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Development and characterization of corn lines with new starch properties

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Development and characterization of corn lines with new starch properties

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Yulin Ji

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ABSTRACT

The objectives of this research were to evaluate functions and structures of starches from exotic x adapted inbred lines and exotic breeding crosses (exotic populations x adapted lines), and to establish relationships between the fine structure and functional properties of the starches. A secondary objective was to confirm the advancement of selected functional traits into the next generation of corn.

A small-scale corn-starch extraction procedure optimized in this research was used to extract starch from corn kernels. To optimize the starch extraction procedure, effect of different starch extraction procedures on starch yield, protein content, and thermal properties were studied. Starch yield and protein content were significantly altered by the experimental treatments. Treatments involving more kernels and/or sedimentation rather than centrifugation, yielded starch with the lowest protein content. Soaking the seeds for less than 24 hrs is preferred if minimizing annealing is desired.

Starches from several developmental lines with unusual thermal properties as measured on a Differential Scanning Calorimeter (DSC, gelatinization onset temperature (T_{oG}) < 60°C or range of gelatinization (R_G) > 14°C) were selected for further characterization. Two independent gelatinization transitions, located in different starch granules, were found in some starches. All selected starches from the developmental lines had greater peak viscosity as measured on a Rapid Visco Analyser (RVA) than did starch from normal corn inbreds Mo17. Significant differences were observed in starch-granule size distributions and shape distributions of the selected starches. Measurements with high-performance anion-exchange chromatography (HPAEC) revealed that all selected unusual starches had a lower normalized concentration of chains with a degree of polymerization (dp) of 15-24 and/or a greater normalized concentration of chains with dp of 6-12. Overall chains with a low T_{oG} had a higher relative concentration of branch chains shorter than dp 13 than did normal starch. These studies will aid in understanding structure-thermal property relationships of starches, and in identifying corn lines of interest for commercial breeding.

To study the effect of environment and genotype on the gelatinization properties of starches from developmental corn lines, starches from developmental corn lines, grown 1) during three successive generations; and 2) in both temperate and tropical environments, were evaluated. Unusual thermal properties (low T_{oG}) were fixed in some progeny lines.

Environmental factors had a significant effect on the thermal properties of starch, and a significant interaction between environment and genotype was observed. These results suggest that incorporation of exotic alleles into Corn Belt germplasm is an excellent means to obtain value-added traits to produce starch with desirable functions.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Starch is the primary source of stored energy in cereal grains. Although the amount of starch contained in grains varies, it is generally between 60 and 75% of the weight of the grain and provides 70-80% of the calories consumed by humans worldwide (Thomas and Atwell 1999). Starch is a valuable ingredient to the food industry. Apart from the nutritional role, starch products function as thickeners of liquid foods, as binding agents in solid foods, as texturizers (to obtain a certain mouth feeling texture), as "fillers" both in liquid foods and powdered foods and as film forming agents.

To improve starch functionality and expand the usefulness of starch, starch is usually chemically modified (such as by cross-linking and/or substitution). However, due to increased consumer and environmental concern, no new derivatives or degree of substitution will be allowed anytime soon even for nonfood applications (BeMiller 1997). A greater potential for the manufacture of modified starches with improved functionalities lies in the use of raw materials from previously uncharacterized plant genotypes. This approach might be especially valuable to the food industries because the corn starch could be used in the manufacture of "all natural" foods.

Exotic (non-Corn Belt Dent) germplasm may be an excellent source for improving quality and, possibly, improved agronomic performance, because much of the corn grown outside the United States is consumed directly by humans and has undergone centuries of selection for flavors, aromas, and textures (Tracy 1990). Exotic germplasm is usually considered to include unadapted domestic populations and foreign temperate, tropical, and semi-tropical populations. The introgression of adapted germplasm with useful genes from exotic corn has successfully altered corn traits and broadened the genetic base through the Germplasm Enhancement of Maize (GEM) project (Pollak and Salhuana 1999).

Several novel GEM adapted by exotic breeding crosses were targeted in our research, because these new crosses can produce starches with improved functional properties, which may have potential applications in food industry. To fully utilize the potential of these GEM materials, it is essential to further characterize the structural and functional properties of starch produced. To produce inbred lines that can be released and used commercially as

breeding lines from these GEM crosses, it is necessary to continue to develop and select lines to genetically "fix" the unusual thermal properties, and evaluate the performance of the selected lines at the different environments.

In this dissertation, development and characterization of corn lines with new starch properties will be presented.

Dissertation Organization

This dissertation is composed of seven parts. The general introduction and a literature review are followed by four papers. The first paper "Optimizing a single-kernel corn-starch extraction method for use in the laboratory" discusses the effect of different starch extraction procedures designed for use in the laboratory on yield, protein content, and thermal properties of corn starch. The procedure optimized in this paper was used in research conducted within the rest of the dissertation. The second and third papers, "Thermal and structural properties of unusual starches from developmental corn lines" and "Structure and function of starch from advanced generation of new corn lines" respectively, focus on characterization of functions and structures of starches from exotic and exotic x adapted inbred lines, and establishment of relationships between the fine structure and functional properties of the starches. The objectives of the fourth paper, "Gelatinization properties of starches from three successive generations of six new corn lines grown in two locations", were to evaluate the intra- and interpopulation variability in thermal properties of starches from exotic and exotic by adapted novel corn lines and their derivatives when grown 1) during two successive years in the same location; and 2) in both temperate (Ames, IA) and tropical (Puerto Rico) environments. The dissertation concludes with a chapter of "General conclusion".

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CHAPTER 2. LITERATURE REVIEW

Starch Structure

Starches from different botanical sources and even from different varieties within a source differ in their properties. The key to understanding the structure-function relationships, the physico-chemical, functional, and even the nutritional properties of starch from various botanical sources is to be found in the chemical structure and composition of the starches, as well as in the organization within the starch granules.

Much progress has been made learning about the understanding of the three dimensional structure of starch. We now have the basic structural information that explains many of its characteristics. One of the low energy conformations of the flexible amylosic chain leads to single strands that readily form rigid double helices. In turn, these pairs associate in pairs that nest together, stabilized by hydrogen bonding and *van der Waals* forces. Finally, these pairs associate to give the A or B structures, depending on their chain length and water content. The branched amylopectin easily forms the double helices, although the branching itself appears to occur in amorphous zones (Imberty et al 1991). Important aspects of starch structure remain unanswered, and "starch remains a beautifully mysterious substance" (BeMiller 1997a).

Amylose and Amylopectin, the Constituent Polymers

Starch granules are typically composed of two types of glucose polymers with different sizes and shapes: amylose and amylopectin. Amylose, the smaller of the two fractions (10^5-10^6 Da; DP 500-5000), possesses very few branches, 9-20 per molecule with chain lengths of between 4 and >100 glucose units (Hizukuri et al 1981). Amylopectin, the larger (10^7-10^9 Da) fraction, is highly branched, 4-5% of its linkages being α (1->6) branch linkages (Manners 1989, Oats 1997).

Structurally and functionally, amylopectin is the more important of the two fractions, because, alone, it is sufficient to generate granules, as occurs in mutant starches that are devoid of amylose. The fine structure of amylopectin has been the subject of many studies and several models have been proposed. In the last 20 years, the "cluster" model proposed by Nikuni (1969) and French(1972), modified by Robin et al (1974) and later reviewed by Linkback (1984), has generally been accepted. In this model, chains in the amylopectin are

classified according to their linkage to the rest of the molecule. The chains include only one C chain, which carries the sole reducing end group; B chains, which are linked to the C chain via the potential reducing end-groups; and A chains, which are linked to B chains in the same way as are A chains and are unbranched. B chains can participate in more than one crystalline amylopectin side-chain clusters, and be classified further to B1-B4 by the number of side chain cluster in which the chain participates (Hizukuri 1986).

After being debranched by using an enzyme such as isoamylase, many amylopectin molecules give a bimodal distribution of chain lengths with degree of polymerization (d.p.) ~40-60 and 15-20, with the shorter chains being the most abundant (Jane et al 1999). The ratio by weight of short to long chains varies between 3:1 and 12:1 depending on the source of the amylopectin (Hizukuri 1985). The major variations in the fine structure of amylopectin are size of molecules, the type of distribution of chain lengths, the ratio of short to long chains, and the chain length.

The amylopectin molecules are fairly large flat discs. Self diffusion data from pulsed-field gradient 1H-NMR indicated that, in DMSO, amylopectin molecules are planar with an axial ratio greater than 6 and that, in water, they are more spherical (Callaghan and Lelievre 1986). Sedimentation coefficient studies showed that amylopectin has an elongated ellipsoidal, flat-sheet or disc-like structure with semi-major and semi-minor axes of 45 and 1.2 nm, respectively, giving a rather linear shape (Callaghan and Lelievre 1987, Lelievre et al 1986).

Starch Granule Structure

The starch granule is nature's chief way to store energy in green plants over long period of time. The granule is well suited to this role. The constituent macromolecules of granules are packaged in a highly ordered and compact manner, resulting in a inert, insoluble-to-water structure but one which is still accessible to the plant's metabolic enzymes. Native granules have crystallinity between15 and 45% (Zobel 1988) and can yield X-ray diffraction patterns, generally of low quality. The amylopectin is predominantly responsible for the granule's crystallinity. The starch granule is a spherocrystalline assembly of amylopectin molecules oriented radially with their non-reducing ends towards the outer surface (BeMiller 1997b). Granule crystallinity is believed to result from clustered amylopectin chains of DP *ca.* 15 (Hizukuri 1985).

The internal structure of granules can be described by three levels of organization: the granule 'growth ring', the 'super-helical' structure blocklet, and stacks of lamellae (Fig 2) (Gallant et al 1997). At the lowest level of organization, starch granule are a mosaic of alternating semi-crystalline 'soft material' and crystalline shells 'hard material' which are between 120 and 400 nm thick (French 1984, Yamaguch et al 1979). The organization of starch polymers in the semi-crystalline shells is poorly understood. It is evident, however, from solid state ^{13}C NMR studies (Gidley and Bociek 1985) that the level of helical order in starch granules is often significantly greater than the extent of crystalline order. Consequently, it appears that much of the amylopectin in semi-crystalline shells is in the double helical form, although it is not crystalline (Gallant et al 1997). The crystalline shell has been intensively studied and the structure within this part is well understood. It is generally believed that the crystalline shell consists of interleaved amorphous and crystalline 'lamellae' (with average thickness 9-10 nm) (Jenkins et al 1993, Oostergetel and van Bruggen 1989), which represents the crystalline (ordered double helical side chain clusters) and amorphous regions (branching regions) of the amylopectin molecules according to the models of Robin *et al.* (1974) and French (1984). Each amylopectin side-chain cluster can contain between 9-17 double helical chains, each with about three turns of the double helix. The amylopectin side chain cluster within the crystalline lamellae are not uniform with respect to their size (diameter ca 5-15 nm), shape (on average around 10 nm wide by 9-10 nm long) or density (Gallant et al 1997, Jenkins and Donald 1995, Oostergetel and van Bruggen 1993).

With the introduction of high-resolution microscopic techniques, substantial evidence was collected supporting the hypothesis for the existence of a second level of granule structure, between that of the 'growth rings' and the lamellae, termed the 'blocklet' (Baldwin et al 1996, Gallant et al 1997). The 'blocklets' play an important role in the structure and organization of crystalline and possibly semi-crystalline granule shells. The blocklet has more or less spherical structures, possibly corresponding to the 'superclusters' and/or 'super-helices' structure formed by organization of the crystalline and amorphous lamella. The blocklets range in diameter from around 20 to 500 nm depending on starch type, location in the granule and possible amylose. Blocklet size is generally large in the B

crystal pattern and is generally smaller in the semi-crystalline shells of the granule (Gallant et al 1997).

Corn, sorghum, and millet (all in the *Panicoideae* subfamily of the *Graminae* family) starch granules were found to have pores, randomly distributed over their surfaces, which are openings to channels that connect a central cavity to the external environment (Fannon et al 1992). These pores were often observed to be clustered. Pores were also found along the equatorial groove of large granules of wheat, rye, and barley starches. None were seen on any granules of rice, oat, potato, or tapioca (cassava) starches. The channels within starch granules are believed to be comprised of semi-crystalline or amorphous starch polymers, and formed at the junction zones between the more crystalline blocklets (Gallant et al 1997). Channels and related pores are believed to be formed naturally. The pores may be the initial sites of enzyme attack during germination and/or openings that allow enzyme molecules direct access to the granule interior (Fannon et al 1992). The central cavity may be formed by crystallization of amylopectin molecules and concurrent shrinkage of the matrix as the granule grows and develops (Huber and BeMiller 2000).

The exact location of amylose, lipid and protein within granules is still not certain. Results of a series of crosslinking studies indicated that amylose molecules randomly interspersed among the amylopectin molecules as individual radial molecules with an increasing concentration of amylose (in non-mutant starches) towards the granule exterior (Jane and Shen 1993, Kasemsuwan and Jane 1994, Morrison and Gadan 1987). The location of amylose with respect to amylopectin in the amorphous and/or crystalline regions is dependent on the botanical source of the starch. In wheat starch, amylose is mainly found in the amorphous region, but in potato starch it may be partly co-crystallized with amylopectin (Blanshard 1987). Size is an important criterion. Large amylose molecules participate in double helices with amylopectin, whereas smaller amylose molecules, located at the granule periphery, are able to leach from the granule (Jane and Shen 1993, Kasemsuwan and Jane 1994).

Two Types of Crystallite Structures, A and B

Two types of crystallite structures, A and B, have been identified in starch granules by wide angle X-ray scattering (WAXS), which can be distinguished by the packing configuration of double helices and water content. Another proposed type, the C pattern of

native starch, is now thought to be a combination of A and B patterns: the B polymorphs are in the center of the granule and are surrounded by the A polymorphs (Bogracheva et al 1998).

Both unit cells A and B contain two helices (12 glucose residues) and their packing is shown in Figure 1. In both unit cells, double helices associate in pairs that are nested together and stabilized by hydrogen and van der Waals' bonds. The most recent model for A-type structure describes a face-centered monoclinic unit cell incorporating 12 residues located in two left-handed chains that contain four water molecules between the helices (Sarko and Wu 1978). The structure of B-type starches is more clearly defined and composed of a basic repeat unit in which chains are packed in a hexagonal array. The unit cell has two left-handed, parallel-stranded, double helices that are arrayed in parallel, forming a hexagonal unit cell. There is much more space available for water in the unit cell of the B-structure than the A-structure. The unit cell contains 36 water molecules (hydration of 27%), among which half of the water is tightly bound to the double helices and the other half is centered on a 6-fold screw axis that is parallel to the c-axis (Buleon et al 1998, Imbety et al 1991).

The pairing of double helices is a common structural element in both A- and B-polymorphs and the lateral distances between the helices are nearly identical, which suggests a possibility of interconversion of the two structures. Under low humidity and high temperature, an irreversible transition from B starch to the A form can be accomplished by the removal of the water and rearrangement of the pairs of double helices to fill the void left while remaining in the solid state as fibers or granules. On the other hand, it appears that the reverse transition can not take place without a gelatinized intermediate that would disrupt the crystalline architecture of the dense A form (Imbety et al 1991).

These crystalline natures of starch probably depend on both the amylopectin chain length and environmental condition during plant growth (soybean and sweet potato) (Hizukuri 1969). In general, amylopectin molecules of A-types starches have shorter chains in both the long-and short-chain fractions and larger amounts of short-chain fractions than those of the B-type starches (Jane et al 1999, Hizukuri 1985). In addition, Pfannemüller (1987) and Gidley and Bulpin (1987) showed that amylosic fragments with DP < 10 did not crystallize, whereas the A form resulted from chains with DP from 10 to 12; chains longer

than 12 yielded B form crystals. Chains longer than DP 50 did not form the single crystals formed by the shorter chains but instead resulted in tangled networks.. Retrograded starch also gives a B diffraction pattern.

Gidley and Bulpin (1987) have proposed that the chain length effect is a result of the different losses of entropy upon crystallization. An alternate explanation is based on a comparison of the ways that the A and B forms might occur. Single strands of amylose can associate with other single strands as long as their DP is greater that nine. Next, two double helices are paired to form the stable duplex described earlier. If there is enough water to fill the central cavity, and if the duplexes are long enough to organize those water molecules in a stable column, then the B form occurs. Otherwise, the pseudo-hexagonal A form results, with its less favorable packing energy (Imberty et al 1991).

The chain lengths of amylopectin of the C-type starches were intermediate and it is inferred that these starches possibly yield any type of crystalline structure depending on the environment temperature and other factors, whereas the A and B-type starches are insensitive to temperature (Hizukuri 1985).

In *in vitro* experiments, B starch crystallizes in pure water and low temperatures, while A starch requires dehydrating conditions, such as addition of alcohol or salt or/and increased temperature (Buleon et al 1984, Imberty et al 1991, Ring et al 1987)

Functional Properties of Starch

When subject to thermal treatments such as heating or freezing in an aqueous environment starches undergo a range of physicochemical transitions (Fig 3) (Jane 1997). When starch granules are equilibrated in water at room temperature, they undergo limited reversible swelling, which is assumed to be related to swelling of the amorphous areas of the granule (Blanshard 1987, French 1984). When starch-water suspensions are heated, at temperatures specific for a given starch, the crystalline structure melts and the starch granules swell to a high degree (Cooke and Gidley 1992, Doublier 1987). This phase transition is called gelatinization. With continue heating, granules continue to swell, become fragile, and eventually burst, especially if some shear force is applied. The resulting paste is a discontinuous phase of swollen granules and /or granule remnants (fragments) in a continuous phase of polymer solution. On cooling, some starch molecules partially

reassociate to form a network by association of the linear starch fractions, termed gelling. Sometimes the gel starts settling as a precipitate from the solution due to retrogradation. The phase transitions of starch define and explain differences in physical properties of starches and their behavior in food products, and they are extremely important in food processing. For example the application of starch as a raw material usually requires the prior disruption of the inert granule structure. Whereas gelling is desirable in most foods, retrogradation is troublesome, especially when the starch paste is subject to freezing and thawing operations.

Gelatinization

Gelatinization is a cooperative process (Dnovan 1979, Evans and Haisman 1982, Marchant and Blanshard 1978). In excess water content, starch granules begin to swell and imbibe water when energy is applied to break some of the intermolecular hydrogen bonds in amorphous regions. Before the temperature reaches the beginning of gelatinization, amorphous region continue to swell and the segmental mobility of the amorphous phase is greatly increased (Bogracheva et al 1998), which destabilizes the crystallites and results in the disruption of the less stable crystallites. The disruption of crystallinity always begin from the hilum area of the granule, which suggests that the less stable crystallites are arranged in this area (French 1984). The disruption of crystallinity in a particular area of the granule increases the swelling capacity of this area. The swollen disrupted parts of the granule have a much higher water content than the amorphous part of undistributed areas, and these swollen areas will decrease the melting temperature of neighboring crystallites based on the "theory of melting point depression" developed by Flory (1953), It is evident that the swelling of disturbed areas accelerates the process of disruption of neighboring crystallites, and that this process is rapidly propagated along the granule.

Studies on the semi-crystalline synthetic polymers suggest that the changes in the melting temperature can have two major causes: a decrease of crystalline lamellae thickness and an increase of surface free energy for faces of crystalline lamellae due to an increase of structural defects (Protserov et al 2002). The second point is very relevant because it is believed that the dissociation of polymer crystals begins from their defects. Generally, gelatinization temperature is a qualitative measure of starch crystallite structure (effectively double helix length), whereas gelatinization enthalpy is a quantitative measure of the overall crystallinity (quality x quantity) (Morrison 1995, Tester and Morrison 1990a). It is concluded

that the low-gelatinization temperature starches have less crystallinity and less perfect crystallites than the high-gelatinization temperature starches due to minor structural differences in their amylopectin. Factors that will affect the gelatinization temperature of native starches include glass transitions of amorphous regions, amylose/amylopectin ratio, the type of crystalline unit, the length of amylopectin double helices and the surface entropy of starch crystalline lamellae (Gerald et al 2000, Matveev et al 2001, Protserov et al. 2002, Safford et al 1998, Tester et al. 1999, Wang et al 1998).

The swelling power of starch depends on the capacity of starch molecules to hold water via hydrogen bonding (Lee and Osman 1991). Interpretation of the swelling behavior of starches is complicated because so many factors are involved. The intact structure of the amylopectin molecules is very important to the swelling power because swelling power of starch granules was almost completely lost after one day of lintnerization. Both the amorphous and crystalline segments of A- and B-chains will contribute to swelling power when heated beyond the point where crystallinity is lost. Amylose acts both as a diluent and as an inhibitor of swelling, especially in the presence of lipids (natural components of waxy cereal starch granules), which can form insoluble complexes with some of the amylose molecules during swelling and gelatinization (Tester and Morrison 1990b). In addition to the large granule size, the negative charges carried by the phosphate monoester contribute to the high swelling power of potato starch (Jane et al 1999, Lim et al 1994).

In addition to structural factors, swelling and gelatinization of starch also can be affected by annealing. Annealing is a process in which starch granules are held in an excess amount of water at a temperature slightly below the gelatinization temperature for a relatively long time, which allows limited molecular reorganization and formation of a more organized structure of lower free energy (Knutson 1990). Annealing delays the gelatinization (Fisher and Thompson 1997, Knustson 1990) and decreases swelling power and solubility of starch (Eerlingen et al 1997). Krueger et al (1987) studied annealing of commercial corn starch and observed that annealing narrowed the gelatinization temperature range, increased peak gelatinization temperature, and increased the enthalpy of gelatinization. These researchers concluded that annealing caused structural changes in the starch granules that affected their amorphous-crystalline relationships, forcing the granules into a more crystalline orientation. These observations, however, were not supported by X-ray diffraction

studies. Nakazawa et al (1984) studied annealing of starch by holding starch water mixtures (50 and 30% starch) at constant temperature for 5 min to 140 hr, and investigating their thermal behavior using DSC. These researchers observed a gradual shift in the endotherm peak temperature to a higher temperature with progressive annealing. The X-ray diffraction patterns of annealed starch, however, showed gradual loss of crystallinity with progressive annealing. The bi-phasic nature of the endotherm at intermediate hydration was gradually lost on annealing, and a single sharper endotherm was formed. These authors concluded that in a bi-phasic endotherm, the high-temperature endotherm represented melting of starch crystallites, whereas the low-temperature endotherm corresponds to melting of the starch's amorphous region of starch. Marchant and Blanshard (1978) also studied starch annealing and reported loss of birefringence and reduction of X-ray crystallinity, but an increase in enthalpy of gelatinization as measured by DSC.

Retrogradation

Retrogradation is a reorganization process that occurs after heating with water. During the initial phase of retrogradation, two or more starch chains may form a simple juncture point, which may then develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Thomas and Atwell 1999). Retrogradation involves both amylose and amylopectin, with linear amylose molecules having a greater tendency to reassociate and form hydrogen bonds than larger, clustered amylopectin molecules. The linear amylose molecule can be involved in more than one crystallite result in stiffened gel, whereas the cluster shape of amylopectin result in fewer intermolecular interactions and soft gels. Once formed, amylose gels generally require autoclave temperatures (110–160°C) for reversal. Temperatures required to solubilize amylopectin gels can vary from room temperature to 95°C, depending on the degree of molecular association or crystallization that had developed (Zobel 1988).

The rate of retrogradation depends on a number of variables, including the structures of amylose and amylopectin, ratio of amylose to amylopectin, temperature, starch concentration, botanical source of the starch, and presence and concentration of other ingredients.

Effect of Environmental Factors on the Structural and Functional Properties of Starch

Individual properties of starch depend both on the genetic background of the plant and on environmental factors. It is evident that "reliance on a particular cultivar for a given food application (because of perceived quality attributes of the starch) is of limited value, unless the different environmental effects experienced during starch deposition are documented and understood" (Tester 1997). Environmental effects on the granule-size distribution of starch, the crystallization of amylopectin, amount of free and lipid complexed amylose, molecular structure of the starch polymers and associated properties of starch granules (similar genotypes) have been repoted (Asaoka et al 1989, Fergason and Zube 1962, Hizukuri 1969, Shi et al 1994). However, opinions vary on the significance of the role of genetic and environmental factors in the total variability of starch properties. One reason for this might be that those different genotype respond differently to environmental factors.

Effect of Environmental Factors on the Structure of Starch

The environment has a strong effect on the starch-granule size-distribution of wheat and barley. In both wheat and barley, greater temperatures have been associated with reductions in number of B granules, shifting the ratio in favor of A granules (Bhullar and Jenner 1985, MacLeod and Duffus 1988, Tester et al 1991). When other stresses were present, the numbers of A granules also were reduced by high temperatures, but proportionately fewer than those of B granules (Tester et al 1991). Depending on growing conditions, the mean volume of A granules within a cultivar varied one and half times and the mean volume of B granules by two times (Morrison and Scott 1986). Wheat cultivars have showed considerable variation in the sensitivity of B granule fraction to environmental stresses (Blumenthal et al 1995).

The amylose content of starch granules also is affected by the environmental temperature. In maize and rice, higher growth temperatures tended to cause a reduction in amylose contents (Asaoka et al 1985a, Asaoka et al 1985b, Asaoka et al 1987, Asaoka et al 1989, Fergason and Zuber 1962) in potato there is little effect of growth temperature, whereas in wheat, amylase concentration tends to increase with temperature (Tester et al 1995, Shi et al 1994). Environmental effects on the amylose content of sweet potato starch appear to be variable (Tian et al 1991). The composition of barley starch was reported to be affected to some extent by environment. Tester et al. (1991) reported that in barley there was

little effect of growth temperature on the amylose content of normal or waxy cultivars of this cereal. However, they showed that when a high amylose cultivar (*Glacier Pentlandfield*) was grown at 15 rather than 10°C, there was a 27% decrease in the total amylose content of the starch, although there was little change between 15 and 20°C.

The lipid content of starch is also affected by the growth temperature. The lipid content tended to increase in waxy, normal, high amylose barley as a function of temperature. Tester et al. (1991) showed that cultivars grown at 20 rather than 10°C contained about 50% more lipid (as lysophospholipid). This increase was associated with an increase in the LAM to FAM ratio. Similar results were reported for wheat (Shi et al 1994) although there is some seasonal variability (Tester et al 1995).

It has been reported that environmental variation during starch biosynthesis affects the branching pattern of the constituent starch polymers. In rice, an elevated environmental temperature led to a reduction in the size of amylose molecules and increased amylopectin chain lengths (Asaoka et al 1985a, Asaoka et al 1985b, Asaoka et al 1987, Asaoka et al 1989) . Furthermore, the higher temperature increased the amount of long B chains of amylopectin and decreased that of short B chains as compared with the lower temperature (Dehass and Goering 1972, Morrison et al 1986). In wheat, elevated growth temperatures reportedly increased the proportion of amylopectin unit chains with a DP of 10-16 but reduced the proportions of unit chains with a DP of 17-21(Shi et al 1994).

Environmental temperature was reported to have large effects on the X-ray diffraction pattern for sweet potato (Hizukuri 1969). It was clearly shown that a change in crystalline structure from A type to B type occurred with decreasing environmental temperature.

A possible reason for the changes in the composition and structure of starch polymers is the regulated starch biosynthesis, which results from the different stability or temperature preference of starch metabolizing enzymes, including starch synthase (Jenner et al 1993, Keeling et al 1993) or starch branching enzymes (Inouchi et al 2000, Lu et al 1996, Takeda et al 1993). For example, isoforms of the branching enzyme, BE I and BE iI, in maize endosperm showed different optimum temperatures as well as a different preference of chain length they transfer (i.e., BE II has a lower optimum temperature and transfers shorter chains than BE I) (Guan et al 1997, Lu et al 1996). Lowering the temperature could increase

the relative activity of BE II to BE I, which leads to the increase in the proportion of shorter chains in the amylopectin.

The reason for the increase of lipid content with higher temperature in barley is not clear, nor indeed is any role of lipid in the biosynthesis of starch granules in cereal grains. Whereas it is tempting to speculate that the lipid controls and regulates the biosynthesis of amylose, and maintains the amylose to amylopectin ratio, this ratio is largely independent of temperature, for example, potato starches which contain no lipid (Tester 1997).

Effect of Environment on Functional Properties of Starch

The functional properties of starch were reported to be sensitive to the environmental temperature under which the starch granule was produced. The gelatinization temperatures and enthalpies of gelatinization of starches from waxy, normal and high amylose barley (Tester et al 1991), wheat (Shi et al 1994, Tester et al 1995) and rice starches are all increased as growth temperature is elevated. Similar effects have been reported for maize starch where planting dates, environmental temperature and day length have been studied as variables (Campbell et al 1994). Pasting temperatures also increased for potato, sweet potato and soybean seedling starch as a function of increasing environmental temperature.

It is not yet certain if environmental conditions directly affect the formation of double helices or, more probably, the association of these double helices into a crystalline region. Increased gelatinization temperature may be caused by changes in double helix lengths. The hypothesis has been, in part, confirmed by work (Moates et al 1997), where it was shown that the increase in the degree of polymerisation of amylose in spherolitic crystals leads to an increase in their dissociation temperature. In addition, it is hypothesized that elevated growth temperature directly enhances crystallite formation in viro. Double helix formation during starch synthesis is probably driven by thermodynamic forces alone, with similarities to the physical processes, that operate during retrogradation in food systems, and will, therefore, be operating in conjunction with, but not driven by, specific synthesis steps active during the deposition of starch. If we assume that double helix formation is spontaneous upon starch synthesis because this is the most thermodynamically favorable state, it seems unlikely that growth temperature increases the number of double helices, but probably more likely influences conformational reorganization within both the

crystalline and amorphous zones as a consequence of growth temperatures to facilitate the association of the double helices. Firm evidence to support these hypotheses is lacking (Tester 1997).

GEM Project

Corn is the major source of starch produced worldwide. The center of origin was probably Mexico or Guatemala, with domestication occurring about 5000 B.C (Pollak and White 1995). Corn is extremely important to the U.S. economy because of the amount produced, and its value for domestic and export use.

In corn, like in all crops, improvement by line-hybrid development is usually followed by a decrease in genetic diversity. Goodman (1985) reported that < 1% of the U. S. commercial maize germplasm base could be considered as foreign exotic germplasm (tropical plus temperate) and even less may be expected from the domestic exotic germplasm (open-pollinated varieties). The narrowing genetic base of maize leads to concerns about corn's genetic vulnerability to changes in environmental and agronomic conditions, and new insect and disease pressures (Crossa and Gardner 1987, Kuckuck et al 1991). Tracy emphasized the importance of exploring exotic (non-Corn Belt Dent) germplasm for improving quality and, possibly, improved agronomic performance, because much of the corn grown outside the United States is consumed directly by humans and has undergone centuries of selection for flavors, aromas, and textures (Tracy 1990). Exotic germplasm is usually considered to include unadapted domestic populations and foreign temperate, tropical, and semi-tropical populations. Geadelmann (1984) suggested that incorporation of exotic strains into adapted germplasm would increase the available genetic variability and give rise to additional heterotic vigor, lessening chances for a yield plateau. The introgression of adapted germplasm with useful genes from exotic corn has successfully altered corn traits and broadened the genetic base through the GEM project (Pollak and Salhuana 1999).

The GEM project, a coordinated and cooperative effort among public and private sectors, was launched with the objective of providing the corn industry with materials developed by using germplasm enhancement of useful exotic germplasm and ultimately improving and broadening the germplasm base of corn hybrids grown by American farmers.

Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics (Pollak and Salhuana 1999).

GEM is the successor to the Latin American Maize Project (LAMP) (Salhuana et al 1998), which was the first coordinated international effort to deal with the evaluation of the genetic resources of a major world crop (Salhuana et al 1998). The LAMP was launched in 1987 by the U.S. Department of Agriculture, Agriculture Research Services (USDA-ARS) and 11 Latin American countries with funding from Pioneer Hi-Bred International (Johnston, IA). The primary goal of LAMP was to evaluate and maintain the irreplaceable corn germplasm bank of Latin America and the United States. LAMP evaluated over 12,000 accessions grown at 70 locations in the United States and Latin America. Screening used in LAMP was based on the yield potential and agronomic characteristics. The results of LAMP indicate that there are accessions (native and foreign) that show good yield potential on which enhancement can be initiated in order to improve yield, agronomic characteristics and adaptability before being incorporated into breeding programs. Of these LAMP accessions, 270 were selected as potential sources of high yields, with 51 chosen to initiate GEM (Singh et al 2001a).

Starch represents nearly 70% of the dry weight of the mature corn kernels and is the most economically important component. Therefore, to further evaluate the starch quality of the GEM materials is essential to the fully utilize these materials. Only a small amount of data has been collected on the compositional, thermal, milling and structural properties of seed and starch from GEM material (Campbell et al 1995, Singh et al 2001a, Singh et al 2001b, White et al 1990). Significant variability in these properties of starch among the GEM accessions was found because of the diverse genetic background. The GEM accessions possessing unusual properties might be useful in developing new lines with unique starches for added value or additional uses.

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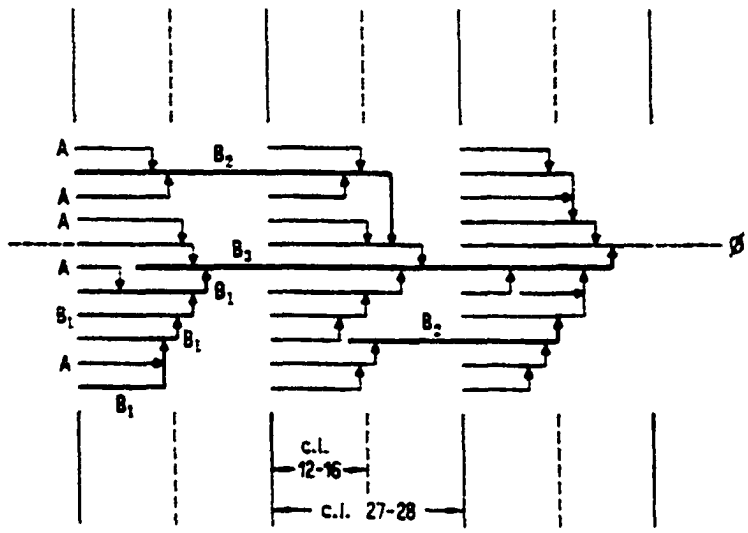

Fig 1. Model of the cluster structure of amylopectin (taken from Hizukuri 1996)

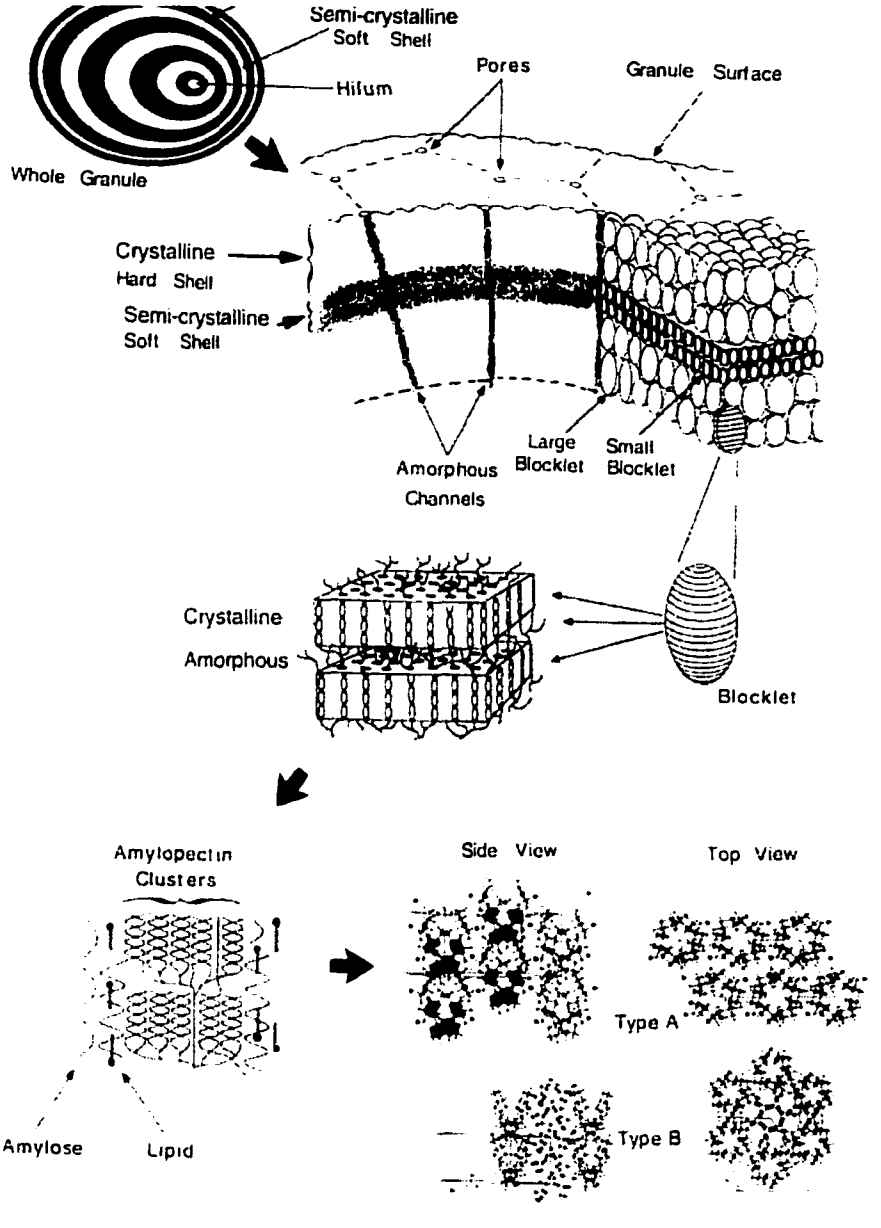

Fig 2. Overview of starch granule structure. At the lowest level of granule organization, the alternating crystalline (hard) and semi-crystalline (soft) shells are shown (dark and light colors, respectively). The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the hilum is shown off center. At a higher level of structure the blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semi-crystalline shells than in the crystalline shells. At the next highest level of structure one blocklet is shown containing several amorphous crystalline lamellae. In the next diagram the starch amylopectin polymer in the lamellae is shown. The next image (from Blanshard, 1987) reminds us that amylose-lipid (and protein) feature in the organization of the amylopectin chains. At the highest level of order, the crystal structures of the starch polymers are shown (taken from Gallant et al. 1997).

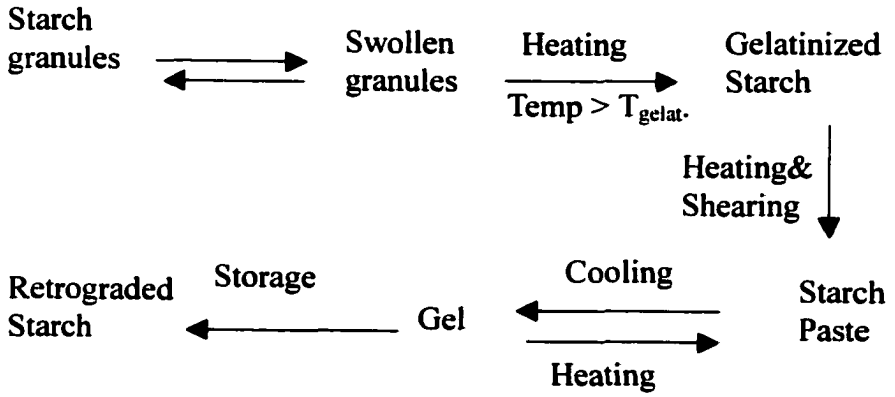

Fig 3. Transformation of starch physical structure (taken from Jane 1997).

CHAPTER 3. OPTIMIZING A SMALL-SCALE CORN-STARCH EXTRACTION METHOD FOR USE IN THE LABORATORY[1]

A paper to be submitted to *Cereal Chemistry*

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Abstract

The objective of this experiment was to determine the effect of different starch extraction procedures designed for use in the laboratory on starch yield, protein content, and thermal properties. The effects were examined of starch extraction methods differing in steeping time (24, 48, or 72 hrs), in the numbers of corn kernels extracted (2, 5, or 10 kernels), or in isolation method (sedimentation or centrifugation). Starch yield, protein content of the extracted starch, and starch thermal properties obtained by using a Differential Scanning Calorimeter (DSC) were determined. Starch yield and protein content were significantly altered by the experimental treatments. Starch extracted following steeping for 24 hr and purified by the sedimentation method had the lowest gelatinization onset temperature and the widest gelatinization temperature range among the treatments examined. The energy required to gelatinize starch did not differ among the treatments. The differences in onset temperature and temperature range were probably caused by annealing of starch that occurs over time, during steeping. Treatments involving more kernels and/or sedimentation rather than centrifugation, yielded starch with the lowest protein content. Therefore, this study suggests that sedimentation is preferred over centrifugation, 10 kernels is preferred over 2 or 5, and soaking the seeds for less than 24 hrs is preferred if minimizing annealing is desired.

Introduction

Common to all starch research is the initial step of extracting starch granules from the plant material. The extraction must be accomplished without significant modification of the starch granules and in sufficient quantities to permit various analyses. Wet-milling, the industrial process for extracting starch from cereal grains, involves chemical, biochemical, and mechanical operations to separate corn into relatively pure fractions of starch, gluten, germ, and fiber. The process involves softening the kernel in steep water, followed by grinding. Fractions are separated by taking advantage of differences in the physical properties including density and particle size of the fractions (Singh et al 1997).

During extraction of corn starch, the grain is steeped in dilute sulphur dioxide (SO_2) or bisulfite solution (a form of aqueous SO_2) for more than 20 hr at 48 to 52°C. The SO_2 disrupts the protein matrix that surrounds starch granules by breaking inter- and intra-molecular disulfide bonds, thus making the physical separation of starch and protein easier. The SO_2 also activates endogenous protease activity in the endosperm, which helps solubilize the protein matrix (Wahl 1969). The degree of protein peptidization in whole kernels increases over the 24-hr steeping period with increasing SO_2 concentrations (up to 0.4% tested) and higher steeping temperature (up to 55°C tested), resulting in increased starch granule release from the surrounding protein matrix. In commercial steeping, kernel degradation for starch release does not occur until kernels are exposed to SO_2 (Wagoner 1948). Bisulfite ions also can form sulfo-protein complexes (Boundy et al 1967). The naturally occurring *Lactobacillus* sp. can propagate at low (20-200 ppm) levels of SO_2 and will consume soluble materials that leach into the steep water directly from the steeped corn or enter with the recycling of process water (Watson 1984). Lactic acid, arising from fermentation of corn by *Lactobacillus* spp. in commercial operations, further enhances separation of starch and proteins.

Laboratory wet-milling procedures can be used to evaluate wet-milling characteristics of new corn hybrids, the effect of harvest and drying methods on the milling efficiency of corn, and the use of different steeping and processing techniques on product yields. Laboratory procedures generally mill between 50 g and 2 kg of corn. These quantities, however, are still too large to screen corn germplasm or developmental lines to identify unusual properties for selection of desirable lines for breeding purposes. White et al

(1990) designed a single-kernel wet-milling starch-extraction process for use in the laboratory, which uses 1 to 10 corn kernels (0.2-3.5 g). The corn kernels were steeped in sodium metabisulfite solution, the pericarp and germ removed by hand, and the endosperm blended with a microblender to further enhance the separation of starch and protein. A modification to the procedure saves time by utilizing a tissue homogenizer rather than a microblender (Krieger et al, 1997). The study showed that starch granules separated by using the tissue homogenizer were intact and undamaged, and that their thermal properties measured with a Differential Scanning Calorimeter (DSC) were similar to those of starch separated by using the microblender (Krieger et al, 1997). Additional evaluation of this procedure could further optimize conditions to obtain maximum starch yields with minimal residual protein in the shortest amount of time.

Different extraction procedures will have different effects on the chemical composition and physical properties of starch. The changes in starch properties resulting from the extraction procedure were explained as a reflection of the non-rigid organization of starch granules (Singh et al 1997). According to these authors, starch granule structure could undergo alteration as a result of extraction. As an example, the reduction in yield of starch from samples pretreated with potassium metabisulphite, an accepted pretreatment in some extraction procedure, could be a result of mild oxidative degradation during pretreatment, because sulphite is known to cleave the amylolytic linkages owing to its prooxidant activity (John et al 1999).

In the current study, the effects of starch extraction methods differing in steeping time (24, 48, or 72 hr), number of kernels (2, 5, or 10 kernels), or in isolation method (sedimentation or centrifugation) were examined. The objectives of this research were to obtain the maximum starch yield, with minimal protein content, in a short time, while still retaining the thermal properties characteristic of native starch, thus optimizing the process conditions designed for use in the laboratory.

Materials and Methods

Materials

The corn inbred, Mo17, was grown and self-pollinated near Ames, IA, in 1998. Ears were harvested at full physiological maturity and dried at 37.5°C until the moisture content

reached ~12%. All seeds were stored at 4°C and 10% relative humidity until analyzed.

Starch Extraction

Corn kernels were hand-picked and cleaned to remove foreign material, mold, and broken kernels before analysis. Kernels of whole corn (2, 5 or 10 kernels) were steeped in 5 mL 1% sodium metabisulfite solution (~ 0.67% SO_2) at 45°C for 24, 48 or 72 hr, followed by manual removal of the pericarp and germ with forceps. The separated endosperm was placed in a 50 mL centrifuge tube with 10 mL of distilled water and homogenized by using a Tekmar Vortex type tissue homogenizer (Ultra-Turrax T25, 600W, Cincinnati, OH) at 20,500 rpm for 30 sec. The homogenized slurry was filtered by using a 30-micron nylon filter under vacuum with several washes, for a total wash-water volume of 500 mL. Coarse and fine fibers, and part of the protein, were removed during filtration. The starch-protein mixture from the filtrate was further separated by either centrifugation or sedimentation. Each sample was separated three times, with 250 mL of distilled water used for each of the three separations. All treatments were performed in replication of five.

Centrifugation Procedure

The starch slurry was centrifuged (Sorvall RC 5B Plus Newtown, CT) at 5,219 x g force for 30 min. The supernatant was decanted, the protein layer was scraped off, and more water (250 mL) added to the partly cleaned starch, with centrifugation and decanting repeated three times. The resulting sediment was air-dried.

Sedimentation Procedure

The starch slurry was allowed to settle in a refrigerator for 2 hr and the supernatant drained. The starch was rinsed with 250 mL water, drained twice, and the resulting sediment air-dried.

Starch Yield

In this paper, the dry flour will be called starch. Starch yield was determined as follows:

% yield = (weight of dry matter recovered from extraction*100/weight of dry whole corn kenels). The dry matter recovered after wet-milling still contained very little amounts of protein, fiber and other residues. The weights of dry starch and corn kernels were measured using the same balance (±0.01 gram accuracy) by using the same balance (Mettler AE 104, Toledo, OH).

Differential Scanning Calorimetry (DSC)

Transition temperatures and enthalpies associated with the gelatinization process of starch were determined by using differential scanning calorimetry (Perkin Elmer DSC 7, Norwalk, CT). A Perkin-Elmer DSC-7 analyzer (Norwalk, CT) equipped with thermal analysis software (Perkin-Elmer Corp., Norwalk, CT) was used. All experiments were run at a scanning rate of 10 °C /min from 30 °C to 110 °C. The samples were prepared with a water to starch ratio of 2:1. The actual dry weight of starch used ranged from 3.96 to 4.02 mg. All enthalpy calculations were based on the dry-starch weight. Thermal transitions for gelatinization were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and ΔH (enthalpy of gelatinization).

Nitrogen Analyzer

Protein content was determined by using a Perkin Elmer Series II Combustion Nitrogen Analyzer 2410 (Norwalk, CT). Combustion and reduction temperature used in the experiment were 930°C and 640°C, respectively. CO_2 was used as purge gas. The compound, Ethenediaminetetracetic Acid, was used as a standard, which was analyzed every three starch samples to calibrate the Nitrogen Analyzer. Starch (40 to 50 mg) was used for each measurement and each sample was analyzed in replicate of three, with the results averaged. The protein conversion factor used was 6.25.

Statistical Analysis

A 3 x 3 x 2 complete factorial experimental design was used with three steeping times (24, 48, or 72 hrs), three levels of kernel number (2, 5, or 10 kernels), and two isolation methods (sedimentation or centrifugation). Unless otherwise noted, samples were processed and analyzed in five replicates. Analysis of variance (ANOVA) was used to test the hypothesis that means were not different for the starch yield, protein content, and each of the DSC properties, and to test for main effects and interactions (including two-way and three-way interactions) between steeping time, numbers of kernels, and isolation method. Tukey's multiple range test was used to test for differences between groups (α=0.05). Calculations were performed with SAS version 8.2 (SAS Institute, Cary, NC) for the Unix Operating system.

Results and Discussion

Overall Evaluation of Extraction Procedures on the Starch Yield and Composition

The main effects of steeping time, number of kernels, isolation method, and two-way interactions (steeping time x number of kernels, steeping time x isolation method, and number x isolation method) were analyzed by using ANOVA for General Linear Model (GLM). Three main factors (steeping time, number of kernels, and isolation method) affected starch yield and protein content significantly ($p < 0.01$). Only the interaction of steeping time and number of kernels was significant for starch yield ($p < 0.05$) indicating that the impact of number of kernels on the starch yield was affected by the steeping time.

The mean yield and protein content of starches extracted by using the various treatments are shown (Table I). Starch yield, ranging from 45.0 to 63.8%, and protein content of the starch, ranging from 0.92 to 4.34%, were significantly different among treatments ($p < 0.05$). Starch extracted from 10 kernels following soaking for 72 hr and separated by using sedimentation resulted in the greatest starch yield with lowest protein content in starch, whereas starch extracted from 2 kernels following soaking for 24 hr and separated by using centrifugation resulted in the lowest starch yield and greatest protein content in starch.

Effect of Steeping Time on Starch Yield and Protein Content

Starch yield increased and the protein content decreased as the steeping time increased for both centrifugation and sedimentation isolation methods (Table I). The average starch yield ranged from 48.5 to 55.2% with centrifugation and 55.5 to 58.9% with sedimentation , and the starch protein content ranged from 3.65 to 2.82% with centrifugation and 2.16 to 1.28% with sedimentation, respectively for the three levels of kernels used in the extractions. The protein was probably more completely separated from starch when steeping time was greater than 48 hr, resulting in both higher starch yields and lower protein content (Wang and Johnson, 1992).

Effect of Number of Kernels on Starch Yield and Protein Content

Increasing the number of kernels extracted at one time resulted in lower protein content in the starch and higher starch yields when using either isolation methods (Table I). Increasing the number of kernels from 2 to 5 increased starch yield from 48.3 to 58.9% for centrifugation and 53.8 to 57.8% for sedimentation (data averaged for all steeping times shown in Table I). No significant increase for starch yield was observed with a further

increase in the number of kernels from 5 to 10. As the number of kernels decreased from 10 to 2, the protein content increased from 2.85 to 3.78% for centrifugation, and 1.33 to 2.01% for sedimentation (data averaged for all steeping times shown in Table I). A possible reason for the increased starch yield and decreased protein content as number of kernels increased was that corn kernels were not uniformly blended within 30 seconds, resulting in some unbroken large particles. And starches could not be released from these large particles, which might affect starch yield and its protein content. This possibility was ruled out because homogenizer is a very efficient blender. No large particles were found from the residue left after filtration or starch. Another possible reason might be the partial separation of starch and protein occurred with the 30-μm filter used in the methods. Starch particles are approximately in the order of 10 to 30 μm in diameter, whereas, protein particles are typically about 5 to10 μm in diameter (Singh 1994). Although the size of the starch particles is greater than the size of the protein particles, the starch should pass through the filter, whereas the stickier proteins would coat the filter cloth and bind with fiber that had not been adequately separated (Singh and Eckhoff, 1996). Corn gluten is also known to agglomerate under certain conditions, which also would account for the protein being retained by the filter (Singh and Eckhoff, 1996). The same amount of water was used to wash all the starch slurries, thus more protein may have been forced through the filter when fewer kernels were extracted, resulting in greater protein contents. In addition, starch might be drained out with wash water during sedimentation and centrifugation process, while this loss of milling product would have a bigger effect on the 2 kernels extraction than 10 kernels extraction because of small initial weight used in the previous extraction procedure. This may also contribute to the low starch yield in the two-kernel extraction procedure.

Effect of Isolation Method on Starch Yield and Protein Content

Sedimentation resulted in starch with lesser protein content and greater starch yield than starch extracted by using centrifugation (Table I). For centrifugation, the average values of yield and protein content of starch over three steeping times and three levels of kernels were 51.1% and 3.29%, respectively, whereas for sedimentation, the corresponding average values were 56.9% and 1.65%, respectively. Milled starch consists primarily of starch and corn protein particles that can be further separated by particle density differences in the isolation procedure. In commercial practices, centrifuges are used for primary starch-

protein separations because of the greater average density of starch granules (1.5g/cm^3) than of protein particles (1.1g/cm^3) (Gausman et al 1952, Biss and Cogan 1988, and Steinke and Johnson 1991). After centrifugation, the heavy starch fraction and the lighter protein fraction both formed sedimentation. The protein layer, which lies above the starch, is scraped off. On the other hand, centrifugation is not an appropriate separation method when the amount of starch sample is small, especially for single- or small multi-kernel extractions. In such extractions, the protein fraction was dispersed within the starch fraction, and no clear separation of two fraction layers was observed, making it very difficult to remove the protein fraction. In addition, some starch might also be removed with protein fraction because of the dispersion. In contrast, during sedimentation, the heavy starch fraction settled to the bottom of the beaker and the lighter protein fraction remained suspended in the water, being removed during decanting. Further, smaller starch granules, which might be excluded at the centrifugation speed used in the centrifugation method, were recovered in the sedimentation method, thus resulting in greater starch yields.

Effect of Extraction Procedures on the Thermal Properties of Starch

Among all thermal properties studied in this research (Table II), only T_o and R were significantly affected by the steeping time. Starch from kernels steeped for 48 hr or more had greater T_o and a narrower R than did starch from kernels steeped for 24 hr. It is likely that the starch underwent annealing which decreased swelling power and solubility of starch and delayed the gelatinization (Fisher and Thompson 1997, Krueger et al 1987). No significant main effects of number of kernels and isolation method on the DSC properties were found.

Conclusions

This study suggests that the sedimentation procedure is preferred for laboratory starch extractions especially when quantity of sample is small because of the lowered protein content in starch and higher starch yields. With total wash-water volume of 500 mL, using 10 corn kernels in the extraction would produce starch with highest yield and lowest protein content compared to 2 and 5 corn kernels. Longer steeping time yielded starch with lower protein content and higher yield. Soaking seeds for less than 48 hr is preferred to minimize annealing of starches and, thus, altering starch thermal properties.

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Table I.
Effect of Extraction Methods on Starch Yield and Protein Content

Isolation Method	Steeping Time	Number of kernels	Starch yield	Protein Content
Centrifugation				
	24	2	45.0a[a]	4.34a
	24	5	52.8a-d	3.32ab
	24	10	47.2a-c	3.30a-c
	48	2	45.3ab	3.86ab
	48	5	50.7a-d	3.31a-c
	48	10	53.5b-d	3.05a-d
	72	2	49.7a-d	3.15a-d
	72	5	56.9de	3.11a-d
	72	10	59.1e	2.21b-e
average			51.1	3.29
Sedimentation				
	24	2	52.2a-d	2.45b-e
	24	5	57.4de	1.90c-e
	24	10	56.8de	2.13c-e
	48	2	55.1cd	1.89c-e
	48	5	57.8de	1.74d-e
	48	10	56.7de	0.92e
	72	2	54.0cd	1.70de
	72	5	58.2de	1.21e
	72	10	63.8e	0.92e
average			56.9	1.65

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Table II.
Effect of Steeping Time on Gelatinization Characteristics of Starch as Determined by DSC

	Steeping time (hr)	T_o (°C)[a]	T_p (°C)	T_c (°C)	ΔH (J/g)[b]	R (°C)[c]
Centrifugation	24	67.93[b]a[c]	72.02a	77.01b	11.88a	9.08c
	48	68.81b	72.53b	77.01b	11.74a	8.20a
	72	68.47b	72.44b	77.02b	11.69a	8.55ab
Sedimentation	24	67.84a	71.86a	76.52a	11.80a	8.69bc
	48	68.03a	71.88a	76.30a	11.79a	8.27a
	72	68.59b	72.47b	76.94b	11.61a	8.35ab

[a] T_o = Gelatinization onset temperature; T_p = Gelatinization peak temperature; T_c = Gelatinization conclusion temperature; ΔH = Enthalpy of gelatinization; R = Range of gelatinization temperature.
[b] Each value represents an average over the number of kernels (2, 5 and 10 kernels), because no significant effect of number of kernels on the DSC properties was observed.
[c] Values followed by the same letter in the same column are not significantly different ($P < 0.05$)

CHAPTER 3. THERMAL AND STRUCTURAL PROPERTIES OF UNUSUAL STARCHES FROM DEVELOPMENTAL CORN LINES [1]

A paper accepted by the *Carbohydrate Polymers*

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Abstract

Starches from exotic corn lines were screened by using Differential Scanning Calorimetry (DSC) to find thermal properties that were significantly different from those exhibited by starches from normal Corn Belt lines. Two independent gelatinization transitions, one corresponding to the melting of a peak at ~ 66°C and the other to a peak melting at ~ 69°C, were found in some starches. The melting characteristics were traced to two separate types of granules within the endosperm. Strong correlations were found between DSC properties and proportion of large granules with equivalent diameter ≥ 17 μm. Starches with a lower peak onset gelatinization temperature (T_{oG}), had a lower normalized concentration of chains with a degree of polymerization (dp) of 15 to 24 and/or a greater normalized concentration of chains with a dp of 6 to 12. These studies will aid in understanding structure-thermal property relationships of starches, and in identifying corn lines of interest for commercial breeding.

Keywords: Maize starch; Corn starch; Starch structure; Starch function; Starch thermal properties; Starch granules; Starch molecular weight; Starch branch chain length.

Introduction

Although corn starch is a valuable ingredient to the food industry, the cooking characteristics of native, unmodified corn starches are undesirable for many applications. To overcome these problems and expand the usefulness of starch, chemical modifications (such as cross-linking and/or substitution) and physical modifications (such as pregelatinization) often are made. With more restrictive food regulations and little hope for the introduction of new modification processes, a greater potential for the manufacture of modified starches with improved functionalities lies in the use of raw materials from previously uncharacterized plant genotypes. This approach might be especially valuable to the food industries because the corn and its starch could be used in the manufacture of "all natural" foods.

As the domestic gene pool becomes more genetically homogeneous, exotic (non-Corn Belt Dent) germplasm may be an excellent source for improving quality and, possibly, improved agronomic performance, because much of the corn grown outside of the United States is consumed directly by humans and has undergone centuries of selection for flavors, aromas, and textures (Tracy, 1990). Exotic germplasm is usually considered to include unadapted domestic populations and foreign temperate, tropical, and semitropical populations. Geadelmann (1984) suggested that incorporation of exotic strains into adapted germplasm would increase the available genetic variability and give rise to additional heterotic vigor, lessening chances for a yield plateau. The introgression of adapted germplasm with useful genes from exotic corn has successfully altered corn traits and broadened the genetic base through the Germplasm Enhancement of Maize (GEM) project (Pollak & Salhuana, 1999). The small amount of data that has been collected suggests the presence of significant variability in the thermal properties of starch from these corn genetic resources (White, Abbas, Pollak, & Johnson, 1990; Campbell, White, & Pollak, 1995).

The genetic background of corn can have substantial effects on the physical and chemical properties of starch. This influence may be attributable to changes in granule-size distribution (Katz, Furcsik, Tenbarge, Hauber, & Friedman, 1993; Campbell, Li, Berke, & Glover, 1996), chemical structure (Lim, Kasemsuwan, & Jane, 1994), crystallinity (Stering, 1962), organization of the molecules within the granule (Shannon & Garwood, 1984), and/or molecular structure of the starch polymers (Sanders, Thompson, & Boyer, 1990; Jane, Chen,

Lee, Mcpherson, Wong, Radosavljevic, & Kasemsuwan, 1999).

We previously identified six novel corn lines, of which two are exotic germplasm lines, and four are derived from breeding crosses developed by crossing exotic genotypes with Corn Belt lines. These lines have starches with significantly different (and potentially useful) thermal properties from those found in starch from normal Corn Belt corn. The objective of this study was to evaluate the thermal properties of novel corn lines regrown and self-pollinated in 1998 and to establish the relationship between the fine structure and physicochemical properties of the starch.

Materials and Methods

Corn Populations

Lines and their advanced progeny from six exotic by adapted breeding crosses from the GEM project and two exotic Plant Introductions, plus public Corn Belt inbred lines B73 (Stiff Stalk heterotic pattern) and Mo17 (non-Stiff Stalk heterotic pattern) as controls, were studied (Table 1). The S_n designation defines the number of times the line has been self-pollinated, starting with the breeding cross (the S_0 population), in the development of the line (Simmonds, 1974). For example, an S_3 line has been self-pollinated three times after starting in the S_0 population. With each self-pollination the numbers of heterozygous genes are reduced by half, thus increasing inbreeding, genetic purity, and repeatability of a trait when it is replanted. The original exotic lines and populations used in this study are maintained at the North Central Regional Plant Introduction Station in Ames, Iowa. The breeding crosses were developed by crossing the exotic populations with inbreds of the Stiff Stalk heterotic pattern. The Stiff Stalk inbreds belong to companies that cooperate in GEM (Pollak et al., 1999). All S_3 families for the exotic breeding crosses, S_1 families for the exotic inbreds, and B73 and Mo17 as controls were grown and self-pollinated in the same environment near Ames, Iowa, in 1998, to reduce the effect of differences caused by environment. Ears were harvested at full maturity and dried at 37.5°C until the moisture content reached 12%. All seeds were stored at 4°C and 10% relative humidity until analyzed.

Single Kernel Starch Extraction

Starch was extracted from single kernels using the method described by White et al. (1990), with modifications (Krieger, Duvick, Pollak, & White, 1997). For the initial

screening, at least 10 randomly selected kernels from up to 21 progeny lines from each of the eight exotic families were individually evaluated for starch characteristics after extraction. Thermal analysis by using Differential Scanning Calorimetry (DSC, described below) was conducted on these starch samples. Based on the results of the initial screening, 32 progeny lines from eight exotic families, plus one line each from Mo17 and B73 as controls, were selected for further characterization. After extraction, starch was stored at 4°C until evaluated.

DSC

A Perkin-Elmer DSC-7 analyzer (Norwalk, CT), equipped with thermal analysis software (Perkin-Elmer Corp., Norwalk, CT), was used to analyze starch thermal properties following procedures of White et al. (1990). All experiments were run at a scanning rate of 10°C /min from 30°C to 110°C, and samples were prepared using a water-to-starch ratio of 2:1. The actual dry weight of starch used ranged from 3.96 to 4.02 mg. DSC parameters recorded for this study included change in enthalpy (ΔH), peak onset (T_o), peak (T_p), and range of gelatinization (RG). A subscript $_G$ after the parameter denotes a gelatinization property. The parameters T_o, T_p, T_c (peak end), and ΔH were given directly by the DSC software. The RG was calculated as T_c-T_o, and peak height index (PHI) was calculated from the change in enthalpy of gelatinization divided by half the range. All enthalpy calculations were based on the dry-starch weight, and all analyses were conducted in duplicate and values averaged. The same scanning method was used for retrogradation of the gelatinized samples kept at 4°C at 7 days. A subscript $_R$ after the DSC parameter denotes a retrogradation property.

Microscopy

AnalysesBirefringence of individual starch granules was evaluated with a polarized microscope equipped with cross-polarizers at 40x magnification (Nikon, Japan). To prepare the partly gelatinized starch sample, 33% water suspensions of a starch sample were sealed in an aluminum DSC pan and heated to 66°C (the temperature between two gelatinization peak maxima previously identified on the DSC thermograms of some starches) at a heating rate of 10°C/min, and then cooled in an ice bath. The sealed DSC pan was opened with tweezers, and the partly gelatinized starch was removed and applied to a glass microscope slide with a drop of mineral oil added to enhance the vision. The presence of ungelatinized

starch granules was determined.

Granule-size distributions of native starches were obtained by following the procedure described by Jane and Chen (1992). Native starch was suspended in 100% ethanol to aid in spreading the particles into a monolayer, mounted on a glass microscope slide, and viewed using a Zeiss axiophot microscope (Zeiss-Kontron, Thornwood, NY) at 50x magnification (20x by 2.5x optivar). Three slides from each sample were analyzed separately, with 200 particles measured from one slide and 400 particles measured from each of two additional slides, to give a total of 1000 starch granules analyzed per starch type. The starch granules selected for the measurement were randomly chosen from each slide. For each granule, area, perimeter, radial S.D., and major axis were determined. The radial S.D. is a measure of the "roundness" of a particle. A perfect circle would have a radial S.D. measurement of 0. The less round the particle, the bigger its radial S.D. number. The equivalent diameter was assessed by: equivalent diameter = sqrt(4*area/π).

Molecular Size of Amylose

A starch components profile, indicating the molecular size of amylose, was determined by gel-permeation chromatography on a sepharose CL-2B column (Chen & Jane, 1992). Distribution coefficients, K_o, of amylose were used for comparison between samples. The peak retention volume of amylopectin was used as the void volume, $K_o = 0$, and the peak retention volume of glucose was used as the total permeation volume, $K_o = 1$. The results are the average of three replicate analyses of each starch type.

X-ray Diffraction

The X-ray diffraction patterns of starches were obtained with copper-nickel foil-filtered, Kα radiation by using a diffractometer (D-500, Siemens, Madison, WI) following the procedure described by Jane et al. (1999). The diffractometer was operated at 27mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4° to 40° at 0.05° increments with a count time of 2 s.

Branch-Chain-Length Distribution of Whole Starch

Branch-chain-length distribution of starch was determined following the procedure described by Jane and Chen (1992). Starch extracted as previously described for single-kernel extracting was debranched by using isoamylase, and the branch-chain-length distributions were analyzed by using a high-performance anion-exchange chromatography

system equipped with an enzyme column reactor and a pulsed amperometric detector (Dionex, Sunnyvale, CA) (HPAEC-ENZ-PAD) by using the method reported by Wong and Jane (1997). The results reported are an average of at least two replicates for each sample.

Statistical Analysis

Granule size and shape distribution of starches were analyzed by using software entitled S-plus 6 (Insightful Corporation, Seattle, WA). Between-sample variations of granule size and shape parameters, which included mean area, equivalent diameter, perimeter, radial S.D., and major axis, were assessed by using the mixed effects Analysis of Variance (ANOVA) model for nested design (i.e., three plates were nested within a starch sample) with unbalanced replicates. The Tukey multiple comparison test was used to calculate differences in means of these parameters among starch samples. Relationships between starch DSC properties and granule size and shape distribution were analyzed by using the Pearson correlation test in the SAS system (release 8.2, SAS Institute, Cary, NC). The coefficients (K_o) of amylose were analyzed by using the general linear model (GLM) procedure on the SAS system. Multiple comparison procedures of the Tukey test were used to calculate the differences among starch samples. The family-wise confidence level used for calculating the differences among starch samples was 95% (i.e., $\alpha = 5\%$).

Results and Discussion

DSC

Starch from 77 progeny S_{n+1} lines of the eight exotic breeding crosses and exotic inbred S_n families (1 to 21 S_{n+1} lines per S_n family) and two Corn Belt inbred lines used as controls (Mo17 and B73) were screened by using DSC to identify unusual thermal properties. A starch was considered to have different and useful thermal properties based on criteria given by Seetharaman et al. (2001). Each of eight exotic S_n families (Table 1) had at least one progeny S_{n+1} line whose kernels contained starch exhibiting one or more useful DSC properties. For example, 9 out of 16 progeny lines of the family Chis-37 that we analyzed contained at least one kernel having starch with a specific useful DSC property.

The frequency of kernels within each S_{n+1} line with starch exhibiting a specific DSC property varied from 1/10 to 36/37 (data not shown). Progeny lines from Chis-37, Cuba-23, Cuba-34, Cuba-38, and PI-83 had a high frequency of kernels (7/10 to 36/37) with both low

T_{oG} (56.6 to 61.6°C) and wide RG (13.6 to 17.8°C). Among them, 7 of 10 kernels of one progeny line from Chis-37 and 8 of 10 kernels of one progeny line from Cuba-38 also contained starches with low T_{pG} (66.1 to 66.2°C). All selected starches (except one progeny line from Cuba-23) had a relatively lower ΔH_G (9.2 to 11.9 J/g) than did Mo17 (12.4 J/g). None of the starches exhibited unusual retrogradation properties.

Some starches from exotic lines also exhibited gelatinization thermogram shapes with shoulders or double peaks (Fig. 1), suggesting either two different independent cooperative transitions, or one time-dependent two-stage process during gelatinization of starches. To distinguish between these possibilities, a two-stage heating experiment was used. The DSC gelatinization curve for starch from one kernel of Cuba-23-1-12 is shown in Fig. 2a, demonstrating the double peak with a large shoulder at the lower temperature. During the experiment, another sample of the starch suspension was heated to 66°C, just beyond the peak temperature of the first peak (Fig. 2b), cooled, and then reheated beyond the gelatinization temperature of the second peak (Fig. 2c). During the second complete heating (Fig. 2c), only a small single peak was found. The position of the peak in Fig. 2c was similar to that of the second peak of the original gelatinization curve (Fig. 2a). This finding clearly indicated that the original curve of gelatinization represents two independent transitions, one corresponding to the melting of the lower temperature peak (T_p~66°C) and the other to a higher temperature peak (T_p~69°C). Different ratios of the two transitions may cause the different shapes of gelatinization peaks shown in Fig. 1.

Two transitions for these starches still existed when the DSC was performed in excess water. No significant differences in gelatinization parameters were observed when the starch:water ratio was increased from 1:2 to 1:4 for two different starches with different curve shapes, Cuba-23-1-12 and PI-83-9-5 (Fig. 3a and b). This observation excluded the possibility that these two transitions were caused by inadequate water content in the DSC run (Donovan, & Mapes, 1979). Possibly, these two transitions might correspond to two different starch structures. Furthermore, all starches exhibited a typical A-type diffraction pattern, indicating the starch molecules were packed in a similar fashion within the granule (data not shown).

Based on these initial data, 13 kernels derived from seven exotic corn lines (the eighth exotic line, DK-8, was not further evaluated) were selected for further structural

studies of their starch. The exact DSC properties for starch extracted from the specific kernels studied are summarized in Table 2. The data are the average of duplicate DSC analyses of the same starch.

Polarized Microscopy Study

The two independent transitions noted during gelatinization of some of the unusual starches were further studied by observing granular birefringence with polarized microscopy (Figs. 4a and b). After being heated beyond the temperature of the first transition (66°C) and below the temperature of the second transition (69°C), some granules were completely gelatinized, whereas others still showed the intact "Maltese Cross" indicating crystalline structure (Fig. 4b). It can be concluded that these two transitions were located in different granules, which differentiates them from those reported in C-type granules characteristic of pea starch (Bogracheva, Morris, Ring, & Hedley, 1998). The two independent gelatinization transitions in C-type starch granules are caused by the coexistence of A and B polymorphs within the same granule. The B polymorphs are arranged centrally with the A polymorphs located peripherally within the granules. During heating in excess salt solution the polymorphs in the two regions melt independently, giving a double peak in heat capacity; the B polymorphs melting at a lower temperature than the A polymorphs.

X-ray Diffraction Pattern of Starch

All starches exhibited a typical A-type diffraction pattern, indicating the starch molecules were packed in a similar fashion within the granule. These data are not shown.

Granule-size Distribution

Significant differences were observed in the mean granule-size parameters among the selected starches (Table 3). Granules of starch from Cuba-34-1-2 had the smallest mean area, equivalent diameter, and major axis, whereas granules of starch from B73 had the greatest values for the same parameters. The mean granule-shape parameters also showed significant differences. Granules from B73 tended to deviate least from a spherical shape (radial S.D. = 7.4), whereas granules from PI-83-9-11 tended to deviate most from spherical shape (radial S.D. = 11.6).

Starch granules were divided into five groups according to their equivalent diameters: < 5 μm, ≥ 5 and < 9 μm, ≥ 9 and < 13 μm, ≥ 13 and < 17 μm, and ≥ 17 μm, and reported as a percentage of the total number of granules measured (Table 4). Significant

differences were observed in the percentage distribution profiles of some of these selected starches. In general, Chis-37-5-3 had the lowest proportion of granules lower than 5 μm in size (2.3%), whereas Cuba-38-5-5 had the greatest proportion of granules lower than 5 μm in size (13.1%). Cuba-38-5-5 tended to have the lowest proportion of large granules (0.1% of granules ≥ 17 μm), and B73 had the highest proportion of large granules (12.5% of granules ≥ 17 μm). Generally, the granule size and shape distributions of all exotic lines studied were different from both B73 and Mo17 starches.

Even though Mo17 and B73 starches had similar DSC properties, granules from these two lines were different in size and shape distributions: granules from B73 were larger and more spherical than granules from Mo17. In addition, B73 starch had a lower percentage distribution of small granules (< 5 μm) and greater percentage distribution of larger granules (≥17 μm) than did Mo17. No significant differences were observed in percentage distribution of granules within the ranges of 5 to 9 μm, 9 to 13 μm, and 13 to 17 μm.

Relationships between starch DSC properties and granule size and shape distributions were analyzed by using the Pearson correlation test (Table 5). The DSC parameters of T_{oG}, ΔH_G and PHI were positively correlated with percentage of granules having an equivalent diameter of ≥ 17 μm ($r = 0.73$, 0.64, and 0.76, respectively), whereas RG was negatively correlated with percentage of granules having an equivalent diameter of ≥ 17 μm ($r = -0.66$). Previous studies have noted a negative correlation between T_{oG} and granule size distribution (Campbell et al., 1996). It is likely that the T_{oG} is influenced by many factors, including that of granule size. For example, small crystals (granules) are more thermodynamically unstable than are large ones (Atkins, 1998), because of the larger surface area, given that both have the same internal structure. Internal structural differences in starch granules also cause size variations in the granule, thus influencing the ultimate granule size. Thus, if only granule size is related to T_{oG}, it might appear that a correlation exists, whereas the correlation is actually a secondary effect of internal structural composition. Another example of differences in composition of large and small granules is the lipid content, which tends to be greater in smaller granules (Banks, Greenwood, & Muir, 1973). No strong correlation was found between DSC properties and mean areas of starch granules.

Molecular Size of Amylose

The Sepharose CL-2B gel permeation chromatograms showed no significant differences in the molecular sizes of amylose among the starches analyzed. The average distribution coefficients (K_o) of amylose for all starches ranged from 0.733 to 0.750 (data not shown).

Branch-Chain-Length Distribution of Amylopectin

Normalized branch-chain-length distributions of the selected starches and of Mo17 and B73 starches were measured by HPAEC-ENZ-PAD (Fig. 5 a-c, Table 6). In Fig. 5 a-c, three starch types representing slightly different patterns of peak concentrations compared with the control starches are presented. The results are expressed as means ± standard deviation of a minimum of duplicate analyses, and the height of each bar at each degree of polymerization (dp) represents its relative concentration to peak I at dp 13 or 14. All starches showed a bimodal distribution with the first peak at dp 13 to 14 and the second peak at dp 42 to 46. Chains with dp ~13 to 14 were most frequent in the distribution, explaining why all starches displayed an A-type diffraction pattern (Jane et al., 1999). In general, A-type starches contain branches with shorter chain lengths (first peak at dp 12 to 14, second peak at dp 41 to 51) than do B-type starches (first peak at dp 14 to 16, second peak at dp 48 to 53). All exotic starches had either lower normalized concentrations of long branch-chain at dp 15 to 24 or higher normalized concentration of short branch-chain at dp 6 to 12 (or both these characteristics) than did Mo17 and B73 (Fig. 5 a-c). To classify the starches, the chains were recorded as belonging to one of four fractions: dp 6 to 12, 13 to 24, 25 to 36, or > 37, corresponding to A, B1, B2, and B3 or longer chains, respectively, based on Hizukuri's model (1986). All exotic starches had relatively lower average chain lengths (dp 23.2 to 24.8) than did Mo17 and B73 starches (dp 25), and contained a higher proportion of A chains at dp 6 to 12 (16.40-18.01%) than did Mo17 (15.64%) and B73 (16.34%) starches (Table 6). Cuba-23-1-12 starch had the shortest average chain length (dp 23.2) and the highest proportion of A chains (dp 6 to 12) and B1 chains (dp13 to 24) among all the starches.

Relationship Between Gelatinization Behavior and Granular Structure

Gelatinization properties are influenced by the crystalline structure of the starch granule. Results suggest that exotic starches with a shoulder peak or double gelatinization peaks are composed of two different kinds of granules. Even though the starch molecules

were packed in an A-type crystalline structure, the granules that gelatinized at a lower temperature may have fewer stable crystallites than those granules that gelatinized at a higher temperature.

Amylopectin is the main component responsible for the crystallinity of starch. Therefore, exotic starches with low T_{oG}, wide RG, and unsymmetrical gelatinization peaks may have different amylopectin structures than do normal starches. This theory is supported by the fact that amylopectin from exotic starches had different amylopectin branch-chain-length distribution patterns than did normal starch (Fig. 5 a-c and Table 6). According to Hizukuri's model (1986), A-chains and B1 chains (those chains that make up the first peak in the distribution profiles of Fig. 5a-c) are primary participants in the crystalline regions. All starches studied contained a greater proportion of A chains with dp 6 to 12 and fewer B1 chains with dp 15 to 24 than did starch from Mo17 and B-73. Also, relative intensities of the shoulder at dp 15 to 24 to peak I in unusual starches were lower than those in Mo17 and B73, which implies that fewer chains in the exotic starches would be long enough to go through the crystalline region, thus resulting in defects in the crystallites. That is to say, crystalline regions in the starches were not as tightly packed as in Mo17 and B73 starch. This observation is corroborated by the DSC data that show lower than normal gelatinization onset temperatures ($T_{oG} \leq 63^{\circ}C$) for all exotic starches of interest (Table 2).

Conclusions

Exotic corn germplasm is a valuable source for producing starches with useful unique characteristics. The starches from the corn lines identified in this study are of interest because of unusually low T_{oG} and wide RG. Two independent gelatinization transitions, one corresponding to the melting of a peak at a lower temperature ~ 66 °C and the other to a peak melting at a higher temperature ~ 69 °C, located in different granules were found in some starches. All starches exhibited a typical A-type X-ray diffraction pattern. Significant differences were observed in starch-granule size-distributions and shape-distributions of the selected starches. No strong correlation was found between DSC properties with mean areas of starch granules. However, strong correlations were found between DSC properties and proportion of large granules with equivalent diameter ≥ 17μm. No significant differences were observed in the starch component profiles, as measured by gel-permeation

chromatography. The low T_{oG} is consistent with the branch chain-length pattern of the amylopectin reported here. Starches with a lower T_{oG} had a lower normalized concentration of chains with a degree of polymerization (dp) of 15-24 and/or a greater normalized concentration of chains with a dp of 6-12. Overall starches with a low T_{oG} had a higher relative concentration of branch chains below dp 13 than did normal starch. Work is in progress to genetically "fix" the unusual thermal properties in each line of corn so that succeeding generations exhibit the property, and to better understand the structure-function relationships in these starches.

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Table 1
Exotic Breeding Crosses and Exotic Inbred Corn Lines and Their Origins.

Exotic Parent[a]	Pedigree for S_n Lines[b]	Source Identification[c]	Number of S_{n+1}[d] Lines Analyzed	Number of Unusual S_{n+1} Lines[e]	Origin of Exotic Parent
		Exotic Breeding Crosses			
PI 576258	CHIS775:S1911b-37-1-2	Chis-37	16	9	Mexico
PI 489361	CUBA164:S2008a-23-1	Cuba-23	1	1	Cuba
PI 489361	CUBA164:S1511b-34-1-3	Cuba-34	8	2	Cuba
PI 489361	CUBA164:S1511b-38-1-3	Cuba-38	8	2	Cuba
Ames 23670	DK212T:S0610-8-1-3	DK-8	5	3	Thailand
Ames 23670	DK212T:S0610-10-1-3	DK-10	21	3	Thailand
		Exotic Inbreds			
PI186182	PI 186182	PI-82	8	4	Uruguay
PI186183	PI 186183	PI-83	10	8	Uruguay

[a] Original corn populations as maintained at the North Central Region Plant Introduction Center, Ames, IA.
[b] Regrown corn ears, maintained as lines to preserve a specific starch characteristic. n = 3 for exotic breeding crosses except Cuba-23 for which n = 2, n = 1 for exotic inbreds.
[c] Abbreviated source identification for use within this paper, representing the S_n lines.
[d] S_{n+1} line means first generation of corn after self-pollination of S_n lines.
[e] Number of S_{n+1} lines having at least one kernel containing starch with unusual thermal properties according to criteria given by Seetharaman et al. (2001).

Table 2
Differential Scanning Calorimetry (DSC) Data of Starches from Single Kernels of Selected Corn Lines Used in Structural Analysis.

Source of Starch (S_{n+1} line[a]-Kernel)	DSC Parameter							
	T_{oG} [b] (°C)	T_{pG}(°C)	ΔH_G(J/g)	RG(°C)	PHI	ΔH_R(J/g)	R%	Shape of DSC Gelatinization Curve[c]
Mo17	66.2	70.8	12.6	9.2	2.7	5.6	44.5	Curve Shape 1
B73	67.2	71.1	11.6	7.6	3.1	6.0	51.6	Curve Shape 1
Chis-37-5-3	**60.3**	**66.5**	10.5	**14.0**	1.5	5.7	54.5	Curve Shape 2
Chis-37-11-1	62.1	70.6	9.3	**13.5**	1.4	3.6	38.6	Curve Shape 3
Cuba-23-1-12	**58.4**	70.1	10.3	**18.3**	1.1	4.7	45.8	Curve Shape 4
Cuba-34-1-2	**60.9**	70.4	11.7	**16.0**	1.5	4.5	38.0	Curve Shape 5
Cuba-38-5-5	**57.9**	**66.1**	9.1	**16.3**	1.1	4.9	54.6	Curve Shape 6
DK-10-1-2	63.2	71.6	11.3	**13.7**	1.6	6.3	56.3	Curve Shape 3
PI-82-1-1	**60.4**	69.4	10.5	**15.1**	1.4	6.3	59.9	Curve Shape 3
PI-83-2-2	**59.6**	69.9	10.8	**16.1**	1.3	5.6	51.4	Curve Shape 3
PI-83-2-5	**60.4**	69.1	10.5	**16.0**	1.3	5.4	51.6	Curve Shape 2
PI-83-2-10	**60.5**	67.6	10.4	**15.3**	1.4	5.5	52.6	Curve Shape 2
PI-83-9-5	**60.6**	70.3	9.6	**15.8**	1.2	4.9	51.3	Curve Shape 7
PI-83-9-8	**59.4**	71.2	9.3	**16.5**	1.1	4.6	49.9	Curve Shape 3
PI-83-9-11	**58.9**	70.7	11.0	**17.2**	1.3	5.9	53.2	Curve Shape 3

[a] See Table 1 for an explanation of S_{n+1} line. For example, Chis-37-5 designates the S_{n+1} line. The -3 designates the kernel number.
[b] T_{oG} = Gelatinization onset temperature; T_{pG} = Gelatinization peak temperature; R_G = Range of gelatinization temperature;
ΔH_G = Enthalpy of gelatinization; ΔH_R = Enthalpy of retrogradation; PHI = Peak height index (enthalpy of gelatinization divided by half the range);
R = Retrogradation. **Bold** numbers indicate values of interest.
[c] See Fig. 1 for definition of curve shapes.

Table 3
Mean Granule Size and Shape Parameters of Starches from Single Kernels of Selected Corn Lines Used in Structural Analyses.

Source of Starch (S_{n+1} line[a]-Kernel)	Mean Area (μm^2)	Mean Diameter (μm)	Mean Perimeter (μm)	Mean Radial S.D. (μm)	Mean Major Axis (μm)
Mo17	88.9b-d[b]	9.8bc-e	41.1b	8.8bc	10.8b
B73	118.8g	11.5g	47.6b	7.4a	12.6c
Chis-37-5-3	91.3b-d	10.4de	43.4c	7.9a	11.3d
Chis-37-11-1	83.1b	9.8b	40.6b	8.3ab	10.6b
Cuba-23-1-12	110.2fg	11.3g	47.6b	8.0a	12.3e
Cuba-34-1-2	62.4a	8.4a	35.3a	8.2ab	9.1a
Cuba-38-5-5	111.9fg	11.4g	48.3d	9.4cd	12.6e
DK-10-1-2	86.3b-d	10.1bc-e	42.7bc	9.5dc	11.1bcd
PI-82-1-1	108.6fg	11.2g	47.6d	9.6de	12.4e
PI-83-2-2	84.8bc	9.8b-d	42.1bc	10.5f	11.6b-d
PI-83-2-5	106.1ef	11.0fg	47.1d	10.5f	12.3e
PI-83-2-10	94.4cd	10.4de	44.2c	10.1d-f	11.6d
PI-83-9-5	82.5	9.8b	41.8b	11.1g	11.1b-d
PI-83-9-8	98.0de	10.7ef	34.8a	10.2ef	12.3e
PI-83-9-11	82.9b	9.6b	41.9bc	11.4g	10.9bc

[a] See Table 1 for an explanation of S_{n+1} lines.
[b] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 4

Distribution Profiles of Starch from Selected Corn Lines Used in Structural Analyses.

Source of Starch (S_{n+1} line[a]-Kernel)	Distribution Profiles (%)[b]				
	< 5(μm)	5-9(μm)	9-13(μm)	13-17(μm)	≥ 17 (μm)
Mo17	12.0c[c]	35.2ab	29.0a	18.3b-d	5.5bc
B73	2.7a	30.3ab	33.3ab	21.2b-e	12.5d
Chis-37-5-3	2.3a	30.8ab	48.1de	17.8b-d	1.0ab
Chis-37-11-1	7.4a-c	35.4ab	39.1a-e	16.4a-d	1.7a-c
Cuba-23-1-12	3.3ab	30.7ab	50.6e	15.0a-c	0.4a-c
Cuba-34-1-2	4.1ab	27.2a	39.0ae	22.8c-e	6.9a
Cuba-38-5-5	13.1c	45.5b	34.7a-c	6.6a	0.1a-c
DK-10-1-2	4.2ab	22.2a	38.1a-e	31.1e	4.4a
PI-82-1-1	3.2ab	21.8a	44.0b-e	26.0de	5b
PI-83-2-2	6.6a-c	34.9ab	40.2a-e	16.1a-d	2.2a-c
PI-83-2-5	3.6ab	27.8a	37.8a-d	23.8c-e	7.0c
PI-83-2-10	7.0a-c	32.8ab	40.2a-e	16.7b-d	3.3a-c
PI-83-9-5	4.4ab	37.0ab	46.0c-e	11.4ab	1.2a-c
PI-83-9-8	2.8ab	26.6a	47.2c-e	21.3b-e	2.1a-c
PI-83-9-11	9.3bc	30.7ab	39.1a-e	18.1b-d	2.8a-c

[a] See Table 1 for an explanation of S_{n+1} lines.
[b] Starch granules are divided into different groups according to equivalent diameters.
[c] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 5

Pearson Correlation Coefficients (r) of Differential Scanning Calorimetry (DSC) Properties with Granule Size and Shape Distribution

DSC Properties	Mean Area (μm^2)	Mean Radial S.D. (μm)	Distribution Profiles (%)[a]				
			< 5μm	5-9(μm)	9-13(μm)	13-17(μm)	>17(μm)
T_{oG}[b] (°C)	0.04	-0.44*[c]	-0.02	-0.15	-0.62**	0.37	0.73**
T_{pG} (°C)	-0.27	0.05	-0.22	-0.45*	-0.11	0.50*	0.38
ΔH_G(J/g)	-0.22	-0.26	0.03	-0.34	-0.51*	0.48*	0.64**
RG(°C)	-0.17	0.50*	-0.07	0.00	0.63**	-0.21	-0.66**
PHI	0.18	-0.50*	0.06	-0.05	-0.65**	0.24	0.76**
ΔH_R(J/g)	0.27	0.18	-0.10	-0.40	-0.20	0.46*	0.38
R%	0.49*	0.40	-0.11	-0.19	0.13	0.15	-0.05

[a] Starch granules are divided into different groups according to equivalent diameter.

[b] See Table 2 for DSC parameter descriptions.

[c] *, ** : where p-values for test Ho ($\rho = 0$) vs. Ha ($\rho \neq 0$) are smaller than 0.10 and 0.05, respectively.

Table 6
Relative Branch-Chain-Length (CL) Distributions of Whole Starch from Single Kernels of Selected Corn Lines

Source of Starch (S_{n+1} line[a]-Kernel)	Peak dp[b]		Average	% Distribution[c]			
	I	II	CL	dp 6-12	dp 13-24	dp 25-36	dp ≥ 37
Mo17	14	45	25.0	15.64	47.21	15.79	21.36
B73	14	47	25.0	16.34	45.94	16.03	21.09
Chis-37-5-3	14	46	24.5	17.57	46.90	14.35	21.17
Chis-37-11-1	14	45	23.8	17.88	48.66	14.04	19.41
Cuba-23-1-12	13	44	23.2	18.01	50.33	14.02	17.63
Cuba-34-1-2	13	46	23.7	17.81	47.92	16.13	18.15
Cuba-38-5-5	13	45	24.3	17.08	46.12	16.83	19.97
DK-10-1-2	14	44	24.4	16.98	48.41	13.67	20.91
PI-82-1-1	14	46	24.5	16.73	47.78	14.89	20.61
PI-83-2-2	14	46	24.4	17.10	47.85	14.90	20.16
PI-83-2-5	14	43	24.5	16.69	47.12	15.80	20.39
PI-83-2-10	14	46	24.3	16.65	47.83	15.95	19.56
PI-83-9-5	14	45	24.8	16.40	47.65	14.28	21.67
PI-83-9-8	14	45	24.4	16.72	46.70	20.49	24.42
PI-83-9-11	14	45	23.9	17.68	48.45	14.73	19.14

[a] See Table 1 for an explanation of S_{n+1} lines.
[b] dp = degree of polymerization
[c] Grouping of dp numbers followed that of Hanashiro, Abe, & Hizukuri. (1996).

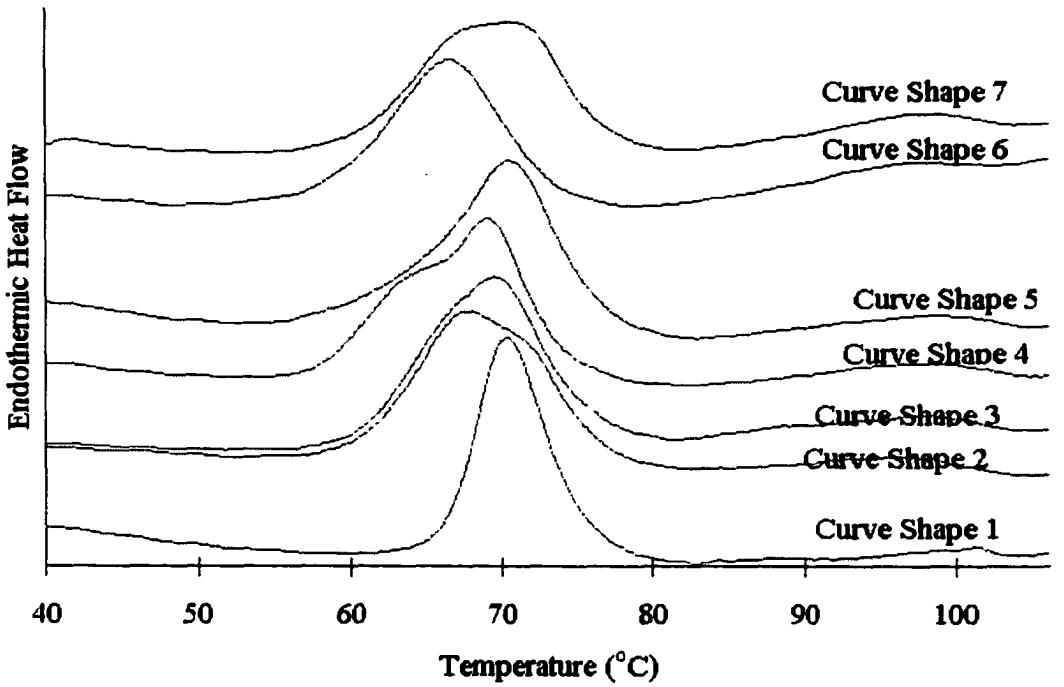

Fig. 1. Typical Differential Scanning Calorimetry gelatinization thermograms of starches listed in Table 3, illustrating the different curve shapes.

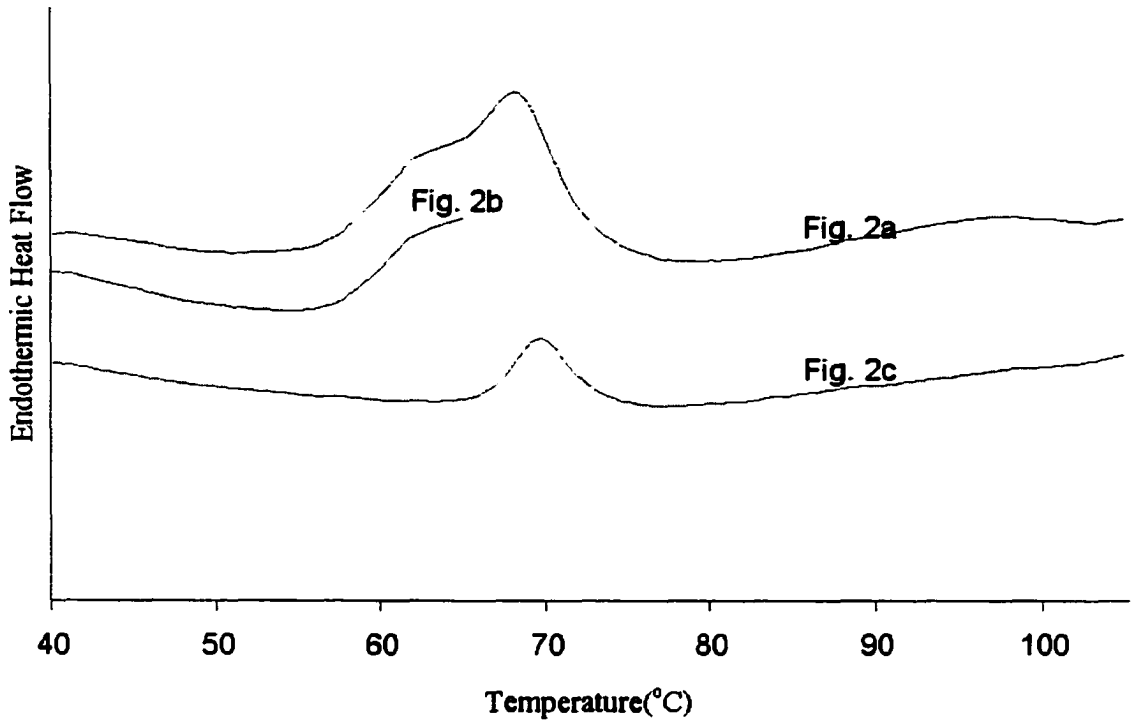

Fig. 2. Differential Scanning Calorimetry gelatinization thermograms of Cuba-23-1-12 starch. a, Sample was heated from 30°C to 110°C. b, Starch was heated from 30°C to 66°C (just after the temperature of the first peak). c, Starch from b was cooled to 30°C and then heated to 110°C.

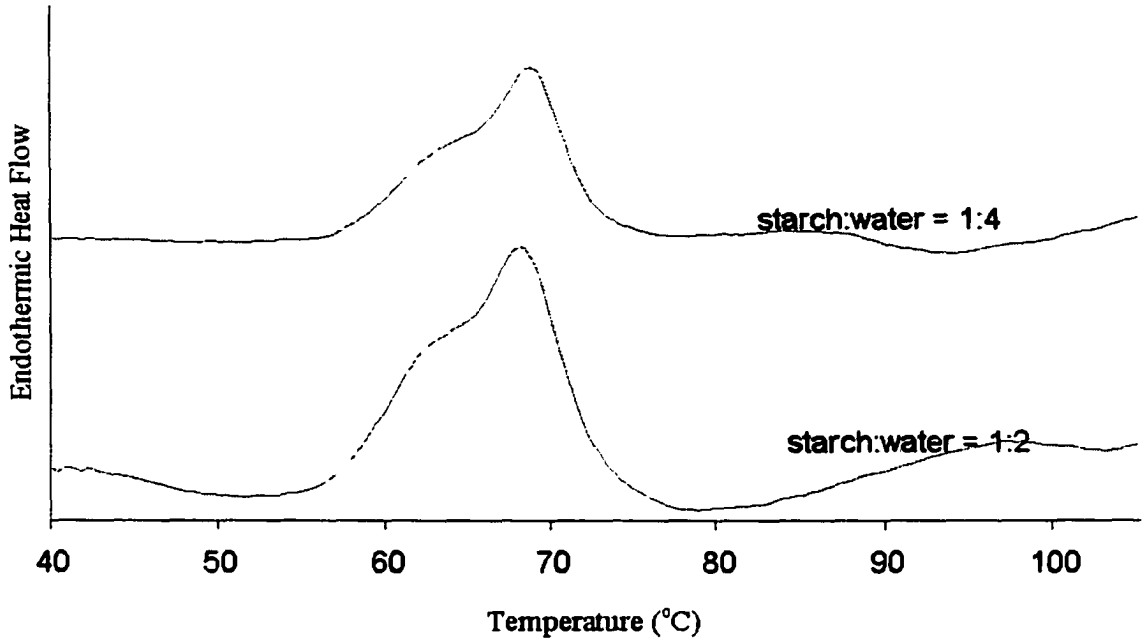

Fig 3a.

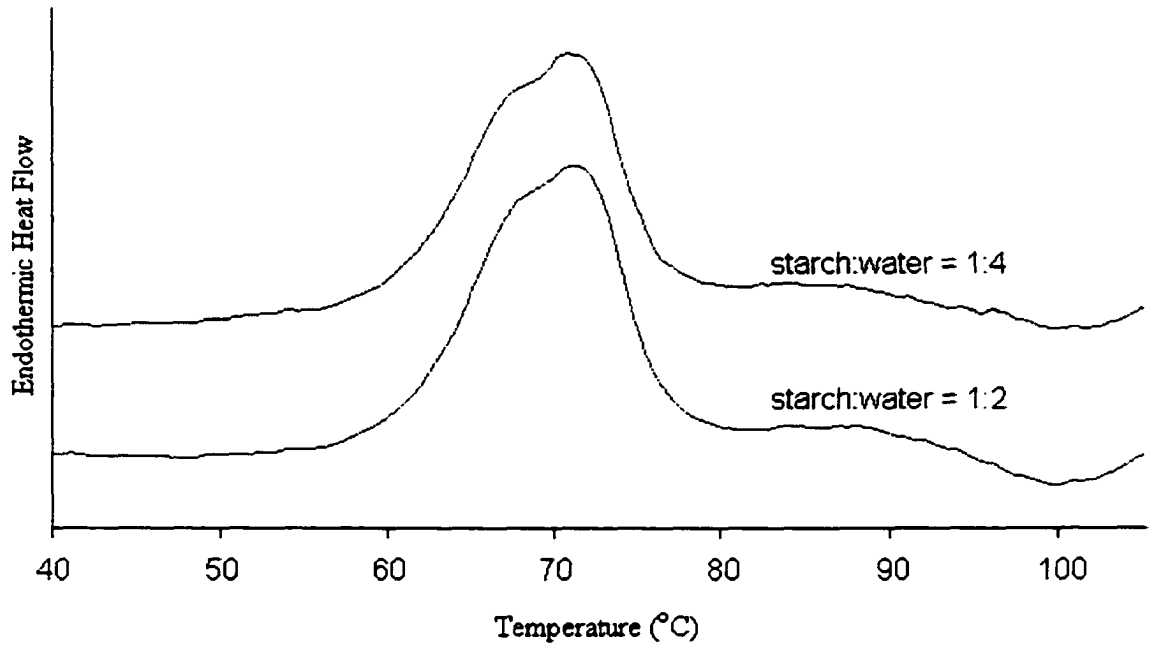

Fig. 3b

Fig. 3. The effects of water content on Differential Scanning Calorimetry parameters and curve shapes of starches from (3a) Cuba-23-1-12 and (3b) PI-83-9-5.

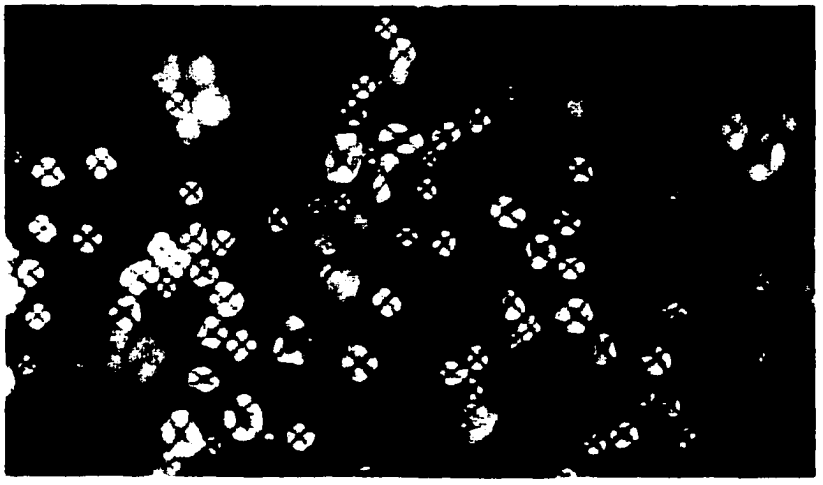

Fig. 4a

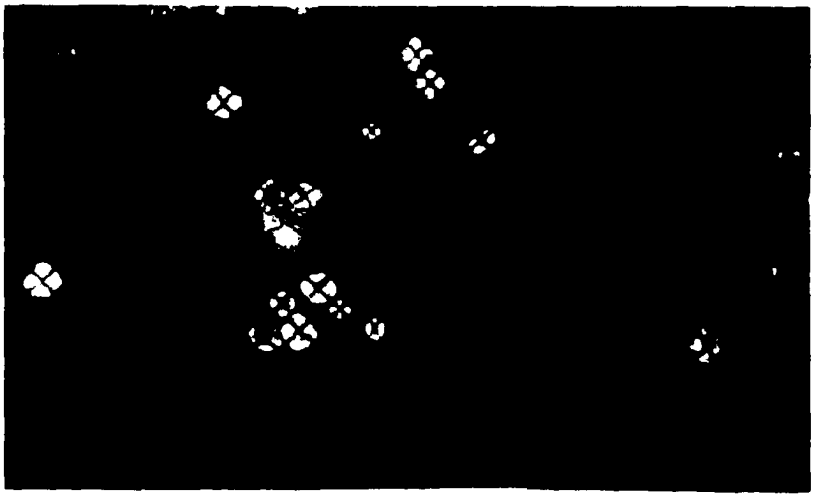

Fig. 4b

Fig. 4. Polarized-light micrographs of starch from Cuba-23-1-12. a, native starch granules, b, starch granules after heating to 66°C, just after reaching the temperature of the first peak shown in Fig. 2a.

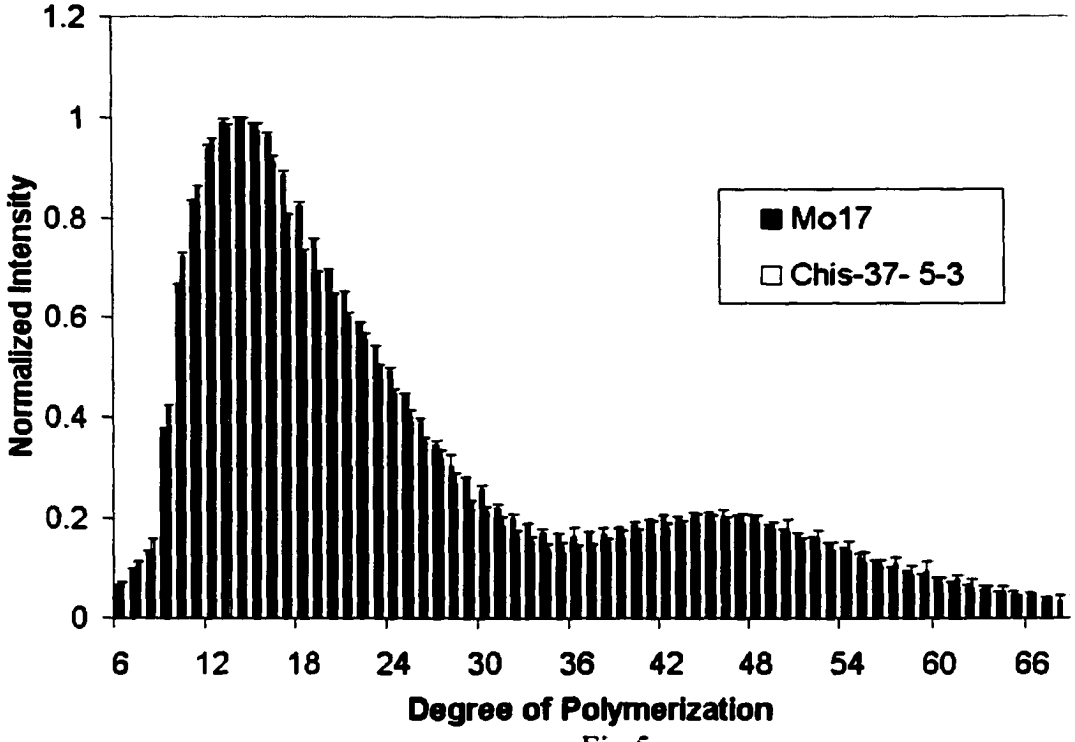
1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
Mo17
Chis-37- 5-3
6
12
18
24
30
36
42
48
54
60
66
Degree of Polymerization

Fig 5a

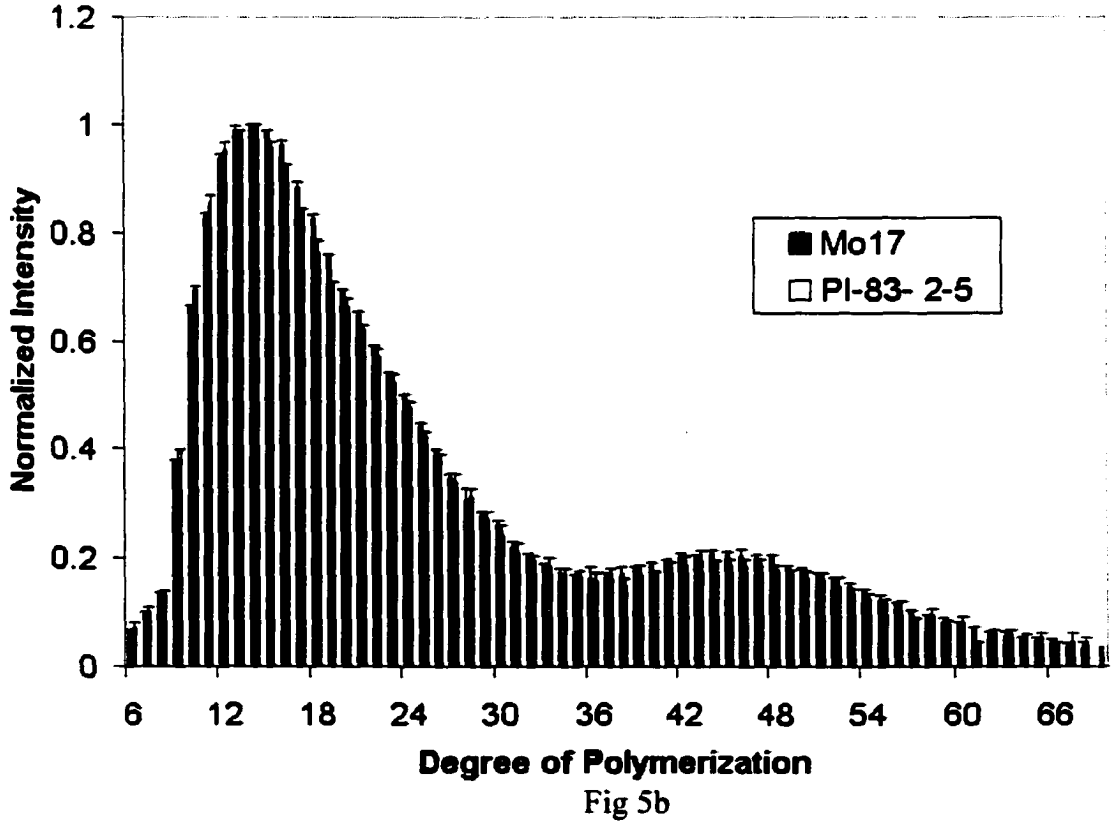
1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
Mo17
PI-83- 2-5
6
12
18
24
30
36
42
48
54
60
66
Degree of Polymerization

Fig 5b

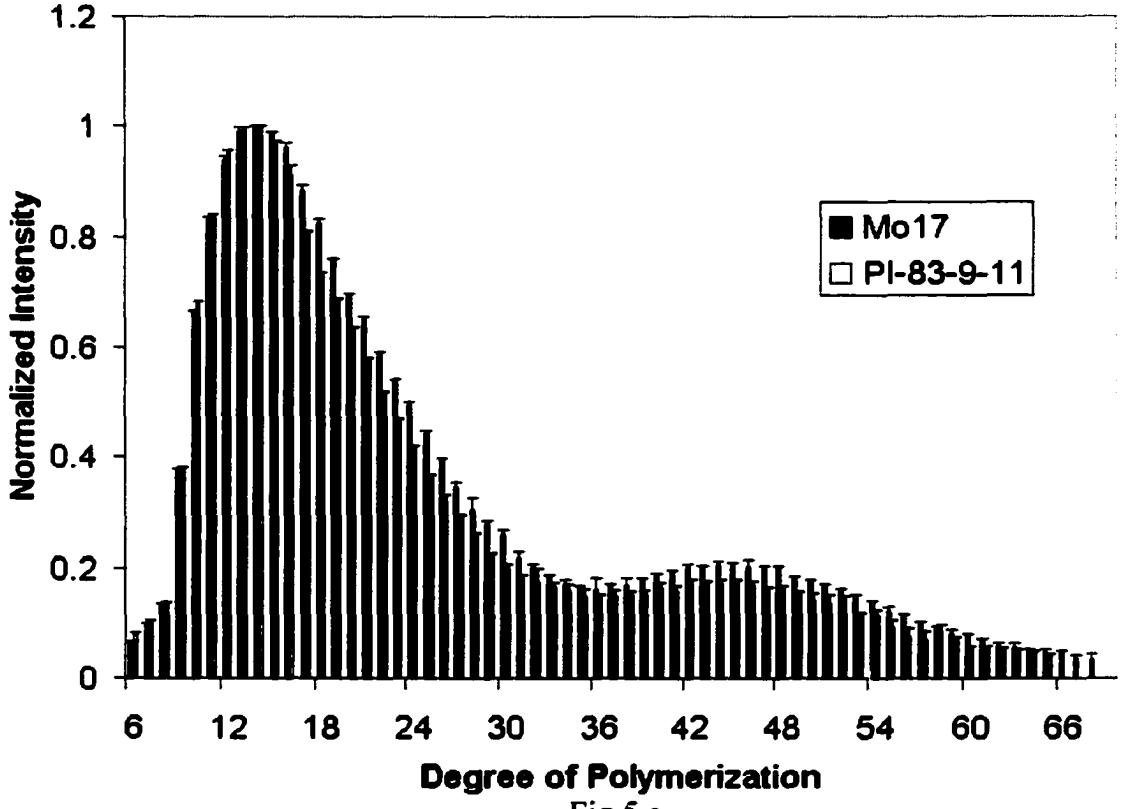

Fig 5 c

Fig. 5 a-c. Normalized branch-chain-length distributions of selected exotic starches compared with Mo17 starch determined by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector. A Carbopac PA100 column composed of immobilized amyloglucosidase was used for the analysis.

CHAPTER 5. STRUCTURE AND FUNCTION OF STARCH FROM ADVANCED GENERATION OF NEW CORN LINES [1]

A paper to be submitted to the *Carbohydrate Polymers*

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Abstract

The objectives of this research were to evaluate functions and structures of starches from exotic x adapted inbred lines and exotic lines, to confirm the advancement of functional traits into the next generation of core, and to establish relationships between the fine structure and functional properties of the starches. Several lines were characterized from the forth and fifth successive generations of exotic crosses and from the third generation of exotic inbreds containing kernels with unusual and potentially useful, thermal properties as measured by a differential scanning calorimetry (DSC, gelatinization onset temperature < 60°C or range of gelatinization temperature > 14°C). The frequency of these traits increased with succeeding generations, when selection of the plants was based on the desired trait. Significant differences were observed in starch-granule size distributions and shape distributions of the selected starches. Measurements with high-performance anion-exchange chromatography (HPAEC) revealed that all selected unusual starches had a lower normalized concentration of chains with a degree of polymerization (dp) of 15 to 24 and/or a greater normalized concentration of chains with a dp of 6 to 12. Overall, chains with a low T_{oG} had a greater relative concentration of branch chains shorter than dp 13 than did normal starch. Strong correlations were found between DSC and RVA properties and the granular structure (granular size distribution and branch-chain-length distribution of amylopectin).

Keywords: Maize starch; Corn starch; Starch structure; Starch function; Starch thermal properties; Starch granules; Weight-average molecular weight; Starch branch chain length.

Introduction

Corn is extremely important to the U.S. economy because of the amount produced, and its value for domestic and export use. Less than one percent of the U.S. germplasm base, however, consists of exotic germplasm (Goodman, 1985) leading to concerns about the genetic vulnerability of corn. The Latin American Maize Project (LAMP) was the first coordinated international effort to deal with the evaluation of the genetic resources of a major world crop (Salhuana, Pollak, Ferrer, Paratori, & Vivo, 1998). The project, involving the cooperative efforts of 12 countries in evaluating their native germplasm accessions, identified accessions (native and foreign) with good yield potential that could be incorporated into breeding programs. The next step in this overall program, entitled the Germplasm Enhancement of Maize (GEM) project, was to provide the corn industry with materials developed from LAMP by germplasm enhancement. The ultimate objective was to improve and broaden the germplasm base of corn hybrids grown by American farmers. Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics (Pollak & Salhuana, 1999).

Starch represents nearly 70% of the dry weight of the mature corn kernels and is the most economically important component. Therefore, it was essential to understand the starch characteristics of the GEM materials. From previous research, we targeted several corn lines containing starches with thermal properties of potential use to the food industry, and that were significantly different from those of normal corn starches. In addition to providing new corn lines with value-added properties, the starches from the corn provide an excellent vehicle to study structure-function relationships. The starches from the targeted corn lines showed unusually low gelatinization onset temperature (T_{oG}, Ji et al, 2002). In some starches, two independent gelatinization transitions were found, one corresponding to the melting of a peak at a lower temperature ~ 66 °C and the other to a peak melting at a higher temperature ~ 69 °C. The two peaks represented two different populations of granules, which gelatinized at different temperatures. All starches exhibited a typical A-type X-ray diffraction pattern. The low T_{oG} was consistent with the branch chain-length pattern of the amylopectin. For example, starches with a lower T_{oG}, had a lower normalized concentration of chains with a degree of polymerization (dp) of 15 to 24 and/or a greater normalized concentration of chains with a dp of 6 to 12.

Previous work was accomplished by using starches from an early developmental stage, including S_3 and S_4 generations for exotic breeding crosses (exotic populations x adapted lines), and S_2 generation for exotic x adapted inbred lines. The S_n designation defines the number of times the line has been self pollinated starting with the breeding cross (the S_0 population), in the development of the line. The frequency of kernels within some lines containing starch with low T_{oG} was relatively low (1/10), which indicated that the genetic background controlling low T_{oG} was still heterogeneous. Therefore, all thermal and structural analysis were performed by using starch extracted from single kernels. By advanced self-breeding and selecting for a specific trait, which was low T_{oG}, this specific trait was fixed in the progeny generation. Furthermore, more materials were produced to allow us to do more complete structural and functional analyses.

The objectives of this research were to evaluate functions and structures of starches from advanced generations of developmental lines from exotic x adapted inbred lines and exotic breeding crosses (exotic populations x adapted lines), and to establish relationships between the fine structure and functional properties of the starches. A secondary objective was to confirm the advancement of selected functional traits into the next generation of corn.

Materials and Methods

Corn Populations

Lines and their derivatives from three exotic by adapted breeding crosses from the Germplasm Enhancement of Maize (GEM) project and four exotic inbreds, plus public inbred lines B73 and Mo17 as controls, were studied (Table 1). Mo17 has a non-Stiff Stalk heterotic pattern and B73 has a Stiff Stalk heterotic pattern. The original exotic lines and populations used in this study are maintained at the North Central Regional Plant Introduction Station in Ames, IA. The breeding crosses were developed by crossing the exotic populations (DK212T is a tropical 3-way commercial hybrid developed by DeKalb Genetics in Thailand) with inbreds of the Stiff Stalk heterotic pattern. The Stiff Stalk inbreds belong to companies that cooperate in GEM. All parent lines for regrowing were selected for the desirable trait, low T_{oG}. All S_4 progeny derived from the exotic x adapted lines, CHIS-37, CUBA-38, and for the Mo-17 control, were grown and self-pollinated in the same environment near Ames, IA in 1998. All S_5 progeny derived from the exotic x adapted lines,

DK-8-1, DK-8-4, DK-8-5, DK-10-1, and DK-10-25, S_2 progeny for the exotic inbreds, PI82-3, PI82-6, PI82-8 and PI82-18, and the B73 control, were grown and self-pollinated in the same environment near Ames, IA in 1999. Ears were harvested at full physiological maturity; they were dried at 38°C for five days, shelled and stored at 4 °C and 45% relative humidity until the kernels were needed for analysis.

Single Kernel Starch Extraction

Starch was extracted from single kernels using the method described by White et al. (1990), with modifications (Krieger, Duvick, Pollak, & White, 1997). Corn kernels were hand-picked and cleaned to remove foreign material, mold, and broken kernels before analysis. One kernel of whole corn was steeped in 5 mL 1% sodium metabisulfite solution at 45°C for 48 hr, followed by manual removal of the pericarp and germ with forceps. The separated endosperm was placed in a 50-mL centrifuge tube with 10 mL of distilled water and homogenized by using a Tekmar tissue homogenizer (Ultra-Turrax T25, 600W, Cincinnati, OH) at 20,500 rpm for 30 sec. The homogenized slurry was filtered by using a 30-micron nylon filter under vacuum with several washes, for a total wash-water volume of 300 mL. The starch slurry was allowed to settle in a refrigerator for 2 hr and the supernatant drained. The starch was rinsed with 250 mL water, drained twice, and the resulting sediment air-dried.

For the initial screening, at least five randomly selected kernels from each of the 55 S_{n+1} lines were individually evaluated for starch characteristics after extraction. Thermal analysis by using Differential Scanning Calorimetry (DSC, described below) was conducted on these starch samples. Based on the results of the initial screening, 11 progeny lines from 11 exotic families, plus one line each from Mo17 and B73 as controls, were selected for further characterization.

Bulk Starch Extraction

Five to ten (depending on the availability of seeds) separate starch extractions, of ten kernels each, were made by using the ten-kernel extraction procedure as for screening, except that the homogenized slurry was filtered by using a nylon filter for a total wash-water volume of 500 mL. Starches were combined and mixed well to produce the large quantities of starch needed for the functional and structural studies described in this paper. After extraction, starch was stored at 4°C until evaluated.

Moisture Content of Starch

Moisture content of starch was measured by heating starch at 110°C for 3 hrs. Triplicate analyses were done for each sample.

DSC

Thermal properties of the isolated starch were analyzed with a DSC7 analyzer (Perkin-Elmer Corp., Norwalk, CT) equipped with a thermal analysis data station. Starch was gelatinized as described by White et al (1990). Starch (4.00 mg) was weighed into an aluminum pan (Perkin-Elmer 0219-0062) and 8 μL of distilled water was added to the starch in the pan. All experiments were run at a scanning rate of 10°C /min from 30°C to 110°C (Ng, Duvick, & White, 1997). DSC parameters recorded for this study included change in enthalpy (ΔH), peak onset temperature (T_o), peak temperature (T_p), and range of gelatinization temperature (R_G). A subscript $_G$ after the parameter denotes a gelatinization property. The parameters T_o, T_p, T_c (peak conclusion temperature), and ΔH were given directly by the DSC software. The R_G was calculated as T_c-T_o, and peak height index (PHI) was calculated from the change in enthalpy of gelatinization divided by half the range. The same scanning method was used for retrogradation of the gelatinized samples kept at 4°C for 7 days. A subscript $_R$ after the DSC parameter denotes a retrogradation property. The T_o, T_p, T_c and H of retrogradation were calculated automatically. Range of retrogradation (R_R) was calculated as (T_c - T_o). All enthalpy calculations were based on the dry-starch weight, and all analyses were conducted in duplicate and the values averaged. Percentage of retrogradation (%R) was calculated from the ratio of H of retrogradation to H of gelatinization.

Pasting Properties

The pasting properties of starches were analyzed by using a Rapid Visco Analyser (RVA, model 4, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) and an STD2 temperature profile equipped with Thermocline for Windows software version 1.2. At least two RVA profiles were obtained for each sample and the results for each sample were averaged. An 8% (dwb) starch in water slurry at a final weight of 28 g was used for each RVA analysis. The following time-temperature profile was used: heat the starch-water mixture for 1 min at 50°C to equilibrate the sample, increase the temperature to 95°C in 7.5 min, hold at 95°C for 5 min, decrease to 50°C in 7.5 min, and hold at 50°C for 2 min. Pasting temperature (P_{temp}), peak time (P_{time}), peak viscosity (PV), trough or hot paste

viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV-HPV), and setback (CPV – HPV) were recorded.

Gel Strength

The texture properties of starch pastes from the RVA analysis were analyzed with a texture analyzer (Stable Micro Systems TA.XT2, Texture Technologies Corp., Scarsdale, NY) equipped with Texture Expert for Windows software (V1.11) (Takahashi & Seib, 1988). After each RVA run, the hot starch paste was poured into an aluminum container (27 mm diameter and 27mm depth) with aluminum foil surrounding the top to increase its depth by 10 mm. The top of the container was covered with aluminum foil to prevent dehydration. Gel strength was tested after two storage conditions: one day at 25°C and seven days at 4°C. Samples stored at 4°C for seven days were equilibrated at room temperature for 1 hr before analysis.

For a gel strength measurement, the aluminum foil cover was removed and the extra gel was removed with a cheese cutter to produce a fresh surface for analysis (Takahashi & Seib, 1988). The gels were compressed at a speed of 0.9 mm/sec to a distance of 7.5 mm using a stainless steel punch probe (P/4, 4 mm diameter). The peak at 7.5 mm (8.2 sec) was reported as the firmness of the gel, and the negative portion of the peak was reported as the stickiness of the gel (Takahashi & Seib 1988). From each of two replicates, gel strength was measured at five different locations on each gel sample and results per replicate averaged.

Microscopy Analyses

Granule-size distributions of native starches were obtained by following the procedure described by Jane and Chen (1992). Native starch was suspended in 100% ethanol to aid in spreading the particles into a monolayer, mounted on a glass microscope slide, and viewed by using a Zeiss axiophot microscope (Zeiss-Kontron, Thornwood, NY) at 50x magnification (20x by 2.5x optivar). Three slides from each sample were analyzed separately, with 400 particles measured from each slide, to give a total of 1200 starch granules analyzed per starch type. The starch granules selected for the measurement were randomly chosen from each slide. For each granule, area, perimeter, radial S.D., and major axis were determined. The radial S.D. is a measure of the "roundness" of a particle. A perfect circle would have a radial S.D. measurement of 0. The less round the particle, the bigger its radial S.D. number. The equivalent diameter was assessed by: equivalent diameter = sqrt

(4*area/π).

Apparent Amylose Content

Starch samples were defatted by dispersing starch in 90% DMSO solution, with stirring in boiling water bath for 1 hr and stirring at 25°C for another 24 hr. Dispersed starch was precipitated with three volumes of ethanol and collected by centrifugation. Precipitated sample was washed with ethanol, recovered by filtration, and dried in a convection oven at 35°C for 24 hr. Iodine affinities of defatted whole starch were determined by using a potentiometric autotitrator equipped with Meterodata recording software (702 SM Titrino, Brinkman Instrument, Westbury, NY) following the procedure by Kasemsuwan, Jane, Schnable, Stinard & Robertson (1995). Apparent amylose contents were calculated by dividing the iodine affinity of starch by 19.0% (Takeda & Hizukuri, 1987). Iodine affinities of the samples were replicated four times and the results averaged.

Branch-Chain-Length Distribution of Whole Starch

Branch-chain-length distribution of starches was determined following the procedure described by Jane and Chen (1992). Starch was debranched by using isoamylase, and the branch-chain-length distributions were analyzed by using a high-performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (Dionex, Sunnyvale, CA) (HPAEC-ENZ-PAD) by using the method reported by Wong and Jane (1997). A CarboPac PA1 anionexchange column (250 x 4 mm) and a CarboPac PA1 guard column (25 x 3 mm) were used for sample separation. The results reported are an average of at least two replicates for each sample.

Molecular Weight Distribution of Amylopectin by HPSEC-MALLS-RI

The weight-average molecular weight (M_W) and z-average radius (R_z) of gyration of amylopectin were measured by using HPSEC (HP1050 series isocratic pump) equipped with multiangle laser lightscattering (MALLS, model Dawn-F, Wyatt Tech. Co., Santa Babara, CA) and refractive index (RI) detectors (HP1047A) following the procedure of Yoo & Jane (2002). In the procedure, a Shodex OHpak KB-G guard column, and KB-806 and KB-804 analytical columns (Shodex Denko, Tokyo, Japen) were used for the separation of amylopectin from amylose.

Statistical Analysis

Between-sample variations of granule size and shape parameters, which included

mean area, equivalent diameter, perimeter, radial S.D., and major axis, were assessed by using the Analysis of Variance (ANOVA) for mixed effects model with nested design (i.e., three plates were nested within a starch sample). The Tukey multiple comparison test was used to calculate differences in means of these parameters among starch samples. The apparent amylose content, molecular weight of amylopectin, starch paste properties, and starch texture properties were analyzed by using the general linear model (GLM) procedure. Multiple comparison procedures of the Tukey test were used to calculate the differences among starch samples. The family-wise confidence level used for calculating the differences among starch samples was 95% (i.e., $\alpha = 5\%$). Relationships between starch functional properties and structural properties were analyzed by using the Pearson correlation test. Calculations were performed by using SAS version 8.2 (SAS Institute, Cary, NC) for the Unix Operating system.

Results and Discussion

Selection and Verification of Trait by Using DSC

Starches from 55 S_{n+1} lines (Table 1) were screened for unusual thermal properties by using DSC. Among these 55 S_{n+1} lines, 24 exotic breeding cross lines were from the S_4 generation (CHIS-37 and CUBA-38), 18 exotic crosses were from the S_5 generation (DK-8-1, DK-8-4, DK-8-5, DK10-1 and DK10-25), and 13 exotic inbreds were from the S_3 generation (PI82-3, PI82-6, PI82-8, PI82-18). Five to thirteen randomly selected single kernels per line were individually extracted and then analyzed by using DSC. Twenty-seven out of 55 lines were identified as lines for potential research and commercial interest, because these lines contained at least one kernel whose starch exhibited thermal properties that are significantly different from that of normal Corn Belt lines (Mo17 and B73), such as a low T_{oG} and wide R_G. For example, 9 out of 16 progeny lines of CHIS-37 contained at least one kernel having starches with unusual gelatinization properties.

Based on the screening (represented in Table 1), 16 S_{n+1} lines containing kernels with unusual thermal properties ($T_{oG} < 60°C$ or $R_G > 14°C$) were selected for further characterization (Table 2). The frequency of kernels within each S_{n+1} line with starch exhibiting the specific DSC properties varied from 1/10 to 10/10. Starch gelatinization properties of individual kernels within an S_{n+1} line exhibited considerable variability. The

mean, minimum and maximum values for T_{oG}, T_{pG}, T_{cG} and ΔH_G of all kernels analyzed within a progeny line are noted (Table 2). These selected lines have the promise for developing inbred lines with fixed unusual thermal properties. None of the starches exhibited unusual retrogradation properties as measured by DSC.

To obtain large quantities of starch for further functional and structural analyses, all corn lines listed in Table 2 underwent starch extraction by using a bulk extraction procedure for 50 to 100 kernels depending on the availability of the seeds. The DSC properties of each starch type, bulked for each sample are listed in Table 3. The values are average of at least two DSC analyses of the bulked starch.

Some starches from exotic lines also exhibited gelatinization thermogram shapes with shoulders or double peaks (Fig. 1), suggesting two independent cooperative transitions located in different populations of starch granules starting to gelatinize at different temperatures (Ji et al, 2002). This finding verifies the presence of the two populations first noted in the previous paper, and demonstrates that their presence is genetic. A food industry might be able to take advantage of a starch having two separate starch population within one source

Pasting and Textural Properties of Starch from Selected Corn Lines

Significant differences were observed in the pasting properties of starch from different lines (Table 4). The B73 starch had the greatest P_{temp} (79.2°C), whereas DK-8-4-5 had the lowest P_{temp} (71.1 °C). All starches from exotic lines had greater peak viscosity values (184.0 to 222.7 RVU), greater breakdown (57.3 to 114.9 RVU), and greater setback values (86.2 to 108.5 RVU) than did Corn Belt inbred line Mo17 (152.4, 48.4 and 77.1 RVU, respectively). Among all exotic lines, DK-8-4-6 had the greatest PV (222.7 RVU), DK-10-1-4 had the greatest breakdown (114.9 RVU) and PI82-6-1 had the greatest setback value (108.5 RVU). The values for starch from the two Corn Belt inbred lines, B73 and Mo17, were quite different from each other in their pasting properties. B73 starch exhibited relatively high PV values (195.2 RVU), high breakdown (77.5RVU) and high setback values (97.2 RVU), compared with Mo17 starch.

Data for the firmness and stickiness of the gels measured with a TA after the two storage treatments are summarized in Table 5. Within a starch, seven days of storage at 4°C gave significantly firmer gels than one day of storage at 25°C (Significance data for this

comparison is not listed in the table). The increase in gel firmness over time is mainly caused by retrogradation of starch gels, which is associated with syneresis of water and crystallization of amylopectin, leading to denser gels (Miles, Morris, Orford, & Ring, 1985).

Among the different lines, significant differences were observed in the textural properties. Starch from B73, CHIS-37-5 and PI82-18-1 required the least amount of force to break the gel among all starch types after both 1 and 7 days storage and starch from DK-8-4-6 required the greatest amount of force to break the gel among all starch types after both 1 and 7 days storage. Gel formed from starch from DK-8-4-5 had the greatest stickiness value (21.99 g x sec) after one day of storage at 25 °C, whereas gel formed from starch PI82-6-1 had the greatest stickiness value (27.57 g x sec) after seven days of storage at 4 °C. Gel formed from starch from DK-10-1-4 had the least stickiness value (14.12 g x sec) after one day of storage at 25 °C, whereas gel formed from starch DK-8-4-3 had the least stickiness value (18.8 g x sec) after seven days of storage at 4 °C. Textural properties of starch gels are very important criterion used to evaluate the performance of starch in a food system. For example, a firm and short (low stickiness) starch gel is desirable in a pudding or salve-like product whereas long (great stickiness) starch gel is desirable in syrup-like products (Thomas & Atwell, 1999b). The variability of the gel properties of starches from developmental lines makes it possible that these starches from developmental lines can be used in a wide variety of foods.

Apparent Amylose Content

No significant differences were observed in the apparent amylose content among the starches analyzed. The average apparent amylose content for all starches ranged from 29.0 to 32.4% (data not shown). Because of the limited quantities of starch available from each line, we did not measure the absolute amylose content for starch. It was difficult to make any conclusion about absolute amylose content from apparent amylose content, because long branch chains of amylopectin, like amylose, could bind iodine to form a single helical complex during potentiometric titration, and consequently inflate the iodine affinity and the apparent amylose content of starch (Jane et al 1999, Kasemsuwan et al, 1995).

Granule-size Distribution

Significant differences were observed in the mean granule-size parameters among the

selected starches (Table 6). Granules of starch from DK-8-5-2 had the smallest mean area, equivalent diameter, and major axis, whereas granules of starch from B73 had the greatest values for the same parameters. The mean granule-shape parameters also showed significant differences. Granules from B73 starch tended to deviate least from a spherical shape (radial S.D. = 7.7), whereas granules from CUBA-38-5 starch tended to deviate most from a spherical shape (radial S.D. = 9.4).

Starch granules were divided into five groups according to their equivalent diameters: < 5 μm, ≥ 5 and < 9 μm, ≥ 9 and < 13 μm, ≥ 13 and < 17 μm, and ≥ 17 μm, and reported as a percentage of the total number of granules measured (Table 7). Significant differences were observed in the percentage distribution profiles of some of these selected starches. In general, B73 had the lowest proportion of granules smaller than 5 μm in size (2.3%), whereas 4726 had the greatest proportion of granules lower than 5 μm in size (13.1%). Also, CUBA-38-5 tended to have the least proportion of large granules (0.1% of granules ≥ 17 μm), and B73 had the greatest proportion of large granules (8.17% of granules ≥ 17 μm).

Similar to results previously noted (Ji et al, 2002), Mo17 and B73 were significantly different from each other in granular size and shape distributions: granules from B73 were larger and more spherical than granules from Mo17. In addition, B73 starch had a lower percentage distribution of small granules (< 5 μm) and greater percentage distribution of larger granules (≥ 17 μm) than did Mo17.

Branch-Chain-Length Distribution of Amylopectin

Normalized branch-chain-length distributions of the selected starches and of Mo17 and B73 starches were measured by HPAEC-ENZ-PAD. The comparison of normalized chain length distribution of exotic starches with starch from control line B73 are shown in Fig. 2a-c. The results are expressed as means ± standard deviation of a minimum of duplicate analyses, and the height of each bar at each degree of polymerization (dp) represents its relative concentration to peak I at dp 13 or 14. All starches showed a bimodal distribution with the first peak at dp 13 to 14 and the second peak at dp 42 to 46. Chains with dp ~13 to 14 were most frequent in the distribution. These three graphs represent three different chain length distribution patterns than the control starches: all exotic starches had

either lower normalized concentrations of long branch-chain at dp 15 to 24 (Fig 2a) or higher normalized concentration of short branch-chain at dp 6 to 12 (Fig 2b), or both these characteristics (Fig 2c) than did B73. Starches from two controls, Mo17 and B73, had slightly different normalized distribution patterns (Fig 3). The Mo17 starch contained lower relative intensities of A-chains at dp 6-12, and higher relative intensities of B1 chains at dp 15-18 than did B73 (Fig 3).

To classify the starches, the chains were recorded as belonging to one of four fractions: dp 6 to 12, 13 to 24, 25 to 36, or > 37, corresponding to A, B1, B2, and B3 or longer chains, respectively, based on Hizukuri's model (1986) (Table 8.). All exotic starches had relatively lower average chain lengths (dp 23.4 to 25.3) than did Mo17 and B73 starches (dp 25.0 and dp 25.5, respectively) (Table 8.). All exotic starches contained a higher proportion of A chains at dp 6 to 12 (16.55-18.77%) than did Mo17 (16.11%) and B73 (16.32%) starches, and a lower proportion of long B-chains (B2 or B3) at dp ≥ 37 (17.79-21.68 %) than did Mo17 and B73 (22.27 and 22.50%). Among all exotic starches, CUBA-38-5 starch had the shortest average chain length (dp 23.4), the greatest proportion of A chains at dp 6 to 12 (18.58%), and the lowest proportion of B2 or B3 chains at dp ≥ 37 (17.79%) (Table 8).

Molecular Weight and Gyration Radii of Amylopectin

Weight-average molecular weight (Mw) and Z-average radius of gyration (Rz, related to the volume occupied by the molecule in a solution) of the amylopectin molecules, determined by using an HPSEC-Malls-RI system, showed significant differences in Mw and Rz among all starches (Table 9). Starch from DK-10-1-3 had the highest Mw ($12.4x10^8$) and Rz (398.1nm), whereas starches from DK-8-5-2 had the lowest Mw ($7.9x10^8$), and starch from DK-8-4-5 had the lowest Rz (351.1nm). A Pearson correlation analysis showed a strong correlation between Mw and Rz ($r=0.72$ with $p < 0.0001$).

Relationship between Starch Function and Granule Size Distribution

Relationships between starch functional behavior and granule size and shape distributions were analyzed by using the Pearson correlation test. Significant correlations at $p < 0.10$ and $p < 0.05$ are listed (Table 10). The correlation coefficient (r) values were fairly low, because functional properties of starch depend on many variables, and the effects of each variable might interfere with each other. Thus, even a low r value, when accompanied

by a statistically significant value may be important.

The DSC parameter of T_{oG} was positively, and RG was negatively correlated with mean area. In addition, T_{oG} and T_{cG} were positively correlated with percentage of granules having an equivalent diameter of ≥ 17 μm, whereas T_{oG} was negatively correlated with percentage of granules having an equivalent diameter of 5 to 9 μm.

The effect of granular size on the gelatinization properties of starch has been the subject of many research papers. Some investigators used A- and B- type starch granules from the bimodal distribution of granules from wheat and barley to show the impact of granule size, but the results are contradictory. The A- (larger) and B- (smaller) type starch granules were reported to have different T_{pG} and T_{cG} with similar T_{oG}, the T_{pG} and T_{cG} of B-type starch granules were higher by 1-2°C than those of A-type starch granules (Eliasson & Karlsson 1983, Soulaka & Morrison 1985, Peng, Gao, Abdel-Aal, Hucl, & Chibbar 1999). According to Seib (1994), B-type granules started gelatinizing at a lower T_o than did A-type granules, but had greater T_p and T_c. But Ghiasi, Hoseney, & Varriano-Marston (1982) reported that A- and B-type starch granules had similar gelatinization temperature regimes. Further, Eliasson & Karlsson (1983), Soulaka & Morrison (1985) and Peng et al (1999) reported that A-type starch granules from wheat had greater gelatinization enthalpy than B-type starch granules, whereas Stevens & Elton (1971) found the gelatinization enthalpies of wheat starch granules of different size classes to be similar. In contrast, Wootton & Bamunuarachchi (1979) reported that A- type wheat starch granules had a lower gelatinization enthalpy than did B-type starch granules. Actually, gelatinization properties of starch are influenced by many factors, some of which inherently affect granule size. For example, the lipid content is greater and amylose content is lower in small wheat starch granules (Evers, Greenwood, Muir, & Venables, 1974), and the saturated fatty acids are present in the greatest quantities in the smallest granules (Meredith, Dengate, & Morrison, 1978). Thus, it might seem that a correlation between granule size and gelatinization temperatures (including T_{oG}, T_{pG} and T_{cG}) exists, whereas the correlation is actually a secondary effect of the internal structural composition.

Other correlations indicated that R% was positively correlated with size of starch granules. The correlation coefficients between R% with starch mean area and major axis both were 0.54. The reason for this might be that larger granules typically a contain higher

content of amylose than do small granules (Duffus & Murdoch, 1979). During the cooling and storage of gelatinized starch, starch molecules begin to reassociate, with amylose retrograding faster than amylopectin. Thus the greater amylose content causes more junction zones and a higher R%.

The PV by RVA was negatively correlated with the percentage of small granules having diameter < 5μm. At the same degree of swelling, small granules occupy less volume than larger granules, resulting in the low PV (Medcalf & Gilles, 1968). The breakdown values were positively correlated with mean major axis of starch granules, meaning that paste stability decreased as the granules size increased. Highly swollen large granules are more fragile and more easily broken by stirring because of their large volume, resulting in a greater decrease in viscosity than for small granules (Medcalf & Gilles, 1968).

Relationship between Starch Functional Behavior and Chain-Length Distribution

A-chains and B1 chains of amylopectin are primary participants in the crystalline regions. All exotic starches studied contained a greater proportion of A-chains with dp 6 to 12 than did starch from Mo17 and B73 (Table 8). Also, relative intensities of the shoulder at dp 15 to 24 to peak I in unusual starches were lower than those in Mo17 and B73 (Fig 2 a-c). Crystalline structure in the exotic starch was not as perfect as in Mo17 or B73, because fewer chains in the exotic starches would be long enough to go through the crystalline region, thus resulting in defects in the crystallites. This observation is corroborated by the DSC data that show lower than normal gelatinization onset temperatures ($T_{oG} \leq 62°C$) for all exotic starches of interest (Table 3).

The values of T_{pG} and ΔH_G were positively correlated with average CL, and negatively correlated with the proportion of A-chains at dp 6 to 12 (Table 10). Tester and Morrison (Tester & Morrison, 1990) suggested that T_{pG} represents a measure of starch crystallite perfection whereas ΔH_G represents the amount of crystalline amylopectin. A high proportion of A-chains at dp 6-12 may lead to the less perfect and smaller quantities of crystalline structure in starch granules.

The ΔH_R values were positively correlated with the average CL, and negatively correlated with the proportion of A-chains at dp 6-12, because A-chains at dp 6-12 will inhibit the retrogradation (Shi & Seib, 1992).

The proportions of A-chains at dp 6-12 also were negatively correlated with P_{temp} by

RVA, because more short chains at dp 6-12, leading to less tightly packed crystalline structures, also promoted swelling.

Correlation among Functional Properties of Starches

The PV and breakdown from RVA were negatively correlated with T_{pG} and T_{cG} (Table 11). High gelatinization temperatures reflected by T_{pG} and T_{cG} will slow the gelatinization process and lead to low PV. Proportion of short A chains of amylopectin could be the reason for the negative correlation between breakdown values and T_{pG} and T_{cG}. A great proportion of A-chains at dp 6-12 will result in a less perfect crystalline structure in the granule and lead to a low gelatinization temperature. At the same time, a great proportion of A-chains at dp 6-12 will promote swelling and result in high breakdown values (Jane et al, 1999).

Firmness and stickiness of gels by the TA were strongly correlated with retrogradation of starch (Table 11). As the starch became more crystallized, water was squeezed out of the gel, causing a firmer, more concentrated gel. With less water, the gels also had greater stickiness (Thomas, & Atwell, 1999a).

Comparison of Functional and Structural Properties of Two Corn-Belt Lines Mo17 and B73

Starch from Corn Belt inbreds, Mo17 and B73, used as control lines in this study were significantly different from each other in function and structure. The Mo17 corn belongs to the non-Stiff Stalk heterotic pattern and B73 belong to the Stiff Stalk heterotic pattern. The Mo17 starch contained lower relative intensities of A-chains at dp 6-12 and higher relative intensities of B1 chains at dp 15-18 than did B73 (Fig 3). This observation is corroborated by the DSC data in that Mo17 showed higher T_{oG}, T_{pG} and ΔH_G than B73 starch. In addition to the structural differences in the chain-length distributions, starch from Mo17 and B73 had significantly different granular size and shape distribution. Starch granules from B73 were larger in size and more spherical in shape than were starch granules from Mo17. Because of the larger size, more spherical shape and higher proportions of A-chains at dp 6-12, starches from B73 had greater PV, and larger breakdown values than did starch from Mo17.

Conclusion

The exotic lines were selected because of a low gelatinization onset temperature and a wide range of gelatinization. The different gelatinization and pasting properties could be explained by the branch chain-length pattern of the amylopectin. Starch granule-size distribution profiles of exotic starches were different than those of normal starch. These results suggest that incorporation of exotic alleles into Corn Belt germplasm is an excellent means to obtain value-added traits to produce starch with desirable functions.

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Table 1
Exotic breeding crosses and exotic inbred corn lines and their origins.

Exotic Parent[a]	Pedigree for S_n Lines[b]	Source Identification[c]	Number of S_{n+1} Lines Analyzed[d]	Number of Unusual S_{n+1} Lines[e]	Origin of Exotic Parent
		Exotic Breeding Crosses			
PI 576258	CHIS 775:S1911b-37-1-2	CHIS-37	16	9	Mexico
PI 489361	CUBA164:S1511b-38-1-3	CUBA-38	8	2	Cuba
Ames 23670	DK212T:S0610-8-1-3-1	DK-8-1	1	1	Thailand
Ames 23670	DK212T:S0610-8-1-3-4	DK-8-4	6	6	Thailand
Ames 23670	DK212T:S0610-8-1-3-5	DK-8-5	3	3	Thailand
Ames 23670	DK212T:S0610-10-1-3-1	DK-10-1	4	4	Thailand
Ames 23670	DK212T:S0610-10-1-3-25	DK-10-25	4	2	Thailand
		Exotic Inbreds			
PI186182	PI186182-3	PI82-3	4	4	Uruguay
PI186182	PI186182-6	PI82-6	3	3	Uruguay
PI186182	PI186182-8	PI82-8	4	4	Uruguay
PI186182	PI186182-18	PI82-18	2	2	Uruguay

[a] Original corn populations as maintained at the North Central Region Plant Introduction Center, Ames, IA.
[b] Regrown corn ears, maintained as lines to preserve a specific starch characteristic. n=4 for exotic breeding crosses CHIS-37 and CUBA-38. n=5 for exotic breeding crosses DK-8-1, DK-8-4, DK-8-5, DK-10-1 and DK-10-25. n=2 for exotic inbreds.
[c] Abbreviated source identification for use within this paper.
[d] S_{n+1} line means first generation of corn after self-pollination of S_n generations.
[e] Number of S_{n+1} lines having at least one kernel containing starch with unusual thermal properties according to criteria given by Seetharaman et al (2001).

Table 2
Differential scanning calorimetry (DSC) data of starches from single kernels of selected corn S_{n+1} lines[a].

Corn Source[b]	Frequency[c]	T_{oG}[d] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Mo17	0/18	66.2	65.2	67.3	70.5	69.7	71.2	75.8	74.5	76.3	12.5	12.1	12.9
B73	0/10	64.9	64.5	65.3	68.7	68.2	69.3	72.8	71.9	73.8	10.9	10.4	11.2
CUBA-38													
CUBA-38-5	8/10	59.7	56.9	62.1	66.4	64.4	67.8	72.7	71.7	74.2	9.4	9.0	9.7
CHIS-37													
CHIS-37-5	5/13	62.2	59.2	63.9	67.9	66.3	69.4	74.9	74.1	75.5	10.8	10.3	11.5
DK-8-1													
DK-8-1	4/5	60.3	59.0	63.2	68.1	67.5	69.7	73.6	72.6	74.7	11.4	10.0	10.5
DK-8-4													
DK-8-4-3	10/10	57.1	52.8	59.1	67.6	66.9	68.5	73.0	72.3	74.2	11.7	9.9	11.4
DK-8-4-5	10/10	59.2	55.7	60.6	68.2	67.3	68.6	73.1	73.7	72.3	12.1	10.4	12.8
DK-8-4-6	10/10	57.5	55.1	59.9	67.3	66.8	68.2	72.5	71.9	73.5	11.2	9.6	11.4
DK-8-4-8	10/10	58.5	55.7	60.6	67.7	67.0	68.3	73.2	72.2	74.4	11.0	9.7	12.2
DK-8-5													
DK-8-5-1	9/10	58.4	54.8	62.8	68.6	67.6	69.7	73.6	72.7	74.9	10.8	10.5	11.8
DK-8-5-2	9/10	57.0	51.7	65.1	68.3	67.8	68.8	73.4	72.3	74.3	10.9	9.6	12.1
DK-10-1													
DK-10-1-3	9/10	60.1	58.3	61.7	69.1	68.0	69.8	74.0	73.4	74.8	11.2	10.7	11.9
DK-10-1-4	7/10	60.2	58.5	61.7	69.1	68.2	69.7	73.8	72.7	74.4	10.8	9.9	11.7
DK-10-25													
DK-10-25-1	9/10	59.4	57.2	61.5	67.4	67.0	67.7	71.9	70.8	72.7	10.6	9.2	11.4
PI82-3													
PI82-3-1	8/10	60.0	57.4	65.1	68.4	67.3	69.0	73.0	71.6	73.5	10.8	10.1	11.2
PI82-6													
PI82-6-1	5/10	60.4	58.6	62.4	67.1	66.0	67.9	73.2	72.5	74.1	9.4	8.6	10.6
PI82-8													
PI82-8-5	7/10	60.6	57.9	64.4	67.8	66.4	69.0	73.2	72.3	73.8	11.0	10.1	11.9
PI82-18													
PI82-18-1	1/10	62.0	59.5	64.3	68.8	67.3	70.0	74.1	73.0	75.4	11.1	10.8	11.3

[a] See Table I for the definition of S_{n+1} lines.
[b] See Table I for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[c] Frequency of kernels whose starch exhibited the unusual DSC properties, $T_{oG} \leq 61°C$ or $R_G \geq 14°C$.
[d] T_{oG} = Gelatinization onset temperature; T_{pG} = Gelatinization peak temperature; ΔH_G = Enthalpy of gelatinization. The results reported are an average of at least two replicates for each sample, and standard deviations are less than 0.38.

Table 3
Differential scanning calorimetry (DSC) data of starches from 50 to 100 kernel extraction of selected corn lines.

Source of Starch (S_{n+1} line[a])	DSC Parameter									
	T_{oG}[b] (°C)	T_{pG}(°C)	T_{cG} (°C)	ΔH_G(J/g)	R_G(°C)	PHI	T_{oR} (°C)	R_R(°C)	ΔH_R(J/g)	R%
Mo17	67.7	72.0	76.5	13.0	8.6	3.0	43.3	19.3	6.9	52.8
B73	65.0	69.8	75.0	11.6	10.1	2.3	43.6	19.0	6.4	54.9
CUBA-38										
CUBA-38-5	61.2	68.1	74.3	11.2	13.0	1.7	42.1	20.8	6.4	57.1
CHIS-37										
CHIS-37-5	61.4	68.6	76.2	11.5	14.9	1.5	42.5	19.6	5.6	48.8
DK-8-1										
DK-8-1	58.7	68.7	74.6	12.4	15.9	1.6	42.3	19.7	6.2	50.2
DK-8-4										
DK-8-4-3	56.2	68.2	73.8	11.1	17.6	1.3	42.2	19.5	4.6	42.0
DK-8-4-5	60.0	67.7	72.6	12.2	12.6	1.9	40.4	21.1	6.2	51.3
DK-8-4-6	56.7	67.7	73.5	12.1	16.8	1.4	41.0	20.6	5.6	46.6
DK-8-4-8	57.2	68.0	73.8	12.1	16.6	1.3	42.5	18.7	5.4	49.0
DK-8-5										
DK-8-5-1	55.3	68.8	74.9	11.3	19.7	1.1	41.6	20.7	5.8	51.6
DK-8-5-2	53.5	69.3	75.5	12.1	22.1	1.1	41.2	21.3	6.2	51.0
DK-10-1										
DK-10-1-3	60.3	69.5	74.8	12.3	14.5	1.7	42.7	18.7	6.7	54.4
DK-10-1-4	62.2	69.2	74.2	11.6	12.0	1.9	41.1	21.6	6.4	55.5
DK-10-25										
DK-10-25-1	56.7	67.3	72.0	11.6	15.3	1.5	39.7	22.0	6.5	56.6
PI82-3										
PI82-3-1	61.4	68.8	74.1	10.6	12.7	1.7	43.5	18.6	5.2	49.4
PI82-6										
PI82-6-1	59.7	67.8	74.2	10.8	14.5	1.5	42.4	19.8	6.5	59.8
PI82-8										
PI82-8-5	59.2	67.3	73.7	11.4	14.4	1.6	40.9	20.5	6.2	54.6
PI82-18	62.0	69.8	75.9	12.2	13.8	1.8	42.9	19.4	5.5	45.0
PI82-18-1	62.0	69.8	75.9	12.2	13.8	1.8	42.9	19.4	5.5	45.0

[a] See Table I for an explanation of S_{n+1} line.

[b] T_{oG} = Gelatinization onset temperature; T_{pG} = Gelatinization peak temperature; T_{cG} = Gelatinization end temperature; ΔH_G = Enthalpy of gelatinization; R_G = Range of gelatinization temperature; PHI = Peak height index (enthalpy of gelatinization divided by half the range); T_{oR} = Retrogradation onset temperature; R_R = Range of retrogradation temperature; ΔH_R = Enthalpy of retrogradation; R% = Percentage of retrogradation. The results reported are an average of at least two replicates for each sample, and standard deviations are less than 0.59.

Table 4
Pasting properties of starches from selected corn lines measured with a Rapid Visco Analyser (RVA).

Source of Starch (S_{n+1} line[a])	P_{temp}[b]	P_{time}	Viscosity(RVU)				
			PV	HPV	CPV	Breakdown	Setback
Mo17	75.5	8.2	152.4	104.0	181.1	48.4	77.1
B73	79.2	8.3	195.2	117.7	214.9	77.5	97.2
CUBA-38							
CUBA-38-5	71.6	8.1	198.3	105.7	202.2	92.6	96.5
CHIS-37							
CHIS-37-5	72.3	8.3	210.0	136	230.6	74.0	94.6
DK-8-1							
DK-8-1	72.9	8.1	204.9	131.3	220.1	73.6	88.8
DK-8-4							
DK-8-4-3	72.1	8.3	184.0	111.6	209.9	72.4	98.3
DK-8-4-5	71.1	8.0	198.4	117	212.9	81.4	95.9
DK-8-4-6	71.2	8.2	222.7	140.1	232.4	82.6	92.3
DK-8-4-8	75.3	8.2	195.9	123.8	215.2	72.1	91.4
DK-8-5							
DK-8-5-1	73.4	8.3	200.3	123.4	221.3	76.9	97.9
DK-8-5-2	77.9	8.5	186.1	128.8	216.8	57.3	88.0
DK-10-1							
DK-10-1-3	72.7	8.1	205.7	119.3	208.8	86.4	89.5
DK-10-1-4	73.9	7.7	222.5	107.6	211.3	114.9	103.7
DK-10-25							
DK-10-25-1	77.1	8.4	204.2	122.4	229.2	81.8	106.8
PI82-3							
PI82-3-1	77.7	8.1	191.2	106.1	202.4	85.1	96.3
PI82-6							
PI82-6-1	71.9	8.0	207.2	105.4	213.9	101.8	108.5
PI82-8							
PI82-8-5	77.7	8.3	208.4	131.8	233.9	76.6	102.1
PI82-18							
PI82-18-1	78.1	8.3	192.8	127.7	213.9	65.1	86.2
S.D.	1.78	0.13	7.66	7.15	12.00	8.82	9.01

[a] See Table I for an explanation of S_{n+1} line.
[b] P_{temp}= pasting temperature (°C), P_{time} = peak time (min), PV = peak viscosity, HPV = hot paste viscosity, CPV = cool paste viscosity, breakdown = PV-HPV, setback = CPV-HPV. S.D. = standard deviations. The results reported are an average of at least two replicates for each sample.

Table 5
Gel properties of starches from selected corn lines measured with a Texture Analyzer.

Source of Starch (S_{n+1} line[a])	Firmness (g)		Stickness (g x sec)	
	25°C, 1 Day	4°C, 7 Days	25°C, 1 Day	4°C, 7 Days
Mo17	14.86ab[b]	19.20ed	18.53bd	24.43a-e
B73	10.46ij	16.44fg	14.40g	22.52a-f
CUBA-38				
CUBA-38-5	12.54d-g	20.46b-d	20.53ab	21.25c-f
CHIS-37				
CHIS-37-5	10.26j	16.40fg	20.33ab	20.44
DK-8-1				
DK-8-1	12.12e-h	20.31b-d	21.87a	26.68ab
DK-8-4				
DK-8-4-3	10.77h-j	14.28h	18.26be	18.8f
DK-8-4-5	13.51b-e	21.67ab	21.99a	18.94f
DK-8-4-6	16.24a	23.42a	18.90bc	21.87b-f
DK-8-4-8	13.39b-e	17.68fe	20.62ab	20.58d-f
DK-8-5				
DK-8-5-1	12.42d-g	16.39fg	20.09ab	19.68ef
DK-8-5-2	11.85f-i	17.36fg	17.42cf	25.46a-d
DK-10-1				
DK-10-1-3	15.3a	21.18bc	15.74eg	22.41a-f
DK-10-1-4	13.63b-d	21.21bc	14.12g	27.14a-c
DK-10-25				
DK-10-25-1	14.10a-c	NA[c]	15.44fg	NA
PI82-3				
PI82-3-1	11.17g-j	15.53hg	18.94bc	20.50d-f
PI82-6				
PI82-6-1	12.27d-h	19.65cd	20.17ab	27.57a
PI82-8				
PI82-8-5	13.09c-f	18.65ed	20.22ab	24.47a-e
PI82-18				
PI82-18-1	10.03j	17.36fg	16.08dg	20.06

[a] See Table I for an explanation of S_{n+1} line.
[b] The results reported are an average of at least five replicates for each sample. Standard deviations are not listed, because of the unbalance data. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).
[c] Not available. Data is missing because of the limited quantities.

Table 6
Mean granule size and shape parameters of starches from selected corn lines used in structural analyses.

Source of Starch (S_{n+1} line[a])	Mean Area (μm^2)	Mean Diameter (μm)	Mean Perimeter (μm)	Mean Radial S.D. (μm)	Mean Major Axis (μm)
Mo17	88.9D	9.8D	41.1C	8.8A-C	10.8D
B73	123.6A	12.0A	49.9A	7.7F	13.1A
CUBA-38					
CUBA-38-5	113.7B	11.3A	48.5A	9.5A	12.5A
CHIS-37					
CHIS-37-5	91.3CD	10.4C	43.4C	7.9EF	11.3C
DK-8-1					
DK-8-1	85.1D	9.9D	41.2C	8.1D-F	10.9D
DK-8-4					
DK-8-4-3	69.1E	8.9F	36.8F	8.2D-F	9.6E
DK-8-4-5	83.7D	9.9D	41.6C	8.2DE	10.8D
DK-8-4-6	86.7D	10.0CD	41.9C	7.8EF	10.9CD
DK-8-4-8	74.2E	9.3EF	38.7DE	7.9EF	10.1E
DK-8-5					
DK-8-5-1	85.7D	10.0D	41.6C	8.5CD	10.9CD
DK-8-5-2	70.7E	9.0F	37.5EF	8.3DE	9.8E
DK-10-1					
DK-10-1-3	71.3E	9.1F	37.9EF	8.5CD	9.9E
DK-10-1-4	94.4C	10.5C	43.9B	8.3DE	11.4C
DK-10-25					
DK-10-25-1	81.9D	9.7DE	40.4CD	8.9AC	10.7D
PI82-3					
PI82-3-1	83.2D	9.9D	41.8C	9.3A	10.9CD
PI82-6					
PI82-6-1	105.7B	10.9B	45.7B	8.6BD	11.9B
PI82-8					
PI82-8-5	83.5D	9.8D	41.2C	8.9A-C	10.7D
PI82-18					
PI82-18-1	85.6D	9.8D	41.2C	9.1AB	10.7D

[a] See Table I for an explanation of S_{n+1} lines.
[b] The results reported are an average of 1200 granules for each sample. Please refer to Statistical Analysis for the detail experimental design. Values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Table 7
Distribution profiles of starch from selected corn lines.

Source of Starch (S_{n+1} line[a])	Distribution Profiles (%)[b]				
	< 5(μm)	5-9(μm)	9-13(μm)	13-17(μm)	≥ 17 (μm)
Mo17	12a[c]	35.2c-e	29e	18.3c-e	5.5a-c
B73	0.92f	21.33f	37.92b-e	31.67a	8.17a
CUBA-38					
CUBA-38-5	4.23b-f	22.25f	38.14b-e	31.06ab	4.32b-d
CHIS-37					
CHIS-37-5	2.3ef	30.8d-f	48.1a	17.8c-g	1.0e
DK-8-1					
DK-8-1	2.92d-f	39.5bd	40.67a-c	14.42d-h	2.5c-e
DK-8-4					
DK-8-4-3	7.67a-d	49.58a	31.25d-e	10.67f-h	0.83e
DK-8-4-5	3.08ef	40.25a-d	41.25a-c	14.33d-h	1.08e
DK-8-4-6	2.08ef	40a-d	38.75a-c	17.67c-g	1.5de
DK-8-4-8	4b-f	47ab	37.33b-e	10.92e-h	0.75e
DK-8-5					
DK-8-5-1	4.42b-f	36.25c-e	42a-c	15.08d-h	2.25de
DK-8-5-2	7a-e	46.33ab	35.75b-e	10.42gh	0.5e
DK-10-1					
DK-10-1-3	8.42a-e	41.83a-c	39.92a-d	9.58h	0.25e
DK-10-1-4	3d-f	33.08c-e	41.75a-c	19.17cd	3c-e
DK-10-25					
DK-10-25-1	3.42c-f	42.67a-c	38.42b-e	14.08d-h	1.42d-e
PI82-3					
PI82-3-1	2.33ef	38.33b-d	44.5ab	13.92d-h	0.92e
PI82-6					
PI82-6-1	6.33b-e	28.33e-f	34.58c-e	23.83bc	6.92ab
PI82-8					
PI82-8-5	4.5b-f	37.83b-e	41.83a-c	14.5d-h	1.33de
PI82-18					
PI82-18-1	9.17ab	35.92c-e	34.33c-e	18c-f	2.58c-e

[a] See Table I for an explanation of S_{n+1} lines.
[b] The results reported are an average of three replicates for each sample.
[c] Values followed by the same letter in the same column are not significantly different ($P < 0.05$)

Table 8
Relative branch chain-length (CL) distributions of whole starch.

Source of Starch (S_{n+1} line[a]-Kernel)	Peak dp[b]		CL	% Distribution[c]			
	I	II		dp 6-12	dp 13-24	dp 25-36	dp ≥ 37
Mo17	14	46	25.0	16.11	47.54	14.07	22.27
B73	14	44	25.5	16.32	45.92	15.25	22.50
CUBA-38							
CUBA-38-5	13	46	23.4	18.58	48.57	15.05	17.79
CHIS-37							
CHIS-37-5	13	46	23.7	18.00	48.12	15.06	18.82
DK-8-1							
DK-8-1	14	46	24.5	17.08	47.01	15.54	20.37
DK-8-4							
DK-8-4-3	14	44	23.7	18.53	48.53	14.34	18.60
DK-8-4-5	14	45	24.3	17.84	47.91	14.40	19.85
DK-8-4-6	14	45	24.2	17.40	47.63	15.57	19.39
DK-8-4-8	14	45	23.9	18.77	48.06	14.08	19.09
DK-8-5							
DK-8-5-1	14	44	24.5	17.66	47.09	15.64	19.61
DK-8-5-2	14	44	24.4	17.98	47.63	14.47	19.92
DK-10-1							
DK-10-1-3	14	46	25.3	16.96	46.25	15.11	21.68
DK-10-1-4	14	44	24.1	17.69	48.13	15.07	19.11
DK-10-25							
DK-10-25-1	14	45	24.4	16.55	47.14	16.69	19.60
PI82-3							
PI82-3-1	14	47	24.0	17.34	47.97	15.97	18.71
PI82-6							
PI82-6-1	13	44	23.5	18.26	49.80	13.95	18.00
PI82-8							
PI82-8-5	13	46	24.0	17.44	48.02	15.86	18.69
PI82-18							
PI82-18-1	14	46	23.6	17.28	49.60	15.20	17.92

[a] See Table 1 for an explanation of S_{n+1} lines.
[b] dp=degree of polymerization
[c] The results reported are an average of at least two replicates for each sample. Grouping of dp numbers followed that of Hanashiro et al (1996).

Table 9
Amylopectin molecular weights and gyration radii of starches from Selected Corn Lines.

Source of Starch (S_{n+1} line[a])	M_w (x 10^8)[b]	R_z (nm)[c]
Mo17	11.5ab	386.8ab
B73	8.6d-f	365.7bc
CUBA-38		
CUBA-38-5	8.3ef	368.3a-c
CHIS-37		
CHIS-37-5	8.5d-f	359.1bc
DK-8-1		
DK-8-1	10.2b-d	366.6bc
DK-8-4		
DK-8-4-3	8.0f	351.1c
DK-8-4-5	9.0d-f	365.7bc
DK-8-4-6	9.4c-f	368.3a-c
DK-8-4-8	8.9d-f	374a-c
DK-8-5		
DK-8-5-1	8.5d-f	367.6bc
DK-8-5-2	7.9f	361.9bc
DK-10-1		
DK-10-1-3	12.4a	398.1a
DK-10-1-4	9.8c-e	376.6a-c
DK-10-25		
DK-10-25-1	9.3c-f	361.1bc
PI82-3		
PI82-3-1	9.1d-f	360.8bc
PI82-6		
PI82-6-1	8.9d-f	368.8a-c
PI82-8		
PI82-8-5	8.3ef	368.5a-c
PI82-18		
PI82-18-1	10.9a-c	385.2ab

[a] See Table I for an explanation of S_{n+1} lines.
[b] Weight –average molecular weight. The results reported are an average of three replicates for each sample.
[c] z-average radius of gyration. The results reported are an average of three replicates for each sample.

Table 10
Pearson correlation coefficients (r) of starch functional properties with structural properties.

Functional Properties	Granule Size Parameter		Granule Size Distribution Profiles [a]				
	Mean Area	Mean Major Axis	≥ 17 (μm)	13-17 (μm)	9-13 (μm)	5-9 (μm)	< 5 (μm)
DSC Parameter [b]							
T_{oG}	0.56**[d]	0.53**	0.48**	0.50**		-0.60**	
T_{pG}							
T_{cG}			0.50**		-0.48**		
H_G							
R_G	-0.56**	-0.54**		-0.44*		0.52**	
H_R							
R%	0.54**	0.54**					
RVA Parameter [c]							
Setback							-0.41*
Breakdown		0.40*[c]					
PV					0.56**		-0.55**

[a] Starch granules are divided into different groups according to equivalent diameter.
[b] See Table 2 for DSC parameter descriptions. See Table 4 for RVA parameter descriptions.
[c] See Table 4 for RVA parameter descriptions.
[d] *, ** : p-values for test Ho: $\rho = 0$ vs. Ha: $\rho = 0$ are smaller than 0.10 and 0.05, respectively. We used 0.1 instead of 0.005 here, because starch is a very complicated system which results in small correlation coefficients. If we use 0.005, we may lose some information here.

Table 10 (continued)

Functional Properties	Branch Chain Length Distribution		
	CL	dp 6-12 (%)	dp ≥ 37 (%)
DSC Parameter[b]			
T_{oG}		-0.49**	
T_{pG}	0.50**	-0.52**	-0.59**
T_{cG}			
H_G	0.49**	-0.42*	0.58**
R_G	0.47*		
H_R	0.48**	-0.48**	0.49**
R%			
RVA Parameter[c]			
Setback			-0.46*
Breakdown			
PV			

Table11.
Pearson correlation coefficient (r) among functional properties of starch.

	DSC Parameter						
	T_{oG}[a]	T_{pG}	T_{cG}	R_G	H_G	T_{oR}	R_R
Gel Parameter							
S7							
F7					0.42*	-0.47*	
S1							
F1					0.44*	-0.42*	
Setback		-0.71**	-0.64**		-0.77**	-0.45*	0.42*
Breakdown		-0.45*	-0.46*	-0.54*			
PV		-0.65**	-0.42*			0.45*	
P_{temp}							
DSC Parameter							
R%							
ΔH_R							
R_R			-0.43*			-0.88**	
T_{oR}	0.57**	0.62**	0.67**			-0.88**	
R_G	-0.94**						
ΔH_G		0.50**					
T_{cG}	0.43*	0.79**					
T_{pG}	0.62**						

[a] See Table 2 for DSC parameter descriptions.
[b] See Table 4 for gel RVA parameter descriptions. S7 = Stickiness after seven days of storage at 4°C. F7 = Firmness after seven days of storage at 4°C. S1 = Stickiness after one days of storage at 25°C. F1 = Firmness after one days of storage at 25°C.
[c] *, ** : p-values for test Ho: $\rho = 0$ vs. Ha: $\rho = 0$ are smaller than 0.10 and 0.05, respectively. We used 0.1 instead of 0.005 here, because starch is a very complicated system which results in small correlation coefficients. If we use 0.005, we may lose some information here.

Table 11 (continued)

	DSC Parameter		Gel Parameter			
	H_R	R%	P_{temp}[b]	PV	Breakdown	F1
Gel Parameter						
S7	0.63**[c]	0.59**				
F7	0.57**		-0.49**	0.43*		0.78**
S1			-0.49**			
F1	0.45*					
Setback		0.44*		0.59**	0.74**	
Breakdown		0.48**		0.73**		
PV						
P_{temp}						
DSC Parameter						
R%	0.83**					
ΔH_R						
R_R						
T_{oR}						
R_G						
ΔH_G						
T_{cG}						
T_{pG}						

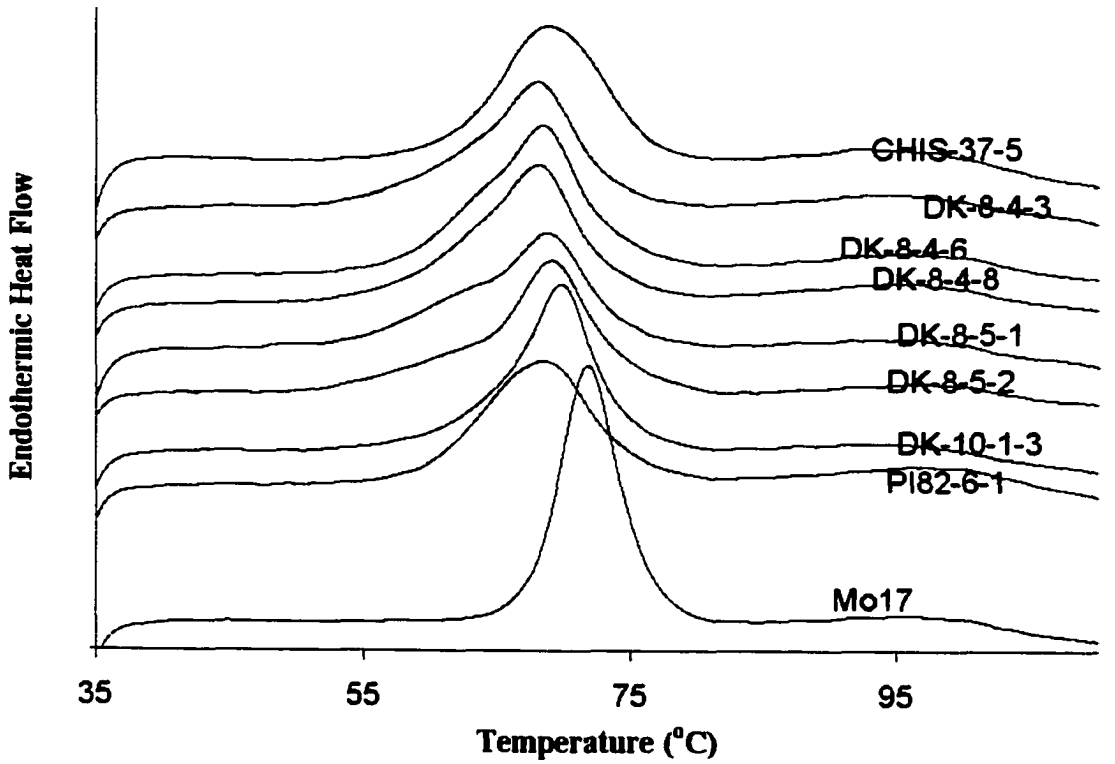

Fig1. Gelatinization differential scanning calorimetry thermographs of starches in Table 3.

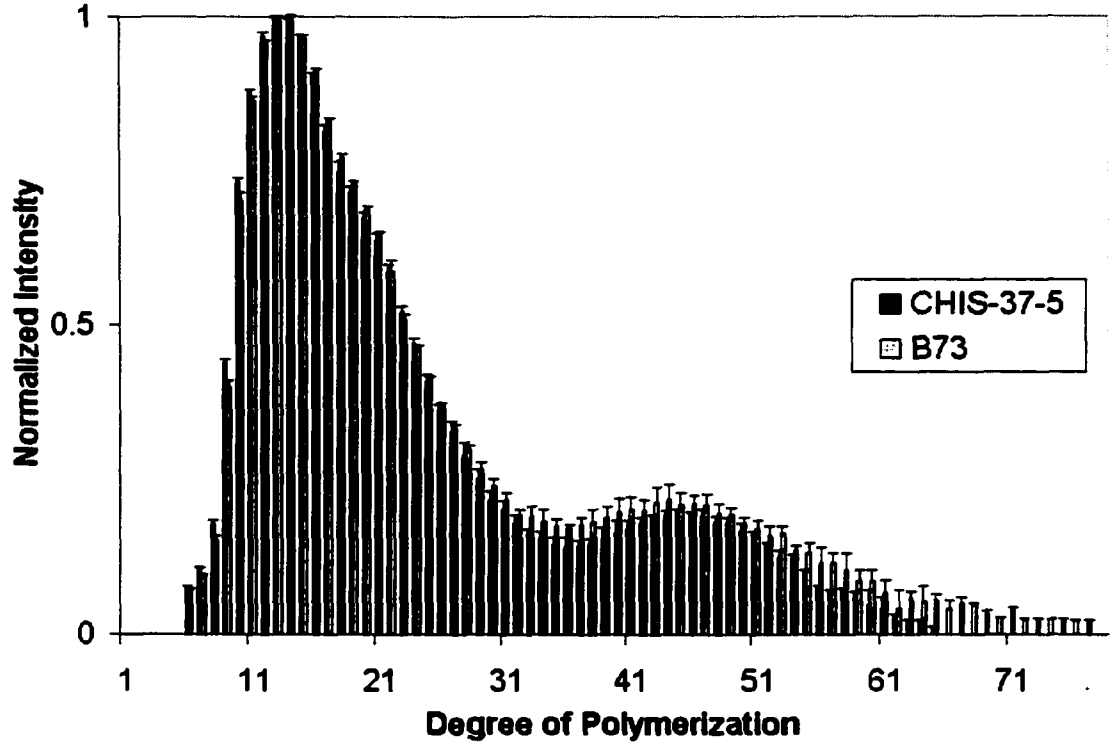
1
0.5
0
Normalized Intensity
1
11
21
31
41
51
61
71
Degree of Polymerization
CHIS-37-5
B73

Fig. 2a

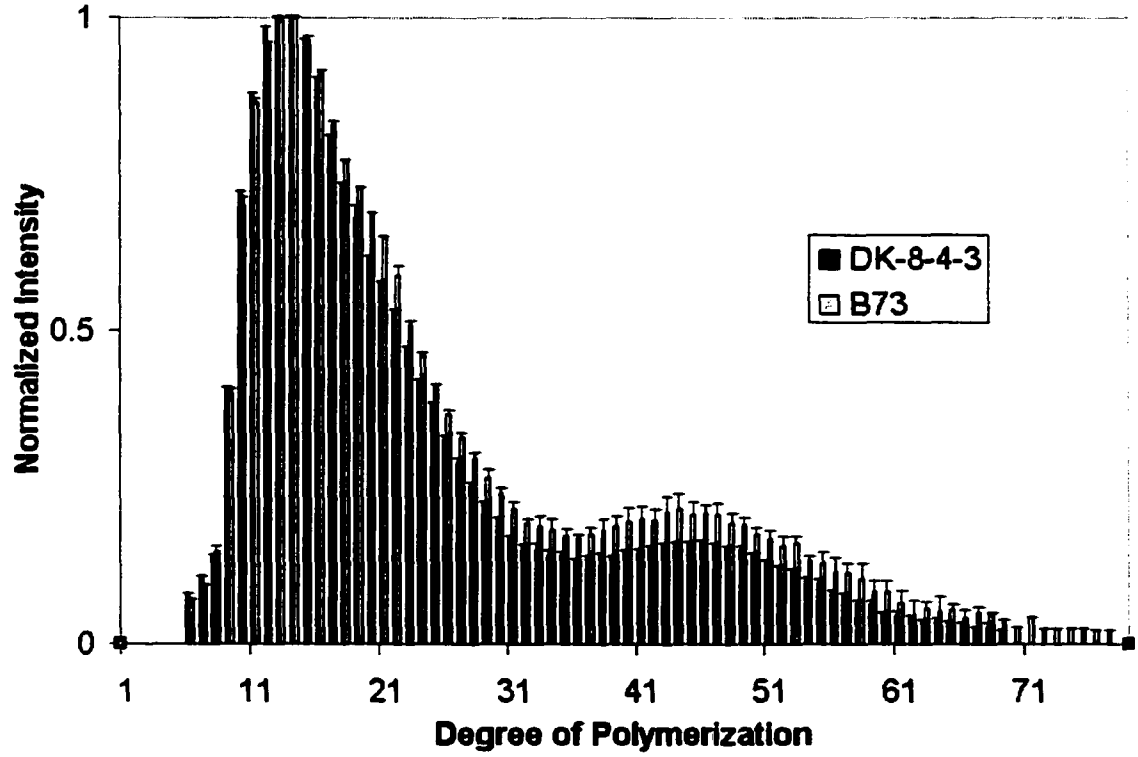
1
0.5
0
Normalized Intensity
1
11
21
31
41
51
61
71
Degree of Polymerization
DK-8-4-3
B73

Fig. 2b

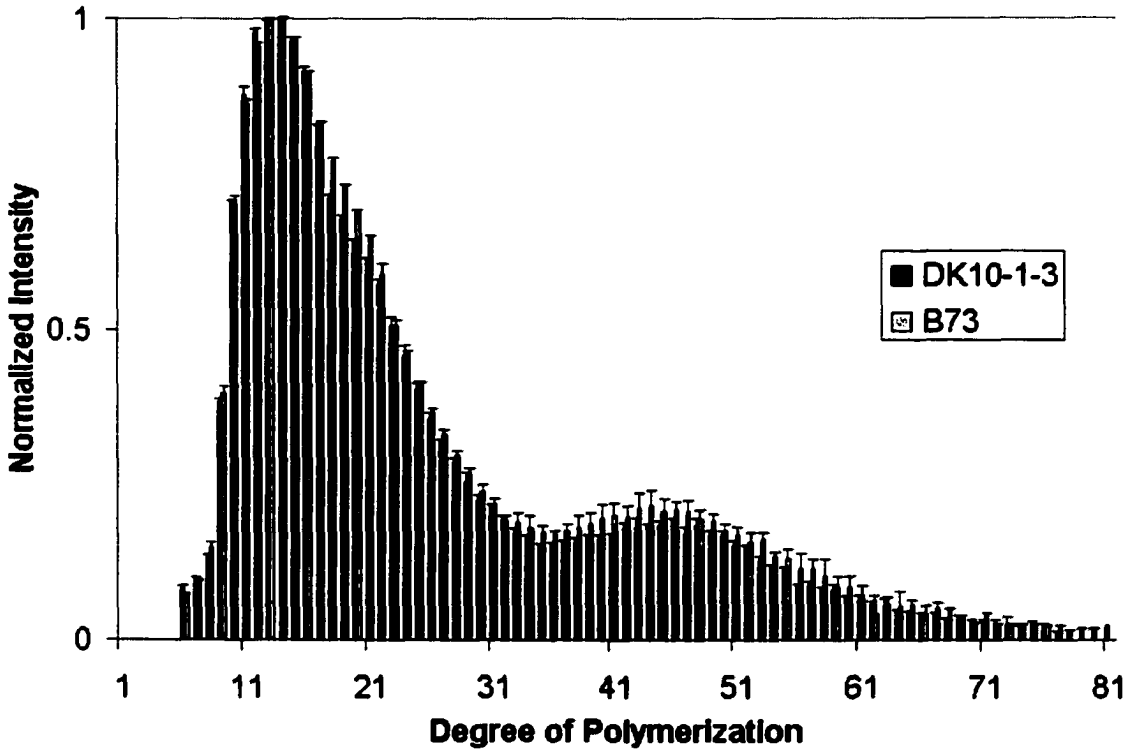

Fig. 2c

Fig 2a-c. Comparison of normalized branch-chain length distributions of exotic starches with B73 starch determined by using a high performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD). A Carbopac PA100 column an immobilized amyloglucosidase column were used for the analysis.

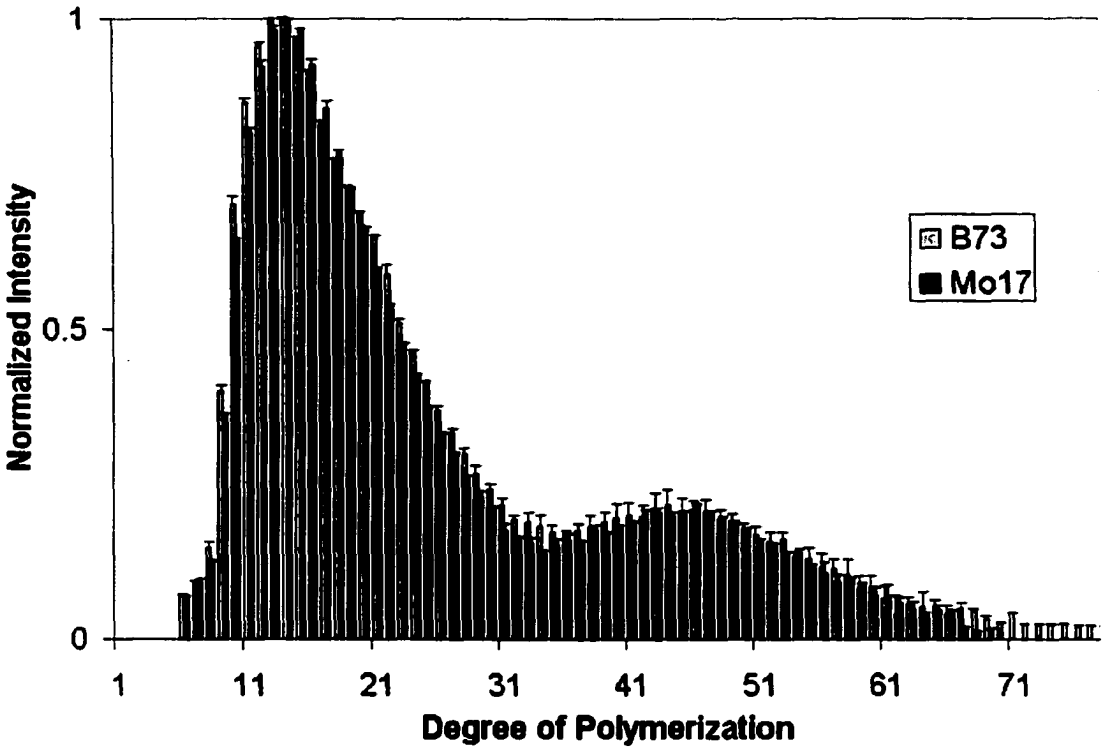

Fig 3. Comparison of normalized branch-chain length distributions of starches from two control line B73 and Mo17, determined by using a high performance anion-exchange chromatography system equipped with an enzyme column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD). A Carbopac PA100 column an immobilized amyloglucosidase column were used for the analysis.

CHAPTER 6. GELATINIZATION PROPERTIES OF STARCHES FROM THREE SUCCESSIVE GENERATIONS OF SIX EXOTIC CORN LINES GROWN IN TWO LOCATIONS [1]

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Abstract

The objectives of this research were to evaluate the intra- and interpopulation variability in gelatinization properties of starches from exotic corn lines and their derivatives when grown 1) during two successive years in the same location; and 2) in both temperate and tropical environments. Six novel corn lines (two exotic lines and four derived from a breeding cross developed by crossing an exotic hybrid with Corn Belt lines) were selected for this research because heir starches have significantly different (and potentially useful) thermal properties than those found in starch from normal Corn Belt corn. The S_n (n=3 for exotic cross population and n=1 for exotic inbred population) generations of the six exotic lines were self-pollinated and grown in the winter nursery in Puerto Rico. Two successive generations (S_{n+1} and S_{n+2}) of selected (for low onset gelatinization temperature T_o) lines were self-pollinated and grown in the same environment near Ames, Iowa. To evaluate the effect of environment, the S_{n+2} generation also was self-pollinated and grown in the winter nursery in Puerto Rico. Thermal properties of starches from ten single kernels from each line were analyzed by using Differential Scanning Calorimetry (DSC). After subsequent generations, the differences in gelatinization properties between selected kernels within each progeny line narrowed, suggesting increased homogeneity of starch structural properties within each line. Unusual thermal properties were fixed in some progeny lines. Environmental factors also affected the thermal properties of starch, and a significant interaction between environment and genotype was observed. These results suggest that introgression of adapted germplasm with useful genes from exotic corn would increase the available genetic variability for starch functionality and allow the development of hybrids with important value-added traits.

Introduction

Less than one percent of the U.S. germplasm base of corn consists of exotic germplasm (Goodman 1985), leading to concerns about corn's genetic vulnerability to changes in environmental and agronomic conditions, and new insect and disease pressures (Crossa and Gardner 1987, Kuckuck et al 1991). The Germplasm Enhancement of Maize Project (GEM), a coordinated and cooperative effort among public and private sectors, was launched with the objective of providing the corn industry with materials developed by using germplasm enhancement of useful exotic germplasm and ultimately improving and broadening the germplasm base of corn hybrids grown by American farmers. Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics (Pollak and Salhuana 1999). Starch represents nearly 70% of the dry weight of the mature corn kernels and is the most economically important component. Therefore, to further evaluate the starch quality of the GEM materials is essential to the fully utilize these materials for food application.

We have currently developed several corn lines derived from breeding crosses of exotic genotypes with Corn Belt lines. Our research has shown that these new lines can produce starches with improved functional properties, which may have potential applications in food industry. The desired functional properties of starches from the new developmental lines (Ji et al 2002b) and their possible food applications are listed in Table I. To produce inbred lines from the early-generation GEM lines that can be released and used commercially as breeding lines, it is necessary to continue to develop and select lines to genetically "fix" the unusual thermal properties, and to evaluate the performance of the selected lines grown under different environments.

Individual properties of starch depend both on the genetic background of corn genotype and the environment. The effect of the genetic background of corn and environmental factors on the starch during the development of plants may be attributable to changes in granule-size distribution, chemical structure, crystallinity, organization of the molecules within the granule, and/or molecular structure of the starch polymers (Asaoka et al 1989, Fergason and Zube 1962, Hizukuri 1969, Morrison and Scott 1986, Shi et al 1994, Tester et al 1991). It is not know the roles of genetic (variety and level of breeding) and environmental (year and location) factors in the total variability of starch properties. One

reason for this might be that different genotype responds differently to environmental factors.

In the current study, S_n generations of selected lines were self-pollinated and grown in the winter nursery in Puerto Rico in 1996. The S_n designation defines the number of times the line has been self-pollinated, starting with the breeding cross or the Plant introduction (the S_0 population), in the development of the line (Simmonds 1974). Two successive generations (S_{n+1} and S_{n+2}) of the selected lines were self-pollinated and grown in same environment near Ames, Iowa in 1998 and 1999. To evaluate the effect of environment, the S_{n+2} generation also was self-pollinated and grown in the winter nursery in Puerto Rico in 1999-2000. The objectives of this research were to evaluate the intra- and interpopulation variability in thermal properties of starches from exotic novel corn lines and their derivatives when grown 1) during two successive years in the same location; and 2) in both temperate and tropical environments.

Materials and Methods

Materials

Three successive generations (S_n, S_{n+1}, and S_{n+2}) of selected lines derived from an exotic by adapted breeding cross from the Germplasm Enhancement of Maize (GEM) project and two exotic inbred Plant Introductions were studied (Table II). The breeding crosses were developed by crossing an exotic 3-way hybrid with two commercial inbreds of the Stiff-Stalk heterotic pattern. Two successive generations (S_{n+1} and S_{n+2}) of selected lines were self-pollinated and grown in the same environment near Ames, Iowa. To evaluate the effect of environment, the S_{n+2} generation also was self-pollinated and grown in the winter nursery in Puerto Rico. Ears were harvested at full physiological maturity and dried at approximately 37.5°C until the moisture content reached approximately 12%. All seeds were stored approximately at 4°C and 10% relative humidity until analyzed.

Single Kernel Starch Extraction

Starch was extracted from single kernels by using the method described by White et al (1990), with modifications (Krieger et al 1997). Starch from each of 10 randomly selected kernels from each line was evaluated separately for starch characteristics after extraction. After extraction, starch was stored at 4°C until evaluated.

Differential Scanning Calorimetry (DSC)

For DSC analysis, a Perkin-Elmer DSC 7 analyzer equipped with a thermal-analysis data station (Perkin-Elmer Corp., Norwalk, CT) was used. Analysis of starch gelatinization was conducted as described by White et al (1990). Starch (~4.0 mg, dwb) was weighed into aluminum sample pans with 8 mg of distilled water. Samples were heated from 30 to 110°C at a rate of 10°C/min. Thermal transitions for gelatinization were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and ΔH (enthalpy of gelatinization). These parameters were calculated directly by the DSC software. All enthalpy calculations were based on the dry-starch weight. The retrogradation parameters are not discussed in this paper because no significant differences among genotypes and environments were observed.

Statistical Analysis

Effect of genotype and environment and their interactions on the gelatinization properties of starch of S_{n+2} generation were analyzed by using an analysis of variance procedure for a mixed model with nested design for unbalanced data. The model was:

$$Y_{ijklm} = \mu + En_i + P_j + L_{kj} + (EnP)_{ij} + (EnL)_{ijk} + O_{lijk} + E_{ijklm}$$

In this equation, Y_{akima} is the gelatinization values for single kernel, μ is the overall average, En_i is the "fixed" environmental effect, P_j is "fixed" source effect, $(EnP)_{ij}$ is the interaction between environment with source, L_{kj} is random line effect (S_{n+1}, nested within the population), $(EnL)_{ijk}$ is the interaction between environment with S_{n+1} line, O_{lijk} is the random progeny line effect (nested within each S_{n+1} line), and E_{ijklm} is the random error. Effects from environment and source were treated as "fixed" effects here, because only two environments (Ames, 1999 and Puerto Rico 1999 winter nursery) and six sources were evaluated in this study. Because of the unbalance data, Type III sum of square was used to test whether the contribution from each item (except μ and E_{ijklm}) in the equation equaled to zero or not.

Results and Discussion

Genetic Variability among and within S_{n+2} Lines Grown at Ames, IA 1999

The degree of genetic variability of developmental lines can be evaluated quantitatively by measuring variations in starch gelatinization properties with DSC (Brockett

et al 1988, Pollak and White 1997, Sanders et al 1990). The gelatinization properties of starches from 66 S_{n+2} lines from exotic sources, DK8, DK10, DK14, DK34, PI82 and PI83, grown in Ames, IA 1999, were determined by using DSC and summaries of their values are listed in Tables III-VIII.

The thermal properties of starch from different lines exhibited considerable variability. Among 66 S_{n+2} lines (from six sources) analyzed, the mean T_o ranged from 55.8 °C (DK8-S_3-4-1, Table III) to 66.7 °C (PI83-S_1-7-1, Table VIII), the mean T_p ranged from 66.8 °C (PI82-S_1-6-3, Table VII) to 70.0 °C (PI83-S_1-7-1, Table VIII), the mean T_c range from 71.4°C (DK10-S_3-3-2, Table IV) to 75.6°C (PI82-S_1-7-1, Table VII), and mean ΔH range from 9.3J/g (PI83-S_1-1-4, Table VIII) to 12.3J/g (DK10-S_3-18-4, Table IV). Within each line, a high degree of variability for different gelatinization properties was also observed among ten single kernels analyzed. Among 66 lines analyzed, line DK8-S_3-5-2 showed the highest variability in T_o, ranging from 51.7-65.1 °C with standard deviation 3.95 (Table III), line PI82-S_1-8-2 showed the highest variability on T_p and T_c, ranging from 66.3-70.3 °C with standard deviation 1.20 and ranging from 71.4-76.1 °C 1with standard deviation 1.69 respectively (Table VII). Line DK10-S_3-7-3 showed the highest variability on ΔH, ranging from 7.9 to 12.3 J/g with standard deviation 1.7 (Table IV).

Sufficient variability in thermal properties within corn germplasm is important in breeding programs aimed at screening maize germplasm for desired starch properties, because the outliers reveal accessions possessing traits of potential use and make it possible to develop corn lines with unusual thermal properties. Several studies have indicated genetic variability among nonmutant sources of maize by DSC. For example, White et al (1990) observed significant variations for onset temperature (T_o), range of gelatinization (R_G), and ΔH values within and among several genetically variable, open-pollinated populations of maize. Similarly, Li et al (1991) observed variability within several exotic populations, suggesting that selection might be possible within populations to obtain genotypes with specific starch properties.

Because the 66 lines in this paper were all grown in the same location during the same year, growing conditions of the kernels were alike. Therefore, the significant differences in gelatinization properties observed suggest that the different genetic backgrounds of corn have a major effect on starch gelatinization behavior. The differences

in gelatinization properties of starch from various corn lines could be attributed to some structural variations of the starch. It has been suggested that gelatinization temperature represents a measure of starch crystallite perfection and ΔH represents the amount of crystalline structure (Tester and Morrison 1990). Previous studies defined the structures of these and other corn lines at S_{n+1} (Ji et al 2002a) and S_{n+2} (Ji et al 2002b) generations.

Significant differences were observed among their granule size and shape distribution, amylopectin molecular weight distribution, and branch-chain length distribution of amylopectin. The variations in these structural properties would lead to the variation in the crystalline structures of starch granule and the observed gelatinization properties.

Comparison among Three Successive Generations

The gelatinization values (mean, range and standard deviation) for starches from derived lines of the exotic breeding cross and the two exotic inbreds populations over three successive growing seasons (S_n in 1996 at Puerto Rico, S_{n+1} in 1998 at Ames, and S_{n+2} in 1999 at Ames) are also listed in Table III-VIII.

In general, each subsequent generation resulted in a narrowing of the differences in gelatinization properties between kernels within each progeny line, suggesting increased homogeneity of starch structural properties within each line. Such a trend of increased homogeneity (or decreased s.d. of gelatinization properties) was clear following the generation DK8-S_3 -> DK8-S_3-1 -> progeny lines of DK8-S_3-1 (Table III). A decrease in the range of T_o values was found over three successive generations (S_n -S_{n+2}) of population DK8 (Table 3). Among ten single kernels analyzed for the S_n generation, the T_o had a range of 6.0°C and s.d. (standard deviation) 1.92, whereas for the S4 generation, the range decreased to 2.6 - 4.9°C with an accompanying decrease of s. d. to 1.01-1.64. The 10 progeny S_{n+2} lines of DK8-S_3-1 showed continued reduction in the range of T_o and s. d., with the range decreased from 4.9°C to 2.3-4.5°C, and the s.d. decreased from 1.64 to 0.70-1.27. A similar trend of increased homogeneity on gelatinization values over successive generations were also observed for some lines from population DK10, DK14, DK34, PI82 and PI83.

The S_3 generation (staring from S_1 generation) of exotic inbreds PI82 and PI83 still exhibited large variability on gelatinization temperatures and enthalpy still exited at. For S_3 generation of PI82 inbred, the largest variability on T_o was shown up among the ten seeds analyzed from PI82-S_1-5-1, with a range of 8.8°C and s.d. of 2.86. For S3 generation of PI83

inbred, the largest variability on T_o was shown up among the ten seeds analyzed from PI83-S_1-4-1, with a range of 9.2 °C and s.d of 3.55. The reason for this might be that inbreds PI82 and PI83 was not true inbreds or the trait was not fixed in them.

For some lines, the change of variability of gelatinization properties over successive generations became complicated. Even among progeny lines from the same parent, some progeny lines showed larger variability, whereas other progeny lines showed smaller variability than their parent did. For example, the s.d. of T_o for parent line DK10-S_3-1 was 1.27. Among the next generation, two lines DK10-S_3-1-1 and DK10-S_3-1-3 had small s.d.s (1.19 and 1.01, Table IV), whereas two lines DK10-S_3-1-2 and DK10-S_3-1-4 had larger s.d.s (1.37 and 1.93). This complication might be caused by many reasons. One possible reason might be that gelatinization properties of starch are controlled by many genes and their interactions. The line with small s.d. might be more homozygous for its gene controlling the trait than the line with large s.d. Another possible reason might be the environmental effect and its interaction with genotype, which will be discussed in detail in the following section.

Gelatinization thermograms of starch from progeny lines of DK8-S_3-4 and DK8-S_3-5, which were grown at Ames, IA 1999, started to separate into a single peak with a big shoulder at low temperature (Fig 1). This separation in gelatinization thermograms may also contribute to the increased variability of gelatinization values among some progeny lines.

Selection and Verification of Trait with Potential Application in Food Industry

We started the selection by using DSC at the S_3 generation for GEM exotic cross lines with the goal to increase the desirable gene frequency (producing starch with $T_o <$ 61.0°C) in the progeny. Before S_3 generation, selection was for agronomic traits and yield. The frequency of kernels containing starch with desirable gelatinization properties ($T_o <$ 61.0°C) and their potential food application are listed in Table III-VIII. Unusual thermal properties ($T_o < 61.0$°C) were fixed in some progeny lines S_{n+2} grown at Ames, 1999. For example, one out of ten kernels of the S_{n+1} line DK8-S_3-1 contained starch with $T_o < 61.0$°C, whereas this frequency increased to 9 out of 10 kernels in the S_{n+2} line of DK8-1-1. Zero out of ten kernels of the S_{n+1} line DK10-S_3-7 contained starch with $T_o < 61.0$°C with one kernel containing starch with $T_o = 61.6$°C,, whereas this frequency increased to 8 out of 10 kernels in the S_{n+2} line of DK10- S_3-7-1(Table IV). In addition, it could be concluded that selection for these traits using the Puerto Rico winter nursery was successful.

Starch Variability between Two Locations

The average values and ranges of gelatinization parameters (To, Tp, Tc and ΔH) of starch from the S_{n+2} generations of the corn grown at two locations (Ames and Puerto Rico) revealed significant environmental effects and some significant interactions between environment and genotype (Table IX). The significant environmental and genotype interactions indicated that different genotypes responded differently to environmental factors and that environment affects expression of these traits.

The environment and genotype interactions in this study include environment and source interaction and environment and S_{n+1} line interaction. And these significant interactions can be specified from the gelatinization data in Table III-VIII. For progeny lines of DK10- S_3-4, DK10-S_3-6, DK10-S_3-10, no significant differences were caused by the effect of environment. Whereas for progeny lines of DK10-S_3-15 and DK10-S_3-18, there was a significant effect of environment on the T_o of starch. Eight out of nine progeny lines of DK10-S_3-15 grown in Puerto Rico exhibited lower mean T_o (61.2-63.5°C) than did two progeny lines grown in Iowa (mean T_o 64.4-64.7 °C). For DK10-S_3-18, one progeny line out of two grown in Puerto Rico exhibited lower mean T_o (61.6 °C) than did two progeny lines grown in Iowa (mean T_o 63.0-64.2 °C), whereas one progeny line out of two progeny lines of DK10-18 grown in Puerto Rico exhibited higher mean T_o (65.7 °C) than did two progeny lines grown in Iowa (mean T_o 63.0-64.2 °C). Similar complications were also observed when comparing progeny lines of DK34-S_3-9 grown at two locations. In contrast, the T_o of starches from progeny lines (S_{n+2} generation) of the exotic accession PI82 grown in the tropical environment were higher than were those grown in the temperate environment.

Conclusion

The degree of genetic variability among and within developmental lines for gelatinization properties was characterized by analyzing starch gelatinization properties on 10 randomly selected single kernels by using DSC. Large intra- and interline variability was observed, and unusual thermal properties were fixed in some progeny lines over two successive generations. Unusual thermal properties (T_o < 61.0°C) were fixed in some progeny lines. These results indicate that these lines might be useful for developing corn breeding lines with unusual thermal properties. Environmental factors also affected the

thermal properties of starch, and a significant interaction between environment and genotype was observed for some lines.

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Table I
Desired Functional Properties of Unusual Starches from New Hybrids and Their Application in Food Industry

Parameters	Desired Characteristics	Potential Application in Food Industry
Gelatinization temperature	Low onset and peak gelatinization temperature by Differential Scanning Calorimeter (<61°C and 66°C respectively)	Energy savings, and nutrient and flavor retention during the cooking process.
Viscosity development	High peak viscosity (>200 RVU) by Rapid Visco Analyzer (RVA)	Good swelling behavior, good thickener, less starch in a formula, formula savings.
Paste Properties	High set back value by RVA	Good gelling properties.
Gel properties	High firmness by Texture Analyzer (TA)	Good gelling properties.

Table II
Exotic Breeding Crosses and Exotic Inbred Corn Lines and Their Origins.

Exotic Parent[a]	Pedigree for S_n Lines[b]	Source Identification[c]	Origin of Exotic Parent
		Exotic Breeding Crosses	
Ames 23670	DK212T:S0610-8-1-3	DK8-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-10-1-3	DK10-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-14-1-2	DK14-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-34-1-1	DK34-S_3	DeKalb Genetics Hybrid from Thailand
		Exotic Inbreds	
PI186182	PI 186182	PI-82-S_1	Inbred 378 from Uruguay
PI186183	PI 186183	PI-83-S_1	Inbred 378 from Uruguay

[a] Original corn populations as maintained at the North Central Region Plant Introduction Center, Ames, IA.
[b] Regrown corn ears, maintained as lines to preserve a specific starch characteristic. In S_n generations, n=3 for exotic breeding crosses and n=1 for exotic inbreds.
[c] Abbreviated source identification for use within this paper.

Table III

Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the DK8 Source

Corn Source[a]	Freq[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S_3 generation, grown in Puerto Rico 1996 winter nursery													
DK8-S_3	0/10	66.8	62.4-68.4	1.92	70.7	69.7-71.5	0.67	74.7	73.5-75.5	0.71	12.2	11.3-12.8	0.58
S4 generation, grown in Ames 1998													
DK8-S_3-1	1/10	63.6	61.5-66.4	1.64	71.4	70.6-72.4	0.49	76.9	76.5-77.6	0.46	12.4	11.7-13.2	0.44
DK8-S_3-2	0/10	65.5	64.7-67.3	1.08	70.2	69.8-70.7	0.32	74.8	74.4-75.9	0.57	11.9	11.3-12.4	0.47
DK8-S_3-3	0/10	64.4	62.3-66.8	1.51	69.9	68.1-70.8	0.85	74.9	73.9-75.4	0.50	12.5	11.4-13.9	0.91
DK8-S_3-4	0/10	66.0	64.1-67.3	1.01	71.1	69.6-72.5	0.78	75.3	73.9-76.1	0.61	12.4	11.5-13.6	0.73
DK8-S_3-5	0/10	65.3	63.8-67.4	1.17	70.1	69.0-71.2	0.73	74.6	74.0-75.7	0.56	12.5	10.8-14.3	1.18
S_5 generation, grown in Ames 1999													
DK8-S_3-1-1	9/10	60.2	59-63.2	1.16	68.1	67.5-69.7	0.64	73.6	72.6-74.8	0.69	11.4	10.4-12.6	0.63
DK8-S_3-4-1	10/10	55.8	52.5-57.8	1.54	67.8	67.2-68.7	0.52	73.2	72.3-74.5	0.63	11.6	10.9-12.3	0.45
DK8-S_3-4-2	10/10	57.0	52.8-59.2	2.16	67.6	66.9-68.5	0.54	73.0	72.3-74.3	0.60	11.7	9.46-13.0	1.01
DK8-S_3-4-3	10/10	59.2	55.7-60.6	1.57	68.2	67.3-68.6	0.38	73.1	72.3-73.7	0.42	12.1	11.5-12.8	0.44
DK8-S_3-4-4	10/10	57.5	55.1-59.9	1.72	67.3	66.8-68.2	0.41	72.6	71.9-73.7	0.68	11.2	8.8-12.7	1.25
DK8-S_3-4-5	9/10	58.6	55.1-61.4	1.64	68.0	66.7-69.6	0.92	72.8	71.5-74.6	0.95	12.1	10.7-13.3	0.67
DK8-S_3-4-6	10/10	58.2	55.7-60.6	1.64	67.8	67-68.3	0.41	73.44	72.2-75.6	1.11	11.4	9.2-12.4	1.07
DK8-S_3-5-1	9/10	58.5	54.8-62.8	2.39	68.7	67.6-69.9	0.66	73.8	72.9-75.0	0.70	11.2	10.0-12.1	0.67
DK8-S_3-5-2	9/10	56.6	51.7-65.1	3.95	68.3	67.8-68.8	0.34	73.2	72.3-74.2	0.73	10.9	9.23-13.7	1.44
DK8-S_3-5-3	5/10	61.5	58.7-64.1	2.13	67.7	67-68.9	0.64	72.8	71.3-74.5	0.97	11.3	9.7-12.5	0.76
S_5 generation, grown in Puerto Rico, 1999 winter nursery													
DK8-S_3-1-2	0/10	64.7	61.8-65.7	1.11	69.2	68.7-69.7	0.35	73.8	72.8-75.0	0.62	11.9	10.9-12.5	0.44
DK8-S_3-1-3	0/10	65.2	64.4-66.7	0.70	69.8	69-70.8	0.52	74.0	73.0-75.0	0.63	11.7	10.5-12.4	0.59
DK8-S_3-1-4	0/10	66.2	64.2-67.7	1.19	69.9	68.7-71	0.74	73.8	72.7-74.8	0.65	12.2	11.4-13.8	0.81
DK8-S_3-1-5	0/10	65.1	63.6-66.3	0.79	69.1	68.2-70.0	0.64	73.4	72.3-74.9	0.81	11.7	10.8-12.8	0.68
DK8-S_3-1-6	0/10	65.0	63.4-66.2	0.93	69.0	68.2-69.8	0.57	73.0	72.0-74.1	0.79	11.2	10.2-12.3	0.75
DK8-S_3-1-7	0/10	66.0	64.0-67.8	1.27	70.1	69.0-71	0.61	74.2	73.6-74.7	0.34	12.4	10.8-13.7	1.01
DK8-S_3-1-8	0/10	64.7	63.4-66.2	0.95	69.4	68.7-70.4	0.60	73.8	72.6-74.6	0.69	11.4	9.8-12.8	0.94
DK8-S_3-1-9	0/10	64.5	62.2-66.7	1.33	69.6	68.4-70.3	0.61	73.8	72.5-74.4	0.67	11.6	10.3-13.0	1.03
DK8-S_3-1-10	0/10	69.3	67.9-70.4	1.11	73.0	72.3-74	0.62	75.9	75.0-77.1	0.77	11.2	10.1-12.4	0.88

[a] See Table I for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.

[b] Freq. = Frequency, number of kernels containing starch with $T_o < 61$°C / total number of kernels analyzed.

[c] T_{oG} = Gelatinization onset temperature; T_{pG} = Gelatinization peak temperature; ΔH_G = Enthalpy of gelatinization.

[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table IV
Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the DK10 Source

Corn Source[a]	Freq.[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S3 generation, grown in Puerto Rico 1996 winter nursery													
DK10-S_3	0/10	66.5	65.0-68.2	0.86	69.5	68.5-70.8	0.73	73.4	72.3-74.0	0.55	12.2	11.2-12.7	0.44
S4 generation, grown in Ames 1998													
DK10-S_3-1	0/10	64.9	63.2-66.7	1.27	71.6	71.0-72.2	0.45	76.2	74.9-77.0	0.72	11.6	11.0-12.1	0.39
DK10-S_3-2	0/10	62.2	61.2-62.5	0.26	69.1	68.3-70.2	0.66	73.5	68.3-70.2	0.66	11.4	10.7-12.0	0.57
DK10-S_3-3	0/10	64.8	64.1-65.9	0.54	69.2	68.7-70	0.39	73.7	73.0-74.6	0.52	11.7	11.4-12.0	0.17
DK10-S_3-4	0/10	64.7	62.7-66.0	1.09	69.2	68.7-69.7	0.33	73.6	73.0-74.2	0.44	11.4	10.2-12.3	0.58
DK10-S_3-5	0/10	64.7	63.0-66.4	1.21	69.9	69.3-70.5	0.37	74.4	73.7-75.1	0.41	11.5	10.7-12.5	0.55
DK10-S_3-6	0/10	66.1	65.2-67.9	0.94	70.4	69.5-71.9	0.70	74.4	73.7-75.0	0.39	11.1	10.4-11.5	0.39
DK10-S_3-7	0/10	63.8	61.6-65.4	1.02	69.6	68.8-70.1	0.41	73.9	73.3-74.5	0.38	11.0	10.3-11.5	0.41
DK10-S_3-10	0/10	63.4	61.2-64.9	1.17	69.0	68.5-69.9	0.45	73.4	72.5-74.3	0.62	11.8	11.0-11.4	0.54
DK10-S_3-12	1/10	63.7	60.7-65.2	1.76	69.5	69.1-70.2	0.52	74.1	73.6-74.6	0.39	11.2	11.0-11.4	0.17
DK10-S_3-13	0/10	63.3	61.9-64.0	0.86	69.2	68.7-69.7	0.34	74.2	73.8-74.7	0.35	11.2	10.4-11.9	0.57
DK10-S_3-15	2/10	61.9	60.6-62.9	0.75	68.3	67.7-69.5	0.45	73.0	72.1-73.7	0.455	11.1	9.9-12.6	0.87
DK10-S_3-18	1/10	62.9	60.9-64.6	1.11	69.7	69.4-70.1	0.21	74.2	73.7-74.7	0.36	11.5	10.6-12.5	0.55
S5 generation, grown in Ames 1999													
DK10-S_3-1-1	4/10	61.6	60-63.5	1.19	69.8	69.3-70.4	0.34	74.3	73.7-75.9	0.66	10.9	9.4-12.8	1.08
DK10-S_3-1-2	4/10	61.8	58.7-64.5	1.93	69.0	67.9-70.0	0.61	73.1	72.0-74.2	0.60	11.1	10.4-12.1	0.60
DK10-S_3-1-3	8/10	60.3	58.3-61.7	1.01	69.2	68-69.8	0.57	73.9	73.4-74.6	0.46	11.4	10.4-12.7	0.68
DK10-S_3-1-4	4/10	60.7	59-63.1	1.37	69.0	68.2-69.7	0.39	73.6	72.7-74.4	0.55	10.9	10.4-11.7	0.39
DK10-S_3-3-1	0/10	62.9	61.5-65.0	1.41	68.2	67.3-69.8	0.98	72.5	71.0-74.3	1.20	11.0	10.3-11.7	0.48
DK10-S_3-3-2	0/10	64.3	63.8-65.0	0.46	67.6	67.0-68.2	0.48	71.4	70.6-71.9	0.55	11.8	11.4-12.3	0.34
DK10-S_3-4-1	0/10	64.5	62.5-65.8	0.98	69.9	69.3-70.7	0.43	74.2	73.3-75.2	0.69	10.8	10.0-11.5	0.46
DK10-S_3-4-2	0/10	64.2	63.2-65.5	0.66	68.3	68.1-68.6	0.16	72.8	72.1-73.3	0.38	11.6	10.4-12.6	0.62

[a] See Table II for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[b] See Table II for an explanation of freq.
[c] See Table III for Differential Scanning Calorimetry (DSC) parameter descriptions.
[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table IV (Continued)

Corn Source[a]	Freq.[b]	T_{oG}[c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
DK10-S_3-6-1	0/10	64.2	62.5-66.3	0.68	68.3	67.1-69.4	0.20	73.0	71.6-74.1	0.41	11.0	9.9-12.6	0.64
DK10-S_3-6-2	0/10	63.2	62.0-64.5	0.87	67.6	66.9-68.6	0.46	72.5	71.9-73.7	0.60	11.1	10.0-12.1	0.75
DK10-S_3-6-3	1/10	62.7	60.4-64.8	1.23	67.7	67.0-69.1	0.60	72.9	72.0-73.9	0.59	10.0	8.2-10.9	1.06
DK10-S_3-7-1	8/10	60.0	57.2-63.3	1.77	67.5	67-67.8	0.25	71.9	70.8-72.7	0.66	10.6	9.0-11.5	0.85
DK10-S_3-7-3	0/10	63.5	62.8-64.6	0.68	67.7	67-68.1	0.43	71.7	70.8-72.1	0.49	9.9	7.9-12.3	1.7
DK10-S_3-7-4	0/10	64.4	63.6-65.2	0.75	67.7	67.3-68.2	0.46	71.5	70.7-72.1	0.59	11.0	10.1-11.7	0.72
DK10-S_3-10-1	0/10	64.1	63.6-64.8	0.41	67.4	66.9-67.7	0.27	72.0	71.6-72.4	0.34	11.5	8.9-12.9	1.22
DK10-S_3-10-2	0/10	62.8	61.5-64.0	0.87	67.2	66.3-67.9	0.53	71.7	71.2-72.3	0.38	10.4	9.7-11.2	0.60
DK10-S_3-10-3	0/10	62.7	61.4-64.0	0.79	67.6	67.2-67.9	0.25	72.6	71.3-74.5	1.04	11.4	9.6-12.9	1.21
DK10-S_3-10-4	3/10	61.7	58.9-64.0	1.51	67.8	67.5-68.4	0.36	71.9	71.5-72.6	0.39	10.7	9.9-11.1	0.39
DK10-S_3-10-5	0/10	64.5	64.1-65.3	0.39	67.7	66.8-68.3	0.44	71.6	69.6-72.4	0.87	12.2	11.1-12.4	0.44
DK10-S_3-13-1	0/10	64.7	64.3-65.2	0.38	68.2	67.7-68.5	0.31	72.3	71.8-72.7	0.36	11.35	10.7-12.7	0.80
DK10-S_3-13-2	6/10	61.0	59.1-63.6	1.49	67.9	66.9-68.5	0.47	72.9	71.7-74.0	0.75	11.4	10.8-11.8	0.37
DK10-S_3-15-1	0/10	64.7	62.8-66.4	1.08	68.3	67.2-69.5	0.75	72.4	71.1-73.4	0.60	11.1	9.9-11.9	0.64
DK10-S_3-15-2	0/10	64.4	62.9-67.1	1.30	68.0	66.8-70.4	1.29	73.1	71.5-75.6	1.31	10.8	9.0-11.7	0.83
DK10-S_3-18-1	0/10	64.2	61.6-65.9	1.12	68.2	66.8-70.5	0.98	72.4	71.6-75.4	1.08	10.8	9.4-11.8	0.73
DK10-S_3-18-2	0/10	63.0	61.9-64.4	0.94	67.3	66.8-67.7	0.38	71.8	71.4-72.5	0.35	10.8	9.3-11.9	0.98
S5 generation, grown in Puerto Rico, 1999 winter nursery													
DK10-S_3-4-3	0/10	64.8	64.1-65.5	0.73	68.5	67.7-69.3	0.83	72.5	71.4-73.6	1.04	11.1	10.9-11.4	0.22
DK10-S_3-4-4	0/10	62.6	61.7-63.6	0.72	68.6	68.0-69.0	0.32	73.8	72.7-76.5	1.03	11.3	10.0-12.4	0.66
DK10-S_3-4-5	1/10	64.1	61.0-65.3	1.2	69.0	68.3-69.5	0.35	73.4	73.0-74.0	0.32	11.6	10.6-12.3	0.48
DK10-S_3-6-4	0/10	64.7	63.7-66.0	0.77	69.2	68.5-70.2	0.55	74.0	73.2-74.9	0.49	10.3	9.1-11.2	0.64
DK10-S_3-6-5	0/10	64.7	63.9-65.6	0.52	69.3	68.3-69.9	0.48	73.8	73.4-74.2	0.24	11.4	10.6-12.5	0.62
DK1-$S_3$0-10-6	0/10	63.9	63.5-64.7	0.46	67.5	66.7-68.2	0.61	71.8	71.2-72.6	0.62	11.4	10.4-13.1	1.09
DK10-S_3-10-7	0/10	64.3	62.4-65.6	0.99	68.5	67.9-68.9	0.33	72.9	71.8-74.5	0.73	11.8	10.6-13.2	0.71
DK10-S_3-10-8	0/10	64.2	63.0-65.2	0.68	67.7	66.7-68.2	0.43	72.4	70.9-74.8	1.05	11.8	10.3-13.1	0.91
DK10-S_3-10-9	3/10	62.2	59.3-64.3	1.51	66.6	65.2-67.4	0.60	71.2	70.0-72.9	0.77	12.0	10.8-13.2	0.79
DK10-S_3-15-3	0/10	63.5	61.6-64.4	0.87	67.7	67.2-68.2	0.35	72.1	70.9-72.7	0.54	12.0	10.6-12.6	0.65
DK10-S_3-15-4	1/10	62.8	60.9-63.8	0.77	66.3	64.9-67.0	0.58	71.1	69.7-72.3	0.77	11.7	10.8-12.8	0.76
DK10-S_3-15-5	3/10	61.4	60.3-62.7	0.92	66.6	66.1-67.4	0.52	71.5	70.3-72.4	0.77	11.4	10.8-12.4	0.59

Table IV (Continued)

Corn Source[a]	Freq.[b]	T_{oG} [c](°C)			T_{pG} (°C)			T_{eG} (°C)			ΔH_G(J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
DK10-S_3-15-6	3/10	61.2	60.2-62.2	0.61	66.4	66-66.7	0.23	71.6	70.7-72.5	0.51	11.8	11.1-12.2	0.36
DK10-S_3-15-7	0/10	62.8	61.9-64.0	0.79	67.4	66.5-67.9	0.44	71.9	70.9-72.3	0.41	11.7	11.0-12.4	0.52
DK10-S_3-15-8	0/10	62.3	61.3-63.4	0.63	67.2	66.6-67.9	0.38	71.7	70.5-72.5	0.71	11.6	10.5-12.5	0.62
DK10-S_3-15-9	0/10	66.0	64.7-68.3	0.99	69.8	69.0-71.7	0.79	73.8	72.9-75.8	0.95	11.8	7.2-12.8	1.76
DK10-S_3-15-10	3/10	61.5	60.0-62.9	0.91	66.9	66.3-67.6	0.41	71.7	71.0-72.3	0.41	12.0	11.5-12.5	0.34
DK10-S_3-15-11	1/10	62.0	60.0-63.3	1.03	67.4	67.1-67.7	0.19	72.4	71.7-73.0	0.39	11.9	11.4-12.8	0.48
DK10-S_3-18-3	3/10	61.6	57.5-63.6	1.68	68.1	67.1-69.3	0.65	72.9	72.2-73.9	0.49	11.4	10.5-12.3	0.58
DK10-S_3-18-4	0/10	65.7	64.0-66.4	0.65	69.8	69.5-70.4	0.27	74.3	73.9-75.4	0.44	12.3	11.9-12.7	0.27

Table V
Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the DK14 Source

Corn Source[a]	Freq.[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S3 generation, grown in Puerto Rico 1996 winter nursery													
DK14-S_3	0/10	67.5	65.7-69.7	1.23	70.8	69.5-72.7	1.07	74.3	72.7-75.9	1.22	12.2	11.3-12.5	0.34
S4 generation, grown in Ames 1998													
DK14-S_3-1	0/10	66.3	65.4-66.8	0.40	70.2	69.8-70.5	0.20	74.4	74.0-74.9	0.28	11.7	10.3-12.6	0.73
DK14-S_3-3	0/10	64.6	62.9-66.4	1.04	69.2	68.8-69.7	0.34	73.3	72.9-73.9	0.48	11.5	11.1-12.3	0.44
DK14-S_3-4	0/10	65.9	64.5-67.0	0.81	70.3	70.1-70.7	0.19	73.9	73.5-74.4	0.33	11.7	10.9-12.1	0.33
DK14-S_3-6	0/10	65.1	62.9-66.5	1.28	69.2	68.6-69.9	0.57	73.5	72.9-74.3	0.48	10.9	10.7-11.4	0.26
DK14-S_3-7	0/10	67.7	65.8-68.7	0.95	71.0	70.2-71.5	0.37	74.8	73.5-75.7	0.65	11.1	9.5-12.1	0.84
S5 generation, grown in Ames 1999													
DK14-S_3-7-1	0/10	65.2	64.2-66.1	0.60	68.8	68.4-69.2	0.31	72.9	71.8-73.8	0.52	11.4	9.8-12.2	0.75
DK14-S_3-7-2	1/10	61.9	56.6-64.0	2.52	68.5	68.1-69.2	0.35	72.8	72.3-73.3	0.34	10.3	9.2-11.7	0.74
S5 generation, grown in Puerto Rico, 1999 winter nursery													
DK14-S_3-7-3	0/10	69.2	68.5-70.2	0.61	72.1	71.3-73.1	0.72	76.0	75.1-77.1	0.72	11.5	10.4-12.3	0.68
DK14-S_3-7-4	0/10	66.0	63.0-67.3	1.31	69.7	68.8-70.2	0.40	73.1	68.8-74.7	1.60	11.2	9.5-12.2	0.81
DK14-S_3-7-5	0/10	68.1	66.4-70.6	1.16	71.5	70.1-73.7	1.07	75.4	74.0-77.6	1.12	11.3	10.3-12.6	0.69

[a] See Table II for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[b] See Table II for an explanation of frequency.
[c] See Table III for Differential Scanning Calorimetry (DSC) parameter descriptions.
[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table VI
Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the DK34 Source

Corn Source[a]	Freq.[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S3 generation, grown in Puerto Rico 1996 winter nursery													
DK34-S_3		65.4	58.8-67.9	2.50	70.3	68.8-71.3	0.71	74.4	73.7-75.2	0.46	12.2	11.6-13.0	0.60
S4 generation, grown in Ames 1998													
DK34-S_3-1	0/10	67.0	65.6-68.8	0.98	70.3	68.9-72.9	1.08	73.9	72.6-74.9	0.71	12.5	11.7-13.1	0.50
DK34-S_3-2	0/10	67.5	67.0-68.3	0.42	70.7	70.4-71.2	0.28	74.7	74.4-75.3	0.31	12.4	12.0-13.2	0.38
DK34-S_3-3	0/10	66.5	65.4-68.2	0.83	70.2	69.0-72.4	0.94	74.3	73.1-77.0	1.13	12.0	11.6-12.7	0.36
DK34-S_3-4	0/10	65.9	65.1-66.9	0.58	69.9	69.3-70.3	0.33	74.2	73.5-74.8	0.37	11.6	10.8-12.5	0.51
DK34-S_3-5	0/10	67.4	66.2-68.4	0.86	71.2	70.6-72.2	0.49	75.4	74.8-76.1	0.45	11.9	11.7-12.3	0.21
DK34-S_3-6	0/10	66.7	65.5-67.7	0.83	70.4	69.7-71.1	0.50	74.7	74.0-75.2	0.37	11.22	8.7-12.6	1.41
DK34-S_3-7	0/10	67.1	66.0-68.0	0.64	70.7	69.6-71.2	0.51	74.7	73.8-75.3	0.44	11.69	11.2-12.0	0.25
DK34-S_3-8	0/10	65.9	64.8-67.1	0.71	70.3	69.5-71.6	0.54	74.0	73.0-74.7	0.48	11.9	11.3-12.9	0.51
DK34-S_3-9	2/10	61.9	59.4-63.7	1.31	68.6	68.0-69.1	0.33	73.9	73.2-75.0	0.55	9.7	7.7-10.8	0.88
S5 generation, grown in Ames 1999													
DK34-S_3-9-1	0/10	64.0	62.7-65.3	0.76	68.0	67.6-68.8	0.39	72.2	71.5-72.9	0.51	11.0	10.2-11.9	0.61
DK34-S_3-9-2	6/10	61.0	58.3-65.2	1.97	68.2	67.3-69.3	0.65	73.1	72.1-74.2	0.64	10.3	9.6-11.1	0.54
S5 generation, grown in Puerto Rico, 1999 winter nursery													
DK34-S_3-9-4	0/10	66.5	65.4-67.8	0.71	70.0	69.4-70.9	0.49	73.9	73.2-75.0	0.58	12.0	11.4-13.4	0.57
DK34-S_3-9-5	0/10	63.8	62.5-64.9	0.79	68.8	68.1-69.6	0.43	73.3	72.7-74.0	0.41	11.6	10.2-12.4	0.62
DK34-S_3-9-6	1/10	63.2	59.6-66.2	2.00	69.2	68.2-70.8	0.88	74.0	72.0-75.4	1.19	11.1	9.9-12.1	0.80
DK34-S_3-9-7	0/10	63.8	62.4-67.1	1.86	69.9	69.5-71.2	0.62	74.9	73.5-76.1	0.84	11.6	10.9-12.4	0.56

[a] See Table II for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[b] See Table II for an explanation of frequency.
[c] See Table III for Differential Scanning Calorimetry (DSC) parameter descriptions.
[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table VII
Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the PI82 Source

Corn Source[a]	Freq.[b]	T_{oG} [c](°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G(J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S1 generation, grown in Puerto Rico 1996 winter nursery													
PI82-S_1	2/10	64.7	57.6-68.6	3.48	70.0	67.2-72.2	1.49	74.6	70.8-76.9	1.68	11.1	10.3-12.2	0.61
S 2 generation, grown in Ames 1998													
PI82-S_1-1	1/10	64.0	60.4-65.8	1.80	70.4	69.4-71.0	0.45	75.8	75.2-77.3	0.59	10.3	9.2-11.1	0.61
PI82-S_1-2	1/10	63.2	60.4-65.1	1.57	69.5	68.4-70.8	0.74	74.9	73.3-75.8	0.72	10.8	9.6-11.7	0.54
PI82-S_1-3	0/10	66.3	64.1-68.3	1.30	70.9	69.5-72.1	0.84	76.4	75.5-77.4	0.64			
PI82-S_1-4	2/10	63.2	60.7-65.3	1.32	69.1	67.4-71.2	1.09	75.7	74.6-77.1	0.66	11.35	10.2-12.2	0.55
PI82-S_1-5	0/10	64.7	62.0-66.5	1.37	69.5	67.2-70.4	0.87	75.3	73.5-77.0	1.13	10.9	8.9-12.5	0.94
PI82-S_1-6	0/10	64.4	62.2-66.5	1.52	69.7	68.5-70.7	0.78	74.5	73.3-76.0	0.97	10.6	10.1-11.4	0.46
PI82-S_1-7	0/10	64.5	62.3-66.4	1.32	69.9	68.7-70.8	0.65	74.6	72.9-75.4	0.77	11.3	9.8-12.1	0.63
PI82-S_1-8	0/10	64.1	61.8-65.1	1.18	69.9	69.1-70.6	0.51	75.1	73.5-76.6	0.93	11.1	10.3-12.2	0.61
S3 generation, grown in Ames 1999													
PI82-S_1-1-1	2/10	61.5	57.1-63.1	1.83	68.9	68.3-69.5	0.45	73.7	72.9-74.6	0.57	11.3	9.9-12.1	0.68
PI82-S_1-1-2	6/10	60.4	58-63.8	1.91	67.4	66.7-68.4	0.63	73.1	72.1-73.9	0.54	10.2	8.5-11.3	0.90
PI82-S_1-2-1	7/10	60.6	58.3-65.1	2.30	68.5	67.8-69.0	0.35	73.2	72.1-73.7	0.52	11.0	9.9-12.3	0.77
PI82-S_1-2-2	6/10	60.9	58.3-63.3	1.70	68.4	67.4-69.8	0.82	73.3	72.6-75.0	0.75	10.7	9.7-11.9	0.67
PI82-S_1-2-3	2/10	62.4	60.2-64.3	1.27	68.5	67.0-69.3	0.63	73.6	71.8-74.5	0.81	10.1	9.0-11.3	0.72
PI82-S_1-2-4	7/10	60.6	58.6-63.0	1.51	67.4	65.9-68.7	0.91	72.4	71.0-73.5	0.85	9.7	8.4-11.7	0.99
PI82-S_1-4-1	5/10	60.4	58.6-62.4	1.33	67.2	66.0-67.9	0.55	73.3	72.6-74.1	0.49	10.1	8.7-11.0	0.62
PI82-S_1-4-2	6/10	60.7	58.6-63.0	1.58	67.8	67-68.8	0.61	74.0	73.0-75.5	0.83	10.5	8.8-11.9	1.11
PI82-S_1-4-3	6/10	60.8	58.3-64.6	1.69	68.4	67.3-69.8	0.75	74.3	73.2-75.4	0.96	11.2	9.6-12.3	0.82
PI82-S_1-5-1	1/10	64.4	58.8-67.6	2.86	69.2	67.4-70.7	1.13	73.9	72.7-75.2	0.82	11.0	8.9-12.0	1.05
PI82-S_1-5-2	2/10	62.0	59.8-63.7	1.34	69.1	68.5-69.9	0.47	74.9	74.1-76.1	0.61	10.7	9.9-11.9	0.68
PI82-S_1-5-3	3/10	61.9	58.4-64.8	2.48	68.9	67.9-69.9	0.81	74.4	73.5-75.2	0.63	10.2	8.35-12.6	1.50

[a] See Table II for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[b] See Table II for an explanation of frequency.
[c] See Table III for Differential Scanning Calorimetry (DSC) parameter descriptions.
[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table VII (continued)

Corn Source[a]	Freq.[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
PI82-S_1-5-4	6/10	61.1	58.2-64.3	2.80	68.4	67.1-69.1	0.79	74.1	73.1-76.1	1.17	11.1	9.8-12.4	0.94
PI82-S_1-6-1	4/10	62.1	59.7-64.3	1.67	68.6	67-69.7	0.73	73.3	72.5-74.3	0.68	10.8	9.7-12.5	0.80
PI82-S_1-6-2	6/10	60.6	59.1-63.0	1.24	67.8	67.1-68.6	0.58	73.8	72.7-74.7	0.64	11.2	10.2-12.5	0.66
PI82-S_1-6-3	9/10	60.3	58.7-61.3	0.77	66.8	66.2-67.7	0.55	72.7	70.8-74.1	1.04	10.7	8.0-12.3	1.23
PI82-S_1-6-4	6/10	60.1	58.5-64.4	1.77	67.4	66.2-69	0.90	73.2	72.3-74.0	0.56	11.2	10.30-11.6	0.48
PI82-S_1-7-1	0/10	65.2	61.9-67.0	1.70	70.8	68.9-71.7	0.81	75.6	74.2-76.3	0.63	10.3	9.6-11.2	0.51
PI82-S_1-5-2	0/10	64.7	63.0-66.5	1.04	70.1	68.5-71.7	1.14	74.5	73.3-76.6	1.21	10.1	8.6-12.1	1.02
PI82-S_1-8-2	3/10	62.0	69.5-64.3	1.49	68.8	67.3-70	0.87	74.1	73.0-75.3	0.83	11.2	10.7-11.7	0.38
PI82-S_1-8-2	3/10	62.4	60.4-65.0	1.48	67.9	66.3-70.3	1.20	73.7	71.4-76.1	1.69	10.3	9.2-12.0	0.81
S3 generation, grown in Puerto Rico, 1999 winter nursery													
PI82-S_1-1-3	0/10	64.7	61.8-66.6	1.37	68.5	66.7-77.3	0.93	73.1	72.4-74.5	0.69	10.9	10.4-11.9	0.48
PI82-S_1-1-4	0/10	65.2	63.5-66.3	0.89	69.3	68.3-70	0.53	73.5	72.4-74.7	0.63	10.8	8.2-12.3	1.12
PI82-S_1-2-5	0/10	67.3	65.6-69.4	1.37	70.8	69.3-72.5	1.24	74.7	72.8-76.9	1.54	11.0	7.7-13.2	1.7
PI82-S_1-2-6	0/10	67.9	65.5-69.9	1.74	71.6	69.3-73.5	1.51	75.9	73.2-78.0	1.80	10.6	8.0-12.9	1.69
PI82-S_1-4-4	0/10	65.4	64.5-65.9	0.62	69.7	69.5-70	0.21	74.0	73.6-74.9	0.63	10.1	9.3-10.6	0.61
PI82-S_1-5-3	0/10	66.1	64.9-67.8	0.88	70.5	69.1-71.7	0.74	75.2	73.7-77.1	1.02	11.0	9.8-12.4	0.73
PI82-S_1-5-4	0/10	65.4	63.3-67.1	1.28	70.6	69.6-71.4	0.53	75.3	74.7-76.8	0.69	11.1	10.3-12.0	0.51
PI82-S_1-5-5	0/10	64.8	62.1-67.4	1.59	70.6	69.3-71.9	0.81	75.1	73.4-76.0	0.77	10.8	7.9-12.3	1.67
PI82-S_1-5-6	0/10	65.6	62.1-68.3	1.98	70.3	69.4-71.4	0.79	75.2	73.7-76.3	0.84	10.5	9.3-11.5	0.73
PI82-S_1-5-7	0/10	66.6	65-67.2	0.66	70.9	69.4-71.7	0.64	75.3	73.8-77.1	0.88	11.1	9.2-12.0	0.86
PI82-S_1-5-8	0/10	65.7	61.0-67.6	2.06	71.3	69.8-72.7	0.84	75.5	74.3-76.5	0.80	10.9	8.5-12.5	1.26
PI82-S_1-6-5	0/10	66.6	64.5-69.1	1.59	70.2	68.3-72.6	1.35	74.6	72.4-77.2	1.46	11.2	10.3-11.9	0.57
PI82-S_1-6-6	0/10	65.7	64.2-67.1	0.94	69.6	68.3-70.7	0.67	74.1	72.4-74.7	0.71	11.2	9.5-12.32	0.78
PI82-S_1-6-7	0/10	65.0	63.6-66.6	0.36	69.8	68.7-70.9	0.75	74.8	74.0-75.5	0.54	11.4	10.9-12.9	0.59
PI82-S_1-6-8	0/10	66.6	64.3-67.8	1.13	70.2	69.0-71.1	0.77	74.9	73.6-76.3	0.88	11.3	10.1-12.5	0.81
PI82-S_1-8-3	0/10	65.6	61.1-67.7	1.96	70.3	69-71.5	1.01	75.5	73.8-77.0	1.27	9.8	7.8-11.3	1.12
PI82-S_1-8-4	0/10	64.9	62.1-67.5	1.66	69.7	68.1-71.3	0.92	75.0	73.5-76.9	1.06	10.3	9.2-11.3	0.81
PI82-S_1-8-5	0/10	65.6	63.5-67.4	1.18	70.2	68.3-72.2	1.07	74.6	72.4-76.4	1.30	11.3	9.9-12.4	0.75
PI82-S_1-8-6	0/10	68.0	65.8-71.2	1.91	72.5	70.0-74.7	1.61	76.2	74.3-77.8	1.00	10.6	7.5-11.6	1.40
PI82-S_1-8-7	0/10	65.2	63.9-67.7	1.18	70.4	69.7-71.2	0.81	74.8	73.2-77.1	1.33	11.2	9.8-12.2	0.70

Table VIII
Gelatinization Data of Starches from Single Kernels of Selected Corn Lines from the PI83 Source

Corn Source[a]	Freq.[b]	T_{oG} [c] (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	s.d.[d]	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
S1 generation, grown in Puerto Rico 1996 winter nursery													
PI83-S_1	1/10	62.6	61.0-64.4	1.00	69.0	65.8-70.6	1.51	74.3	73.0-75.3	0.81	11.0	10.3-11.8	0.39
S2 generation, grown in Ames 1998													
PI83-S_1-1	8/10	60.9	59.1-64.6	1.69	69.3	67.3-70.9	1.15	75.9	75.2-76.4	0.35	11.0	10.4-12.3	0.64
PI83-S_1-2	5/10	61.3	59.5-63.5	1.38	71.1	70.5-71.6	0.36	76.7	75.9-77.4	0.54	11.5	11.0-12.1	0.32
PI83-S_1-3	1/10	62.3	60.0-65.7	1.53	70.2	68.9-71.7	0.87	76.3	75.2-78.2	0.96	11.1	9.2-13.1	1.29
PI83-S_1-4	1/10	62.8	60.0-64.4	1.5	70.2	69-70.8	0.55	76.3	75.0-77.0	0.74	10.6	9.9-11.3	0.48
PI83-S_1-5	6/10	61.1	59.3-63.6	1.37	70.3	68.8-71.1	0.84	75.8	74.9-77.7	0.84	10.9	10.5-11.4	0.37
PI83-S_1-6	2/10	62.8	58.1-66.6	2.68	70.3	68.7-71.1	0.69	75.4	74.0-76.1	0.76	10.1	9.0-11.8	0.81
PI83-S_1-7	9/10	60.6	58.9-62.6	1.15	70.9	70.3-71.8	0.48	76.0	74.9-77.0	0.59	11.2	10.5-12.3	0.57
PI83-S_1-8	1/10	61.6	59.2-62.6	0.98	69.8	69.2-70.7	0.55	76.1	74.7-77.8	0.83	11.0	10.5-11.7	0.36
S3 generation, grown in Ames 1999													
PI83-S_1-4-1	8/10	60.0	56.9-66.1	3.55	69.5	68.3-70.4	0.75	73.4	72.9-74.2	0.62	10.6	9.5-12.4	1.14
PI83-S_1-4-2	0/10	65.0	62.1-66.2	1.68	69.2	68.7-69.8	0.53	73.3	73.0-73.6	0.28	10.3	9.5-11.0	0.63
PI83-S_1-4-3	0/10	65.1	64.2-65.9	0.62	69.3	68.8-70.0	0.46	73.1	72.5-73.6	0.51	11.0	10.7-11.2	0.20
PI83-S_1-7-1	0/10	66.7	65.7-67.6	0.72	70.0	69.2-71	0.72	73.8	72.8-74.5	0.69	11.9	10.9-12.8	0.69
PI83-S_1-8-1	6/10	61.5	59.5-66.4	1.92	68.8	67.8-69.8	0.60	73.9	73.8-74.5	0.39	11.5	9.9-12.3	0.84
S3 generation, grown in Puerto Rico, 1999 winter nursery													
PI83-S_1-1-1	0/10	63.3	62.2-65.2	0.96	68.3	67.5-69.0	0.48	73.2	72.4-73.7	0.44	10.4	7.9-11.8	1.10
PI83-S_1-1-2	0/10	63.5	62.5-65.0	0.69	68.3	67.7-69.1	0.48	73.2	72.1-74.1	0.79	10.3	8.2-12.0	1.16
PI83-S_1-1-3	0/10	65.7	62.8-71.2	2.98	71.4	68.2-74.8	2.59	75.6	73.2-78.2	2.01	10.3	9.16-11.7	0.89
PI83-S_1-1-4	0/10	68.2	66.7-69.7	1.2	73.0	72-73.7	0.67	76.5	75.9-77.7	0.85	9.4	8.9-9.8	0.33
PI83-S_1-1-5	0/10	62.9	62.0-63.8	0.63	68.2	67.7-68.5	0.33	72.8	71.8-73.1	0.59	10.6	9.9-11.6	0.62
PI83-S_1-4-3	0/10	65.7	63.8-66.8	1.45	70.0	69-70.7	0.71	74.1	73.0-74.7	0.67	10.4	8.6-11.3	1.01
PI83-S_1-7-2	0/10	64.7	63.1-65.9	0.96	69.9	69.6-70	0.17	74.5	73.7-75.1	0.51	11.4	10.5-11.9	0.49
PI83-S_1-7-3	0/10	64.4	63.5-65.9	0.87	69.4	69.3-69.7	0.18	74.8	73.9-76.7	1.33	11.5	9.9-12.6	0.51

[a] See Table II for an explanation of source identification. The number after the dash signifies the ear number for the pedigree of the Sn line.
[b] See Table II for an explanation of frequency.
[c] See Table III for Differential Scanning Calorimetry (DSC) parameter descriptions.
[d] s.d. = Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

Table IX
Significance of Genotype Effects and Environment Effects and their Interaction from Analysis of Variance of Starch Gelatinization Properties

Source of Variation	df[a]	Starch Gelatinization Properties			
		T_o [b](°C)	T_p (°C)	T_c (°C)	ΔH(J/g)
Environment	1	**[c]	**	**	*
Source	5		*		
Environment x Source	5	*			
S_{n+1} Line (Source)	9				
Environment* S_{n+1} Line (Source)	9	**	**	**	
S_{n+2} Line (S_{n+1} Line (Source))	64	**	**	**	**

[a] df = degree of freedom.
[b] See Table III for Differential Scanning Calorimeter (DSC) parameter descriptions.
[c] *, ** indicate significance at $P < 0.10$ and $P < 0.05$, respectively.

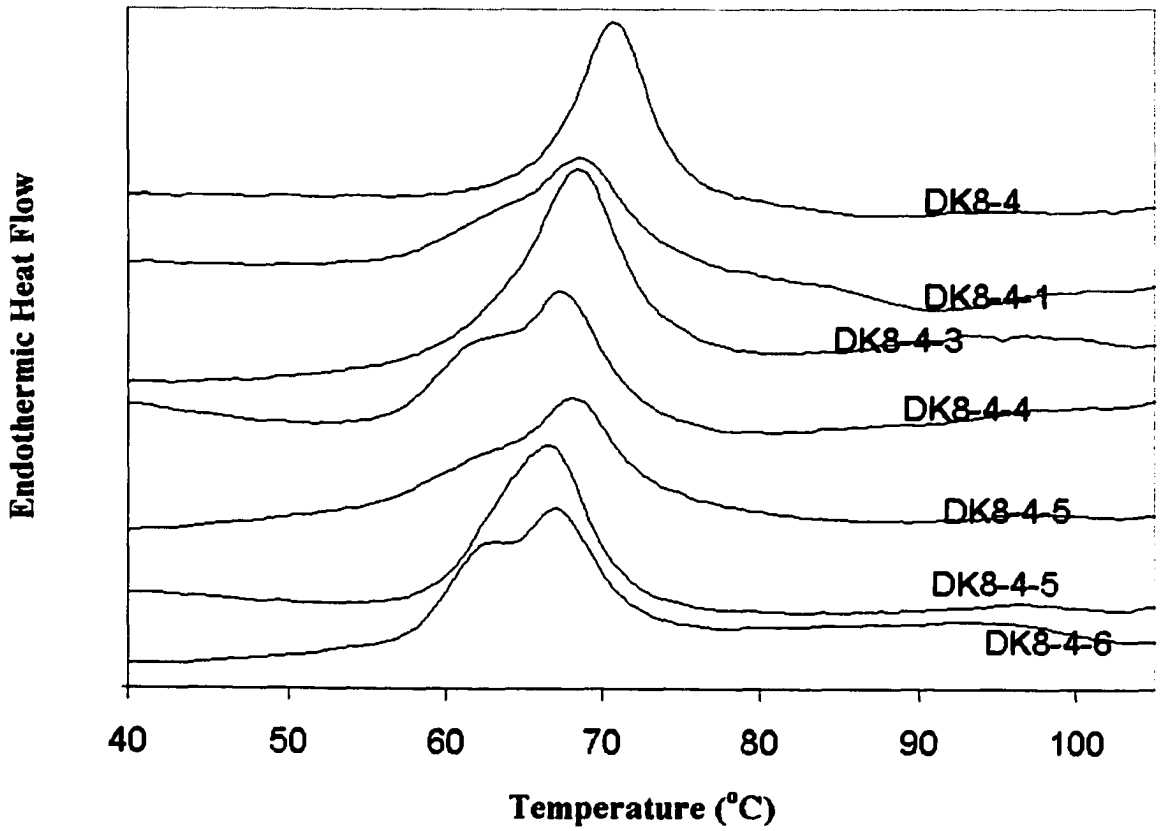

Fig 1. Gelatinization thermograms of starches from progeny lines of DK8-4.

CHAPTER 7. GENERAL CONCLUSION

We have currently developed several novel GEM corn lines, adapted by exotic breeding crosses, which can produce starches with improved functional properties. Starches from these new lines will also provide a good vehicle for structure-function study. The underlying objectives of this research work were to evaluate functions and structures of starches from developmental lines, and to establish relationships between the fine structure and functional properties of the starches. A secondary objective was to confirm the advancement of selected functional traits into the next generation of corn. The work published in this dissertation successfully meets the objectives.

The effect of different starch extraction procedures designed for use in the laboratory on starch chemical composition and physical properties were studied in the chapter 3. From this study, it was suggested that the sedimentation procedure is preferred for laboratory starch extractions especially when quantity of sample is small because of the lowered protein content in starch and higher starch yields. 10 kernels is preferred over 2 or 5 kernels with the same total volume of wash water. Longer steeping time yielded starch with lower protein content and higher yield. Soaking seeds for less than 48 hr is preferred to minimize annealing of starches and, thus, altering starch thermal properties. The procedure optimized in this paper was used in research conducted within the rest of the dissertation.

Chapter four and chapter five evaluated functional and structural properties of starches from exotic x adapted inbred lines and exotic breeding crosses (exotic populations x adapted lines), and to establish relationships between the fine structure and functional properties of the starches. The starches from the corn lines identified in this study are of interest because of unusually low T_{oG}. Two independent gelatinization transitions, located in different granules, were found in some starches. All selected starches from the developmental lines had significant different pasting properties and gel properties than did starch from normal corn inbreds Mo17. Significant differences were observed in starch-granule size-distributions and shape-distributions of the selected starches. The different gelatinization and pasting properties could be explained by the branch chain-length pattern of the amylopectin. Starches with a lower peak onset gelatinization temperature (T_{oG}), had a lower normalized concentration of chains with a degree of polymerization (dp) of 15 to 24

and/or a greater normalized concentration of chains with a dp of 6 to 12. These studies will aid in understanding structure-thermal property relationships of starches, and in identifying corn lines of interest for commercial breeding.

Effect of environment and genotype on the gelatinization properties of starches from developmental corn lines was studied in chapter six. Gelatinization properties of starches from exotic corn lines and their derivatives when grown 1) during three successive years; and 2) in both temperate and tropical environments were evaluated.Unusual thermal properties (low T_{oG}) were fixed in some progeny lines. Environmental factors had a significant effect on the thermal properties of starch, and a significant interaction between environment and genotype was observed. These results suggest that introgression of adapted germplasm with useful genes from exotic corn would increase the available genetic variability for starch functionality and allow the development of hybrids with important value-added traits.

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