism for the effect of acetylation. The introduction of the acetyl group reduces the bond strength between starch molecules and thereby increases the swelling power and solubility of the starch granule, decreases the coagulation of the starch, and gives improved clarity and freeze–thaw stability.

Much research has been reported on acetylated starches. Acetylation increased viscosity, swelling power and solubility, but decreased gelatinization temperature of rice starch, while hardness, adhesiveness and cohesiveness of gels made from the starches were increased (Jae *et al.*, 1993). Pereira-Pacheco *et al.* (1994) studied *Xanthosoma violaceum* starch and found that acetylation increased solubility and reduced gelatinization temperature, viscosity and swelling power. Acetylated cassava starch was shown to be more soluble than native cassava starch, while the clarity and sol stability of the derivatives were improved and viscosity was reduced (Aiyeleye *et al.*, 1993). Hoover & Sosulski (1985) found that acetylation increased the stability of legume starch under low temperature storage. Studies on smooth pea and waxy maize starches (Biliaderis, 1982) showed that acetylation occurred exclusively in certain parts of the granule. A study of functional properties of modified black gram starch showed that acetylated starch had lower swelling capacity (at 95°C) than isolated starch (Deshpande *et al.*, 1982). Acetylation of Great Northern bean starch decreased the solubility, but increased swelling over a temperature range 60–90°C, and reduced swelling over a pH range 2–10 (Sathe *et al.*, 1981).

Gene mutation can influence the total starch content and the amylose–amylopectin ratio. The *ae* mutant of maize is associated with a high amylose content of the endosperm starch, whereas the *wx* starch mutant has essentially no amylose (Shannon & Garwood, 1984). In

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differential scanning calorimetry (DSC) analyses, *wx* starch showed thermal behavior similar to that of normal corn starch. The *ae* starch, however, did not exhibit a clear peak, and the endotherm extended beyond 100°C (Wang *et al.*, 1992). The special properties of different mutants, such as thermal behavior during gelatinization, and the starch structures have been reported (e.g. Sanders *et al.*, 1990; Wang *et al.*, 1993). However, very little has been reported on properties of chemically modified maize starches, especially *ae* mutants. As mutant starches represent extremes in biological variation in amylose–amylopectin ratio, it is worthwhile to study the effect of chemical modification on them. In this way, we hope to identify useful directions for future biotechnological modification of starch to achieve precise control of desired physical properties by interaction of biological variation with chemical modification.

The specific objective of this study was to evaluate the effect of acetylation on the properties of two high amylose, one waxy, and one normal maize starch including thermal and pasting properties, swelling power and solubility, gel texture, clarity, and enzymatic digestibility.

MATERIALS AND METHODS

Starch samples

All native starch samples were supplied by Starch Australasia Limited (Lane Cove, Australia), and amylose contents were confirmed with an amylose/amylopectin assay kit (Megazyme Pty Ltd., Warriewood, Australia). Samples were two high amylose maize starches, i.e. Hi-Maize™ (HM) with 66% amylose, and GELOSE 50 (G50) with 47% amylose; one waxy i.e. MAZACA 3401X with 3.3% amylose; and one normal (MAIZE CORNFLOUR 3401C with 22.4% amylose).

Starch modification by acetylation

The method of Hoover & Sosulski (1985) was followed with minor modification. About 100 g of starch was dispersed in 225 ml distilled water, and stirred for 60 min at 25°C to obtain a uniform suspension. The pH was adjusted to 8.0 with 3.0% NaOH. Acetic anhydride (0.1 moles per treatment) was added dropwise to the stirred slurry while maintaining the pH within the range 8.0 to 8.4 using 3.0% NaOH solution. The reaction was allowed to proceed for 10 min after completion of acetic anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 N HCl and centrifuged for 3 min at 2000 rpm. It was washed free of acid twice with distilled water and once with 95% ethanol then oven dried at 40°C.

The degree of substitution (DS) for the acetylated starches was determined according to the method of Wurzburg (1964). Blanks with unmodified starches were analyzed concurrently. The DS is defined as the average number of acetyl groups per glucose unit in the starch molecule:

$$\text{Acetyl percent} = \frac{(\text{ml blank} - \text{ml sample}) \times \text{normality of HCl} \times 0.043 \times 100}{\text{weight of starch (g, d.b.)}}$$

$$\text{DS} = \frac{162 \times \text{acetyl percent}}{4300 - (42 \times \text{acetyl percent})}$$

The effects of acetylation on the acetyl content and degree of substitution of maize starches are shown in Table 1.

Viscoamylography

A Rapid Visco-Analyzer model 3D (RVA) (Newport Scientific Pty. Ltd., Narrabeen, Australia) was employed to determine the pasting properties of starch samples. 3.0 g starch sample (d.b.) and a weighed amount of distilled water were combined and stirred in the aluminum RVA sample canister to make a 10.7% starch suspension (w/w). A programmed heating and cooling cycle was used, where the sample was held at 50°C for 1 min, heated to 95°C in 7.5 min, held at 95°C for 5 min, cooled to 50°C in 8.5 min, and then held at 50°C for 3 min. Triplicate tests were used in each case. Parameters recorded were temperature at which peak viscosity was attained (P_{temp}); peak viscosity (*PV*); hot paste viscosity (*HPV*) (minimum viscosity at 95°C, or for starches which do not show shear thinning, the viscosity after 5 min at 95°C); cool paste viscosity (*CPV*) (final viscosity at 50°C at the end of the 25 min cycle); breakdown (*BD*) (= *PV* − *HPV*, an indicator of shear thinning); and setback (*SB*) (= *CPV* − *HPV*).

Differential scanning calorimetry

Thermal analysis was performed with a Mettler DSC 20 instrument (Mettler, Naenikon-Uster, Switzerland) equipped with a Mettler TC 11 data analysis station.

Table 1. Effect of acetylation on the acetyl content and degree of substitution (DS) of maize starch

Starch	Percent amylose (native starch)	Acetyl group percent	DS
Waxy	3.3	4.22	0.165
Hi-Maize™	66.1	3.35	0.130
Normal	22.4	2.71	0.105
GELOSE 50	47.1	3.83	0.149

2.5 mg starch (d.b.) was weighed directly into a 40 μl pan and then 7.5 mg of deionized water (i.e. excess water for hydration of the starches in this system) was directly added into the pan by a microsyringe and mixed for initial sample homogenization. After sealing, the pan was left for one hour to allow the sample to equilibrate. Then the sample was heated from 30°C to 120°C at a heating rate of $10°C\,min^{-1}$. An empty pan was used as a reference. Onset temperature (T_o), intermediate temperature (T_p), completion temperature (T_c) and endothermic energy (ΔH) were recorded.

Swelling power and solubility

Starch swelling power was determined following Subramanian *et al.* (1994) with minor modifications. Starch (0.6 g, d.b.) was heated with 40 ml of water to the desired temperature for 30 min. Lump formation was prevented by stirring. The mixture was centrifuged at 3 000 rpm for 15 min. The supernatant was carefully removed, and the swollen starch sediment was weighed. Swelling power was the ratio of weight of the wet sediment to the initial weight of dry starch. An aliquot of supernatant was evaporated overnight at 130°C and weighed. Solubility was calculated as the ratio in weight of the dried supernatant to the initial weight of the dry starch.

Texture analysis

After RVA testing, the starch pasted in the sample canister was covered and kept at 25°C for 4 h to allow cooling and gelation. Gel texture was determined from triplicate measurements using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, England). The gel was compressed at a speed of 1.0 $mm\,s^{-1}$ to a distance of 10 mm with a cylindrical flat-ended probe of 6 mm diameter. The peak height at 10 mm compression was termed firmness, and the negative area of the curve during retraction of the probe was termed stickiness. Because of their extreme softness, waxy maize and acetylated waxy maize were also tested with larger diameter (20 mm) probe.

Clarity

Clarity of starches was determined as described by Wu & Seib (1990). 1% starch paste was heated in a boiling water bath for 30 min and cooled to 25°C. The clarity (T) was evaluated spectrophotometrically as percent transmittance at 650 nm against a water blank.

Enzymatic digestibility using α-amylase

The method reported by Zhang *et al.* (1995), slightly modified, was used. 30 ml phosphate buffer (0.2 M, pH 6.9) was mixed with 1.0 g (d.b.) starch in a 50 ml test tube. After heating in a water bath at 95°C for 30 min and cooling to 25°C, 320 Units bacterial α-amylase (catalogue item A-6380, Sigma Chemical Co., St. Louis, MO) was added. After incubation at 30°C in a shaking water bath for 14 h digestion, the reaction was stopped by addition of 5 ml 1.0% (w/v) sulphuric acid. Samples were centrifuged, and the resulting pellet of undigested flour residue was washed with 80% ethanol, re-centrifuged and dried to constant weight. For each sample, a blank of starch without enzymatic hydrolysis was included to correct for initial concentration of soluble sugars. Starch digestibility was expressed as percent weight loss after α-amylase digestion.

RESULTS AND DISCUSSION

Paste viscosity of native and modified maize starches

The pasting properties of the native and acetylated Hi, G, waxy and normal maize starches are shown in Fig. 1 and Table 2. After acetylation all the starches began to paste at a lower temperature and had higher subsequent viscosities than the corresponding unmodified starch. The magnitude of the effect of acetylation differed considerably among the different starches. Normal maize starch showed least difference; there was little change in the peak viscosity (PV) and a slight rise in the cool paste viscosity (CPV) though gelatinization took place at a temperature 7°C lower. Waxy starch showed an appreciable increase in PV and a similar 7°C reduction in gelatinization temperature. The most dramatic changes were observed with the high amylose starches where PV and CPV were greatly increased.

Acetylation can influence the interactions between the starch chains through three possible mechanisms; 1) by simple steric hindrance preventing close association of chains to allow formation of hydrogen bonds, 2) by altering the hydrophilicity of the starch and thus affecting bonding with water molecules, or 3) by participation of the acetyl groups in improved hydrogen bonding with other starch chains. The observed effects of acetylation are consistent with an overall reduction in bonding between starch chains and a consequent increase in the ease of hydration of the starch granule. Gelatinization can thus commence at a lower temperature and greater swelling of the granule will lead to an increased peak viscosity. Hot paste viscosity would not, however, be expected to show much change as starch–starch interactions are already reduced by thermal and shear forces. Cool paste viscosity is largely determined by associations between amylose chains to form the starch gel and here acetylation would lead to a decrease in viscosity and gel strength.

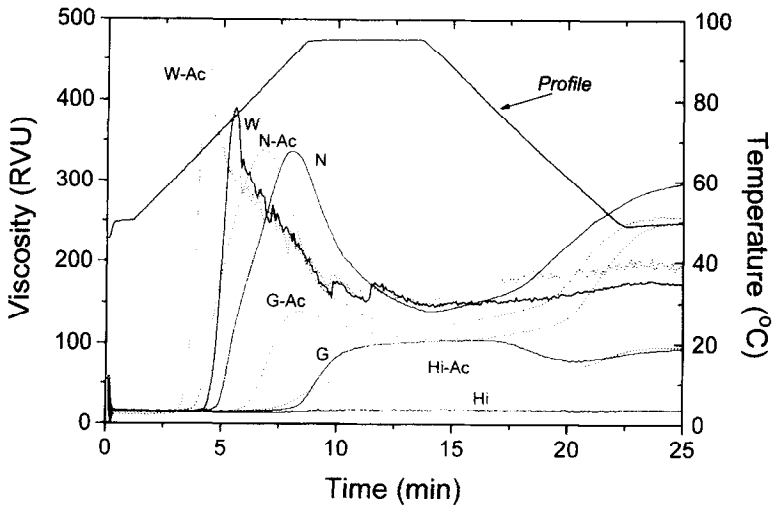

Fig. 1. RVA pasting curves of native and acetylated (-Ac) maize starches.

Table 2. Pasting characteristics of native and acetylated (-Ac) maize starches

Starch	P_{temp} (°C)	PV (RVU)	HPV (RVU)	CPV (RVU)	BD (RVU)	SB (RVU)
Waxy	76.15	391	148	172	243	24
Waxy-Ac	69.25	442	150	200	292	50
Hi-Maize™	–	–	17	18	–	1
Hi-Maize™-Ac	–	–	57	89	–	32
Normal	90.35	337	142	270	195	128
Normal-Ac	83.35	339	119	243	220	124
GELOSE 50	–	–	103	86	–	−17
GELOSE 50-Ac	92.40	140	106	214	34	108

DSC analysis

DSC thermograms of the acetylated and native maize starches are shown in Fig. 2. Decreases were recorded for gelatinization temperatures of all the starches after acetylation (Table 3). The magnitude of the changes corresponded with those observed on pasting and follow from the reduction in starch chain interactions which reduce the energy required for hydration and disruption of starch structure. An exothermic peak was observed in some samples at 110°C, in starch systems this is normally the result of amylose–lipid complexation.

Swelling and solubility

The swelling power of all the starches showed similar increases after acetylation (Fig. 3). The solubility of normal and high amylose starches was greatly increased after acetylation but there was only a minor increase in the solubility of waxy starch (Fig. 4). Increased swelling of the starch granules is an expected consequence of the more rapid hydration permitted by acetylation. An increase in solubility can be attributed to the increased hydrophilicity of the starch, though in the case of less hydrophilic amylopectin this effect would be limited, as is observed for the waxy starch.

Gel texture properties

After acetylation the hardness of all the starch gels was significantly decreased (Table 4). This is in agreement with the reduced CPV already observed and can be attributed to the inhibition of amylose chain interactions reducing the formation of junction zones leading to the formation of a weaker gel. This weakening of gel structure is further indicated by the decreases in adhesiveness and gumminess (data not shown) after acetylation.

Clarity

The clarity of normal and high amylose starches increased after acetylation while that of waxy starch was decreased (Table 3). Loss of clarity in starch is associated with increasing crystallinity during retrogradation which is largely due to amylose association. In the acetylated normal and high amylose starch inhibition of chain association favoured the retention of an amorphous character and high clarity. Waxy starches have a low tendency to retrograde and

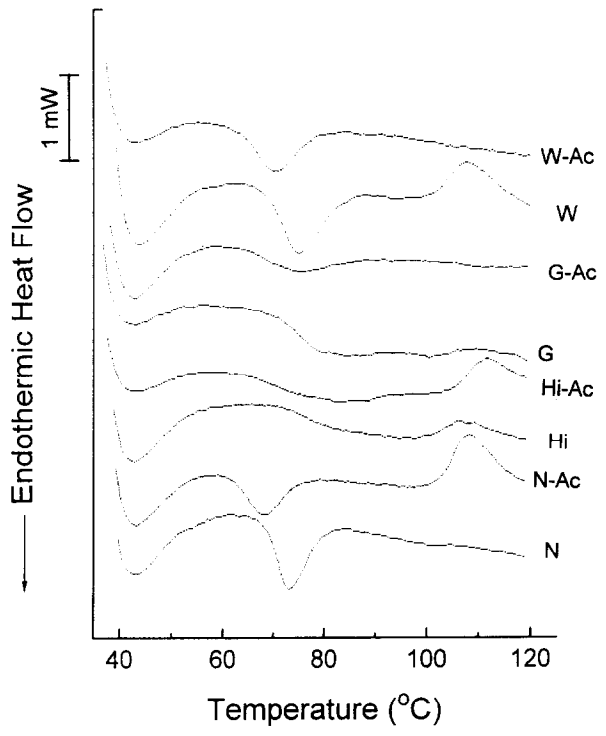

Fig. 2. Differential scanning calorimetry thermograms of native and acetylated (-Ac) maize starches.

normally possess high clarity, despite the reduction in clarity observed the acetylated waxy starch still gave the highest value.

Enzymatic digestibility

The digestibility of normal and waxy starches showed little change due to acetylation. High amylose starch, however, was digested much more rapidly in the acetylated form (Table 3). High amylose starch also showed the biggest increase in solubility of any starch tested and this would be reflected in the increased accessibility of the starch to enzyme attack. The high amylose starches still showed the lowest overall digestibility.

CONCLUSION

The general effect of acetylation on starch has been reported as causing a reduction in gelatinization temperature, increase in peak viscosity, decrease in cool paste viscosity and gel strength, increase in solubility, swelling power and clarity (Biliaderis 1982, Jae *et al.*, 1993). The starches tested here showed similar trends

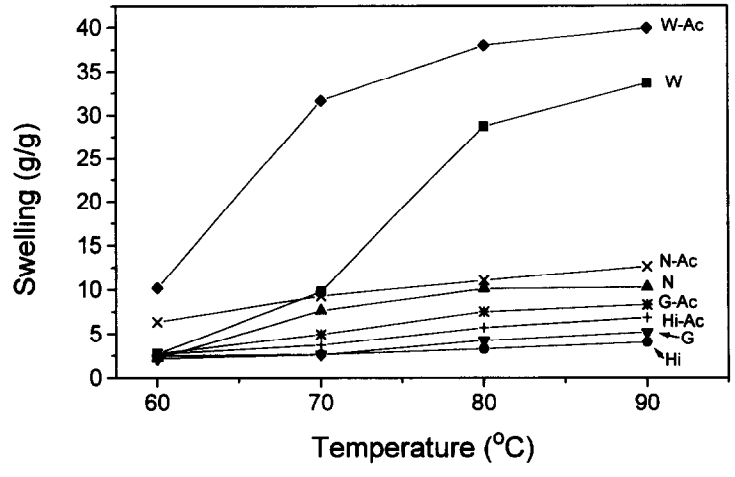

Fig. 3. Swelling power of native and acetylated (-Ac) maize starches.

Table 3. Thermal properties, clarity and digestibility of native and acetylated (-Ac) maize starches. In some cases (see Figure 2), start and end points could not be unambiguously determined.

Starch	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J g^{-1})	Clarity (T)	Digestibility %
Waxy	62.9	72.8	84.3	13.6	45.2	98.9
Waxy-Ac	59.6	67.3	76.9	8.5	35.1	99.6
Hi-Maize™	69.9	92.3	104.5	13.7	1.6	40.1
Hi-Maize™-Ac	63.4	79.8	90.8	6.2	10.0	66.5
Normal	65.3	71.3	80.9	11.0	16.6	90.3
Normal-Ac	59.3	66.3	74.7	7.1	25.7	93.9
GELOSE 50	66.7	77.3	104.0	14.7	2.7	55.7
GELOSE 50-Ac	63.3	73.4	84.2	5.1	14.8	80.0

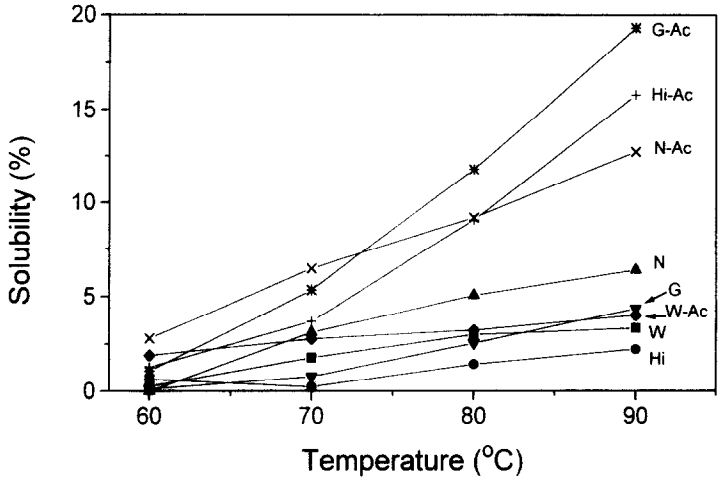

Fig. 4. Solubility of native and acetylated (-Ac) maize starches.

Table 4. Gel texture of native and acetylated (-Ac) maize starches

Probe	Sample	Hardness (g)	Adhesiveness (g s)	Springiness	Cohesiveness
5 mm	Waxy	–	–	–	–
	Waxy-Ac	–	–	–	–
	Hi-Maize™	14.84	18.77	0.52	0.32
	Hi-Maize™-Ac	5.30	17.52	0.92	0.59
	Normal	66.32	65.65	0.94	0.48
	Normal-Ac	14.76	0	1.00	0.76
	GELOSE 50	45.31	44.67	0.86	0.35
	GELOSE 50-Ac	11.06	30.79	0.93	0.56
20 mm	Waxy	23.27	4.60	1.00	0.85
	Waxy-Ac	13.30	21.37	0.88	0.88

but with considerable differences in magnitude between different starches.

Acetylation has been shown to differ profoundly in its influence on starch performance dependent on the amylose/amylopectin ratio of the native starch. While amylose is considered to be the prime determinant of starch hydration and gelling, amylopectin can also have considerable significance, as is demonstrated by the results with the waxy starch. What is less clear is whether any difference in the ease of acetylation of amylose and amylopectin would lead to preferential acetylation of one component in normal starches. Were this to occur, modifications in starch behaviour could only be ascribed to the component which was predominantly acetylated. More detailed investigation of the relationship between acetylation and the modified behaviour of starch components is required to enable a full understanding and reliable prediction of starch pasting in modified starches of diverse origin.

ACKNOWLEDGEMENTS

We thank Dr. Diane Miskelly (Goodman Fielder Mills, Sydney, Australia) for arranging supply of the native starch samples.

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