

# Starch-Lipid Interactions in Common, Waxy, *ae du*, and *ae su2* Maize Starches Examined by Differential Scanning Calorimetry<sup>1</sup>

V. Kurtis Villwock,<sup>2,3</sup> Ann-Charlotte Eliasson,<sup>2</sup> José Silverio,<sup>2</sup> and James N. BeMiller<sup>3,4</sup>

## ABSTRACT

Cereal Chem. 76(2):292–298

Four maize genotypes (common, waxy, *ae du*, and *ae su2*) were examined by differential scanning calorimetry (DSC) in the presence of four surfactants (sodium dodecyl sulfate [SDS], dodecyltrimethylammonium bromide [DOTAB], sodium hexadecyl sulfate [SHS], 1-monolauroyl-*rac*-glycerol [ML]) to characterize the starch-lipid complexes produced and provide evidence of differences in starch structure. The ionic surfactants with a C<sub>12</sub> hydrocarbon tail reduced the gelatinization onset temperature, a phenomenon that does not occur typically with neutral surfactants or with surfactants with longer hydrocarbon tails. Subtracting the DSC curves, the

exotherm we suspected was caused by starch-lipid complexation, which occurs concomitantly with the gelatinization endotherm, was identified and provided evidence of the existence of amylopectin-lipid interactions. Apparent starch amylose content correlated well with enthalpies of amylose-lipid complexes. Complexes formed from DOTAB produced DSC endotherms that were broad and shallow and that shifted to lower temperatures as the DOTAB concentration increased. This was in contrast to other surfactants that normally produce amylose-lipid complex endotherms at temperatures independent of surfactant concentration.

Starch is synthesized in various tissues of many species of plants to serve as the major source of reserve carbohydrate, each species forming unique starch granules. Starch granules from various sources may have very similar basic chemical compositions yet still exhibit wide differences in behavioral characteristics, due not only to factors such as the amylopectin-to-amylose ratio, but perhaps equally important, the arrangement of these components in the amorphous and crystalline areas of the granules. However, a thorough understanding of the arrangement of granular components and the effect on starch properties has been elusive. Furthermore, the most promising aspects for introducing new types of functional starches will not arise from new chemical modifications alone but rather from current chemical modifications following biological manipulation (BeMiller 1997). Unfortunately, no one approach can answer all questions about starch granule structure.

One approach is examination of starch polymer-lipid complexes. The formation of inclusion complexes between starch chains and a hydrophobic guest molecule has long been reported (Mikus et al 1946). A large body of work has characterized amylose-lipid complexes in terms of lipid type and, to a lesser extent, starch type. It is known that differences occur between the complexing ability of amylose and amylopectin in solution versus the same molecules in the granular matrix. Evidence has been presented which suggests that cetyltrimethylammonium bromide (CTAB) may interact to a greater degree with isolated amylopectin than with granular amylopectin (Eliasson and Ljunger 1988). After heating aqueous suspensions of wheat and potato starch granules in the presence of SDS, only the interior of granules stained blue with iodine, while the outer granular region stained brown (Svensson et al 1997). Thus, the structural arrangement of amylose and amylopectin within the granule is probably a major factor in determining the formation and nature of amylose and amylopectin-lipid interactions.

The objective of this study was to examine differences between the complexing behaviors of starches of two double-mutant maize genotypes, *ae du* and *ae su2* (Friedman et al 1988, 1989). The structures of the constituent polymers and the physicochemical properties of the granules and pastes have been examined (Ikawa et al 1981, Inouchi et al 1991, Katz et al 1993, Wang et al 1993) but not

in terms of lipid-complexing characteristics. Common corn and waxy maize starches were examined in the same way as controls.

## MATERIALS AND METHODS

### Materials

Starches used were of commercial quality and were used without further purification. Common corn and waxy maize starches were obtained from Lyckeby-Stärkelsen (Kristianstad, Sweden). The genetically modified *ae du* and *ae su2* maize starches were a gift of Cerestar USA (Hammond, IN). The latter starches were specially prepared and presumed to be free of contamination, while the former two contained the usual 3–5% contamination of one with the other. Water used for all experiments was distilled twice and passed through a Millipore filtering system. Dodecyltrimethylammonium bromide (DOTAB), sodium dodecylsulfate (SDS), and 1-monolauroyl-*rac*-glycerol (ML) were 99% pure and obtained from Sigma Chemical Co. (St. Louis, MO). Sodium hexadecylsulfate (SHS) was 99% pure and obtained from Lancaster Synthesis, Ltd. (Lancashire, England). Precautions were taken to protect surfactants from heat and oxygen.

### Differential Scanning Calorimetry (DSC)

Starch samples were weighed accurately (2,000–5,000 mg) using a Cahn C-30 microbalance (Cahn Instruments, Madison, WI) into coated aluminum sample pans (PLD Instruments AB; Upplands Väsby, Sweden). Each analysis was done at least in duplicate. For most samples, either water or a surfactant solution of known concentration was added carefully with a micropipet in the amount needed to obtain a 3:1 weight ratio of water to dry starch. An exception was ML, which was added in dry form before the starch and water. SDS and DOTAB existed as micelles under the conditions used in the study (Small 1986, Wang and Olofsson 1995). SHS melted and presumably became micellar with mild heating (38–44°C). ML melts at ≈40°C in water to a lamellar phase, suitable for starch-lipid complexation (Larsson et al 1978, Larsson and Quinn 1994). Melting of ML was complete before any gelatinization events took place (determined by DSC). The pans were sealed and allowed to rest for ≈1 hr. Samples were analyzed using a calorimeter (DSC 6200, Seiko Instruments) at heating and cooling rates of 10°C/min. The temperature regime consisted of heating from 10 to 130°C with a 3-min hold, cooling back to 10°C with a 3-min hold, and reheating to 140°C. A pan containing Al<sub>2</sub>O<sub>3</sub> was used as the reference. Pans were subsequently punctured, dried overnight at 105°C, and reweighed to calculate the weight of dry starch. Data from the DSC scans were analyzed using the calorimeter's software. Enthalpies are reported on a dry starch weight basis, taking the weight of surfactant into consideration.

<sup>1</sup> Journal paper No. 15867 of Purdue Agricultural Research Programs.

<sup>2</sup> Food Technology, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden S221 00.

<sup>3</sup> Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907-1160.

<sup>4</sup> Corresponding author. E-mail: bemiller@purdue.edu

The results are reported as onset temperature of gelatinization ( $T_{oG}$ ), temperature at gelatinization endotherm peak ( $T_{pG}$ ), temperature at peak of complex melting endotherm ( $T_{pC}$ ), enthalpy of gelatinization ( $\Delta H_G$ ), and enthalpy of complex melting endotherm ( $\Delta H_C$ ). To calculate molar ratios, the moles of surfactant were divided by the moles of  $\alpha$ -D-glucopyranosyl units.

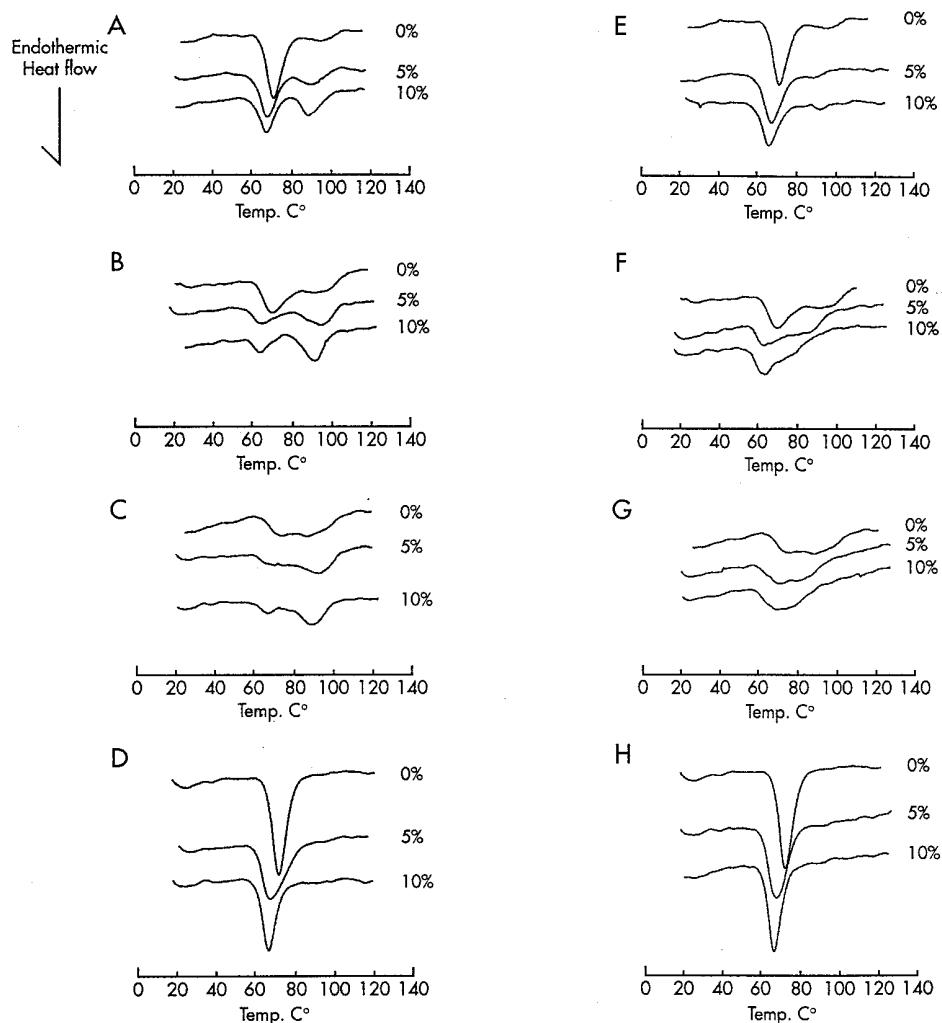
### Native Lipid Analysis

Lipids were extracted from common, *ae du*, and *ae su2* maize starches (duplicate samples) with boiling methanol (100%) for 24 hr in a Soxhlet apparatus (Kugimiya et al 1980). Lipids were recovered by rotary evaporation at 50°C under reduced pressure. Extracted lipids were stored at -10°C in a nitrogen atmosphere until further analysis. A second portion of starch was analyzed for moisture content just before lipid extraction to calculate lipid yields on a dry starch basis (AACC 1995).

Lipid extracts spotted (10  $\mu$ L; 20 mg/mL in  $\text{CHCl}_3$  and MeOH, 2:1 v/v) on activated (120°C for 30 min) glass, thin-layer chromatography (TLC) plates (Kieselgel 60, 0.25-mm; Merck, Darmstadt, Germany) in equal weight quantities included various lipid class standards: triolein (Sigma), dilinolein (Sigma), monoolein (Sigma), oleic acid (Sigma), L- $\alpha$ -lysophosphatidyl choline (Larodan Fine Chemicals, Malmö, Sweden), and L- $\alpha$ -lysophosphatidyl ethanolamine (Larodan). Plates were developed with 65:25:4 v/v  $\text{CHCl}_3$ , MeOH, and  $\text{H}_2\text{O}$ . The lipid class of the extracts was identified by

comparison with the standards after spraying with a cupric acetate-phosphoric acid solution and heating (180°C, 15 min). A second series of TLC was done using the same solvent system used for preparation for gas chromatography-mass spectrometry (GC-MS) analysis. A 250- $\mu$ L syringe was used to apply 75 mg of lipid extract dissolved in 2:1 v/v  $\text{CHCl}_3$  and MeOH in the form of long bands. After separation, the bands were identified by spraying with a solution of 2', 7'-dichlorofluorescein in ethanol (Gunnlaugsdottir and Sivik 1995). The bands containing free fatty acid (FFA), which were by far the major class, were scraped off and placed into screw-cap centrifuge tubes. Tetrahydrofuran (1.0 mL) and 1.0 mL of 7% boron trifluoride reagent (Sigma) (14% solution in MeOH diluted further to 7% before use) were placed into the tubes. The mixture was flushed with nitrogen and vortex mixed before heating 30 min in a heating block at 100°C. After cooling, 1.0 mL of water and 1.0 mL of hexane was added and the mixture was vortex mixed. The hexane phase was removed and the aqueous phase was washed again with hexane. The combined fatty acid methyl ester (FAME) extracts in hexane were then injected into a GC-MS unit.

GC-MS was done on a Hewlett-Packard system (GC model 6890; MS model 5972A). The conditions used were: injector temperature 240°C; column (Rtx-50% phenyl-50% methyl polysiloxane, 30.0  $\times$  0.32 mm); injection volume 1  $\mu$ L; He carrier 1.2 mL/min;  $\text{H}_2$  flow 40 mL/min; air flow 450 mL/min; makeup gas flow 45 mL/min; split ratio 100:1; MS interface temperature 280°C. The GC oven



**Fig. 1.** Differential scanning calorimetry (DSC) thermograms of maize starch-surfactant blends (0–10% surfactant, dsb) during initial heating. Normalized to constant weight. **A–D**, SDS with common, *ae du*, *ae su2*, and waxy starches, respectively. **E–H**, dodecyltrimethylammonium bromide (DOTAB) with common, *ae du*, *ae su2*, and waxy starches, respectively.

was programmed to increase from 200 to 250°C at a heating rate of 5°C/min followed by a 12-min hold. Peaks were identified both by using the spectrometer's MS-spectra library and by comparison of retention times with those of FAME standards of 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, and 20:0, which were run separately.

## RESULTS AND DISCUSSION

Examples of representative DSC thermograms during initial heating and during the reheat portion of the program are shown in Figs. 1 and 2. Waxy maize starch showed no endotherms during the reheat cycle. A possible exception was in the presence of 15% SHS, which reproducibly showed small but defined peaks at 104°C (cooling) and 115°C (reheated) with enthalpies of 0.2–0.5 J/g of dry starch (data not shown). It is not known if this represents a phenomenon occurring with the amylopectin of waxy maize starch or, more likely, if it is a function of contamination from common corn starch granules, which typically are present in commercial samples of waxy maize starch. This waxy maize starch sample was determined to be 98.4% amylopectin (Fredrikson et al 1997); common maize starch contamination was confirmed using iodine staining and light microscopy.

The DSC data in Table I shows that a leveling-off in endotherm values for reheated pastes occurred as surfactant concentration was increased. Complexes formed with DOTAB were exceptions to this observation. The leveling suggests that saturation had occurred. The maximum amount of complexed surfactant was typically between 5% (0.027 molar ratio) and 10% surfactant (0.054 molar ratio).

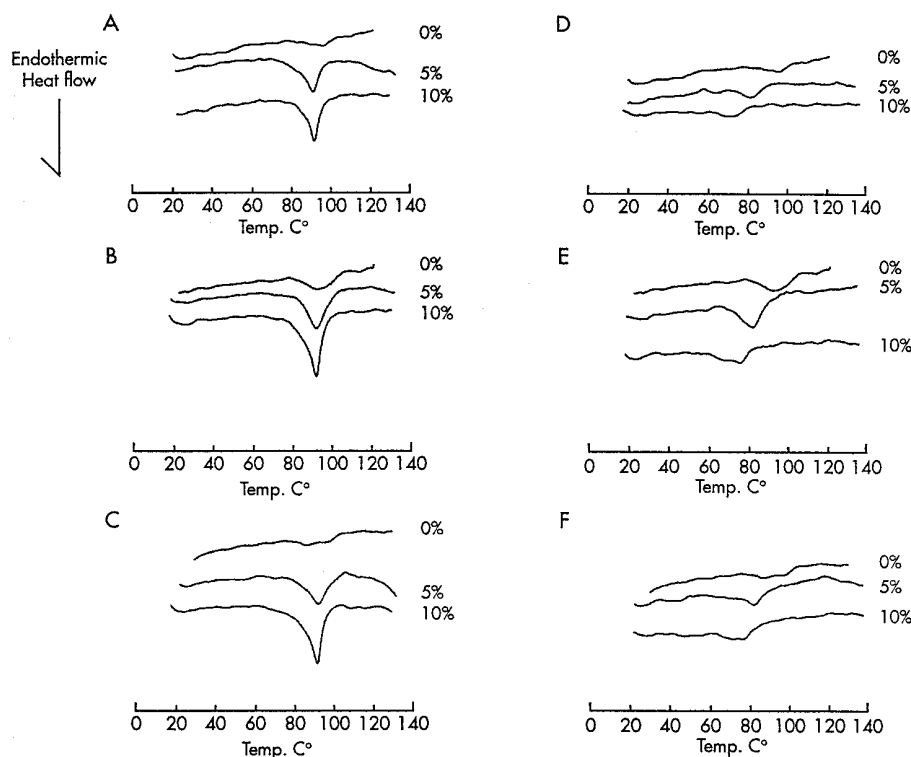
Variation of the mean value was typically <5%, although in certain cases the deviation became considerable (≈20%). It was sometimes difficult to achieve replicates with precise lipid content and, consequently, the deviations are reflected in the enthalpy values obtained. Furthermore, variation in temperature values tended to be high with *ae du* and *ae su2* starches. In *ae du* starch, two types of

granules are present (Obanni and BeMiller 1995); in *ae su2* starch, the gelatinization endotherm was shallow and broad, lacking well-defined temperature parameters. Samples containing DOTAB demonstrated small ill-defined starch-lipid complex endotherms that occurred at different temperatures depending on the DOTAB concentration. These endotherms also overlapped the gelatinization endotherm at higher DOTAB concentrations. Because of these factors, high variability was unavoidable in some experiments.

These results are interpreted in light of previous studies of both starch-lipid complexes and characterization of starch polymers of the various maize genotypes with a goal of both providing evidence related to the structure of starch granules and an understanding of the nature of starch-lipid complexes.

### Influence of Starch Type on Gelatinization

Gelatinization curves of the four starches are presented in Fig. 1. Gelatinization enthalpy and onset and peak endotherm temperatures for the starches are listed in Table II. The thermogram of common corn starch had a natural shoulder, which is produced by melting of the starch-lipid complex present naturally and which becomes larger as surfactant is added. The thermogram of *ae du* maize starch had the largest shoulder, which disappeared when the starch was defatted before DSC (data not shown). Of the starches examined, *ae du* maize starch gave the highest yield of lipids after extraction with boiling methanol. Gelatinization of very dilute suspensions without agitation shows that *ae du* maize starch is composed of two types of granules, one of which is swollen resembling common corn starch granules, and the other which is not swollen and resembles high-amylose maize starch (Obanni and BeMiller 1995). We found no evidence of two separate gelatinization peaks, but that does not necessarily mean that both populations gelatinize within a similar range (Obanni and BeMiller 1997). It does appear that both populations gelatinize under the employed conditions, otherwise the enthalpies would be considerably lower due to a diluting effect from the nongelatinizing granules (Tables I and II). However, the



**Fig. 2.** Differential scanning calorimetry (DSC) thermograms of maize starch-surfactant blends (0–10% surfactant, dsb) during reheating. Normalized to constant weight. **A–C,** SDS with common, *ae du*, and *ae su2* starches, respectively. **D–F,** dodecyltrimethylammonium bromide (DOTAB) with common, *ae du*, and *ae su2* starches, respectively.

DSC and rheological properties of blends of different starch granules cannot be predicted from what is known about the characteristics of the individual starch granule populations (Obanni and BeMiller 1997).

There appeared to be a shoulder on the DSC endotherm of *ae su2* starch, but this shoulder remained after defatting (data not shown). The *ae su2* starch appeared to have low crystallinity (Inouchi et al 1991) and melted over a wide range of temperatures. Higher melting crystallites (e.g., 80°C) may result from amylopectin short chains that are longer than those of other maize genotypes, with an average of 21  $\alpha$ -d-glucopyranosyl units, compared to 16 for that of common corn starch (Ikawa et al 1981). Starches from maize plants with the *su2* gene are particularly sensitive to heating rate (Inouchi et al 1991). Conversely, waxy maize starch lacked a shoulder, melted over a relatively narrow temperature range, and had the highest  $\Delta H_G$  value, indicating the presence of many crystalline regions.

### Native Lipid Composition

Soxhlet extraction with methanol yielded lipid contents of between 10 and 12 mg/g of dry starch. DSC evaluation of the defatted starches (3:1 water and starch) revealed that common and *ae du* maize starches lost the shoulder that appeared next to the gelatinization endotherm in nondefatted starch, whereas in *ae su2* maize starch, the shoulder area remained and no endotherm from any complexed lipid present could be discerned from the gelatinization endotherm (data not shown). In all cases, defatted starches showed no endotherms upon reheating a cooled paste, signifying the adequacy of the extraction method used in this study, despite the fact that other methods with higher extraction efficiency have been reported (Morrison and Coventry 1985).

TLC analysis showed FFA was the major lipid class, as expected (Tan and Morrison 1979, Morrison 1983, Padley et al 1994). Fatty acid analysis of the FFA portion showed only 16:0, 18:0, 18:1, 18:2, and 18:3 fatty acids present in the following proportions, respectively: common (23: 1.6: 11: 62: 2.8), *ae du* (32: 2.3: 20: 44: 1.4), and *ae su2* (33: 1.9: 13: 50: 2.0). The starches of the two mutant genotypes had a considerably higher proportion of saturated FFA than did common maize starch. A spot not observed in the lipid extracts of common maize starch was seen in *ae du* and *ae su2* starches just below that of the monoglyceride standard.

The effect of native lipids on complexation endotherms is difficult to judge based on the  $T_{pc}$  values in Table I because of the similar endotherm temperature range produced by both native and added lipids with SDS, and the concentration-dependent shifting of  $T_{pc}$  with DOTAB. Data in Fig. 2 suggest that added surfactants govern the

location of the complex endotherm because no small endotherm appears at the location where one would expect the native lipid-amylose complex endotherm. However, examination of the  $\Delta H_C$  values (Table I) at the molar ratio of  $\approx 0.008$  shows that enthalpy increased with added SDS in similar increments for each genetic variant: 1.7, 1.9, and 1.2 J/g for common, *ae du*, and *ae su2* maize starches, respectively. Notably,  $\Delta H_C$  of *ae du* starch increased by the same approximate amount despite the fact that it already had a high enthalpy from complexed native lipids (3.8 J/g). This suggests that the resultant enthalpy consists of additive effects of both native and added lipids, even though two separate endotherms were not observed.

### Influence of Lipid Type on Gelatinization

In both the SDS and DOTAB series and in all starches studied, a trend was seen:  $\Delta H_G$  magnitudes decreased as the surfactant concentration increased. This reduction has been thought to be the result of an exothermic effect from the formation of starch-lipid complexes occurring simultaneously as the granule gelatinizes (Evans 1986, Eliasson 1994). With the presence of starch-lipid complexes which yield endotherms that tend to overlap the gelatinization temperature range, it was difficult to quantify accurately the magnitude of the  $\Delta H_G$  reduction in all but a few mixtures used in this study. Using the subtraction software included with the DSC unit, the gelatinization endotherm without surfactant was subtracted from the gelatinization endotherm at  $\approx 10\%$  surfactant. Due to the shift in the  $T_{oG}$ , the curves had to be shifted to match  $T_{oG}$  before subtraction. The  $\Delta H_G$  reduction results for waxy-SDS, waxy-DOTAB, waxy-SHS, and common-SHS were 5.3, 2.7, 2.3, and 2.6 J/g, respectively. The corresponding exotherms are shown in Fig. 3 to illustrate their shapes. The low temperature endotherm seen in the DSC curves of SHS (Fig. 3D and E) is due to melting of SHS. Unfortunately, other starch-surfactant combinations contained too much interference from amylose-lipid melting endotherms (e.g., common-SDS, Fig. 3A). The fact that the  $\Delta H_G$  reduction occurs in waxy maize starch has been taken as evidence for the existence of an amylopectin-lipid interaction (Eliasson 1994). Huang and White (1993) actually obtained a precipitate from waxy maize starch and monoglycerides, which could have been an amylopectin-lipid complex, based on the reduced iodine affinity as compared to that of a control.

Although evidence for the existence of an amylopectin-lipid complex of some sort is becoming stronger, very little is known about the specific nature of such interactions. In these results, a comparison between the  $\Delta H_G$  reductions with waxy maize starch and SDS, DOTAB, or SHS suggests that SDS interacted to a greater degree with

TABLE I  
Melting Enthalpy of Amylose-Surfactant Complexes ( $\Delta H_C$ ) and Peak Endotherm Values ( $T_{pc}$ ) from Reheating Maize Starch-Surfactant Blends at Various Molar Ratios of Surfactant to Starch Anhydroglucose Units<sup>a</sup>

Genotype	SDS				DOTAB <sup>b</sup>			
	<i>n</i>	Molar Ratio	$\Delta H_C$ (J/g)	$T_{pc}$ (°C)	<i>n</i>	Molar Ratio	$\Delta H_C$ (J/g)	$T_{pc}$ (°C)
Common	2	0.0000 $\pm$ 0.0000	2.0 $\pm$ 0.0	95.7 $\pm$ 0.5	2	0.0000 $\pm$ 0.0000	2.0 $\pm$ 0.0	95.7 $\pm$ 0.5
	3	0.0080 $\pm$ 0.0004	3.7 $\pm$ 0.3	93.8 $\pm$ 0.6	3	0.0074 $\pm$ 0.0005	2.7 $\pm$ 0.3	89.9 $\pm$ 1.6
	3	0.0271 $\pm$ 0.0002	7.1 $\pm$ 0.5	91.8 $\pm$ 0.1	5	0.0250 $\pm$ 0.0005	2.8 $\pm$ 0.4	82.0 $\pm$ 0.8
	3	0.0566 $\pm$ 0.0011	7.7 $\pm$ 0.3	92.3 $\pm$ 0.4	5	0.0525 $\pm$ 0.0026	4.0 $\pm$ 0.8	70.5 $\pm$ 1.5
	4	0.0887 $\pm$ 0.0072	6.8 $\pm$ 0.3	93.7 $\pm$ 0.4	2	0.0757 $\pm$ 0.0010	5.8 $\pm$ 0.0	63.5 $\pm$ 1.4
<i>ae du</i>	2	0.0000 $\pm$ 0.0000	3.8 $\pm$ 0.7	94.0 $\pm$ 0.0	2	0.0000 $\pm$ 0.0000	3.8 $\pm$ 0.7	94.0 $\pm$ 0.0
	3	0.0081 $\pm$ 0.0002	5.7 $\pm$ 0.6	94.8 $\pm$ 0.5	2	0.0075 $\pm$ 0.0003	4.0 $\pm$ 0.1	88.3 $\pm$ 0.3
	3	0.0268 $\pm$ 0.0007	11.1 $\pm$ 1.0	93.4 $\pm$ 0.1	5	0.0237 $\pm$ 0.0026	6.2 $\pm$ 1.1	83.7 $\pm$ 1.4
	3	0.0526 $\pm$ 0.0010	14.3 $\pm$ 0.5	93.5 $\pm$ 0.5	3	0.0486 $\pm$ 0.0013	5.2 $\pm$ 1.1	77.4 $\pm$ 0.4
	5	0.0878 $\pm$ 0.0085	12.3 $\pm$ 1.2	93.8 $\pm$ 0.7	2	0.0780 $\pm$ 0.0008	7.9 $\pm$ 0.5	62.2 $\pm$ 0.3
<i>ae su2</i>	4	0.0000 $\pm$ 0.0000	2.3 $\pm$ 0.5	94.1 $\pm$ 2.2	4	0.0000 $\pm$ 0.0000	2.3 $\pm$ 0.5	94.1 $\pm$ 2.2
	4	0.0083 $\pm$ 0.0002	3.5 $\pm$ 0.3	93.8 $\pm$ 1.7	2	0.0077 $\pm$ 0.0002	4.0 $\pm$ 0.3	87.2 $\pm$ 1.6
	3	0.0265 $\pm$ 0.0006	8.6 $\pm$ 0.2	92.8 $\pm$ 0.3	4	0.0249 $\pm$ 0.0001	4.5 $\pm$ 0.5	81.7 $\pm$ 0.4
	3	0.0543 $\pm$ 0.0004	14.5 $\pm$ 1.9	92.8 $\pm$ 0.1	2	0.0488 $\pm$ 0.0004	8.1 $\pm$ 0.1	76.6 $\pm$ 0.2
	5	0.0799 $\pm$ 0.0018	12.8 $\pm$ 1.2	94.1 $\pm$ 0.2	3	0.0729 $\pm$ 0.0007	8.4 $\pm$ 0.8	65.6 $\pm$ 1.3

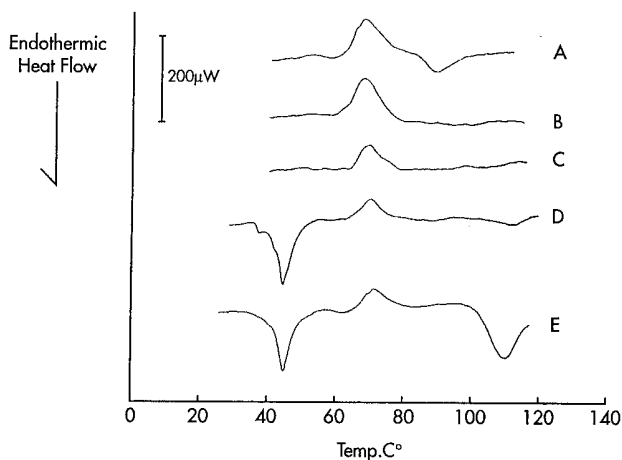
<sup>a</sup> Values  $\pm$  standard deviations.

<sup>b</sup> Dodecyltrimethylammonium bromide.

amylopectin than did the other surfactants. SDS has demonstrated a special ability to enhance the  $\Delta H_G$  reduction and  $\Delta H_C$  in starches, most likely because of its ability to disrupt and swell granules (Gough et al 1985, Svensson et al 1997). It could be that SDS destabilizes granules by expanding amylopectin crystallites and thereby physically causes the granule to swell, perhaps by like-charge repulsion of the polar head groups that project from the ends of amylopectin short chains (Evans 1986). If amylopectin A and B chains are, in fact, complexing with SDS, an unraveling of the starch double helix must occur before complexation can occur, which should also expand the granule tangentially.

Enthalpy reductions effected from using SDS and CTAB with wheat starch and, especially, with potato starch are much larger in comparison to waxy maize and other maize starches (Eliasson 1986, Evans 1986). If the effects are considered to be dependent on repulsion of the polar head groups, one would expect that longer fatty acid chains could be less granule destabilizing due to their greater flexibility and ability to separate like charges. There is evidence that ionic complexing ligands form complexes equivalent to a neutral ligand of shorter length, suggesting that a shorter portion of the hydrocarbon tail of an ionic surfactant participates in the inclusion complex as compared to its nonionic analog (Kowblansky 1985). Neutralization or charge screening of ionic complexing agents also reduces the decrease in  $\Delta H_G$  (Evans 1986). Support for this observation was found in this study in that the  $\Delta H_G$  reduction upon addition of SHS ( $C_{16}$  tail) to waxy maize starch was 2.3 J/g as compared with SDS ( $C_{12}$  tail, 5.3 J/g). Furthermore, the  $\Delta H_G$  decrease effected by the neutral  $C_{12}$  surfactant ML (0% ML minus 10% ML) was only 0.2 J/g. The Kowblansky hypothesis explains why the enthalpy drops diminish substantially when using the  $C_{16}$  chain lengths of CTAB and SHS and nearly disappear with longer lengths ( $C_{18}$ ), e.g., sodium stearyl-2-lactylate (Eliasson 1986, Evans 1986), although there are some inconsistencies with this trend (Eliasson et al 1988).

Upon addition of either SDS and DOTAB to all starches studied,  $T_{oG}$  and  $T_{pG}$  decreased with increasing surfactant concentration. This effect is well known with SDS (Eliasson 1985, Eliasson 1994) and was formerly thought to be due to an ion effect specific for SDS. However, the same effect is now observed for DOTAB ( $C_{12}$ ) but does not typically occur with the  $C_{16}$  analog CTAB (Eliasson 1994). With this new evidence, it was suspected that perhaps lipid chain length played a role in the destabilization of starch granules, which is why the SHS, the  $C_{16}$  analog of SDS, was included.



**Fig. 3.** Differential scanning calorimetry (DSC) thermograms of maize starch-surfactant blends after subtracting the curve obtained with 0% surfactant from the corresponding curve at 10% surfactant. Normalized to constant weight and adjusted to match  $T_{oG}$ . Common starch with SDS (A); waxy starch with SDS (B); waxy starch with dodecyltrimethylammonium bromide (DOTAB) (C); waxy starch with sodium hexadecyl sulfate (SHS) (D); common starch with SHS (E).

$T_{oG}$  depressions are illustrated in Fig. 4A–D, including ML as a neutral surfactant in Fig. 4A. This data suggests that both ionic surfactant types of  $C_{12}$  chain length are granule destabilizing, whereas ionic surfactants of longer chain length and neutral surfactants are not destabilizing and may be even slightly stabilizing, with the possible exception of the waxy maize starch-SHS system. This finding concerning the behavior of neutral surfactants agrees with the report of Eliasson (1993), although Huang and White (1993) reported a slight decrease in  $T_{oG}$  with neutral monoglycerides as the fatty acyl chain length was reduced from  $C_{18}$  to  $C_{12}$ . Kowblansky (1985) proposed that, when amylose complexes are formed with an anionic complexing group, the complex can be considered a pseudopolyelectrolyte and, as such, its behavior is dominated by electrostatic effects. If this principle is applied to amylopectin-lipid complexes, one can imagine that electrostatic repulsion between the head groups could assist in destabilizing amylopectin crystallites (Evans 1986).

### Influence of Starch Type on Complexation

Figure 2 presents the DSC scans taken during reheating and shows the melting of amylose-lipid complexes. Waxy maize starch, as expected, did not produce an endotherm during reheating. The shapes of the endotherms for common, *ae du*, and *ae su2* maize starches are similar, which suggests that the complexes of these three starches are somewhat similar, as is also evident in the data in Table I.  $T_{pC}$  values for the three starches are quite similar when compared at a constant surfactant-to-starch molar ratio. The deviations are greater with DOTAB because of the sensitivity of concentration dependence. Note that, at a molar ratio of zero, the values reported are from complexes with native starch lipids only (i.e., phospholipids and free fatty acids).

If the saturation point is taken as a molar ratio of  $\approx 0.054$ , the  $\Delta H_C$  values obtained for the three starches with SDS were proportional to amylose contents determined by gel filtration (Ikawa et al 1981). The comparison did not hold for DOTAB, probably because saturation did not exist judging from  $\Delta H_C$  and, possibly,  $T_{pC}$  values. The differences seen between the enthalpies of DOTAB complexes with *ae du* and *ae su2* maize starches at various molar ratios of DOTAB are interesting and could be an indication of structural differences between the granules of these two genetic variants, although both starch types have similar amylose contents.

A strong correlation exists between lipid and amylose contents (South et al 1991), and methods for the quantitation of amylose by saturating it with lysolecithin and determining the enthalpy of the resultant complex have been reported (Siefert and Holm 1993, Mestres et al 1996). It is interesting to note that the  $\Delta H_C$  values found in this study were proportional to the percentages of amylose present in the starches (Ikawa et al 1981), without consideration of the amounts of intermediate fraction (defined by Ikawa et al (1981) as iodine complexes having  $\lambda_{max}$  between 600 and 620 nm). Data for *ae su2* maize starch, which contains a significant amount (8.3%) of intermediate fraction, could indicate that the intermediate fraction is not taking part in lipid-complexation.

In addition, it was found qualitatively that the material leached from *ae su2* and *ae du* maize starches at 50, 60, and 70°C ( $2 \times 1$ -hr extractions in  $H_2O$ ) stained purple in the presence of  $KI/I_2$ , indi-

**TABLE II**  
Gelatinization Events for Starches from Maize Genotypes  
in Excess Water Without Added Surfactants<sup>a</sup>

Genotype	<i>n</i>	$\Delta H_G$ (J/g)	$T_{oG}$ (°C)	$T_{pG}$ (°C)
Common	2	15.7 ± 0.5	64.3 ± 0.2	70.2 ± 0.1
waxy	3	17.9 ± 0.4	65.7 ± 0.0	71.4 ± 0.3
<i>ae du</i>	2	16.4 ± 0.7	61.8 ± 0.0	69.6 ± 0.7
<i>ae su2</i>	4	14.0 ± 0.4	63.8 ± 0.2	72.1 ± 0.9

<sup>a</sup> Enthalpy of gelatinization ( $\Delta H_G$ ) (including shoulders if applicable), gelatinization endotherm onset temperature ( $T_{oG}$ ), and gelatinization endotherm peak temperature ( $T_{pG}$ ). Values ± standard deviations.

cating that at least a significant portion of the leached material had a higher degree of branching; the same material from common corn starch stained blue.

### Influence of Lipid Type on Complexation

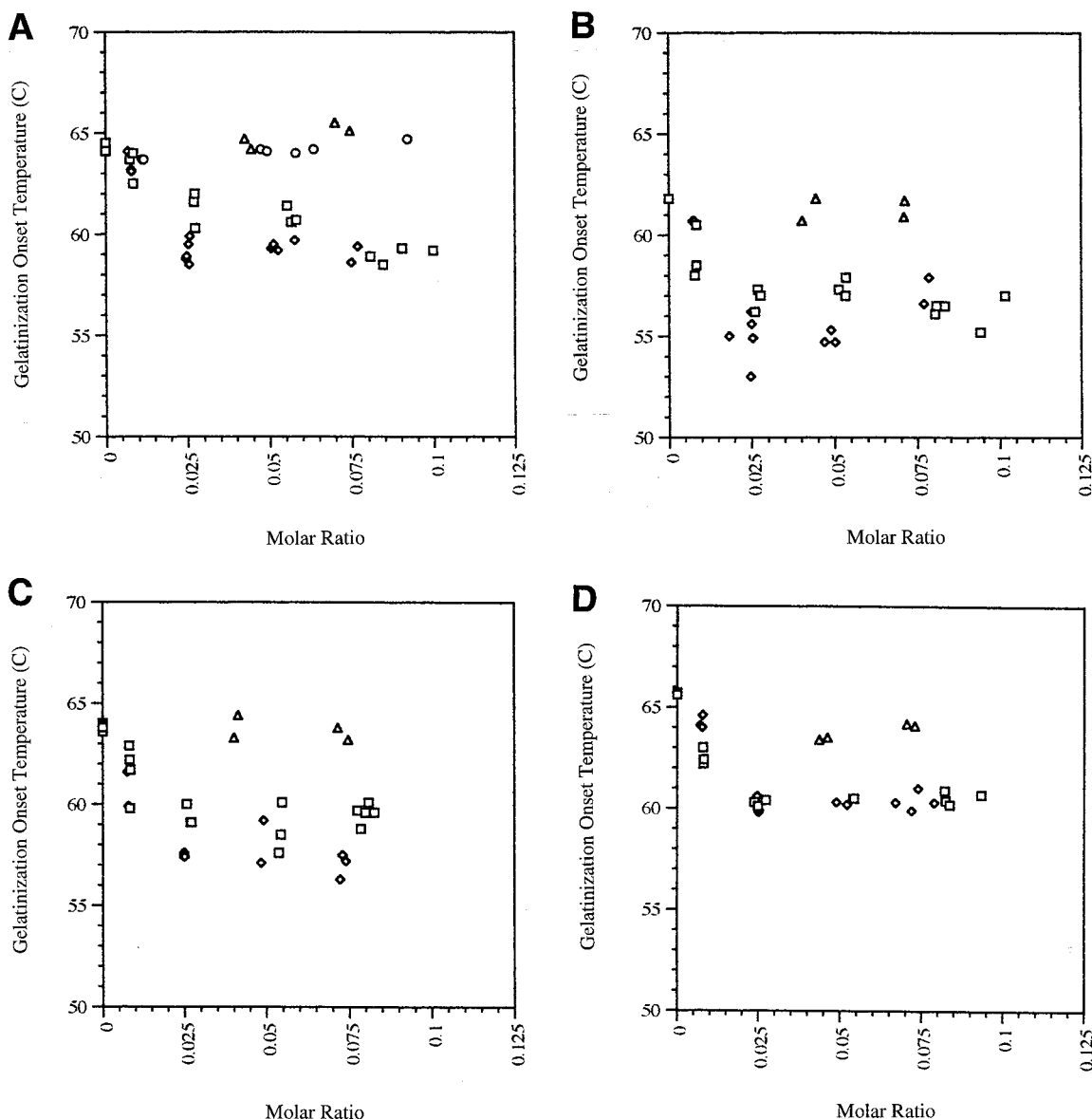
Mixtures containing SDS yielded complexes with high enthalpies and sharp peaks. In contrast, the DOTAB complex endotherms were shallow and not well defined. When DOTAB was present, the complex melting peak ( $T_{pc}$ ) decreased dramatically as DOTAB concentration increased to a value much lower than the 90–120°C range where most starch-lipid complexes are expected to melt (Table I).

Certain ions are particularly disruptive toward the crystalline regions of granular starch, namely  $I^-$ ,  $Br^-$ , and  $CNS^-$ , whereas  $SO_4^{2-}$  and most cations help, to some degree, to maintain granule integrity (Villwock 1996). Biliaderis and Seneviratne (1990) demonstrated that this is also true for amylose-monostearate complexes in the presence of various salts. So it is not surprising that DOTAB, which contributes destabilizing bromide ions and a not-so stabilizing trimethylammonium group, destabilizes granules and amylose-lipid complexes. Alternatively, SDS contributes a somewhat stabilizing sodium ion and a very stabilizing sulfate half-ester group.

The Biliaderis and Seneviratne (1990) results may explain the trend observed with DOTAB. However, the magnitude of destabilization found here was much greater when data of the two studies were compared at equimolar concentrations. A plausible explanation is that the bromide ion, due to its inherent affiliation to the head group of the surfactant because of the requirement for ion pairing, is closely associated with the overall complex, bringing about greater instability of the complex. Reductions in  $T_{pc}$  with increasing DOTAB concentration were linear in nature ( $R > 0.98$ ). The slopes of the regression lines were  $-2.15$ ,  $-1.86$ , and  $-1.96$  for common, *ae su2*, and *ae du* maize starches, respectively.

Although at 10% concentration, saturation is usually achieved for most surfactants, DOTAB systems resulted in continuously larger  $\Delta H_c$  and decreasing  $T_{pc}$  values, at least up to  $\approx 15\%$  DOTAB. So it appears that DOTAB may be driven to some degree by a concentration gradient to form complexes and, as DOTAB concentration increases, the complexes become more unstable.

This instability could be produced from either the ionic conditions resulting from excess DOTAB in solution, or from the greater difficulty of packing of amylose-lipid complex helices due to like-charge repulsion as the amylose becomes more saturated with complexes.



**Fig. 4.** Gelatinization onset temperature ( $T_{og}$ ) as a function of molar ratio of surfactant to dry maize starch. **A**, common; **B**, *ae du*; **C**, *ae su2*; **D**, waxy.  $\square$  = SDS;  $\diamond$  = dodecyltrimethylammonium bromide (DOTAB);  $\circ$  = 1-monolauroyl-*rac*-glycerol (ML);  $\Delta$  = sodium hexadecyl sulfate (SHS).

Kowblansky (1985) reported that reducing the hydrocarbon chain length reduces the complex melting temperature and that the effect is accentuated with greater ionic character of the surfactant head group as it effectively reduces the length of the nonpolar tail that participates in the complex. That study, which used concentrations much greater than those used in this work, did not find any effects due to concentration of complexing agent.

## CONCLUSIONS

The nature of starch-lipid complexation varied considerably between surfactants, whereas differences between starches were less easily discernible. Ionic surfactants, SDS and DOTAB, lowered the gelatinization onset temperature, possibly through an interaction with amylopectin because the lowering occurred in waxy maize starch. Comparison of decreases in  $\Delta H_G$  could provide useful information that could be related to starch granule structure if interference from overlapping amylose-lipid complex endotherms is eliminated. SDS tends to give the largest decrease in  $\Delta H_G$  values, whereas longer chain or neutral surfactants give much smaller decreases. Higher concentrations of DOTAB destabilized complexes, as indicated by dramatic reductions of  $T_{pc}$  as DOTAB concentration increased. This could be due to actual properties of the amylose-DOTAB complex or to the nature of the surrounding ionic media or both. Exotherms produced by starch-lipid complexation could, in some cases, be determined by subtracting DSC curves. Accurate determination of this exotherm could be valuable in characterizing structural differences between amylopectin-type starch polymers, but overlapping endotherms of amylose-lipid complexes makes it difficult in other cases.

## LITERATURE CITED

American Association of Cereal Chemists 1995. Approved Methods of the AACC, 9th ed. Method 44-19, approved April 1961, revised Oct. 1975, reviewed Oct. 1982. The Association: St. Paul, MN.

BeMiller, J. 1997. Starch modification: Challenges and prospects. *Starch/Staerke* 49:127-131.

Biliaderis, C., and Seneviratne, H. 1990. Solute effects on the thermal stability of glycerol monostearate-amylose complex superstructures. *Carbohydr. Res.* 208:199-213.

Eliasson, A.-C. 1985. Starch gelatinization in the presence of emulsifiers. A morphological study of wheat starch. *Starch/Staerke* 12:411-415.

Eliasson, A.-C. 1986. On the effects of surface active agents on the gelatinization of starch—A calorimetric investigation. *Carbohydr. Polym.* 6:463-476.

Eliasson, A.-C. 1994. Interactions between starch and lipids studied by DSC. *Thermochim. Acta* 246:343-356.

Eliasson, A.-C., Finstad, H., and Ljunger, G. 1988. A study of starch-lipid interactions for some native and modified maize starches. *Starch/Staerke* 40:95-100.

Eliasson, A.-C., and Ljunger, G. 1988. Interactions between amylopectin and lipid additives during retrogradation in a model system. *J. Sci. Food Agric.* 44:353-361.

Evans, I. 1986. An investigation of starch/surfactant interactions using viscosimetry and differential scanning calorimetry. *Starch/Staerke* 38:227-235.

Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A.-C., and Åman, P. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydr. Polym.* 35:119-134.

Friedman, R., Gottneid, D., Faron, E., Pustek, F., and Katz, F. 1988. Food stuffs containing starch of an amylose extender dull genotype. U.S. patent 4,790,997.

Friedman, R., Gottneid, D., Faron, E., Pustek, F., and Katz, F. 1989. Foodstuffs containing starch of an amylose extender sugary-2 genotype. U.S. patent 4,798,735.

Gough, B., Greenwell, P., and Russell, P. 1985. On the interaction of sodium dodecyl sulphate with starch granules. Pages 99-108 in: *New Approaches to Research on Cereal Carbohydrates*. R. Hill and L. Munck, eds. Elsevier: Amsterdam.

Gunnlaugsdottir, H., and Sivik, B. 1995. Lipase-catalyzed alcoholysis of cod liver oil in supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* 72:399-405.

Huang, J., and White, P. 1993. Waxy corn starch: monoglyceride interaction in a model system. *Cereal Chem* 70:42-47.

Ikawa, Y., Glover, D., Sugimoto, Y., and Fuwa, H. 1981. Some structural characteristics of starches of maize having a specific genetic background. *Starch/Staerke* 33:9-13.

Inouchi, N., Glover, D., Sugimoto, Y., and Fuwa, H. 1991. DSC characteristics of gelatinization of starches of single-, double-, and triple-mutants and their normal counterpart in the inbred Oh43 maize (*Zea mays* L.) background. *Starch/Staerke* 43:468-472.

Katz, F., Furcsik, F., Tenbarga, F., Hauber, R., and Friedman, R. 1993. Behavior of starches derived from varieties of maize containing different genetic mutations: effects of starch genotype on granular morphology. *Carbohydr. Polym.* 21:133-136.

Kowblansky, M. 1985. Calorimetric investigation of inclusion complexes of amylose with long-chain aliphatic compounds containing different functional groups. *Macromol.* 18:1776-1779.

Kugimiya, M., Donovan, J., and Wong, R. 1980. Phase transitions of amylose-lipid complexes in starches: A calorimetric study. *Starch/Staerke* 8:265-270.

Larsson, K., and Quinn, P. 1994. Physical properties: Structural and physical characteristics. Pages 401-485 in: *Lipid Handbook*, 2nd ed. F. Gunstone, J. Harwood and F. Padley, eds. Chapman and Hall: New York.

Larsson, K., Gabrielsson, K., and Lundberg, B. 1978. Phase behaviour of some aqueous systems involving monoglycerides, cholesterol and bile acids. *J. Sci. Fd. Agric.* 29:909-914.

Mestres, C., Matencio, F., Pons, B., Yajid, M., and Fliedel, G. 1996. A rapid method for the determination of amylose content by using differential scanning calorimetry. *Starch/Staerke* 48:2-6.

Mikus, F., Hixon, R., and Rundle, R. 1946. The complexes of fatty acids with amylose. *J. Am. Chem. Soc.* 68:1115-1123.

Morrison, W., and Coventry, A. 1985. Extraction of lipids from cereal starches with hot aqueous alcohols. *Starch/Staerke* 37:83-87.

Obanni, M., and BeMiller, J. 1995. Identification of starch from various maize endosperm mutants via ghost structures. *Cereal Chem.* 72:436-442.

Obanni, M., and BeMiller, J. 1997. Properties of some starch blends. *Cereal Chem* 74:431-436.

Padley, F., Gunstone, F., and Harwood, J. 1994. Cereal lipids. Occurrence and characteristics of oils and fats. Pages 47-223 in: *Lipid Handbook*, 2nd ed. F. Gunstone, J. Harwood, and F. Padley, eds. Chapman and Hall: New York.

Sievert, D., and Holm, J. 1993. Determination of amylose by differential scanning calorimetry. *Starch/Staerke* 45:136-139.

Small, D. 1986. The physical chemistry of lipids. Pages 90-91 in: *Handbook of Lipid Research*. Vol. 4. D. Hanahan, ed. Plenum Press: New York.

South, J., Morrison, W., and Nelson, O. 1991. A relationship between the amylose and lipid contents of starches from various mutants for amylose content in maize. *J. Cereal Sci.* 14:267-278.

Svensson, E., Autio, K., and Eliasson, A.-C. 1998. The effect of sodium dodecyl sulphate on gelatinization and gelation properties of wheat and potato starches. *Food Hydrocoll.* 12:151-158.

Svensson, E., Gudmundsson, M., and Eliasson, A.-C. 1996. Binding of sodium dodecylsulphate to starch polysaccharides quantified by surface tension measurements. *Colloids Surfaces B* 6:227-233.

Tan, S., and Morrison, W. 1979. The distribution of lipids in the germ, endosperm, pericarp and tip cap of amylomaize, LG-11 hybrid maize and waxy maize. *J. Am. Oil Chem. Soc.* 56:531-535.

Villwock, V. 1996. Role of salts in starch modification. MS thesis. Purdue University: West Lafayette, IN.

Wang, G., and Olofsson, G. 1995. Ethyl(hydroxyethyl)cellulose and ionic surfactants in dilute solution calorimetric and viscosity study of the interaction with SDS and some cationic surfactants. *J. Phys. Chem.* 99:5588-5596.

Wang, Y.-J., White, P., and Pollak, L. 1993. Physicochemical properties of starches from mutant genotypes of the Oh43 inbred line. *Cereal Chem.* 70:199-203.

Yeh, J., Garwood, D., and Shannon, J. 1981. Characterization of starch from maize endosperm mutants. *Starch/Staerke* 33:222-230.

[Received July 7, 1998. Accepted December 17, 1998.]