Thermal properties of starch from new corn lines as impacted by environment and during line development

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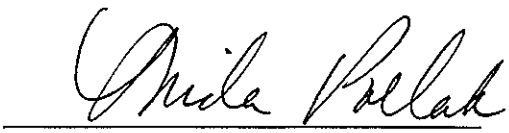

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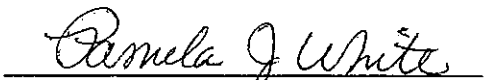

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Abstract

The objectives of this research were to further characterize exotic by adapted corn inbreds by studying the impact of environment on their starch thermal properties, and investigating the development of starch thermal properties during kernel maturation by using differential scanning calorimetry (DSC).

A method to expedite identification of unusual starch thermal traits was investigated by examining five corn kernels at a time, instead of one kernel, which our previous screening methods used. Corn lines with known thermal functions were blended with background starch (control) in ratios of unique starch to control starch, and analyzed by using DSC. Control starch was representative of typical corn starch. The values for each ratio within a mutant type were unique ($\alpha<0.01$) for most DSC measurements. These results supported the five-kernel method for rapidly screening large amounts of corn germplasm to identify unusual starch traits.

The effects of 5 growing locations on starch thermal properties from exotic by adapted corn and Corn Belt lines were studied using DSC. The warmest location, Missouri, generally produced starch with greater gelatinization onset temperature (T_{oG}), narrower range of gelatinization (R_G), and greater enthalpy of gelatinization (ΔH_G). The coolest location, Illinois, generally resulted in starch with lower T_{oG}, wider R_G, and lower ΔH_G. Starch from the Ames 1 farm had thermal properties similar to those of Illinois, whereas starch from the Ames 2 farm had thermal properties similar to those of Missouri. The temperature at Ames 2 may have been warmer since it was located near a river; however, soil type and quality also were different.

Final corn starch structure and function change during development and maturity. Thus, the changes in starch thermal properties during 5 stages of endosperm development from exotic by adapted corn and Corn Belt lines at two locations were studied by using DSC. The T_{oG} tended to decrease during maturation of the kernel, whereas the ΔH_G tended to not to change. Retrogradation parameters did not vary greatly among days after pollination (DAP) and between locations. Genotypes were affected differently by environments and significant interactions were found between genotype, environment, and DAP.

Chapter 1. General Introduction

Introduction

Starch is an abundant polymer of anhydroglucose units that can be found in different parts of plants, including the roots, tubers, seeds, and leaves. It serves as the plant's primary storage unit for energy and provides 70-80% of the calories consumed by humans worldwide (Whistler and Bemiller 1997). Commercial starches are obtained from seeds, especially corn, waxy corn, high-amylose corn, wheat, and rice; and from roots and tubers, such as potato and cassava. For food and industrial purposes, starch is used unmodified and modified in many products, including applications for adhesives, films, glazes, and thickeners (Whistler and Bemiller 1997). In the United States, 95% of starch is processed from corn (White 2001).

No new corn starch derivatives have been introduced to the industry in about 40 years, because of restrictions related to consumer safety, consumer concerns, worker safety, environmental concerns, and economics (Bemiller 1997). Therefore, there has been interest to develop non-mutant corn starches that naturally possess properties similar to those of chemically modified corn starches.

Exotic germplasm is usually considered to include all sources of unadapted germplasm, including domestic, temperate, and tropical (Goodman 1985). Exotic populations and lines have been reported to have a high variation in thermal traits, suggesting the use of these lines in further breeding trials to develop varieties with unusual traits (Li et al 1994, Campbell et al 1995, Pollak and White 1997, Singh et al 2001). Campbell et al (1995) reported that thermal properties measured by DSC could be used to predict functional properties of starches among nonmutant sources of maize.

The Germplasm Enhancement of Maize (GEM) project has developed and identified exotic by adapted lines, partially from germplasm foreign to corn races grown in the United States, that may be useful for agronomic, nutritional, and/or industrial reasons (Pollak 2002). Ji et al (2003a, 2003b) identified exotic by adapted corn inbred lines that exhibit unusual properties, such low gelatinization onset temperature (T_{oG}) and wide range of gelatinization (R_G), and characterized their functional and structural properties. Some lines exhibited gelatinization thermogram shapes with shoulders or double peaks demonstrating two independent transitions. They also were able to develop lines in which unusual starch thermal traits were "fixed", meaning they were inherited consistently from generation to generation and, therefore, are available to the industry as lines that naturally possess unique properties as alternatives to chemically or physically modified starches.

In our studies, we continued to characterize these new corn lines by studying the development of thermal properties during maturation of the kernel, and we evaluated the effect of environment on the thermal properties. It is important to study the effect of growing location on the heritability of "fixed" starch thermal traits in order to understand the stability of the traits when produced at different locations. Rapid screening methods also were studied, because there is a need to expedite the process for detecting future lines of interest.

Thesis Organization

This thesis is organized into five parts. Three papers to be submitted to journals follow the general introduction and literature review. The first paper "Rapid screening to identify unusual thermal starch traits from bulked kernels" investigates the use of bulking corn kernels for extraction, rather than single-kernel extraction, in order to expedite detection of unusual thermal starch traits in new corn populations. The second paper "Thermal

properties of starch from exotic corn (*Zea mays* L.) lines grown in five locations" examines the effect of growing location on the thermal properties of starch from exotic inbred corn lines. The third paper "Thermal properties of starch from exotic corn (*Zea mays* L.) lines during kernel development at two locations" describes the development of thermal properties of exotic corn inbreds after pollination and at two locations. Following the papers is the final chapter "General Conclusions".

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Chapter 2. Literature Review

A. General Introduction

Starch is an abundant polymer of anhydroglucose units that can be found in different parts of plants, including the roots, tubers, seeds, and leaves. It serves as the plant's primary storage unit for energy and provides 70-80% of the calories consumed by humans worldwide (Whistler and Bemiller 1997). Commercial starches are obtained from seeds, especially corn, waxy corn, high-amylose corn, wheat, and rice; and from roots and tubers, such as potato and cassava. For food and industrial purposes, starch is used unmodified and modified, including applications for adhesives, films, glazes, and thickeners (Whistler and Bemiller 1997). In the United States, 95% of starch is processed from corn (White 2001).

Starch is unique because it occurs in granules and in two polymeric forms, amylose and amylopectin. Amylose is an α-D (1->4) linked glucose polymer with a few branch points and normally constitutes about 25% of the starch. Amylopectin constitutes about 75% of the starch and consists of α-D (1->4) linked glucose units with α-D (1->6) branch points. The size and specific structure of these polymers varies with several factors, including source and growing environment.

B. Starch Structure

1. Granule Structure

The starch granule serves as the primary storage organ of carbohydrates in plants. In the granular form, starch is semi-crystalline, insoluble in water, and dense (French 1984). The size and shape of the granule depends on its origin (Lineback 1984). Maize starch granules are typically round and 15 nm in diameter, whereas potato starch granules are oval

and have a 40 nm diameter. Rice starch is smaller (3-8 nm in diameter) and polygonal in shape. These characteristics are easily discernible under a light microscope.

Under a polarized light microscope, granules are birefrigent and exhibit a typical Maltese cross. At the center of the cross is the hilum, which is the original growth point of the granule. The birefrigerence implies a high degree of molecular orientation in the granule and is a result of the semi-crystallinity of the starch (Lineback 1984). The crystalline component is known to be amylopectin, with short-branched chains forming local organization (French 1984). There is agreement that the chains within the granule are radially arranged with their non-reducing ends pointing towards the surface, and are organized into alternating crystalline and amorphous lamellae 9 nm apart (Jenkins et al 1993). Amylose is essentially amorphous and interdispersed with amylopectin (Jane et al 1992). Lipids are present mainly in cereal starch granules as phospholipids or fatty acids (Swinkels 1985). The lipids complex with amylose and can have a profound effect on the functionality of the starch.

The preferred method to study the crystallinity of starch granules is X-ray diffraction analysis. Four types of unit cells have been identified, which are A, B, C, and V (Lineback 1984). The A type is common to cereal starches, such as maize, wheat, and rice, whereas B type is common to tuber, fruit and stem starches, such as potato and sago. The C type is a mixture of A and B granules (Bogracheva et al 1998) and is common to smooth pea and various beans. The V pattern is caused by amylose complexes, usually with lipid, within the granule.

The most accepted conformations of the unit cells are as follows. The A unit cell consists of 12 glucose residues in double helix formation and a few water molecules (Imberty

et al 1988). Hydrogen bonds and Van der Waals' forces stabilize the helix. The chains in B-type starch are also arranged in double helices, but there are 30 to 40 water molecules present (Wu and Sarko 1978). Overall, the arrangement of molecules in both A- and B-type starches are essentially identical. The double helices are connected through hydrogen bonds that leave a channel in the center of a hexagonal arrangement of six double helices (Imberty et al 1991). In A-type starch, a seventh double helix lies in this channel and in B-type starch a column of water is present in the channel (Imberty et al 1991).

2. Amylopectin Fine Structure

Amylopectin is the major component of most starch and plays a major role in the functionality of the starch. It is a polymer of anhydroglucose units, linked as α-D-1->4 bonds with α-D-1->6 branch points at 4-5% of the glucose linkages. There are typically several thousand chains, with an average degree of polymerization (DP) of 20 in one molecule, which results in a high molecular weight (10^7-10^9 Da). The most probable structure is the cluster model, proposed by or modified by French (1973), Robin et al (1974), Nikuni (1978), and Hizukuri (1986). Each amylopectin molecule has one C chain, which carries the only reducing group. The B chains are linked to the C chain through their sole reducing group and can participate in one or more clusters. They are, therefore, classified by B1 to B4, according to the number of clusters the chain passes through. The A chains are linked to B chains via their sole reducing group. The A chains are short and not branched. The A chains and exterior B chains form double helices, therefore causing crystallinity of the starch.

The branch points in the cluster model are amorphous, whereas the clusters are crystalline. The chains in the clusters, particularly the A chains, form double helices leading to the semi-crystalline nature of the starch. The branch points, however, differ specifically

with A- and B-type starches (Jane et al 1997). Starches with A-type x-ray diffraction patterns tend to scatter the branch points throughout the amorphous and crystalline regions, whereas B-type starch branch points tend to have most branch points in the amorphous region. A-type starches tend to have more short A-chains (DP 6-12) than B-type starches, causing the chains to be tightly packed and, therefore, have more branch points in the crystalline region (Jane et al 1997).

3. Amylose Fine Structure

Amylose is a polymer of anhydroglucose units linked as α-D-1->4 bonds with very few α-D-1->6 branch points (9-20 chains per molecule of amylose). The branches are either very long or very short and are separated by long distances, which allows amylose to act as essentially linear molecules.

In neutral, aqueous solution, amylose behaves as a random coil (Banks and Greenwood 1971). The presence of lipids, such as phospholipids and fatty acids, causes the formation of amylose-lipid inclusion complexes. Amylose also forms inclusion complexes with iodine and several organic compounds, such as butanol, phenols, and hydrocarbons. The complexes are formed by amylose coiling around the complexing agent in a single helix conformation. These complexes are essentially insoluble in water and are slightly crystalline in nature. The complexes with lipid in native starches, such as in corn or wheat, melt around 90-100°C (Kugimiya et al 1980).

C. Functional Properties

1. Gelatinization

Gelatinization is the disruption of molecular order within starch granules as they are heated in the presence of water. Water is absorbed through the amorphous regions and heat

provides the energy to break existing hydrogen bonds. Crystallinity dissipates as noted by the loss of the Maltese cross with use of a polarized light microscope equipped with a hot stage. Amylose tends to leach out of the granule and there is an increase in viscosity and clarity of the starch/water mixture. It should be noted that gelatinization can occur in the presence of certain solvents, such as sodium hydroxide, calcium chloride, lithium chloride, and others, through the disruption of hydrogen bonds that hold the integrity of the granule in place (Jane 1993).

The gelatinization process with heat generally occurs within a narrow temperature range, with larger granules gelatinizing first, followed by the smaller granules. Methods to study gelatinization and quantify these temperatures, and other parameters, include light and electron microscopy, light transmission, swelling and solubility determinations, enzymatic analysis, nuclear magnetic resonance, x-ray diffraction, and differential scanning calorimetry (DSC). DSC has been used extensively since Stevens and Elton (1971) first reported its use for studying starch gelatinization. Parameters that are calculated precisely from DSC include onset, peak, and conclusion gelatinization temperatures, temperature range, and enthalpy of gelatinization. These parameters can also be measured in retrogradation studies.

Results of DSC are affected by a number of factors. Under normal operating conditions, including excess water (about one and one half to two parts or more water to one part starch), two endothermic transitions occur. The first peak corresponds to the gelatinization of the starch and the other is a result of the melting of the amylose complex if amylose and/or lipid are present, such as occurs in normal wheat or maize starch (Kugimiya et al 1980). To attain complete gelatinization, a minimum water to starch ratio must be present (Donovan 1979, Wooton and Bamunurachchi 1979, Biliaderis et al 1980, Marchant

and Blanshard 1980, Man et al 2001). The amorphous regions must be maximally hydrated to solvate the crystalline regions and subsequently melt them (Biliaderis et al 1980). At intermediate moisture, less than 67%, two endotherms are present with respect to the melting of crystallites (Donovan 1979). The first endotherm is a result of the melting where excess water is located; the second, higher temperature endotherm, occurs as water is redistributed (Donovan 1979). If there is less than 20% moisture, the gelatinization endotherm enthalpy lessens and the glass transition temperature of the starch is detected, which increases in temperature with lower amounts of water (Zeleznak and Hoseney 1987, Zobel et al 1988, Thiewes and Steeneken 1997).

Also affecting the DSC results is annealing of starch, which is defined as incubation of starch in excess water above the glass transition temperature, but below the gelatinization temperature (Jacobs et al 1998). Gough and Pybus (1971) demonstrated that heating in this manner, similar to conditions in the corn wet-milling process (Krueger et al 1987a), caused the range of gelatinization to narrow and the onset temperature to increase. Krueger et al (1987a) showed there is an increase in gelatinization temperature that reaches a maximum that is not exceeded by any further annealing treatments. They also demonstrated an increase in enthalpy that occurs with annealing. Krueger et al (1987b) suggested that annealing increased the crystallinity of the starch sample, mostly amylose, therefore causing the starch to gelatinize at a higher temperature and undergo a larger change in enthalpy. Jacobs et al (1998), however, demonstrated that crystallinity does not increase as studied through small angle X-ray scattering. They postulated that double helices pack closer together, which enhances stability of the semi-crystalline state causing a higher gelatinization temperature and enthalpy. Tester et al (1998) also supported the observation that no new crystals are

formed, but the existing crystals are perfected by the hydration and expansion of the amorphous regions, which initiates the reorganization of the double helices.

In food systems, certain ingredients, such as sugars, salts, fats, and oils, affect the characteristics of the starch gelatinization. Sugars lower the amount of starch gelatinization that occurs because sugars tend to compete with starch for water (White 2001). The onset temperature of starch thickening is also increased. Salts have little effect on gelatinization unless their concentration reaches the point that they begin to lower water activity (Whistler and Bemiller 1997). Polar lipids, such as monoglycerides and diglycerides, restrict gelatinization (Whistler and Bemiller 1997). The added lipids form an inclusion complex with amylose and cause an increase in gelatinization temperature. An application for the use of these components as surfactants is to reduce staling in bread.

The fine structure of starch, specifically the amylopectin and amylose structures, affect the gelatinization properties. Longer branch-chain lengths increase the gelatinization temperature (Yuan et al 1993, Jane et al 1997). Therefore, B-type starches tend to have higher gelatinization temperatures. An exception, among others, is potato starch (Jane et al 1997). This starch contains phosphate monoesters, which enhance the dissociation of the double helix when heat and water are applied. *Waxy* varieties of starches have nearly 100% amylopectin and, therefore, are almost entirely crystalline. The high crystallinity results in a higher enthalpy of gelatinization than normal varieties of the same starch.

2. Pasting

Pasting entails the events that occur after gelatinization, including granular swelling, leaching of molecular components from the granule, and total disruption of the granules (Atwell et al 1988). Usually, a Brabender viscoamylograph is utilized to study starch pastes

and measure the viscosity throughout the process. A starch and water mixture is heated and stirred, at a uniform rate, to create a paste and then cooled. During the process, the granules swell with amylose leaching out of the granule. After peak viscosity, at which the granules are swollen to their maximum, the granule structure disintegrates causing the viscosity to drop, which is referred to as shear-thinning. Upon cooling, pastes may gel and harden depending on the source of the starch. This last step is referred to as setback viscosity or retrogradation.

Pasting properties are affected by amylose and lipid contents and also by the branch chain-length distribution of amylopectin. Amylose and lipids inhibit the swelling of granules, whereas amylopectin contributes to the swelling (Tester and Morrison 1990). Amylose also contributes to the setback viscosity of the paste. *Waxy* varieties of starch have high peak viscosities, because of the elevated concentration of amylopectin, but little setback because of the near absence of amylose (Jane et al 1999). Amylose lipid complexes tend to increase the pasting temperature and increase resistance to shear-thinning (Jane et al 1999). Long branch-chains of amylopectin may lead to low shear-thinning and high setback viscosity because the long chains may mimic amylose to form helical complexes with lipids and entwine with other branch chains to hold the integrity of starch granules during heating and shearing (Jane et al 1999).

3. Retrogradation

Upon cooling, starch pastes retrograde. Retrogradation is complex and depends on many factors, such as type of starch, starch concentration, cooking procedure, temperature, storage time, pH, and the presence of other substances (Swinkels 1985). Low temperatures and high starch concentrations favor starch retrogradation.

Amylose association is the primary cause of retrogradation. Dissolved amylose molecules orient themselves in a parallel alignment and form hydrogen bonds between chains. This bonding causes aggregates, which precipitate in dilute solutions. In more concentrated solutions, a gel will form because the amylose associations form a network, entrapping water. The rate of retrogradation decreases with longer and shorter amylose molecules (Swinkels 1985). Long amylose molecules do not have high molecular mobility, whereas short amylose molecules are too short to associate completely. The minimum chain length required for retrogradation is 8 to 9 glucose units (Gidley et al 1986). The maximum potential for retrogradation is for amylose molecules of approximately 100 DP (Gidley et al 1986).

Amylopectin is less likely to retrograde than amylose. Association is inhibited because of its highly branched structure. However, long chains of amylopectin can participate in networking with amylose (Jane and Chen 1992, Yuan et al 1993). The outer chains of amylopectin may also form double helices, which may associate and organize into crystallites under the appropriate conditions (Ring et al 1987).

Retrogradation can be measured from the cooling and storage of starch pastes created with a Brabender viscoamylograph or Rapid Visco Analyzer. It can also be studied by using DSC (White et al 1989). Gels made by DSC are usually stored from 1 to 2 weeks at 4°C and then analyzed on the DSC. Peak retrogradation temperature is usually lower than peak gelatinization temperature because the crystals of the gel are less perfect than the original crystals of the starch. The percentage of retrogradation (%R) is calculated by dividing the enthalpy of retrogradation by the enthalpy of gelatinization and multiplying by 100%.

D. Development of the starch granule and functional properties

Most starch resides in the endosperm but significant levels are found in the embryo, bran, and tip cap. Because the majority of storage starch is located in the endosperm, discussion will be limited to the endosperm starch development.

The actual path of starch synthesis is not entirely clear and much research is presently being conducted concerning the subject. Generally, the substrate for starch is ADP glucose, which is synthesized by ADPglucose pyrophosphorylase from sucrose (Smith et al 1997). The nature and location of the enzyme varies within and between organs and species. Starch synthase (SS) forms α-D-1->4 linkages, whereas starch branching enzyme (BE) catalyzes the formation of α-D-1->6 branch points. There are a number of isoforms for each enzyme, but the purposes of all these forms are still not clear.

Reserve starch granules in higher plants develop in organelles called amyloplasts. An amyloplast may contain one starch granule, termed a simple granule, or several granules, called compound granules (Shannon and Garwood 1984). Maize, wheat, and peas contain simple granule starches. Oats, rice, and cassava contain compound starch granules. Wheat grain also contains two populations of granules: large, lenticular granules (known as A-type) and smaller, spherical granules (known as B-type). The A-type granules are produced during the early development of the endosperm, whereas B-granules develop later in maturity.

At about 8 to 10 days after pollination (DAP), cells in the central crown begin to accumulate starch first; the lower endosperm cells begin starch synthesis much later (Boyer et al 1977). At about 12 DAP, sugars are relatively high in concentration and starch is low. As the amount of cells accumulating starch increases, kernel sugar content decreases.

In a study by Wolf et al (1948) moisture content of corn kernels decreased from about 87% at 12 to 13 DAP to 9 to 11% at maturity. At the same time, starch content increased. The rise in starch content was most rapid between 12 and 20 DAP. Dent corn starch also increased in granule diameter rapidly during this time. The most rapid changes in starch properties were found to occur during the first 35 DAP.

Brown et al (1971) studied the development of corn mutants and reported that at 12 DAP the crystalline organization of the mutant and normal maize starches were similar. The differentiation from the normal starch occurred over the period of 12 to 24 DAP, indicating that mutant genes modified granule properties over this period.

During growth, apparent amylose percentage increases and molecular size of amylose and amylopectin increases (Banks and Greenwood 1975). Inouchi et al (1983) found that at three stages of growth (21, 28, and 35 DAP) certain maize starches had increased amylose contents compared to earlier DAP starches. They also reported that in the starch of normal, *amylose-extender* (*ae*), *sugary-1* (*su1*), and *sugary-2* (*su2*) maize, the amylopectin content decreased from 11 to 20 DAP. Boyer et al (1976) also reported that apparent amylose percentage increased during maturation of the kernel (18 to 36 DAP) in the starch of normal and mutant (*ae*, *ae su*) maize. Overall, the changes in fine structure in the starch are dependent upon genetic background and source of the starch.

Analyses have been performed on the DSC properties of starch during development (Biliaderis 1982, Inouchi et al 1984, Ng et al 1997). Ng et al (1997) studied the thermal properties of starch from *ae*, *su2*, and *waxy* (*wx*) mutants during development, sampling at 12, 18, 24, 30, and 36 DAP. They reported that within a genotype, DSC values of starches at 24, 30, and 36 DAP were similar to each other, but often were significantly different from the

values at 12 DAP. They postulated that this difference indicated that changes in the fine structure of starch occurred during endosperm development.

E. Environmental effect on starch structural and functional properties

1. General effect of environment on different grains

Starches from the same cereal cultivar grown at various sites and in different crop years can vary significantly in composition and properties. Crops give their highest yield, and lowest risk of failure, when they are grown as close as possible to their respective temperature optima (Keeling et al 1994). In cereal crops, the optimum temperature for maximum grain yield lies between 20 and 30°C (Chowdhury and Wardlaw 1978). The main effect of high temperature after pollination is a reduction in grain size. Grain yield, kernel weight, and kernel density were less for maize ears at 35°C than for those at 25 °C (Lu et al 1996). Singletary et al (1994) found that the rate of starch accumulation in maize reached a maximum at about 32 °C. However, at that temperature the actual levels of the enzymes responsible for synthesis of starch declined causing a lower kernel weight to result. The reduction in grain weight is caused by decreased production of starch, because starch accounts for 70% of the dry weight of the grain. Protein (Denyer et al 1994) and sucrose content (Nicolas et al 1984, Bhullar and Jenner 1986) are affected less by high temperatures.

The effects of high temperature on starch synthesis and yield may be a result of the elevated heat sensitivity of starch synthase, specifically soluble starch synthase (Denyer et al 1994). The soluble starch synthase has a temperature optimum between 20 and 25°C (Keeling et al 1993). In wheat ears heated to 40 °C for 2 h the soluble starch synthase activity was reduced to 3% of that of the unheated wheat (Keeling et al 1993). Others (Boyer and Preiss 1978, Takeda et al 1993) have also reported that branching enzyme (BE) activities

differ with temperature. BEI, with minor branching activity, preferentially transfers long chains and has a temperature optimum of 35 °C. BEIIa and BEIIb, with major branching activities, transfer short chains and have temperature optimums of 25 and 20 °C, respectively.

Bhullar and Jenner (1985) and Blumenthal et al (1995) found that during high temperatures B-type wheat starch granules were reduced in number. There was no apparent reduction in the number of A-type granules, which may be because A-type granules are produced early in the development and therefore the high temperatures did not occur early enough to affect the A-type granule development. Shi et al (1994) found that high temperatures also resulted in deformed wheat starch granules smaller in size. This difference concerning A- and B-type granule size also occurred in barley starch (Tester et al 1991).

Precipitation also affects the grain filling period. Nicolas et al (1984) found that drought, and drought in addition to high temperatures, reduced the storage capacity of the wheat grain, with a decrease in the number of cells and starch granules in the endosperm. Brooks et al (1982) also found that fewer B-type granules were produced and the size of A-type granules was reduced under water stress. They also reported that water stress did not affect the initial grain-filling period but reduced the final dry matter of both wheat and barley as a result of early termination of growth.

2. Effect of environment on starch structure

A number of studies have been conducted on the effect of environment temperature on amylose and amylopectin content and structures. Asaoko et al (1984) and Asaoko et al (1985) reported that in Japanese rice cultivars, amylose content decreased with higher temperatures (30°C) during early stages of development. Higher temperatures also increased the amount of long B chains in the amylopectin and decreased the number of short chains

compared to rice grown at a lower temperature (25°C). Inouchi et al (2000) also found the same differences and reported that the environmental temperature between 5 and 10 d after pollination strongly influenced the structural characteristics of rice starch.

Contrary to rice starch, Goering et al (1957) found no differences in amylose content of barley starch grown at different geographical and seasonal conditions. Tester et al (1991) also reported similar results and also concluded that amylopectin characteristics of the barley starch did not differ with higher developing temperature and the lipid contents increased.

At elevated temperatures, wheat starch lipid levels increased (Shi et al 1994, Blumenthal et al 1995, Tester et al 1995) and amylose levels increased slightly (Shi et al 1994). Shi et al (1994) reported that short chains (DP 10-15) of amylopectin increased at high developmental temperatures, whereas longer chains (DP 17-21) decreased.

Fergason and Zuber (1962) found that amylose content of maize decreased at higher growing temperatures, especially in high-amylose corn varieties. Lu et al (1996) also found that the true amylose content of maize decreased with high growing temperatures (35°C), along with the molecular size of the amylose. The amylopectin structure, however, varied with both variety and developmental temperature of the maize. In general, medium branch-chains were increased and short branch-chains were decreased at high development temperatures. These differences can be attributed to the temperature optimums of branching enzymes. BEI transfers long branch-chains and has a temperature optimum near 35 °C, whereas BEIIa and BEIIb transfer short-chains and have maximum activity near 25 °C and 20 °C, respectively. Therefore, at high grain-filling temperatures starch would be expected to contain a larger number of longer chains of amylopectin and fewer short branch-chains, than at low grain-filling temperatures.

It is clear from the preceding information that the effect of environmental stress, specifically high temperature, affects starch structure. The specific change, or lack of it, is dependent on the source and genotype of the starch.

3. Effect of environment on starch functional properties

Because different environmental factors affect the structural properties of starch, it also is expected that there may be an effect on the functional properties. High temperatures increase the starch lipid level in wheat, therefore causing reduced solubility and swelling power (Shi et al 1994). High grain-filling temperatures also resulted in higher onset temperature of gelatinization (T_{oG}) for wheat starch (Shi et al 1994, Tester et al 1995). These differences in T_{oG} values could be a result of different degrees of crystallite perfection or structural differences. Shi et al (1994) annealed the samples, and because the differences in T_{oG} remained, they concluded the variations were a result of a fundamental difference in granular structure. Increase in T_{oG} values also have been reported for rice starch (Asaoka 1984, Asaoka 1985) and barley starch (Tester et al 1991) developed at higher temperatures.

White et al (1991) found that starches from corn grown in tropical conditions gave an elevated and narrow gelatinization range (R_G) when compared to the same populations grown in temperate regions. Lu et al (1996) reported that maize starch developed at 35 °C had higher T_{oG} and wider R_G than starch developed at 25 °C. The gelatinization enthalpy (ΔH_G) did not change with elevated temperature. Ng et al (1997) examined the thermal properties of 62 exotic corn inbreds planted in Georgia and Puerto Rico. The starch from Georgia had higher T_{oG}, ΔH_G, and peak height index (PHI) than the starch grown in Puerto Rico. The temperature was higher in Georgia during the grain-filling period and may have caused perfection of the crystals or raised the chain length of the medium branch-fractions of

amylopectin, as reported by Lu et al (1996). Campbell et al (1994) found increases in peak gelatinization temperature (T_{pG}), ΔH_G, and R_G from starches planted at later dates. These differences were most likely due to variations in daily high temperatures and day length during the grain-filling period. Krieger et al (1998) studied maize starch thermal properties from two locations, both only 24 km apart. The T_{oG} values were different at both locations and were attributed to soil and/ or precipitation differences.

The effect of environment on starch properties also is affected by the genotype of the line. Ji et al (2003b) found significant environment and genotype interactions in thermal properties studied by DSC when exotic corn inbreds were grown in Ames, IA and Puerto Rico. These results show that different genotypes respond differently to environmental factors.

F. Effect of mutations on functional properties

Variations in DSC measurements have been demonstrated for a variety of maize mutants, including *amylose-extender* (*ae*), *dull* (*du*), *sugary-1* (*su1*), *sugary-2* (*su2*), and *waxy* (*wx*) (Inouchi et al 1984, Brockett et al 1988, Ninomya et al 1989, Sanders et al 1990, Inouchi et al 1991, Wang et al 1992, Campbell et al 1995a, Perera et al 2001, Tziotis 2001). These particular mutants cause changes from normal corn starch in amylose percentage and phytoglycogen accumulation (Shannon and Garwood 1984). The *ae* mutation results in starch with 50-70% apparent amylose content (Ikawa et al 1981, Yeh et al 1981, Shannon and Garwood 1984), which may dilute the crystalline regions thus causing a loss of cooperative melting (Wang et al 1992). Therefore, the *ae* starch typically has a broad gelatinization peak that is not complete until around 100°C and a high ΔH_G (Brockett et al 1988, Inouchi et al 1991). The *du* and *su1* genotypes also are reported to increase apparent amylose percentage

(Ikawa et al 1981, Yeh et al 1981), but do not have broad gelatinization peaks typical of *ae* starch (Inouchi et al 1984, Brockett et al 1988, Wang et al 1992). They both, however, typically possess a lower ΔH_G value and a T_{oG} a few degrees below that of normal starch, which may be due to slightly lower crystallinity in the starch (Inouchi et al 1984). Starch from the *su2* genotype also has a higher apparent amylose content than normal starch, but results in gelatinization at a much lower temperature and ΔH_G, which may be due to the very low percentage of crystallinity in *su2* starches (Inouchi et al 1984, Perera et al 2001). The *wx* genotype does not increase amylose content, unlike the other mutants presented here, but merely eliminates it (Inouchi et al 1984). This variance results in starch with nearly 100% amylopectin, the crystalline component of starch, which then requires more energy to gelatinize (Inouchi et al 1984).

G. GEM Project

No new corn starch derivatives have been introduced to the industry in about 40 years because of restrictions of consumer safety, consumer concerns, worker safety, environmental concerns, and economics (Bemiller 1997). Therefore, there has been interest to develop corn starches that naturally possess properties similar to those of chemically modified corn starches.

Exotic germplasm is usually considered to include all sources of unadapted germplasm, including domestic, temperate, and tropical (Goodman 1985). Mungoma and Pollak (1988) found that some exotic crosses have higher yields than the common Corn Belt Dent heterotic pattern cross, 'Reid' X 'Lancaster'. Holley and Goodman (1988) reported that crosses developed from 100% tropical inbred lines crossed with U.S. lines were agronomically competitive with commercial U.S. hybrids. Tracy (1990) reported that some

exotic sweet corn lines crossed to domestic lines improved agronomic traits, such as yield, stalk breakage, and ear appearance factors.

Exotic materials have been reported to have a high variation in thermal traits, suggesting the use of these lines in further breeding trials to develop varieties with unusual traits (Li et al 1994, Campbell et al 1995b, Pollak and White 1997, Singh et al 2001). Campbell et al (1995b) reported that thermal properties measured by DSC could be used to predict functional properties of starches among nonmutant sources of maize.

The Germplasm Enhancement of Maize (GEM) project has developed and identified exotic by adapted lines, partially from germplasm foreign to corn races grown in the United States, that may be useful for agronomic, nutritional, and/or industrial reasons (Pollak 2002). Our laboratory routinely screens corn sources for starch traits that may be useful to the starch industry such as low T_{oG} or low percentage of retrogradation (%R), and other criteria as described by Seetharaman et al (2001). Ji et al (2003a) identified exotic by adapted corn inbred lines that exhibit unusual properties such as low T_{oG} and wide R_G. Some lines also exhibited gelatinization thermogram shapes with shoulders or double peaks demonstrating two independent transitions. Distinctive lines are then further developed by inbreeding to increase the heritability of the traits. Lines that naturally possess unique properties are potentially available to industry as alternatives to chemically or physically modified starches.

H. Screening starch for unique thermal properties

DSC is an excellent method for researching starch gelatinization, because it allows for use over a wide range of starch/water ratios, is not limited to temperatures below 100°C, and estimates transition enthalpies (Biliaderis et al 1980). Also, DSC requires only a small amount of sample, is easy to operate, and is relatively rapid compared with other methods

(Sanders et al 1990, Campbell et al 1995). These factors make it conducive for breeding programs in which large numbers of corn genotypes are screened for desirable starch properties, such as low T_{oG} or low ΔH_G.

Earlier methods of screening typically involved extracting starch from single corn kernels, such as utilized by Ji et al (2003c), for a total of up to 10 kernels from one source. Obanni and Bemiller (1995) described a technique of screening via ghost structures, which are the remnants of starch after autoclaving. Much interest, however, is in the thermal properties of the starch via DSC. It would be advantageous to expedite the single-kernel procedure by bulk-extracting starch from a pool of kernels instead of only 1 kernel, while still being able to recognize the presence of starch with different properties via a DSC analysis. Obanni and Bemiller (1997) studied the thermal properties of starch blends with the DSC and the Brabender Viscoamylograph. DSC results did not resemble either of the two components and the amylograph data suggested that some mixtures behaved like a chemically modified starch. Preliminary data in our laboratory, however, suggested that the DSC results of certain mixtures of starches did display two separate peaks and their original properties were unchanged. Therefore, it would be advantageous to investigate the detection limits of unique properties in blends of different starches.

Because the consumer perception of genetically modified corn is presently low, it is advantageous to develop and investigate the properties of corn that naturally possesses desirable traits, such as lines available from the GEM project. The development of thermal properties and the effect of environment on thermal properties are important examinations that will further characterize present lines of interest. Rapid screening methods also need to be developed in order to expedite the process of detecting future lines of interest. A first

objective of this research was then to investigate rapid screening methods through use of a bulk-starch extraction rather than a single kernel extraction. This research also included examining the impact of growing location on the thermal properties of starch from exotic by adapted corn inbreds, and studying the development of thermal properties of starch from these lines.

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Chapter 3. Rapid screening to identify unusual thermal starch traits from bulked corn kernels

A paper to be submitted to *Cereal Chemistry*

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Abstract

Differential scanning calorimetry (DSC) is used routinely to screen for starch thermal properties when heated in water. In early generations of line development, the established analysis uses starch extracted from single kernels, which is timely. The objective of the current work was to expedite selection by examining five corn kernels at a time, instead of one. Corn lines, all from the same genetic background (ExSeed68 or Oh43), with known thermal functions (*amylose-extender*, *dull*, *sugary-1*, *sugary-2* (*su2*), and *waxy*) were blended with normal starch (control) in ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0, and analyzed using DSC. The values for each ratio within a mutant type were unique ($\alpha<0.01$) for most DSC measurements, especially for gelatinization onset temperature (T_{oG}), change in enthalpy of gelatinization, and range of gelatinization. Also, *su2* lines were identifiable in all ratios, because their T_{oG} was low (~50°C) compared with that of the control starch (~65°C). The

su2 starches had two separate thermal peaks, one from *su2* starch and another from control starch, even when the starch ratio blend had just one part *su2*. These results support the five-kernel method for rapidly screening large amounts of corn germplasm to identify kernels with unusual starch traits.

Introduction

Differential scanning calorimetry (DSC) was first utilized by Stevens and Elton (1971) to study starch gelatinization. DSC is an excellent method for researching starch gelatinization, because it allows the use of a wide range of starch/water ratios, is not limited to temperatures below 100°C, and estimates transition enthalpies (Biliaderis et al 1980). Also, DSC requires only a small amount of sample, is easy to operate, and is relatively rapid compared with other methods (Sanders et al 1990, Campbell et al 1995). These factors make it conducive for breeding programs in which large numbers of corn genotypes are screened for desirable starch properties, such as low gelatinization onset temperature (T_{oG}) or low change in enthalpy of gelatinization (ΔH_G).

Variations in DSC measurements have been demonstrated for a variety of maize mutants, including *amylose-extender* (*ae*), *dull* (*du*), *sugary-1* (*su1*), *sugary-2* (*su2*), and *waxy* (*wx*) (Inouchi et al 1984, Brockett et al 1988, Ninomya et al 1989, Sanders et al 1990, Inouchi et al 1991, Wang et al 1992, Campbell et al 1995, Perera et al 2001, Tziotis 2001). These particular mutants cause changes from normal corn starch in amylose percentage and phytoglycogen accumulation (Shannon and Garwood 1984). For example, the *ae* mutation results in starch with 50-70% apparent amylose content (Ikawa et al 1981, Yeh et al 1981, Shannon and Garwood 1984),which may dilute the crystalline regions thus causing a loss of cooperative melting (Wang et al 1992). The *ae* mutation also was reported to increase the

chain length of amylopectin (Ikawa et al 1981), which would then require a higher temperature to gelatinize (Wang et al 1992). Therefore, the *ae* starch typically has a broad gelatinization peak that is not complete until up to 120°C, and a high ΔH_G (Brockett et al 1988, Inouchi et al 1991). The *du* and *su1* genotypes also are reported to increase apparent amylose percentage (Ikawa et al 1981, Yeh et al 1981), but do not have broad gelatinization peaks typical of *ae* starch (Inouchi et al 1984, Brockett et al 1988, Wang et al 1992). They both, however, typically possess a lower ΔH_G value and a T_{oG} a few degrees below that of normal starch, which may be a cause of slightly lower and less perfect crystallinity in the starch (Inouchi et al 1984). Starch from the *su2* genotype also has a higher apparent amylose content than normal starch, but gelatinizes at a much lower temperature and ΔH_G, which may be a result of the very low percentage of crystallinity and higher amount of short branch-chains of amylopectin in *su2* starches than in normal starches (Inouchi et al 1984, Perera et al 2001). The *wx* genotype causes an elimination of amylose content, unlike the other mutants presented here (Inouchi et al 1984). This mutant results in starch with nearly 100% amylopectin, the crystalline component of starch, which then requires more energy to gelatinize (Inouchi et al 1984).

Recently, there has been interest in developing corn starches that naturally possess properties similar to those of chemically modified corn starches. The Germplasm Enhancement of Maize (GEM) project has developed and identified exotic by adapted lines that are partially from germplasm foreign to corn races grown in the United States, and which may be useful for agronomic, nutritional, and/or industrial reasons (Pollak 2002). Our laboratory routinely screens corn sources for starch traits that may be useful to the starch industry, such as low T_{oG} or low percentage of retrogradation (%R), as well as other criteria

as described by Seetharaman et al (2001). As described earlier, DSC is one of the most rapid methods available for such screening. Earlier methods, however, typically involved extracting starch from single corn kernels, such as utilized by Ji et al (2003), for a total of up to 10 kernels from one source. Obanni and BeMiller (1995) described a technique of screening corn via ghost structures , which are the remnants of starch granules after autoclaving small amounts of starch. The greatest value for starch, however, is in the thermal properties. It would be advantageous to expedite the procedure by bulk-extracting starch from a pool of kernels instead of only 1 kernel, while still being able to recognize the presence of starch with different properties via a DSC analysis. Obanni and BeMiller (1997) studied properties of blends of different types of starches, such as normal corn, *waxy* corn, *amylose-extender* corn, potato, and wheat. They reported that the DSC output did not resemble either of the components in the mixture. Preliminary data in our laboratory, however, indicated that a unique DSC starch characteristic was retained when two starch types were blended together. For example, we used a mixture of normal and *su2* starch in different ratios in the DSC pan, which resulted in independent peaks on the DSC, both similar to their respective starch type.

The objectives of this study were to investigate the use of DSC as a screening method for detecting unique thermal properties in a blend of two starch types, and to determine whether the starches gelatinized independently when mixed in different ratios in the DSC pan. The practical purpose for this work would to be able to detect the presence of a recessive gene affecting starch gelatinization within a segregating corn type. We created model systems with different ratios of mutant (*ae*, *du*, *su1*, *su2*, and *wx*) to normal starches to

determine the number of kernels containing starch with different functional properties needed in a bulk extraction to be detectable by DSC.

Materials and Methods

Materials

Corn (*Zea mays* L.) lines, all from the same genetic background (ExSeed68), with known thermal functions (*amylose-extender 25* (*ae*), *dull 39* (*du*), *sugary-1* (*su1*), *sugary-2* (*su2*), and *waxy 55* (*wx*)) were obtained from ExSeed Genetics (Ames, IA) along with the wild type of the ExSeed68 background (WT), having normal corn starch. The genes of corn lines from the same background were identical, except for those modified by the genetic mutation. The *ae*, *du*, *wx*, and WT lines were grown and harvested in Ames, IA during summer of 1999. The *wx*, *su1*, and a second WT control were grown in the summer of 2000. Another *su2* line in the Oh43 background, Oh43*su2*, was obtained from the USDA-ARS (Ames, IA) along with the Oh43 parent line. These lines were grown and harvested in Ames, IA during the summer of 1989. All ears were harvested at physiological maturity and dried at 35°C until the moisture content reached 12%. All seeds were stored at 4°C and 45% relative humidity until analyzed.

Starch Extraction

Corn starch was extracted as bulked 5-kernel samples from each line, according to the method of White et al (1990) with modifications by Krieger et al (1997) and Ji et al (in press). Each starch type was extracted in triplicate with replicates analyzed separately on the DSC.

Differential Scanning Calorimetry

Gelatinization characteristics of starch samples were determined by using differential scanning calorimetry (DSC) (Perkin Elmer DSC 7, Norwalk, CT) equipped with thermal analysis software (Perkin Elmer Corp., Norwalk, CT).

Samples were weighed on the same balance (Mettler AE 104, Toledo, OH). The starches with known thermal properties (*ae*, *du*, *wx*, *su1*, *su2*, Oh43*su2*) were blended in an aluminum DSC pan with their respective background starch, either WT or Oh43 grown in the same season as the respective mutant, in ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0 [mutant starch (dry weight): background starch (dry weight)] to give a total starch weight of 3.50 mg. These ratios are reported in this paper as mutant starch ratios 0, 1, 2, 3, 4, and 5, respectively. This plan allowed visualization of the effect of one kernel out of five being unique, two out of five, and so on, up to five out of five kernels. Water was added to the blended starch sample in a water to starch ratio of 2:1 and the sample was allowed to equilibrate for at least 30 min before DSC analysis. All samples were analyzed by DSC from 30°C to 110°C at a rate of 10°C per minute. Data parameters collected from the computer included gelatinization onset temperature (T_{oG}), gelatinization peak temperature (T_{pG}), gelatinization conclusion temperature (T_{cG}), and change in enthalpy of gelatinization (ΔH_G). The enthalpy was calculated on a starch dry-weight basis. Also calculated were the range of gelatinization (R_G) (T_{cG} minus T_{oG}) and peak height index (PHI) [ΔH_G (dry basis)/ ($1/2xR_G$)].

Statistical Analysis

Calculations were performed with SAS version 8.02 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to test the hypothesis that means were not different within each DSC parameter for each ratio within a mutant type. Tukey's multiple range test

was used to test for differences between ratios within a DSC parameter (α=0.01). Pearson correlation coefficients also were calculated for each DSC parameter within each mutant starch.

Results and Discussion

The model systems were based on a total of 5 kernels, because a recessive trait occurs 25% of the time in a heterozygous population (Poehlman 1959). Therefore, bulk-extracting 5 kernels greatly increased the probability of detecting a recessive trait. Each unique starch (*ae*, *du*, *su1*, *wx*, *su2*, and Oh43*su2*) imparted properties in the starch mixtures in their own specific ways, such as lowering T_{oG}, decreasing ΔH_G, or increasing T_{cG}, explained as follows.

ae

Because *ae* starch typically gelatinizes as a broad DSC peak that has only minimal impact in the gelatinization range of amylopectin (Figure 1), when measured alone as ratio 5, its T_{cG} and R_G are very high (Table I). The data for ratios 0 to 4 measured the contribution of the WT peak as peak 1a, whereas the *ae* was measured as peak 1b. The presence of *ae* starch was visible in ratio 1 through 4 as a long, broad peak following the peak of the WT, similar to the typical amylose-lipid peak and created some significant differences in the DSC values. For ratio 0, which contained 100% *ae* starch, the amylose-lipid complex was also measured as peak 1b in order to demonstrate the increase in parameters with the presence of *ae* starch. The *ae* starch had greater T_{oG} and greater T_{cG}, but lower ΔH_G, than WT starch, agreeing with previous work (Brockett et al 1988). When the *ae* starch was blended with WT starch, the peak 1a (WT starch) decreased in ΔH_G ($r=-0.99$, $p<0.0001$) with increased amounts of *ae* starch (Figure 1). The R_G of peak 1a also decreased ($r=0.65$, $p<0.0001$) from ratios 0 to 4 and the T_{cG} of peak 1a decreased from ratio 0 to 4 ($r=-0.87$, $p<0.0001$). The PHI of peak 1a

also decreased as the ratio of *ae* to WT starch increased from ratios 0 to 4 (r=-0.99, $p<0.0001$). The T_{oG} of peak 1b decreased with increased amounts of *ae* starch (r=-0.86, $p<0.0001$). The T_{oG} of peak 1b, however, was probably lower than measured in the presence of WT starch because its onset occurred within the range of the WT starch. The R_G of peak 1b increased with higher amounts of *ae* starch (r=0.86, $p<0.0001$). The ΔH_G of peak 1b also increased with higher amounts of *ae* starch (r=0.82, $p<0.0001$) along with the PHI of peak 1b (r=0.82, $p<0.0001$).

These results indicated that the low crystallinity of the *ae* starch (Wang et al 1992), which altered thermal properties, such as increasing the R, were detectable by DSC. The detection limit in the *ae* model system was a 1:5 kernel ratio for ΔH_G and PHI of peak 1a, a 2:5 kernel ratio for T_{cG} of peak 1a and ΔH_G of peak 1b, a 3:5 kernel ratio for R_G and PHI of peak 1b, and a 4:5 kernel ratio for R_G of peak 1a. In practical terms, the detection limit is the ratio of kernels needed to create a difference in a specific DSC parameter. The presence of *ae* starch was visible on a DSC curve and measurable by ΔH_G and PHI for the WT peak at ratio 1. These results suggested that an *ae* type starch could be detected in a blend of bulked-extracted starch from 5 kernels, even if only one unique kernel was present. The DSC operator could then return to the original line source of the bulked sample and extract using single-kernel methods to locate a single-kernel source of the *ae*-like starch.

du

The T_{oG} and ΔH_G of 100% *du* starch (ratio 5) were lower than those values for 100% WT starch (ratio 0), which agreed with previous work (Brockett et al 1988, Inouchi et al 1991, Wang et al 1992) (Table II). The T_{oG} decreased with increased amounts of *du* starch (r=-0.89, $p<0.0001$). The decrease in T_{oG} was accompanied by an increase in R_G (r=0.83,

$p<0.0001$). The PHI also decreased with increased ratios ($r=-0.83$, $p<0.0001$). The ΔH_G, however, did not become significantly different from the WT starch until ratio 5 (100% *du* starch), but the value did decrease with an increase in ratio of *du* starch ($r=-0.70$, $p<0.0001$). Inouchi et al (1984) reported that *du* starch was less crystalline than normal starch, thus causing changes in thermal DSC properties. In the current work, all parameters decreased, except for R_G, with increased amounts of the *du* starch. The detection limit for *du* starch was a 2:5 kernel ratio with respect to T_{oG}, R_G, and PHI and 3:5 for T_{pG}. These results suggest that the presence of a *du* type starch could be detected in a bulk extraction of 5 kernels. For example, if screenings were being performed on bulked samples, and a sample was detected with a lower T_{oG} and ΔH_G than normal, the investigator may want to return to the original corn line and re-extract by single-kernel methods in order to identify additional kernels with less crystalline starch.

sul

The *sul* starch had lower T_{oG}, T_{pG}, ΔH_G, and wider R_G than the WT starch, which was similar to previous reports of *sul* starch in various backgrounds (Brockett et al 1988, Inouchi et al 1991, Wang et al 1992) (Table III). T_{oG} decreased with increased amounts of *sul* starch ($r=-0.88$, $p<0.0001$). There were few differences, however, among blends at higher ratios of *sul* starch (3, 4, and 5). The decrease in T_{oG} was accompanied by an increase in R_G ($r=0.78$, $p<0.0001$). Similarly, the differences were not significant at higher ratios (3, 4, and 5). The ΔH_G decreased significantly with increased increments of *sul* starch ($r=-0.81$, $p<0.0001$). The PHI also decreased significantly ($r=-0.83$, $p<0.0001$). Overall, these results indicated that small amounts of *sul* starch significantly affected the DSC results. The *sul* starch was lower in crystallinity than normal starch, which resulted in lower T_{oG} and ΔH_G

(Inouchi et al 1984). These differences were significant in the model systems at a 1:5 kernel ratio for T_{oG}, R_G, and PHI, a 2:5 kernel ratio for ΔH_G, and a 4:5 kernel ratio for T_{pG}, the latter which decreased significantly with the addition of *su1* starch ($r=-0.79$, $p<0.0001$). Similar to the other starch blends, the DSC operator could return to the original source of the corn kernels if a lower T_{oG} and lower ΔH_G were detected within a bulked kernel starch sample.

wx

The thermal properties of the *wx* starch had lower T_{oG}, greater T_{cG}, wider R_G, greater ΔH_G, and lower PHI than the WT starch (Table IV). These results agreed with previous findings of *wx* starch in another background (Inouchi et al 1984), and were a consequence of the higher crystallinity of the *wx* starch (Inouchi 1984). In the current study, the T_{oG} tended to decrease ($r=-0.78$, $p<0.0001$), whereas the T_{cG} increased ($r=0.93$, $p<0.0001$), causing a wider R_G ($r=0.98$, $p<0.0001$) with greater ratios of *wx* starch. The ΔH_G also increased with increased amounts of *wx* starch, but only became significantly different from the WT starch at ratio 5 (100% *wx* starch, $r=0.74$, $p<0.0001$). The PHI also decreased significantly as the ratio of *wx* starch increased ($r=0.95$, $p<0.0001$). As the ratio of *wx* starch to WT increased, the crystallinity of the starch blend increased (Inouchi et al 1984), causing a rise in ΔH_G and R_G, which resulted in a lower PHI. The differences were significant at a 1:5 kernel ratio for R_G, a 2:5 kernel ratio for PHI, T_{oG}, and T_{cG}, and a 4:5 kernel ratio for T_{pG}, the latter which decreased with the addition of *wx* starch ($r=0.64$, $p<0.0001$). Similar to results just discussed, if the DSC investigator detected such thermal properties as the *wx* sample ratios, they could return to the original kernel samples and extract with single-kernel methods to further locate the source of *wx*-like thermal properties.

su2* and Oh43*su2

Because the T_{oG} and T_{cG} of *su2* and Oh43*su2* starch were much lower than those of their background starches, two separate gelatinization peaks were formed when they were blended (Figures 2 and 3). The first peak, 1a, was caused by the presence of either *su2* or Oh43*su2* starch and the second peak, 1b, was contributed by the background starch, either WT or Oh43.

With greater proportions of *su2* starch, the ΔH_G of peak 1a increased ($r=0.94$, $p<0.0001$), whereas the ΔH_G of peak 1b decreased ($r=-0.98$, $p<0.0001$) with greater proportions of *su2* starch (Table V). The R_G of peak 1a increased ($r=0.96$, $p<0.0001$), whereas the R_G of peak 1b decreased ($r=-0.66$, $p<0.0001$) with greater proportions of *su2* starch. This pattern resulted in increased PHI of peak 1a ($r=0.97$, $p<0.0001$) and decreased PHI of peak 1b ($r=-0.94$, $p<0.0001$). Total ΔH_G of the *su2* model system decreased with an increased proportion of *su2* starch ($r=-0.94$, $p<0.0001$). The detection limit was a 1:5 kernel ratio for ΔH_G and R_G of peak 1b, and for R_G and PHI of the total starch sample. A 2:5 kernel ratio was needed to detect significant differences in T_{oG}, T_{cG}, R_G,ΔH_G, and PHI of peak 1a, and R_G of peak 1b. To detect differences in T_{oG} and T_{pG} of peak 1b, and ΔH_G of the total starch sample, a kernel ratio of 3:5 was required.

Similar to the *su2* results, the ΔH_G of peak 1a increased with greater proportions of Oh43*su2* starch, ($r=0.94$, $p< 0.0001$), whereas the ΔH_G of peak 1b decreased ($r=-0.99$, $p<0.0001$) (Table VI). The R_G of peak 1a also increased ($r=0.89$, $p<0.0001$), whereas the R_G of peak 2a decreased ($r=0.62$, $p<0.0001$) with increased proportions of the *su2* starch. This pattern caused the PHI of peak 1a to also increase ($r=0.95$, $p<0.0001$) and the PHI of peak 1b to decrease ($r=-0.98$, $p<0.0001$). The total ΔH_G decreased significantly as the ratio of the

Oh43*su2* starch increased (r=-0.91, p<0.0001). These differences were significant at a 1:5 kernel ratio for ΔH_G and PHI of peak 1b, and for R_G and PHI of the total, combined peaks. A 2:5 kernel ratio was needed to identify differences in the T_{cG}, R_G, ΔH_G, and PHI of peak 1a, and ΔH_G of the total combined peaks. A 3:5 kernel ratio was needed to achieve significant differences in T_{cG} and R_G for peak 1b, and a 4:5 kernel ratio was needed to identify differences in T_{oG} of peaks 1a and 2a.

Overall, detection of unique properties for the Oh43*su2* and *su2* model systems was visible at a 1:5 kernel ratio, because a separate peak occurred that was a result of the *su2* starch (Figures 2 and 3). These ratio studies demonstrated how the thermal properties of a starch mixture could be affected by having just 20% of a starch that is different from the background starch. The *su2* and Oh43*su2* starches were lower in crystallinity than normal starches (Inouchi et al 1984, Perera et al 2001), and therefore resulted in lower T_{oG} and ΔH_G, which then produced a peak that was entirely separate from that created from the gelatinization peaks of the WT or Oh43 starch.

Conclusions

This study demonstrated that a five-kernel bulk extraction might be used to screen corn starch for the presence of only one in five kernels having different thermal properties. If one kernel out of five were different from the normal starch it was detected by DSC, as demonstrated with model systems created by blending different ratios of mutant starches previously shown to possess thermal properties different from those found in normal corn (*ae*, *du*, *su1*, *wx*, *su2*, and Oh43su2). A five-kernel bulk extraction was very helpful in expediting the screening process, because it reduced the extraction time and DSC analyses by a factor of five. This method can be applied to screening unknown germplasm for starch

thermal properties that are different from the properties of normal corn starch. The study also demonstrated that starch blends do impart the thermal properties of the independent starch components, suggesting conclusions that differed from those of Obanni and BeMiller (1997). In future work, it would be useful to study the impact of these starch blends on pasting properties of the mixtures, which could lend further insight into the thermal interactions of starch types.

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Table I: Thermal properties of corn starch blends with ratios of *ae* starch and wild type (WT) starch

	Thermal Property											
	Peak 1a[d]						Peak 1b					
Ratio[a]	T_{oG} (°C)[b]	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)
0	65.1ab	69.1a	73.1a	8.0a	11.6a	2.9a	87.1a	95.9a	102.4ab	15.3d	1.6d	0.21c
1	65.0a	69.1a	72.8b	7.7ab	8.9b	2.3b	83.7a	96.0a	102.7ab	19.0cd	1.9cd	0.20c
2	65.0a	69.1a	72.6bc	7.6b	6.5c	1.7c	84.0a	95.9a	103.2a	19.2cd	2.5c	0.27bc
3	65.0a	69.1a	72.5c	7.4b	4.3d	1.2d	82.8a	96.0a	103.1a	20.2c	3.4b	0.34b
4	65.4b	69.1a	72.0d	6.6c	1.7e	0.5e	76.5b	93.8b	102.6ab	26.1b	4.2b	0.32b
5							68.2c	84.9c	101.9b	33.8a	11.3a	0.67a
r[c]	0.17	0.04	-0.87	-0.65	-0.99	-0.99	-0.86	-0.70	-0.19	0.86	0.82	0.82
p-value	0.3746	0.838	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.27	<0.0001	<0.0001	<0.0001

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

[d] Peak 1a is the WT peak and Peak 1b is the *ae* peak, except ratio 0 for peak 1b is the amylose-lipid complex thermal properties

Table II: Thermal properties of corn starch blends with ratios of *du* starch and wild type (WT) starch

	Thermal Property					
Ratio[a]	T_{oG} (°C)[b]	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)
0	64.8a	69.0a	73.2a	8.4c	11.7a	2.80a
1	64.3ab	68.8a	73.2a	8.9bc	11.8a	2.69a
2	63.7bc	68.8a	73.3a	9.6ab	10.9ab	2.28b
3	63.3cd	68.4b	73.3a	10.0a	10.4ab	2.09bc
4	62.9d	68.2b	73.2a	10.3a	10.7ab	2.08bc
5	62.8d	67.7c	73.1a	10.3a	9.8b	1.90c
r[c]	-0.89	-0.86	-0.07	0.83	-0.69	-0.83
p-value	<0.0001	<0.0001	0.7021	<0.0001	<0.0001	<0.0001

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

Table III: Thermal properties of corn starch blends with ratios of *su1* starch and wild type (WT) starch

	Thermal Property					
Ratio[a]	**T_{oG} (°C)[b]**	**T_{pG} (°C)**	**T_{cG} (°C)**	**R_G (°C)**	**ΔH_G (J/g)**	**PHI (ΔH/°C)**
0	66.0a	69.7a	73.7c	7.7c	12.0a	3.12a
1	65.3b	69.8a	73.9bc	8.6b	11.8a	2.74b
2	63.7c	70.0a	74.4a	10.6a	11.9b	2.09c
3	63.0d	69.9a	74.3ab	11.3a	10.9bc	1.93cd
4	63.1d	68.3b	74.3ab	11.2a	10.4cd	1.85d
5	63.1d	67.3c	73.8c	10.7a	9.9d	1.86d
r[c]	-0.88	-0.79	0.13	0.78	-0.81	-0.89
p-value	<0.0001	<0.0001	0.4282	<0.0001	<0.0001	<0.0001

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

Table IV: Thermal properties of corn starch blends with ratios of *wx* starch and wild type (WT) starch

	Thermal Property					
Ratio[a]	T_{oG} (°C)[b]	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)
0	66.1a	69.9c	73.9e	7.8f	12.0b	3.06a
1	66.0ab	69.9c	74.3e	8.3e	12.3b	2.98ab
2	65.8bc	70.0c	74.9d	9.1d	12.8ab	2.82b
3	65.5cd	70.1c	75.7c	10.2c	12.9ab	2.53c
4	65.4de	70.4b	76.4b	11.0b	13.1ab	2.39cd
5	65.2e	70.9a	77.4a	12.2a	13.6a	2.22d
r[c]	-0.78	0.64	0.93	0.98	0.74	0.95
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

Table V: Thermal properties of corn starch blends with ratios of *su2* starch and wild type (WT) starch

	Thermal Property														
	Peak 1[d]						Peak 1b						Total		
	T_{oG}[b]	T_{pG}	T_{cG}	R_G	ΔH_G	PHI	T_{oG}	T_{pG}	T_{cG}	R_G	ΔH_G	PHI	ΔH_G	R_G	PHI
Ratio[a]	(°C)	(°C)	(°C)	(°C)	(J/g)	(ΔH/°C)	(°C)	(°C)	(°C)	(°C)	(J/g)	(ΔH/°C)	(J/g)	(°C)	(ΔH/°C)
0							65.0a	69.1a	73.3a	8.3a	11.7a	2.82a	11.7a	8.3a	2.82a
1	53.7a	57.4a	59.9a	6.2a	0.4a	0.11a	65.2ab	69.3ab	73.3a	8.1ab	7.9b	1.98b	10.6a	19.6b	1.09b
2	52.9b	57.3a	61.2b	8.3b	1.1b	0.27b	65.4ab	69.4abc	73.1a	7.7bc	5.5c	0.94c	10.2ab	20.2bc	1.01bc
3	52.6b	57.5a	61.9c	9.4c	2.2c	0.47c	65.8b	69.6bc	73.2a	7.4c	3.2d	0.58d	8.9bc	20.6c	0.86cd
4	52.5b	57.5a	62.6d	10.2d	3.4d	0.66d	66.6c	69.8c	73.2a	6.6d	1.6e	0.30e	7.7cd	20.7c	0.74d
5	51.8c	57.5a	64.0e	12.3e	6.8e	1.10e							6.8d	12.3d	1.10b
r[c]	-0.57	0.16	0.87	0.96	0.94	0.97	0.55	0.55	-0.13	-0.66	-0.98	-0.94	-0.94	0.24	-0.66
p-value	0.0011	0.3858	<0.0001	<0.0001	<0.0001	<0.0001	0.0015	0.0014	0.5041	<0.0001	<0.0001	<0.0001	<0.0001	0.1673	<0.0001

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

[d] Peak 1a is the *su2* peak, peak 1b is the Exseed 68 (background starch) peak and the total is the results from the start of peak 1a to the end of peak 1b

Table VI: Thermal properties of corn starch blends with ratios of Oh43*su2* starch and wild type (WT) starch

	Thermal Properties														
	Peak 1[d]						Peak 1b						Total		
Ratio[a]	T_{oG}[b] (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH/°C)	ΔH_G (J/g)	R_G (°C)	PHI (ΔH/°C)
0							67.2a	71.8a	77.0a	9.8a	11.9a	2.41a	11.5a	9.8a	2.35a
1	51.4a	55.7a	59.8a	8.5a	0.7a	0.16a	67.1a	71.7a	76.8ab	9.7a	9.0b	1.87b	11.5a	25.4b	0.90b
2	51.3a	56.1a	60.9b	9.5b	1.6b	0.35b	67.4a	71.9a	76.9ab	9.5ab	6.2c	1.30c	9.6b	25.5b	0.76bc
3	50.9ab	55.9a	60.9b	10.0b	2.5c	0.51c	67.2a	71.6a	76.3bc	9.1b	3.8d	0.83d	8.2c	25.4b	0.65c
4	50.8b	56.0a	61.8c	11.0c	3.9d	0.70d	68.2b	71.7a	75.9c	7.8c	1.5e	0.38e	7.3c	25.1b	0.59c
5	50.6b	56.3a	62.9d	12.3d	7.5e	1.22e							7.5c	12.3c	1.22d
r[c]	-0.33	0.17	0.83	0.89	0.94	0.95	0.44	-0.11	-0.70	-0.79	-0.99	-0.98	-0.91	0.08	-0.53
p-value	0.0724	0.3687	<0.0001	<0.0001	<0.0001	<0.0001	0.0147	0.5764	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6421	0.0008

[a] Each ratio is a proportion of unusual starch to normal starch; i.e. ratio 0 is 0 parts unusual starch to 5 parts normal starch

[b] T_{oG}=gelatinization onset temperature; T_{pG}=gelatinization peak temperature; T_{cG}=gelatinization conclusion temperature; R_G=range of gelatinization temperature; ΔH_G=Enthalpy of gelatinization; PHI=peak height index (enthalpy of gelatinization divided by half the range). Values in a column with different letters are significantly different (α= 0.01)

[c] r is Pearson's correlation coefficient of means within a column, p-value tests Ho: r=0

[d] Peak 1a is the *su2* peak, peak 1b is the Exseed 68 (background starch) peak and the total is the results from the start of peak 1a to the end of peak 1b

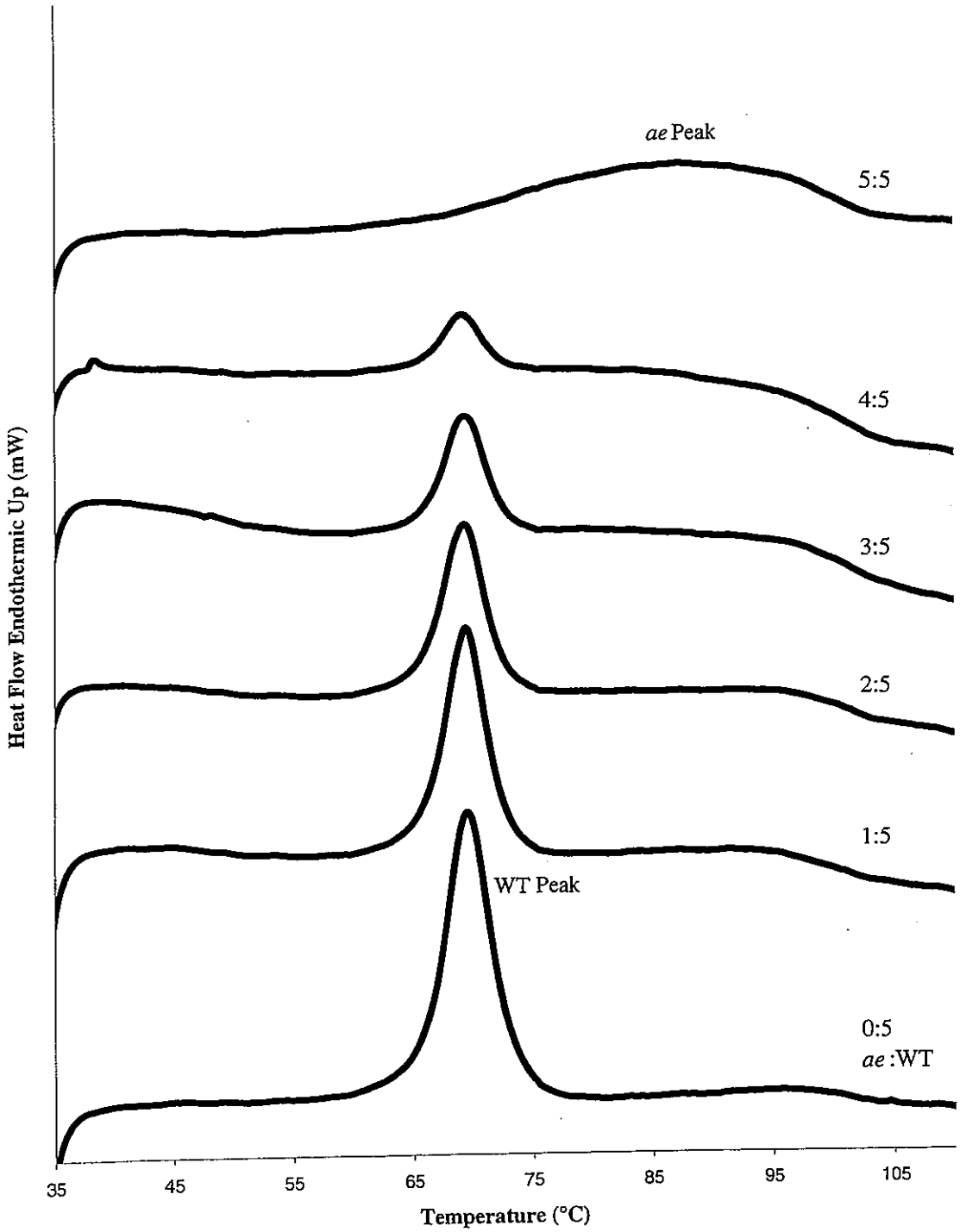

Figure 1: Differential Scanning Calorimetry (DSC) thermogram of ratios of *ae* and wild type (WT) corn starch

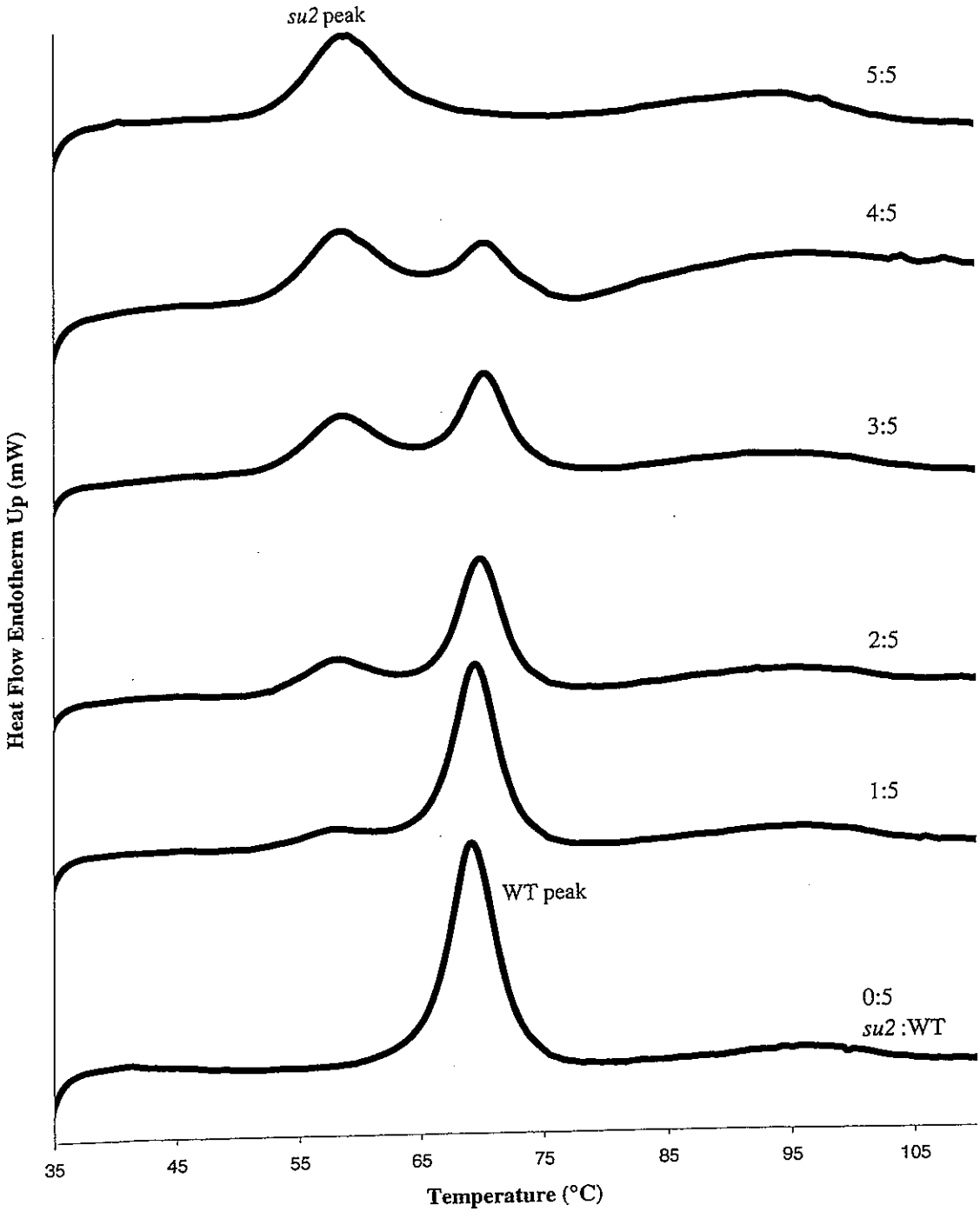

Figure 2: Differential Scanning Calorimetry (DSC) thermogram of ratios of *su2* and wild type (WT) corn starch

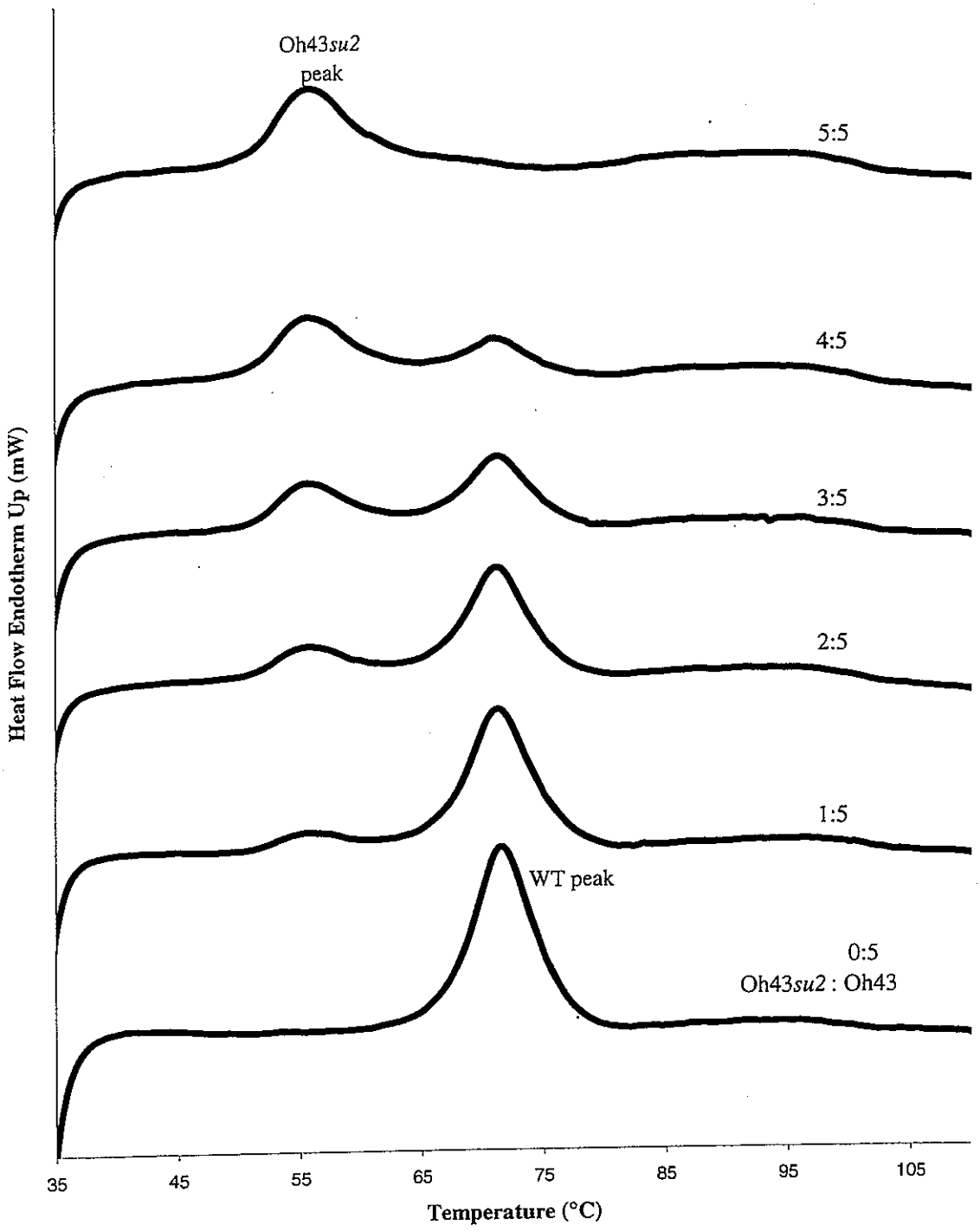

Figure 3: Differential Scanning Calorimetry (DSC) thermogram of ratios of Oh43*su2* and Oh43 corn starch

Chapter 4. Thermal properties of starch from exotic corn (*Zea mays* L.) lines grown in five locations

A paper to be submitted to *Cereal Chemistry*

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Abstract

The effect of 5 growing locations (Ames, IA (2), Clinton, IL, Columbia, MO, and Puerto Rico) on the thermal properties of starch from 5 exotic by adapted corn inbred lines (Chis37, Cuba34, Cuba38, Dk8, Dk10) and two control lines (B73 and Mo17) were studied using differential scanning calorimetry (DSC). The variations in thermal properties were similar between the exotic by adapted lines and control lines. Missouri was the warmest location and generally produced starch with greater gelatinization onset temperature (T_{oG}), narrower range of gelatinization (R_G), and greater enthalpy of gelatinization (ΔH_G). Illinois was the coolest location and generally resulted in starch with lower T_{oG}, wider R_G, and lower ΔH_G. These differences were attributed to higher temperatures in Missouri during grain-filling months either increasing the amount of longer branches of amylopectin or perfecting amylopectin crystalline structure. The Ames 1 farm produced starch with thermal properties

generally similar to those of Illinois, whereas the Ames 2 farm produced starch with thermal properties similar to those of Missouri. Ames 2 was located near a river bottom, which tends to be warmer, and may have caused higher grain-filling temperatures. Differences between locations may also be a result of differences in soil type and quality.

Introduction

Non-mutant corn starches may be developed to naturally possess properties similar to those of chemically modified corn starches. Exotic lines, grown in locations such as Argentina, Chile, Cuba, Mexico, and Puerto Rico, have high variations in their starch thermal traits, suggesting the use of these lines to create lines with unusual traits (Li et al 1994, Campbell et al 1995a, Pollak and White 1997, Singh et al 2001). The Germplasm Enhancement of Maize (GEM) project has developed and identified exotic by adapted lines, partly from germplasm foreign to corn races grown in the United States, that may be useful for agronomic, nutritional, and/or industrial reasons (Pollak 2002). Our laboratory routinely screens corn sources for starch traits that may be useful to the starch industry, such as low gelatinization onset temperature (T_{oG}) or low percentage of retrogradation (%R), and other criteria as described by Seetharaman et al (2001). Ji et al (2003a) identified unusual exotic corn inbred lines that exhibit unique properties, such low T_{oG} and wide range of gelatinization (R_G). Ji et al (2003b) examined the thermal properties of exotic lines grown in Ames, IA and Puerto Rico and found significant environment and genotype interactions in the thermal properties measured by differential scanning calorimetry (DSC). These results demonstrated that all genotypes do not respond the same to environmental factors. This variance, which is phenotypic, is actually the sum of three components, the effects of

genotype, environment, and genotype and environment interaction (Poehlman and Sleper 1995).

Crops give their highest yield and lowest risk of failure when they are grown as close as possible to their respective temperature optima (Keeling et al 1994). In cereal crops, the optimum temperature for maximum grain yield lies between 20 and 30°C (Chowdhury and Wardlaw 1978). Grain yield, kernel weight, and kernel density were less for corn ears at 35°C than for those at 25 °C (Lu et al 1996). The reduction in grain weight is caused by decreased production of starch, because starch accounts for 70% of the dry weight of the grain. Protein (Denyer et al 1994) and sucrose content (Nicolas et al 1984, Bhullar and Jenner 1986) are affected less by high temperatures than is starch.

The effects of high temperature on starch synthesis and yield may result from the elevated heat sensitivity of starch synthase, specifically soluble starch synthase (Denyer et al 1994). The soluble starch synthase has a temperature optimum of between 20 and 25°C (Keeling et al 1993). In wheat heated to 40 °C for 2 h the soluble starch synthase activity was reduced to 3% of that of the unheated wheat (Keeling et al 1993). Also, amylose content decreased at higher growing temperatures for corn (Fergason and Zuber 1962, Lu et al 1996) and rice (Asaoko et al 1984, Asaoko et al 1985, Inouchi et al 2000). However, no differences in amylose content (Goering et al 1957, Tester et al 1991) and amylopectin characteristics (Tester et al 1991) were found in starch from barley grown at different geographical and seasonal conditions.

Others (Boyer and Preiss 1978, Takeda et al 1993) also reported that branching enzyme (BE) activities differed with temperature. BEI, with minor branching activity, preferentially transfers long chains and has a temperature optimum of 35 °C. BEIIa and

BEIIb, with major branching activity, transfer short chains and have temperature optima of 25 and 20 °C, respectively. Lu et al (1996) found that, in general, medium branch-chains were increased and short branch-chains of corn starch were decreased at high development temperatures. Similar results were reported for rice starch (Asaoko et al 1984, Asaoko et al 1985, Inouchi et al 2000). Therefore, at high grain-filling temperatures starch would be expected to contain a larger number of longer chains of amylopectin and fewer short branch-chains, than at low grain-filling temperatures.

Moisture also affects the grain-filling period. Nicolas et al (1984) found that drought, and drought in addition to high temperatures, reduced the number of cells and starch granules in the endosperm of wheat. Brooks et al (1982) also found that fewer B-type granules were produced and the size of A-type granules was reduced under water deficit. They also reported that water deficit did not affect the initial grain-filling period, but reduced the final dry matter of both wheat and barley as a result of early termination of growth.

Because different environmental factors affect the structural properties of starch, there also may be an effect on the functional properties. White et al (1991) found that starches from corn grown in tropical conditions gave an elevated and narrow R_G when compared to the same populations grown in temperate regions. Lu et al (1996) reported that corn starch developed at 35 °C had higher gelatinization temperatures and wider R_G than starch developed at 25 °C. The ΔH_G did not change with elevated temperature. Ng et al (1997) examined the thermal properties of starch from 62 exotic corn inbreds planted in Georgia and Puerto Rico. The starch from Georgia had greater T_{oG}, ΔH_G, and peak height index of gelatinization (PHI) than did the starch from corn grown in Puerto Rico. The temperature, being higher in Georgia during the grain-filling period, may have caused

perfection of the crystals or raised the chain length of the medium branch-fractions of amylopectin, as reported by Lu et al (1996). Krieger et al (1998) studied corn starch thermal properties from corn grown at two locations, both only 24 km apart. The T_{oG} values were different at both locations, which were attributed to soil and/or precipitation differences.

The purpose of the present study was to examine the thermal properties, using DSC, of exotic by adapted developed from GEM breeding crosses and selected for unusual starch characteristics that were grown in four different locations in the U.S. Corn Belt and in Puerto Rico. The effect of genotype, environment, and genotype and environment interactions were examined to further understand the role of environment on the thermal properties of corn starch.

Materials and Methods

Materials

Corn (*Zea mays* L.) exotic by adapted inbreds GEM breeding crosses were used in this study, along with public inbred lines B73 and Mo17 as controls. B73 had a Stiff Stalk heterotic pattern, whereas Mo17 had a non-Stiff Stalk heterotic pattern. The exotic by adapted inbred lines were developed by crossing exotic populations (DK212T is a tropical 3-way commercial hybrid developed by Dekalb Genetics in Thailand) with inbreds of the Stiff Stalk heterotic pattern (Pollak 2002). These crosses were further developed by inbreeding through self-pollination. Five inbred generations [CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4, (Dk8), DK212T:S0610-10-1-3-6 (Dk10)] were chosen because data generated by our laboratory indicated that these lines possessed unusual desirable thermal properties as detected by differential scanning calorimetry (DSC) (Table I). The

Chis37, Cuba34, Cuba38, and Dk8 lines were grown during the summer of 2001 in Ames, IA, whereas Dk10 was grown during the summer of 1998 at the same location in Ames, IA. Unusual desirable thermal properties were previously defined by Seetharaman (2001). Examples included T_{oG} less than 61°C, ΔH_G less than 9.5 J/g or greater than 14.5 J/g, and %R less than 20% or greater than 80%. The selected lines were related to lines studied by Ji et al (2003a) in our laboratory, in that lines in the previous study were sister or parent lines of the genotypes selected for this study. Cuba34 and Cuba38 were of the same exotic origin, but derive from different self-pollinations (S_1 lines) of the breeding cross. The T_{oG} of Cuba34 and Cuba38 parent lines were similar, 60.8°C and 60.6°C, respectively, but differed in R_G, 15.2°C and 12.8°C, respectively. The same derivation scheme applies to Dk8 and Dk10. Both the T_{oG} and R_G of the Dk8 and Dk10 parent lines differed. The data shown in Table I were not previously published, but were produced independently in our laboratory.

Planting Locations

The five inbred lines, plus the two controls, were planted in a randomized complete block design at four locations in the Midwestern United States during the summer of 2002 with 3 blocks at each location. Rows were the experimental units. Two farms were located in Ames, IA were only 9 km apart. The first farm, the North Central Regional Plant Introduction Farm, referred to as Ames 1, had Clarion-Webster-Nicollet association soils, which are formed in loamy glacial till and glacial sediments, moderately drained and permeable and are found on uplands. The association consisted of 35% Clarion, 22% Webster, 10% Nicollet, and 33% minor soils. It was well suited for crops if it was properly drained and erosion was controlled. The second farm, the Iowa State University Hinds Farm, referred to as Ames 2, was near a river bottom and had soils classified as a Coland-Spillville-

Zook association. These soils are nearly level, moderately well to poorly drained, loamy and silty soils formed in alluvium, and found on bottom lands. The association at Ames 2 consisted of 40% Coland, 32% Spillville, 15% Zook, and 13% minor soils. This association was well suited for cultivation. The lines also were planted in Columbia, MO and Clinton, IL. Columbia, MO soils are classified as Freeburg silt loam, which are formed in silty alluvial sediments, very deep, somewhat poorly drained and moderately permeable. The Missouri farm was also located near a river bottom, similar to Ames 2. The Clinton, IL farm has a mixture of two closely related soils, Ipava silty loam (43%) and Sable silty clay loam (68%). Ipava is a very deep, somewhat poorly drained, and moderately permeable soil formed in uplands. Sable is a mesic Typic Endoaquoll, which is a very deep, poorly drained and moderately permeable soil formed in loess on nearly leveled summits of moraines and stream terraces. The progeny from the Ames 2 farm of summer 2002 were planted in Ponce, Puerto Rico because of low seed count of the original seed planted in the summer of 2002. Thus, the seed planted in Puerto Rico was the progeny, rather than sibling, of the other lines. The harvested seed allowed insight into the effect of a tropical environment on the thermal properties of starch from the exotic lines grown in the Midwest. The Puerto Rico farm soils were of the San Anton association and consist of very deep, well-drained, moderately permeable soils on alluvial fans and flood plains. They formed in alluvium that weathered from volcanic rock and limestone.

All Midwestern fields were planted in May 2002, whereas the Puerto farm was planted November 30, 2002. The Missouri farm was harvested on September 16, 2002, Illinois on October 2, 2002, Ames 1 on October 7, 2002, Ames 2 on October 22, 2002, and Puerto Rico on March 24, 2003. Ears were harvested at physiological maturity and dried at

38°C for 5 days, shelled, and then stored at 4°C and 45% relative humidity until the kernels were needed for analysis.

Bulk Starch Extraction

Two ears from each row were chosen for analysis. Corn starch was extracted as bulked 5-kernel samples from each ear, according to the method of White et al (1990) with modifications by Krieger et al (1997) and Ji et al (2003c). Each ear was extracted in duplicate, and 2 replicate analyses from each duplicate were analyzed with DSC.

Differential Scanning Calorimetry (DSC)

Starch (3.50 mg, with 10% assumed moisture content) was weighed into an aluminum pan on the same balance (Mettler AE 104, Toledo, OH) (White et al 1990). Water was added to the starch sample in a water to starch ratio of 2:1; the sample was hermetically sealed and allowed to equilibrate for at least 30 min before DSC analysis. All samples were analyzed by DSC equipped with thermal analysis software (Perkin Elmer DSC 7, Norwalk, CT) from 30°C to 110°C at a rate of 10°C per min. Data parameters collected from the computer included onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and change in enthalpy (ΔH). The enthalpy was calculated on a starch dry-weight basis. Also calculated were the range (R) (T_c minus T_o) and peak height index for gelatinization (PHI) (ΔH_G (dry basis)/(1/2 x R_G)). A subscript "G" after a parameter denotes a gelatinization property. Samples were stored at 4°C for 7 days to study the retrogradation characteristics. Stored samples were analyzed by DSC from 30°C to 90°C at a rate of 10°C per minute. The same parameters for gelatinization were calculated for retrogradation and are denoted by a subscript "R" after the parameter. Percentage of retrogradation (%R) also was calculated from the ratio of ΔH_R divided by ΔH_G.

Statistical Analysis

The effect of location, line, and their interactions on the thermal properties of starch of inbreds from 2002 was analyzed by using an analysis of variance procedure for a mixed model computed with SAS version 8.02 (SAS Institute, Cary, NC). Fixed factors were farm, line, and farm*line. Random effects were included for ear, row, block, extraction, and DSC. Contrast statements were used to determine significant differences ($p<0.05$) between locations within the same line and same DSC parameter.

Because the seed planted in Puerto Rico was the progeny of the seed from Ames 2 and, therefore, not identical genetically to the Midwest locations, the Puerto Rico results were only compared to the Ames 2 farm results.

Results and Discussion

Climate Variations

The Puerto Rico farm was infested with insects during the growing season, which decreased crop production. Incomplete replications resulted and no Mo17 samples from Puerto Rico were produced for analysis. Production at the Ames 1 farm also was low due to poor growing conditions, including poor soil quality, primarily a result of low levels of erosion control, which was necessary for good crop cultivation for the soil association of Clarion-Webster-Nicollet at Ames 1.

The climate summaries of the 4 different environments are shown in Table II. In general, Missouri and Puerto Rico had greater average mean temperatures than Ames and Illinois during the grain filling months (July and August for the Midwestern locations and January and February for Puerto Rico). The Ames locations essentially received the same weather patterns, but Ames 2 may have been warmer because it was located near a river

bottom and, therefore, more similar to Missouri, which also was located near a river bottom. Puerto Rico received a low amount of precipitation compared to the other farms, but the location was irrigated. Ames had a lower amount of precipitation in August, a grain-filling month, than did the Missouri and Illinois locations.

Variations between locations for each line

B73

B73 did not perform well agronomically at several locations. The Illinois and Missouri farms both produced 3 complete blocks, whereas Ames 2 and Puerto Rico both lost a block. The Ames 1 farm had only 1 block that produced B73.

The T_{oG} at Missouri and Ames 1 were greater than the T_{oG} at Illinois, which produced B73 starch with lower T_{oG} than the other locations (Table III). The T_{oG} at Ames 2 was similar to the T_{oG} at Ames 1 but lower than the T_{oG} at Missouri. The R_G of B73 grown in Missouri was narrower than the R_G at Ames 2 and Illinois. The R_G at Illinois was wider than the R_G at Ames 2 and Missouri. The ΔH_G at Illinois and Ames 1 were lower than the ΔH_G at Ames 2 and Missouri. The PHI at Missouri was greater than the PHI at Ames 2 and Illinois. The PHI at Illinois was lower than the PHI at Ames 2 and Missouri, indicating that the peaks of starch from B73 grown in Illinois were wider (as also indicated in the R_G) than starch from B73 grown at Ames 2 and Missouri.

The T_{oR} of B73 starch did not differ among locations. However, the R_R at Ames 2 was greater than the R_R at Ames 1 and Illinois. The R_R at Missouri did not differ from the R_R at the other B73 locations. The ΔH_R at Ames 2 was greater than the ΔH_R at other farms. The ΔH_R at Illinois was lower than the ΔH_R at Missouri. The %R at Illinois and Missouri were lower than the %R at Ames 2.

The Ames 2 farm and Puerto Rico farm results were compared for all lines because the seed grown in Puerto Rico was the progeny of seed grown in Ames 2. The starch from B73 cultivated in Puerto Rico was only significantly different from the B73 starch grown in Ames 2 with respect to PHI and ΔH_R. The PHI was greater for the B73 starch grown in Puerto Rico, whereas the ΔH_R was lower.

Statistical tests for interaction revealed that B73 x location interaction ($p<0.01$) occurred for T_{oG}, R_G, ΔH_G, PHI, and ΔH_R, and B73 x location interaction ($p<0.05$) for %R (Table IV). Therefore, for these parameters, the relative outcome depended on the location of cultivation of B73. The starch from the Illinois section tended to have lower T_{oG} and wider R_G, which indicated less perfect crystallization of the starch (Inouchi et al 1984). This could have been a result of lower grain-filling temperatures in Illinois causing the crystallites to be less perfect than those developed at greater temperatures as suggested by Lu et al (1996). However, Ames had temperature patterns similar to those of Illinois and, therefore, the results between the two farms in Ames and the farm in Illinois should be similar, if only temperature were considered. These expected results did not occur, perhaps because of differences in soil type, precipitation differences, or interactions between soil types, precipitation, and temperature between the Illinois and Ames locations. Missouri, however, had the highest average temperatures during the grain filling period and resulted in B73 starch with the greatest T_{oG} and tightest R_G. This was partly consistent with results reported by Lu et al (1996), who found that starch from corn developed at higher temperatures (35°C) had greater T_{oG} and wider R_G. Ng et al (1997) also found starch developed at higher temperatures had greater T_{oG}, ΔH_G, and PHI. This increase may have resulted from an increase in the medium branch-chains of amylopectin, caused by the higher temperature

optimum of BEI, and/or the perfection of crystallites at higher temperatures. The Ames 2 farm also produced B73 starch with greater ΔH_R and %R, which may have been caused by more fractions of longer amylopectin chains that were able to recrystallize.

Mo17

In Puerto Rico, no Mo17 kernels were produced due to extreme infestation of insects. Illinois only produced two blocks of Mo17, however, the Mo17 replicates were complete at all other farms.

The T_{oG} of Mo17 was greater at the Ames 2 farm than at Illinois and Ames 1 farms (Table III). The T_{oG} at Missouri was not different than the T_{oG} at Ames 2 and Ames 1. The T_{oG} at Illinois was the lowest and was different from the T_{oG} at all other farms. The R_G at Illinois was greater than the R_G at the other farms, which all had no differences in R_G. The ΔH_G at Ames 2 and Missouri were greater than the ΔH_G at other farms, whereas the ΔH_G Illinois was greater than the ΔH_G at Ames 1 farm. The PHI at Missouri was greater than the PHI at Illinois and Ames 1. The PHI at Ames 1 and Ames 2 were not significantly different. The PHI at Illinois was lower than the PHI at all other farms.

The T_{oR} and ΔH_R of Mo17 grown at Ames 2 were greater than the T_{oR} and ΔH_R of Mo17 grown at the other three farms. The R_R at Ames 2 was greater than the R_R at Ames 1, and the R_R at Illinois and Missouri were similar to the R_R at other farms. The %R at Missouri was lower than the %R at other farms. There were no differences in %R among Mo17 starch from the other farms.

Significant interactions ($p<0.01$) between Mo17 and location were found for the T_{oG}, R_G, ΔH_G, PHI, T_{oR} ΔH_R, and %R. Similar to the B73 starch, lower T_{oG} and wider R_G resulted at Illinois compared with Mo17 starch from other locations, indicating a less perfect

crystalline structure of the amylopectin (Inouchi et al 1984). This difference was, again, most probably a result of lower temperatures at the time of development of the starch. Similar to B73 starch, the Missouri location produced Mo17 starch with greater T_{oG}, narrower R_G, and greater ΔH_G, which could indicate more perfect crystallization of the amylopectin strands or more crystallization overall and may have been a result of higher mean average temperatures during the grain-filling period. However, the Ames locations had similar weather to Illinois, but did not produce results similar to that farm or similar to each other. This latter difference may be a result of the actual location of Ames 2, which was near a river bottom, causing higher temperatures than actually reported at Ames 2 and producing Mo17 with starch that had a greater T_{oG}, ΔH_G, T_{oR}, ΔH_R, and %R than from the Ames 1 location, indicating more perfect crystals. The two farms also had different types of soil, as noted in the Material and Methods section, which may also have affected the results. The Ames 1 farm had soil with a Clarion-Webster-Nicollet association and also had poor growing conditions, including poor soil quality. The Ames 2 farm soil was a Coland-Spillville-Zook association. The different soil types and quality likely interacted with the environment, causing these differences in starch quality.

Chis37

Chis37 did not perform well agronomically due to late silk dates, thereby interfering with pollination. The Illinois, Missouri, and Puerto Rico farms had complete replications, whereas Ames 2 lost one block and Ames 1 lost two blocks.

Chis37 was chosen as a line of interest because its starch previously was reported to have low T_{oG} (59.7°C) (Table I). In the present study, progeny seed only partly retained the properties, especially T_{oG} and R_G, of the original line (Table III). The R_G decreased from

13.5°C in the parent line to an average of 9.5°C for the successions. The Illinois farm, however, produced Chis37 starch with a R_G of 11.5°C, which was closer to that of the parent line. The Illinois farm also produced Chis37 starch with a T_{oG} of 62.8°C, which was the closest of the progenies to the T_{oG} of the parent line. The parent Chis37 was produced during the summer of 2001, at a third Iowa farm location, Iowa State University Agronomy and Agricultural Engineering Farm. The farm was about 10 km from Ames, IA in Boone County and had soils that were Canisteo-Clarion-Nicolet associations. The association had 29% Canisteo, 27% Clarion, 14% Nicollet, and 30% minor soils. This type of soil was very good for cultivation. These soils were loamy soils, which are nearly level to moderately sloping, poorly drained to well-drained, and are found on uplands. The temperature was relatively similar to the temperature during the summer of 2002, during the grain-filling months. There was considerably less precipitation, however, during July and August of 2001 in Ames (4.2 cm and 6.8 cm, respectively) than July and August of 2002 in Ames (11.7 cm and 12.2 cm, respectively). The lesser precipitation and farm location may have affected the results. None of the 2002 locations, however, resulted in starch with T_{oG} as low or R_G wide as that of the Chis37 parent line. Because the lines were progeny of self-pollinated generations, it may be that the traits are genetically complex, and genes of the inbreds were still segregating, producing ears with kernels that were not completely homogenous in thermal properties. This segregation may then have caused the progeny of summer 2002 to produce starch that did not resemble the starch from the parent lines because of minor differences in the genes and also genetic modifiers, as suggested by Campbell et al (1995b), controlling starch production. This explanation is somewhat unlikely, however, because the progeny was highly inbred. For example, in Table I, the full name of each line is displayed. Each number

after a dash is a generation of self-pollination. With each self-pollination, homozygous genes increase by 25%. Chis37 of 2002 is 99% homozygous because it had been self-pollinated 6 times. Therefore, it is likely that the phenotypic outcome of Chis37 was affected more by environment and the interaction of genotype and environment variations from year rather than by genotypic variations.

Starch from Chis37 grown at Missouri had greater T_{oG} than starch from Chis37 grown in Illinois and Ames 1 (Table III). The T_{oG} at Illinois was lower than the T_{oG} at Missouri and Ames 2. The R_G at Illinois was greater than the R_G at the other three farms. The ΔH_G at Ames 2 and Missouri were greater than the ΔH_G at Illinois and Ames 1. The PHI at Ames 2 and Missouri were greater than the PHI at Illinois. The PHI at Ames 1 was not different from that at any farm.

Retrogradation results showed that the T_{oR} of starch from Chis37 grown at Ames 2 and Missouri were greater than the T_{oR} at Illinois. The T_{oR} at Ames 1 did not differ from that at other farms. The ΔH_R at Ames 2 was greater than the ΔH_R at other farms, and the ΔH_R at Illinois was lower than the ΔH_R at other farms. The R_R at Ames 2 was greater than the R_R at Illinois and Missouri. The R_R at Ames 1 did not differ from the R_R at other farms. The %R at Ames 1 and Ames 2 were greater than the %R at Illinois. The %R at Missouri did not differ from the %R at other locations.

The starch from Chis37 grown in Puerto Rico had lower ΔH_G, T_{oR}, ΔH_R, and R_R than starch from Chis37 grown in Ames 2. The average temperatures in Puerto Rico (Table II) were greater than the temperatures in Ames. Hypothetically, the T_{oG} should have been greater for the starch from Puerto Rico based on previous results from Lu et al (1996). However, this did not occur and could be a result of insect infestation in Puerto Rico,

different soil types, and/or the interactions of these factors. Statistical testing revealed that a significant interaction ($p<0.01$) existed between the genotype, Chis37, and location of cultivation for T_{oG}, R_G, ΔH_G, PHI, ΔH_R, and %R (Table IV), and interaction between Chis37 and location ($p<0.05$) also existed for T_{oR} and R_R. Similar to the Mo17 and B73, the controls, results, Chis37 starch grown at Illinois had lower T_{oG}, wider R_G, and lower ΔH_G, whereas the Chis37 starch from Missouri had greater T_{oG}, narrower R_G, and greater ΔH_G. Again, these differences may be a result of higher temperatures in Missouri than in Illinois during the grain-filling period. The Ames 1 farm produced starch similar to that from the Illinois farm, whereas the Ames 2 farm produced starch similar to that of Missouri. Similar to the results for B73 and Mo17, the Ames 2 farm tended to produce Chis37 starch with the greatest ΔH_R, T_{oR} and %R. The differences between Ames 1 and Ames 2 may be caused by differences in temperature, with Ames 2 being warmer because of its location near a river bottom. Differences in soil type and quality may also have affected the results.

Cuba34

Cuba34 performed very well agronomically, with complete replications at all farms. Cuba34 was chosen as a line of interest because its starch previously had a low T_{oG} (60.8°C) (Table I). Starch from Cuba34 at Ames 1 resulted in T_{oG} of 61.6°C and a broad R_G of 14.4°C (Table III), which were relatively close to those of the starch from the parent line.

The T_{oG} at Missouri was greater than the T_{oG} at all other farms (Table III). The T_{oG} at Ames 2 was the second greatest. The T_{oG} at Illinois and Ames 1 were not different from each other. The R_G at Ames 1 was greater than the R_G at other farms. The R_G at Illinois was the second greatest, whereas the R_G at Ames 2 was the second lowest. The R_G at Missouri was

the lowest. The ΔH_G at Ames 2 and Missouri farms were greater than the ΔH_G at Ames 1 and Illinois.

The T_{oR} at Ames 1 was greater than the T_{oR} at Illinois and Ames 1. The T_{oR} at Missouri was not different from the T_{oR} at the other 3 farms. There was no difference in R_R among all farms. The ΔH_R at Ames 2 and Missouri were greater than the ΔH_R at Ames 1 and Illinois. The %R at Ames 2 was greater than the %R at Illinois and Ames 1. The %R at Ames 1 was lower than the %R at Ames 2 and Missouri.

Cuba34 starch from Puerto Rico had lower T_{oG}, R_G, ΔH_G, PHI, and T_{oR} than Cuba34 starch from Ames 2. These results are similar to the Chis37 results and may have been a result of the insect problems, soil differences, and/or interactions of these factors.

Significant interactions ($p<0.01$) were found between the genotype, Cuba34, and location for T_{oG}, R_G, ΔH_G, PHI; ΔH_R, and %R, and interactions ($p<0.05$) were also found for T_{oR} (Table IV). Similar to the results of the genotypes just discussed, starch from Cuba34 grown in Illinois and Ames 1 had lower T_{oG}, wider R_G, and lower ΔH_G than starch from Cuba34 produced in Missouri and Ames 2. With respect to retrogradation results, the Ames 2 farm again produced starch with greater T_{oR}, ΔH_R, and %R than Cuba34 starch from other farms. Again, the differences between Missouri and Illinois may be a result of higher temperatures in Missouri than in Illinois. The phenotypic variations between Ames 1 and Ames 2 may have been caused by differences in soil type and quality of growing conditions and also the possibility of higher temperatures at Ames 2 as a result of its location near a river bottom.

Cuba38

The Cuba38 corn line performed well, agronomically, with complete blocks obtained at all 5 farms. It was chosen as a line of interest because its starch exhibited a low T_{oG} (60.6°C) and moderately wide R_G (12.8°C) (Table I). No progeny planted at the 5 farms exhibited these interesting thermal properties of the parent Cuba38 line (Table III). The R_G of starch from the progeny were narrower, 9.2°C, than the starch of parent line Cuba38. The parent Cuba38 line (Table I) was grown in Ames during the summer of 2001 at a different farm than in the present study. As noted in the discussion about the Chis37 line, the temperature during 2001 was relatively similar to the temperature during the summer of 2002 in Ames during the grain-filling months; however, there was considerably less precipitation during July and August of 2001 in Ames. The lesser precipitation and farm location may have affected the results. As noted in the discussion of the Chis37 line, these results may suggest that the genes of the inbreds were still segregating and produced Cuba38 kernels that were not completely homogenous in thermal properties. The Cuba38 produced in the summer of 2002, similar to the Chis37 line, had also been self-pollinated 6 times. Therefore, the genes were 99% homogenous and less likely to be segregating. The phenotypic variation in starch properties between parent Cuba38 and progeny Cuba 38 starches were most likely a result of the effects of environment and interaction of environment and genotype rather than genotypic effects alone.

The T_{oG} at Illinois was greater than the T_{oG} at the other 3 farms (Table III). The R_G did not differ among the 4 farms. The ΔH_G at Missouri and Ames 2 was greater than at Ames 1. The ΔH_G at Illinois did not differ from ΔH_G at the other farms. The PHI at the 4 different farms were not different from each other.

The T_{oR} at Ames 2 was greater than the T_{oR} at Ames 1 and Illinois. The T_{oR} at Missouri did not differ from the T_{oR} at other farms. The R_R did not differ between the locations. The ΔH_R at Ames 2 was greater than the ΔH_R at other farms. The ΔH_R at Illinois was lower than the ΔH_R at Ames 2 and Missouri. The %R at Ames 2 was greater than the %R at other farms. No differences existed with respect to %R among the other locations.

The T_{oR} at Puerto Rico was lower than the T_{oR} at Ames 2, which was the only difference between the two farms. These results differ from the results reported for B73, Chis37, and Cuba34, which all had more differences between properties. Starch from Cuba38, however, did not differ greatly between these two locations with respect to thermal properties.

There was a significant interaction ($p<0.01$) between the genotype, Cuba38, and growing location for T_{oR}, ΔH_R, and %R, and interaction ($p<0.05$) also was found for T_{oG} (Table IV). The T_{oG} at Missouri was greater than the T_{oG} at Illinois, which is similar to the results for B73, Mo17, Chis37, and Cuba34. The Ames 1 and 2 farms, however, were similar to each other and to Missouri, with respect to gelatinization. The T_{oR}, ΔH_R, and %R were generally greater for starch produced at the Ames 2 farm than starch from the other farms, which was similar to the other lines previously discussed. Fewer parameters for the Cuba38 starch were affected by location than for the previously discussed genotypes; however, no location produced Cuba38 starch that retained the thermal properties of the parent line. These results suggest either an overall interaction between the 2001 location and 2002 locations, or that the genes were still segregating and the kernels were not homogenous with respect to genes and genetic modifiers affecting starch traits. As previously mentioned regarding the Chis37 progeny, this latter suggestion is not likely because the inbreds were

highly inbred. Therefore, the genes were nearly all homogenous and, therefore, the phenotypic variations from year to year were more likely caused by variations in environment and genotype by environment interactions.

Dk8

Dk8 performed well agronomically, resulting in only one missed block at the Illinois location. Successions produced starch that only partly retained the original unique property of T_{oG} 59.4°C (Table I & Table III). The Dk8 seed planted in the present study came from parents produced in the summer of 2001 at the same location as the Chis37, Cuba34, and Cuba38 parents. Again, the Dk8 progeny from the summer of 2002 were seventh inbred successions. Therefore, the genes were over 99% homogenous and the phenotypic variation between the parent and progeny starch thermal properties were likely a result of environmental and interaction of environment and genotype effects rather than only genotypic variation.

The T_{oG} at Missouri was greater than the T_{oG} at Illinois and Ames 1 (Table III). The T_{oG} at Ames 1 was lower than the T_{oG} at Ames 2 and Missouri. The R_G of Dk8 starch did not differ among the 4 farms. The ΔH_G at Ames 2 and Missouri were greater than the ΔH_G at Illinois and Ames 1. The PHI at Missouri was greater than the PHI at Ames 1. PHI at Ames 2 and Illinois did not differ from the PHI at the other farms.

The T_{oR} at Ames 2 was greater than the T_{oR} at the other 3 farms. The R_R of Dk8 starch did not differ among the 4 locations. The ΔH_R at Ames 2 was greater than the ΔH_R at the other 3 farms. The ΔH_R at Missouri was greater than the ΔH_R at Ames 1 and Illinois. The %R at Ames 2 and Missouri were greater than the %R at Ames 1.

Dk8 starch from Puerto Rico only differed with respect to T_{oR} when compared to Dk8 starch from Ames 2, which was similar to the results of the Cuba38 starch. The T_{oR} at Puerto Rico was lower than the T_{oR} at Ames 2.

Significant interactions ($p<0.01$) were found between the genotype, Dk8, and location T_{oG}, ΔH_G, T_{oR}, and ΔH_R (Table IV). The genotype Dk8, like Cuba38, was less affected by location than were the other lines. However, no progeny retained the unique properties of the parent line, similar to Cuba38, which was likely a result of an overall location effect between summer 2001 and summer 2002 in Ames, IA.

Similar to results with the genotypes previously discussed, Dk8 starch tended to have greater T_{oG} and ΔH_G when grown in Ames 2 and Missouri than when grown at Ames 1 and Illinois. Also similar to other results, the Ames 2 farm produced Dk8 with starch having greater T_{oR} and ΔH_R, than did the other farms.

Dk10

Dk10 produced only 1 block in Missouri and 2 blocks in Puerto Rico. All other farms produced 3 full blocks of Dk10. Dk10 was chosen as a line of interest because although it was a sibling of Dk8, its starch had gelatinization properties that were more typical of traditional starches, with a T_{oG} of 65.3°C and a narrow R_G (9.4°C) (Table I). The T_{oG} and R_G of successions produced in the present study were similar to the parent line starch with T_{oG} ranging from 64.3°C to 65.8°C and R_G ranging from 8.6°C to 10.1°C (Table III).

The T_{oG} at Missouri was greater than the T_{oG} at Ames 1 and Illinois. The T_{oG} at Ames 2 was greater than the T_{oG} at Ames 1. The R_G at Ames 1 was greater than the R_G at Illinois. The R_G at Ames 2 and Missouri did not differ from each other. The ΔH_G at Ames 1 was

lower than the ΔH_G at the other 3 locations. The PHI at Illinois and Missouri were greater than the PHI at Ames 1.

The T_{oR} at Ames 2 and Missouri were greater than the T_{oR} at Ames 1 and Illinois. The R_R at Missouri was greater than the R_R at the other 3 farms. The ΔH_R at Ames 2 was greater than the ΔH_R at Ames 1 and Illinois. The %R at Ames 2 was greater than the %R at Illinois. The %R at Ames 1 and Missouri neither differed from each other nor from the %R at the other 2 locations.

Dk10 starch from Puerto Rico had significantly lower R_G and ΔH_G than did starch from Dk10 grown at the Ames 2 location. The magnitude of the differences, however, were less than for other lines suggesting the Dk10 genotype may have been less affected by growing location.

Significant interactions ($p<0.01$) occurred between the Dk10 genotype and location for T_{oG}, ΔH_G, T_{oR}, ΔH_R and %R, and interaction ($p\leq0.05$) was also found for PHI, R_R, and %R. The Dk10 starch from Missouri and Ames 2 had greater T_{oG} and ΔH_G than did starch from corn grown in Illinois and Ames 1 and, similar to other genotypes, was likely a result of differences in temperatures, soil types, and quality. Missouri had the highest growing temperatures, whereas Illinois had the lowest. Ames 1 and Ames 2 differed in soil types and general growing condition qualities. Ames 2 also may have experienced higher growing temperatures as a result of its location near a river bottom. Also, similar to the other lines, Ames 2 produced starch that had greater T_{oR}, R_R, ΔH_R and %R, than did all other locations except for Missouri, which had greater T_{oR}.

Conclusions

Puerto Rico was the warmest location, but could not be directly compared to the lines grown at the other 4 locations because the seed planted in Puerto Rico was actually progeny of the Ames 2 location. Therefore, the corn grown in Puerto Rico was not of the same genetic background as the corn grown in the summer of 2002 in the U.S. Corn Belt. Variations in thermal properties were similar between exotic by adapted lines and control lines. The corn from Puerto Rico generally did not produce results that were consistent, which may be a result of the low level of replication caused by extreme insect infestation. Missouri was the warmest location during the summer of 2002, whereas Illinois was the coolest. The Missouri location generally produced starch that had greater T_{oG}, narrower R_G, and greater ΔH_G, whereas the Illinois location generally resulted in starch that had lower T_{oG}, wider R_G, and lower ΔH_G. These results are relatively consistent with the results of previous studies (White et al 1991, Lu et al 1996, Ng et al 1997) and the DSC parameters have been attributed to the temperature differences either increasing the amount of longer branches of amylopectin or perfecting the amylopectin crystalline structure. Evidence from the other farms, however, suggests that soil type and quality may affect the thermal properties of starch from these inbred lines. The Ames 1 and 2 farms produced starch with consistently different thermal properties. The Ames 2 farm was located in a river bottom, which is generally warmer. This increase in temperature may have caused the results of the Ames 2 farm to be more similar to that of the Missouri location, the warmest of all locations in this study. The significant variations between the Ames farms may have also been a result of differences in soil type and quality. The Ames 1 farm, with poorer quality soil of the Clarion-Webster-Nicollet association, produced starch with thermal properties generally similar to those from

Illinois. The Ames 2 farm, with better quality soil of the Coland-Spillville-Zook association, generally produced starch with thermal properties similar to those of the starch from Missouri. Starch from the Ames 2 farm also consistently had greater T_{oR}, R_R, ΔH_R and %R than did starch from the other 3 farms in the Midwest. These results may be a result of increased numbers of longer amylopectin chains in the Ames 2 corn starches that were able to form a stronger network during retrogradation.

There was evidence of genotype and location interactions. The location effect included factors such as temperature, precipitation, soil type, and growing conditions.

The successions in this study only partly retained the interesting thermal properties present in the parent lines; however, because the lines studied were inbreds there was a chance that the genes were still segregating and the kernels on an ear were not homogenous with respect to genes and genetic modifiers as described earlier. The high level of homogeneity (99%), however, makes this scenario unlikely. Therefore, it is likely that the phenotypic variation of the lines were affected more by environmental and the interaction of environment and genotype variations than by genotypic variations alone. Previously, related lines were successfully developed that retained their unique thermal characteristics with further selfing (Ji et al 2003b). As mentioned earlier, the difference between parent and progeny starch thermal traits was likely a result of temperature, precipitation, and location variations. As a related note, both Cuba lines had excellent agronomic performance in this study. The estimated yields were even higher than that of the control lines, B73 and Mo17, suggesting the use of Cuba lines for agronomic strength in addition to its unusual thermal traits.

Overall, a number of factors can affect the thermal properties of corn starch from a particular growing location, including temperature, precipitation, soil type, and growing conditions. In the current study, the strongest relationships were between temperature and soil type. There also were highly significant interactions between the growing location and genotype therefore complicating the prediction of the effect of a growing location on the thermal properties of corn. Future studies could include controlled growing environments in a greenhouse to further elucidate the effects of temperature, precipitation, and soil type on the thermal properties of starch from these exotic lines. Another study could be conducted on the inheritance of unusual starch traits in inbred lines because the majority of the lines in this study did not inherit the unusual thermal starch trait they were selected for.

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Table I: The gelatinization characteristics and source locations of original parent lines of corn used in the current study

Line[a]	Source Identification[b]	Source Origin	DSC Parameters[c] T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH_G/°C)	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
CHIS775:S1911b-37-1-2-8-7	Chis37	Mexico	59.7	13.5	11.6	1.7	39.7	21.2	6.4	55.1
CUBA164:S1511b-34-1-3-1-11-2	Cuba34	Cuba	60.8	15.2	10.9	1.4	41.6	19.8	5.3	49.2
CUBA164:S1511b-38-1-3-5-13	Cuba38	Cuba	60.6	12.8	12.5	2.0	42.8	18.5	5.8	46.4
DK212T:S0610-8-1-3-4-6-4	Dk8	Thailand	59.4	14.0	11.4	1.6	42.2	18.6	5.6	49.0
DK212T:S0610-10-1-3-6	Dk10	Thailand	65.3	9.4	11.7	2.5	42.9	19.7	5.8	49.5

[a] Exotic inbreds from Germplasm Enhancement of Maize (GEM) project; data produced independently in our laboratory

[b] Abbreviated identification for use within this paper

[c] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2)$]; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_R=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$ x100%)

Table II: Average Monthly Temperatures (°C) and Total Precipitation near Ames, IA (2002), Clinton, IL (2002), Columbia, MO (2002), and Ponce Puerto Rico (2002-2003)

Location	Month	Temperature			Total Precipitation (cm)
		Maximum Mean (°C)	Minimum Mean (°C)	Average Mean (°C)	
Ames[a]					
	May	21.6	8.6	15.1	10.2
	June	29.4	17.3	23.3	8.4
	July	31.1	19.4	25.2	11.7
	August	27.8	16.4	22.1	12.2
	September	26.2	12.7	19.4	3.0
	October	12.9	3.3	8.1	30.7
Illinois[b]					
	May	21.3	8.6	14.9	17.7
	June	29.3	17.5	23.4	9.9
	July	31.4	19.4	25.4	10.3
	August	28.9	17.7	23.3	24.3
	September	27.6	13.3	20.4	4.2
	October	16.5	5.2	10.8	7.2
Missouri[a]					
	May	22.7	10.4	16.6	27.0
	June	30.0	19.0	24.5	8.8
	July	32.6	21.1	26.9	9.9
	August	31.2	20.2	25.7	19.8
	September	28.4	15.9	22.2	4.3
Puerto Rico[a]					
	October	32.8	22.9	27.9	1.8
	November	32.3	22.4	27.4	5.7
	December	31.8	20.8	26.3	2.7
	January	30.9	21.1	26.0	5.2
	February	30.9	20.7	25.8	5.0
	March	31.3	20.9	26.1	6.8

[a] National Climatic Data Center (www.ncdc.noaa.gov: Asheville, NC.)

[b] Central Golden Harvest Research (Clinton, IL)

Table III: Thermal and retrogradation properties of 5 maize lines[a] grown at 5 locations[b]

		DSC Parameters[c]							
Line	Location	T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
B73	Ames 1	65.8ab[d]	8.4abc	12.6b	3.0abc	41.9a	19.9b	5.6bc	45.0ab
	Ames 2	66.2b	9.0b	13.3a	3.0b	42.1a	21.7a	6.9a	50.6a
	Illinois	63.7c	10.2a	12.6b	2.5c	41.0a	20.3b	5.7c	44.5b
	Missouri	67.2a	7.7c	13.2a	3.4a	41.0a	21.1ab	6.1b	46.3b
	Puerto Rico	66.0[e]	7.8	13.2	3.4*	41.0	20.5	6.4*	49.3
Mo17	Ames 1	67.7b	8.5b	12.9c	3.1b	41.4b	20.5b	6.5b	50.1a
	Ames 2	69.0a	8.7b	14.0a	3.2ab	42.6a	21.9a	7.3a	53.0a
	Illinois	65.4c	10.5a	13.5b	2.6c	40.7b	20.9ab	6.5b	49.4a
	Missouri	68.7ab	8.3b	14.0a	3.4a	41.3b	21.0ab	6.3b	44.6b
Chis37	Ames 1	64.3bc	9.5b	12.3b	2.6ab	40.9ab	20.6ab	6.2b	52.5a
	Ames 2	66.0ab	9.9b	13.3a	2.7a	42.3a	21.8a	7.1a	51.9a
	Illinois	62.8c	11.5a	12.4b	2.2b	40.7b	20.1b	5.3c	42.9b
	Missouri	66.8a	9.2b	13.1a	2.9a	42.0a	20.0b	6.0b	46.2ab
	Puerto Rico	66.2	8.7	12.6*	2.9	40.8*	20.5	6.3*	50.0

[a] Exotic corn inbreds obtained from the Genetic Enhancement of Maize (GEM) project: B73, Mo17, CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4 (Dk8),DK212T:S0610-10-1-3-6 (Dk10)

[b] Ames 1, Ames 2, (each 9km apart), Clinton, IL, Columbia, MO, and Ponce, Puerto Rico

[c] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2)$]; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_G=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$x100%)

[d] Means followed by different letters (a-d) are significantly different ($p<0.05$) within one set of locations within a line within a DSC parameter

[e] Puerto Rico means followed by a * are significantly different ($p<0.05$) from Ames 2 means within a location within a DSC parameter

Table III: (continued)

		DSC Parameters[c]							
Line	Location	T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
Cuba34	Ames 1	61.6c	14.4a	11.7b	1.6c	40.9b	20.0a	5.1b	42.5b
	Ames 2	64.4b	11.3c	12.5a	2.2b	42.2a	21.0a	6.2a	49.2a
	Illinois	62.2c	13.1b	11.8b	1.8c	40.8b	20.2a	5.3b	44.2bc
	Missouri	65.9a	10.1d	12.6a	2.5a	41.3ab	20.5a	5.9a	47.5ac
	Puerto Rico	63.2*	13.0*	11.9*	1.8*	40.8*	20.3	6.0	50.8
Cuba38	Ames 1	65.9a	9.1a	12.5b	2.8a	41.1b	20.6a	5.8bc	48.3b
	Ames 2	65.8a	9.3a	12.9a	2.8a	42.4a	21.2a	6.8a	52.9a
	Illinois	64.5b	9.0a	12.6ab	2.8a	40.5b	20.8a	5.6c	45.4b
	Missouri	66.0a	9.4a	12.9a	2.8a	41.5ab	20.3a	6.0b	47.1b
	Puerto Rico	64.8	9.3	12.6	2.7	40.9*	20.4	6.5	52.8
Dk8	Ames 1	63.3c	10.3a	12.0b	2.4b	40.9b	20.2a	5.6c	46.3b
	Ames 2	64.7ab	10.6a	13.1a	2.5ab	42.8a	21.0a	6.6a	50.4a
	Illinois	64.2bc	9.9a	12.1b	2.5ab	40.5b	20.4a	5.7c	47.5ab
	Missouri	65.7a	9.7a	13.3a	2.8a	41.2b	20.8a	6.2b	46.7a
	Puerto Rico	64.7	10.1	13.0	2.6	40.8*	20.5	6.5	50.0
Dk10	Ames 1	64.3c	10.1a	12.4b	2.5b	40.7b	20.9a	6.1b	49.2ab
	Ames 2	65.7ab	9.7ab	13.2a	2.7ab	42.2a	21.6a	6.8a	52.1a
	Illinois	64.7bc	8.8b	12.8a	2.9a	40.6b	20.9a	6.0b	47.1b
	Missouri	66.5a	9.2ab	13.3a	3.0a	43.5a	19.0b	6.4ab	48.8ab
	Puerto Rico	65.8	8.6*	12.7*	3.0	41.0	20.6	6.7	53.4

Table IV: Significance of genotype (line) source[a] by location[b] interaction effects on each line from analysis of variance of starch gelatinization properties by DSC

	DSC Parameters[c]							
Line	T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
B73	**	**	**	**	NS	NS	**	*
Mo17	**	**	**	**	**	NS	**	**
Chis37	**	**	**	**	*	*	**	**
Cuba34	**	**	**	**	*	NS	**	**
Cuba38	*	NS	NS	NS	**	NS	**	**
Dk8	**	NS	**	NS	**	NS	**	NS
Dk10	**	NS	**	*	**	*	**	*

[a] B73, Mo17, CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4 (Dk8), DK212T:S0610-10-1-3-6 (Dk10)

[b] Ames 1, Ames 2, (each 9km apart), Clinton, IL, Columbia, MO, and Ponce, Puerto Rico

[c] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2)$]; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_G=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$x100%)

[f] *, ** indicates significance at $p<0.05$ and $p<0.01$, respectively; NS = no significance

Chapter 5. Thermal properties of starch from exotic corn (*Zea mays* L.) lines during kernel development at two locations

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Abstract

The changes in thermal properties of corn starches during five stages of endosperm development [12, 18, 24, 30, and 36 days after pollination (DAP)] from 3 exotic corn lines (Chis37, Dk8, and Dk10) and two control lines (B73 and Mo17) at two locations in Ames, IA were studied by using differential scanning calorimetry (DSC). Chis37 and Mo17 were not included in final analyses because of poor agronomic performance. The onset gelatinization temperature (T_{oG}) tended to decrease during maturation of the kernel, whereas enthalpy of gelatinization (ΔH_G) tended to not to change. The range of gelatinization (R_G) of Dk10 increased during maturation, whereas the R_G of B73 and Dk8 were variable. Retrogradation parameters did not vary greatly among DAP and between locations. Differences between DAP samples within the same genotype were likely a result of variations in starch structure during maturation. Starch from Dk8 varied the most with location, especially with respect to

T_{oG} and R_G, whereas starch from Dk10 was less affected by location. The locations in the present study, however, were only about 9 km apart, therefore weather patterns were very similar. These differences between DSC parameters were likely a result of differences in soil type and quality.

Introduction

The exact path of starch synthesis in corn is not entirely clear and much research is presently being conducted concerning the subject. Generally, the substrate for starch is ADP glucose, which is synthesized by ADPglucose pyrophosphorylase from sucrose (Smith et al 1997). The nature and location of the enzyme varies within and between organs and species. Starch synthase (SS) forms α-D-1->4 linkages, whereas starch branching enzyme (BE) catalyzes the formation of α-D-1->6 branch points. There are a number of isoforms for each enzyme, but the purposes of all these forms are still not clear.

At about 8 to 10 days after pollination (DAP), cells in the central crown of a corn kernel begin to accumulate starch first; the lower endosperm cells begin starch synthesis much later (Boyer et al 1977). At about 12 DAP, sugars are relatively high in concentration and starch concentration is low. As the amount of cells accumulating starch increases, kernel sugar content decreases.

In a study by Wolf et al (1948), moisture content of corn kernels decreased from about 87% at 12 to 13 DAP to 9 to 11% at maturity. At the same time, starch content increased. The rise in starch content was most rapid between 12 and 20 DAP. Dent corn starch also increased in granule diameter rapidly during this time. The most rapid changes in starch properties occurred during the first 35 DAP.

Brown et al (1971) studied the development of corn mutants and reported that at 12 DAP the crystalline organization of the mutant and normal maize starches were similar. The differentiation from the normal starch occurred over the period of 12 to 24 DAP, indicating that mutant genes modified granule properties over this period.

During growth, apparent amylose percentage increases and molecular size of amylose and amylopectin increases (Banks and Greenwood 1975). Inouchi et al (1983) found that at three stages of growth (21, 28, and 35 DAP) certain maize starches had increased amylose contents over time. They also reported that in the starch of normal, *amylose-extender* (*ae*), *sugary-1* (*su1*), and *sugary-2* (*su2*) maize, the amylopectin content decreased from 11 to 20 DAP. Boyer et al (1976) also reported that apparent amylose percentage increased during growth in the starch of normal and mutant (*ae*, *ae su*) maize. Overall, the changes in fine structure in the starch are dependent upon genetic background and source of the starch.

Analyses have been performed on the differential scanning calorimetry (DSC) properties of corn starch during development (Biliaderis 1982, Inouchi et al 1984, Ng et al 1997). Ng et al (1997) studied the thermal properties of corn starch from *ae*, *su-2*, and *waxy* (*wx*) mutants during development, sampling at 12, 18, 24, 30, and 36 DAP. They reported that within a genotype, DSC values of starches at 24, 30, and 36 DAP were similar to each other, but often were significantly different from the values at 12 DAP. They postulated that this difference indicated that changes in the fine structure of starch occurred during endosperm development.

Exotic and exotic by adapted materials have been reported to be highly variable in thermal traits, suggesting the use of these lines in further breeding to develop lines and varieties with unusual traits (Li et al 1994, Campbell et al 1995, Pollak and White 1997,

Singh et al 2001). The Germplasm Enhancement of Maize (GEM) project has identified and developed exotic lines, partly from germplasm foreign to corn races grown in the United States, that may be useful for agronomic, nutritional, and/or industrial reasons (Pollak 2002). Our laboratory routinely screens corn sources for starch traits that may be useful to the starch industry, such as low onset of gelatinization (T_{oG}) or percent of retrogradation (%R), and other criteria as described by Seetharaman et al (2001). Ji et al (2003a) identified unusual exotic corn inbred lines that exhibit properties such as low T_{oG} and wide range of gelatinization (R_G). Some lines also exhibited gelatinization thermogram shapes with shoulders or double peaks demonstrating two independent transitions. Distinctive lines are then further developed by inbreeding to increase the inheritability of the traits. Lines that naturally possess unique properties are potentially available to industry as alternatives to chemically or physically modified starches.

It is of interest to determine whether or not development of thermal properties of corn starch from exotic germplasm is similar to adapted corn lines. Exotic by adapted lines of interest (Table 1) were self-pollinated at two farms in Ames, IA. Samples from each line were obtained at 12, 18, 24, 30, and 36 DAP and starch was extracted from the samples. DSC was used to evaluate the difference in thermal properties of starch from different DAP of exotic corn lines and also at different locations.

Materials and Methods

Materials

Corn (*Zea mays* L.) exotic by adapted inbreds developed from GEM breeding crosses were used, along with public inbred lines B73 and Mo17 as controls. B73 has a Stiff Stalk heterotic pattern, whereas Mo17 has a non-Stiff Stalk heterotic pattern. The exotic inbred

lines were developed by crossing exotic populations (DK212T is a tropical 3-way commercial hybrid developed by Dekalb Genetics in Thailand) with inbreds of the Stiff Stalk heterotic pattern. These crosses were further developed by inbreeding through self-pollination (Pollak 2002). Three inbred generations [CHIS775:S1911b-37-1-2-8-7 (Chis37), DK212T:S0610-8-1-3-4-6-4, (Dk8), DK212T:S0610-10-1-3-6 (Dk10)] were chosen because data generated by our laboratory indicated that these lines possessed desirable thermal properties as detected by DSC. Unusual desirable thermal properties were previously defined by Seetharaman (2001). Examples included T_{oG} less than 61°C, ΔH_G less than 9.5 J/g or greater than 14.5 J/g, and %R less than 20% or greater than 80%. The selected lines were related to a study by Ji et al (2003a) produced in our laboratory. Lines in the previous study were sister lines or direct parent lines of the genotypes selected for this study. Dk8 and Dk10 were of the same exotic origin, but derive from different self-pollinations (S_1 lines) of the breeding cross. Both the T_{oG} and R_G of the Dk8 and Dk10 parent lines differ. The data on which the lines were selected on have not been published, but have been produced independently in our laboratory (Table I).

Kernels were grown in a randomized complete block design in Ames, IA during the summer of 2002 at 2 farms, approximately 9 km apart, with rows serving as experimental units and a total of 3 blocks at each farm. The first farm, North Central Regional Plant Introduction Farm, referred to as Ames 1, had soils that are a Clarion-Webster-Nicollet association, which are formed in loamy glacial till and glacial sediments, are moderately drained and permeable and are found on uplands. The second farm, the Iowa State University Hinds Farm, referred to as Ames 2, had soils identified as Coland-Spillville-Zook association

types. These soils are nearly level, moderately well drained to poorly drained, have loamy and silty soils formed in alluvium and are found on bottom lands.

The materials were harvested at five different stages of maturity: 12, 18, 24, 30, and 36 DAP. Kernels were removed immediately after harvesting and stored below 0°C before starch extraction and analysis. Chis37 did not perform well agronomically because of late silk dates and, therefore, was removed from statistical analysis. Mo17 also did not perform well agronomically and was removed from statistical analysis. No sampling was complete because of a combination of poor growing conditions at the Ames 1 farm and beetles at both farms.

Starch Extraction

Corn starch was extracted as bulked 2-g samples from each ear, according to the method of White et al (1990) with modifications by Krieger et al (1997) and Ji et al (2003b). Each ear was extracted in duplicate with replicates analyzed separately.

Differential Scanning Calorimetry

Gelatinization characteristics of starch samples, such as onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH), were determined by using Differential Scanning Calorimetry (DSC) (Perkin Elmer DSC 7, Norwalk, CT) equipped with thermal analysis software (Perkin Elmer Corp., Norwalk, CT).

Starch (3.50 mg, with 10% assumed moisture content) was weighed into an aluminum pan on the same balance (Mettler AE 104, Toledo, OH). Water was added to the starch sample in a water to starch ratio of 2:1; the sample was hermetically sealed and allowed to equilibrate for at least 30 min before DSC analysis. All samples were analyzed by DSC from 30°C to 110°C at a rate of 10°C per minute. Data parameters collected from the computer

included T_o, T_p, T_c, and ΔH. The enthalpy was calculated on a starch dry-weight basis. Also calculated were the range of gelatinization (R_G) (T_{cG} minus T_{oG}) and peak height index (PHI) (ΔH_G (dry basis)/(1/2xR_G)). A subscript "G" after a parameter denotes a gelatinization property. Samples were stored at 4°C for 7 days to study the retrogradation characteristics. Stored samples were analyzed by DSC from 30°C to 90°C at a rate of 10°C per minute. The same parameters for gelatinization were calculated for retrogradation and are denoted by a subscript "R" after the parameter. Percentage of retrogradation (%R) was calculated from the ratio of ΔH_R divided by ΔH_G.

As mentioned earlier, sampling was not complete due to poor growing conditions. Dk8 had only two complete DSC analyses out of a possible total of 24 (6 blocks, 2 ears per row, 2 DSC replicates per ear) for starch from 12 DAP. All other sampling was complete for Dk8. Dk10 did not have complete DSC analyses for 12 DAP (missing 6), 18 DAP (missing 4), 24 DAP (missing 8), and 36 DAP (missing 4). B73 also did not have complete DSC analyses for 12 DAP (missing 8), 18 DAP (missing 10), 24 DAP (missing 2), 30 DAP (missing 6), and 36 DAP (missing 4).

Statistical Analysis

The effect of DAP and location and their interactions on the thermal properties of starch of inbreds from 2002 was analyzed by using an analysis of variance procedure for a mixed model. Fixed factors were DAP, farm, line, DAP*farm, DAP*line, farm*line, and DAP*farm*line. Random effects were included for ear, row, block, extraction, and DSC. Contrast statements were used to determine significant differences ($p<0.05$) between DAP's within the same line and location and between locations within the same DAP.

Results and Discussion

The DSC properties of starches of B73, Dk8, and Dk10 at five different stages of maturity and two locations are shown in Table II.

B73

The T_{oG} of B73 starch at the Ames 1 and the Ames 2 farm decreased significantly from 12 DAP to 36 DAP (Table II). At Ames 2, however, the T_{oG} at 18 DAP was lower than all other DAP samples. The decline in T_{oG} may be a result of enzyme degradation of starch during maturation. R_G did not show a definite pattern between DAP at both farms. At the Ames 1 farm, the R_G at18 DAP was wider than the R_G at 12, 30, and 36 DAP. There was no significant difference between the R_G at 12 DAP and 24, 30, and 36 DAP indicating the R_G of B73 was variable throughout the grain-filling period. At the Ames 2 farm, the R_G of starch from 18, 24, and 30 DAP were wider than the R_G at 36 DAP. The R_G at 12 DAP did not differ significantly from the R_G at other DAP's. There was no difference in ΔH_G between samples at different DAP at the Ames 1 farm. At the Ames 2 farm, the ΔH_G at 18 DAP was greater than the ΔH_G at 12 and 36 DAP. At the Ames 1 farm, the PHI at 12 and 30 DAP was greater than the PHI at 18 DAP. The PHI did not differ between DAP samples at the Ames 2 farm.

At the Ames 1 farm, there were no differences between DAP samples with respect to retrogradation parameters. At the Ames 2 farm, however, T_{oR} and R_R differed. The T_{oR} at 12 DAP was greater than the T_{oR} at 24 and 36 DAP. The R_R at 24 DAP was greater that the R_R at 12 and 18 DAP.

There were a few differences between farms at the same DAP of B73 starch for the same DSC parameter (Table II). The T_{oG} at Ames 1 at 18 DAP was greater than the T_{oG} at

18 DAP at Ames 2. The R_G at Ames 2 at 30 DAP was wider than the R_G at Ames 1 at 30 DAP. Similarly, the PHI at Ames 1 at 30 DAP was greater than the PHI at Ames 2 at 30 DAP. The sole difference between the retrogradation parameters of B73 was at 24 DAP for R_R.

Overall for B73 starch, there were detectable differences between DAP samples for DSC parameters, especially with respect to T_{oG}. The T_{oG} at 18 DAP from the Ames 2 farm was especially low, and may be a result of the lack of replication for 18 DAP at Ames 2 because out of a possible 12 DSC analyses, only 2 were obtained.

Dk8

Dk8 was chosen as a line of interest because its starch has an unusually low T_{oG} (59.4°C) (Table I). However, the 36 DAP thermal analyses from both farms did not exhibit this unusual property. In an additional study by the present authors, similar results occurred with fully mature kernels from the same source, location, and year. Therefore, at 36 DAP the starch was fully mature as suggested by Wolf et al (1948), but likely because of genetic segregation and/or location differences between years, the unusual thermal properties were not retained.

At the Ames 1 farm, the T_{oG} at 12, 18, 24, and 30 DAP were greater than the T_{oG} at 36 DAP (Table II). Similar to B73, this decline may be a result of starch degradation by enzymes. At the Ames 2 farm, the T_{oG} at 18 DAP was greater than the T_{oG} at 24 and 30 DAP. The R_G was variable at both farms. At the Ames 1 farm, R_G at 18, 24, and 36 DAP were wider than the R_G at 30 DAP. At the Ames 2 farm, R_G at 24 DAP was wider than all other DAP samples. The R_G at 30 DAP was second widest and followed by 36 DAP. The R_G at 12 and 18 DAP were the most narrow. The ΔH_G at Ames 1 farm at 18 and 24 DAP

were greater than the ΔH_G at 12 and 36 DAP. At the Ames 2 farm, ΔH_G at 12 DAP was lower than the ΔH_G at 18, 24, 30, and 36 DAP. There were no differences among higher DAP. At the Ames 1 farm, the PHI was greater at 30 DAP than the PHI at 36 DAP. The PHI at 12, 18, and 24 DAP were not different neither from each other nor from PHI at 30 and 36 DAP. At the Ames 2 farm, the PHI at 18 DAP was greater than the PHI at all other DAP. The PHI at DAP 36 was greater than the PHI at 24 DAP.

The T_{oR} at 12 DAP at Ames 1 and Ames 2 was greater than the T_{oR} at 18, 24, 30, and 36 DAP. At the Ames 1 farm, the R_R at 18 DAP was greater than the R_R at 12 and 24 DAP. The R_R at 12 DAP at both farms was lower than the other DAP. The ΔH_R at Ames 1 at18 DAP was greater than the ΔH_R at 12 and 36 DAP. The ΔH_R at 12 DAP was lower than the ΔH_R at 18, 24, and 30 DAP. The ΔH_R at 12 DAP at Ames 2 was lower than the ΔH_R at other DAP. The %R did not differ between DAP at Ames 1 farm. At the Ames 2 farm, the %R at 12 DAP was lower than the %R at other DAP.

Between the two farms, the T_{oG} at Ames 2 for Dk8 starch at 12, 18, 24, and 30 was greater than the T_{oG} at Ames 1 for the same DAP (Table II). The R_G at 24 and 30 DAP at Ames 1 was greater than the R_G at 24 and 30 DAP and Ames 2. The ΔH_G at 12 DAP at Ames 1 was greater than the ΔH_G at 12 DAP at Ames 2. Similar to R_G, the PHI at 24 and 30 DAP from Ames 1 was greater than the PHI at 24 and 30 DAP from Ames 2. The differences between farms with respect to retrogradation was between 12 DAP samples. The T_{oR} at 12 DAP from Ames 1 was greater than the T_{oR} at 12 DAP from Ames 2. The ΔH_R at Ames 2 at 12 DAP was greater than the ΔH_R at Ames 1 for 12 DAP.

Overall, for Dk8 starch, there were measurable differences between different DAP samples, but no definite patterns were visible. A factor affecting the results may be that at 12 DAP for both farms, there was a low level of replication.

Dk10

Dk10 was chosen as a line of interest because its starch had a narrow R_G (9.4°C) (Table I). The R_G at 36 DAP at both locations were close to this value, 9.3°C at Ames 1 and 8.9°C at Ames 2.

During maturation of the Dk10 kernel, the T_{oG} of the starch tended to decrease (Table II). For example, at both farms, the T_{oG} at 12 DAP were greater than the T_{oG} at other DAP. Specifically, the T_{oG} at 18 DAP was greater than the T_{oG} at 30 and 36 DAP at both farms and the T_{oG} at 36 DAP was lower than the T_{oG} at other DAP for Ames 1. At the Ames 2 location, however, the T_{oG} at 36 DAP was lower than 12, 18, and 24 DAP. Similarly, this decline may be caused by the degradation of starch necessary for maturation of the kernel. The R_G at both farms tended to widen during maturation of the kernel. At Ames 1, the R_G at 36 DAP was wider than the R_G at other DAP. At Ames 2, the R_G at 36 DAP was wider than R_G at 12, 18, and 24 DAP. Also at Ames 2, the R_G at 12 DAP was narrower than R_G at other DAP. The ΔH_G at both farms did not differ between DAP. At the Ames 1 farm, the PHI at 12, 18, and 24 DAP was greater than the PHI at 30 and 36 DAP. The PHI at 30 DAP was greater than the PHI at 36 DAP. At the Ames 2 location, the PHI at 12 DAP was greater than the PHI at other DAP. At 18 and 24 DAP, the PHI was greater than the PHI at 30 and 36 DAP. There were no differences between DAP at both farms with respect to retrogradation parameters.

Between farms, the Dk8 starch differed little within the same DAP (Table II). The only difference found was for ΔH_G at 18 DAP. The Ames 2 ΔH_G at 18 DAP was greater than that for Ames 1.

Significant Interactions

Significant interactions were found between the fixed factors for all DSC parameters (Table III). Significant differences ($p<0.01$) occurred for T_{oG} for farm, line, and DAP. The results were also affected by the interactions of DAP and farm ($p<0.05$), line and DAP ($p<0.01$), and farm, line, and DAP ($p<0.05$). Specifically, the T_{oG} of B73 and Dk8 were affected by location and the T_{oG} of all lines were affected by DAP. The T_{oG} of all genotypes were also affected by the interaction of farm and DAP.

Highly significant differences ($p<0.01$) were found for line and DAP for R_G. Interactions also were found between line and farm ($p<0.05$), DAP and farm ($p<0.01$), line and DAP ($p<0.01$), and farm, line, and DAP ($p<0.01$). The R_G of Dk8 was affected by farm location, whereas the R_G of all lines were affected by DAP. The R_G of all lines were also influenced by the interaction of DAP and farm.

There were fewer significant differences and interactions for ΔH_G than for other gelatinization parameters. The ΔH_G differed among DAP ($p<0.01$). Interactions also occurred between DAP and farm ($p<0.05$). Specifically, the ΔH_G of Dk8 was influenced by DAP and the interaction of farm and DAP.

PHI differed between line ($p<0.01$) and DAP ($p<0.01$). The results also were affected by the interactions of DAP and farm ($p<0.05$) and line and DAP ($p<0.01$). Specifically, the PHI of Dk8 was affected by farm location, whereas the PHI of Dk8 and Dk10 were

influenced by DAP. The PHI of Dk8 and Dk10 also were influenced by the interaction of farm and DAP.

The T_{oR}, R_R, and ΔH_R each differed for line ($p<0.01$) and DAP ($p<0.01$). Significant interactions for each were found between line and DAP ($p<0.01$). The T_{oR} of B73 and Dk8 were influenced by DAP. The T_{oR} of Dk8 also was influenced by the interaction of DAP and farm. The R_R of Dk8 was affected by location of farm. The R_R of all three lines were influenced by DAP and also the interaction of farm and DAP. The ΔH_R of Dk8 was influenced by DAP and also the interaction of location and DAP.

The %R differed between line ($p<0.01$) and DAP ($p<0.05$). No significant interactions were observed for %R.

Conclusions

The influences on DSC properties of corn starch varied with the stages of endosperm development, location, genetic background, and interactions of these factors. Ng et al (1997) found that starches varied with stages of corn endosperm development, and with genetic background. Ji et al (2003c) found that the starch exotic inbreds varied with location, genotype, and interactions of these factors. The T_{oG} tended to decrease with maturity of the kernel for most samples. The decrease in T_{oG} during maturation may be a result of enzyme degradation of the starch. The ΔH_G tended not to change during maturity. Ng et al (1997) found that the ΔH_G did not decrease during the development of normal, *ae*, *su2*, and *wx* starches and the T_{oG} of *su2* starch was higher at 12 DAP than other DAP samples. Less noticeable differences were detected for retrogradation parameters. Overall, the differences between DAP suggested variations in the fine structure of starch during the endosperm development, as also suggested by Ng et al (1997a). Environmental interactions depended

on the genotype of the starch source. For example, starch from Dk8 varied the most with location, especially with respect to T_{oG} and R_G. Starch from Dk10 was less affected by location. Ng et al (1997b) found that exotic corn lines grown in a hotter environment had greater T_{oG} and narrower R_G, among other DSC parameters, than the same lines grown in a cooler environment, which may a result of perfection of the amylopectin crystals. The locations in the present study, however, were only about 9 km apart, therefore essentially experiencing identical weather patterns. The differences between farms then may be a result of differences in soil types and general quality of the farms. Future studies may include controlled growing environments in a greenhouse, providing more stabilized temperatures, rainfall, and soil types to give further insight into the effect of temperature and precipitation on maturity of the endosperm of these new exotic inbreds.

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Table I: The gelatinization characteristics and source locations of original parent lines of corn used in the current study

Line[a]	Source Identification[b]	Source Origin	DSC Parameters[c] T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI (ΔH_G/°C)	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
CHIS775:S1911b-37-1-2-8-7	Chis37	Mexico	59.7	13.5	11.6	1.7	39.7	21.2	6.4	55.1
DK212T:S0610-8-1-3-4-6-4	Dk8	Thailand	59.4	14.0	11.4	1.6	42.2	18.6	5.6	49.0
DK212T:S0610-10-1-3-6	Dk10	Thailand	65.3	9.4	11.7	2.5	42.9	19.7	5.8	49.5

[a] Exotic inbreds from Germplasm Enhancement of Maize (GEM) project; data produced independently in our laboratory

[b] Abbreviated identification for use within this paper

[c] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2)$]; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_R=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$ x100%)

Table II: Mean Differential Scanning Calorimetry (DSC) parameters for three maize genotypes[a] at five stages of kernel maturity[b] at two different locations[c]

			DSC Parameters[d]							
Genotype	Farm	DAP	T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
B73	Ames 1	12	68.2a y[e,f]	8.1bc y	13.2a y	3.3a y	41.6a y	21.3a y	7.1a y	51.9a y
		18	66.9b y	9.0a y	13.1a y	2.9b y	40.8a y	21.6a y	7.5a y	57.5a y
		24	65.5c y	8.6ab y	13.0a y	3.0ab y	40.7a y	20.0a z	7.3a y	56.5a y
		30	66.6bc y	7.7c z	12.9a y	3.4a y	40.6a y	21.4a y	7.3a y	56.3a y
		36	65.4c y	8.5bc y	13.3a y	3.1ab y	40.4a y	22.2a y	7.4a y	57.5a y
B73	Ames 2	12	67.4a y	8.3ab y	12.4b y	3.0a y	41.9a y	20.0b y	6.5a y	49.7a y
		18	63.2c z	9.5a y	14.2a y	3.0a y	42.2ab y	19.6b y	7.3a y	50.0a y
		24	65.8b y	8.6a y	13.1ab y	3.1a y	39.8b y	22.6a y	7.3a y	55.8a y
		30	64.9b z	8.9a y	13.1ab y	3.0a z	40.7ab y	21.6ab y	7.2a y	55.0a y
		36	65.6b y	7.7b y	12.7b y	3.3a y	40.4b y	21.5ab y	6.9a y	54.2a y

[a] B73, DK212T:S0610-8-1-3-4-6-4 (Dk8), and DK212T:S0610-10-1-3-6 (Dk10)

[b] Values at each day after pollination (DAP) are the means of 2 differential scanning calorimetry (DSC) replicates from each of 2 extraction replicates from 2 separate ears, for a total of 12 DSC runs.

[c] Ames, IA; each farm was about 9 km apart

[d] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2)$]; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_G=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$*100%)

[e] Means followed by different letters (a-d) are significantly different ($p<0.05$) within one set of DAP within a genotype

[f] Means followed by different letters (y-z) are significantly different ($p<0.05$) between different locations within a DAP and a genotype

Table II: (continued)

			DSC Parameters							
Genotype	Farm	DAP	T_{oG} (°C)	R_G (°C)	ΔH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	ΔH_R (J/g)	%R
Dk8	Ames 1	12	67.2a y	8.3ab y	12.7ab y	3.0ab y	49.0a y	13.9c z	5.2c y	40.0a y
		18	66.7a y	9.0a y	13.4a y	3.0ab y	39.9b y	22.2a y	7.4a y	50.0a y
		24	66.1a y	8.8a z	13.5a y	3.1ab y	40.8b y	20.2b y	7.0ab y	47.5a y
		30	66.0a y	8.0b z	13.0ab y	3.3a y	40.6b y	21.2ab y	6.8ab y	49.1a y
		36	64.5b y	8.9a y	12.6b y	2.9b y	40.1b y	21.7ab y	6.4bc y	45.0a y
Dk8	Ames 2	12	64.9ab z	7.7d y	10.5b z	2.7bc y	43.7a z	17.6b y	4.1b y	35.0b y
		18	65.5a z	8.3d y	13.3a y	3.2a y	40.4b y	21.2a y	7.0a y	47.5a y
		24	64.2b z	11.4a y	13.2a y	2.3c z	40.3b y	20.0a y	6.6a y	45.0a y
		30	64.4b z	10.2b y	13.0a y	2.5bc z	40.6b y	21.7a y	7.0a y	50.0a y
		36	64.7ab y	9.2c y	12.9a y	2.7b y	40.9b y	21.1a y	6.9a y	48.3a y
Dk10	Ames 1	12	69.6a y	7.2b y	13.3a y	3.7a y	40.5a y	22.1a y	7.5a y	54.6a y
		18	67.7b y	7.4b y	13.5a y	3.7a y	40.9a y	21.6a y	7.2a y	51.2a z
		24	66.9bc y	7.3b y	13.0a y	3.6ac y	39.9a y	22.0a y	7.1a y	55.8a y
		30	66.2c y	8.0b y	12.9a y	3.2b y	40.9a y	21.3a y	7.4a y	55.8a y
		36	64.8d y	9.3a y	13.0a y	2.8c y	40.3a y	22.2a y	7.3a y	55.8a y
Dk10	Ames 2	12	69.4a y	6.3c y	12.7a y	4.1a y	40.5a y	21.5a y	7.6a y	59.1a y
		18	67.1b y	7.3b y	12.6a z	3.5b y	40.4a y	21.7a y	7.4a y	59.2a y
		24	66.0bc y	7.4b y	13.0a y	3.5b y	40.5a y	21.5a y	7.6a y	58.5a y
		30	65.4cd y	8.6a y	12.9a y	3.0c y	40.4a y	21.8a y	7.0a y	56.7a y
		36	64.4d y	8.9a y	12.7a y	2.9c y	40.4a y	21.3a y	7.0a y	55.0a y

Table III: Significance of genotype source (line)[a], days after pollination (DAP)[b], and location (farm)[c] effects and their interactions from analysis of variance of starch gelatinization properties by DSC

		DSC Parameters[e]							
Source of variation	df[d]	T_{oG} (°C)	R_G (°C)	DH_G (J/g)	PHI	T_{oR} (°C)	R_R (°C)	DH_R (J/g)	%R
Farm	1	**[f]	NS	NS	NS	NS	NS	NS	NS
Line	2	**	**	NS	**	**	**	**	**
DAP	4	**	**	**	**	**	**	**	*
line*farm	2	NS	*	NS	NS	NS	NS	NS	NS
DAP*farm	4	*	**	*	*	NS	NS	NS	NS
line*DAP	8	**	**	NS	**	**	**	**	NS
line*DAP*Farm	8	*	**	NS	NS	NS	NS	NS	NS

[a] B73, DK212T:S0610-8-1-3-4-6-4 (Dk8), and DK212T:S0610-10-1-3-6 (Dk10)

[b] 12, 18, 24, 30, and 36 days after pollination (DAP); Values at each DAP are the means of 2 differential scanning calorimetry (DSC) replicates of 2 extraction replicates from 2 separate ears

[c] Ames, IA; each farm was about 9 km apart

[d] df = degrees of freedom

[e] T_{oG}=gelatinization onset temperature; R_G=range of gelatinization temperature; ΔH_G=enthalpy of gelatinization; PHI=peak height index [$\Delta H_G/(R_G/2]$; T_{oR}=retrogradation onset temperature; R_R=range of retrogradation; ΔH_G=enthalpy of retrogradation; %R=percent of retrogradation ($\Delta H_R/\Delta H_G$*100%)

[f] *, ** indicates significance at $p<0.05$ and $p<0.01$, respectively; NS = no significance

Chapter 6. General Conclusions

The overall objective of this research was to further characterize exotic corn inbred lines that had been previously researched. The first paper researched a rapid method for detection of unusual thermal properties of starch from new corn sources by using differential scanning calorimetry (DSC). Utilization of a bulked-kernel extraction method was shown to allow detection of unique thermal properties, especially low onset gelatinization temperature (T_{oG}). This method can be used to expedite kernel selection process, which previously utilized a time-consuming single-kernel method.

The second paper evaluated the impact of growing location on the thermal properties of starch from exotic corn inbreds by using DSC. Missouri was the warmest location and generally produced starch with greater T_{oG}, narrower range of gelatinization (R_G), and greater enthalpy of gelatinization (ΔH_G). Illinois was the coolest location and generally resulted in starch with lower T_{oG}, wider R_G, and lower ΔH_G. These differences were attributed to higher temperatures in Missouri either increasing the amount of longer branches of amylopectin or perfecting amylopectin crystalline structure. The Ames 1 farm produced starch with thermal properties generally similar to those of Illinois, whereas the Ames 2 farm produced starch with thermal properties similar to those of Missouri. The temperature at Ames 2 may have been warmer as a result of its location near a river bottom. However, soil type and quality were also different between the two Ames locations and may have been a cause of the differences. The successions generally did not exhibit the interesting thermal properties of the parent lines, which was attributed to environemtal variations rather than genotypic variations, because of the high level of homogeneity (99%) of the inbreds.

The third paper studied the development of thermal properties of starch from exotic corn inbreds at two locations in Ames, IA during kernel development by sampling at 12, 18, 24, 30, and 36 days after pollination (DAP) and by using DSC. The T_{oG} tended to decrease during maturation of the kernel, whereas ΔH_G tended to not to change. The decrease in T_{oG} may have been caused by enzyme degradation of the starch during maturation. The R_G differed among DAP depending on the genotype. Retrogradation parameters did not vary greatly among DAP and between locations. Differences between DAP samples within the same genotype were likely a result of variations in starch structure during maturation. Thermal properties varied with location, but this variation depended on the genotype. The locations in the present study, however, were only about 9 km apart, therefore weather patterns were very similar. These differences between DSC parameters were likely a result of differences in soil type and quality. Significant interactions ($p<0.01$, $p<0.05$) were found between genotype, location, and DAP.

Overall, these studies further elucidated the knowledge of the thermal traits of starch from exotic corn inbreds. The environmental impact depended not only on growing temperature, but also on precipitation, soil type, farm quality, and interactions of these factors. Significant interactions were found between genotype and growing location for several DSC factors, meaning the parameter could change depending on the growing environment of a certain genotype. Significant interactions were also found in starch from developing kernels between genotype, location, and DAP. Collectively, these studies show that the thermal traits of starch from exotic corn inbreds vary especially with location. The location factors, such as temperature, soil type, and precipitation, can interact with the genotype and have an effect on the thermal traits of starch from exotic corn lines.

Future studies could include controlled growing environments in a greenhouse to further investigate the effects of temperature, precipitation, and soil type on the thermal properties of starch from these exotic lines and also to study the effects of these factors on the development of starch during kernel maturity. Another study could be conducted on the heritability of unusual starch traits in inbred lines, because the majority of the lines in this study did not inherit the unusual thermal starch trait for which they were selected. It would be advantageous to return to the original sources of the lines in these papers, in order to fully understand the effect of environment on the starch thermal traits and also understand the heritability of unusual starch traits.

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