

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

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### Abstract

The physical properties and enzymatic digestibility of hydroxypropylated starches prepared from high amylose (Hi-Maize™, 66% amylose; GELOSE 50, 47% amylose), waxy (MAZACA 3401X, 3.3% amylose), and normal (22.4% amylose) maize starches were studied. For normal and high amylose starch, hydroxypropylation decreased the temperature at peak viscosity and caused a large increase in both the peak and cool paste viscosities. Hydroxypropylation had little effect on the pasting properties of waxy starch. All the hydroxypropylated starches had lower gelatinization parameters ( $T_o$ ,  $T_p$ ,  $T_c$ , and  $\Delta H$ ) than their unmodified and control starches, but hydroxypropylation increased swelling power and solubility. After hydroxypropylation, the hardness and adhesiveness of all the starch gels decreased. Hydroxypropylation increased the clarity and enzymatic digestibility of all the starches. © 1999 Elsevier Science Ltd. All rights reserved.

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### 1. Introduction

Functional groups can be introduced into starches by a number of chemical modification techniques in order to provide starches with improved or specific properties to extend their usefulness in food or industrial application. Chemically modified starches generally have markedly altered physicochemical properties, compared to their parent starches, primarily depending on the molar substitution or the degree of substitution (DS) and the type of functional groups employed (Rutenberg & Solarek, 1984; Kim, 1988).

Hydroxypropylation is a widely used method for making starch derivatives. Hydroxypropylation of starches imparts extended shelf-life, freeze–thaw stability, and cold storage stability to formulated food products. Hydroxypropyl groups are hydrophilic in nature and when introduced into starch granules, weaken the internal bond structure holding the granule together and also prevent water in the starch paste from separating through syneresis when subjected to freeze–thaw cycling. Hydroxypropyl groups also prevent retrogradation resulting in more fluid paste with improved clarity. This modification can impart desired textural properties to the product (El-Hinnawy, El-Saied, Fahmy, El-

Shirbeeney, & El-Sahy, 1982; Fleche, 1985; Tuschhoff, 1986).

Wu and Seib (1990) compared the paste properties of hydroxypropylated starch with those of starch cross-linked by  $\text{POCl}_3$  in waxy barley starch. They pointed out that hydroxypropylation was more effective than acetylation in improving freeze–thaw stability. The structural changes in potato starch granules, caused by hydroxypropylation, were studied using light microscopy by Kim, Hermansson, and Eriksson (1992). The results suggested that the central region of the potato starch granule is where the hydroxypropyl groups are mainly distributed. The results can provide an explanation for the altered pasting properties of hydroxypropylated potato starches depending on their DS. Seow and Thevamalar (1993) found that the introduction of propyl groups in starch molecules through hydroxypropylation acted as an internal plasticizer and reduced the energy of gelatinization. Biliaderis (1982) in a study on physical characteristics, enzymatic digestibility, and structure of chemically modified smooth pea and waxy maize starches found that hydroxypropylation occurred uniformly throughout the starch granule.

The amylose extender (*ae*) mutant is associated with a high amylose content of the endosperm starch, whereas the waxy mutant (*wx*) starch has essentially no amylose (Shannon & Garwood, 1984). In differential scanning calorimetry (DSC) analyses, the *wx* starch showed thermal behaviour

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similar to that of normal maize starch. The *ae* starch, however, did not exhibit a clear peak, and the endotherm extended beyond 100°C (Wang, White, & Pollak, 1992). The special properties of different mutants, such as thermal behaviour during gelatinization and starch structures have been described elsewhere (Sanders, Thompson, & Boyer, 1990; Wang, White, Pollak, & Jane, 1993). However, there appear to be no systematic comparative reports of properties of chemically modified *ae* and *wx* mutant starch.

The specific objective of this study was to evaluate the effect of hydroxypropylation on 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.

## 2. Materials and methods

### 2.1. 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 Pvt. Ltd., Bray, Ireland). Samples were two high amylose maize starches, Hi-Maize™ (H) with 66% amylose, and GELOSE 50 (G) with 47% amylose; one waxy, MAZACA 3401X with 3.3% amylose (W); and one normal (maize cornflour 3401C with 22.4% amylose) (R).

### 2.2. Starch modification by hydroxypropylation

Hydroxypropylated maize starches of varying DS were prepared according to the method of Kesler and Hjermstad (1964) with slight modification. Native maize starch (50 g) was made up into 125 g of water suspension. A mixture of 2.5 g 30% (w/v) NaOH and 17.5 g 26% (w/v) NaCl was added to the starch suspension slowly with vigorous agitation. The mixture was degassed with nitrogen for 15 min before liquid propylene oxide (3 or 5 ml, 0 ml as a control) was added with stirring. The suspension was reacted in a closed vessel for 24 h at 40°C with sufficient agitation to prevent settling. The suspension was then adjusted to pH 5.0 with 1 M HCl and the slurry was centrifuged for 5 min at 2000 rpm. The pellet was washed twice with water and once with 95% EtOH followed by oven drying at 40°C. The molar substitution of hydroxypropylated starches was analysed following Johnson (1969).

### 2.3. Viscoamylography

A Rapid Visco-Analyser Model 3-D (RVA) (Newport Scientific Pvt. Ltd., Warriewood, Australia) was used to determine the pasting properties of starch samples. Starch (3.0 g d.b.) and a weighed amount of distilled water were combined and stirred in the aluminium 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 performed in each case.

### 2.4. 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. Starch (2.5 mg d.b.) was weighed directly into a 40 µl pan and then 7.5 mg of deionized water was directly added into the pan by a microsyringe and mixed. After sealing, the pan was left for 1 h to allow the sample to equilibrate. Then the sample was heated from 30 to 120°C at a heating rate of 10°C/min. An empty pan was used as a reference. Onset temperature ( $T_o$ ), intermediate temperature ( $T_p$ ), completion temperature ( $T_c$ ) and endothermic energy ( $\Delta H$ ) were recorded.

### 2.5. Swelling power and solubility

The swelling power of starches was determined as described by Subramanian, Hosney, and Bramel-Cox (1994) with minor modifications. Starch (0.5 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 3000 rpm for 15 min. The supernatant was decanted and the swollen starch sediment weighed. Swelling power was the ratio in weight of the wet sediment to the initial weight of dry starch. An aliquot of supernatant was evaporated overnight at 130°C and weighed. The solubility was the ratio in weight of the dried supernatant to the initial weight of the dry starch.

### 2.6. Texture analysis

After RVA testing, the starch pastes were covered and kept at 25°C for 24 h. Then the gel texture (10.7%) was determined in triplicate using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, England). The gel was compressed at a speed of 1.0 mm/s to a distance of 15 mm with a cylindrical plunger (diam = 5 mm). The peak force at 15 mm compression was termed hardness, and the negative area of the curve during retraction of the probe was termed adhesiveness. Because waxy maize and hydroxypropylated Hi-Maize were so soft the probe reached the bottom of the container under the test conditions, they were also tested with a larger probe of 20 mm diameter.

### 2.7. Clarity

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

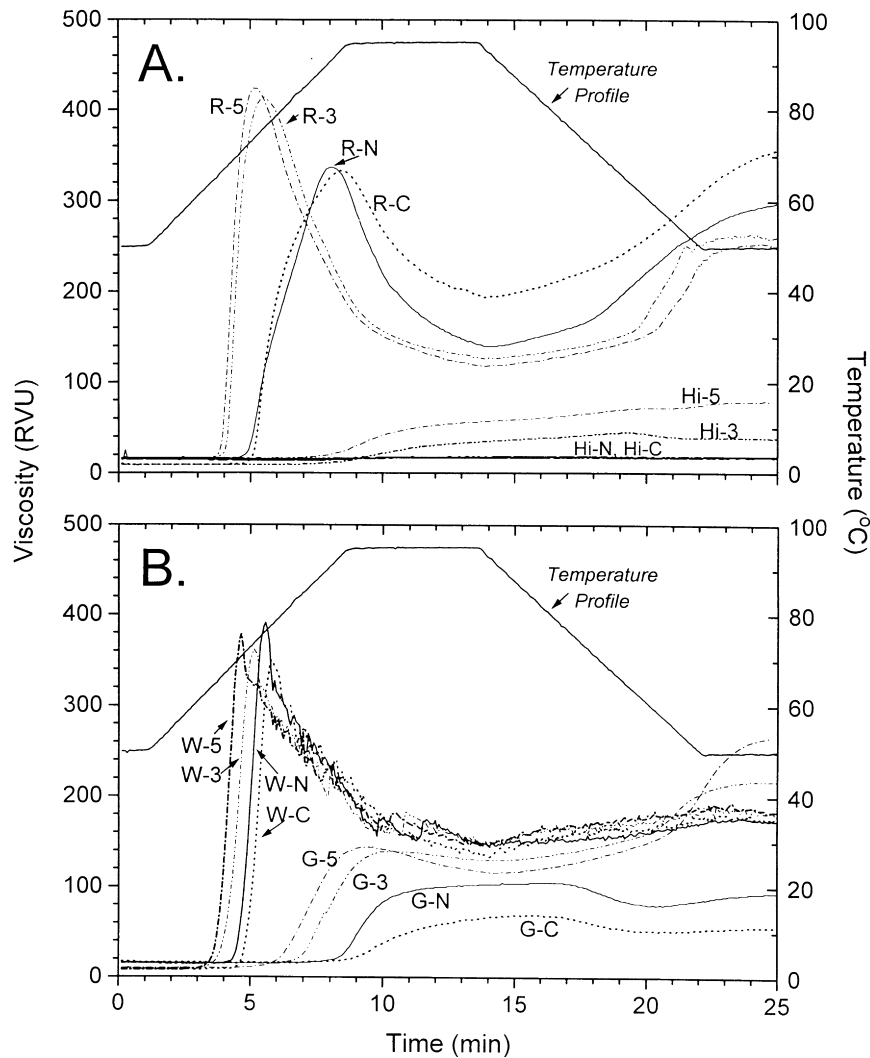


Fig. 1. RVA pasting profiles of native and hydroxypropylated maize starches: (A) Regular (R) and Hi-Maize (H); and (B) Waxy (W) and Gelose-50 (G). (N = native; C = control; 3,5 = prepared with 3 or 5 ml propylene oxide respectively.)

### 2.8. Enzymatic digestibility using $\alpha$ -amylase

The method of Zhang, Collins, and Andrade (1995), with modification, was used.  $\text{KHPO}_4/\text{K}_2\text{PO}_4$  buffer (30 ml, 0.2 M, pH 6.9) was mixed with 1.0 g of starch (d.b.) in a 50 ml test tube. The starch was heated in a water bath at 95°C for 30 min. Lump formation was prevented by stirring. After cooling to 25°C, 320 Units bacterial  $\alpha$ -amylase (Sigma, St. Louis, MO) was added. Tubes were placed in a shaking water bath and incubated at 30°C. Shaking was adjusted to maintain the starch in suspension. After 14 h digestion, 5 ml of 1.0% (w/v)  $\text{H}_2\text{SO}_4$  was added to stop enzymatic hydrolysis. Samples were centrifuged at 4000 rpm for 20 min. The resulting pellet containing the undigested flour residue was washed with 15 ml of 80% ethanol and centrifuged again under the same conditions. The residue was then oven dried at 80°C to constant weight. A starch blank was included for each sample to correct for the presence of any soluble sugar in the samples. Starch

digestibility was expressed as percent weight loss after  $\alpha$ -amylase digestion.

## 3. Results and discussion

### 3.1. Paste viscosity of native and modified maize starches

The pasting properties of the native and hydroxypropylated Hi, G, waxy and normal maize starches are shown in Fig. 1 and Table 1. After hydroxypropylation, the shapes of the pasting curves differed markedly. Normal and high amylose starches all showed increases in peak viscosity, whereas though high amylose starches also showed increased CPV, the CPV of normal starch was reduced. Waxy maize starch was little affected by modification with no change in paste viscosity and only a slight reduction in pasting temperature.

The control starches of *ae* and normal maize had pasting

Table 1  
 Pasting parameters for native and acetylated maize starch. (N = native; C = control; 3.5 = prepared with 3 or 5 ml propylene oxide respectively)

Starch	MS	Pasting onset (°C)	Peak temperature (°C)	Peak viscosity (RVU)	Viscosity at 95°C (RVU)	Viscosity after 5 min at 95°C (RVU)	Final viscosity at 50°C (RVU)	Breakdown (RVU)	Setback (RVU)
W-N	–	67.7	76.2	391	203	148	172	243	24
W-C	–	68.3	77.5	350	214	140	178	210	38
W-3	0.067	61.6	73.4	362	191	149	184	213	35
W-5	0.127	60.6	70.9	379	203	152	187	227	35
Hi-N	–	–	–	–	16	17	18	–	1
Hi-C	–	–	–	–	16	17	17	–	0
Hi-3	0.078	88.2	–	–	14	34	39	–	5
Hi-5	0.119	87.0	–	–	27	56	75	–	19
R-N	–	69.4	90.4	337	301	142	270	195	128
R-C	–	70.0	92.9	333	329	197	313	136	116
R-3	0.061	64.8	75.9	414	195	128	253	286	125
R-5	0.094	62.7	73.5	425	183	120	236	305	116
G-N	–	90.1	–	–	33	103	86	–	–17
G-C	–	92.9	–	–	20	65	53	–	–12
G-3	0.076	80.7	95.0	139	115	130	206	9	76
G-5	0.139	72.9	95.0	144	140	118	207	26	89

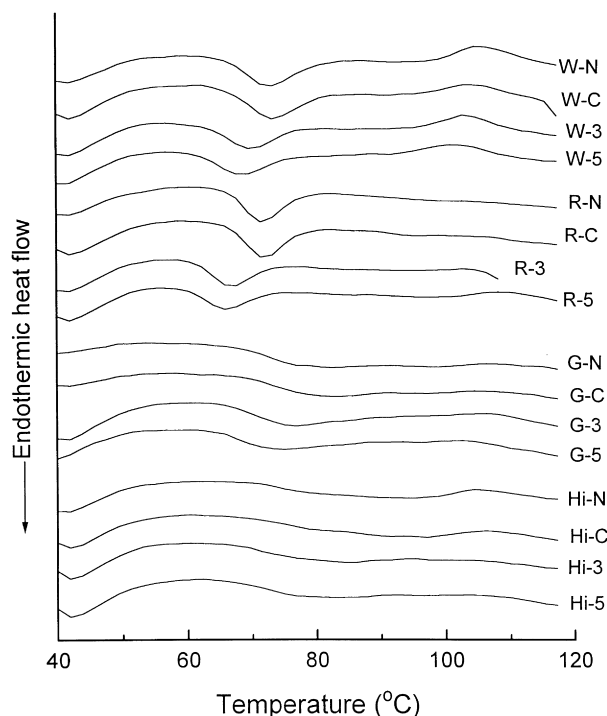


Fig. 2. Differential scanning calorimetry thermograms of native and hydroxypropylated maize starches: Regular (R), Hi-Maize (H), Waxy (W) and Gelose-50 (G). (N = native; C = control; 3,5 = prepared with 3 or 5 ml propylene oxide respectively).

properties that differed somewhat from those of native maize starches (Fig. 1). These differences may be ascribed to the structural changes of starch granules taking place under the reaction conditions used, or possibly, to the removal of either damaged starch granules or metal ions during the purification process of the sample. However the magnitude of these changes was much less than those observed when hydroxypropylation took place.

Table 2

Differential scanning calorimetry, thermal analysis, clarity, and digestibility of native and modified maize starches. (N = native; C = control; 3,5 = prepared with 3 or 5 ml propylene oxide respectively)

Starch	$T_0$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$\Delta H$ (J/g)	Clarity, $T$ (%)	Digestibility (%)
W-N	62.9	72.8	84.3	13.6	45.7	98.9
W-C	62.8	73.2	83.0	13.2	45.9	99.1
W-3	61.7	69.6	78.7	8.7	50.7	99.2
W-5	60.8	68.7	77.4	8.0	56.4	99.2
R-N	65.3	71.3	80.9	11.0	19.2	90.3
R-C	65.0	72.1	80.8	11.2	22.8	91.8
R-3	60.0	66.6	75.4	8.4	33.4	93.7
R-5	58.7	66.1	74.3	7.1	34.4	94.1
G-N	66.7	77.3	104.0	14.7	2.8	55.7
G-C	67.0	80.6	103.7	14.1	4.2	56.0
G-3	65.1	76.6	92.0	11.2	13.1	76.9
G-5	63.0	72.7	88.3	9.5	17.3	81.7
Hi-N	69.9	92.3	104.5	13.7	1.7	40.1
Hi-C	68.3	92.2	105.9	12.6	2.1	38.7
Hi-3	66.0	83.7	94.6	8.4	6.4	56.9
Hi-5	63.9	76.1	92.0	6.4	10.6	63.1

Hydroxypropylation can influence the interactions between the starch chains through different possible mechanisms: (1) by steric hindrance preventing close association of chains restricting formation of inter-chain hydrogen bonds; (2) by changing the hydrophilicity of the starch and thus altering bonding with water molecules; and (3) by participation of the hydroxypropyl groups in hydrogen bonding with other starch chains. The observed effects of hydroxypropylation 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 increased peak viscosity.

### 3.2. Differential scanning calorimetry analysis

DSC thermograms of the hydroxypropylated, native and control maize starches are shown in Fig. 2. Decreases were recorded for gelatinization temperatures and gelatinization enthalpy ( $\Delta H$ ) of all the starches after hydroxypropylation (Table 2). The higher the DS of the starch the larger the decrease in gelatinization temperature and enthalpy. The magnitude of the changes corresponded with those observed on pasting and follow from the reduction in starch chain interactions that reduce the energy required for hydration and disruption of the starch structure.

### 3.3. Swelling and solubility

The swelling power of all the starches showed similar increases after hydroxypropylation (Fig. 3). The higher the MS of the hydroxypropylated starches, the more the swelling power increased. The solubility of normal and high amylose starches was greatly increased after hydroxypropylation but there was only a minor increase in the solubility of waxy starch (Fig. 4). The behaviour of the starch granule during heating is that water penetrates into the more

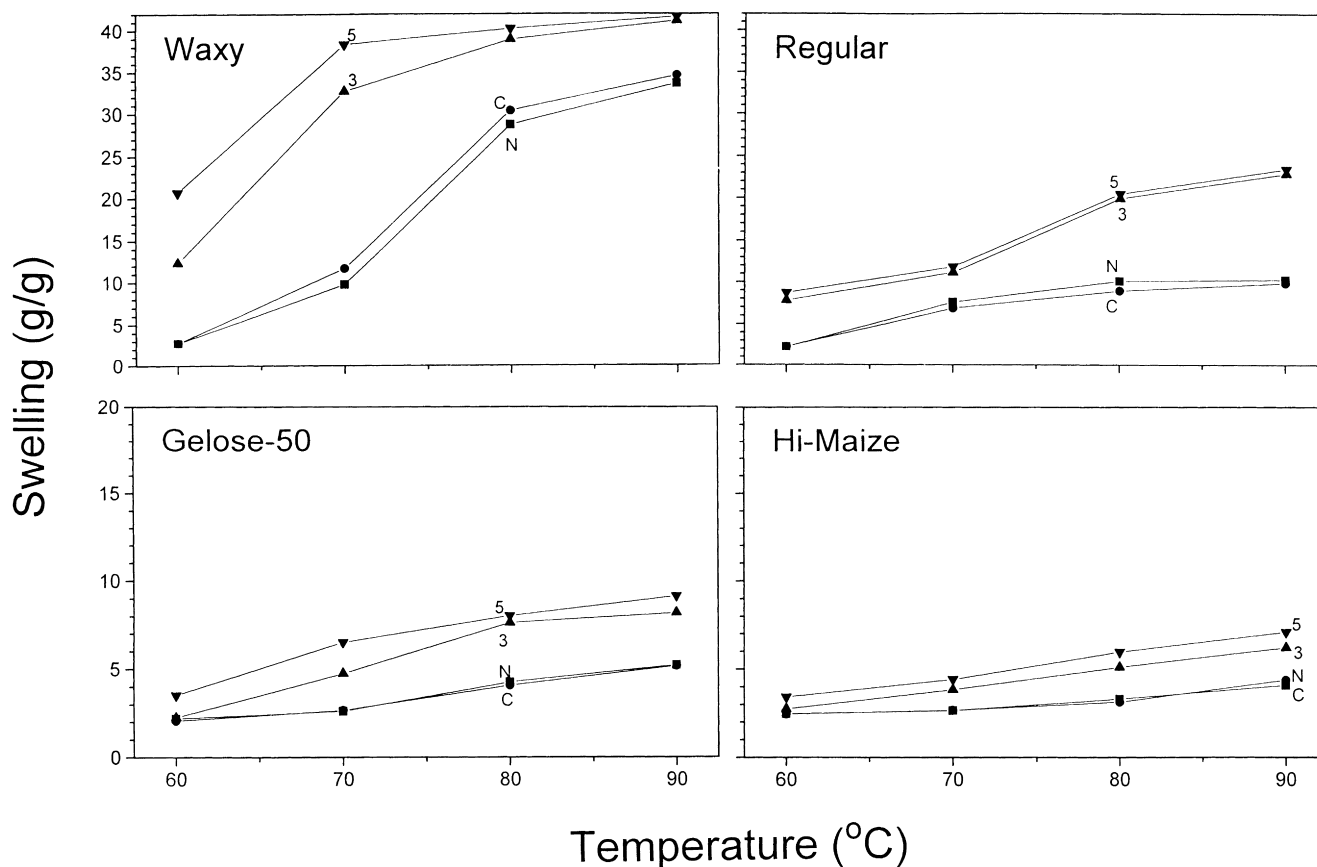


Fig. 3. Swelling power of native and hydroxypropylated maize starches. (N = native; C = control; 3,5 = prepared with 3 or 5 ml propylene oxide respectively.)

accessible amorphous region of the starch granule, resulting in hydration and limited swelling. As the temperature increases to the point of gelatinization (pasting), the swelling of the amorphous phase (water-penetrated phase) accelerates the disruption of the crystalline region. The consistency development during this process is primarily related to the swelling properties of the starch granule. Increased swelling of starch granules is an expected consequence of the more rapid hydration permitted by hydroxypropylation. 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.

### 3.4. Gel texture properties

After hydroxypropylation the hardness and adhesiveness of all the starch gels decreased significantly (Table 3). This can be attributed to the inhibition of amylose chain interactions reducing the formation of junction zones leading to the formation of a weaker gel. Hydroxypropylation increased the springiness and cohesiveness of high amylose starches (Gelose, Hi), but decreased that of normal starch and did not affect that of waxy starch.

### 3.5. Clarity

The clarity of all the starches increased after hydroxypropylation (Table 2). The higher the DS of the starch, the greater the clarity. Loss of clarity in starch is associated with increasing crystallinity during retrogradation that is largely due to amylose association. In hydroxypropylated starch inhibition of chain association favoured the retention of an amorphous character and high clarity.

### 3.6. Enzymatic digestibility

Normal and waxy starches are already highly digestible and therefore showed little change due to hydroxypropylation. High amylose starch has much lower digestibility but this was greatly increased by hydroxypropylation (Table 2). This would reflect the increased accessibility of the starch to enzyme attack after this modification. The higher the DS of the starch the greater the increase in digestibility.

## 4. Conclusion

There is an appreciable difference in the performance of modified starches depending on the substituent group. Acetylation of maize starches modifies the pasting properties of both high amylose and waxy starches (Liu, Ramsden, &

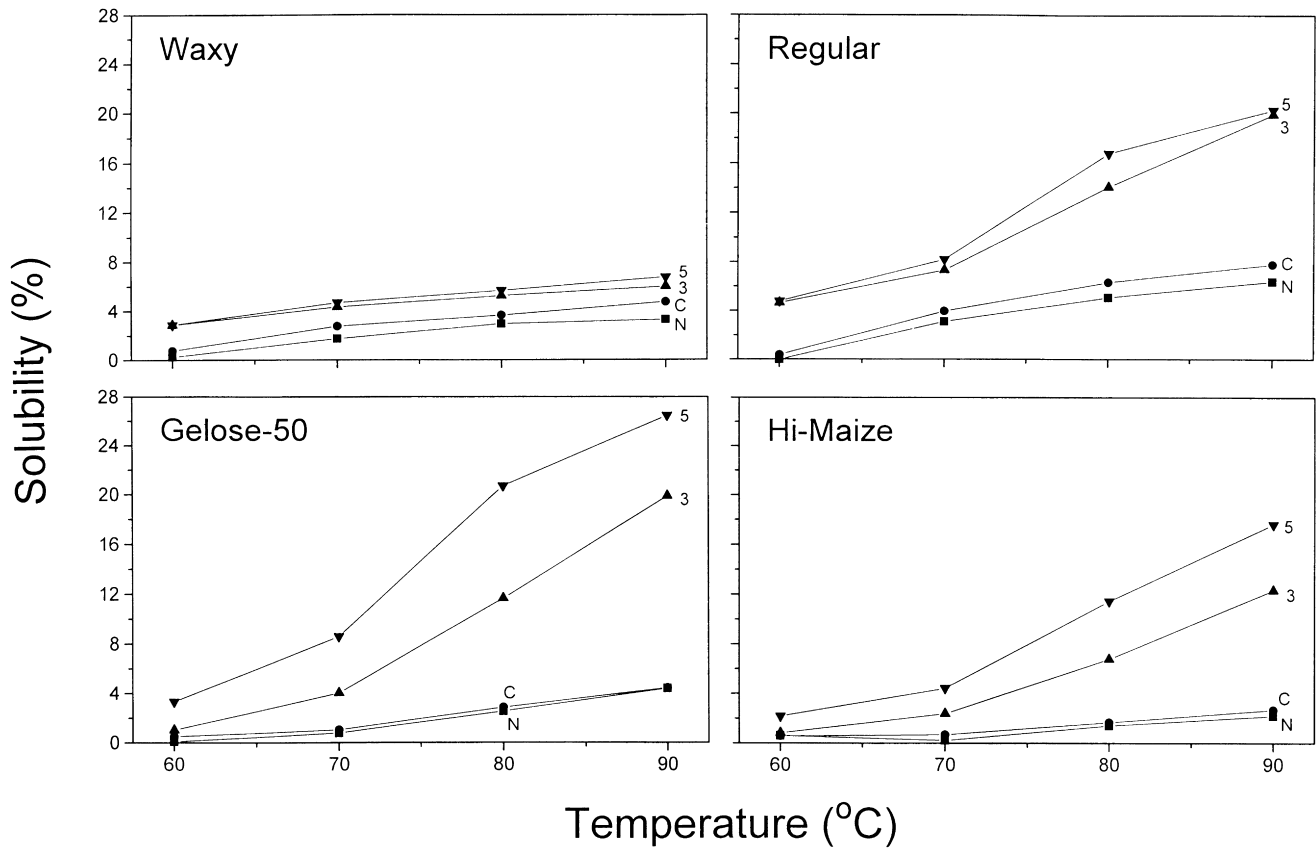


Fig. 4. Solubility of native and hydroxypropylated maize starches. (N = native; C = control; 3,5 = prepared with 3 or 5 ml propylene oxide respectively.)

Table 3  
Gel texture of native and hydroxypropylated maize starches

Probe	Sample	Hardness (g)	Adhesiveness (g s)	Springiness	Cohesiveness
5 mm	W-N	—	—	—	—
	W-C	—	—	—	—
	W-3	—	—	—	—
	W-5	—	—	—	—
	R-N	75	219	0.94	0.51
	R-C	75	215	0.95	0.52
	R-3	35	46	0.76	0.42
	R-5	23	39	0.75	0.42
	G-N	44	102	0.89	0.32
	G-C	25	54	0.79	0.27
	G-3	11	50	0.96	0.62
	G-5	11	47	0.96	0.61
	Hi-N	19	18	0.61	0.31
	Hi-C	15	17	0.62	0.28
	Hi-3	—	—	—	—
Hi-5	—	—	—	—	
20 mm	W-N	28	18	0.94	0.81
	W-C	18	15	0.94	0.83
	W-3	15	6	0.95	0.86
	W-5	15	5	0.94	0.86
	Hi-N	128	160	0.85	0.35
	Hi-C	94	131	0.89	0.34
	Hi-3	21	47	0.93	0.75
	Hi-5	17	46	0.94	0.73

Corke, 1997). Hydroxypropylation, however, has little effect on waxy maize starch. This difference cannot be attributed to differing efficiency of reaction with amylose or amylopectin since the measured DS of both starches were comparable. It is possible that the hydroxypropyl group, because of its greater flexibility is less of a hindrance to the association between starch chains than the more rigid acetyl group. Thus the failure to observe any effect with waxy starch is simply a consequence of the low magnitude of any effect being obscured in a starch that already hydrates rapidly to give a high peak viscosity.

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