

# Enthalpic Transitions in Native Starch Granules<sup>1</sup>

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## ABSTRACT

Cereal Chem. 76(3):444–448

Crystalline properties of native starch granule fractions that varied in apparent density and size were investigated using differential scanning calorimetry (DSC). Endotherms obtained at 80% hydration showed significant variations in enthalpy between the six fractions. Typical bi-phasic endotherms exhibiting significant variation in start temperature were obtained for the six fractions at 50% hydration. However, on annealing at 50°C/50% hydration for 48 hr, all fractions showed a single endotherm with-

out any significant variability in endotherm characteristics. At 10% hydration, the six fractions exhibited single high-temperature endotherms with significant differences in their peak temperatures. It was observed that mechanically damaging starch, resulted in the disappearance of any enthalpic transition. These observations could not be satisfactorily explained on the basis of prevailing concepts about DSC enthalpic transitions.

The crystalline order in starch granules is often the basic underlying factor influencing its functional properties. Collapse of crystalline order within starch granules manifests itself as irreversible changes in properties such as granule swelling, pasting, loss of birefringence, and starch solubility (Atwell et al 1988). The order-disorder transitions that occur on heating an aqueous suspension of starch granules has been extensively investigated using differential scanning calorimetry (DSC). In starch-water mixtures containing more than 60% water, generally, a single symmetrical endotherm is observed in DSC. As the water content decreases, however, the size of this transition is progressively reduced with a concomitant development of a second high-temperature endothermic transition. The nature of this bi-phasic transition is still not completely understood, however, several models have been proposed.

It was suggested by Donovan (1979) that crystallites in a granule melt cooperatively, exhibiting a single DSC endotherm as long as excess water is available for starch gelatinization. However, when the amount of water becomes insufficient for gelatinization, due to hydration-swelling of the amorphous parts of the granule, and due to its coupling with the starch crystallites, the remaining crystallites melt at a higher temperature. This results in a bi-phasic endotherm, where the second endotherm is dependent on the water content and the thermal stability of the remaining intact crystallites.

An alternate explanation for the existence of bi-phasic endotherms at intermediate water levels was given by Evans and Haisman (1982). These researchers observed that starch granules with less stable crystallites melt first cooperatively, absorbing water, and thus reducing its availability for the remaining granules. Consequently, the granules with more stable crystallites melt at a higher temperature thus giving rise to the secondary enthalpic transition.

Nakazawa et al (1984) studied annealing of starch by holding starch water mixtures (50 and 30% starch) at constant temperatures for 5 min to 140 hr, and investigating their thermal behavior using DSC. These researchers observed a gradual shift in the endotherm peak temperature to a higher temperature with progressive annealing. The X-ray diffraction patterns of annealed starch, however, showed gradual loss of crystallinity with progressive annealing. The bi-phasic nature of the endotherm at intermediate hydration was gradually lost on annealing, and a single sharper endotherm was formed. These authors concluded that in a bi-phasic endotherm, the high-temperature endotherm represented melting of starch

crystallites, while the low-temperature endotherm, corresponds to melting of the starch's amorphous region. Marchant and Blanshard (1978) also studied starch annealing and reported loss of birefringence and reduction of X-ray crystallinity, but an increase in enthalpy of gelatinization as measured by DSC.

Krueger et al (1987) studied annealing of commercial corn starch and observed that annealing narrowed the gelatinization temperature range, increased peak gelatinization temperature, and increased the enthalpy of gelatinization. These researchers concluded that annealing caused structural changes in the starch granules that affected their amorphous-crystalline relationships, forcing the granules into more crystalline orientation; these observations, however, were not supported by X-ray diffraction studies.

Biliaderis et al (1986) observed that at a low moisture content, the DSC thermal curve is not representative of the initial crystallite profile of starch granules. Instead, it describes the composite thermal effect of several processes that occur simultaneously during heating in DSC: melting, annealing, and recrystallization. These researchers observed that water at levels <30% essentially acted as a plasticizer and decreased the glass transition temperature of the amorphous regions of the starch granules. At a slow heating rate in the DSC (5°C/min.), immediately after the onset of the first endotherm and due to the increased molecular mobility, there was a greater opportunity for chain rearrangement to take place in the crystallites. These authors thus suggested that the multiple melting transition profile of intermediate moisture starch systems is a consequence of partial melting followed by recrystallization and final melting of crystallites.

Liu et al (1991) investigated starch gelatinization using DSC and X-ray diffraction. They suggested that in a semicrystalline polymer structure such as starch, amorphous regions are maintained by crystallites that function as cross-links. These cross-links hold the amorphous regions in a metastable strained state. With a strained state, there is a higher melting temperature. On partial melting, the strain relaxes and the melting temperature of the remaining crystallites is decreased. Thus, the crystallites melt over a narrower range than occurs when no strain is present. In addition, with a reduction in the number of cross links, a flux of diluent into the amorphous zones take place that depresses the melting temperature further. According to these researchers, the relationship between loss of X-ray crystallinity and DSC temperature of crystal melt, suggested that this is a two-step process. There was an initial decrease in crystallinity with increase in DSC temperature, an inflection leading to an intermediate plateau section (stabilization of crystallites with increasing temperature), followed by rapid crystallinity loss as DSC temperature was further increased. This model suggests that the bi-phasic order disorder transition is due to a water gradient within the samples and requires that crystallinity is lost in parallel with the progress of gelatinization as detected by DSC.

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Cooke and Gidley (1992) used cross-polarization magic angle sample spinning nuclear magnetic resonance spectroscopy, X-ray diffraction, and DSC to study loss of crystalline and molecular order during starch gelatinization. They concluded that melting of molecular helical order was the primary determinant of the endothermic enthalpy of gelatinization. It was also suggested by these researchers that there are two types of double helices in a starch granule, those involved in crystallites that are large and perfect enough to diffract X-rays and those that are not. Based on these observations, Zobel (1992) inferred that the forces holding the starch granule together were largely at the double helical level, and that the observed starch "crystallinity" functioned as a means of achieving dense packing rather than as a primary provider of structure.

It is evident from these diverse and often contradictory explanations, that starch researchers are far from a consensus on the nature of DSC transitions observed on starch gelatinization. Nevertheless, the limitations of DSC and the X-ray diffraction techniques as a true measures of crystallinity become quite obvious.

Fractionation studies show that starch granules are not uniformly homogenous in character, but display wide variations in functional properties (Sahai and Jackson 1994). As starch granules contain both crystalline and amorphous regions, each granule is likely to possess its own degree of crystallinity and its own unique energy characteristics (Larsson and Eliasson 1991). The present research was designed to elicit information about heterogeneity in crystalline nature of native corn starch granules by DSC analysis of fractionated starch. We were interested in determining whether existing theories explaining starch crystallinity can rationalize existence of bi-phasic endotherms, starch annealing, or behavior of mechanically damaged starch.

## MATERIALS AND METHODS

### Corn Starch

Commercially isolated normal dent corn starch (American Maize-Products Company, now CereStar USA, Inc., Hammond, IN) was used as the primary raw material for the study.

### Fractionation of Starch Granules

Starch granules were separated into six fractions according to apparent density and size by the reverse-flow sedimentation system designed for granule fractionation as described previously (Sahai and Jackson 1994). Fraction 1 is considered to be the most dense, while fraction 6 the least dense.

### DSC Analysis of Starch Granule Fractions

DSC endotherms of starch fractions were obtained using a Du Pont 2000 DSC (910 DSC cell, TA Instruments Inc., New Castle, DE). Starch samples were hydrated to 10, 50, and 80% moisture contents by adding calculated amounts of water. Samples were sealed in aluminum pans and equilibrated overnight before heating. Sample pans were heated at the rate of 5°C/min from 30–125°C (from 30–250°C for 10% hydrated samples) to obtain the endotherms. From the endotherms, peak start, peak onset, peak maximum, and peak end temperatures were recorded. Each starch fraction was also annealed at 50% hydration/50°C for 48 hr, and endotherms obtained.

A small sample (0.5 g) of normal unfractionated corn starch was mechanically damaged by grinding with a mortar and pestle, until granular birefringence as observed under a polarizing microscope was completely lost. DSC endotherms were obtained of the damaged starch sample at 80 and 50% hydration.

### Statistical Analysis

All DSC observations were made in duplicate and mean values reported. To establish whether any statistical differences existed between the starch fractions, single factor analysis of variance (ANOVA) was performed on the DSC data using statistical software (NCSS 97, Kaysville, UT).

Heterogeneity in native starch granules fractionated on the basis of size and apparent density has been previously demonstrated, differences in molecular weight profiles, X-ray crystallinity, and extent of solubility in dimethyl sulfoxide have been elucidated (Sahai and Jackson 1994).

### DSC Endotherms of Starch Fractions

DSC profiles of starch fractions obtained at 80% hydration, showed differences in endotherm shape, peak start, peak onset, peak maximum, and peak end temperatures and enthalpy values (Fig. 1). Differences in peak start, peak onset, peak maximum, and peak end temperatures between the six fractions were statistically insignificant. However, enthalpy ( $\Delta H$ ) values exhibited significant differences ( $P = 0.0317$ ) between the granule fractions. The  $\Delta H$  of crystal melt for the intermediate density granule fraction 3 showed the lowest value ( $\Delta H = 7.0$  J/g), while the most dense fraction 1 had the highest value ( $\Delta H = 11.8$  J/g). Enthalpy values, however, could not be correlated with granule density. These significant differences in  $\Delta H$  values obtained at 80% hydration between the six fractions indicate differences in extent of crystallinity.

At intermediate levels of hydration (50% hydration), starch granule fractions exhibited typical bi-phasic endotherms (Fig. 2). Variations within the six fractions in the nature of the bi-phasic peaks are evident from endotherm profiles (Fig. 2). The endotherm start temperatures were statistically significantly different for the six fractions ( $P < 0.0001$ ), however, the peak temperatures did not exhibit significant differences. Due to the emergence of the high-temperature secondary endotherm, it was difficult to judge the peak end temperature, hence,  $\Delta H$  values could not be calculated using DSC software. It is, however, apparent from the distinctly different shapes of the endotherms, that granule fractions must have different crystalline helical or amorphous structures because they exhibited such distinctly different endotherms at 50% hydration.

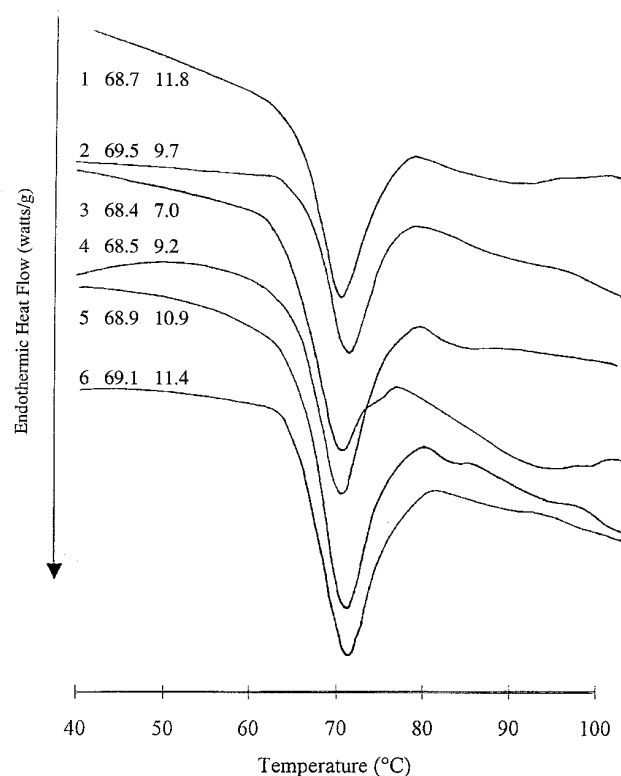
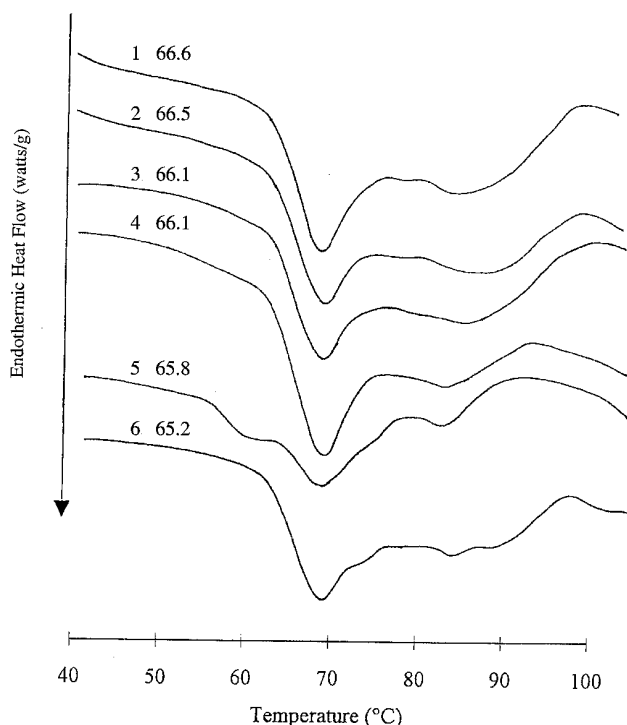


Fig. 1. Differential scanning calorimetry (DSC) endotherms of native starch fractions at 80% hydration. Fraction numbers 1–6 followed by melting temperatures ( $T_m$ , °C) and enthalpies ( $\Delta H$ , J/g).

A high-temperature endotherm in the range of 140–180°C is observed when low moisture starch (10–25% moisture) is heated during DSC (Zobel 1992). Little information is available in the literature regarding the nature of these high-temperature endotherms. These endotherms, however, may represent melting and uncoiling of helices (Zobel 1992). Endotherms obtained at 10% hydration levels for the six fractions exhibited wide variations in shapes (Fig. 3). Endotherm peak temperatures exhibited highly significant differences ( $P < 0.0001$ ) between the fractions. Endotherm peak onset and peak end temperatures did not exhibit significant differences. If the high-temperature endotherms are considered to be representative of helical uncoiling and melting, as suggested by Zobel (1992), the six starch fractions with significantly different peak melt temperatures must have differences at the helical level.

### Nature of the Bi-Phasic Endotherm

According to the hypothesis proposed by Donovan and Mapes (1980) and Evans and Haisman (1982) explaining bi-phasic DSC endotherms in a limiting water situation, the least stable crystallites melt first and use most of the water. Therefore, the remaining crystallites have insufficient water and thus melt at higher temperatures. It appears from this hypothesis that, on reducing the hydration levels from 80 to 50% during DSC, the endotherm start and peak temperatures would remain the same, while the peak end temperature would be increased due to the emergence of the secondary endotherm. The endotherm start and peak temperatures obtained by us at 50% hydration (Fig. 2), however, were always lower than those obtained at 80% hydration (Fig. 1). Reducing the level of starch hydration from 80 to 50%, apparently facilitated the melting of the least stable crystallites, lowering the endotherm start and peak temperatures, besides increasing the melting temperatures of the stable crystallites. It appears that the hypothesis proposed by Donovan and Mapes (1980) and Evans and Haisman (1982) cannot satisfactorily explain the behaviors of the “least stable crystallites” during DSC with reduced hydration levels.

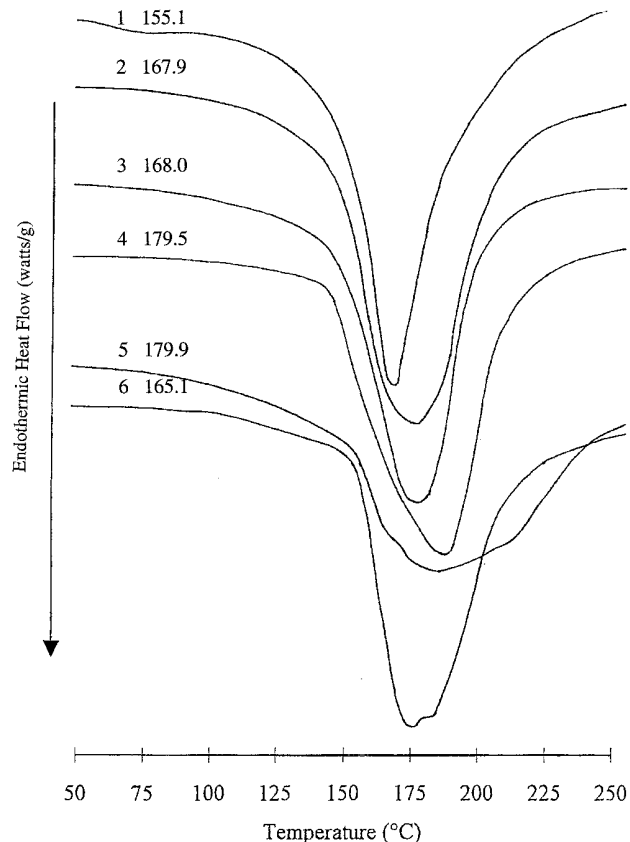


**Fig. 2.** Differential scanning calorimetry (DSC) endotherms of native starch fractions at 50% hydration. Fraction numbers 1–6 followed by melting temperatures ( $T_m$ , °C).

### DSC of Annealed Starch Fractions

Annealing of starch is usually observed when starch-water suspensions are maintained at temperatures slightly below the starch gelatinization temperature range. DSC endotherms obtained after annealing the starch fractions at 50% hydration/50°C for 48 hr showed total or partial disappearance of the secondary high temperature endotherm (Fig. 4). There were also no significant differences in peak start, peak onset, or peak maximum temperature values within the six fractions (Fig. 4).

Biliaderis et al (1986) attributed the existence of bi-phasic endotherms at intermediate hydration levels to annealing, lipid complexation, and recrystallization taking place during DSC heating. These researchers have proposed a three-phase model, incorporating two distinct types of amorphous material and crystalline domains of amylopectin short-chain clusters to account for the thermal properties of granular starch-water mixtures. Our starch fractions at 50% hydration showed typical bi-phasic endotherms with considerable variability in DSC patterns and significant differences in endotherm start temperatures (Fig. 2). However, on annealing, the granule fractions exhibited a single endotherm with no significant variability in peak start, peak onset, or peak maximum temperature values (Fig. 4). Nakazawa et al (1984) also reported the gradual disappearance of the secondary high-temperature endotherm on progressive annealing. If the appearance of the secondary endotherm is assumed to be due to annealing, recrystallization, and amylose-lipid complex formation during DSC analysis as suggested by Biliaderis et al (1986), such phenomenon apparently do not occur in annealed starch. It is logical to question why recrystallization or amylose lipid complexation would not occur in annealed starch. It can be concluded that the hypothesis proposed by Biliaderis et al (1986) cannot adequately explain the disappearance of these secondary endotherms in annealed starch.



**Fig. 3.** Differential scanning calorimetry (DSC) endotherms of native starch fractions at 10% hydration. Fraction numbers 1–6 followed by melting temperatures ( $T_m$ , °C).

Liu et al (1991) proposed that due to the semicrystalline nature of starch granules, the amorphous regions are maintained by crystallites in a metastable strained state and function as cross-links. If annealing of starch is a phenomenon where granules are forced into a more crystalline orientation (Krueger et al 1987), the crystalline cross-links and strain that hold the amorphous regions in a granule must continue to exist in annealed starch, creating bi-phasic endotherms. Our data, however, show that the bi-phasic nature of starch endotherms at intermediate moisture levels was lost on annealing, suggesting that the model proposed by Liu et al (1991) cannot adequately explain starch annealing. Liu et al (1991) also emphasized the fact that a bi-phasic order disorder transition is due to a water gradient within the starch sample. They suggest that a flux of diluent in the sample depresses the melting temperature. It appears that annealing of starch at 50% hydration for 48 hr should equilibrate starch granule moisture, reduce water gradients, and thus depress the melting temperature. However, increases in gelatinization and melting temperatures on annealing have been well documented (Nakazawa et al 1984, Krueger et al 1987).

### Crystallinity in Mechanically Damaged Starch

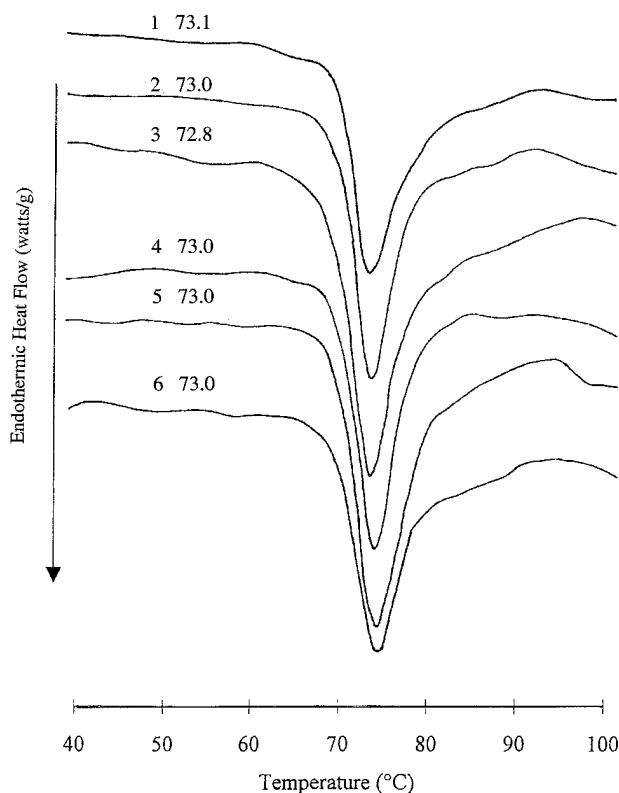
Mechanical damage to starch granules causes loss of birefringence and crystallinity, resulting in an amorphous material (Morrison et al 1994). DSC analysis of damaged starch that had completely lost birefringence did not show any endotherms at 80 or 50% hydration levels (Fig. 5).

Cooke and Gidley (1992) proposed that melting of molecular helical order was the primary event occurring during DSC and is responsible for enthalpy of gelatinization. Zobel (1992) suggested that the first and the second peaks of a bi-phasic endotherm may reflect melting of crystallites and helices, respectively. As damaged starch granules did not exhibit any enthalpic transitions, it appears

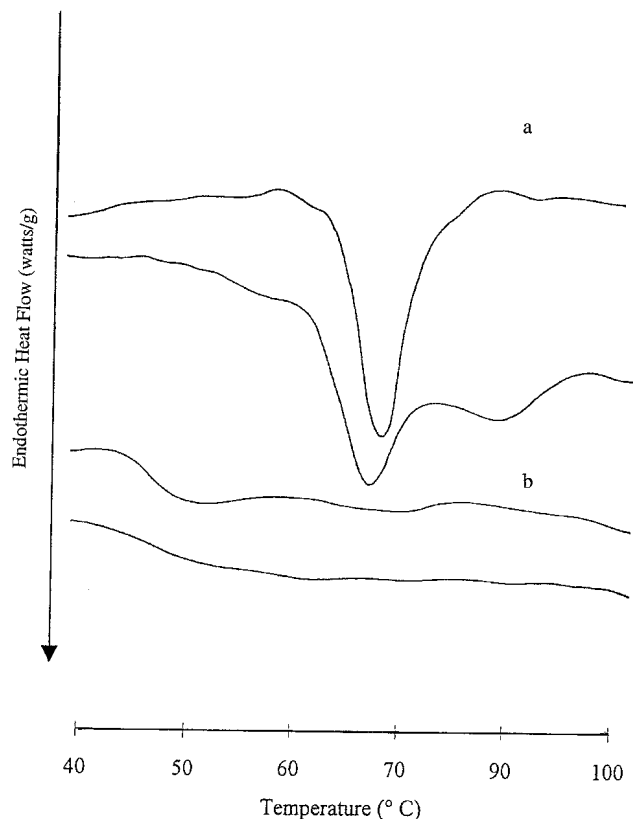
that mechanical damage not only destroyed crystallinity but also disrupted the helical order in starch granules. On the basis of Zobel's hypothesis, it can be argued that annealed starch that did not exhibit a bi-phasic endotherm is devoid of helical structures. Zobel (1992) has also suggested that "the forces holding a starch granule together are largely at the double helical level." We could create starch granules that do not exhibit any enthalpic transition and annealed starch granules that do not exhibit a secondary DSC endotherm (helical melt). Were granules we created granules "held together" without helices? Limitations of the hypothesis by Zobel (1992) and Cooke and Gidley (1992) become obvious because it can be argued that enthalpic transitions registered during DSC may not reflect melting of starch double helical structures.

### CONCLUSIONS

Failure of the prevailing models in explaining behavior of starch fractions during DSC at various hydration levels, starch annealing, and the nature of mechanically damaged starch granules, highlights an inadequate understanding of the complexities in starch granule design. Researchers have often pointed out such anomalies. For example, Nakazawa et al (1984) observed that transitions associated with DSC peaks were due to changes not always detectable by X-ray diffraction techniques. It appears that the enthalpic transitions at various moisture levels do not represent a single phenomenon but may represent numerous changes occurring simultaneously, where one or more of these changes is more prominently evident by DSC at given moistures. Information presented in this article may not be sufficient to develop new hypothesis or explain starch behavior during DSC. Our data, however, does emphasize the inconsistencies in prevailing models and the need for further investigation.



**Fig. 4.** Differential scanning calorimetry (DSC) endotherms of annealed (50°C/48 hr) native starch fractions at 50% hydration. Fraction numbers 1–6 followed by melting temperatures ( $T_m$ , °C).



**Fig. 5.** Differential scanning calorimetry (DSC) endotherms of normal dent corn starch at 80 and 50% hydration (a) and mechanically damaged starch at 80 and 50% hydration (b).

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[Received August 20, 1998. Accepted February 15, 1999.]