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Effect of starch structures on the efficiency of enzyme hydrolysis

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LIST OF ABBREVIATIONS

ae Amylose extender

AAG Aspergillus niger glucoamylase

BAA Bacillus species alpha amylase

Ca^{+2} calcium ion

°C degrees centigrade

cp centipoise

DDGS distillers dried grains with solubles

DE Dextrose Equivalent

DMSO dimethyl-sulfoxide

DP degree of polymerization

DSC differential scanning calorimeter

du dull

FFA free fatty acids

g gram

g acceleration due to gravity

GPC gel permeation chromatography

G1 glucose

G2 maltose

G3 malto-triose

G4 malto-tetraose

G5 malto-pentaose

G6 malto-hexaose

G7 malto-heptaose

hylon V high amylose corn starch V

hylon VII high amylose corn starch VII

ΔH enthalpy change

HPAEC-ENZ-PAD high performance anion exchange chromatography equipped with an enzyme reactor and a pulsed amperometric detector

HPSEC high performance size-exclusion chromatography

h hour

H_2O water

HB hull-less barley

J/g joules/gram

μm micrometer

μl microliter

mg milligram

ml milliliter

mM millimolar

mV millivolts

min minute

M molarity

$Na_2S_2O_5$ sodium meta bisulphate

NaCl sodium chloride

NaOH sodium hydroxide

PAA Porcine pancreatic alpha amylase

RVA rapid visco analyzer

RVU rapid visco analyzer units

RI refractive index detector

rpm rotations per minute

sec second

su2 sugary-2

TLC thin-layer chromatography

To onset gelatinization temperature

Tp peak gelatinization temperature

Tc conclusion temperature

Tc-To gelatinization range

U units

v/v volume/volume

INTRODUCTION

Starch is widely used for the production of glucose, high-fructose corn syrup, fuel alcohol and beverage alcohol. Amylases convert starch to dextrins in the initial stages of liquefaction and then to glucose in the saccharification process. *Bacillus licheniformis* α-amylase has a high thermo-stability and a broad pH range (pH 5 – 8), which leads to its wide use in the starch processing industries (Blackeney and Stone 1985). Different methods have been introduced to decrease the amounts of by-products produced during the starch-enzyme conversion process. Glucoamylase-catalyzed reverse reaction products like iso-maltose contribute to bitter taste and were found to be detrimental in the high-fructose corn syrup industry. Also, these by-products cannot be used by yeast for alcohol fermentation. Use of modified enzymes was successful to some extent in increasing the glucose yields, but could not achieve complete conversion of starch to glucose. There is not enough data relating the starch structures and the efficiency of enzyme hydrolysis.

Large capital investments and high energy costs are required for the extraction of starch and production of glucose and high fructose corn syrups. Also, a considerable amount of starch is lost during the extraction and purification steps of the large-scale process (Arasaratnam et al 1998). Researchers have tried to introduce many cost effective methods of starch extraction but could not eliminate the losses incurred in the processes (Cao et al 1996). Direct conversion of corn flour to alcohol eliminates the extraction and purification costs of glucose and thus is a cost-effective process. In addition, the by-products such as the protein, mucilage and other complex polysaccharides serve as good sources for animal feed. Oil and protein contents of corn flour were found to interfere with the enzyme hydrolysis rate of starch. Protein contents were responsible for color and odor changes in addition to foam

formation during the starch hydrolysis process; on the other hand, oil contents had a negative effect on the starch hydrolytic rate because of the formation of amylose-lipid complexes (Bowler et al 1985, Konieczny-Janda and Hannover 1991, Slominska et al 2003). However, the effects of starch structures and oil and protein contents of dry-milled corn flour on the enzyme hydrolytic rate are not known.

Chemically modified starches have been extensively used at the industry level replacing most of the native starches for specific product functionality. Use of chemicals to produce desired modified starch might cause environmental pollution in addition to extra processing costs. Different types of corn with tailored properties can be produced by channeling genetic engineering aspects through starch biosynthesis mechanisms. Researchers are trying to produce different types of genetically modified corn starch with specific functionalities that fit various industrial needs. In this study, pasting properties of the population of recombinant inbred corn lines were analyzed to identify any specific starch traits.

The objectives of this research were (a) to study the effect of starch structures on the efficiency of enzyme hydrolysis, (b) to study the effect of oil and protein contents of dry-milled corn flour on the enzyme hydrolytic rate, and (c) to study the starch pasting properties of the population of recombinant inbred lines.

LITERATURE REVIEW

Starch

Plants utilize solar energy for the synthesis of carbohydrates and store them in the form of starch in plant organs like seeds, fruits, leaves, stems, roots and tubers (Gallant et al 1992, Perera et al 2001). Starch granules are semi-crystalline in nature and are composed of two glucose polymers, amylose and amylopectin. Amylose is essentially a linear molecule of (1→4) linked α-D-glucopyranose residues, and amylopectin is a highly branched molecule with many short chains of (1→4) α-D-glucose residues connected by (1→6) α-D-glucosidic linkages (Hizukuri 1986, Jane et al 1999). Most starches contain 20 – 30% amylose and 70 – 80% amylopectin, depending on their botanical sources. Amylopectin contributes to the granule crystallinity, and the branch chains form double helical crystallites to produce a structure of alternating crystalline and amorphous lamellae. (Gerard et al 2001, Jane 1992, Jenkins and Donald 1995). Amylose molecules exist as random coils interspersed among the amylopectin molecules and are found to be more concentrated at the periphery of the granule (Jane and Shen 1993, Jane 1992, Pan and Jane 2000). The variations in amylose-to-amylopectin ratios, molecular weights and amylopectin branch chain lengths greatly affect the starch functional properties (Perera et al 2001, Yoo and Jane 2002).

Susceptibility of starches to enzymes

Susceptibility of starches to amylase attack mainly depends on the type of enzyme and the structure of starch (Fuwa et al 1977, Jane et al 1997, Williamson et al 1992). Uncooked A-type granular starches (maize, waxy maize, rice, wheat, taro and tapioca) in general, are more susceptible to enzyme hydrolysis than the B-type starches (high-amylose

maize, ae-waxy maize and potato) (Planchot et al 1995). The A-type starches have shorter average branch chain-lengths than the B-type starches (Hizukuri 1985). Jane et al (1997) have proposed that A-type starches have amylopectin of more short branch chains with scattered branch points that would eventually generate a defective crystalline structure of weak points susceptible to enzyme hydrolysis. On the other hand, B-type starches have fewer short branch chains and have clustered branch points, which develop a superior crystalline structure resistant to enzyme hydrolysis.

Starch granular structures play an important role in amylase hydrolysis of different mutant starches like sugary-2 (su2), amylose extender (ae) and dull (du) maize endosperms (Fujita et al 1989). Starch granules with the A-type polymorphism hydrolyzed to a greater extent than the starch granules with the B-type polymorphism. Further it is shown that the resistance to amylolysis increased as the percentage of B-type crystallites increased (Gerard et al 2001 & 2002, Planchot et al 1997). Sanroman et al (1996) reported that the increase in the molecular weight increased the branch structure of the molecule. However, the branch structure not only increases the points of enzyme attack but also induces a stearic effect on the enzyme. As the amylose content increases in the different mutant starches and amylopectin decreases, the non-reducing ends decrease, thus reducing the sites for glucoamylase attack and yielding glucose at a slower rate (Kim and Robyt 1999).

The rate of amylase hydrolysis of starch is dependent on the starch granule size and the molecular weight of the components of the starch (Chang Rupp and Schwartz 1988). Interesting results were reported when waxy, normal and high-amylose hull-less barley (HB) starches were hydrolyzed by porcine pancreatic (PPA), bacillus species α-amylase (BAA) and Aspergillus niger glucoamylase (AAG). In the initial stages, high-amylose barley starch

was hydrolyzed to a greater extent compared with normal barley starch. Even though the average granular size (6.7-6.8 μm) of both starches were similar, the percentage of granular size ≤ 10 μm in high-amylose starch was greater (38% of total weight) than that of the normal barley starch, which contributed to a larger surface area and resulted in a greater rate of hydrolysis (Li et al 2001).

Amylases

Leloup et al (1991) reported that enzymes first diffuse on to the crystalline starch substrates and adsorb on to the susceptible sites followed by the final catalysis step. Enzymes hydrolyze both amorphous and crystalline layers of the starch granules unlike acid that preferentially hydrolyzes the amorphous parts of the granule (Gerard et al 2002).

Hydrolysis of dispersed starch depends on the enzyme unique action pattern. The action pattern of *Bacillus amyloliquefaciens* alpha amylase (BAA) differs from porcine pancreatic alpha amylase (PPA) in their glucose binding subsites and their formation of distinct products from amylose, amylopectin and maltodextrins. BAA has dual product specificity with nearly twice the glucose binding sites and relatively greater hydrolysis rate (3-14%) on maize, waxy maize and amylomaize-7 starches compared to PAA (Cheol and Robyt 2002). *Bacillus amyloliquefaciens* enzyme shares an 80% homology with *Bacillus licheniformis*, but differs in thermostability and composition of their hydrolysis products (Ivanova et al 1991).

Production of malto-oligosaccharides of a specific degree of polymerization (DP) by product-specific amylases contribute to the ease in their preparation and use as fine chemicals and diagnostic kits (Duedahl-Olesen et al 2000). Saito (1973) discovered a strain

of *Bacillus licheniformis* that displayed unusual thermal stability and wide alkaline pH range (pH 5 – 8) that largely produced maltopentose (G5) oligosaccharide. The enzyme's high thermal stability, above the gelatinization temperatures (95-105 °C), proved to be advantageous to convert starch to soluble dextrins and subsequent hydrolysis by other amylases at lower temperatures (Ivanova et al 1991). When potato amylose was subjected to *Bacillus licheniformis* (Termamyl 60L) hydrolysis in water and in ethanol-water (7:3, v/v) produced oligosaccharides predominant in DP 3 & 5 and DP 1 & 2, respectively (Blakeney and Stone 1985).

Hydrolysis of extruded and autoclaved potato starches using bacterial α-amylase from *Bacillus licheniformis* and fungal α-amylase from *Aspergillus niger* showed that the bacterial α-amylase was efficient to a greater extent and produced greater amounts of polymeric oligosaccharides (DP 6- 8) than the fungal α-amylase (Krzyzaniak et al 2003). Calcium (Ca^{+2}) ions stabilizing effect contributed to the activity of bacterial and fungal α-amylase thermo-stability. However, the activity of glucoamylase was not dependent on any metal ions (Kearsley et al 1980, Richardson et al 2002).

The enzyme pullulanase specifically acts on the α-1-6 branch linkages of pullulan connected by maltotriose units and is useful in producing light beer because it hydrolyzes the α-1-6 branch linkages of starch, facilitates the production of glucose, and thus converts most of the carbohydrates to ethanol (Sreenath and BeMiller 1990). Hydrolysis of waxy corn starch with α-amylase (BAN-120L) followed by pullulanase under controlled reaction conditions (low enzyme concentrations and short time periods) produced maltodextrins of low glucose concentrations with a potential of making low-calorie bulking agents. The hydrolysis rate of waxy starch was enhanced with successive or simultaneous addition of α-

amylase and pullulanase in the production of maltodextrins (Sreenath and BeMiller 1990). Glucoamylase slower rate of action on the α-1-6 linkages of starch leads to the possibility of formation of reverse by-products like iso-maltose. Subsequent use of pullulanase with glucoamylase would accelerate the glucose production and reduce the effect of formation of reverse products (Maarel et al 2002).

Starch gelatinization

Gelatinization is the process that starch granules in water lose crystalline molecular structures and birefringence over a narrow temperature range at a particular temperature. Pre-wetting of starch granules and complete gelatinization of starch greatly increase the enzyme hydrolysis rate (Krzyzaniak et al 2003). Gelatinization temperatures of starches are generally affected by the environmental and processing conditions and various starch isolation procedures. Jane et al (1999) reported that the waxy amaranth starch isolated by strong alkaline method displayed greater onset gelatinization temperature compared with that isolated from mild-alkaline and enzymatic method. Onset gelatinization temperature varies with the type of starch and other minor components like phospho-monoesters (Kimura and Robyt 1996, Tester and Morrison 1990). High amylo-maize starch granules with a wide gelatinization temperature range are resistant to amylase hydrolysis. The resistance to enzyme hydrolysis is attributed to its long branch chains that derive to large crystallites (Jane et al 1997).

Starches with larger proportion of short chains (DP 6-12), fewer long chains with obvious shoulders at DP 18-21 displayed lower gelatinization temperatures, whereas starches with long branch-chain lengths which developed into larger crystallites displayed higher

gelatinization temperatures. However, potato starches displayed lower gelatinization temperatures because of the presence of phosphate monoester groups. In general, the B-type polymorph starches have lower gelatinization temperature than the A-type. Starches with long-branch chain lengths, including high-amylose maize, ae-waxy maize and potato starches displayed larger gelatinization enthalpy changes. On the other hand, waxy starches, with a higher percentage of crystallinity, also displayed larger gelatinization enthalpy changes (Jane et al 1999). Waxy barley starch gelatinized at a higher temperature than normal barley reflecting the more ordered crystallinity of waxy amylopectin (MacGregor and Balance 1980, Song and Jane 2000).

Starch pasting

Tester and Morrison (1990) reported that amylopectin contributes to swelling, whereas amylose and lipids under favorable conditions form complexes and retard swelling. For example, waxy starch, having mainly amylopectin, is not affected by amylose-lipid complexes, therefore displays large peak viscosities at a low pasting temperature. Phospholipids in the starch complex with amylose and restrict granule swelling and thus contribute to low peak viscosity and high pasting temperature (Jane et al 1999). Jane and Chen (1992) reported that amylopectin long- branch chain lengths and amylose molecular size produced synergistic effects on the viscosities of the starch pastes. It was further postulated that the long branch-chains of amylopectin, like amylose, can form helical complexes with lipid molecules and interact with other branch-chains thus restricting starch swelling.

Corn flour hydrolysis

Enzyme conversion of dry-grind corn flour to alcohol reduces the production costs, and the by-product of the process known as distillers dried grains with solubles (DDGS) that is rich in fiber and protein, can be used as a ruminant animal diet (Arasaratnam and Balasubramaniam 1993, Kearsley and Nketsia-Tabiri 1979, Singh et al 2005). The usage of appropriate enzyme concentrations during hydrolysis might affect the hydrolysis rate at different stages in the processes. Cao et al (1996) used an enzyme dosage in the liquefaction of corn flour that produced dextrose equivalent (DE) values ideal for the saccharification process. Alpha-amylase hydrolyzes starch and produces smaller molecules with more non-reducing ends, which is more susceptible for the glucoamylase hydrolysis. Subsequent action of glucoamylase on liquefied starch (DE ~ 10) to produce glucose displays synergicity between glucoamylase and α-amylase (Fujii and Kawamura 1984, Leloup et al 1991).

Effect of enzyme hydrolytic products on the hydrolysis rate

Commercial corn and cassava starches were subjected to bacterial α-amylase (*Bacillus subtilis*) and fungal glucoamylase (*Aspergillus niger*) hydrolysis for 36 h at 37 °C with and without removal of hydrolysis products. Results showed that corn starch hydrolyzed at a faster rate than cassava starch prior to the elimination of hydrolytic products, but the hydrolysis rate of cassava starch increased after the removal of hydrolytic products developed during hydrolysis (Franco et al 1987). Oligosaccharides like maltose and maltotriose formed during the starch hydrolysis process acted as competitive inhibitors in solution and retarded the rate of formation of glucose (Fujii and Kawamura 1984, Planchot et al 1994).

Lipids

Amylose-lipid complexes that exist in the native starch granule are termed as type I complex with dissociation temperatures at 94 –100°C, whereas complexes formed after starch gelatinization are generally referred to as type II complex with dissociation temperatures at 100 – 125 °C (Morrison 1995). When starches are gelatinized, lipids interfere with the starch swelling and rheological characteristics (South et al 1991). The swelling characteristic is the property of the whole amylopectin component with a minor contribution from lipid-free amylose, but is strongly inhibited by lipid-complexed amylose (Morrison et al 1994). High-amylose cereal starches possess larger lipid contents than waxy counterparts. Free fatty acids (FFA) and lyso-phospholipids in maize starch form complexes with amylose molecules and thus resist oxidation and solvent extraction (Morrison, 1995). Tufvesson et al (2003) reported that the amylose-lipid complexes formed during heating can be amorphous (Form I) or crystalline (Form II). The dissociation temperatures of amylose-fatty acid complexes increase with an increase in chain-length of the fatty acid. Studies have reported that the Form II complexes formed with fatty acids of chain length $\geq$ 12 displayed a V-type X-ray diffraction. Also, the type of complexes formed depended on the heating conditions, and the longer heating cycles favored the formation of Form II complexes (Raphaelides and Karkalas 1988, Tufvesson et al 2003). The complexing ability of monoglycerides and other lipids during starch gelatinization has been exploited in various processed foods to retard bread staling, prevent stickiness in instant mashed potato and to control texture in extruded starch products (Raphaelides and Karkalas 1988).

MATERIALS AND METHODS

Materials

Normal maize, waxy maize, ae-waxy maize, Hylon V and Hylon VII maize starches were gifts from Cerestar, USA (Hammond, IL). Alpha amylase from *Bacillus licheniformis*, amyloglucosidase from *Aspergillus niger*, Pullulanase from *Bacillus acidopullulyticus* and glucoamylase from *Rhizopus* mold were purchased from Sigma Chemical Co. (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Whatman Silica K5 silica gel plates (20 x 20 cm) were purchased from Fischer Scientific (Chicago, IL). Homologous series of maltodextrins were prepared by hydrolysis of corn starch with α-amylase followed by isoamylase. Other chemicals (reagent grade) were used without further processing. Selected corn grain samples of varying protein and oil contents were provided by Drs. Charles Hurburgh and Linda Pollak (USDA-ARS, Ames). Recombinant inbred corn lines were provided by Dr. Paul Scott (Iowa State University).

Methods

Experimental design and hypothesis:

The objectives of the research are to understand the optimal structures of the different corn starches for enzyme hydrolysis. There is no sufficient data relating the effects of starch structures on the efficiency of enzyme hydrolysis. The hypothesis of this objective is that an increase in the branch chain length and a decrease in the number of α-1-6 branch-linkages will increase the efficiency of enzyme hydrolysis of starch. To test our hypothesis, we

selected two sets of starch samples for this study. The first set of sample is normal and waxy maize starch, which have similar amylopectin structures but differ in amylose contents. Normal maize has 23 % absolute amylose content and waxy maize starch has 0 %. The second set of samples include ae-waxy maize, hylon V and hylon VII maize starches with apparent amylose contents of 34, 52 and 70 % respectively and absolute amylose contents of 0, 27 and 40 % respectively (Table 1). Waxy maize starch amylopectin has short branch chains with its branch linkages located in close vicinity, whereas ae-waxy and hylon maize starches have long external branch chains.

Table 1. Apparent and absolute amylose contents of maize starches.

Maize Starch	Apparent Amylose (%)	Absolute Amylose (%)	Amylopectin (%)
Normal	29	23	77
Waxy	0	0	100
ae-waxy	34	0	100
Hylon V	52	27	73
Hylon VII	70	40	60

Enzyme hydrolysis of corn starches

The starch sample (50 mg) was wetted with 0.1ml of water and dispersed in 0.9ml of dimethyl-sulfoxide (DMSO) solution. The suspension was subjected to heating and stirring for an hour followed by stirring overnight. The dispersed DMSO solution was mixed with 3 volumes of ethanol and centrifuged at 5200 × *g* for 20 min. The precipitated starch was dissolved in boiling water (5ml) and stirred for 30 min in a boiling water bath. The starch

aqueous solution (1%) was then equilibrated to 70°C in a water bath. *Bacillus licheniformis* α-amylase (0.05U/mg of starch) was added to starch solution and the hydrolyzed samples were collected at different time intervals (15, 30, 60 and 90 min). Samogyi Nelson (Samogyi, 1952) and phenol-sulfuric acid (Dubois et al 1956) methods were used respectively to determine the reducing sugars and total carbohydrate of the enzyme hydrolysates. Dextrose equivalents of the hydrolyzed samples are calculated as the ratio of reducing sugars to the total carbohydrate.

Separation and quantitative determination of sugars using Thin Layer Chromatography (TLC)

The enzyme-hydrolyzed starch samples were analyzed for sugar contents by using thin layer chromatography and quantitatively determined following the procedure reported by Robyt and Mukherjea (1994). The silica plate was first marked with a pencil and the starch hydrolysate samples were spotted on the TLC plate at a distance of 2cm apart. Each sample of aliquots (2 µl) was spotted drop wise with a diameter of 2mm followed by air-drying. The plate was irrigated for three ascents in a pre-equilibrated chamber containing an acetonitrile/ethylacetate/1-propanol/water (85/20/50/50, v/v/v/v) solvent system. Proper caution was taken so that the sample spots were above the solvent. Completion of each ascent was followed by a thorough air-drying step to avoid samples being washed off. The irrigated plate was dipped into a methanol solution containing 0.5% (w/v) α-naphthol and 5% (v/v) sulfuric acid followed by air drying and baking at 110°C oven temperature for 10min. The densities of the separated carbohydrate spots on the TLC plate were quantitatively determined using TLC densitometric image analysis software.

Molecular weight distribution of starch α-amylase hydrolysates determined by HPSEC-RI system

Molecular weight distributions of starch hydrolyzed by *Bacillus licheniformis* α-amylase were determined by using high-performance size-exclusion chromatography equipped with a refractive index detector (RI, HP1047A, Hewlett Packard) (HPSEC-RI) (Yoo and Jane 2002). The sample concentration for injection was 0.4 mg/ml. A shodex OH pak KB-803 analytical column and a KB-G guard column (Showa Denko K.K., Tokyo, Japan) were used for the sample separation. The temperature of the injector and columns was maintained at 50 °C using a CH-460 column heater and a TC-50 controller (Eppendorf, Madison, WI). Temperature of the RI detector was set at 30 °C. The mobile phase was distilled-deionized water passed through on-line membrane filters (0.2 and 0.1 μm, Millipore, Bedford, MA) at a flow rate of 0.5 ml/min). The molecular weights of starch enzyme hydrolysates were calculated by using a calibration curve constructed on the basis of a series of pullulan molecular weight standards (0.58, 1.22, 2.37, 4.80, 10.0 and 18.6 ×104) (Showa Denko K. K., Tokyo, Japan).

Chain-length distribution of starch α-amylase hydrolysates analyzed by HPAEC-ENZ-PAD system

The chain-length distributions of starch α-amylase hydrolysates were analyzed by using a high-performance anion-exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Dionex, Sunnyvale, CA) following the conditions reported by Wong, & Jane (1997). A PA-100 anion-exchange analytical column and a guard column (Dionex, Sunnyvale, CA,

USA) were used for sample separation. The profile of separation eluent gradient composed of eluent A (100 mM sodium hydroxide) and eluent B (100 mM sodium hydroxide and 300 mM sodium nitrate) was: 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.

Enzyme hydrolysis of corn flours to produce glucose

Selected corn grains were ground using a Udy Cyclone mill with a 0.5mm sieve (Udy Corporation, Fort Collins, Colorado). Corn flour suspensions (containing 5% starch, dry basis) were heated from 30°C to 105°C with the presence of α-amylase (126U/g starch) at pH 6.0. The corn flour-enzyme mixture was maintained at 105°C for 5min. The reaction was continued with an additional α-amylase (63U/g starch) and incubating the mixtures in a water bath at 90°C for 30min. The resultant hydrolysates were subjected to saccharification using glucoamylase (4.5U/g starch) and pullulanase (2.1PNU/g starch) at 60°C, pH 4.5 for 24h. Samogyi Nelson (Samogyi, 1952) and phenol-sulfuric acid (Dubois et al 1956) methods were used respectively to determine the reducing sugars and total carbohydrate of the enzyme hydrolysates.

Starch isolation procedure from corn hybrids

Corn grains were steeped in sodium meta-bisulphite ($Na_2S_2O_5$) (0.45%) solution for overnight in a refrigerator. The steeped grains were ground in a micro blender and filtered through a 53μm nylon screen. This step was repeated until most of the starch granules were passed through the screen. The starch slurry was then suspended in 0.1M NaCl containing 10% toluene and stirred for an hour and allowed to stand to remove the protein layer. The

protein layer was siphoned off and the step was repeated until the top yellow layer was not visible. The starch layer was then washed with distilled water and ethanol, recovered by filtration using Whatman No. 4 filter paper and dried in a convection air oven at 31°C for 48h.

Amylose content of starch

The amylose content of starch was determined by using gel permeation chromatography (GPC), following the method of Jane and Chen (1992). Starch samples (1%) were dispersed in 90% DMSO solution, precipitated with ethanol, and redissolved in distilled water. An aliquot (2ml) containing 6mg of starch was injected into a Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ) column in an ascending mode. The eluent consisted of NaCl (25 m*M*) and NaOH (1 m*M*) with a flow rate of 0.5 mL/min. Fractions of 2.5mL each were collected and subjected to total carbohydrate and amylose content analyses using phenol-sulfuric acid (Dubois et al 1956) and iodine staining methods (Juliano 1971), respectively. The amylose content was calculated as the ratio of the total carbohydrate content of the amylose peak area to the sum of the amylopectin and amylose peak areas.

Thermal Properties using Differential Scanning Calorimeter (DSC)

Starch thermal properties were determined by using a differential scanning calorimeter equipped with an Intracooler II system and Pyris thermal analysis software (DSC-7, Perkin-Elmer, Norwalk, CT). Starch samples (2mg, dry starch basis) and distilled water (6mg) were sealed in aluminum pans (Perkin-Elmer) and equilibrated at room temperature for 2hr before analysis. An empty aluminum pan was used as the reference. The samples were heated at 10°C/min over a temperature range of 25 - 120°C. Indium and zinc

were used as the reference standards. Enthalpy Change (ΔH), gelatinization onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc) were determined. The thermal properties of the retrograded starches were analyzed following the same procedure after the gelatinized starch samples were stored at 4°C for 7 days.

Pasting Properties using Rapid Visco Analyzer (RVA)

Starch pasting properties were analyzed using a Rapid Visco Analyzer (RVA-4, Foss North America, Eden Prairie, Minnesota, USA). Starch suspensions (8%, w/w, dsb: 28g of total weight) were equilibrated at 50°C for 1min, heated to 95°C at 6°C/min, held at 95°C for 5min, and then cooled down to 50°C at 6°C/min. Paddle speed was set at 960 rpm for the first 10 sec and then 160 rpm for the remainder of the analysis.

Data analysis

Proc Corr was run in SAS to determine the Pearson correlation coefficients.

RESULTS AND DISCUSSION

Part I. Enzyme hydrolysis of starch mutants

Bacillus licheniformis α-amylase hydrolysates of maize starch mutant dispersions showed that the non–waxy starch varieties were digested to greater extents compared with waxy mutants. Waxy and ae-waxy maize starches displayed slower hydrolysis rates than the normal and high-amylose maize starches (Table 2). Previous studies have reported that the total solubility of the starch in dimethyl sulfoxide (DMSO) increased with the increase in amylose content (Han et al 2004, Jackson 1991). High-amylose starches with larger

proportions of amylose contents were solubilized to a greater extent compared with other starch mutants. Waxy starches, on the other hand, having 100% amylopectin, were not solubilized unless subjected to a shaking induced shear conditions (Jackson 1991, Millard et al 1997). In our experiments, normal, hylon V, hylon VII with 23, 27 and 40% absolute amylose contents, respectively, were hydrolyzed to a greater extent than waxy starch. Among the waxy starches, ae-waxy maize starch hydrolyzed to a slightly larger degree than waxy maize starch.

Aberle et al (1994) reported that the DMSO/H_2O solvent mixtures often formed incomplete starch dissolutions and that the fully dispersed amylose molecules had a high tendency to form crystalline helical complexes on cooling resulting in retrogradation. In our experiments, after DMSO dissolution and prior to enzyme hydrolysis, cloudy aqueous suspensions of high-amylose starches were observed that could be attributed to slight retrogradation in the samples. Care was taken to avoid time lag in subjecting the starch solutions to enzyme hydrolysis after DMSO dissolution.

Recent studies by Han et al (2004) have proposed that structural changes occurred when starches were subjected to heating and stirring in dimethyl-sulfoxide (DMSO) solution. Starches with greater percentage of amylopectin were much harder to dissolve and, on the other hand, excessive boiling (2h or longer), mechanical stirring and autoclaving (121°C, 15 min) caused starch chain degradation (Han et al 2004). Morrison (1995) reported that high-amylose corn starches have higher lipid contents than waxy starches. Dimethyl-sulfoxide (DMSO) is an efficient solvent to remove lipids and thus the formation of amylose-lipid complex in starch solution had been eliminated.

Table 2. Dextrose Equivalent (DE) values of five starch hydrolysates of *Bacillus licheniformis* α-amylase at different time intervals

Time	Dextrose Equivalent (DE)				
(min)	Normal	Waxy	*ae* Waxy	Hylon V	Hylon VII
15	1.9±0.1[a]	1.5±0.1	1.8±0.0	1.9±0.0	1.9±0.0
30	3.6±0.1	2.7±0.0	3.0±0.1	3.1±0.1	3.5±0.1
60	6.7±0.3	5.3±0.1	5.6±0.1	6.0±0.8	5.9±0.0
90	8.9±0.1	7.2±0.3	7.4±0.4	8.2±0.3	8.4±0.4

[a] Mean of duplicate ± Standard deviation.

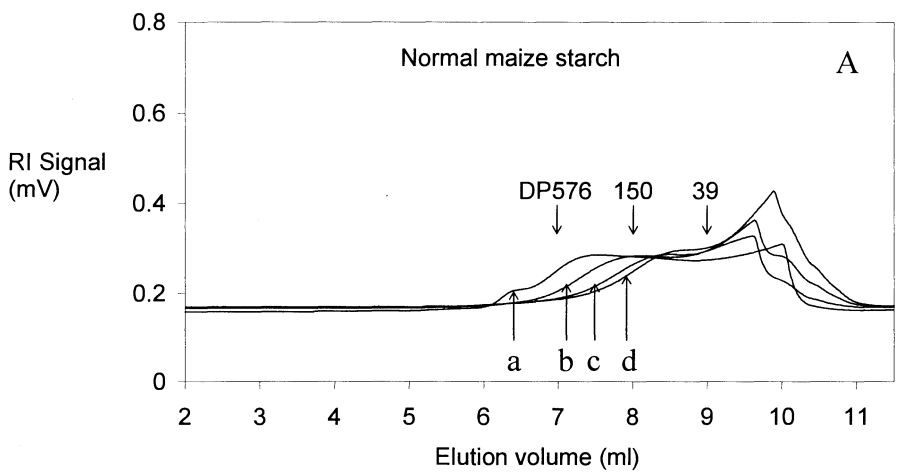
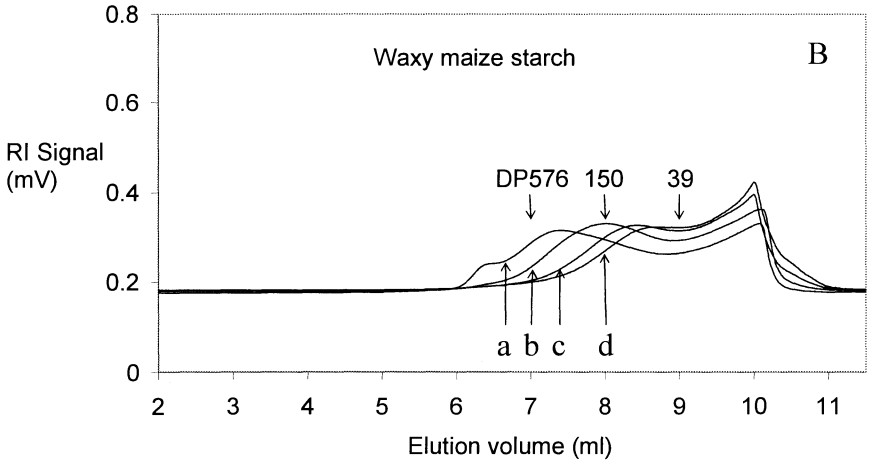


Figure 1. HPSEC profiles of maize starch mutants produced by *Bacillus licheniformis* hydrolysis at different incubation times. a: 15 min; b: 30 min; c: 60 min; d: 90 min. A. Normal maize, B. Waxy maize, C. ae waxy maize, D. Hylon V maize & E. Hylon VII maize. (DP: degree of polymerization)



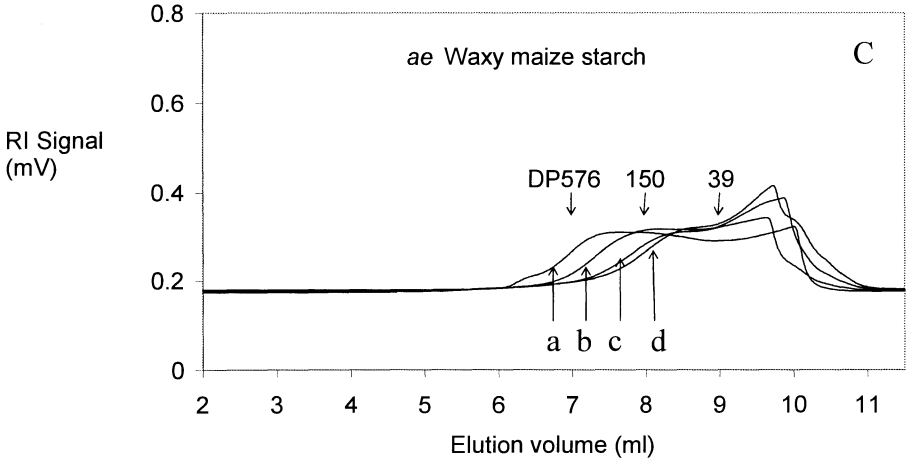
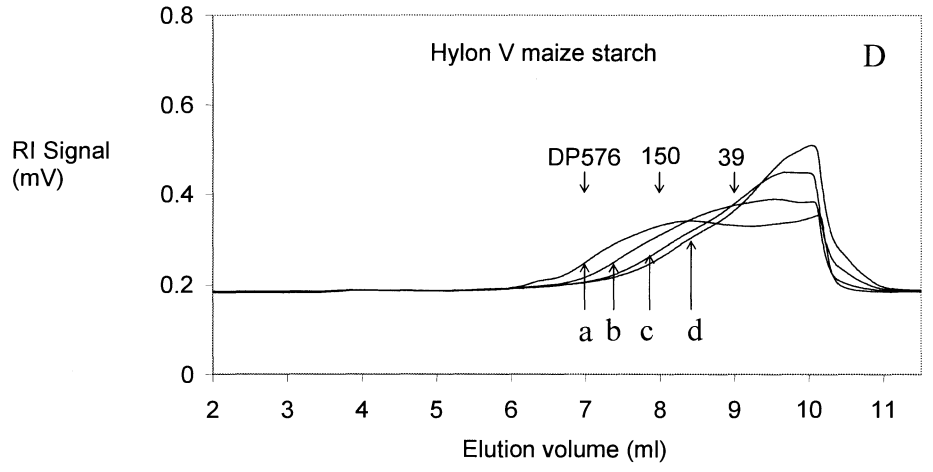


Figure 1. (continued)



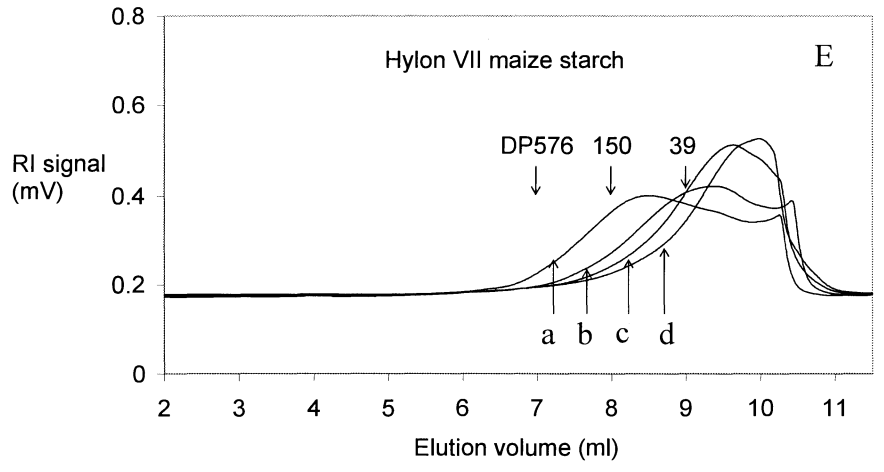


Figure 1. (continued)

HPSEC results showed that the normal, waxy and ae-waxy maize (Figure 1. A, B & C) starches had larger proportions of molecules ranging between DP 39 and DP 150 than did high-amylose starches at the end of 60min hydrolysis time. In high amylose starches, larger molecular weight fragments were digested to a greater extent compared with other starches. Hylon V starch hydrolysates showed a similar chromatographic profile with Hylon VII starch hydrolysates (Figure 1. D & E). This could be attributed to the reason that the highly branched waxy amylopectin consists of more α-1-6 branch linkages that affect α-amylase digestion. Also, it is much harder for the α-amylase to act on the short chains attached to the α-1-6 branch linkages. Amylopectins of waxy, normal and ae-waxy starch had more short chains than did high-amylose starches (Jane et al 1999). On the other hand, high-amylose starches with much longer branch chains could be digested by α-amylase to a greater extent producing lower molecular weight products shown in the figures D & E. Therefore, waxy starches with lower hydrolysis rates had larger proportions of high molecular weight products than did high-amylose starches.

Oligosaccharides present in the α-amylase hydrolysates of starch separated by thin layer chromatography (TLC) ranged from G2 – G17 (Figure 2). Previous studies reported that the *Bacillus licheniformis* enzyme mainly produced malto-triose (G3) and malto-pentaose (G5) (Blackeney and Stone 1985, Saito 1973). Ivanova et al (1991) reported that *Bacillus licheniformis* α-amylase produced five major malto-oligosaccharides ranging from maltose to malto-hexaose with the dominant products as maltose, malto-pentaose (DP5) and malto-triose (DP3).

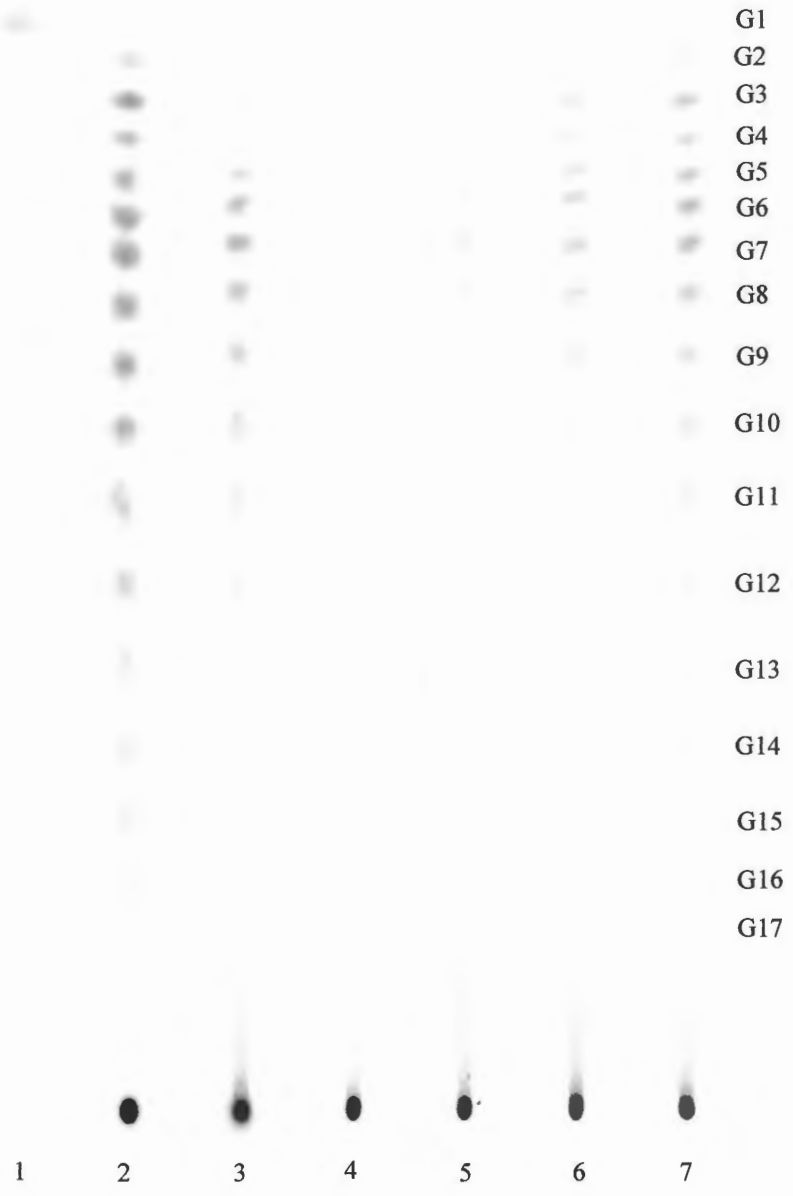
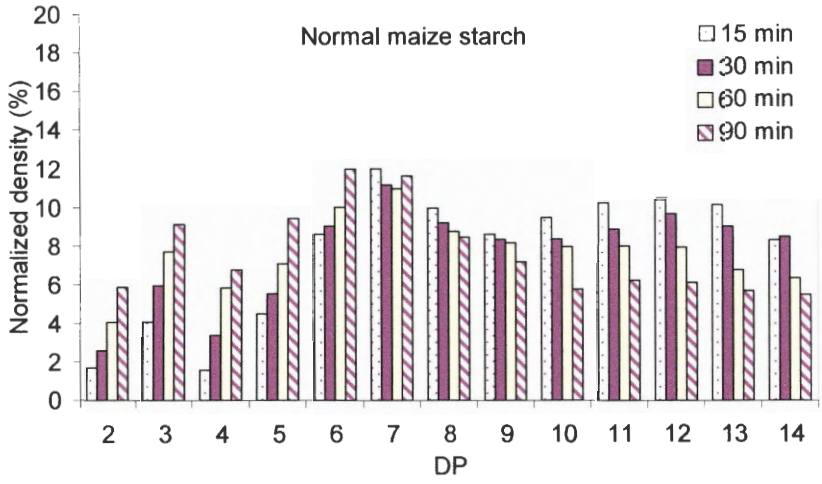


Figure 2. TLC chromatograms of D-glucose (lane 1), debranched maltodextrin (lane 2), maltodextrin (lane 3), and enzyme hydrolysates of normal maize starch at different time intervals (lane 4, 5, 6, and 7 for 15 min, 30 min, 60 min, and 90 min, respectively)

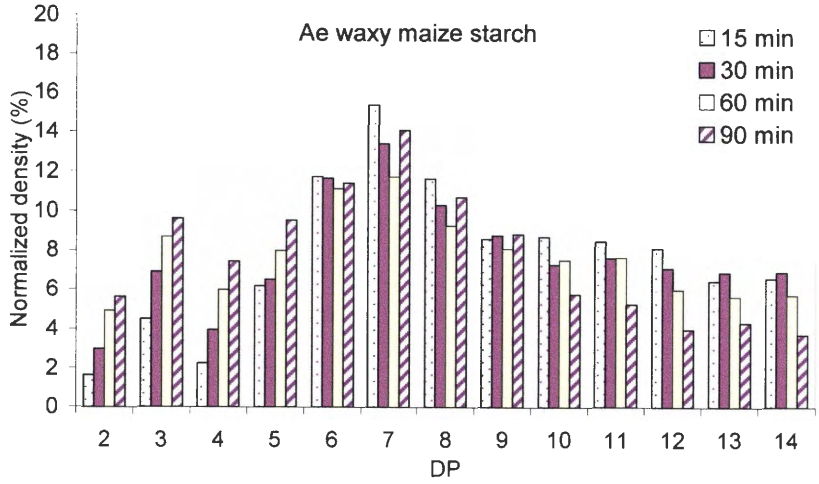
A



B

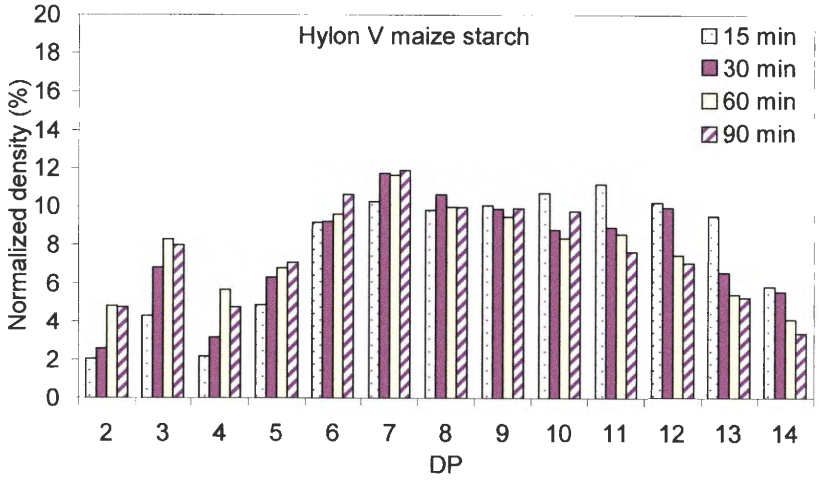
Figure 3. Normalized molecular weight distributions of oligosaccharides produced by *Bacillus licheniformis* hydrolysis of maize starch mutants and separated by TLC. The concentration was determined by using a densitometer. A. Normal maize, B. Waxy maize, C. Ae waxy maize, D. Hylon V maize & E. Hylon VII maize.

C



Degree of Polymerization

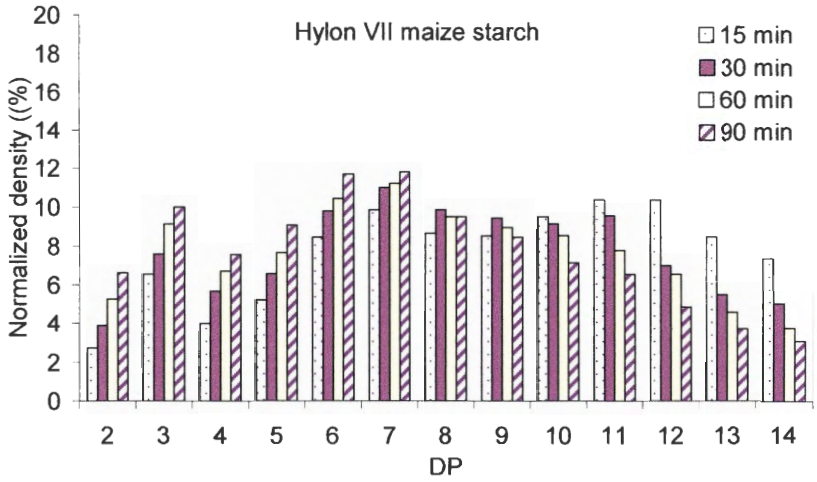
D



Degree of Polymerization

Figure 3. (continued)

E



Degree of Polymerization

Figure 3. (continued)

In this study, TLC results showed that the most dominant products of all the starches with dextrose equivalents (DE) ranging between 7 and 10 were observed to be malto-hexaose (G6) and malto-heptaose (G7). The densitometric analysis showed that the oligosaccharides with larger intensities were G3, G5, G6, G7 & G8 (Figure 3). It has been proposed that the oligosaccharides like maltose and malto-triose complexed with the enzyme and result in change in the adsorption equilibrium. The oligosaccharides acted as competitive inhibitors in solution and retarded the rate of glucose formation (Franco et al 1987, Fujii and Kawamura 1984, Planchot et al 1994).

The concentration of the products ranging between G2 to G6 increased but G8 to G12 decreased as the reaction time progressed from 15min to 90min (Figure 4). The concentration of oligosaccharides from G2 to G5 were to a greater extent in waxy, ae-waxy and normal

starches compared with that of high-amylose starches at the 90 min time interval. This might be because α-amylase has hydrolyzed most of the short chains of waxy starches producing low DP products to a greater extent than the high-amylose starches. Similar product profile has been observed in the HPAEC results. Waxy starch displayed a higher concentration of low molecular weight products (DP 2 – DP 8) than did the other starches (Figure 4. B). HPAEC results of 90 min enzyme hydrolyzed solutions showed that the high-amylose V & VII and ae-waxy starches had a greater proportion of molecules ranging between DP 22 – DP 40 than did normal and waxy starches (Figure 4). This could be attributed to the fact that α-amylase produced larger DP molecules from the long chains of high-amylose starches. However, HPSEC results showed that the waxy starches with a lower degree of hydrolysis displayed larger molecules ranging between DP 39 and DP 150 to a greater extent than high-amylose starches.

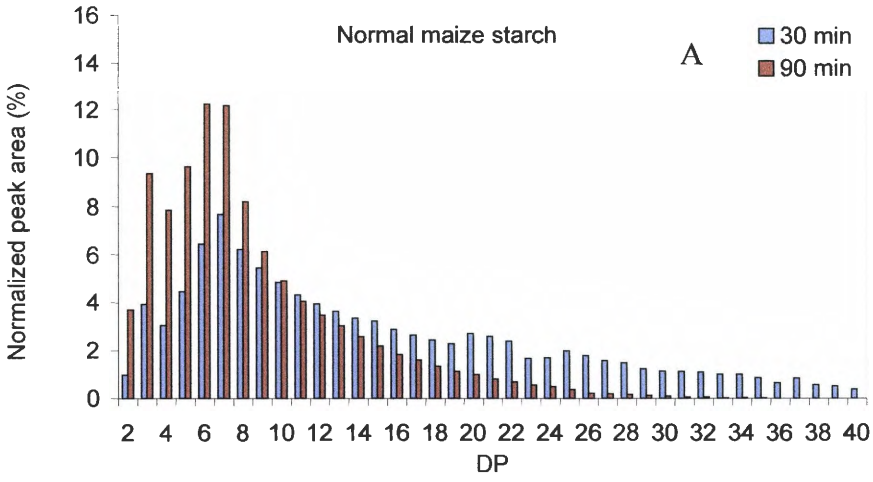
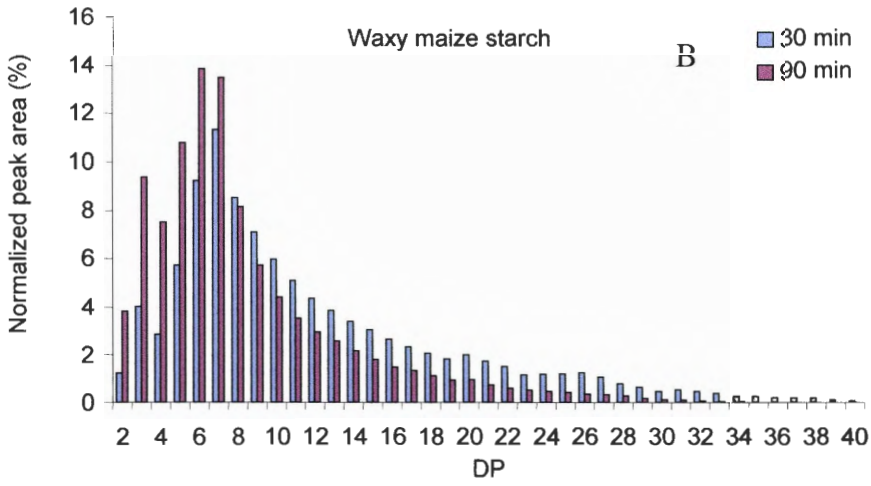
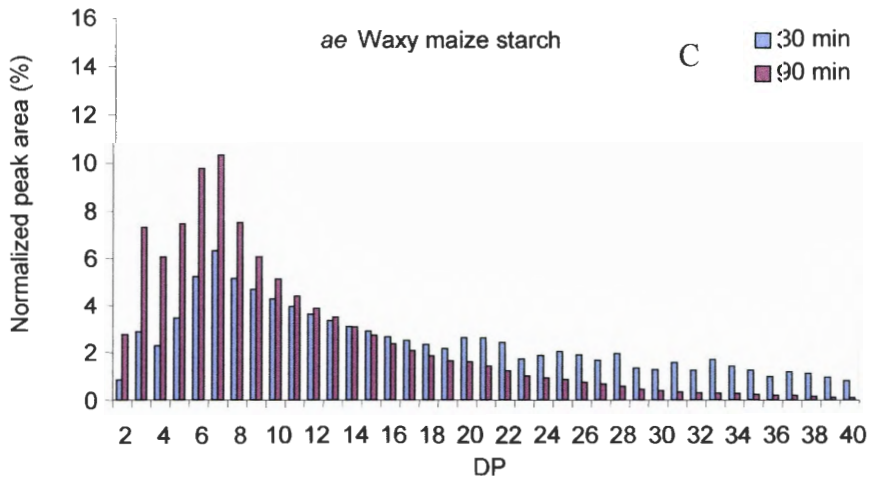


Figure 4. Normalized peak area distributions produced by *Bacillus licheniformis* hydrolysis of maize starch mutants determined by HPAEC-ENZ-PAD. A. Normal maize, B. Waxy maize, C. Ae waxy maize, D. Hylon V & E. Hylon VII.

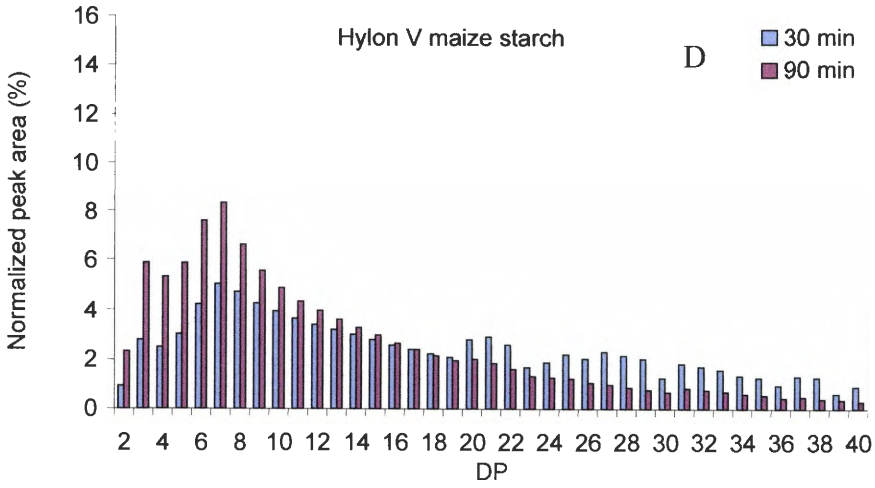


Degree of Polymerization

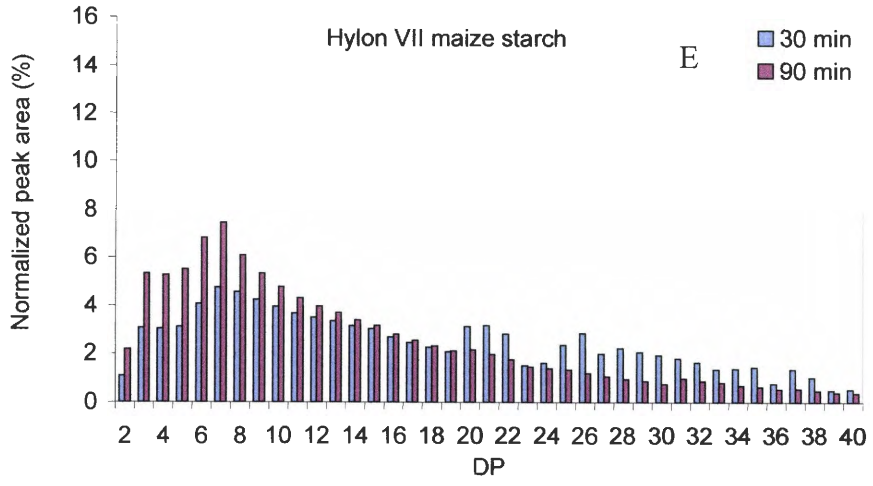


Degree of Polymerization

Figure 4. (continued)



Degree of Polymerization



Degree of Polymerization

Figure 4. (continued)

Part I. Summary

Waxy maize starches displayed lower hydrolysis rates compared to non-waxy starches. Comparatively, ae-waxy starch hydrolyzed to a slightly larger degree than waxy maize starch. HPSEC results showed that waxy maize starches with lower degree of hydrolysis displayed larger molecules ranging between DP 39 and DP 150 to a greater extent than high-amylose starches. TLC results showed that the oligosaccharides present in the α-amylase hydrolysates ranged from G2 – G17. *Bacillus licheniformis* α-amylase hydrolysis of maize starch produced malto-hexaose (G6) and malto-heptaose (G7) as the major products. HPAEC results of 90 min enzyme hydrolyzed solutions showed that the high-amylose V & VII and ae-waxy maize starches had greater proportion of molecules ranging between DP 22 – DP 40 compared with normal and waxy starches.

Part II. Enzyme digestibility of starch in dry-milled corn flours

Dry-milled corn flours of varying protein (5.3-13.0%) and oil contents (3.0-10.9%) were subjected to enzyme hydrolysis using α-amylase, glucoamylase (*Aspergillus niger*) and pullulanase. Effects of oil and protein contents of corn on the enzyme digestibility rate are shown in figures 5 & 6. Glucose yields of the 24h hydrolysates showed that the corn flour with higher oil contents displayed lower glucose yields ($r = -0.66$, p-value = 0.0002). However, varying protein contents did not affect the enzyme hydrolytic rate ($r = 0.06$, p-value = 0.75) (Table 3). Tester and Morrison (1990) reported that substantial amounts of water-soluble lipid-free amylose are released from normal cereal starches when subjected to heat treatment in water. The amylose leached during the heating process can readily complex with the lipid and slows down the enzyme hydrolytic rate (Seneviratne and Biliaderis 1991).

Cao et al (1996) conducted similar enzyme hydrolysis studies and reported that the effect of lipid on the hydrolytic rate was eliminated after corn oil and zein protein were extracted from yellow dent corn flour. Hanna and Lelievre (1975) reported that the starch damaged in the process of wheat flour milling was highly susceptible to formation of lipid-complexes and inhibited the enzyme hydrolysis of starch. Amylopectin contributes to swelling, whereas amylose and lipid complex inhibits swelling (Jane et al 1999, Tester and Morrison 1990). In our experiments the temperature of corn flour-enzyme suspensions was maintained at 105-110°C. It was previously reported that thermo-stable α-amylase (Termamyl 120L) showed higher efficiency at a maximum temperature of 110°C than at 105 °C (Cao et al 1996). The Type II amylose-lipid complexes formed after starch gelatinization usually dissociate at higher temperatures ranging between 100-125°C (Morrison 1995).

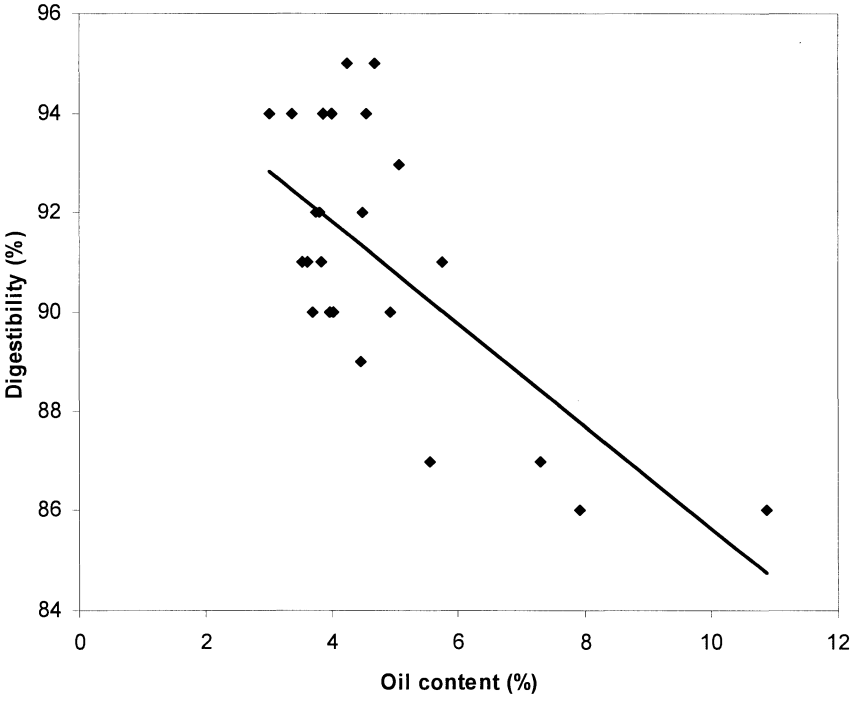


Figure 5. Enzyme digestibility of starch of dry-milled corn with various oil contents

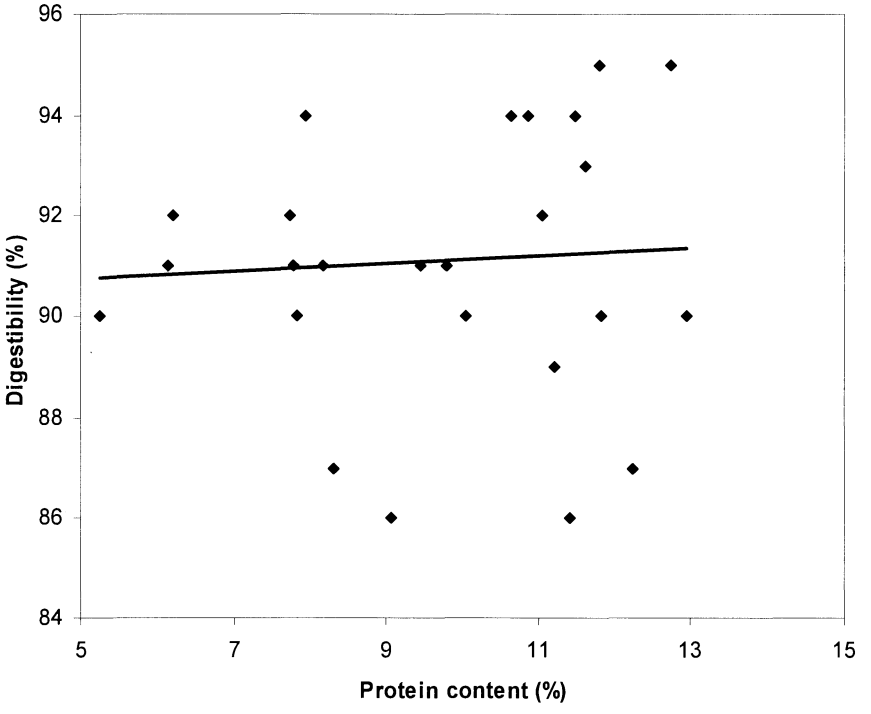


Figure 6. Enzyme digestibility of starch of dry milled corn with various protein contents

Table 3. Protein and oil contents and enzyme digestibility of corn flour[a]

Sample	Protein Content (%)	Oil Content (%)	Amylose Content (%)	Dextrose Equivalent (DE) (%)
091	11.8	4.7	28.8±0.1	95±2.1
093	12.7	4.2	ND[b]	95±2.8
238	6.2	3.7	28.5±0.5	92±2.1
308	9.1	7.9	23.4±0.1	86±4.9
090	11.6	5.1	25.2±0.8	93±2.1
412	6.1	5.8	24.6±1.4	91±0.0
087	12.2	5.6	28.4±1.5	87±4.2
085	11.0	4.5	29.3±1.8	91±0.7
413	8.2	3.6	6.7 ± 0.0	91±1.4
062	5.3	3.7	25.7±0.3	90±1.4
560	8.3	7.3	34.6±0.7	87±0.7
728	11.4	10.9	23.4±1.9	86±0.0
239	9.5	3.8	26.1±0.5	91±1.4

[a] Average of two replicates
[b] Not determined

Minor components like protein and lipids affect the color and flavor of the final glucose syrup quality (Bowler et al 1985). Impurities in whole grain flour can inactivate the enzyme and slow down the hydrolysis rate compared with that of the pure starch (Kearsley and Nketsia-Tabiri 1979). Cellulose fiber in ground corn flour, being hydrophilic in nature, competes with starch for water and impedes the enzyme diffusion rate. Non starch constituents like cell walls and proteins entrap the starch and act as a physical barrier to the enzyme hydrolysis (Seneviratne and Biliaderis 1991).

Effect of starch properties on the glucose yields obtained from enzyme hydrolysis of corn flour

Starch was isolated from selected corn lines that gave different glucose yields by a combination of α-amylase, glucoamylase and pullulanase hydrolysis. The gelatinization and pasting properties of the starch samples were determined and related to the glucose yield. Gelatinization properties of the selected corn starches measured by differential scanning calorimeter (DSC) are shown in Table 4. Starch onset gelatinization temperature (To) had a negative correlation to the enzyme hydrolysis rate ($r = -0.63$, p-value = 0.02). The onset gelatinization temperatures varied from 55.1 to 68.4 °C and the enthalpy changes varied from 9.6 to 13.9 J/g. Among the starches, samples 091 and 093 with lower onset gelatinization temperatures (55.1 and 57.2 °C, respectively) displayed higher glucose yields than other starch samples. The starches also had lower enthalpy changes of 10.1 and 9.6 J/g, respectively, compared with other starches. Lower gelatinization temperature and enthalpy changes suggested that the order and the degree of crystallinity are lower than other starches. Amylopectin long branch chain-lengths affect the starch gelatinization properties. Starches

with a larger percentage of short branch-chains had smaller size crystallites, which resulted in decreased onset gelatinization temperatures and enthalpy changes (Jane et al 1999, Perera et al 2001).

Table 4. Thermal properties of gelatinization of selected corn starches by DSC[a]

Sample	Gelatinization			
	T_o (°C)	T_p (°C)	T_c-T_o (°C)	ΔH (J/g)
091	55.1±0.4	66.7±0.8	18.7	10.1±0.5
093	57.2±0.2	68.3±0.4	17.8	9.6±0.5
238	60.0±0.5	65.8±0.3	11.6	11.5±0.2
308	60.5±0.2	66.4±0.2	11.6	10.8±0.7
090	60.6±0.1	68.3±0.1	12.9	11.4±0.6
412	61.1±0.1	67.6±0.3	12.2	11.8±0.4
087	61.7±0.9	68.8±0.1	12.7	11.7±0.7
085	61.7±0.5	68.0±0.5	11.6	10.7±0.5
413	62.0±0.4	68.6±0.2	13.3	12.1±0.5
062	64.0±0.3	68.7±0.4	9.4	11.4±0.4
560	66.7±0.2	72.2±0.3	10.3	11.4±0.3
728	67.5±0.8	72.4±0.4	10.7	12.9±0.4
239	68.4±0.5	72.5±0.4	9.2	13.9±0.3

[a] T_o, T_p and T_c = onset, peak and conclusion temperatures (°C) of endotherm.
ΔH = enthalpy change of gelatinization.
Values are mean ±standard deviation

Thermal properties of the retrograded corn starches are shown in Table 5. The onset thermal transition temperatures of the dissociation of retrograded starches ranged between 37.2 to 42.5 °C and were lower than the onset gelatinization temperatures of their native counterparts. Sample 413 with zero amylose content displayed the lowest retrogradation rate (R % = 32.2). Enthalpy change of dissociation of retrograded starch for sample 413 was lower than other starches (Table 5).

Table 5. Thermal properties of retrogradation of selected corn starches by DSC[a]

Sample	Retrogradation				
	T_o (°C)	T_p (°C)	T_c-T_o (°C)	ΔH (J/g)	R (%)
091	40.2±0.9	49.0±0.5	19.4	4.2±0.6	41.6
093	40.5±1.1	50.9±0.2	21	5.2±0.5	54.2
238	38.6±0.2	49.1±0.2	21.5	4.3±0.1	37.4
308	38.5±0.5	48.7±0.4	21.5	4.6±0.4	42.6
090	42.5±0.7	52.0±0.6	18.2	4.9±0.7	43.0
412	40.7±0.7	51.4±1.0	19.3	4.8±0.1	40.7
087	39.6±0.8	50.5±0.4	20.4	8.3±0.9	70.9
085	41.8±0.2	51.1±0.4	17.4	5.1±0.6	47.7
413	37.5±0.0	52.7±0.0	23.5	3.9±0.3	32.2
062	37.2±0.5	48.3±0.3	22.1	5.2±0.3	45.6
560	37.2±0.3	49.7±0.4	23.8	5.8±0.3	50.9
728	37.3±0.4	48.7±0.0	23.2	6.2±0.2	48.1
239	36.7±1.4	50.2±0.9	26	7.7±0.9	55.4

[a] T_o, T_p and T_c = onset, peak and conclusion temperatures (°C) of endotherm.
ΔH = enthalpy change of dissociation of retrograded starch.
%R = (enthalpy change of retrograded starch/enthalpy change of native starch) ×100.
Values are mean ±standard deviation

Pasting profiles and viscosity of the corn starches are shown in Figure 7 and Table 6. Starch pasting temperature had a negative correlation to the enzyme hydrolysis rate (r = -0.66, p-value = 0.01). Pasting temperatures varied between 62.6 to 73.6 °C and peak viscosities varied between 127 to 246.6 RVU. Sample 413, a waxy starch, displayed the highest peak viscosity (246.6 RVU) and breakdown (157.7 RVU) and the lowest setback (25.0 RVU) than other starches. Starch samples 560 and 728 with higher onset gelatinization and pasting temperatures displayed lower glucose yields. Tester and Morrison (1990) reported that the extent of swelling of cereal starch granules is highly correlated with the leaching of amylose. Non–starch polysaccharides like hemi-celluloses in flours could compete with starch for moisture and affect the swelling and gelatinization behavior of starches (Jane et al 1992).

Table 6. Pasting properties of selected corn starches

Sample[a]	Pasting Temperature (°C)	Viscosity (RVU)[b]			
		Peak	Breakdown	Final Visc	Setback
091	69.1	127	39.3	178.5	90.8
093	62.6±9.3	139.7±1.1	51.5±1.4	174.8±0.9	86.6±1.6
238	69.4±0.3	132.8±0.7	38.4±0.3	166.1±0.3	71.7±0.7
308	71.3±0.2	148.0±2.7	50.5±0.7	174.1±2.4	76.6±0.4
090	69.4±0.4	170.0±1.5	53.5±0.1	214.0±0.7	97.4±0.8
412	71.5±0.0	145.2±0.5	56.2±1.9	158.2±2.2	69.2±0.2
087	72.0±0.6	149.9±0.1	43.1±0.2	196.0±1.6	89.3±1.3
085	72.5±0.2	145.9±2.8	46.2±0.6	192.7±0.7	93.0±2.9
413	67.4±0.3	246.6±3.1	157.7±7.7	114.0±2.2	25±2.4
062	72.5±0.3	135.5±0.3	42.8±5.8	169.9±2.1	77.1±4.0
560	71.9±0.6	143.7±0.3	51.4±1.1	176.3±0.9	84.0±2.2
728	73.6±0.1	141.0±1.4	43.3±5.0	151.7±4.5	54.0±1.8
239	73.2±0.1	146.2±0.1	40.7±0.8	178.8±0.4	73.3±1.2

[a] Starch sample suspensions were 8% (w/w, dsb).
[b] Measured in Rapid Visco-Analyzer units.

Native starch structures and properties affect the enzyme hydrolytic rate; however, gelatinization and pasting behavior of starches in dry-milled flours varied with the effect of minor components like lipid, protein and fiber.

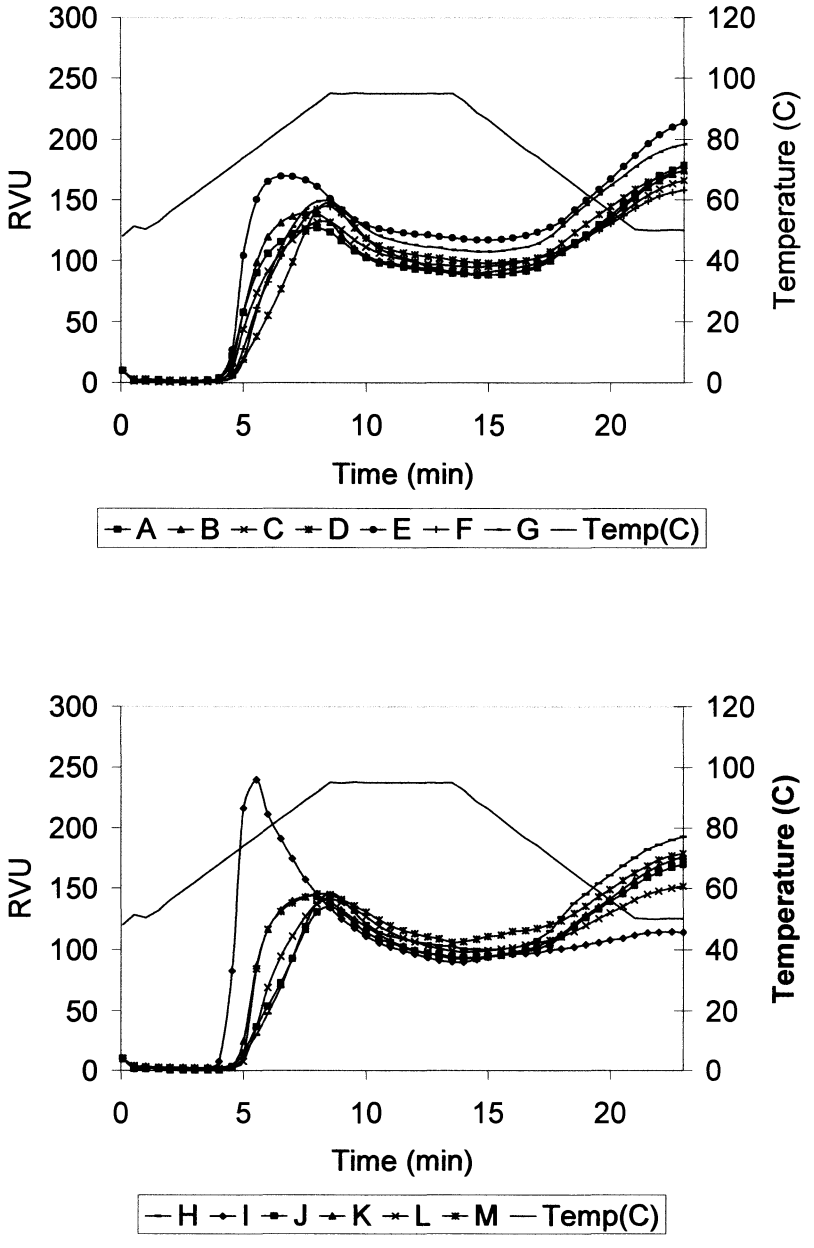


Figure 7. Pasting profiles of corn starch measured by Rapid Visco Analyzer. A. 091, B. 093, C. 238, D. 308, E. 090, F. 412, G. 087, H. 085, I. 413, J. 062, K. 560, L. 728, M. 239.

Part II. Summary

Glucose yields of the 24h hydrolysates showed that the corn flour with higher oil contents displayed lower glucose yields. However, varying protein contents did not affect the enzyme hydrolytic rate. The onset gelatinization temperatures (To) of starches from dry-milled corn varied from 55.1 to 68.4 °C and the enthalpy changes varied from 9.6 to 13.9 J/g. Starch samples 091 and 093 with lower onset gelatinization temperatures (55.1 and 57.2 °C, respectively) displayed higher glucose yields than other starch samples. The onset thermal transition temperatures of the dissociation of retrograded starches ranged between 37.2 to 42.5 °C. Starch pasting temperatures varied between 62.6 to 73.6 °C and peak viscosities varied between 127 to 246.6 RVU. Starch samples 560 and 728 with higher pasting temperature displayed lower glucose yields.

CONCLUSIONS

Dispersed normal, hylon V, hylon VII maize starches, which contain amylose, displayed greater enzyme hydrolysis rates than did waxy starches. In this experiment, *Bacillus licheniformis* α-amylase hydrolysis of maize starch produced malto-hexaose (G6) and malto-heptaose (G7) as the major products. The enzyme hydrolytic rate of starch in dry-milled corn flour was negatively correlated with oil content ($r = -0.66$, p-value = 0.0002). Varying protein contents did not have an effect on the enzyme hydrolytic rate ($r = 0.06$, p-value = 0.75). Starch with lower onset gelatinization temperatures displayed higher enzyme hydrolytic rates in dry-milled corn flour ($r = -0.63$, p-value = 0.02). Starch pasting temperature was negatively correlated to the enzyme hydrolysis rate ($r = -0.66$, p-value = 0.01).

APPENDIX: ADDITIONAL DATA

Corn starch pasting characteristics of the population of genomic inbred lines

Corn starch pasting properties of the population of genomic inbred lines are summarized in Table 6 and Figure 8. The peak viscosity and pasting temperature ranges of the starch samples were 125–173 RVU and 70.8–75.0 °C, respectively. Breakdown, final and setback viscosities ranged from 20–77 RVU, 150–213 RVU and 66–105 RVU, respectively.

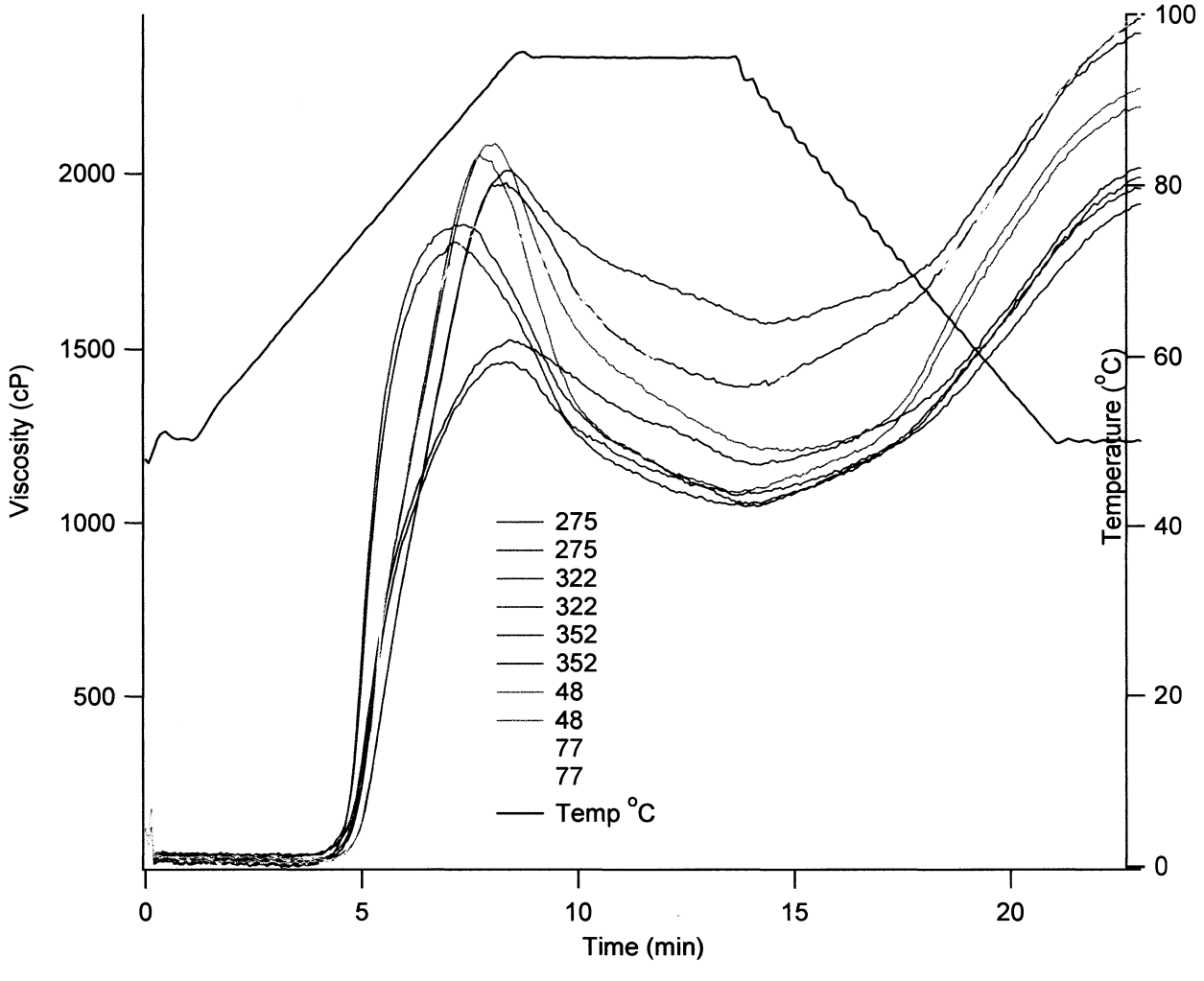


Figure 8. Corn starch pasting profiles of the population of genomic inbred lines measured by Rapid Visco Analyzer

Table 7. Corn starch pasting properties of the population of genomic inbred lines

Sample[a]	Pasting Temperature (°C)	Viscosity (RVU)[b]			
		Peak	Breakdown	Final Visc.	Setback
326-1	72.7±0.0	155.0±0.9	59.0±2.1	175.0±1.4	79.0±2.5
383	73.6±0.6	147.0±1.2	50.0±1.1	176.0±0.6	78.0±0.5
309	74.1±0.3	135.0±0.1	31.0±0.4	187.0±0.8	83.0±1.1
272	75.0±0.3	143.0±0.9	49.0±8.3	179.0±1.7	85.0±5.7
288	72.9±0.3	138.0±0.2	49.0±0.2	167.0±2.1	78.0±2.1
382	71.6±0.6	147.0±1.1	59.0±0.9	172.0±0.8	85.0±1.2
311	72.9±0.2	137.0±0.4	43.0±4.3	173.0±1.1	79.0±3.5
298	73.8±0.3	144.0±0.1	33.0±0.5	201.0±1.3	88.0±2.9
310	74.8±0.1	157.0±1.5	45.0±1.7	185.0±0.3	73.0±0.4
269	74.5±0.3	162.0±0.4	65.0±5.2	177.0±3.3	80.0±1.5
267	70.8±0.6	148.0±0.9	33.0±0.8	199.0±1.1	84.0±2.8
369	74.0±0.1	145.0±0.3	49.0±5.5	168.0±3.5	72.0±2.4
378	74.0±0.0	161.0±3.3	43.0±6.4	213.0±4.4	96.0±5.3
379	71.1±0.6	165.0±2.4	55.0±0.9	199.0±0.8	89.0±0.6
40	73.7±0.3	157.0±3.5	74.0±1.1	174.0±3.2	91.0±0.8
46	73.5±0.0	140.0±2.3	46.0±10.4	173.0±2.2	79.0±6.0
51	71.4±0.2	139.0±1.6	42.0±0.7	172.0±2.5	74.0±0.2
44	74.4±0.0	143.0±0.7	40.0±0.2	185.0±0.9	82.0±0.1
43	73.5±0.2	156.0±0.8	59.0±1.5	177.0±0.7	80.0±0.1
45	74.4±0.0	134.0±0.3	33.0±1.1	171.0±0.2	70.0±1.1
57	73.3±2.5	138.0±0.5	51.0±0.5	170.0±1.5	84.0±1.4
58	72.3±0.6	149.0±2.5	56.0±1.4	174.0±0.1	81.0±1.1
35	73.4±0.4	148.0±3.3	53.0±2.8	177.0±7.4	82.0±1.3
33	73.1±0.0	146.0±0.4	47.0±1.3	178.0±1.2	80.0±2.8
32	72.4±0.0	141.0±0.4	41.0±6.0	177.0±2.8	77.0±2.9
30	73.6±0.0	133.0±2.2	49.0±6.8	150.0±7.1	66.0±1.9
31	71.9±0.5	137.0±1.8	40.0±3.4	167.0±8.2	70.0±3.0
29	72.1±0.8	147.0±3.9	55.0±1.6	172.0±3.5	80.0±1.1
27	74.5±0.3	131.0±1.6	20.0±3.4	192.0±3.5	80.0±1.7
262	73.3±0.3	149.0±0.4	51.0±5.1	181.0±18.2	83.0±13.4
337	73.0±0.3	161.0±0.6	64.0±3.0	194.0±3.1	97.0±0.7

Table 7. (continued)

Sample[a]	Pasting Temperature (°C)	Viscosity (RVU)[b]			
		Peak	Breakdown	Final Visc.	Setback
276	74.8±0.0	142.0±0.9	46.0±6.9	175.0±2.8	80.0±3.2
275	73.8±0.3	166.0±2.1	42.0±8.5	202.0±2.4	79.0±8.2
48	72.1±0.3	173.0±2.1	77.0±4.9	185.0±3.1	89.0±3.9
360	71.8±0.3	151.0±0.3	60.0±0.0	175.0±0.7	83.0±1.0
25	73.4±0.4	147.0±0.8	44.0±0.5	208.0±0.9	105.0±1.2
28	74.2±0.2	153.0±4.5	46.0±2.2	193.0±5.8	86.0±0.9
10	72.8±0.0	138.0±1.1	37.0±5.1	184.0±1.9	83.0±4.2
5	73.3±0.3	158.0±0.1	51.0±0.9	196.0±1.7	89.0±0.9
17	72.3±1.1	133.0±2.9	41.0±4.1	187.0±0.2	95.0±1.1
52	73.1±0.0	136.0±0.9	35.0±1.4	190.0±0.3	88.0±0.7
67	73.0±0.2	138.0±3.3	45.0±2.5	166.0±2.9	74.0±2.9
75	73.1±0.0	126.0±2.4	39.0±0.4	163.0±2.2	76.0±0.5
341	75.0±0.3	152.0±2.5	39.0±2.6	204.0±3.4	91.0±1.7
346	73.6±0.0	166.0±2.7	70.0±2.7	176.0±1.7	80.0±1.7
264	73.1±0.1	149.0±3.2	37.0±8.1	213.0±6.1	101.0±5.2
79	73.7±0.3	140.0±1.5	40.0±7.7	184.0±2.8	84.0±6.4
326-2	73.1±0.0	145.0±1.1	51.0±0.4	177.0±2.0	83.0±0.9
24	72.1±0.3	150.0±1.7	56.0±8.6	178.0±9.1	85.0±1.2
15	72.9±0.2	139.0±0.1	54.0±6.5	158.0±4.8	73.0±1.8
1	72.3±0.1	141.0±0.5	33.0±0.8	189.0±1.9	80.0±2.2
60	73.0±0.3	137.0±0.5	38.0±0.7	177.0±0.2	78.0±1.0
66	71.9±0.5	146.0±1.3	46.0±3.9	182.0±4.8	82.0±0.4
77	72.6±0.8	157.0±0.0	42.0±5.4	205.0±0.4	91.0±6.3
7	74.3±0.0	157.0±3.4	50.0±1.9	186.0±4.2	79.0±3.2
21	72.1±0.3	143.0±3.5	26.0±0.4	203.0±3.8	86.0±0.7
352	71.5±0.0	125.0±3.7	32.0±3.3	163.0±6.7	72.0±1.0
323	72.4±0.0	138.0±0.2	37.0±1.1	186.0±0.1	85.0±1.4
76	73.3±0.3	163.0±7.1	51.0±4.1	204.0±8.3	92.0±2.9
281	73.1±0.0	159.0±3.4	49.0±3.2	196.0±5.0	85.0±1.6
326-3	73.1±0.0	145.0±1.5	51.0±0.4	177.0±2.0	83.0±0.9

Table 7. (continued)

Sample[a]	Pasting Temperature (°C)	Viscosity (RVU)[b]			
		Peak	Breakdown	Final Visc.	Setback
325	73.7±0.4	147.0±1.5	54.0±6.7	172.0±7.7	79.0±0.5
322	71.2±0.5	155.0±0.0	66.0±1.5	165.0±1.9	76.0±3.2
365	72.4±0.1	148.0±2.5	55.0±2.4	168.0±0.9	75.0±0.9
265	72.4±0.0	146.0±4.6	36.0±2.4	187.0±3.2	78.0±1.0
321	73.6±0.0	156.0±0.0	55.0±2.5	190.0±0.3	89.0±2.2
68	73.6±0.6	150.0±1.8	43.0±8.5	190.0±4.9	82.0±5.3
74	73.1±0.0	153.0±1.2	53.0±0.8	187.0±1.5	87.0±0.5
354	70.8±3.5	141.0±3.8	55.0±2.7	164.0±6.3	78.0±0.2
328	73.6±0.6	143.0±3.4	46.0±1.0	169.0±5.9	72.0±1.5
14	73.8±0.3	151.0±1.8	56.0±0.5	173.0±2.1	78.0±0.9
12	71.8±0.3	141.0±2.0	48.0±1.1	163.0±1.7	71.0±0.8
296	74.8±0.0	147.0±2.5	52.0±4.6	176.0±5.5	80.0±1.6
297	72.5±0.2	133.0±0.1	41.0±0.7	168.0±1.8	76.0±1.8
8	70.8±0.0	135.0±1.3	41.0±0.7	172.0±2.5	78.0±3.2

[a] Starch sample suspensions were 8% (w/w, dsb).
[b] Measured in Rapid Visco-Analyzer units.

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