

Amylopectin Nature and Amylose-to-Amylopectin Ratio as Influences on the Behavior of Gels of Dispersed Starch

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ABSTRACT

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The importance of the nature of the amylopectin and the amylose-to-amylopectin ratio in the gelation of high-amylose maize starch was examined by preparing gels of model systems using amylose (AM) from a high-amylose maize starch mixed with an amylopectin (AP): either *wx* starch, acid-hydrolyzed *wx* starch (AH-*wx*), *ae wx* starch, or *wx* β -limit dextrin. Mixtures of 7.5% starch in 20% dimethyl sulfoxide (DMSO) were examined by differential scanning calorimetry and dynamic oscillatory rheometry. Mixtures of 1.88 or 3.75% AM with the remainder either *wx* or AH-*wx* developed a measurable elastic modulus (G') within one day, more quickly than for the corresponding AP without AM. Gels of AM with either *wx* or AH-*wx* starch developed a higher retrogradation enthalpy (ΔH) than corresponding AP without AM when the ΔH values were normalized to the amylopectin content. The G' and ΔH of all gels

containing *ae wx* did not change after one day. During heating of the gels to 80°C, most of the G' at 25°C was lost, indicating that the initial gel structure was not due to a thermally stable AM network. Gelation is proposed to be due to physical junction zones (PJZ) between AM molecules (AM-AM), between AM and amylopectin molecules (AM-AP), and between amylopectin molecules (AP-AP). For *wx*, AH-*wx*, or *ae wx* starch, the higher G' and ΔH of the gels with 3.75% AM compared with the gels with 1.88% AM suggests that AM-AP PJZ are more important in gel formation when AM makes up half the starch. Gels from the mixture of 3.75% AM and 3.75% *ae wx* starch behaved most similarly to gels of 7.5% high-amylose maize starch. The development of a starch gel is affected by both the nature of the amylopectin as well as the amylose-to-amylopectin ratio of the gel.

For nongranular dispersed starch, the contributions of amylose and amylopectin to the viscoelastic properties of mixtures remain unclear. Some studies (Kalichevsky and Ring 1987; Russell 1987; Leloup et al 1991; German et al 1992; Case et al 1998) considered gels composed of nongranular mixtures of amylose and amylopectin to consist of amylopectin-rich and amylose-rich phases. Kalichevsky and Ring (1987) observed phase separation within 1 min at 80°C for aqueous mixtures of equal volumes of amylose (3%) and amylopectin (3%). German et al (1992) observed that an aqueous mixture of 1.5% amylose and 1.9% amylopectin were phase separated at 85°C after 48 hr.

Some studies of gelled mixtures of amylose and amylopectin have used the idea of phase separation of amylose and amylopectin (Kalichevsky and Ring 1987) to explain the effects of increasing the amylose content on gel characteristics (Leloup et al 1991; Doublier and Llamas 1993). Below an amylose-to-amylopectin weight ratio of 17:83, gels containing 4% starch stored for 8 hr at 25°C had a low elastic modulus (G') (Doublier and Llamas 1993). For amylose-to-amylopectin weight ratios between 17:83 and 27:73, the G' of gels increased dramatically (Doublier and Llamas 1993). These authors suggested that the change in these characteristics from amylopectin-like to amylose-like resulted from a phase inversion, with the amylose-rich phase becoming the continuous phase. In a study by Leloup et al (1991), at an amylose-to-amylopectin weight ratio above 30:70, the G' of 8% starch gels retained some network structure after heating in boiling water. Above a weight ratio of 30:70, the susceptibility of the gels to enzymatic and acid hydrolysis became more like that of amylose gels. Leloup et al (1991) suggested that the change in these characteristics from amylopectin-like to amylose-like resulted from the amylose-rich phase becoming the continuous phase at a ratio of 30:70 and above. The difference in the phase inversion ratios suggested by Doublier and Llamas (1993) and Leloup et al (1991) might be attributed to the differing sensitivity of the tests conducted by each or by the difference in starch concentration examined by each. Although phase separation of amylose and amylopectin at 80°C (Kalichevsky and Ring 1987) has been invoked to explain the rheological properties of gels of

mixtures of amylose and amylopectin, the conditions under which a phase separation might occur are not well established. For high-amylose maize starch gels prepared by heating at 121–166°C, Case et al (1998) attributed the lower stress and strain at fracture for gels prepared at the higher temperatures to a decreased ability for amylose and amylopectin to phase separate during cooling of the gelatinized pastes.

Others (Jane and Chen 1992; Parovuori et al 1997; Boltz and Thompson 1999; Klucinec and Thompson 1999) have suggested that interactions between amylose and amylopectin could contribute to the gel properties of their mixtures. Jane and Chen (1992) observed that for 8% aqueous starch dispersions consisting of 20:80 (amylose-to-amylopectin) mixtures of amylose from either potato, normal maize, or *ae* maize starch with amylopectin from either *ae* maize, *wx* maize, or normal rice starch, the gel with the highest resistance to uniaxial compression was formed from the mixture containing amylopectin and amylose from the *ae* starch. Mixtures of any of the amyloses with the *wx* maize or rice amylopectin either did not gel or formed very weak gels during storage (Jane and Chen 1992). Parovuori et al (1997) examined 25:75 or 50:50 (amylose-to-branched molecules) mixtures of amylose with *wx* maize starch or with small, medium, or large dextrans prepared from *wx* maize starch (M_r 0.82 \times 10⁶, 8.4 \times 10⁶, and 18 \times 10⁶, respectively). Gels (8%, w/w) prepared with the intermediate size dextrans had a lower G' than gels prepared with the other dextrans or the *wx* maize starch. Parovuori et al (1997) attributed the G' differences observed with the different branched molecules to variation in the concentration of the amylose due to a difference in the effective excluded volume of the branched molecules, or to differences in the purported ability for the amylose and branched molecules to phase separate, which they considered to be diminished with the small branched molecules. Boltz and Thompson (1999) observed that when high-amylose maize starches (30% w/w starch in water) were initially heated to <140°C, endotherms >140°C, indicative of amylose retrogradation, were observed. When Boltz and Thompson (1999) heated high-amylose maize starches to >160°C, endotherms >140°C were not observed during reheating, suggesting that the relatively independent organization of amylose observed when starches were initially heated to <140°C did not occur when starches were initially heated to >160°C. Klucinec and Thompson (1999) prepared gels from high-amylose maize starches by dilution of dimethyl sulfoxide (DMSO) dispersed starch. Small-angle dynamic oscillatory rheometry of the high-amylose starch gels showed that 80–95% of the G' of the gels was lost on heating from 5 to 70°C. This loss

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was greater than the roughly 50% loss of G' over the same temperature range for gels prepared from the amylose fractionated from these starches. Klucinec and Thompson (1999) attributed the large decrease in the G' of the native high-amylose starch gels to the loss of double-helical interactions between the amylose and the external chains of the amylopectin molecules. They suggested that the dissociation of these mixed helices occurred at temperatures similar to the dissociation of amylopectin double helices because the length, and therefore the thermal stability, of the mixed double helices would be dictated by the length of a participating amylopectin external chain (Klucinec and Thompson 1999). They suggested that interactions between amylose and amylopectin are important in the development of a gel prepared from high-amylose maize starch, and they speculated that these interactions form quickly during the cooling of the gelatinized starch.

Flory (1953) has argued that elastic networks of vulcanized rubber may be described as a system of covalently linked linear polymer chains (Fig. 1a). A direct application of this model to starch gels is complicated by 1) the multiple branching of amylopectin, 2) the two-component nature of starch gels composed of amylose and amylopectin, and 3) the dependence on the formation of physical rather than chemical cross-links for starch gelation to occur.

The purpose of the present study was to understand how the nature of the amylopectin and the amylose-to-amylopectin ratio contribute to the rheological characteristics of gels of dispersed high-amylose maize starches. The gelation of fractionated amylose or amylopectin alone, of mixtures of amylose and amylopectin to simulate the proportion in high-amylose maize starch, and of dispersed normal and high-amylose maize starches were investigated using small deformation oscillatory rheometry and differential scanning calorimetry.

MATERIALS AND METHODS

Native Starches

A commercial amylose-extender starch (*aeVII*, Hylon VII), a commercial waxy maize starch (*wx*, Amioca), and a commercial normal maize starch (*n*, Melojel) were gifts from National Starch and Chemical Co. (Bridgewater, NJ). An amylose-extender waxy

maize starch (*ae wx*) was a gift from Cerestar USA, Inc. (Hammond, IN). A commercial maltodextrin with a reported dextrose equivalent (DE) of 1 prepared from *wx* maize starch (Star-Dri 1) was a gift from A.E. Staley Mfg. Co. (Decatur, IL). The *n* and *aeVII* starches had been previously determined to contain $23.1 \pm 1.4\%$ and $48.9 \pm 1.8\%$ amylose, respectively, by recovery of the 1-butanol complex (Klucinec and Thompson 1998).

Amylose and Amylopectin

Nongranular starches (Banks and Greenwood 1975) free of native lipid were prepared as described by Klucinec and Thompson (1998). The amylose from *aeVII* starch (AM) and the amylopectin from normal maize starch (*n* AP) were fractionated from the nongranular starches (Klucinec and Thompson 1998). The *n* AP, *ae wx* maize, and *wx* maize (either modified or not by acid hydrolysis or by β -amylolysis) will be collectively referred to as amylopectin (AP).

Acid-hydrolyzed *wx* maize starch was prepared using the method of Robyt et al (1996). Granules of *wx* starch (75 g) were suspended in 300 mL of methanol and 3.6 mL of concentrated HCl (12.1M) was added with constant stirring. The suspension was stirred continuously for 36 hr before the starch granules were recovered by vacuum filtration. This acid-hydrolyzed *wx* starch was fractionated to eliminate low molecular weight components. The acid-hydrolyzed *wx* starch granules (65 g) were dispersed in 1.3 L of 90% DMSO by heating in a boiling water bath for 3 hr. An equal volume of 95% ethanol was slowly added while constantly stirring the dispersed starch. The starch was permitted to sediment for four days at room temperature, then the clear upper layer of the mixture was discarded. The sediment was dispersed in 400 mL of 90% DMSO and precipitated and dried using the same methods as for the preparation of nongranular starches. This fractionation

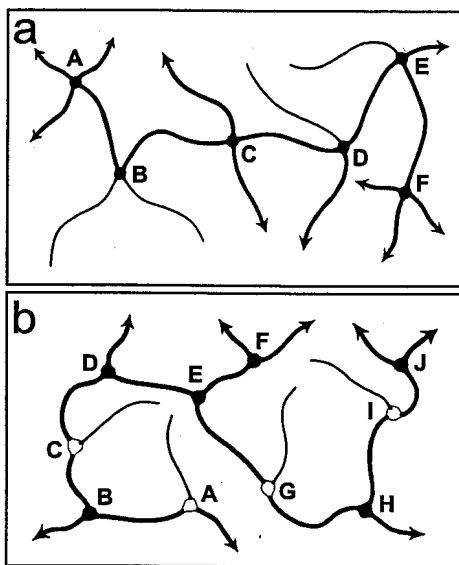


Fig. 1. a, Model network structures (Flory 1953). Cross-linkages (●), internal chains (darker lines), and terminal chains (lighter lines). Arrows indicate continuation of the network toward unshown cross-linkages. b, Modified Flory-type model for general application to starch gel networks. Internal elements (darker lines), terminal elements (lighter lines), network linkages (●) joining three internal elements, and terminal linkages (○) joining a terminal element to an internal element.

TABLE I
Composition of Mixtures of Amylose (AM) and Branched Molecules

Branched Molecule	1.88% AM	3.75% AM
<i>wx</i> Maize	5.63	3.75
<i>wx</i> Maize β -limit dextrin	2.48	1.65
Acid-hydrolyzed (AH) <i>wx</i> maize	5.63	3.75
<i>ae wx</i> Maize	5.63	3.75
None (<i>aeVII</i> amylose control)	0	0

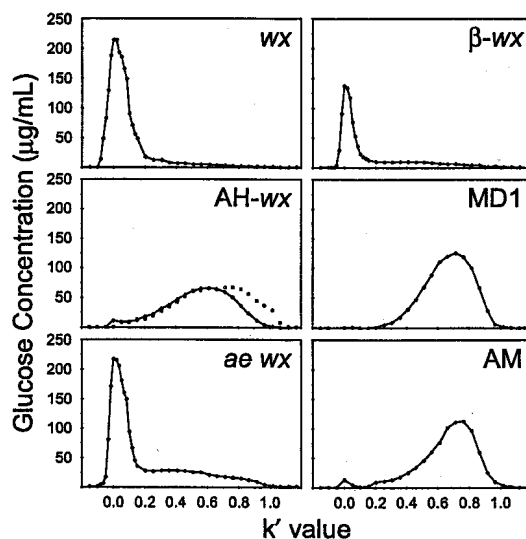


Fig. 2. Size-exclusion chromatograms of starch mixture molecules from *wx* maize starch (*wx*), *wx* maize β -limit dextrin (*wx*- β), acid-hydrolyzed high molecular weight *wx* maize starch (AH-*wx*), maltodextrin (MD1), *ae wx* maize starch (*ae wx*), *aeVII* amylose (AM). Chromatogram of sample before fractionation for AH-*wx* starch (---) is also shown. k' = capacity factor.

procedure eliminated nearly all of the material that eluted at a capacity factor (k') of 0.9–1.0 in preliminary Sepharose CL-2B chromatography as described by Klucinec and Thompson (1998).

The preparation of wx maize β -limit dextrin was initiated by dispersing ≈ 6 g (± 0.1) of wx starch in 200 mL of 90% DMSO for 3 hr with constant stirring in a boiling water bath. The dispersed starch was then precipitated with ethanol (95%, 800 mL) and recovered by centrifugation at $3,500 \times g$ for 10 min at 4°C . Immediately after centrifugation, 450 mL of sodium acetate buffer (0.02M, pH 6.0) at 80°C was added to the precipitate. The mixture was placed in a boiling water bath with constant stirring for 1 hr and then placed in a water bath at 50°C . When the mixture reached 50°C , 5,000 units of barley β -amylase (Megazyme International Ireland, Ltd., County Wicklow, Ireland) in 20 mL of sodium acetate buffer (0.02M, pH 6.0) were added. This mixture was held at 50°C for two days, then the β -amylolysis limit was determined from the concentration of total carbohydrate (Dubois et al 1956) and reducing ends (Robyt and Whelan 1968). The β -amylolysis was considered complete because preliminary experiments with a second β -amylase digestion

or longer incubation times did not increase the reducing capacity of the digest. The solution of β -limit dextrin and maltose was subsequently concentrated to 200 mL by boiling and then precipitated and dried using the same methods as for the preparation of non-granular starches. Four 6-g samples of wx starch were digested simultaneously; another four 6-g samples were subjected to the same treatment. The wx β -limit dextrin recovered from the eight digestions was combined.

The AM (15 mg) was applied to a column of Sepharose CL-2B media. The AM was debranched and analyzed by high-performance size-exclusion chromatography (HPSEC). Both procedures were conducted according to Klucinec and Thompson (1998).

Nongranular wx , AH- wx , and ae wx starches (15 mg) were applied to a column of Sepharose CL-2B media as described by Klucinec and Thompson (1998). For the wx β -limit dextrin, 7.5 mg was loaded onto the column providing an approximately equal number of moles of wx β -limit dextrin relative to the wx starch. The wx , AH- wx , and ae wx starches and the wx β -limit dextrin were debranched and analyzed by HPSEC.

TABLE II
Elastic Modulus (G') of Gels Containing Amylopectin (AP) in 20% DMSO

Sample ^c	Days	AM (%)	Measurements	Elastic Modulus (G') ^{a,b}	
				25°C	80°C
wx Maize	1	0	3	nd ^d	nd
		1.88	3	150 \pm 31c,BC	30 \pm 27a,CD
		3.75	3	1,300 \pm 418b,C	420 \pm 51a,D
	3	0	3	nd	nd
		1.88	3	1,100 \pm 124b,B	50 \pm 39a,BC
		3.75	4	1,200 \pm 194b,D	330 \pm 66a,D
	7	0	3	160 \pm 23B	5 \pm 3B
		1.88	3	1,500 \pm 211a,B	20 \pm 10a,BC
		3.75	3	2,800 \pm 646a,C	350 \pm 78a,D
wx Maize β -limit dextrin	1	0	3	nd	nd
		1.88	3	14 \pm 4a,C	5 \pm 2a,D
		3.75	3	800 \pm 123a,C	590 \pm 14b,C
	3	0	3	nd	nd
		1.88	3	15 \pm 5a,E	4 \pm 2a,D
		3.75	3	800 \pm 147a,D	420 \pm 60a,D
	7	0	3	nd	nd
		1.88	3	15 \pm 3a,D	10 \pm 12a,C
		3.75	3	1,000 \pm 247a,D	460 \pm 74a,CD
Acid-hydrolyzed (AH) wx maize	1	0	3	na ^e	na
		1.88	3	200 \pm 200b,B	57 \pm 8a,BC
		3.75	5	4,700 \pm 811b,A	870 \pm 79a,B
	3	0	3	na	na
		1.88	3	430 \pm 36b,D	18 \pm 4a,D
		3.75	3	5,900 \pm 343a,A	840 \pm 46a,B
	7	0	3	na	na
		1.88	3	800 \pm 138a,C	60 \pm 45a,B
		3.75	3	6,800 \pm 493a,AB	850 \pm 36a,B
ae wx Maize	1	0	3	8,900 \pm 978a	120 \pm 28a
		1.88	3	800 \pm 107a,A	70 \pm 10a,AB
		3.75	3	5,000 \pm 512a,A	700 \pm 116a,C
	3	0	3	10,000 \pm 1099a	133 \pm 19a
		1.88	3	770 \pm 14a,C	73 \pm 2a,B
		3.75	3	5,200 \pm 304a,B	570 \pm 21a,C
	7	0	3	9,600 \pm 355a,A	120 \pm 24a,A
		1.88	3	870 \pm 86a,C	70 \pm 12a,B
		3.75	4	5,900 \pm 710a,B	550 \pm 88a,C
AM (ae VII amylose)	1	1.88	3	50 \pm 35a,BC	30 \pm 24a,D
		3.75	3	1,560 \pm 41a,BC	704 \pm 9a,C
	3	1.88	3	21 \pm 6a,E	8 \pm 2a,D
		3.75	3	1,300 \pm 221a,D	560 \pm 93a,C
	7	1.88	3	23 \pm 5a,D	7 \pm 4a,C
		3.75	4	1,400 \pm 547a,D	500 \pm 174a,CD

^a Elastic modulus (G' , Pa) mean \pm standard deviation.

^b Within each sample, values with the same AM content followed by the same lowercase letter in the same column are not significantly different ($P < 0.05$). For each storage time across all samples with the same AM content, values followed by the same uppercase letter in the same column are not significantly different ($P < 0.05$). Uppercase letters correspond to letters in Table III.

^c Starch content (%) of wx - β samples: $(AM\%) + [7.5\% - (AM\%)] \times 0.44$, where $0.44 = 1 - (\beta\text{-amylolysis limit})$. All other samples contain 7.5% starch.

^d Not determined. Sample could not be transferred intact for rheological analysis.

^e Not applicable. Samples precipitated during storage at 4°C .

Mixtures of AM and AP

The moisture content of the granular and nongranular starch materials was determined in duplicate by air drying 0.5 g (± 0.0001) at 130°C in a convection oven for 2 hr. Mixtures of AM with either *wx*, *ae wx*, or AH-*wx* starch were prepared so that the AM content was either 25% (w/w) or 50% (w/w) of the total starch on a dry weight basis. Mixtures of AM with the *wx* β -limit dextrin were prepared with an amount of *wx* β -limit dextrin equimolar (based on the β -amylolysis limit) to the amount of *wx* starch required for AM content of 25% (w/w) or 50% (w/w) on a dry weight basis. Consequently, the number of branch points was also equimolar to the *wx* starch. AM was mixed with either the *wx* or *ae wx* maize starch by adding AM to the granular *wx*-type starch. The combined material was then dispersed in 90% DMSO, precipitated, and dried using the same methods as for the preparation of nongranular starches. The ratios of AM to AP were chosen for comparison to *n* and *aeVII* starch, which had contained $\approx 23\%$ and $\approx 49\%$ amylose, respectively (Klucinec and Thompson 1998).

Preparation and Examination of Starch Gels

The moisture content of the nongranular starches and the mixtures of AM and AP was determined as described above for the mixture components. Dispersions containing AM, AP, or starch mixtures in 20% DMSO in water (w/w) were prepared by dilution of 90% DMSO dispersions of starch according to the method of Klucinec and Thompson (1999). All dispersions contained 7.5% starch after dilution with water except for those dispersions containing β -limit dextrin which had lower carbohydrate contents to maintain final amylose contents of 1.88 or 3.75%. The composition of each mixture is presented in Table I. The dispersions in 20% DMSO were poured into a casting apparatus (Klucinec and Thompson 1999) and stored at 4°C before rheological examination. In addition, aliquots (30–50 mg) of the dispersions were deposited into 60- μ L stainless steel pans for examination by DSC.

The small-deformation rheological behavior of the prepared starch systems was examined using a controlled strain dynamic oscillatory rheometer (RFS II, Rheometric Scientific, Piscataway, NJ) following the method of Klucinec and Thompson (1999) with slight modification. After storage at 4°C for one, three, or seven days, the elastic modulus (G') of the gels was measured every 15 sec at a strain of 0.2% and a frequency of 1 rad/sec while the gels were heated from 25 to 80°C at 2°C/min. The strain and frequency conditions were within the linear viscoelastic region of every gel at 25°C. Those systems reported were considered true gels at 25°C because, under these conditions, the G' remained above the G'' of the systems. At

least two independently prepared samples of each starch were examined for each storage time. Data were analyzed for significance ($P < 0.05$) using Fisher's least significant difference procedure (v. 10.51 Xtra, Minitab, Inc.). Although the manufacturer reports a nominal instrument sensitivity limit of ≈ 50 Pa for these conditions, we obtained reproducible results below this value.

The enthalpy (ΔH) of starch endotherms of the DMSO-containing systems was determined using differential scanning calorimetry (DSC-7, Perkin-Elmer Corp., Norwalk, CT). Samples stored for one, three, or seven days at 4°C were heated from 5 to 120°C at 10°C/min. An empty stainless steel pan was used as a reference. Enthalpy of the endotherms was calculated (Pyris Software, v. 2.1; Perkin-Elmer). Temperature and enthalpy calibrations were made using indium.

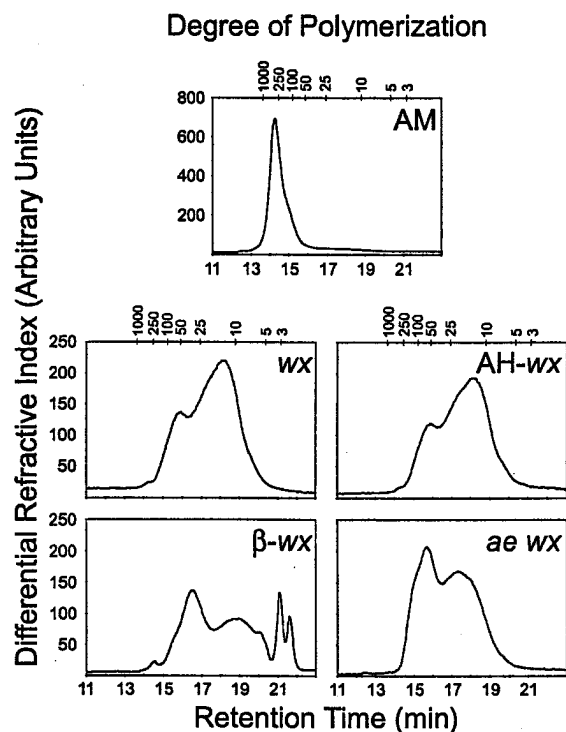


Fig. 3. High-performance size-exclusion chromatograms of molecules after debranching with isoamylase: *wx* maize starch (*wx*), *wx* maize β -limit dextrin (*wx*- β), acid-hydrolyzed HMW *wx* maize starch (AH-*wx*), maltodextrin (MD1), *ae wx* maize starch (*ae wx*), *aeVII* amylose (AM).

TABLE III
Elastic Modulus (G') of Gels of Commercial Starches, AM Mixed with MD1, and AM Mixed with Commercial *n* AP in 20% DMSO^{a-c}

Sample	Days	AM (%) ^d	Measurements	Elastic Modulus (G')	
				25°C	80°C
<i>n</i>	1	1.73	3	nd ^e	nd
	3		3	140 \pm 13a,DE	19 \pm 7a,CD
	7		3	1,600 \pm 76b,B	34 \pm 2b,BC
<i>n</i> AP	1	1.88	2	700 \pm 181a,A	100 \pm 21a,A
	3		2	2,400 \pm 607ab,A	160 \pm 8a,A
	7		2	3,800 \pm 800b,A	150 \pm 46a,A
MD1	1	3.75	2	2,400 \pm 499a,B	368 \pm 35a,D
	3		2	3,530 \pm 34ab,C	400 \pm 23a,D
	7		2	5,000 \pm 846b,B	480 \pm 45a,CD
<i>ae VII</i>	1	3.68	5	4,700 \pm 657a,A	1,400 \pm 81a,A
	3		3	6,200 \pm 457b,A	1,390 \pm 73a,A
	7		3	7,100 \pm 732b,A	1,400 \pm 119a,A

^a Elastic modulus (G' , Pa) mean \pm standard deviation.

^b Within each sample, values followed by the same lowercase letter in the same column are not significantly different ($P < 0.05$). For each storage time across all samples with the same AM content, values followed by the same uppercase letter in the same column are not significantly different at ($P < 0.05$). Uppercase letters correspond to letters in Table II.

^c *n* = Normal maize starch, *n* AP = normal maize amylopectin, MD1 commercial maltodextrin, *aeVII* = *aeVII* starch.

^d AM added to *n* AP and MD1. *n* and *aeVII* contained native amylose (23 and 49%, respectively) as determined by Klucinec and Thompson (1998).

^e Not determined. Sample could not be transferred intact for rheological analysis.

Two independently prepared samples of each starch (7.5% w/w) were analyzed for each storage time. To obtain more readily analyzed thermograms and provide additional support for the DSC data obtained with samples containing 20% DMSO, other samples were prepared for DSC analysis by weighing ≈ 12 mg (± 0.01) of nongranular *wx*, AH-*wx*, and *ae wx* starch into a stainless steel DSC pan. The samples were brought to 30% solids (w/w) with deionized water, sealed, permitted to equilibrate at room temperature for ≈ 1 hr, heated to 180°C in using DSC, quench cooled, then held at 4°C. After one or seven days at 4°C, samples were heated from 5 to 180°C at 10°C/min. Three independently prepared samples of each starch (30% w/w) were analyzed for each storage time. For each set of DSC samples, data were analyzed for significance using LSD.

RESULTS

Characteristics of AM and AP

The major portion of *wx*, *ae wx*, and *wx* β -limit dextrin eluted at the void volume of the Sepharose CL-2B chromatography column

(Fig. 2). Nearly all of the AH-*wx*, maltodextrin, and the AM eluted after the void volume.

Debranched *wx* and AH-*wx* starch had essentially identical chain length distributions that differed from the *ae wx* starch chain length distribution in the lower relative mass in chains at DP > 30 (Fig. 3). The chromatogram of the debranched *wx* β -limit dextrin was consistent with previous work (Yuan et al 1993): the maltose and maltotriose residues of A chains and a bimodal distribution of chains attributable to short and long B chain residues are apparent. After debranching, most chains from AM had DP > 50.

Small Deformation Oscillatory Rheometry

The *wx* starch developed a gel that could be transferred for rheological analysis between three and seven days (Table II, Fig. 4). The *ae wx* starch gelled sufficiently to allow transfer to the rheometer plates for analysis within one day. The *wx* β -limit dextrin sols did not develop a gel over the course of seven days. The AH-*wx* and maltodextrin sols both precipitated rather than forming a gel. For all of the gels examined, phase separation was not visually observed.

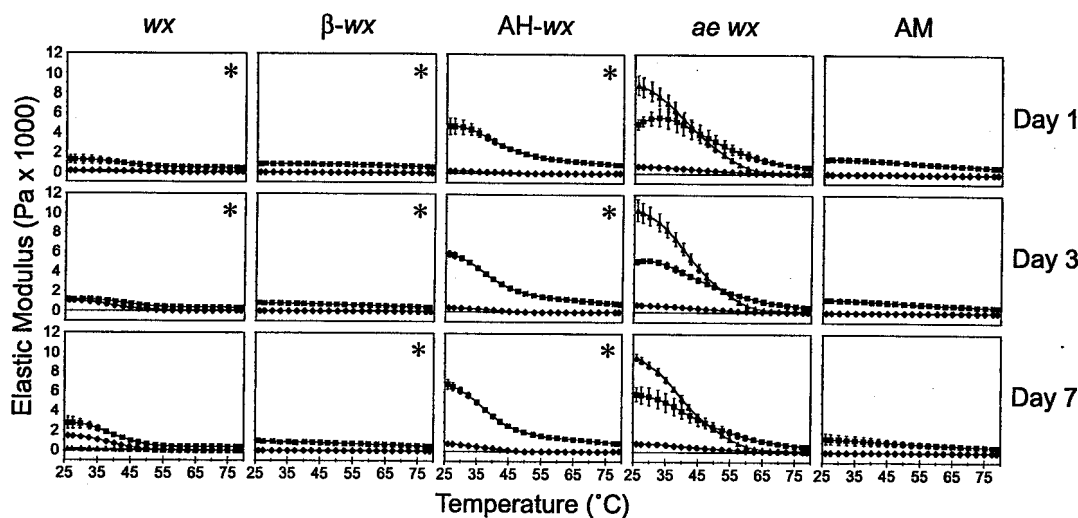


Fig. 4. Elastic modulus [G' average ± 1 standard deviation of at least three measurements (see Table II)] of 7.5% gels of mixtures of amylose (AM) and an amylopectin (AP) and of AM control gels as a function of temperature. AP samples contained either 0% AM (\blacktriangle), 1.88% AM (\blacklozenge), or 3.75% AM (\blacksquare). Samples in 20% DMSO examined after storage for one, three, or seven days at 4°C: *wx* maize starch (*wx*), *wx* maize β -limit dextrin (*wx*- β), acid-hydrolyzed high molecular weight *wx* maize starch (AH-*wx*), maltodextrin (MD1), *ae wx* maize starch (*ae wx*), *aeVII* amylose control gels (AM). * = 0% AM samples that could not be transferred to rheometer for analysis.

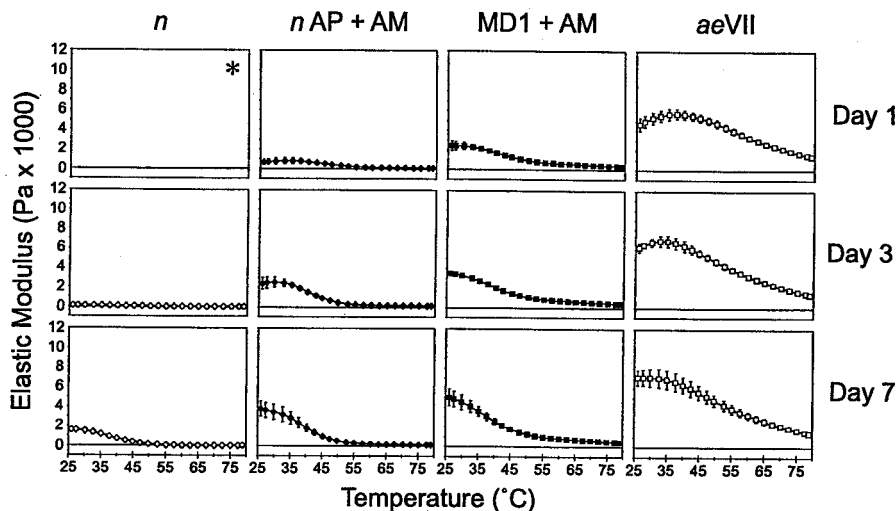


Fig. 5. Elastic modulus [G' average ± 1 standard deviation of at least two measurements (see Table III)] of gels of unfractionated normal maize starch (*n*), 5.63% normal maize amylopectin (*n* AP) mixed with 1.88% amylose (AM), 3.75% commercial maltodextrin (MD1) mixed with 3.75% AM, and unfractionated *aeVII* starch as a function of temperature. Samples contained either 1.88% added AM (\blacklozenge), 3.75% added AM (\blacksquare), or native amylose of starch at 1.73% (\blacktriangleright) or 3.68% (\square) based on values determined by Klucinec and Thompson (1998). Samples in 20% DMSO examined after storage for one, three, or seven days at 4°C. * = sample that could not be transferred to rheometer for analysis.

All dispersions containing AM, including those of AM alone and the *aeVII* starch, developed sufficient gel structure within one day to be transferred to the rheometer (Tables II and III, Figs. 3 and 4). The *n* starch developed a gel that could be transferred between one and three days (Table III, Fig. 5). For 1.88% AM gels and mixtures of 1.88% AM and *wx* β -limit dextrin, the G' at 25°C remained <50 Pa through seven days at 4°C. The G' at 25°C of most gels increased over seven days at 4°C (Tables II and III, Figs. 3 and 4). After seven days at 4°C gels of mixtures of AM and AP with intact external chains had a G' at 25°C, which was higher than the value of the control AM gel at the same temperature.

After heating to 80°C, the G' of the gels of the mixtures and of the native starches had decreased to values similar to those of the G' of the control AM gels at 80°C (Tables II and III, Figs. 3 and 4). For most gelled mixtures, decrease in G' of gels during heating to 80°C occurred in a sigmoidal fashion: an initial decrease during which most of the G' was lost, and a secondary decrease, generally >55–65°C, during which the G' decreased more gradually with temperature. For 3.75% AM and 3.75% AM plus 3.75% *wx* β -limit dextrin, G' steadily decreased during heating from 25 to 80°C. G' first increased and then decreased during heating for 3.75% *ae wx* and 3.75% AM stored for one and three days (Fig. 4), and for gels of *aeVII* starch stored for one and three days (Fig. 5).

Differential Scanning Calorimetry

Of the dispersions without AM in 20% DMSO, only *ae wx* starch exhibited an endotherm after one day of storage at 4°C (Table IV); *wx* and AH-*wx* starches developed endotherms only after longer storage periods. After seven days of storage, endotherms between 20 and 120°C were not observed for AM, *wx* β -limit dextrin, or their mixtures (Fig. 6). When dispersions of mixtures of AP and either 1.88 or 3.75% AM developed endotherms, the endotherms were within the same temperature range as the corresponding preparations without AM (Fig. 6). Endotherms for *n* starch were first observed for samples stored for three days (Table IV), and endotherms of *aeVII* starch were first observed for samples stored for one day. The 30% starch mixtures in water developed endotherms over a temperature range similar to those of the 7.5% starch mixtures in 20% DMSO (Table V, Fig. 7).

DISCUSSION

Structural Elements in Starch Gels

Flory (1953) recognized that some chains may not contribute to the elasticity of a gel network. To differentiate these noncontributing chains from the others, he described vulcanized rubber as a combination of internal chains, terminal chains, and cross-linkages (Fig. 1a).

TABLE IV
Enthalpy (ΔH)^a of 7.5% Starch in 20% DMSO^b

Amylopectin (AP) or Dispersed Sample	AM (%) ^c	Days	ΔH_{total} (J/g of starch)	ΔH_{BR} (J/g of branched starch) ^d	R ^e
<i>wx</i> Maize	0.00	1	nd ^f	nc ^g	na ^h
		3	nd	nc	na
		7	11.8 ± 0.8BC	11.8 ± 0.8BC	na
	1.88	1	nd	nc	nc
		3	10.9 ± 0.0a,B	14.5 ± 0.0B	u ⁱ
		7	11.6 ± 1.1a,BC	15.4 ± 1.4AB	1.3
	3.75	1	4.0 ± 0.2b,D	7.9 ± 0.4B	u
		3	8.7 ± 1.9a,BC	17.3 ± 3.8AB	u
		7	9.4 ± 1.1a,C	18.8 ± 2.3A	1.6
Acid-hydrolyzed (AH) <i>wx</i> maize	0.00	1	nd	nc	na
		3	3.0 ± 0.8a,D	3.0 ± 0.8D	na
		7	5.6 ± 0.4a,D	5.6 ± 0.4D	na
	1.88	1	nd	nc	nc
		3	7.2 ± 0.1b,C	9.6 ± 0.1C	3.2
		7	10.6 ± 0.2a,B	14.1 ± 0.3C	2.5
	3.75	1	2.8 ± 0.2b,D	5.5 ± 0.4B	u
		3	9.7 ± 1.3a,B	19.3 ± 2.7A	6.4
		7	9.0 ± 0.9a,C	17.9 ± 1.9C	3.2
<i>ae wx</i> Maize	0.00	1	15.9 ± 0.5a,A	15.9 ± 0.5A	na
		3	15.6 ± 0.4a,A	15.6 ± 0.4B	na
		7	17.7 ± 2.1a,A	17.7 ± 2.1C	na
	1.88	1	13.0 ± 1.6a,B	17.3 ± 2.2A	1.1
		3	14.0 ± 0.8a,A	18.6 ± 1.0A	1.2
		7	13.6 ± 1.1a,B	18.1 ± 1.4AB	1.0
	3.75	1	7.7 ± 1.6a,C	15.3 ± 3.3A	1.0
		3	10.1 ± 1.0a,BC	20.2 ± 2.0A	1.3
		7	11.0 ± 0.2a,C	21.9 ± 0.4A	1.2
Normal maize starch (<i>n</i>)	1.73	1	nd	nc	us ^j
		3	6.3 ± 0.1b,C	8.4 ± 0.1C	us
		7	9.1 ± 0.4a,C	12.1 ± 0.6BC	us
<i>aeVII</i> Amylose	3.68	1	nm ^k	nc	us
		3	nm	nc	us
		7	nm	nc	us

^a Enthalpy (ΔH) reported as mean ± standard deviation of two measurements.

^b Within each sample, values of gels with the same AM content followed by the same lowercase letter are not significantly different ($P < 0.05$). For each storage time, values of gels followed by the same uppercase letter in the same column are not significantly different ($P < 0.05$).

^c Amylose (AM) added to *wx* starch, AH-*wx* starch, and *ae wx* starch. The *n* starch and *aeVII* starch contained native amylose (23% and 49%, respectively) as determined by Klucinec and Thompson (1998).

^d Enthalpy assuming only the branched molecules contribute to ΔH_{total} determined as $\Delta H_{\text{BR}} = \Delta H_{\text{total}} / [(7.5\% - \text{AM}\%) / 7.5\%]$.

^e Ratio of ΔH_{BR} of samples with AM to ΔH_{BR} of samples without AM analyzed on the same day.

^f Not detected. No endotherm detected for these samples.

^g Not calculable. Values for ΔH_{BR} and R could not be calculated because no endotherm could be evaluated for these samples.

^h Not applicable. Calculation of R does not apply because these samples did not contain amylose.

ⁱ Undefined. In calculation of R, denominator is effectively 0, and R approaches infinity.

^j Unfractionated starch. Calculation of R does not apply because these were unfractionated starches.

^k Not measured. Endotherm extended beyond range of temperatures examined by differential scanning calorimetry (DSC).

Internal chains were defined as a portion of the structure extending from one cross-link to the next one occurring along the given primary molecule (Flory 1953) (Fig. 1a; AB, BC, CD, DE, and EF). Terminal chains were defined as chains bound at one end to a cross-linkage and terminated at the other by an end (free end) of a primary molecule (Flory 1953) (Fig. 1a). Cross-linkages were defined as fixed points of the structure in the sense that at each of them the four (or *f*) chain ends are required to meet, regardless of whatever displacements in space the cross-linkage may sustain (Fig. 1a; A, B, C, D, E, and F). Cross-linkages were considered tetra-functional in the vulcanized rubber system because a cross-link was a covalent linkage of two chains that crossed. Flory (1953) questioned whether a cross-linkage joining only two internal chains (Fig. 1a, B) could be classified as a full-fledged cross-linkage. If starch double helices or crystallites form such cross-links, the Flory model would predict that they would contribute little to the G' of the system.

The gel networks envisioned by Flory (1953) were formed as a result of the formation of covalent bonds between polymer molecules. The development of a starch gel from NG starch requires the formation of physical junction zones (PJZ) between molecules (Ring et al 1987; Cameron et al 1994; Wüsch and Gumy 1994; Klucinec and Thompson 1999). The PJZ could be individual intermolecular double helices (Cameron et al 1994) or crystallites formed from the double helices of more than one molecule (Ring et al 1987; Keetels et al 1996) or combinations of the two. To adapt the rubber elasticity model to account for the special features of starch gels, and to use terminology that will avoid confusion with established starch terminology, the internal chains and terminal chains as defined by Flory (1953) (Fig. 1a) will be considered internal elements and terminal elements, respectively, according to the modified model presented in Fig. 1b. Because this sort of structure could result from starch branching instead of cross-linking, in our modified model for application to starch (Fig. 1b), we have subdivided the cross-linkages as defined by Flory (1953) (Fig. 1a) into two groups: network linkages joining more than two internal elements, and terminal linkages joining a terminal element to an internal element (Fig. 1b). In our modified model, a single internal element (e.g., internal element EH in Fig. 1b) could be viewed as

solely due to covalent structure (Fig. 8a, 1 and 2) or due to a combination of covalent structure and a PJZ formed during retrogradation (Fig. 8a, 3 and 4). During the reorganization of the molecules, double helices and crystallites may form along existing internal elements (Fig. 8a, 2). An internal element may have numerous terminal linkages along its length (e.g., G in Fig. 1) as would occur due to $\alpha(1\rightarrow6)$ covalent branch points in starch. The terminal elements may be as small as an individual external chain of a molecule (Fig. 8b, 1), may be a single cluster of branches (Fig. 8b, 2 and 3) or may be a multicluster unit of branches (Fig. 8b, 4). McEvoy et al (1985) also recognized that terminal elements of gels may be more complex than single linear chains. Unlike rubber, starch chains have the capacity to retrograde along internal elements without the formation of a new network linkage (possibly resulting in Fig. 8a, 2), and starch terminal elements have the capacity to retrograde (Fig. 8b, 3 and 4). In these cases, starch retrogradation would contribute to the ΔH of the gel but not necessarily to the G' of the gel.

Clark et al (1990) argued that an increase in the number of internal elements would increase G' . Nielsen and Landel (1994) showed that the G' is inversely proportional to the DP between cross-linkages. The $\alpha(1\rightarrow6)$ branch points in the molecules may function as natural tri-functional network linkages in the covalent structure of the molecule. PJZ may also be network linkages whether formed from an individual single double helix or from associations of double helices that form during the reorganization of the molecules.

Although chain and cross-linkage terminology have been applied to linear biopolymer gels (McEvoy et al 1985; Clark et al 1989; Moe et al 1993), these designations have not been applied to gels of amylopectin molecules as shown in Fig. 8.

Gels from Mixtures of AM and wx β -Limit Dextrin

The G' of gels of mixtures of AM and wx β -limit dextrin were not different from the corresponding AM control gels, and no DSC endotherms were observed for any of these samples. Because the formation of internal elements as a result of the formation of the PJZ between AM and AP would only be possible between amylose and the external chains of AP (Klucinec and Thompson 1999), the wx β -limit dextrin, having no external chains longer than DP 3, is

TABLE V
Enthalpy (ΔH)^a of 30% Starch in Water^b

Amylopectin (AP) or Dispersed Sample	AM (%)	Days	ΔH_{total} (J/g of starch)	ΔH_{BR} (J/g of branched starch) ^c	R ^d
<i>wx</i> Maize	0	1	nd ^e	nc ^f	na ^g
		7	2.5 ± 0.7D	2.5 ± 0.7D	na
	7.5	1	3.5 ± 0.8b,D	4.7 ± 1.1D	u ^h
		7	6.4 ± 1.4a,C	8.6 ± 1.9BC	3.4
		15	1.9 ± 0.8b,E	3.8 ± 1.7D	u
	Acid-hydrolyzed (AH) <i>wx</i> maize	0	1	nd	nc
7			5.6 ± 0.3C	5.6 ± 0.3CD	na
7.5		1	5.6 ± 0.4b,CD	7.4 ± 0.5C	u
		7	7.2 ± 0.5a,C	9.6 ± 0.7B	1.7
		15	4.1 ± 0.1b,D	8.2 ± 0.1C	u
<i>ae wx</i> Maize		0	1	15.8 ± 1.6a,A	15.8 ± 1.6A
	7		17.4 ± 2.9a,A	17.4 ± 2.9A	na
	7.5	1	11.9 ± 1.3a,B	15.8 ± 1.8A	1.0
		7	12.4 ± 2.6a,B	16.5 ± 3.5A	0.9
		15	5.6 ± 1.0a,C	11.2 ± 2.1B	0.7
			7	7.6 ± 1.6a,C	15.2 ± 3.2A

^a Enthalpy (ΔH) reported as mean ± standard deviation of three measurements.

^b Within each sample, values of gels with the same amylose (AM) content followed by the same lowercase letter are not significantly different ($P < 0.05$). For each storage time, values of gels followed by the same uppercase letter in the same column are not significantly different ($P < 0.05$).

^c Enthalpy assuming only the branched molecules contribute to ΔH_{total} determined as $\Delta H_{BR} = \Delta H_{total} / [(7.5\% - AM\%) / 7.5\%]$.

^d Ratio of ΔH_{BR} of samples with AM to ΔH_{BR} of samples without AM analyzed on the same day.

^e Not detected. No endotherm detected for these samples.

^f Not calculable. Values for ΔH_{BR} and R could not be calculated because no endotherm could be evaluated for these samples.

^g Not applicable. Calculation of R does not apply because these samples did not contain amylose.

^h Undefined. In calculation of R, denominator is effectively 0, and R approaches infinity.

unable to form such internal elements. With no possibility for the development of internal elements between AM and the *wx* β -limit dextrin, the AM could only form a network analogous to that of a gel of AM alone, with the *wx* β -limit dextrin molecules occupying the voids of the gel. Although the AM would be the only direct contributor to the internal elements of the gel network, the *wx* β -limit dextrin might act to indirectly concentrate the AM in the gels of the mixtures (relative to AM alone), resulting in a higher G' than those of AM gels. However, no such effect of *wx* β -limit dextrin was observed with gels containing either 1.88% AM or 3.75% AM (Fig. 4, Table II) as compared with the control AM gels. Parovuori et al (1997) reported that an 8% (w/w) starch gel containing 4% (w/w) β -limit dextrin and 4% (w/w) amylose had a G' between fivefold and 10-fold lower than a control gel of 4% amylose, and an 8% (w/w) starch gel containing 6% (w/w) β -limit dextrin and 2% (w/w) amylose had a G' as much as twofold higher than a control

gel of 2% amylose. In the present work, the weight proportion of *wx* β -limit dextrin was $\approx 50\%$ less than used by Parovuori et al (1997) because the experiment was designed to provide similar moles of branch points as compared with *wx* AP. The higher concentration of *wx* β -limit dextrin (and $\alpha(1\rightarrow6)$ branch points) in the work of Parovuori et al (1997) may have altered the development of AM gels as a result of the greater total excluded volume of *wx* β -limit dextrin as compared with a similar mass of *wx* starch. Under the conditions of the present work, the *wx* β -limit dextrin apparently does not act to further concentrate the AM.

Gels from Mixtures of AM and Other Branched Molecules

The retrogradation enthalpy from endotherms $<100^\circ\text{C}$ is normally attributed solely to amylopectin (Miles et al 1985). When the enthalpy was normalized to the proportion of amylopectin (ΔH_{BR}), the *wx* or AH-*wx* molecules showed greater ΔH_{BR} with AM than for the same concentration of these AP without AM. This difference is expressed $R > 1$ (Tables IV and V), indicating that the AP appear to develop more retrogradation enthalpy in the presence of AM within a given period of time, especially after one and three days. We suggest that the AM forms double helices with the AP to result in $R > 1$. Gudmundsson and Eliasson (1990) and Gudmundsson (1994) also observed that the putative amylopectin enthalpy was higher than expected for mixtures of amylose and amylopectin, but only when amylose contents exceeded 50% of the total starch. The differences between the observations of the present study and those of Gudmundsson and Eliasson (1990) and Gudmundsson (1994) may be in the preparation of the samples. In that work, mixtures of amylose and amylopectin were heated in water to 105°C before storage. Based on the work of Boltz and Thompson (1999), heating mixtures of amylose and amylopectin only to 105°C would be expected to promote more independent retrogradation of AP and AM molecules than if the mixtures were heated to 160 or 180°C . In the present work, the starch preparations were either heated to 180°C (before DSC analysis) or dispersed in 90% DMSO, a good solvent for both amylose (Pffannmüller et al 1971; Colonna and

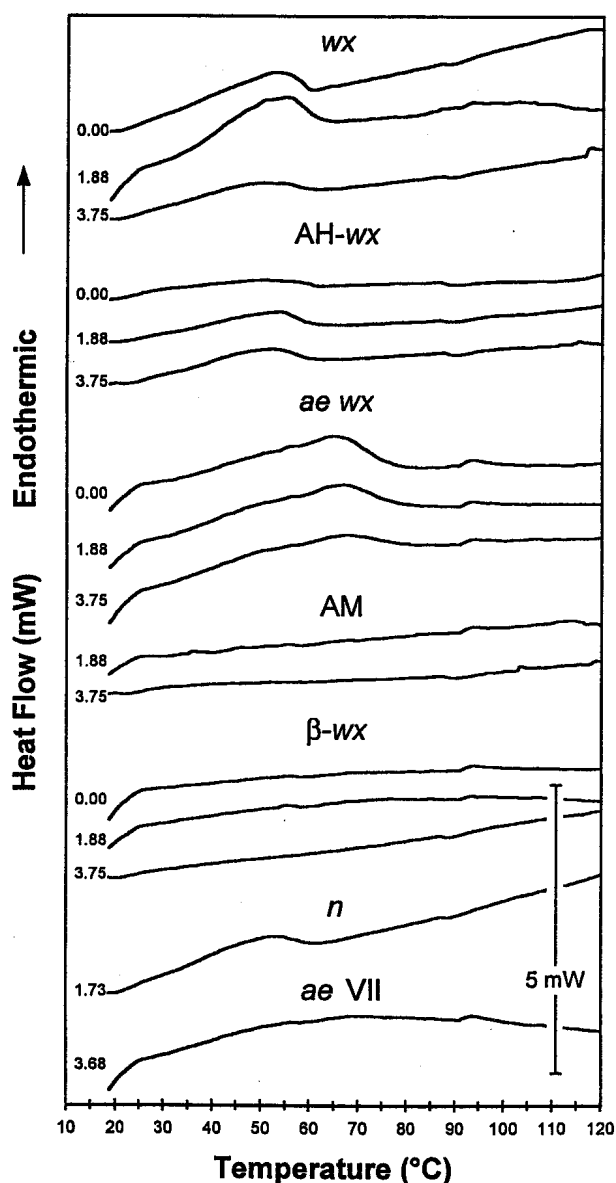


Fig. 6. Differential scanning calorimetry thermograms of starches in 20% DMSO after seven days at 4°C : *wx* maize starch (*wx*), *wx* maize β -limit dextrin (*wx*- β), acid-hydrolyzed high molecular weight *wx* maize starch (AH-*wx*), maltodextrin (MD1), *ae wx* maize starch (*ae wx*), *aeVII* amylose control gels (AM). All gels contained 7.5% (w/w) starch except for those containing β -limit dextrin. Amylose content of each gel (% w/w) is indicated to left of each thermogram. Estimates of amylose content of normal maize starch (*n*) and *aeVII* starch gels based on Klucinec and Thompson (1998).

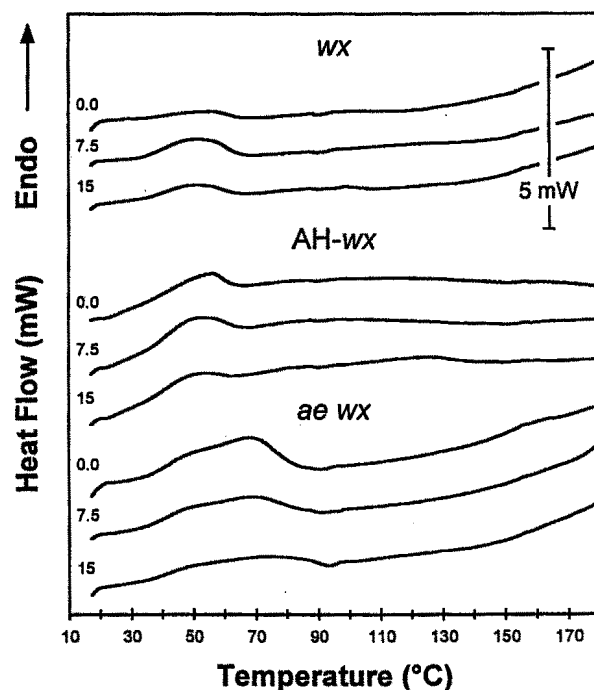


Fig. 7. Differential scanning calorimetry thermograms of 30% (w/w) starch in water after seven days at 4°C : *wx* maize starch (*wx*), acid-hydrolyzed high molecular weight *wx* maize starch (AH-*wx*), *ae wx* maize starch (*ae wx*). Amylose content (% w/w) of each gel is indicated to left of each thermogram.

Morris 1987; Takeyama et al 1994; Mua and Jackson 1998) and amylopectin (Callaghan and Lelièvre 1985; Millard et al 1997). Using these treatments, dispersion of the molecules amongst each other would be more likely than for heating in water at 105°C. Rapid cooling or alteration of the solvent to promote organization within such mixtures would be more likely to result in interactions between AM and AP than quench-cooling mixtures heated to 105°C.

Klucinec and Thompson (1999) suggested that amylose and amylopectin could form double-helical interactions and that the thermal stability of such double helices would be similar to those double helices formed by the amylopectin alone, because the length of an external chain would limit the length of the double helices. Gidley and Bulpin (1987) showed that although solutions of chains below DP 10 did not form double helices and crystallites, chains as short as DP 6 would crystallize in the presence of chains with

DP > 10. By similar logic, one might expect stable PJZ between AM and external chains of AP. Due to the instability of dispersed AM in aqueous environments (Clark et al 1989), when PJZ involving interactions between AM and AP form, they are likely to form rapidly. Because of the longer average external chain length of *ae wx* starch (Klucinec and Thompson 2002), one might anticipate that PJZ between *ae wx* starch and AM would be more likely to form than PJZ between *wx* starch or AH-*wx* starch and AM. However, unlike *wx* and AH-*wx* starch, no substantial increases in the retrogradation ΔH_{BR} of *ae wx* starch were observed with the addition of AM after one and three days of storage (Tables IV and V). This incongruity will be discussed further in relation to the unusual gel behavior for *ae wx* starch with and without added AM.

Kalichevsky and Ring (1987) observed that the amylose-rich phase occupied 1.5 times more volume than the amylopectin-rich phase in phase-separated mixtures of ~6–8% amylose and ~6–8% amylopectin. An alternative explanation for the $R > 1$ for gels of mixtures of AM and AP might be that an AM-rich phase concentrated the AP, hastening its retrogradation and causing it to be more extensive. Liu and Thompson (1998) observed retrogradation ΔH for *wx* starch after one day when the *wx* starch content exceeded 30% (w/w). However, phase concentration to the extent observed by Kalichevsky and Ring (1987) would only lead to an approximate concentration of 7.5% AP, far less than what has been shown to lead to rapid retrogradation of *wx* starch (Yuan and Thompson 1993).

Gels from Mixtures of 3.75% AM and Other Branched Molecules

The higher G' of the gels of 3.75% AM and 3.75% AH-*wx* starch as compared with 3.75% AM could be attributed to additional internal elements established by AM-AP or AP-AP PJZ. A DSC endotherm of AH-*wx* starch was not observed after one day, and since the dispersion did not develop turbidity until three days (data not shown), internal elements involving AP-AP PJZ are not likely to have formed. Based on the ECL of the *wx* starch (Klucinec and Thompson 2002), and thus, presumably, the AH-*wx* starch, one would anticipate that the structural contribution from the AH-*wx* starch in the mixed gels would be eliminated by 80°C, leaving only internal elements created from the formation of AM-AM PJZ. At 80°C the gels of 3.75% AM and 3.75% AH-*wx* have a G' similar to the G' of the gels of 3.75% control AM gels. The additional G' developed by the gel of 3.75% AM and 3.75% AH-*wx* starch at 25°C would appear to be due to the internal elements of AM-AP PJZ.

For gels of 3.75% AM and 3.75% AH-*wx* starch, the G' values at 25°C increased between one and three days (Fig. 4, Tables II and III). The ΔH for these gels also increased between one and three days (Tables IV and V). A gradual increase in the G' over time for gels of 3.75% AH-*wx* starch and 3.75% AM would be consistent with an increase in internal elements due to the develop-

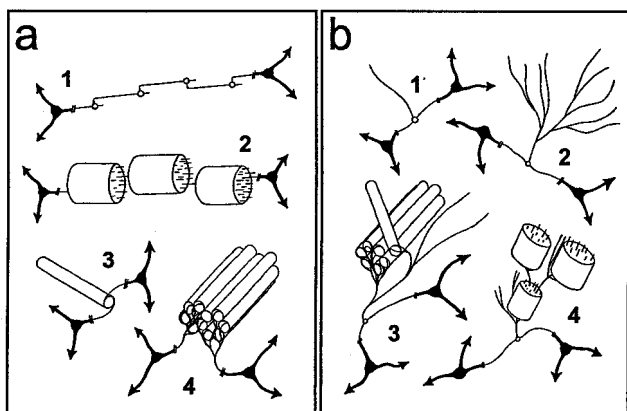


Fig. 8. Modified Flory-type model of network linkages, internal elements, and terminal elements in starch gel. **a** (1) Internal element based on a series of linear portions of chains linked through $\alpha(1\rightarrow6)$ branch points; branch points serving as terminal linkages (\circ). (2) Internal element with organized clusters of short chains along the main chain, with clusters represented as cylinders in the branching structure of the molecule; no new internal elements are formed when the clusters organize. (3) Two terminal elements combine to form a double-helical physical junction zones (PJZ), creating a new internal element transforming the two terminal linkages into network linkages. (4) Two terminal elements combine to form an association of double helices acting as a crystalline PJZ, creating a new internal element and transforming the two terminal linkages into network linkages. **b** (1) Single external chain of starch molecule attached to an internal element by $\alpha(1\rightarrow6)$ terminal linkage (\circ). (2 and 3) Cluster of chains as a complex terminal element shown in varying states of organization. (4) Multicluster grouping of chains as a complex terminal element with clusters represented as cylinders in molecule branching structure.

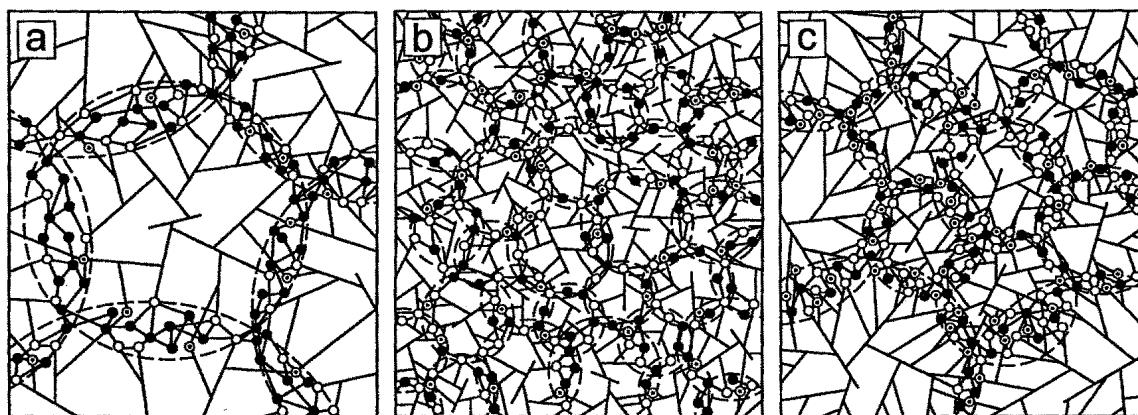


Fig. 9. Two-dimensional representation of physical junction zones (PJZ) in gel network structures: **a**, 3.75% amylose (AM) and 3.75% *wx* maize starch (*wx*); **b**, 3.75% AM and 3.75% acid-hydrolyzed high molecular weight *wx* maize starch (AH-*wx*); **c**, 3.75% AM and 3.75% *ae wx* maize starch (*ae wx*). In **a** and **b**, AM-AM PJZ (\bullet) and AM-AP PJZ (\circ) form within one day, with the formation of AP-AP PJZ (\odot) between one and seven days. For **c**, all PJZ form within one day. Internal elements (solid lines) and approximate limit of covalent structure of individual AP molecules (dashed lines).

ment of AP-AP PJZ, which would be expected to form more slowly than internal elements from AM-AM or AM-AP PJZ. The same logic could be applied to the gradual increase in G' in the gels of mixtures of 3.75% AM and either 3.75% wx starch or 3.75% maltodextrin between one and seven days.

The higher G' of the mixtures of 3.75% AM and 3.75% AH- wx starch than the mixtures of 3.75% AM and 3.75% wx starch might be attributed to a larger number of internal elements formed between AM and the smaller AH- wx molecules. More extensive formation of internal elements from AM-AP PJZ would be a consequence of an increased overlap volume (due to greater interface area of the smaller molecules) of the AH- wx starch as compared with the wx starch. Like the gel of 3.75% AM and 3.75% AH- wx , gels of 3.75% AM and 3.75% maltodextrin also produced a higher G' within one day than gels of 3.75% AM and 3.75% wx starch (Figs. 3 and 4, Table III). Moreover, the presence of the AM prevented the precipitation that occurs with AH- wx or maltodextrin without AM.

Like those gels of mixtures 3.75% AM and either 3.75% wx , AH- wx , or maltodextrin, a subset of the internal elements in the gel of 3.75% AM and 3.75% $ae\ wx$ starch is attributable to AM-AM PJZ because of the residual G' at 80°C. Since the G' at 25°C of the gels of 3.75% AM and 3.75% $ae\ wx$ starch did not change between one and seven days of storage, and the enthalpy of these gels also did not increase with storage, it would appear that formation of internal elements from AM-AP and AP-AP PJZ and the retrogradation of terminal elements of the $ae\ wx$ starch are complete within one day for this mixture. At 25°C, the G' values of the gels of 3.75% AM and 3.75% $ae\ wx$ starch are far greater than for the gels of 3.75% AM and 3.75% wx starch. Thus, we suggest that the gels of 3.75% AM and 3.75% $ae\ wx$ starch may form more internal elements from AM-AP PJZ than do the gels of 3.75% AM and 3.75% wx starch. This outcome could be due to the longer ECL and ICL of $ae\ wx$ starch as well as the increased proportion of molecules of smaller molecule size for $ae\ wx$ starch. At the same time, for 3.75% $ae\ wx$ starch and 3.75% AM, the G' at 25°C was actually lower than for the 7.5% $ae\ wx$ starch. An equal proportion of AM appears to interact with this AP to inhibit network structure slightly, relative to the $ae\ wx$ starch alone. The extent of this inhibition appears to be critically related to the proportion of AM and AP.

Possible structures of gels of mixtures of 3.75% AM and either 3.75% wx , AH- wx , or $ae\ wx$ at 25°C after seven days are depicted in Fig. 9. For the gels of 3.75% AM and either 3.75% wx or AH- wx starch (Fig. 9a and b), AM-AM PJZ (filled circles) and AM-AP PJZ (open circles) would form within the first day to create new internal elements. Between one and seven days, AP-AP PJZ (dotted circles) would form, increasing G' during storage due to additional internal elements. For the gels of 3.75% $ae\ wx$ starch and 3.75% AM (Fig. 9c), AM-AM, AM-AP, and AP-AP PJZ all form within one day; therefore the development of internal elements for this gel would be complete after one day. For both AH- wx and $ae\ wx$ starch, a greater concentration of network linkages or shorter distances between them would account for the higher G' than for wx starch. For each gel, AM-AP and AP-AP PJZ and thus internal elements would dissociate when they are heated to 80°C, leaving only the elastic elements formed from AM-AM PJZ, and a similar G' for each gel. The differences between the gels at 80°C could be related to differences in the residual network structure of the gel from AM-AM interactions.

The development and magnitude of the G' of the gels of 7.5% $aeVII$ starch gels and of gels of 3.75% AM and 3.75% $ae\ wx$ starch were similar and far higher than gels of 3.75% AM and 3.75% wx starch (Figs. 3 and 4, Tables II and III). Compared with wx starch, a higher ECL and a higher average ICL are observed for AP from $aeVII$ starch and for the $ae\ wx$ starch (Klucinec and Thompson 2002). The higher ECL and ICL and the decreased density of branch points indicated by a greater proportion of long B chains would enable formation of more internal elements as a result of AM-AP and AP-AP PJZ. The gels of $aeVII$ starch have a higher G' at 80°C

than the gels of 3.75% $ae\ wx$ starch and 3.75% AM (Figs. 3 and 4, Tables II and III). Klucinec and Thompson (1999) showed that at 75°C, a considerable proportion of the G' remained even for the AP from $aeVII$ starch. Because the ECL of $aeVII$ AP is somewhat larger than the ECL of $ae\ wx$ (Klucinec and Thompson 2002), residual internal elements due to AP-AP and AM-AP PJZ are more likely to exist at 80°C for the gels of $aeVII$ starch than for the gels of 3.75% $ae\ wx$ starch and 3.75% AM.

After one and three days, the G' of gels of 7.5% $aeVII$ starch and of 3.75% $ae\ wx$ starch and 3.75% AM increased as the temperature increased from 25 to 35°C (Figs. 3 and 4). Eliasson and Kim (1992) suggested that an initial increase in complex shear modulus as the temperature increased from ambient temperature was observed for gels that syneresed during centrifugation. Yuan and Thompson (1998) observed a similar initial increase in the G' of gels of 15% wx starches only when stored for 25 days. For $du\ wx$ starch, this phenomenon appeared after seven days and intensified with increasing storage time (Yuan and Thompson 1998). An increasing number of freeze-thaw cycles also intensified this phenomenon (Eliasson and Kim 1992). If extensive retrogradation is responsible, it is puzzling why this behavior is not observed for the 7.5% $ae\ wx$ starch gel, which does not contain AM but also retrogrades extensively within one day. It is also puzzling why for $aeVII$ and for the mixture of 3.75% AM and 3.75% $ae\ wx$, this phenomenon appears most prominently after one day, less so after three days, and is absent for gels stored for seven days. In the present work, the increase in the G' of the gels stored one day might be due the loss of internal elements composed of short, thermally labile PJZ and the reformation of a higher number of internal elements composed of more thermally stable PJZ during heating at 2°C/min. Annealing of the starch during heating, to create more internal elements and increase the G' during heating from 25 to 35°C, would be observable only for these starches because they retrograde so quickly. In other work, the least stable regions of DSC thermograms of $ae\ wx$ starch annealed somewhat on storage (Liu and Thompson 1998). This change could preclude an increase in the G' between 25 and 35°C after storage.

Gels from Mixtures of 1.88% AM and Other Branched Molecules

Like the gels of 3.75% AM and 3.75% AP, the gelation of 1.88% AM and 5.63% AP might be expected to be the result of the formation of internal elements composed of AM-AM, AM-AP, and AP-AP PJZ. In the gels of 1.88% AM and either 5.63% wx starch, AH- wx starch, or $ae\ wx$ starch, the AM would form some AM-AM interactions to establish a weak gel network. Leloup et al (1991) observed that an amylose-to-amylopectin ratio at or above 30:70 was required for a gel to maintain stability during heating in boiling water. A weak AM network and the development of AM-AP PJZ would prevent the AH- wx from precipitating and AM-AP PJZ would result in $R > 1$ for these mixtures (Table IV). However, because of the lower amylose content, the AM may be more likely to form PJZ with the AP without forming an internal element in the network. In $ae\ wx$ gels, the G' with 1.88% AM was far less than for either $ae\ wx$ gels with 0% AM or 3.75% AM. Formation of AM-AP PJZ that do not create internal elements could interfere with the formation of internal elements from AP-AP PJZ and thus inhibit the development of internal elements between the AP molecules.

Dispersions of 1.88% AM and 5.63% n AP gelled after one day, while the 7.5% n starch did not gel (Fig. 5, Table III). This is consistent with previous observations showing amylose from normal maize starch to be less effective than amylose from high-amylose maize starch in the gelation of mixtures of amylose and amylopectin (Jane and Chen 1992). In addition, after the same storage time, the gels of 5.63% n AP and 1.88% AM had a higher G' than those of 5.63% wx starch and 1.88% AM (Figs. 3 and 4, Table III). For 30% starch solids, the n AP previously developed more enthalpy (11.2 J/g) after seven days at 4°C (Klucinec and Thompson

1999) than that produced by 30% *wx* starch (w/w) in the present study (Table V). These differences in the behavior of *wx* starch and the *n* AP are puzzling considering the similar chain length distributions of *wx* maize starch (Fig. 4) and the *n* AP (Klucinec and Thompson 1998). The difference between the *n* AP and *wx* starch may be due to a slightly longer ECL of *n* AP compared with *wx* starch (Klucinec and Thompson 2002), a different higher level organization of AP (Bertoft 1989a,b, 1991; Bertoft et al 1999), or a different relationship among branch points (Thompson 2000). Further investigation of *wx* maize starch and the *n* AP may reveal differences in the way that clusters of chains in these molecules are organized. The results of the present work suggest that both the nature of an amylopectin and the proportion of amylose to amylopectin (at both low amylose [25% w/w dwb] and high amylose [50% w/w dwb] levels) may be important influences on the physical behavior of starch dispersions.

CONCLUSIONS

Starch gels may be described as a combination of internal elements, terminal elements, network linkages, and terminal linkages. New internal elements may form through the development of AM-AM, AM-AP, and AP-AP PJZ. Compared with *wx* and AH-*wx*, the fine structure of *ae wx* starch and AP from *aeVII* starch makes them well-suited for the development of a large number of PJZ.

In gels containing amylose, a portion of the network structure of gels is attributable to a network resulting from AM-AM PJZ. With a high proportion (50% w/w) of amylose, near the amylose content of *aeVII* starch, amylopectin molecules can participate in the network within one day through the formation of internal elements from AM-AP PJZ. At lower amylose contents, approximating the amylose content of normal maize starch, the formation of AM-AP PJZ does not necessarily contribute to the development of new network linkages. Smaller AP molecules and AP molecules with longer external chains may both form more internal elements as a result of the formation of AM-AP PJZ than for *wx* starch.

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