

# Comparison of Physicochemical Properties and Structures of Sugary-2 Cornstarch with Normal and Waxy Cultivars<sup>1</sup>

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## ABSTRACT

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Starches from normal, waxy, and sugary-2 (su2) corn kernels were isolated, and their structures and properties determined. The total lipid contents of normal, waxy, and su2 corn starches were 0.84, 0.00, and 1.61%, respectively. Scanning electron micrographs showed that normal and waxy corn starch granules were spherical or angular in shape with smooth surfaces. The su2 starch granules consisted of lobes that resembled starch mutants deficient in soluble starch synthases. Normal and waxy corn starches displayed A-type X-ray patterns. The su2 starch showed a weak A-type pattern. The chain-length distributions of normal, waxy, and su2 debranched amylopectins showed the first peak chain length at DP (degree of polymerization) 13, 14, and 13, respectively; second peak chain length at DP 45, 49, and 49, respectively; and highest detectable DP of

80, 72, and 76, respectively. The su2 amylopectin showed a higher percentage of chains with DP 6–12 (22.2%) than normal (15.0%) and waxy (14.6%) amylopectins. The absolute amylose content of normal, waxy, and su2 starches was 18.8, 0.0, and 27.3%, respectively. Gel-permeation profiles of su2 corn starch displayed a considerable amount of intermediate components. The su2 corn starch displayed lower gelatinization temperature, enthalpy change, and viscosity; a significantly higher enthalpy change for melting of amylose-lipid complex; and lower melting temperature and enthalpy change for retrograded starch than did normal and waxy corn starches. The initial rate of hydrolysis (3 hr) of the corn starches followed the order su2 > waxy > normal corn. Waxy and su2 starches were hydrolyzed to the same extent, which was higher than normal starch after a 72-hr hydrolysis period.

Starch is the most abundant reserve carbohydrate in plants. It is stored in granular form in many plant organs including seeds, stems, roots, leaves, tubers, and fruits. Normal starch consists of two types of molecules, amylose and amylopectin, in a ratio of  $\approx$ 1:3. Studies have shown that mutations affect starch biosynthesis and alter starch content, molecular structure, and the percentages of amylose and amylopectin (Shannon and Garwood 1984). These changes, in turn, alter the properties of starch. For instance, large reductions in starch content are generally associated with mutations of enzymes early in the starch biosynthetic pathway, whereas small reductions in starch content but significantly altered ratios or structures of amylose and amylopectin are characteristic of starch synthase and starch branching enzyme mutants (Boyer and Liu 1983). Nelson and Pan (1995) categorized mutants with altered amylose-to-amylopectin ratios or structural difference in  $\alpha$ -glucan into three classes: 1) mutants (waxy), starch contains essentially 100% amylopectin; 2) mutants (high amylose) produce starch with high amylose, usually 40–70% of starch weight; 3) mutants (sugary) produce higher levels of sucrose but reduced levels of starch. It is believed that the effect of sugary-2 (su2) locus is primarily on the synthesis of amylopectin rather than amylose (Nelson and Pan 1995).

Several studies have been conducted regarding the properties of su2 starch. Li and Corke (1999) reported that the swelling power of su2 starch was significantly lower than that of normal corn starch. The su2/su2 starches had flatter viscosity peaks than normal corn starch, and also showed extremely low viscosity and almost zero breakdown and setback viscosities. Furthermore, during storage for seven days, there were no significant changes observed in the hardness and adhesiveness of su2 starch gels (Li and Corke 1999). Campbell and Glover (1996) reported that the double-mutant genotypes starchy su2/su2 and sugary-1/su2 produced starches had low gelatinization enthalpy changes (2.2 and 0.8 J/g, respectively) compared with normal (12.1 J/g), su2 (5.2 J/g), starchy sugary-1 (10.9 J/g), and sugary-1 (9.2 J/g). Campbell et al (1995) observed that su2 starch exhibited excellent freeze-thaw stability in acidic foods, and water was effectively retained in gels formed with su2.

A chicken feeding study conducted with isogenic normal, waxy, and su2 corn showed different feed conversion efficiency and starch digestibility values (Lu 1999). The body weight gains of chickens fed with normal, waxy, and su2 corn diets for 0–2 weeks were 289.2, 301.0, and 313.6 g, respectively (Lu 1999). Chickens fed a su2 corn diet showed a higher average daily feed intake and lower feed conversion efficiency. Feed conversion efficiencies were 1.45, 1.43, and 1.49 g of feed/g of body weight, respectively, for normal, waxy, and su2 corn diets. The objective of this study was to acquire an indepth understanding of these three corn starches to find out what structural features relate to the feed conversion efficiency.

## MATERIALS AND METHODS

Seeds of normal, waxy, and su2 corn private, inbred lines with isogenetic background were gifts of ExSeeds Genetics (Ames, IA) and were planted at an Iowa State University experiment farm where they were self-pollinated. Corn ears were harvested at maturity, dried at room temperature (25°C), and stored in sealed plastic bags at 4°C. Isoamylase (EC 3.2.1.68) from *Pseudomonas amyloferm* was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). Porcine pancreatic  $\alpha$ -amylase was obtained from Sigma Chemical (St. Louis, MO).

### Isolation, Fractionation, and Morphology of Starch

Starches from normal, waxy, and su2 corn were isolated according to the method of Kasemsuwan et al (1995) using HgCl<sub>2</sub> as an amylase inhibitor and dried at 32°C for 24 hr. Starch yields were determined on the basis of the amount of starch recovered. Starch content was determined using the method of Umemoto et al (1995) with modifications. Fractionation of starch into amylose and amylopectin followed the procedures of Schoch (1942) with modifications (Jane and Chen 1992). Morphology of starch granules was studied by scanning electron microscopy at the Bessey Microscopy Facility at Iowa State University. Starches were mounted on brass disks with double-sided sticky tape, coated with gold and palladium (60:40), and observed using a scanning electron microscope (JEOL model 1850, Tokyo, Japan).

The total lipid contents of corn starches were determined by using Goldfish solvent extractors (Laboratory Construction, Kansas City, MO). Propanol and water (3:1, v/v) was used as the solvent to extract lipids (Morrison and Coventry 1985).

Starch samples were equilibrated in a relative humidity chamber (rh 100%) at 25°C for 24 hr. X-ray diffraction patterns were obtained using copper, nickel-filtered, K $\alpha$  radiation with a Siemens D-500

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diffractometer (Madison, WI). Starches were scanned from 4° to 37° with a 0.05° step size and a count time of 2 sec.

### Molecular Weight of Starch Molecule

Starch (or amylopectin) samples (100 mg) were dissolved in 90% DMSO (10 mL), precipitated with ethanol, and redissolved in distilled water. An aliquot (5 mL) containing 15 mg of starch (or amylopectin) was injected into a column (2.6 × 80 cm) packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ). A solution containing 10 mM NaOH and 50 mM NaCl was used as the eluent at a flow rate of 30 mL/hr. Fractions were collected and analyzed for the total carbohydrate content and blue value using an ultra-microplate reader (Bio-Tek Instruments, Winooski, VT), following a procedure derived from those reported by Fox and Robyt (1991) and by Jane and Chen (1992).

Molecular weight of amylopectin was determined by HPSEC (HP1050 series isocratic pump) equipped with multiangle laser light scattering (MALLS, model Dawn-F, Wyatt Tech. Co., Santa Barbara, CA) and refractive index (RI) detectors (HP1047A). Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Shodex Denko, Tokyo, Japan) were used for the separation. The temperature of columns and injector was maintained at 55°C using a CH-460 column heater and a TC-50 controller (Eppendorf, Madison, WI). The temperature of the RI detector was set at 30°C. Distilled, deionized, and degassed water (18.2 MΩ cm) was used as the mobile phase, and the flow rate was set at 0.7 mL/min following the method reported by McPherson and Jane (2000).

### Iodine Affinity and Amylose Content

Iodine affinities of defatted whole starch and isolated amylopectin samples were determined using a potentiometric autotitrator equipped with Meterdata recording software (702 SM Titrino, Brinkman Instrument, Westbury, NY). Apparent and absolute amylose contents were determined by the method of Schoch (1964) and Kasemsuwan et al (1995), respectively. Absolute amylose contents were calculated as  $C = (IA_S - IA_{AP+IC}) / [0.2 - (IA_{AP+IC} / 100)]$  where C,  $IA_S$ , and  $IA_{AP+IC}$  represent the percentage of absolute amylose content, iodine affinity of starch, and iodine affinity of amylopectin plus intermediate components, respectively.

### Branch Chain-Length Distribution of Amylopectin

Fractionated amylopectin was debranched using isoamylase according to the procedure of Jane and Chen (1992). Branch chain lengths

were determined using a high-performance anion exchange chromatograph with a postcolumn amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong and Jane 1997). The separation of samples was performed using a PA-100 anion exchange analytical column and a PA-100 guard column (Dionex, Sunnyvale, CA). The mobile phase used for separation consisted of eluent A (100 mM NaOH) and eluent B (100 mM NaOH and 300 mM NaNO<sub>3</sub>) with a flow rate of 0.5 mL/min. The separation gradient was programmed as 0–5 min, 99% A and 1% B; 5–30 min, linear gradient to 8% B; 30–150 min, linear gradient to 30% B; 150–200 min, linear gradient to 45% B.

### Naegeli Dextrins Prepared by Acid Hydrolysis of Starch

Naegeli dextrins of corn starches were prepared following the procedure of Jane et al (1997) using an H<sub>2</sub>SO<sub>4</sub> solution (15%, v/v) under two experimental conditions: hydrolyzing starches at 38°C for 12 days, and at room temperature for 2.5 months. For the starches hydrolyzed at 38°C, the total carbohydrates of the supernatants after 3, 6, 9, and 12 days of hydrolysis were determined (Dubois et al 1956). HPAEC-ENZ-PAD chromatograms of Naegeli dextrins (12 days and 2.5 months of hydrolysis) were obtained following the procedure stated earlier.

### Thermal Properties of Starch

Thermal properties of corn starches and Naegeli dextrins were determined using differential scanning calorimetry (DSC) using an Intracooler II system and Pyris thermal analysis software (DSC-7, Perkin-Elmer, Norwalk, CT). Starch (5 mg) and water (15 μL) were sealed in a stainless steel pan and equilibrated at room temperature for 2 hr. The sample was scanned from 25 to 130°C at a heating rate of 10°C/min using an empty pan as the reference. Melting of

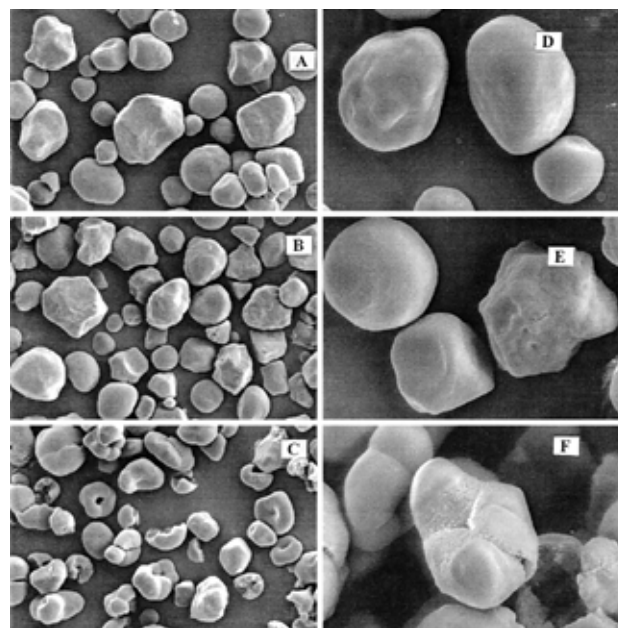


Fig. 1. Scanning electron micrographs of normal corn (A, D), waxy corn (B, E) and sugary-2 (C, F) starch granules.

TABLE I  
Iodine Affinity and Amylose Content of Corn Starches<sup>a</sup>

Starch	Iodine Affinity		Amylose Content	
	Starch	AP and IC <sup>b</sup>	Apparent <sup>c</sup>	Absolute <sup>d</sup>
Normal corn	4.3 ± 0.2	0.7 ± 0.0	21.6 ± 0.3	18.8 ± 0.3
Waxy corn	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sugary-2	6.7 ± 0.2	1.7 ± 0.1	33.5 ± 1.5	27.3 ± 0.4

<sup>a</sup>Values are means of three determinations, mean ± standard deviation.

<sup>b</sup>Amylopectin and intermediate component.

<sup>c</sup>Apparent amylose content determined by dividing iodine affinity by 0.20.

<sup>d</sup>Absolute amylose content:  $C = (IA_S - IA_{AP+IC}) / [0.20 - (IA_{AP+IC} / 100)]$  where C is percentage of absolute amylose content;  $IA_S$  is iodine affinity of starch;  $IA_{AP+IC}$  is iodine affinity of amylopectin and intermediate component.

TABLE II  
Branch Chain-Length Distribution of Amylopectin<sup>a</sup>

Starch	Peak 1	Peak 2	% Distribution (DP)					Highest Detectable DP
			6–9	6–12	13–24	25–36	≥ 37	
Normal corn	13	45	2.9 ± 0.3	15.0 ± 0.4	48.0 ± 0.7	15.5 ± 0.5	21.4 ± 0.2	80
Waxy corn	14	49	3.9 ± 0.2	14.6 ± 0.1	47.9 ± 0.5	18.5 ± 0.5	19.4 ± 0.5	72
Sugary-2	13	49	6.5 ± 0.2	22.2 ± 0.3	44.4 ± 0.2	12.8 ± 0.1	20.4 ± 0.3	76

<sup>a</sup>High-performance anion exchange chromatography with a postcolumn amyloglucosidase reactor and pulsed amperometric detector (HPAEC-ENZ-PAD); mean ± standard deviation. DP = degree of polymerization.

amylose-lipid complex was determined by rescanning the sample after cooling it to 25°C at 80°C/min. The analysis of retrograded starches followed the same method after storing gelatinized samples at 4°C for 7 days.

### Pasting Properties

Pasting profiles of corn starches were obtained using a Rapid Visco Analyser (RVA-4, Newport Scientific). Starch suspensions (8%, dsb) were equilibrated at 50°C for 1 min, heated to 95°C at 6°C/min, held at 95°C for 5 min, and cooled down to 50°C at 6°C/min while stirring the sample at 160 rpm throughout the experiment.

### Enzyme Hydrolysis

Starch (100 mg) was suspended in deionized water (25 mL), vigorously mixed (Vortex Genie 2, Fisher Scientific, Pittsburgh, PA), and divided to 5-mL aliquots. A phosphate buffer solution (0.1M, pH 6.9, 4 mL), containing 120 units of porcine pancreatic  $\alpha$ -amylase (Sigma) was added to each aliquot, and the mixture was incubated at 37°C and 120 rpm in a water-bath shaker. At 0, 3, 24, 48, and 72 hr, samples were removed, and supernatants separated and analyzed for total carbohydrate content (Dubois et al 1956). The samples were analyzed in triplicate, and controls without the enzyme were subjected to the same analysis.

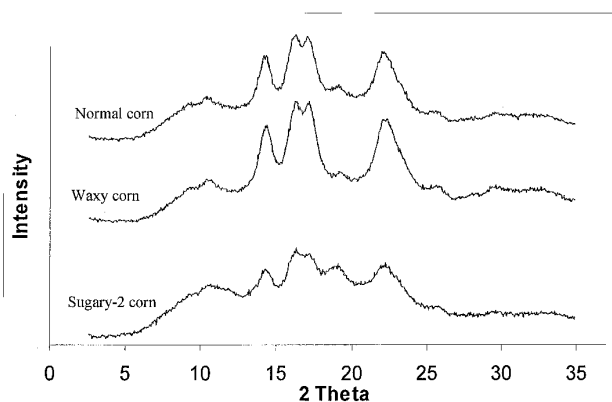
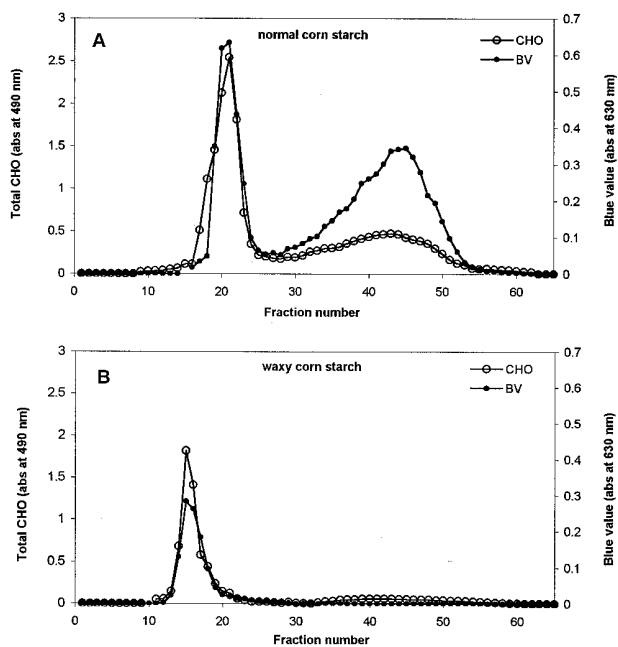


Fig. 2. X-ray diffraction patterns of corn starches.



## RESULTS AND DISCUSSION

Starch contents of the normal, waxy, and su2 corn kernels were 72.1, 67.6, and 63.0%, respectively, and their starch isolation

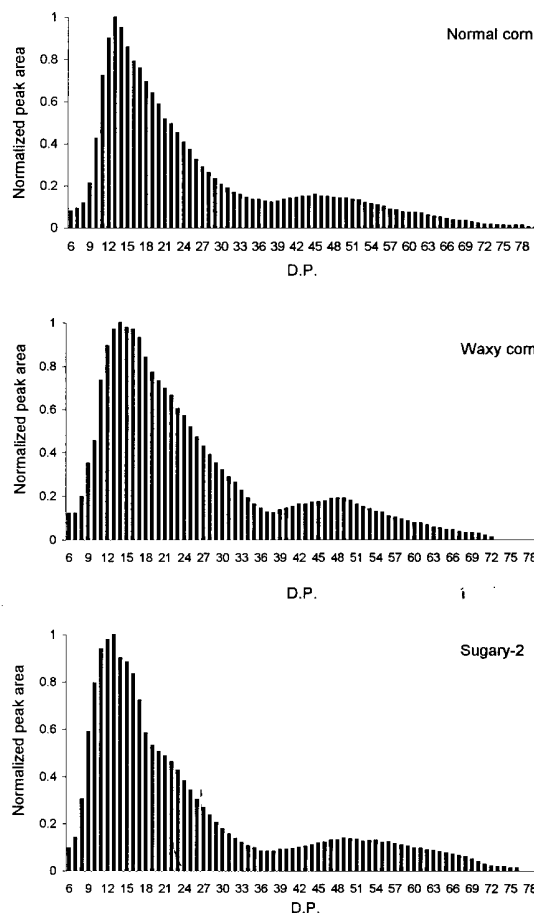


Fig. 4. Branch chain-length distributions of normal corn, waxy corn, and sugary-2 amylopectins determined by using a high-performance anion exchange chromatograph equipped with a postcolumn amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD).

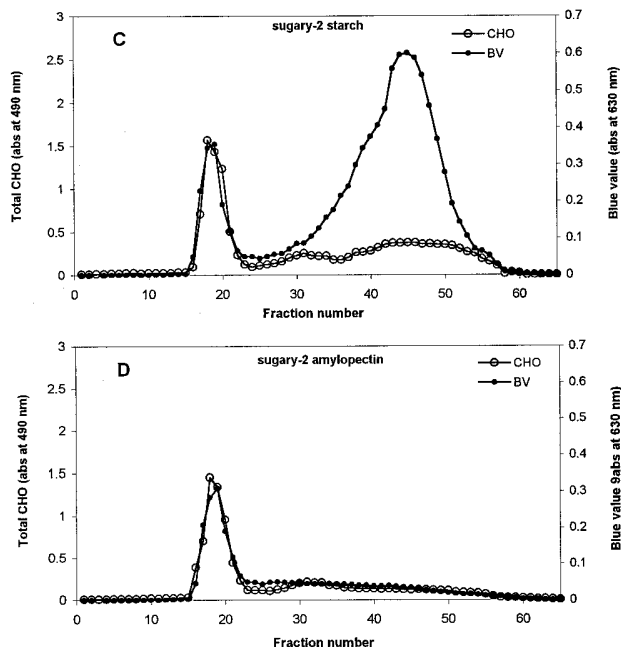


Fig. 3. Gel-permeation chromatography profiles of normal corn starch (A), waxy corn starch (B), sugary-2 corn starch (C), and sugary-2 amylopectin (D).

resulted in yields of 32.3, 39.4, and 19.4%, respectively. The total lipid contents of normal, waxy, and su2 corn starches were 0.84, 0.00, and 1.61%, respectively.

Scanning electron micrographs of starches are shown in Fig. 1. Normal (Fig. 1A) and waxy (Fig. 1B) corn starches displayed granules spherical or angular in shape. The su2 (Fig. 1C) corn starch displayed granules consisting of lobes. Normal and waxy starches showed populations of large and small granules, whereas in su2, the majority of granules were small and uniform in size. Normal (Fig. 1D) and waxy (Fig. 1E) corn starch granules displayed smooth surfaces, a few had uneven surfaces. In contrast, su2 (Fig. 1F) corn starch granules displayed rough surfaces with cracks and holes. Some of these lobed su2 starch granules displayed fragmented pieces that could have resulted from mechanical force applied during the extraction process. The lobed and cracked patterns of su2 corn starch granules resembled those of potato starch granules isolated from potatoes with suppressed soluble starch synthases (SSII and SSIII genes antisensed) (Edwards et al 1999). The resemblance suggested that the su2 mutant could be deficient in soluble starch synthases, which agreed with reports by Nelson and Pan (1995). It is plausible that while amylose is synthesized radially by granular-bound starch synthase at a regular rate, amylopectin biosynthesis is disproportionately reduced (Table I) and fails to fill up the space during the development of starch granules. Consequently, the starch developed lobes and irregular shapes instead of spherical granules.

X-ray diffraction patterns of corn starches are shown in Fig. 2. Both normal and waxy corn starches showed typical A-type X-ray

patterns. Prepared under the same conditions, su2 starch displayed a weak A-type pattern with lower peak intensities than the other two starches, except for a relatively strong peak at  $20^\circ 2\theta$ , indicating an amylose-lipid complex. The weak X-ray pattern and low peak intensities of the A-type polymorphism suggested that su2 starch had low granule crystallinity.

Molecular size distribution of corn starches are shown in Fig. 3. Normal corn starch (Fig. 3A) displayed two peaks representing amylopectin (first peak) and amylose (second peak). Waxy corn starch (Fig. 3B) showed only one peak, which corresponded to amylopectin. In addition to the two major peaks, su2 corn starch (Fig. 3C) displayed another small peak, which appeared in between the amylopectin and amylose peaks (fraction 23–35), suggesting the presence of intermediate components. A gel-permeation profile (Fig. 3D) obtained with isolated amylopectin showed that the molecular weight of the intermediate component overlapped with that of amylose. The low blue value profile (Fig. 3D) showed that the molecules eluted within the molecular weight range of amylose had structures similar to amylopectin. The calculation of areas under the total carbohydrate curve showed that the intermediate component contributed to 30.3% of the total starch and 42.7% of the isolated amylopectin plus the intermediate component. Amylopectin content was calculated to be 40.7% of total starch.

Amylose contents and iodine affinities of starches are presented in Table I. The iodine affinities of normal, waxy, and su2 starches were 4.3, 0.0, and 6.7, respectively, and that of the amylopectins and intermediate components of the three starches were 0.7, 0.0, and 1.7, respectively. The apparent amylose content of su2 starch

TABLE III  
Gelatinization and Properties of Corn Starches<sup>a</sup>

Sample	Peak I <sup>b</sup>				Peak II <sup>c</sup>				$\Delta H$ (J/g) 1 <sup>st</sup> scan	$\Delta H$ (J/g) Rescan
	$T_o$	$T_p$	$T_c$	$\Delta H$ (J/g)	$T_o$	$T_p$	$T_c$			
Normal corn starch	64.4 ± 0.4	69.4 ± 0.2	80.4 ± 0.4	13.2 ± 0.2	92.3 ± 0.5	98.4 ± 0.6	103.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	
Normal corn Naegeli dextrin <sup>d</sup>	75.6 ± 0.9	81.3 ± 0.7	87.3 ± 1.5	5.6 ± 0.4	100.4 ± 0.1	103.9 ± 1.9	109.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.0	
Waxy corn starch	64.2 ± 0.2	69.4 ± 0.1	81.2 ± 0.2	15.8 ± 0.2	nd <sup>e</sup>	nd	nd	nd	nd	
Waxy corn Naegeli dextrin <sup>d</sup>	60.7 ± 1.9	69.5 ± 0.2	77.6 ± 0.1	0.9 ± 0.2	nd	nd	nd	nd	nd	
Sugary-2 corn starch	47.8 ± 0.4	54.2 ± 0.8	63.2 ± 0.3	7.9 ± 0.4	85.2 ± 2.9	96.4 ± 1.1	105.2 ± 1.7	5.3 ± 0.3	2.6 ± 0.3	
Sugary-2 corn Naegeli dextrin <sup>d</sup>	58.0 ± 0.1	64.0 ± 0.3	70.6 ± 0.1	1.8 ± 0.4	93.8 ± 1.8	102.1 ± 0.9	108.8 ± 0.5	1.4 ± 0.2	1.3 ± 0.1	

<sup>a</sup>  $T_o$ ,  $T_p$  and  $T_c$  = onset, peak, and conclusion temperatures (°C) of endotherm.  $\Delta H$  = enthalpy change of gelatinization and melting of amylose-lipid complex. Values are mean ± standard deviation.

<sup>b</sup> Gelatinization.

<sup>c</sup> Melting of amylose-lipid complex.

<sup>d</sup> Prepared at room temperature.

<sup>e</sup> Not determined.

TABLE IV  
Retrogradation Properties of Corn Starches<sup>a</sup>

Starch	$T_o$	$T_p$	$T_c$	$\Delta H$ (J/g)	Retrogradation (%) <sup>b</sup>
Normal corn	40.1 ± 1.9	49.1 ± 0.3	62.8 ± 0.9	5.7 ± 0.8	43.2 ± 0.7
Waxy corn	40.2 ± 0.5	52.0 ± 0.7	62.7 ± 0.1	6.2 ± 0.7	39.2 ± 0.5
Sugary-2	42.5 ± 0.8	49.3 ± 0.8	57.6 ± 0.4	0.5 ± 0.5	6.7 ± 0.5

<sup>a</sup>  $T_o$ ,  $T_p$  and  $T_c$  = onset, peak, and conclusion temperatures of endotherm determined by differential scanning calorimetry.  $\Delta H$  = enthalpy of dissociation of retrograded starch. Average of triplicate analysis, mean ± standard deviation.

<sup>b</sup> % Retrogradation = (enthalpy of retrograded starch/enthalpy of native starch) × 100.

TABLE V  
Pasting Profiles of Corn Starches<sup>a</sup>

Starch	Peak 1	Breakdown	Final Viscosity	Setback	Peak Time (min)	Pasting Temp (°C)
Normal corn	109.9 ± 1.5	30.8 ± 0.4	142.8 ± 1.8	63.5 ± 0.7	8.2 ± 0.1	72.7 ± 0.4
Waxy corn	235.7 ± 2.3	145.7 ± 3.3	118.8 ± 1.4	28.8 ± 0.5	5.8 ± 0.1	69.1 ± 0.4
Sugary-2	7.1 ± 0.2	1.5 ± 0.4	8.0 ± 1.5	2.4 ± 0.9	12.4 ± 0.5	nd <sup>b</sup>

<sup>a</sup> Determined by Rapid Visco Analyser measuring in RVU. Mean ± standard deviation.

<sup>b</sup> Pasting temperature of the starch could not be determined.

(33.5%) was higher than the normal maize starch (21.6%). Waxy corn starch showed 0.0 % apparent amylose, which is in agreement with the findings of other researchers (Blanshard 1987, Jane et al 1999). The absolute amylose content was determined after subtracting the iodine affinity of amylopectin and intermediate components from that of defatted starch. The values were 18.8 and 27.3%, respectively, for normal and su2 starches.

The branch chain-length distributions of corn amylopectins are shown in Fig. 4 and Table II. Normal, waxy, and su2 amylopectins showed the first peak of branch chain length at DP 13, 14, and 13, respectively, and the second peak of branch chain length were at DP 45, 49, and 49, respectively. The distributions showed that su2 contained a higher proportion (22.2%) of short chains (DP 6–12) than that of normal (15.0%) and waxy (14.6%) corn amylopectins. The percentages of branch chains with DP  $\geq$  37 in normal, waxy, and su2 were 21.4, 19.4, and 20.4, respectively. The highest detectable DP values of normal, waxy and su2 amylopectins were 80, 72, and 76, respectively (Table II).

The rates of acid hydrolysis of starches conducted at 38°C from 0 to 12 days are given in Fig. 5. The percentages of acid hydrolysis of normal, waxy, and su2 starches at the end of three days were 33.4, 40.1, and 44.9%, respectively. All three starches displayed higher initial hydrolysis rates (from 0 to 3 days) compared with the later incubation periods. The su2 starch showed the highest rate of hydrolysis during the three-day period, and the rate decreased progressively thereafter, reaching a plateau after nine days. Both waxy and su2 starches were hydrolyzed nearly to the same extent (78.7 and 76.8%, respectively) at the end of 12 days. However, normal corn starch was hydrolyzed to a lower extent (63.8%) than the other two starches at the end of 12 days. Kainuma and French (1971) have postulated that it is necessary for the glucosidic units to undergo a change in conformation from a chair to a half chair during the hydrolysis. If glucosidic units are tightly packed in a crystalline structure, such a conformational change is hindered. Thus, the differences in the rates and the extent of hydrolysis between the above starches seem to reveal the differences in packing of starch molecules into amorphous and crystalline regions in these three starches.

Structures of normal, waxy, and su2 Naegeli dextrans prepared at different temperatures were compared. HPAEC-ENZ-PAD chromatograms of the Naegeli dextrans prepared at room temperature (Fig. 6) showed multiple peaks representing linear, single, double,

and triple branched molecules. Their peak chain lengths were at DP 13, 24, and 37 for normal corn, at DP 13 and 25 for waxy corn, and at DP 13, 25, 37, and 51 for su2 starch. Jane et al (1997) reported that  $\alpha$ -1,6 branch linkages of A-type starches are located in both amorphous and crystalline regions. The branch linkages in the amorphous region are easily hydrolyzed, whereas those in the crystalline region are protected from acid hydrolysis. After isoamylase debranching, the branched molecules of the Naegeli dextrans disappeared and were hydrolyzed to linear chains (Fig. 7). The chromatograms of all three isoamylase debranched Naegeli dextrans had their peak chain lengths at DP 13. Naegeli dextrans prepared at room temperature were used for other characterization.

The chromatograms of normal and waxy corn Naegeli dextrans prepared by acid hydrolysis at 38°C for 12 days (Fig. 8) showed only two peaks with peak chain lengths at DP 14 and 25, and DP 13 and 24, respectively, indicating extensive hydrolysis at the elevated temperature. The su2 Naegeli dextrin had peaks at DP 13 and 34. After isoamylase debranching, the second peak of normal and waxy corn Naegeli dextrans mostly disappeared, but the peak at DP 34 of the su2 chromatogram remained (Fig. 9). The second peak (up to DP 38) shown in debranched su2 and normal Naegeli dextrans are attributed to retrograded amylose that developed during the process of acid hydrolysis (Jane and Robyt 1984). It is likely that starches went through annealing during the process of hydrolysis at 38°C, which facilitated amorphous amylose molecules to retrograde.

The gelatinization properties of corn starches are given in Table III. The onset gelatinization temperature ( $T_0$ ) of normal and waxy corn starches were 64.4 and 64.2°C, whereas that of su2 starch was considerably lower (47.8°C). Gelatinization enthalpy changes for normal, waxy, and su2 corn starches were 13.2, 15.8, and 7.9 J/g,

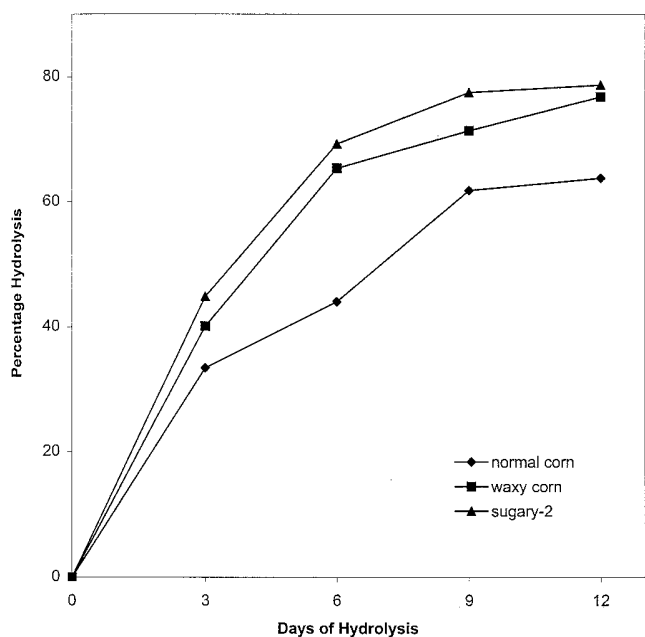


Fig. 5. Acid hydrolysis (15.3% H<sub>2</sub>SO<sub>4</sub>) rates of corn starches.

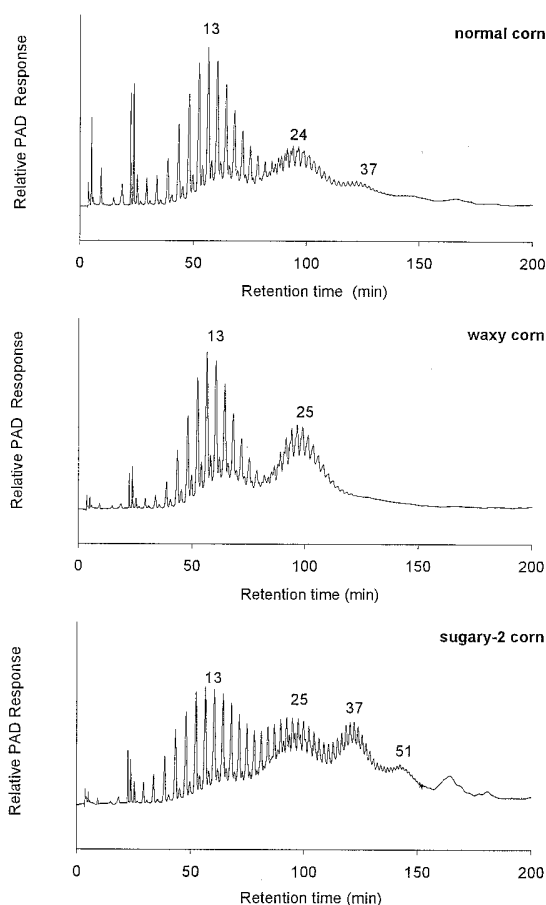


Fig. 6. High-performance anion-exchange chromatograms of Naegeli dextrans obtained after 2.5 months of acid hydrolysis at room temperature (25°C).

respectively. The substantially lower gelatinization temperature and enthalpy change of su2 starch suggest that the order and degree of crystallinity are lower than that of the other two starches.

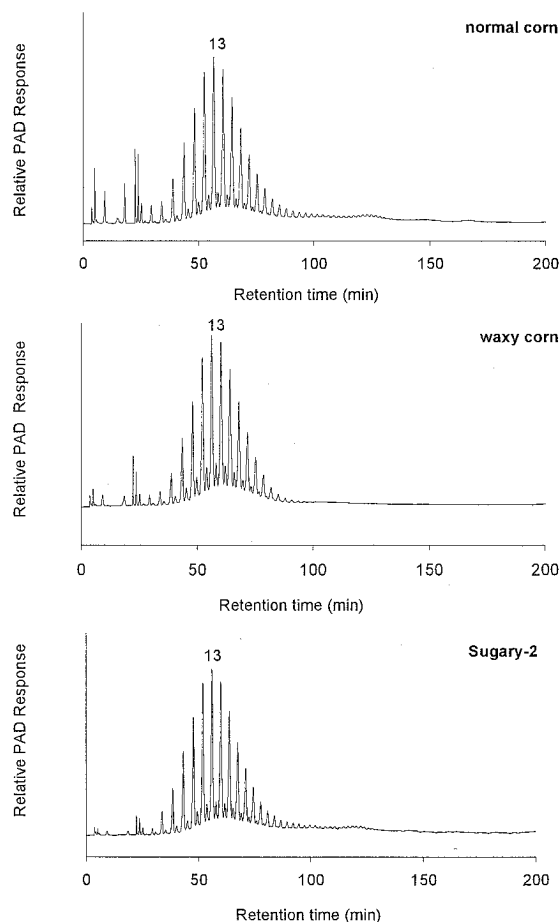
The onset temperatures ( $T_o$ ) of dissociating normal and su2 Naegeli dextrans (75.6 and 58.0°C) were higher than that of the native starches (64.4 and 47.8°C), whereas the  $T_o$  of the waxy Naegeli dextrin (60.7°C) was lower than that of the native waxy starch (64.2°C). These results suggested that amylose interacted with the Naegeli dextrans and increased the dissociation temperature.

Normal and su2 corn starches (Table III) also showed second endotherms that represented melting of the amylose-lipid complex, whereas waxy corn starch did not show it. The amylose-lipid complex was confirmed by its reappearance during rescanning of the samples. For normal and su2 corn starches, the enthalpy changes of the amylose-lipid complex were 0.5 and 5.3 J/g, respectively. For normal corn starch and its Naegeli dextrin, the first scan and the rescan showed similar enthalpy changes (0.5 J/g) for melting of the amylose-lipid complex. However, su2 starch showed a lower enthalpy change (2.6 J/g) for melting of amylose-lipid complex during rescanning. It is likely that in su2 starch, the amylose-lipid complex does not reform to the same extent as it does in the native starch during cooling after gelatinization. These results suggest that there is a significant amount of amylose-lipid complex present in the su2 starch that is resistant to enzyme hydrolysis. It is likely that this amylose-lipid complex results in lower feed conversion efficiency. The su2 Naegeli dextrin showed the presence of an amylose-lipid complex with an enthalpy change of 1.4 J/g. The enthalpy changes for melting of the amylose-lipid complex of su2 starch and its Naegeli dextrin were substantially higher than the other

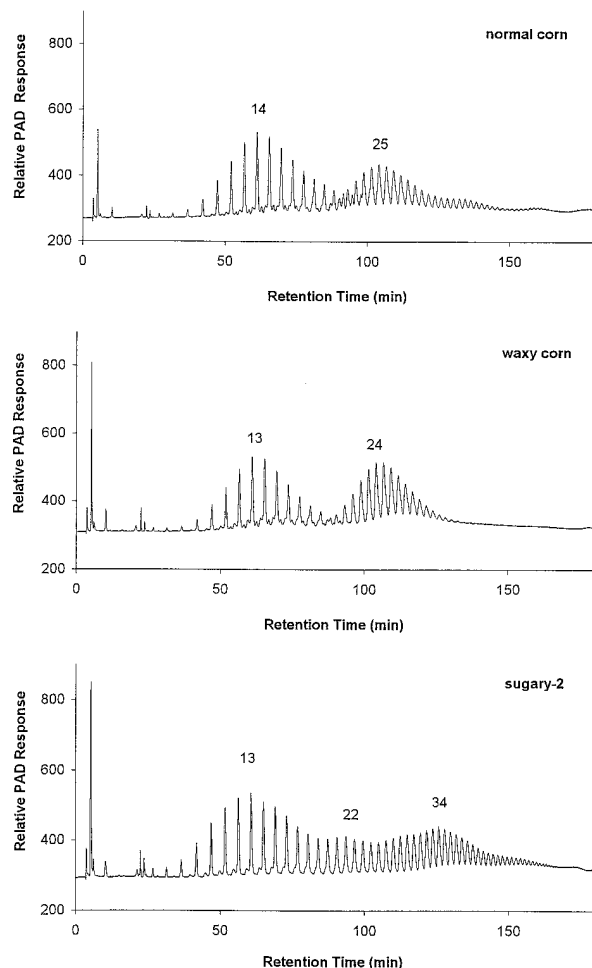
two corn starches. All three Naegeli dextrans prepared at room temperature showed melting endotherms with much lower enthalpy changes (5.6, 0.9, and 1.8 J/g, respectively, for normal, waxy, and su2 corn Naegeli dextrans) than did their native starches.

Thermal properties of retrograded corn starches are summarized in Table IV. All three retrograded corn starches showed lower dissociation temperatures than the gelatinization temperatures of their native starches. Retrograded normal, waxy, and su2 corn starches showed  $T_o$  of 40.1, 40.2, and 42.5°C, respectively. The enthalpy change for melting of retrograded su2 corn starch (0.5 J/g) was significantly lower than that of normal (5.7 J/g) and waxy corn (6.2 J/g) starches. The low retrogradation rate agreed with those reported previously (Campbell et al 1995; Li and Corke 1999). It is likely that the larger percentage of amylopectin short branch chains (22.2%) and the presence of substantially larger amount of lipids (1.61%) in su2 starch resulted in a lower retrogradation (6.7%).

Pasting profiles and viscosity data of the corn starches are given in Fig. 10 and Table V. Amylograms of normal and waxy corn starches showed pasting temperatures of 72.7 and 69.1°C, and peak viscosity of 109.9 and 235.7 RVU, respectively. However, the su2 starch pasting profile displayed a higher pasting temperature ( $\approx 95^\circ\text{C}$ ) and an extremely low peak viscosity (7.1 RVU). The viscosity breakdown (shear thinning) of waxy corn starch was much more severe than that of normal corn starch. The su2 starch did not show an apparent viscosity breakdown. Tester and Morrison (1990) stated that amylopectin contributes to swelling, whereas amylose and lipids inhibit swelling. The high shear thinning shown in the waxy corn profile is attributed to disintegration of swollen starch granules with no amylose and little lipids to maintain granule



**Fig. 7.** High-performance anion-exchange chromatograms of isoamylase debranched Naegeli dextrans obtained after 2.5 months of acid hydrolysis at room temperature (25°C).



**Fig. 8.** High-performance anion-exchange chromatograms of Naegeli dextrans obtained after 12 days of acid hydrolysis at 38°C.

integrity (Morrison et al 1993). It is likely that the significantly high lipid (1.61%) and apparent amylose contents (33.5%) of su2 starch restricted granule swelling. The very low amylopectin content (40.7%) did not provide much swelling power for the starch. The weight average molecular weights ( $M_w$ ) of normal, waxy, and su2 corn amylopectins were  $8.0 \times 10^8$ ,  $7.4 \times 10^8$ , and  $2.8 \times 10^9$ , respectively, which showed that the molecular weight of su2 amylopectin was slightly larger than the other two. The molecular weight of the intermediate component, however, was much smaller, which also contributed to the low viscosity.

The *in vitro* enzyme digestibility of corn starches were determined by hydrolyzing native starch granules with porcine pancreatic  $\alpha$ -amylase. Figure 11 shows that the initial rate of hydrolysis of starches follow the order of su2 > waxy > normal corn. However, at the end of the incubation period (72 hr), waxy, and su2 starches were hydrolyzed nearly to the same extent (84.2 and 86.7%, respectively), whereas normal corn starch was hydrolyzed to 76.1%. Marsden and Gray (1985) have reported that hydrolysis by  $\alpha$ -amylase predominantly occurs in the amorphous regions of the granule. The high initial hydrolysis rate of su2 corn starch suggests that these granules have more amorphous regions and, hence, are more easily attacked by the enzyme. This result is in agreement with the low X-ray diffraction intensity, low gelatinization temperature, and the low gelatinization enthalpy change of su2 starch.

### SUMMARY

This study showed that su2 corn starch structure and properties differ to a great extent in comparison with normal and waxy corn

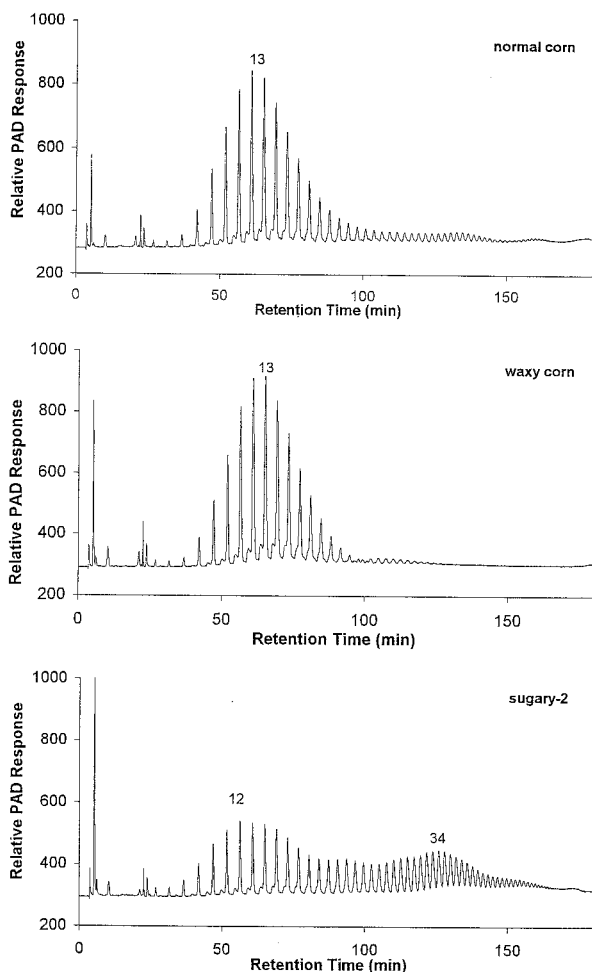


Fig. 9. High-performance anion-exchange of isoamylase debranched Naegeli dextrans obtained after 12 days of acid hydrolysis at 38°C.

starches. These differences may reflect the variation in amylose, amylopectin, and intermediate component contents, amylopectin structures, the magnitude of the interaction between amylose and amylopectin, and the arrangement of these starch chains within the amorphous and crystalline domains of the granules. For su2 starch, it is likely that the larger percentage of amylopectin short branch chains and more amylose content resulted in low granule crystallinity, low gelatinization temperature, and enthalpy change, and low viscosity. The short branch chains and low crystallinity make su2 starch granules highly digestible by  $\alpha$ -amylase. However, the amylose-lipid complex in the su2 starch, which is resistant to enzyme hydrolysis, caused the low feed efficiency that was observed in the chicken feeding experiment.

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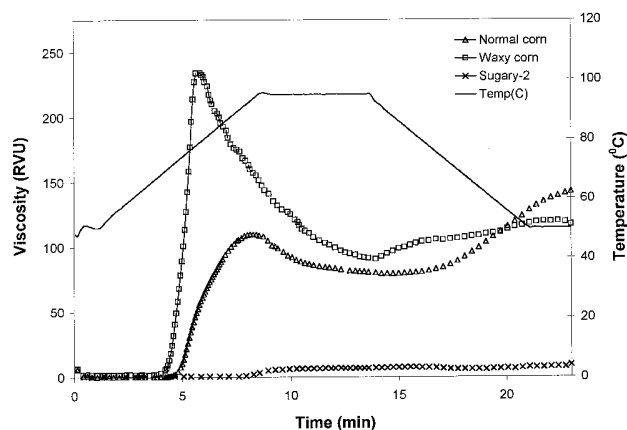


Fig. 10. Rapid Visco Analyser pasting profiles of corn starches (8%, dsb).

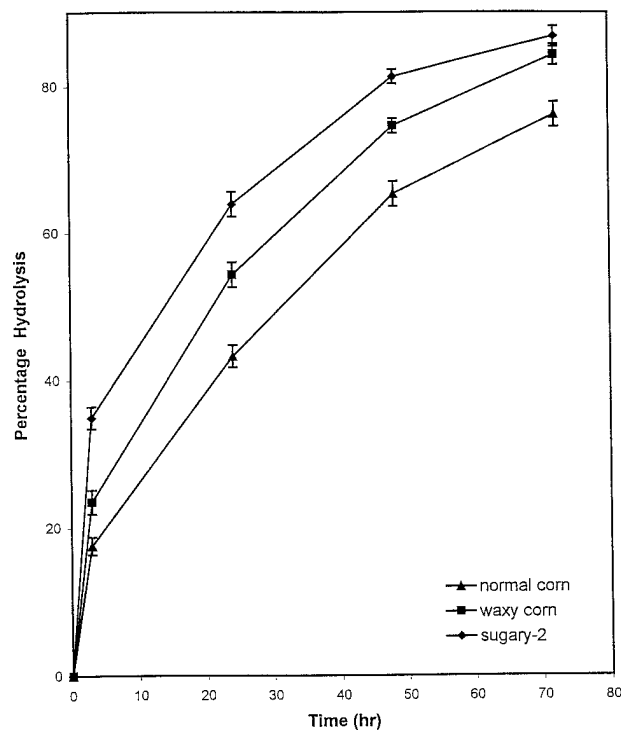


Fig. 11. Enzyme hydrolysis rates of corn starches by porcine pancreatic  $\alpha$ -amylase.

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