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Effect of heat–moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches

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ABSTRACT

Native wheat, oat, lentil, yam, and potato starches were heat treated at 100°C for 16 h at moisture contents between 10 and 30%. The heat treatment did not change granule size and shape. In oat starch, granules were less compactly packed after heat treatment. The X-ray diffraction intensities increased in wheat, oat, and lentil starches, but decreased in potato and yam. The X-ray patterns of wheat and oat starches remained unchanged, while those of lentil, potato, and yam starches became more cereal-like. In all starches, the swelling factor and amylose leaching decreased, being more pronounced in potato. Heat treatment induced complex formation between amylose and native lipids. Differential scanning calorimetry of the heat-treated samples showed a broadening of the gelatinization-temperature range and a shifting of the endothermal transition towards higher temperatures. These changes were more pronounced in potato starch. The gelatinization enthalpy of wheat, oat, and lentil starches remained unchanged, but those of potato and yam starches decreased on heat treatment. Heat treatment increased the 95°C viscosity of wheat starch, but decreased those of oat, lentil, potato, and yam starches. In all starches, thermal and shear stability increased after heat treatment. Acid hydrolysis decreased on heat treatment of wheat and lentil starches, but increased in oat, potato, and yam starches. However, in potato and yam starches, the foregoing trend was evident only during the first seven days of hydrolysis. Thereafter, acid hydrolysis was more pronounced in native than in heat-treated starches. The susceptibility towards hydrolysis by porcine pancreatic alpha amylase decreased on heat treatment of wheat and lentil starches, whereas increases were observed for oat, potato, and yam starches. The results indicated that the extent of starch-chain associations within the amorphous regions and the degree of crystalline order are altered during heat–moisture treatment. The magnitude of these changes were found to be dependent upon the moisture content during heat treatment and on the starch source.

INTRODUCTION

Heat treatment of starches at low water contents (18–27%) and high temperature (100°C) for 16 h has been shown[1–11] to dramatically change the physicochemical properties of wheat, barley, triticale, potato, and cassava starches. In cereal and

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tuber starches, heat–moisture treatment increases enzyme susceptibility[6,11], paste stabilities[7–11], and gelatinization temperatures[4,6,7,9,11], but decreases the swelling power and hot-paste consistencies[4,6,7,9,11]. In cereal starches, X-ray patterns remain unchanged[4,6] and solubilities[6] increase on heat–moisture treatment, whereas, changes in X-ray patterns and decreased solubilities[6] are observed in tuber starches.

It has been postulated[4] that the effects of heat–moisture treatment may be due either to new crystallization or recrystallization and perfection of the small crystalline regions of the granule. However, changes within the amorphous regions still remain an area of uncertainty which merits further investigation. In order to obtain a precise interpretation of the structural changes within the amorphous and crystalline regions of the starch granule, we compared changes in: *1*, X-ray diffraction intensities and *d*-spacings; *2*, swelling factor; *3*, amylose leaching; *4*, thermal transition parameters; *5*, viscosity, and *6*, susceptibility towards alpha amylase and acid hydrolysis during heat–moisture treatment of cereal, legume, and tuber starches.

EXPERIMENTAL

Materials.—Wheat, corn, and potato starches were obtained from Sigma Chemical Co, St. Louis, MO, USA. Seeds of lentil (*Lens culinaris Medicus*) were obtained from a local supplier. AC Hill oat grains (*Avena nuda, var chinensis. Fish. ex link*) which is a spring type, day length-sensitive cultivar, were obtained from the central experimental farm at Ottawa. The extraction of lentil and AC Hill oat starches were carried out by procedures outlined in earlier publications[12,13]. Crystalline porcine pancreatic alpha amylase (EC 3.2.1.1), type 1-A was obtained from Sigma Chemical Co (St. Louis, MO).

Chemical composition of starch.—Quantitative estimations of moisture, ash, nitrogen, and starch damage were performed by the standard AACC procedures. Starch lipids were obtained by Soxhlet extraction (7 h) with 100 mL of 3:1 1-propanol–water. Lipids were also extracted, after acid hydrolysis with 24% HCl for 30 min at 70–80°C, and the hydrolyzate then extracted three times with hexane[15]. The purification and quantification of extracted lipids were carried out by procedures that have been described elsewhere[16]. Apparent and total amylose content was determined by the method of Chrastil[17].

Heat–moisture treatment.—The method of heat–moisture treatment was that of Sair[3], with minor modifications. Starch samples (15 g dry basis) were weighed into glass containers. Starch moisture content was brought to 10, 20, and 30% by adding the appropriate amounts of distilled water into the containers. Starch samples were mixed thoroughly during the addition of water. The containers were sealed, kept for 24 h at ambient temperature, and then placed in a forced-air oven for 16 h at 100°C. Afterwards the containers were opened, and the starch samples air dried to a uniform moisture content (~ 10%). The effect of treatment time

(1–24 h), on gelatinization transition temperatures and enthalpy, was studied on starches heat treated (100°C) at 30% moisture.

X-ray diffraction.—X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm/min. The starch powder was scanned through the 2θ range of 3–35°. Traces were obtained using Cu-$K\alpha$ radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40KV, 50mA, 1°/1° divergence slit/scattering slit, 0.30-mm receiving slit, 1 s time constant, and scanning rate of 3° per min.

Swelling factor.—The swelling factor of the starches when heated to 50–95°C in an excess of water was measured according to the method of Tester and Morrison[18]. This method measures only intragranular water and hence the true swelling factor at a given temperature. The swelling factor is reported as the ratio of the volume of swollen starch granules to the volume of the dry starch.

Extent of amylose leaching.—Various concentrations of the starches (15–20 mg) in water were heated in volume-calibrated sealed tubes (50–95°C) for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 3600 rpm for 10 min. The supernatant liquid (1 mL) was withdrawn and its amylose content determined by the method of Chrastil[17].

Differential scanning calorimetry (DSC).—Gelatinization temperatures were measured and recorded on a Perkin–Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter equipped with a thermal analysis data station. Water (8.0 μL) was added with a microsyringe to starch (2.5 mg) in the DSC pans, which were then sealed, reweighed, and kept overnight at room temperature. The scanning temperature range and the heating rate were 20–120°C and 10°C min^{-1}, respectively. The thermograms were recorded with water as reference.

The transition temperatures reported are the onset (T_o), peak (T_p), and conclusion (T_c) of the gelatinization endotherm. Indium was used for calibration. The enthalpy (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of calories per unit weight of dry starch (cal g^{-1}). All DSC experiments were replicated at least twice.

Pasting properties.—A Brabender viscoamylograph (model VA-V) equipped with a 700 cm-gf cartridge was used to determine the pasting properties at a concentration of 6% w/v (dry basis) and pH 5.5. The starch dispersions were stirred at 75 rpm and heated at 1.5°C min^{-1} to 95°C, kept at this temperature for 30 min, and cooled to 50°C.

Acid hydrolysis.—The starches were hydrolyzed with 2.2 M HCl at 35°C (1.0 g starch/40 mL acid) for 25 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24-h intervals, aliquots of the mixtures were neutralized and centrifuged (3500 rpm) and the supernatant liquid was assayed for total carbohydrate[19]. The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch.

Enzymatic hydrolysis.—The extent of hydrolysis by porcine pancreatic alpha amylase was determined following previously described methods[20].

Scanning electron microscopy (SEM).—Granule morphology and the mode of action of alpha amylase were studied by SEM. Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed with a Hitachi (S 570) SEM at an accelerating potential of 20 kV. Enzyme-digested granules were prepared for SEM by rapidly freezing in liquid nitrogen and freeze drying at 80°C. The dried samples were prepared for viewing as just described.

RESULTS

Proximate composition.—The proximate analyses of the starches are presented in Table I. All starches were of very high purity (< 0.05% nitrogen).

Granule morphology.—Heat–moisture treatment did not alter the size or shape of the granules used in this study. Similar observations have also been made by Kulp and Lorenz[5] on potato and wheat starches. However, in oat starch, many of the granules which were aggregated in the native state (Fig. 1A) were less compactly packed after heat treatment (Fig. 1B).

X-ray diffraction.—The X-ray diffraction patterns and the intensities of the *d* spacings of the major diffraction peaks of native and heat–moisture treated starches are presented in Fig. 2 and Table II respectively. The *d*-spacings of all starches shifted slightly on heat–moisture treatment. However, none of the treated starches exhibited major new *d*-spacings (Table II). Heat treatment at 30% moisture increased the intensities of the major peaks of wheat, lentil and oat starches (wheat > lentil > oat), but decreased those of potato and yam starches

TABLE I

Proximate composition of native starches [a]

Characteristics	Composition (%)				
	Wheat	Oat	Lentil	Potato	Yam
Moisture	9.9	9.4	9.6	9.7	9.6
Ash	0.02	0.03	0.03	0.05	0.02
Nitrogen	0.04	0.05	0.02	0.03	0.03
Lipid					
Solvent extracted					
$CHCl_3$–MeOH [b]	0.04	0.07	0.03	0.02	0.02
1-propanol–water [c]	0.64	1.05	0.11	0.09	0.07
Acid hydrolyzed [d]	0.70	1.13	0.14	0.11	0.09
Amylose content (% of total starch)					
Apparent [e]	21.1	16.7	36.7	21.0	27.0
Total [e]	27.3	19.4	38.0	24.7	27.1

[a] All data reported on dry basis and constitute the mean of three determinations. [b] Lipids extracted from native starch by 2:1 $CHCl_3$–MeOH at 25°C (mainly unbound lipids). [c] Lipids extracted by hot 3:1 1-propanol–water from the residue left after $CHCl_3$–MeOH extraction (mainly bound lipids). [d] Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids). [e] Apparent, and total amylose determined by I_2-binding before, and after removal of bound lipids by hot 1-propanol–water extraction.

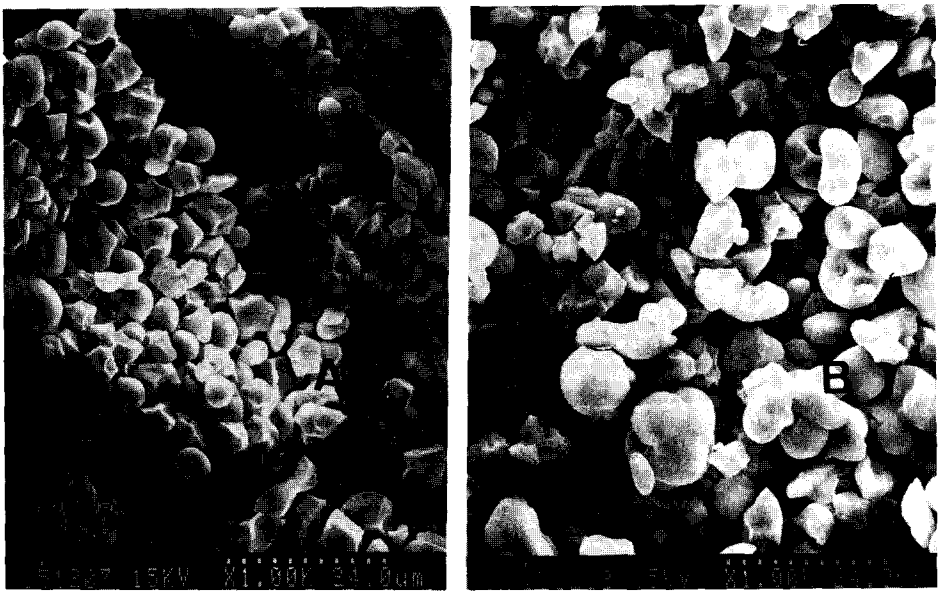

Fig. 1. Scanning electron micrographs of native (A), and heat–moisture treated (B) oat starch granules (16 h, 100°C, 30% moisture).

(potato > yam) (Fig. 2. Table II). The noted changes in intensities in potato and wheat starches were influenced by the level of moisture content during heat treatment (Table II). However, intensity changes in the other starches were only marginally affected by variations in moisture levels (Table II). The X-ray diffraction patterns of native wheat, oat, and lentil starches was retained after treatment. However, alterations ('B' → 'A' + 'B') occurred in potato and yam starches. The 'B' to 'A' + 'B' transformation in potato starch has also been reported by other researchers[7,9].

Swelling factor and amylose leaching.—The swelling factor (SF) and amylose leaching (AML) of native starches increased with rise in temperature, but were decreased on treatment (Figs. 3A–D). The extent of decrease in SF among the starches followed the order: potato > lentil > yam > wheat > oat (Figs. 3A–C). In contrast, the corresponding order in AML was: potato > yam > lentil > oat > wheat (Figs. 3B and D).

Apparent amylose content.—The apparent amylose contents (determined by I_2 binding without lipid removal) are presented in Table III. The results showed that complex formation between native lipids and amylose increased on heat treatment.

Differential scanning colorimetry.—The influence of heat treatment at moisture contents between 10 and 30% on gelatinization transition temperatures [onset (T_o), mid-point (T_p), and conclusion (T_c)] and gelatinization enthalpy (ΔH) are presented in Table IV. The T_o, T_p, and T_c values of potato and oat starches (Table IV) increased on heat treatment (at all moisture levels). The increase was most

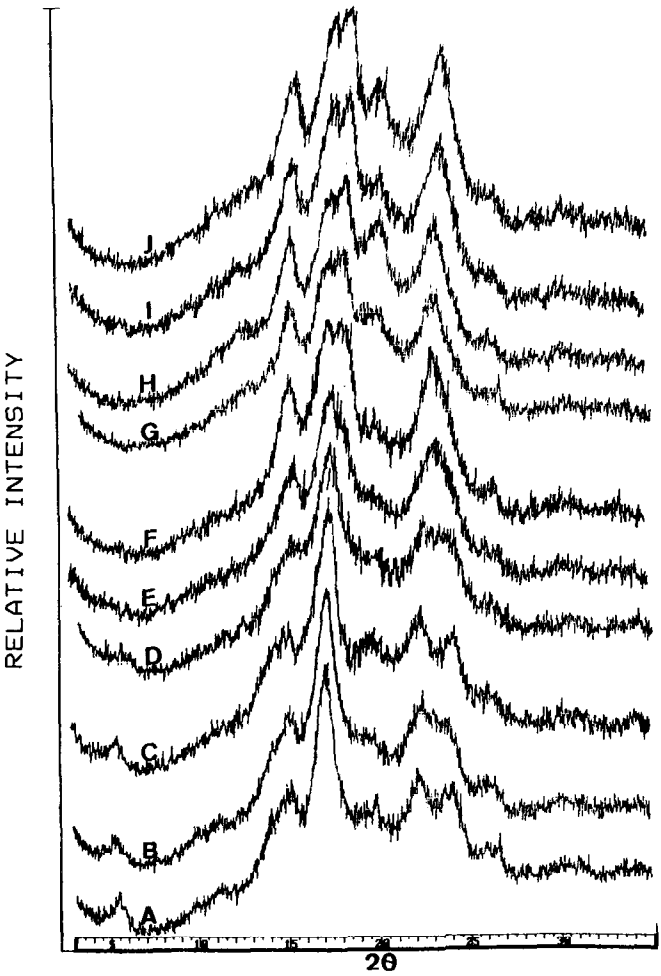

Fig. 2. X-ray diffraction patterns of native, and heat-moisture treated (HMT) starches (16 h, 100°C, 30% moisture). (A) native yam [moisture content (MC) 9.6%], (B) HMT yam (MC 9.8%), (C) native potato (MC 9.7%), (D) HMT potato (MC 9.5%), (E) native lentil (MC 9.6%), (F) HMT lentil (MC 9.9%), (G) native oat (MC 9.4%), (H) HMT oat (MC 9.9%), (I) native wheat (MC 9.9%), and (J) HMT wheat (MC 10.0%).

marked at a moisture level of 30%. In both starches, the extent of increase in transition temperatures followed the order: $T_c > T_p > T_o$. At all moisture levels, the increase in T_o was more marked in potato than in oat starch. At 30% moisture, the gelatinization temperature range ($T_c - T_o$) increased by 5 and 6°C in potato and oat starches, respectively. However, in wheat, lentil, and yam starches, significant increases in T_o, T_p, and T_c were observed only at moisture levels beyond 20% ($T_c > T_p > T_c$). As in potato and oat starches, these increases were more marked at a moisture level of 30%. At 30% moisture, the increase in $T_c - T_o$ in wheat, lentil, and yam was respectively 2, 1 and 2°C. Increases in gelatinization temperatures on

TABLE II

X-ray diffraction spacings, and intensities of the major peaks of native and heat–moisture-treated starches

Starch source and treatment	Interplanar spacings (d) in Å with intensities (cps) [a]						
Wheat							
native [b]	5.94(489)	5.24(523)		4.90(362)	4.45(378)	3.83(435)	380(403)
HMT [c]	5.94(554)	5.19(759)		4.91(818)		3.83(761)	
HMT [d]	5.95(615)	5.15(1115)		4.91(1191)	4.90(1130)	3.88(925)	
Oat							
native [b]	6.03(427)	5.22(576)	5.09(626)	4.90(704)	4.64(362)	4.49(368)	3.83(638)
HMT [c]	5.88(689)	5.15(686)		4.87(744)	4.67(364)	4.49(470)	3.88(551)
Lentil							
native [b]	5.90(404)	5.24(523)	5.10(539)	4.99(527)			3.83(409)
HMT [d]	5.90(700)	5.26(686)		4.99(1079)		4.50(544)	3.88(447)
Potato							
native [b]	16.5(196	5.98(400)	5.20(876)	4.50(306),	4.00(384)	3.70(360)	
HMT [c]	16.8(155)		5.25(703)	3.92(424)	3.80(445)		
HMT [d]		5.96(320)	5.20(569)	4.50(300),	3.98(331)	3.74(290)	
Yam							
native [b]	16.5(288)	5.94(482)	5.20(1067)	4.50(343),	4.00(412)	3.95(430)	
HMT [d]	16.5(150)	5.94(370)	5.20(905)	4.81(338)	4.60(345)	3.74(344)	

[a] Counts per second. [b] Native starches had moisture contents ranging from 9.6 to 9.7%. [c] Heat-treated at 20% moisture for 16 h at 100°C. [d] Heat-treated at 30% moisture for 16 h at 100°C.

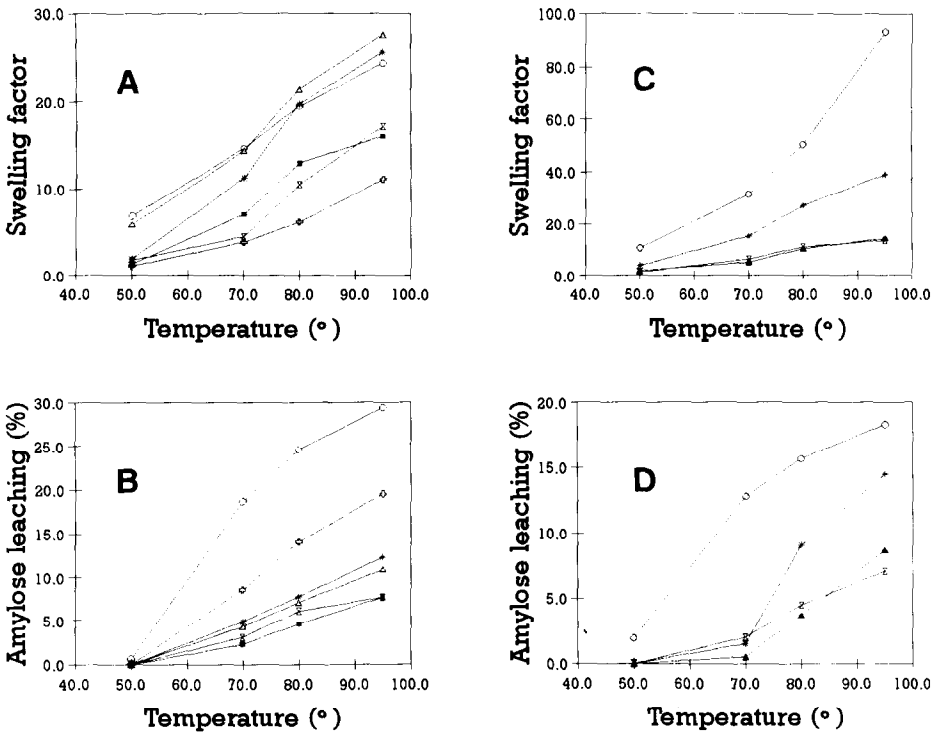

Fig. 3. Swelling factor (A and C) and leached amylose (B and D) of native and heat-moisture-treated (HMT) starches (16 h, 100°C, 30% moisture). (A) and (B) native wheat (△), HMT wheat (⧖) native oat (∗), HMT oat (□), native lentil (○), and HNT lentil (⊕) (C) and(D): native potato (○), HMT potato (⧖), native yam (∗) HMT yam (△).

heat–moisture treatment has also been observed by other researchers in potato[4,6–9] and wheat[4–7] starches. The ΔH of potato and yam starches progressively decreased with an increase in the level of moisture during heat treatment (Table IV). At 30% moisture, this decrease amounted to 1.1 and 1.4 cal/g in potato and yam starches, respectively. However, at all moisture levels of heat treatment, the ΔH of

TABLE III

Apparent amylose content of native, and heat–moisture treated starches

Starch source	Apparent amylose content (%) [a]	
	Native	Heat–moisture treated [b]
Wheat	21.1	19.8
Oat	16.7	14.8
Lentil	36.3	35.4
Potato	22.0	22.0
Yam	27.0	27.0

[a] Determined by I_2 binding without extraction of native lipids. [b] 100°C for 16 h at 30% moisture content.

TABLE IV

DSC characteristics of native, and heat moisture treated starches

Source	Treatment	T_o [c]	T_p [c]	T_c [c]	ΔH [d] (cal/g)
Wheat	Native	56.0	61.0	67.0	2.3
	HMT [b]				
	10%	56.0	62.0	67.0	2.3
	20%	57.0	62.0	67.0	2.3
	30%	65.0	70.0	78.0	2.3
Oat	Native	60.0	64.0	70.0	2.5
	HMT [b]				
	10%	61.0	67.0	72.0	2.5
	20%	61.0	70.0	76.0	2.5
	30%	64.0	75.0	80.0	2.5
Lentil	Native	55.0	61.0	68.0	1.8
	HMT [b]				
	10%	55.0	61.0	70.0	1.8
	20%	55.0	61.0	71.0	1.8
	30%	64.0	71.0	78.0	1.8
Potato	Native	54.0	59.0	64.0	3.8
	HMT [b]				
	10%	59.0	64.0	71.0	3.5
	20%	60.0	64.0	73.0	3.0
	30%	65.0	71.0	80.0	2.7
Yam	Native	72.0	77.0	83.0	5.0
	HMT [b]				
	10%	72.0	77.0	84.0	4.6
	20%	72.0	78.0	85.0	3.6
	30%	77.0	84.0	90.0	3.6

[a] Average standard deviation = 0.11 ($n = 3$). [b] Heat-moisture treatment of all starches were conducted at 100°C for 16 h at moisture contents of 10, 20, and 30%. [c] T_o, T_p, and T_c indicates the temperature of the onset, midpoint, and end of gelatinization. [d] Enthalpy of gelatinization.

wheat, lentil, and oat starches remained unchanged (Table IV). The foregoing changes in ΔH for potato starch agreed with those of Donovan et al.[4] and Stutz[9]. However, the former authors reported a decrease in ΔH for wheat starch (100°C, 16 h, 27% moisture).

The changes in T_o, T_p, T_c, and ΔH as a function of time during heat treatment at 30% moisture content are presented in Figs. 4 and 5, respectively. During the first 5 h, rapid increases were observed in T_o, T_p, and T_c (Fig. 4) for wheat, lentil, potato, and yam starches (potato > lentil > wheat > yam). Thereafter, increases were less pronounced (Fig. 4). Oat starch behaved differently in exhibiting only gradual increases in T_o, T_p, and T_c with time (less than those of the other starches). The increases in T_o ceased after 2 h of heat treatment. In contrast, T_p and T_c continued to increase gradually and then began to level off after 16 h of heat treatment (Fig. 4).

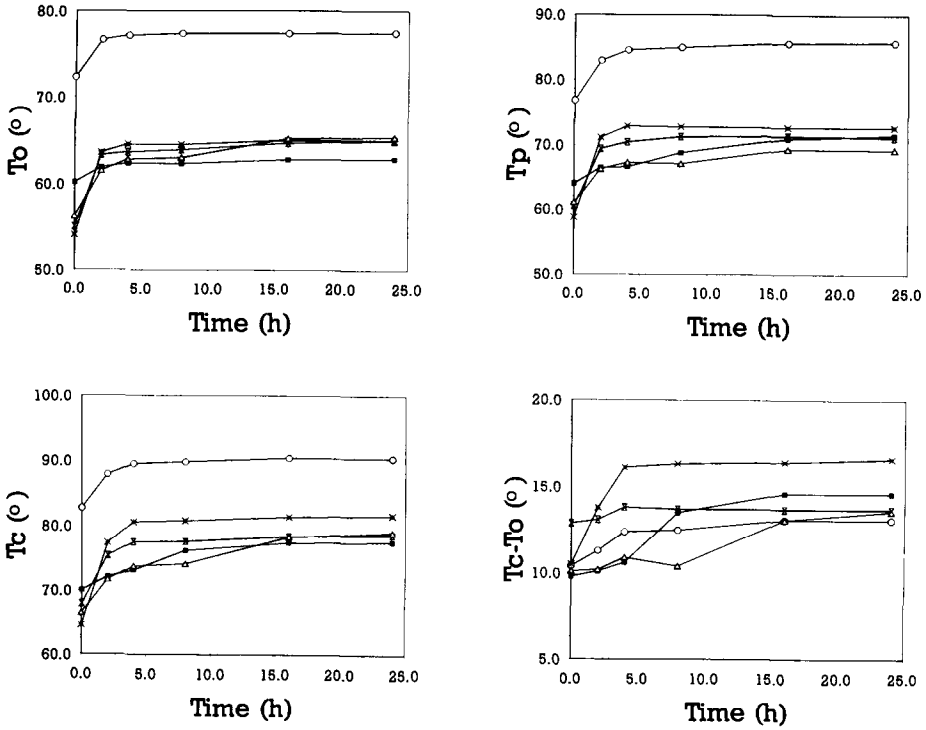

Fig. 4. Gelatinization transition temperatures (T_o, T_p and T_c) and range (T_c-T_o) as a function of time of heat–moisture treatment (100°C, 16 h, 30% moisture). Wheat starch (△), lentil starch (⊠), potato starch (∗), oat starch (■) and yam starch (○———○).

The gelatinization-temperature range (T_c-T_o) of potato starch increased rapidly during the first 5 h of heat treatment (10.2 to 17°C) (Fig. 4), while increases during this time period were only marginal in the other starches. Heat treatment beyond 5 h caused only marginal changes in (T_c-T_o) of potato, lentil, and yam starches. However, (T_c-T_o) of oat and wheat starches showed rapid increases between the 8th and 16th h of heat treatment. Thereafter, changes were only minimal. Rapid increases in ΔH of potato and yam starches occurred during the first 3 h and between the 8th and 16th h of heat treatment (Fig. 5). However, no changes were observed after 16 h (Fig. 5). The changes (decrease) in ΔH (decrease) were only marginal in wheat, whereas those of oat and lentil starches remained unchanged (Fig. 5).

Pasting curves.—The pasting curves of native and heat–moisture treated starches are presented in Fig. 6. Heat–moisture treatment decreased the pasting temperature of wheat starch by 3°C and increased those of lentil, potato, and yam starches by 24, 30, and 16°C, respectively. However, that of oat starch remained unchanged. The 95°C consistency increased by 30 Brabender units (BU) on heat–moisture treatment of wheat starch, but decreased by 130, 220, 370, and 2100 BU, respectively, in heat–moisture treated oat, lentil, yam, and potato starches. In all starches

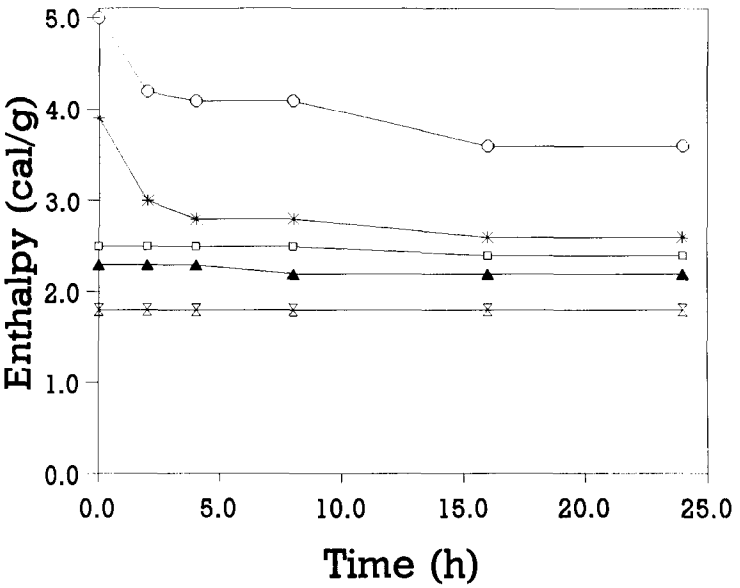

Fig. 5. The influence of time of heat–moisture treatment (100°C, 30% moisture), on gelatinization enthalpy (ΔH). Wheat starch (▲), lentil starch (X), potato starch (*), oat starch (□), and yam starch (o).

the thermal stability (during the holding cycle at 95°C) increased on heat–moisture treatment. Our amylograms on potato starch are in general agreement with reported data[5,7]. However, with respect to wheat starch, discrepancies were observed between our results and those of Kulp and Lorenz[5] and Lorenz and Kulp[7]. The former authors reported a gradual decrease in consistency (at 92°C) with increase in moisture level (21 to 27%) during heat treatment (16 h, 100°C). The decrease being 50 BU at 27% moisture. While the latter authors reported that during heat treatment (16 h, 100°C) the consistency (at 92°C) increased by 70 BU at a moisture level of 21% but decreased by 10 BU at a level of 27% moisture. These conflicting data are rather puzzling, since the variety of wheat used in both studies was identical.

Acid hydrolysis.—The solubilization patterns of native and heat–moisture treated starches (at 30% moisture content) are presented in Fig. 7. The extent of hydrolysis of the amorphous and crystalline regions of wheat and lentil starch granules decreased on heat–moisture treatment. The extent of the decrease was nearly the same in both starches. However, heat–moisture-treated granules of oat starch were hydrolyzed to a slightly greater extent than those of native starch. During the first 7 days of hydrolysis, heat–moisture-treated starches of potato and yam were hydrolyzed to a greater extent than their native counterparts. Thereafter, hydrolysis was more extensive in native starches.

The extent of acid hydrolysis (after 48 h) in potato and yam starches subjected to heat treatment (at 30% moisture content) for 16 h at 100 and 75°C are

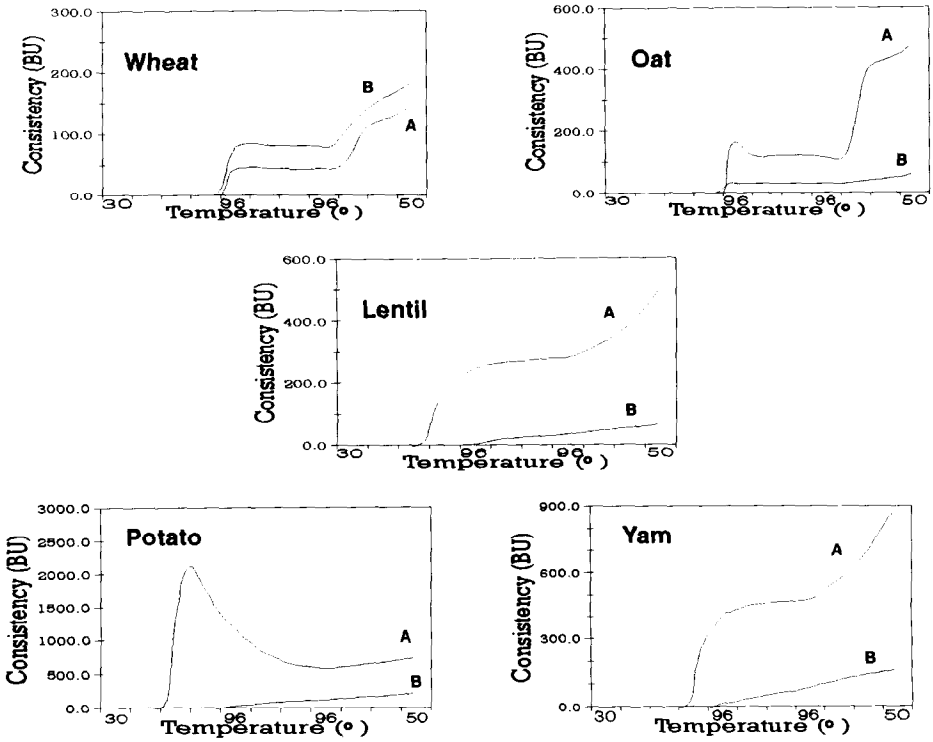

Fig. 6. Pasting characteristics of (A) native and (B) heat–moisture-treated starches (16 h, 100°C, 30% moisture).

presented in Table V. The hydrolysis remained unchanged in starches treated at 75°C, but heat treatment at 100°C increased the susceptibility of both starches to acidic degradation.

Enzyme hydrolysis.—The extent of hydrolysis (after 72 h) of native and heat–moisture treated starches (30% moisture) by porcine pancreatic alpha amylase are presented in Table VI. Among native starches the extent of hydrolysis followed the

TABLE V

Hydrolysis of native, and heat–moisture-treated potato and yam starches

Starch source	Treatment	Hydrolysis [b,c] (%)
Potato	Native	5.8
	[a]HMT at 75°C	5.8
	[a]HMT at 100°C	8.6
Yam	Native	4.3
	[a]HMT at 75°C	4.3
	[a]HMT at 100°C	5.7

[a] Heat–moisture treated at 30% moisture for 16 h. [b] After 2 days in 2.2 M HCl at 35°C. [c] Average standard deviation = 0.5 (n = 3).

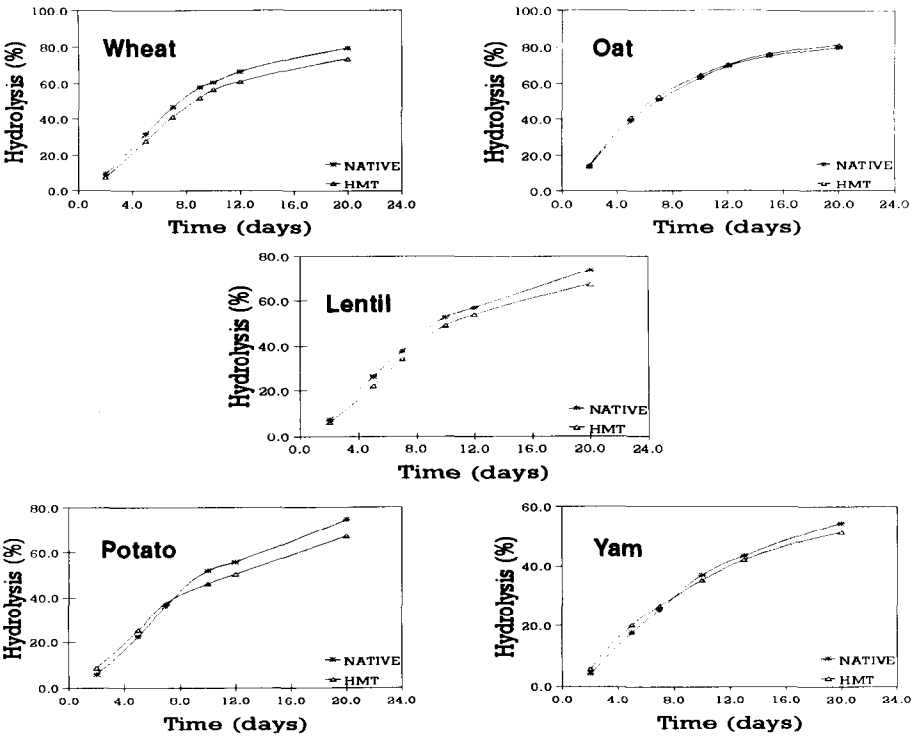

Fig. 7. Acid hydrolysis of native, and heat–moisture-treated (16 h, 100°C, 30% moisture) starches.

order: wheat ~ lentil > oat > potato > yam. The hydrolysis of wheat and lentil starches decreased by 2.1 and 6.5%, respectively on heat–moisture treatment (Table VI). However, heat–moisture treatment increased the susceptibility of oat (56%), potato (300%), and yam (320%) starches towards alpha amylase.

TABLE VI

Hydrolysis of native, and heat–moisture-treated starches by porcine pancreatic alpha amylase

Starch source	Treatment	Hydrolysis [a,b] (%)
Wheat	Native	66.1
	HMT [c]	65.0
Oat	Native	32.0
	HMT [c]	50.0
Lentil	Native	65.0
	HMT [c]	59.0
Potato	Native	5.0
	HMT [c]	21.0
Yam	Native	1.5
	HMT [c]	6.0

[a] Hydrolysis for 72 h. [b] Average standard deviation = 0.75 ($n = 3$). [c] Heat–moisture treated for 16 h at 100°C at 30% moisture.

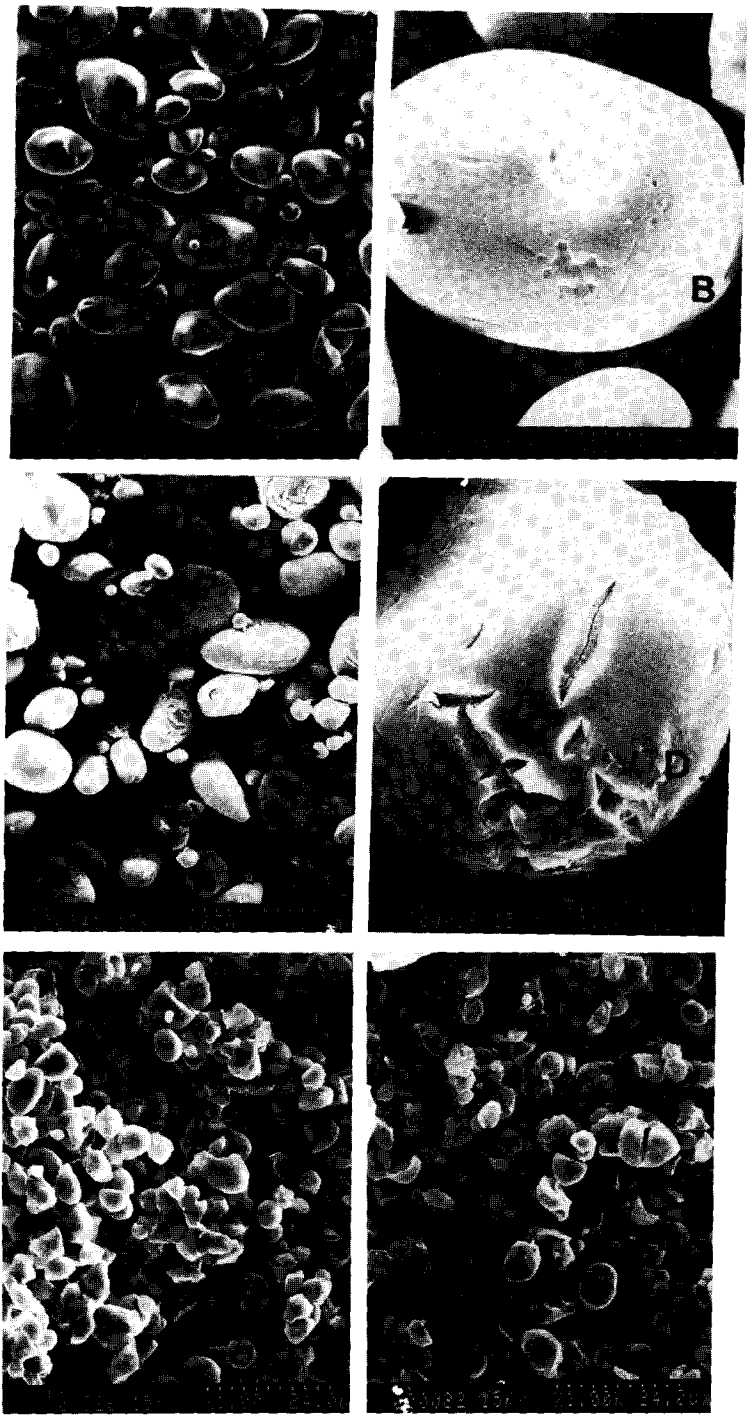

Fig. 8. Scanning electron micrographs of native, and heat–moisture treated (16 h, 100°C, 30% moisture) starches after attack (72 h) by porcine pancreatic alpha amylase: (A) and (B) native potato starch, (C) and (D) heat–moisture-treated potato starch, (E) native oat starch, (F) heat–moisture-treated oat starch.

Our results on wheat and potato starches are not in agreement with those of Lorenz and Kulp[6] who reported that the extent of hydrolysis (in 24 h) of wheat and potato starches by a commercial fungal alpha amylase increased respectively, by 110 and 45% on heat–moisture treatment (16 h, 100°C, 27% moisture). This discrepancy may have been due to differences in enzyme source or purity. The very high degree of susceptibility of potato starch towards alpha amylase after heat–moisture treatment, has also been shown by Kuge and Kitamura[11]. These authors showed that the extent of hydrolysis of potato starch by alpha amylase from *Bacillius subtilis* increased on heat–moisture treatment (1 h, 120°C, 27.3% moisture) from 3% (in 24 h) to 80% (in 6 h), whereas, for corn starch the corresponding increase (in 24 h) was only 11.1%.

Scanning electron micrographs of native and heat–moisture-treated granules of potato starch after attack by alpha amylase (72 h) are presented in Figs. 8A and B and Figs. 8C and D, respectively, while those of native and heat–moisture-treated oat starch granules are presented in Figs. 8E and F respectively. The attack of alpha amylase on native potato starch (Figs. 8A and B) manifested itself in only superficial erosion of the granules. Morphological changes were not discernible, and the result is in agreement with low amylolysis rate (Table IV). However, during the same time period, granules of heat–moisture-treated potato starch were more extensively attacked by alpha amylase (Figs. 8C and D) than those of native starch (Figs. 8A and B). Many of these granules showed deep cracks (Figs. 8C and D). Granules of oat starch were also more degraded after heat–moisture treatment (Fig. 8F). However, unlike potato starch, the mode of attack of alpha amylase on native (Fig. 8E) and heat–moisture-treated oat starch granules were identical.

DISCUSSION

Imberty et al.[21] have shown that in crystallites of both 'A' and 'B' starches double helices are found in pairs and all chains are packed in parallel arrays. The pairing of double helices is the same in both polymorphs and corresponds to the interaction between double helices that have the lowest energy. Starches exhibiting 'A' and 'B' X-ray diffraction patterns differ in their water content and the manner in which the pairs of double helices are packed within their respective crystals. In 'B' starches there are 36 water molecules present in a channel in the center of a hexagonal arrangement of six double helices, while in A starches there are only four water molecules between double helices[21]. Furthermore, the center of 'A' starches is occupied by an amylosic helix rather than a column of water. It has been suggested that adjacent double helices within crystallites of 'A' starches are mainly linked by direct hydrogen-bonding[21,22]. However, in crystallites of 'B' starches, adjacent double helices are mainly linked by hydrate water bridges and to a limited extent by direct hydrogen-bonding[22]. French[23] has suggested that long-range molecular order (crystallinity) in starch granules is the result of regular

packing of double helices formed from adjacent clusters of the short-DP chains of amylopectin.

The absence of new *d*-spacings and the slight shifting of the existing *d*-spacings on heat treatment of wheat, oat, lentil, potato, and yam starches (Table II), suggests that the increases in X-ray intensities (Table II) are largely due to structural changes within the crystalline domains of the granule. This seems plausible, since the increase in *d*-spacing intensities on heat–moisture treatment of waxy corn starch (99% amylopectin) is greater than in native corn (73% amylopectin) starch (unpublished results). The increase in X-ray intensities with increase in moisture content on heat treatment of wheat starch (Fig. 2, Table II), suggests that thermal energy and moisture may have caused double helices to shift within the crystallites and to assume a crystalline array that is more closely packed and ordered (due to an increase in the number of direct hydrogen-bonds linking adjacent helices) than that in native starch. The decrease in X-ray intensities on heat treatment of potato and yam starches (Fig. 2, Table II), suggests a loss of crystalline order. This probably occurs due to rupture of the hydrate water bridges linking adjacent double helices. Although direct hydrogen-bonds between adjacent helices may have remained intact, the overall decrease in magnitude of the bonding forces between adjacent helices would cause them to move apart and assume orientations that are not in perfect parallel crystalline array. As a result, diffraction of X-rays would be less intense after heat treatment. The results for potato starch (Table II), show that moisture level during heat treatment influences the extent of decrease in X-ray intensities. This could be attributed to greater chain flexibility (increases with increase in moisture content) of the loosely packed double helices of heat–moisture-treated potato starch. An increase in chain flexibility would hinder parallel alignment of the double helices.

The transformation of the X-ray pattern of potato and yam starches from 'B' to 'A' + 'B' (Fig. 2) is probably initiated by rupture of the hydrate water bridges, which enable helices to rearrange themselves into a crystalline array that contains an amylosic helix in the central channel of the unit cell. The gradual reduction in intensity of the *d*-spacing at 16.8 Å in potato and yam starches (Table II) with increase in moisture content (this peak disappears at 30% moisture in potato starch) and the disappearance (at 20% moisture) of the doublet centered at 4.0 Å and 3.7 Å in potato and yam starches (Fig. 2), clearly demonstrates that the extent of rearrangement of the helices is influenced by the moisture level during heat treatment.

Gernat et al.[24] have shown that the legume starch 'C' crystalline polymorph is a mixture of 'A' and 'B' unit cells, and that these starches contain pure 'A' and 'B' polymorphs in varying proportions. The X-ray pattern of native lentil starch did not exhibit any spacings that were characteristic of the 'B' pattern. This meant that native lentil starch contained mainly 'A' unit cells. This would then explain the similar responses (increase in X-ray intensities) shown by lentil and cereal starches on heat treatment. The X-ray patterns of wheat, oat and lentil starches are not

altered (Fig. 2) after heat treatment, since the direct hydrogen-bonds linking adjacent double helices within the crystalline domains of these starches are resistant to disruption at the moisture level (30%) prevailing during heat treatment. Consequently, only limited helical rearrangement is possible, and this is probably confined only to those helices that are linked via hydrate water bridges (fewer than in tuber starches). It is, therefore, likely that it is the movement and interaction (via direct hydrogen-bonds) of these helices within the unit cell that is responsible for increased X-ray intensities on heat treatment of wheat, oat, and lentil starches.

Starch lipids are known to form amylose-inclusion complexes in which the ligand resides within the central hydrophobic core of the helix[25,26]. A 'V'-type X-ray pattern is seen when lipid-containing starches are subjected to extrusion cooking[27] and after addition of monoacyl lipids to starch under appropriate conditions[28,29]. Native (untreated) starches do not exhibit a 'V'-type X-ray pattern[30]. This means that either the complexes do not exist in native starch and are formed only on heating or more probably that they do exist but only in partially helical or with insufficient degree of long-range order to generate the necessary X-ray diffraction pattern[30,31]. The decrease in apparent amylose content (Table III) in wheat, oat, and lentil starches indicates, a change in amylose chain conformation (random coil → partial helix) on heat–moisture treatment. This probably, enables lipids which were unbound within the native granule (Table I) to readily form 'V' amylose helices. The results indicate a close relationship between the amount of unbound lipids in the native granules (Table I) and the magnitude of the decrease in apparent amylose content (Table III). The apparent amylose content of potato and yam starches remain unchanged on heat–moisture treatment, due to their low content of unbound lipids (Table I).

Starch granule swelling is known to begin in the bulk, relatively mobile amorphous fraction, and in the more restrained amorphous regions immediately adjacent to the crystalline regions. Tester and Morrison[18] have shown by comparative studies on normal and waxy barley starches, that swelling is primarily a property of amylopectin and that amylose is a diluent. These authors also showed that amylose and lipids in normal starches could also inhibit granule swelling under conditions where amylose–lipid complexes are likely to be formed.

The results from our study suggest that the extent of decrease in swelling factor on heat–moisture treatment of the various starches could be attributed to an interplay of three factors: (*1*) Changes in the packing arrangement of the starch crystallites (Table II, Fig. 2); (*2*) Interaction between or among starch components in the amorphous regions of the granule (Figs. 3B and D) and (*3*) Amylose–lipid interactions (Table III). Decreases in amylose leaching (Figs. 3B and D) could be attributed to an interplay of factors 2 and 3.

In wheat, oat, and lentil starches, the increase in T_o, T_p, and T_c on heat–moisture treatment (Table IV) reflects a decrease in the destabilization effect of the amorphous regions on the melting of starch crystallites during gelatinization. This

is probably due to interactions between amylose chains (within the bulk amorphous regions) and/or between amylose chains and the branched segments (within the intercrystalline regions) of amylopectin, rather than to any increase in crystalline stability. This seems plausible, since T_o, T_p, and T_c of waxy maize starch are unaltered after heat–moisture treatment under similar conditions (unpublished results).

As seen from X-ray diffraction studies (Table II), the crystallites of potato starch are disrupted on heat–moisture treatment. Consequently, if changes in crystalline stability were a factor influencing increases in thermal transition temperatures during heat–moisture treatment, then T_o, T_p, and T_c of heat–moisture-treated potato starch should have been less than that of its native counterpart. The results on potato starch (Table IV), therefore, suggests that, as in wheat, oat, and lentil starches, the increase in T_o, T_p, and T_c on heat–moisture treatment reflects starch interactions within amorphous regions of the granule. The foregoing increases are higher in potato owing to its longer amylose chain length[38]. As may be seen in Table II, crystallite disruption also occurs on heat–moisture treatment of yam starch. Furthermore, the amylose chain-lengths of yam and potato starches are fairly similar. Therefore, the marginal increases in T_o, T_p, and T_c on heat–moisture treatment was rather surprising (Table IV). It is probable that starch chains in the amorphous regions of native yam starch granules may be highly associated [this is reflected in high values for T_o, T_p, and T_c (Table IV)], and as a result only minimal interactions are possible during heat–moisture treatment.

The changes in transition temperatures with time (Fig. 4) indicate that, in all starches, the interactions within the amorphous domains of the granules appears to occur entirely within the first 5 h of heat–moisture treatment. Cooke and Gidley[33] have shown by DSC, ^{13}C CP/MAS NMR spectroscopy, and powder X-ray diffraction studies that ΔH values of gelatinization primarily reflect the loss of double-helical order rather than loss of crystalline register. The lack of influence of heat–moisture treatment on ΔH of wheat, oat and lentil starches (Table IV), therefore, suggest that double helices do not disrupt (do not unravel) under the conditions prevailing during heat–moisture treatment. This suggests that identical amounts of double helices unravel and melt during gelatinization of native and heat–moisture-treated wheat, oat, and lentil starches. These findings prove conclusively that molecular (helical) order and not crystalline order is the primary determinant of the endothermic enthalpy of gelatinization. If crystalline order had influenced ΔH, the ΔH values of wheat, oat, and lentil starches should have then increased [because of increase in crystalline order (Table II)] on heat–moisture treatment. The decrease in ΔH values on heat–moisture treatment of potato and yam starches (Table IV), suggest that some of the original double helices may have disrupted during the polymorphic transformation ('B' to 'A'+ 'B').

The progressive decrease in ΔH with increase in moisture content (Table IV) indicates that the extent of double-helical disruption in potato and yam starches is influenced by double-helical chain motions (which increases with increase in

moisture content). The changes in ΔH with time of heat–moisture treatment (Fig. 5) shows that the movement and reorientation of double helices of potato and yam starches takes nearly 16 h for completion.

The increase in the Brabender 95°C viscosity on heat–moisture treatment of wheat starch (Fig. 6), could be attributed to an increase in granular rigidity resulting from an increase in crystalline order (Table II) and starch chain interactions within the amorphous regions (Figs. 3A and B, Table IV).

Although similar changes [somewhat reduced in magnitude (Figs. 3A and B, Table IV)] also occurs within the amorphous and crystalline regions during heat–moisture treatment of oat starch, the granules of native oat starch are better able to withstand the shear effects (higher 95°C viscosity) than those of their heat–moisture-treated counterparts (Fig. 6). This could be due to oat granules being less compactly packed (increases shear sensitivity) after heat–moisture treatment (Figs. 1A and B). These results suggest that the change in granule packing overrides the influence of increased granular rigidity on viscosity. The decrease in 95°C viscosity on heat–moisture treatment of lentil starch (Fig. 6) could be attributed to a very high decrease in the volume occupied by the swollen granules in the continuous phase (Fig. 3A). This is not surprising, since the magnitude of the changes within the amorphous (Figs. 3A and B, Table IV) and crystalline regions (Table II) during heat–moisture treatment of lentil starch is much higher than in wheat and oat starches (Figs. 3A and B, Table IV). The decrease in volume fraction apparently negates the influence of increased granular rigidity on viscosity. In potato and yam starches, the decrease in 95°C viscosity on heat–moisture treatment (Fig. 6) is to a large extent due to crystallite destruction (Table II) which decreases both granular rigidity and the volume fraction occupied by the swollen granules.

It has generally been accepted[34–39] that heterogenous acid hydrolysis preferentially attacks the more amorphous regions of the granule, whether they be at the surface or interior. In contrast, crystalline regions are less accessible to hydrated protons (H_3O^+) and are attacked only after a period of 10–12 days. The decrease in acid (Fig. 7) and enzyme hydrolysis (Table VI) after heat–moisture treatment, suggests that the structural changes within the amorphous and crystalline regions of the granule on heat–moisture treatment may have rendered these regions less accessible to penetration by H_3O^+ and alpha amylase. These decreases are higher in lentil than in wheat starch (Fig. 7, Table VI) because of stronger starch-chain associations within the amorphous regions of the former (Fig. 3B, Table IV). This seems plausible, since in both starches the extent of increase in crystalline order on heat–moisture treatment were nearly of the same order of magnitude (Table II). The susceptibility of oat starch to attack by H_3O^+ or alpha amylase increases on high-moisture treatment (in spite of starch-chain interactions within amorphous regions) due to a greater degree of contact of the individual granular surfaces with H_3O^+ or alpha amylase (Figs. 1A and B). Crystallite disruption in potato and yam starches during heat–moisture treatment may have been the causative factor

responsible for differences in the extent of acid hydrolysis between native and heat–moisture treated starches (heat–moisture treated > native) during the first 7 days of hydrolysis (Fig. 7). It is plausible that the initial attack of H_3O^+ may have been on the disrupted crystallites present at or near the granular surface. The reversal (native > heat–moisture treated) in the trend of acid hydrolysis after the 7th day (Fig. 7), suggests that the action of H_3O^+ is now confined mainly to the amorphous regions in the granule interior (which are more accessible to hydrolysis in native than in heat–moisture-treated starches).

The results in Table VI shows that crystallite disruption is influenced by the amount of thermal energy imparted to the starch crystallites during heat–moisture treatment.

The four-fold increase in enzyme hydrolysis on heat–moisture treatment of potato and yam starches (Table V) clearly demonstrates that, as in acid hydrolysis, the initial action of alpha amylase could have been on the disrupted starch crystallites near the granule surface. Therefore, it is plausible that if the reaction between alpha amylase and the foregoing starches was monitored over time periods > 72 h, a stage may have been reached when the extent of enzyme hydrolysis would have been greater in native than in heat–moisture-treated starches.

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