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Potential sources of error in the calorimetric evaluation of amylose content of starches

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Abstract

The L-α-lysophosphatidylcholine calorimetric method for determining amylose content of starches was used to characterize an amylose fraction derived from common maize starch using an aqueous leaching process. The enthalpy of amylose-LPC complexation and dissolution was significantly greater for the leached maize starch fraction compared with that for potato amylose, leading to erroneously high values for amylose content when potato amylose was used as the reference standard. This was not likely caused by a difference in molecular weight as previously proposed, but possibly to differences in degree of branching. A spherulitic morphology was observed after rapid cooling from 180 °C at low LPC concentrations, but its formation was inhibited at concentrations of lipid typically used in the LPC test. The mechanism of spherulite formation during rapid cooling in the absence of lipid appears to be different than that observed on slow cooling in the presence of lipid.

Keywords: Differential scanning calorimetry; Amylose; L-α-Lysophosphatidylcholine; Spherulites

1. Introduction

The molecular structure of starch, particularly the relative ratio of nearly linear amylose to highly-branched amylopectin, plays a decisive role in its nutritional and technological functionality. Structural features such as molecular weight and degree of branching depend on botanical origin and may alter the determination of amylose content, the precise value of which depends on the method used to quantify it. The relative proportion of amylose in native starches ranges from 0% in waxy varieties to 80% in wrinkled pea (Gerard, Barron, Colonna, & Planchot, 2001). The presence of "intermediate" material, i.e., lightly branched amylose or atypical amylopectin with long branches, is troublesome since it cannot be clearly differentiated as amylose or amylopectin, and may be assigned to either category depending on the characterization method employed. Intermediate fractions are more prevalent in starches of atypical genotype (Klucinec & Thompson, 2002). Gerard et al. (2001) compared methods for determining amylose content of mutant genotype starches, and concluded that only size exclusion chromatography gave accurate results.

Native starches exhibit complex thermal behavior that depends on their moisture content and botanical origin. A reversible endothermic transition is often observed for lipid-containing cereal starches, which is assigned to the melting of helical amylose–lipid inclusion complexes. This has become the basis of a relatively straightforward, rapid, and quantitative procedure for evaluating amylose content vis-à-vis amylopectin for starches and starch-containing materials (Kugimiya & Donovan, 1981; Sievert & Holm, 1993) that does not require defatting prior to analysis (Mestres, Matencio, Pons, Yajid, & Fliedel, 1996). However, discrepancies are

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often noted when comparing the amylose content determined by thermal analysis of lipid complexes to that obtained by other methods, especially when contrasting potato starches to cereal starches (Gerard et al., 2001).

The calorimetric method for determining percent amylose relies on the assumption that the only enthalpy measured is due to formation of amylose–lipid inclusion complexes. However, these complexes may exist in several forms (Biliaderis, Page, Slade, & Sirett, 1985), and under thermal conditions similar to those used in the L-α-lysophosphatidylcholine (LPC) – differential scanning calorimetry (DSC) method (Sievert & Holm, 1993), we have observed crystallization of starch into the B-allomorph with a spherulitic morphology (Nordmark & Ziegler, 2002a, 2002b). These present potential confounding factors when utilizing the LPC–DSC method, especially since the ability of starch to form spherulites varies with botanical source (Ziegler, Nordmark, & Woodling, 2003). The present study was undertaken to resolve some of the discrepancies in the reported findings of the LPC–DSC method and to more fully understand the possible role of lipid in the formation of starch spherulites.

2. Materials and methods

2.1. Materials

Potato amylose (>99% pure, A-0512), potato amylopectin (10118, Fluka), d_6-DMSO (151874), DMSO (471267) and L-α-lysophosphatidylcholine (L4129, type I, from egg yolk, 99%) were purchased from Sigma Aldrich Corp. (St. Louis, MO). Common maize starch was supplied by the National Starch and Chemical Corporation Food Products Division (Bridgewater, NJ). USP ethyl alcohol was obtained from Pharmco (Brookfield, CT).

2.2. Methods

2.2.1. Amylose fractionation

Amylose was leached from a 4% (w/v) suspension of common maize starch using distilled water at 75 °C with gentle stirring for 45 min (Mua & Jackson, 1995). The suspension was centrifuged at 2950*g* and the supernatant reserved. Starch granules were re-suspended and leached two more times, after which 1.5 vol. of ethanol was added to the combined supernatants, and the resulting precipitate allowed to form overnight at 20 °C. The ethanol was decanted and the precipitate dried at 50 °C. The final yield of precipitate was approximately 6% of the dry weight of the parent starch, with a moisture content of 12.5% (w/w), and essentially lipid-free.

2.2.2. Amylose characterization

The precipitate was characterized using ^{1}H and ^{13}C NMR. Approximately 30 mg of precipitate was dissolved in 1.5 mL of d_6-DMSO using a boiling water bath. The ^{1}H and ^{13}C NMR experiments were performed at 500.13 and 125.76 MHz, respectively, on a Bruker AMX2-500 spectrometer operating in the quadrature mode at 60 °C. ^{1}H and ^{13}C NMR were referenced indirectly to tetramethylsilane. The generated spectra were compared to previously published starch NMR data (Falk, Micura, Stanek, & Wutka, 1996; Falk & Stanek, 1997). This spectra confirmed the precipitate as amylose.

Intrinsic viscosity in DMSO at 25 °C (±1.0 °C) was determined using a Cannon-Ubbelohde dilution viscometer (#50, Cannon Instrument Co., State College, PA). Four concentrations were measured in the range 0.001–0.005 g/mL. Efflux times were recorded in triplicate using a digital stopwatch (Traceable®, VWR International, West Chester, PA). Mark–Houwink–Sakurada values (α and *K*) of Banks and Greenwood (1968) (0.70 and 0.0151, respectively) and Everett and Foster (1959) (0.64 and 0.0306, respectively) for amylose in DMSO were both used to calculate the molecular weight and the results averaged (Gidley & Bulpin, 1989).

2.2.3. Differential scanning calorimetry

Samples were prepared for differential scanning calorimetry as detailed by Sievert and Holm (1993). Potato amylose and amylopectin were used to construct a standard curve by combining them in high-volume (60 μL) sealed stainless-steel DSC pans (Perkin–Elmer Instruments, Norwalk, CT) in ratios from 0 to 100% amylose to a total of approximately 10 mg dry matter with 50 μL of a 3% (w/v) α-L-lysophosphatidylcholine (LPC) solution. Samples were prepared using a Perkin–Elmer DSC 7, operated by Pyris software (Perkin–Elmer Instruments, Norwalk, CT) by heating from 20 to 180 °C at 5 °C/min, cooling at 10 °C/min to 4 °C, holding for 15 min, and reheating to 180 °C at 5 °C/min (Sievert & Holm, 1993). The enthalpy of both amylose lipid complexation (exothermic on cooling) and dissolution (endothermic on reheating) was determined based on the dry matter in each sample using the Pyris software (Perkin–Elmer). Dissolution enthalpy was measured between 80 and 140 °C (or 160 °C for samples containing $\leqslant$5% lipid). Complexation enthalpy was measured between 70 and 100 °C. Tests were performed in duplicate. Evaluation of dissolution enthalpy as a function of lipid content was performed on a Thermal Advantage Q100 DSC using Thermal Advantage Universal Analysis software (TA Instruments, New Castle, DE). Samples were prepared and analyzed as above except that starch, lipid and water were weighed separately into the pan and the samples stored at 20 °C overnight prior to reheating. Both instruments were calibrated with indium and an empty pan was used as the reference.

2.2.4. Microscopy

After cooling the samples to 4 °C at 10 °C/min, the morphology of the resulting material was viewed using bright-field and polarized light microscopy on an Olympus BX-41 microscope (Hitech Instruments, Edgemont, PA) equipped with a SPOT Insight QE camera and analysis completed using SPOT analytical and controlling software (SPOT Diagnostic Instruments, Sterling Heights, MI).

3. Results and discussion

NMR revealed both potato amylose and leached maize fraction to be essentially free of branching by the absence of a peak at 70 ppm (Fig. 1) that is typical of starch containing 1–6 branch points (Falk et al., 1996). Benesi and Brant (1985) assigned a peak at 70.4 ppm to carbon 4 of the glucose bound at carbon 6 of the 1–6 branch points of pullulan. Therefore, we conclude that the leached maize fraction is essentially amylose, free of amylopectin. The viscosity-average molecular weights and degrees of polymerization (DP) of leached maize amylose and potato amylose were approximately 1.5×10^5 (DP = 920), and 1.55×10^5 (DP = 965), respectively, which are on the low end of the molecular weight distributions expected for amylose.

Following the procedure of Sievert and Holm (1993), a calibration curve (Fig. 2) for determination of amylose content was constructed from mixtures of potato amylose and amylopectin. Consistent with Mestres et al. (1996), the exothermic response was less variable ($r^2 = 0.9967$) than the endothermic response ($r^2 = 0.9624$), though the slopes were nearly identical, 0.177 ± 0.003 and 0.178 ± 0.01, respectively. Using this relationship, the amylose content of the parent common maize starch was determined to be 26%. The enthalpy of complexation for the leached maize amylose was found to be 21.7 ± 0.7 J/g, or about 15% higher than that for potato amylose (18.7 J/g). The LPC–DSC method has been shown previously to over-predict amylose content for both maize and wheat starches (Gerard et al., 2001; Kugimiya & Donovan, 1981; Mestres et al., 1996). While such behavior was not observed by Sievert and Holm (1993), they did report erroneously low amylose contents for their potato starches, which they were unable to explain.

Although Mestres et al. (1996) reported that a phospholipid:starch ratio of 1:10 was sufficient to saturate amylose, Kugimiya and Donovan (1981) reported earlier that the enthalpy of complex dissolution continues to increase up to a ratio of 2:10. Since we employed a LPC:starch ratio of 1.5:10, we first hypothesized that the potato starch was undersaturated with respect to the lipid content vis-à-vis the leached maize amylose. Therefore, we measured the endothermic response on reheating of the two amylose samples as a function of lipid content (Fig. 3). Saturation does occur near a lipid:starch ratio of 1.5:10 for both materials, but the leached maize starch fraction exhibited a much higher enthalpic response at all lipid concentrations. Gerard et al. (2001) ruled out complexation of LPC by long amylopectin chains as an explanation for the over-prediction of amylose content in maize and wheat starches by this approach, as well as the presence of endogenous lipids. Instead they suggested that a difference in molecular weight could explain their findings, since Kugimiya and Donovan (1981) reported that a sample of relatively low molecular

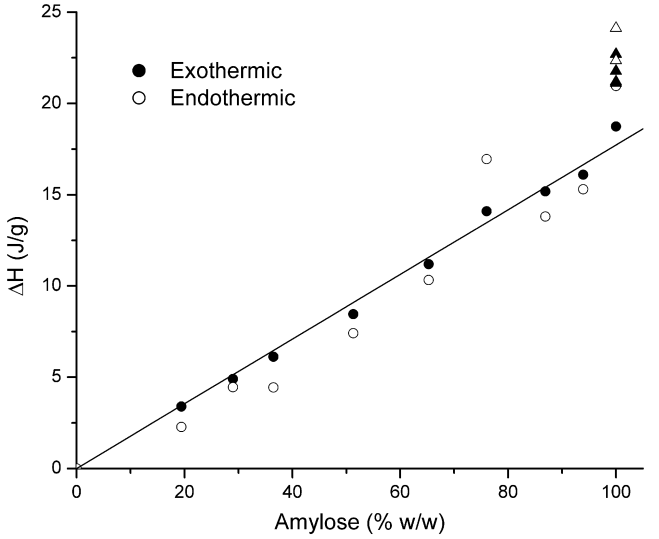

Fig. 2. Standard curve generated from DSC analysis of potato amylose and amylopectin mixtures with excess 3% α-L-lysophosphatidylcholine solution. Closed symbols, exotherm; open symbols, endotherm; Cirlces, potato starch; triangles, leached maize amylose. The line represents a linear regression of the exotherms of potato amylose.

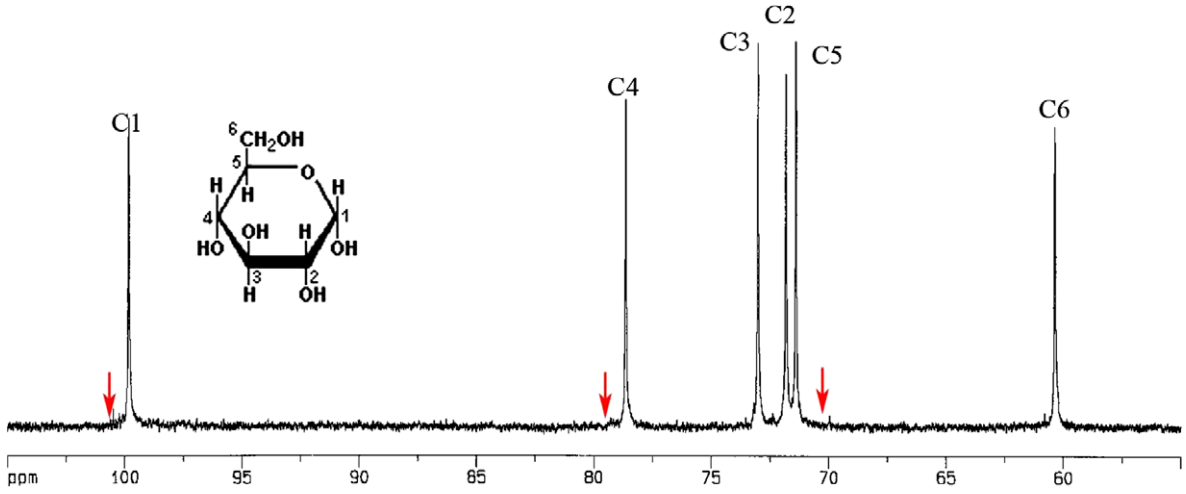

Fig. 1. ^{13}C NMR spectra of leached maize amylose in d_6-DMSO. Red arrows indicate location of peaks expected for branched starch (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

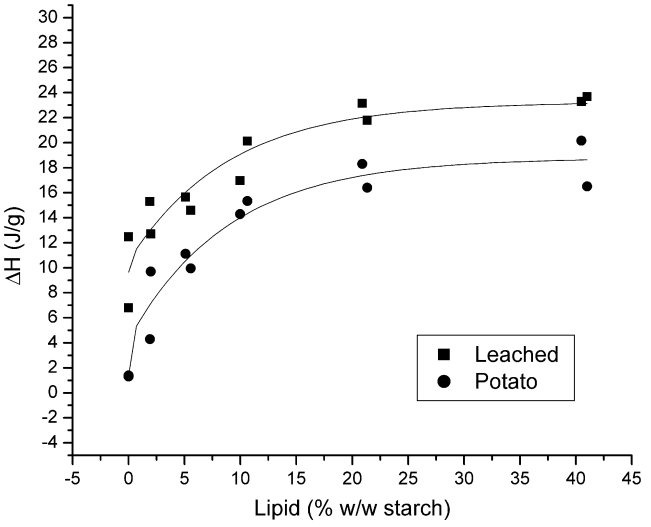

Fig. 3. Dissolution enthalpy for potato amylose and leached maize amylose as a function of LPC concentration. Lines represent an exponential association model for two components.

weight amylose (DP ≈ 300, botanical origin unspecified) yielded about 25% greater dissolution enthalpy than a higher molecular weight (DP > 900, potato) amylose. However, the difference in average molecular weight between the potato and leached maize amylose used in our study is small, although we cannot completely rule out the possibility that the molecular weight distribution of the leached maize amylose contains a relatively low molecular weight component.

Regardless of whether amylose content is derived from a standard curve or referenced to the enthalpy of a "pure" sample, a reference material is required. This has typically been potato amylose, readily available from commercial suppliers. In retrospect, this is an unusual choice, since potato starch contains little native lipid and is phosphorylated (Hoover, 2001). While Kugimiya and Donovan (1981) suggest that LPC, being positively charged, is probably helpful in formation of complexes with starches containing negatively charged phosphate groups, it is equally likely that the interaction of the charged groups may interfere with the incorporation of the lipid into the starch helix, either sterically or by distorting the conformation necessary to exclude the polar head group due to electrostatic attraction. However, it appears that starch phosphate is associated with the portion of the amylopectin present in the amorphous lamellae of the semi-crystalline rings of starch granules (Blennow, Bay-Smidt, Olsen, & Moller, 2000), and therefore would not influence the reaction of lipids with amylose. Furthermore, this would not explain why potato amylose is apparently an appropriate reference for the numerous other starches analyzed by the LPC–DSC method, unless perchance the amylose of those starches contained branches to an extent similar to the degree of phosphorylation of potato amylose.

Gerard et al. (2001) concluded that the DSC technique was more sensitive to branch points than the iodine binding capacity, suggesting that branches interfere with amylose–lipid association. Perhaps it is also more sensitive than ^{13}C NMR. Potato amylose contains from approximately 4–6 branch points/molecule (Hoover, 2001), while no detectable branching was found for amylose samples prepared from pea starch by aqueous leaching at temperatures between 60 and 80 °C (Ring, l'Anson, & Morris, 1985). It is possible that the discrepancy between the LPC binding of leached corn amylose and potato amylose is due to different extents of branching.

Stoichiometric ratios were calculated for both amylose samples from the maximum binding ratio, which was determined from the data in Fig. 3 (per Kugimiya & Donovan, 1981), to be 0.14 for leached maize amylose and 0.1 for the potato amylose. Assuming a molecular weight of 483 g/gmol for LPC and using the molecular weights for amylose determined by viscosity, leached amylose contained 43 moles of LPC per mole of starch, while potato amylose contained 32 moles of LPC per mole of amylose. When divided by the degree of polymerization, these result in a value of 21 glucose residues per molecule of LPC for leached amylose and 30 glucose residues per molecule of LPC for potato amylose. V_h amylose, characteristic of amylose–lipid complexes, corresponds to a left-handed single helix of 6 glucose residues per turn of pitch 0.806 nm (Nuessli, Putaux, LeBail, & Buléon, 2003). Based on the stoichiometric calculations above this would imply approximately two-three turns for each complex at saturation (leaving some room for non-helical flexible segments between complexes), which would correspond to a helix length of about 1.6–2.4 nm. For lipid complexes with DP 30 amylose, Godet, Bouchet, Colonna, Gallant, and Buléon (1996) observed crystal lamella thickness of 1.6 nm, in close agreement with our results.

If the presence of branch points reduces starch–lipid interactions (as it apparently does), then we would expect branched material to saturate at a lower lipid concentration and the enthalpy of dissolution at saturation to be lower vis-à-vis unbranched starch. This would result in figures similar to Figs. 2 and 3 of Kugimiya and Donovan (1981), who demonstrated that the enthalpy of dissolution on the first heating, where there was incomplete complexation, was lower than that observed on reheating. Sievert and Holm (1993) observed incomplete complexation and hence lower enthalpy when starch was initially heated to temperatures below 170 °C. In any case, 0% added lipid should yield no enthalpy of complexation for starches containing no endogenous lipids. However, as seen in Fig. 3 a finite enthalpy is observed for both amyloses in the absence of LPC, particularly the leached maize amylose.

In the case of excess LPC, the endotherm for the maize amylose–lipid complex appeared at a temperature of ~106 to 108 °C with a slight shoulder on the lower temperature side of the endotherm (Fig. 4). As lipid content decreased, a broad shallow endotherm appeared with a peak near 135 °C (Fig. 4). Similar results were obtained for potato amylose, although the peak appeared at 110–112 °C and the overall

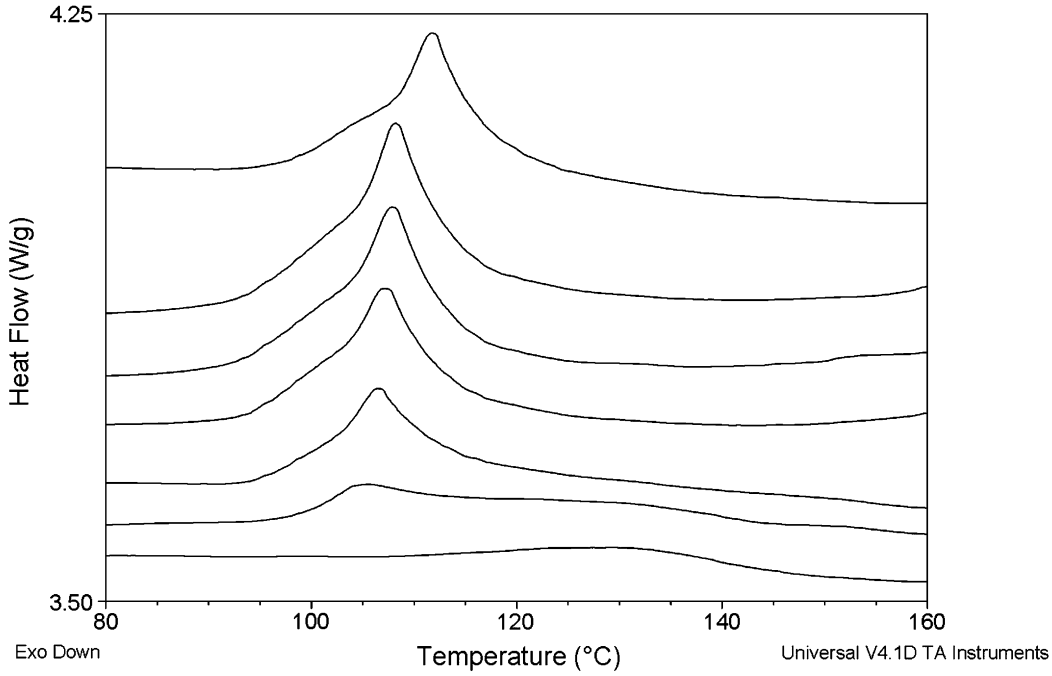

Fig. 4. DSC thermograms for the dissolution of leached maize amylose-LPC complex at varying lipid contents. Lipid concentration 0, 2, 5, 10, 20 and 40% (w/w amylose), from bottom up. Thermogram for potato amylose and 40% LPC, top.

enthalpy was smaller (Fig. 4). The same biphasic nature of the transition was observed previously (Kugimiya & Donovan, 1981; Mestres et al., 1996; Sievert & Holm, 1993). Apparently at least two forms of complex exist with varying peak temperatures. We previously observed one or more endothermic transitions in samples of lipid-free starch that resulted from spherulitic crystallization (Nordmark & Ziegler, 2002a, 2002b; Ziegler, Creek, & Runt, 2005) at approximately the same temperature as that for the amylose–lipid complex, following the thermal regime proposed by Sievert and Holm (1993). We therefore undertook a morphological investigation of the potato and leached maize amylose samples in the presence and absence of LPC following heating to 180 °C and cooling at 10 °C/min to 4 °C.

Spherulites exhibiting the typical "Maltese" cross extinction pattern when viewed between crossed polarizers were observed in both maize and potato amylose samples containing no lipid (Fig. 5). Fewer were observed for potato amylose, and those that formed had a broader distribution of sizes. This is consistent with previous observations (Ziegler et al., 2003), and the idea that potato amylose may be more highly branched, since branching tends to reduce the size and extent of spherulitic crystallization (Mandelkern, Go, Peiffer, & Stein, 1977; Chowdhury, Haigh, Mandelkern, & Alamo, 1998). This also explains the presence of a non-zero intercept in Fig. 3, i.e., as the lipid content approaches zero the amylose begins to crystallize in a different form (likely the B-allomorph).

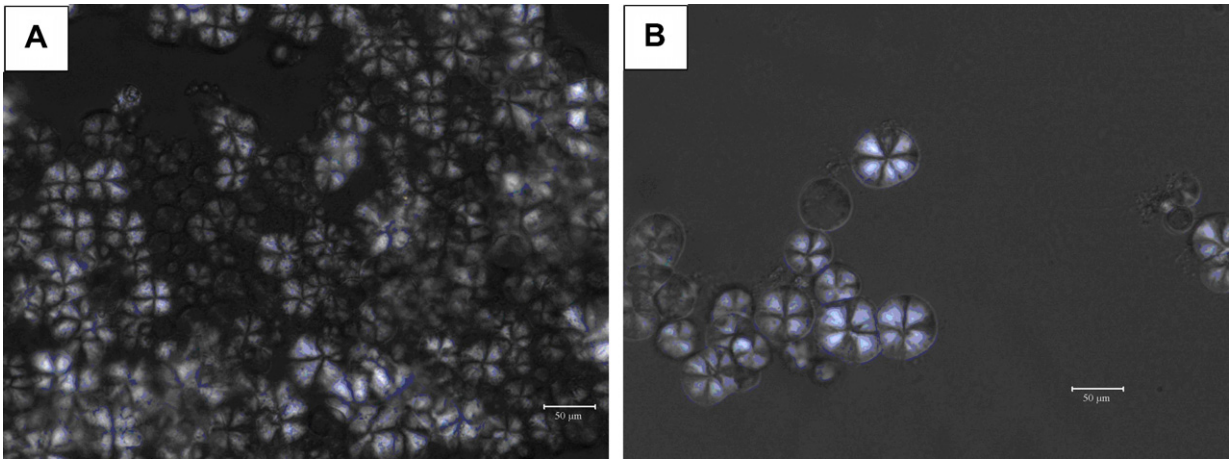

Fig. 5. The morphology of leached maize amylose (A) and commercial potato amylose (B) without added LPC between crossed polarizers. Scale bars represent 50 µm.

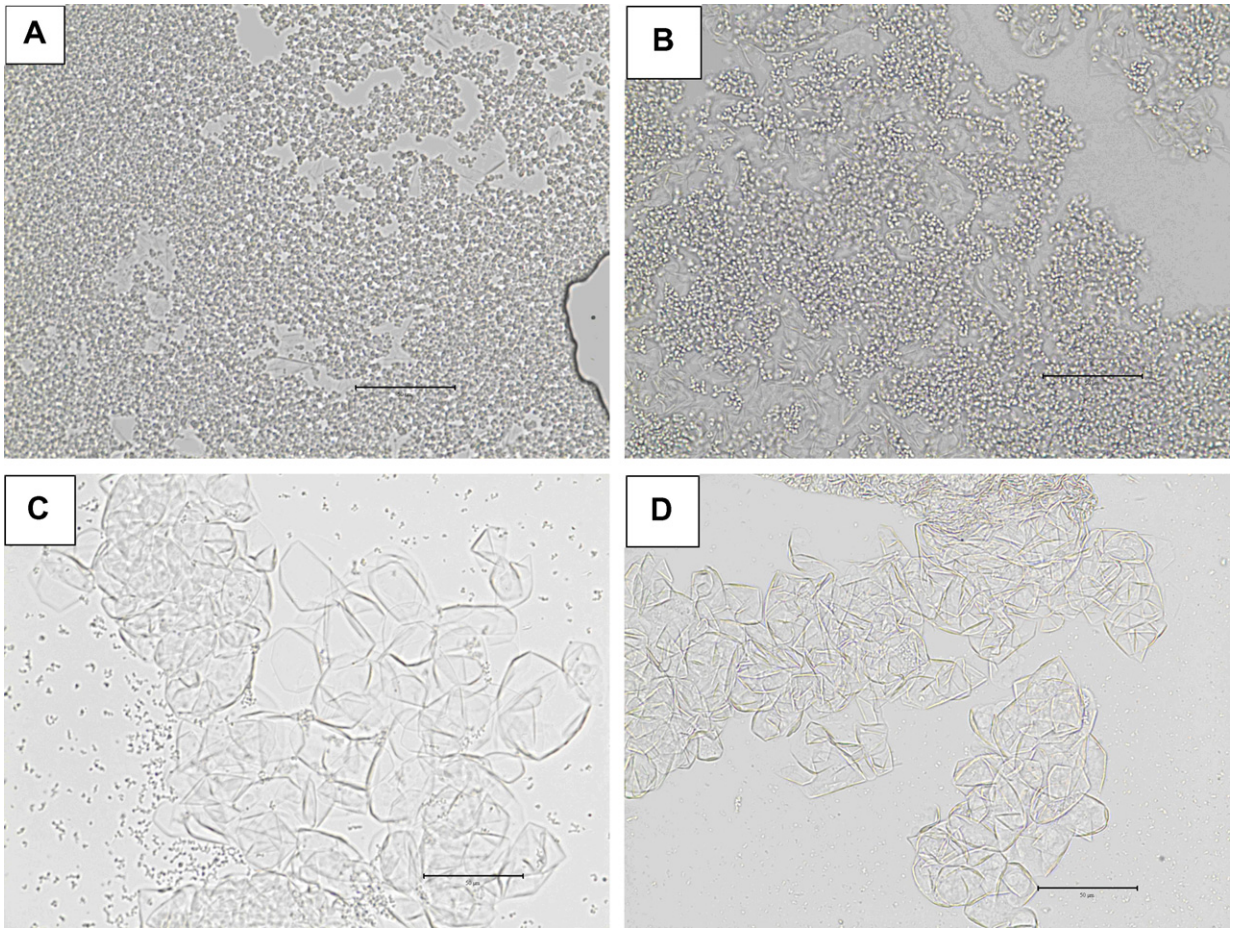

Fig. 6. Brightfield images after heating in the presence of excess LPC: 100% potato amylose (A), 90% amylose/10% amylopectin (B), 20% amylose/80% amylopectin (C), and 100% amylopectin (D). Scale bars represent 50 μm.

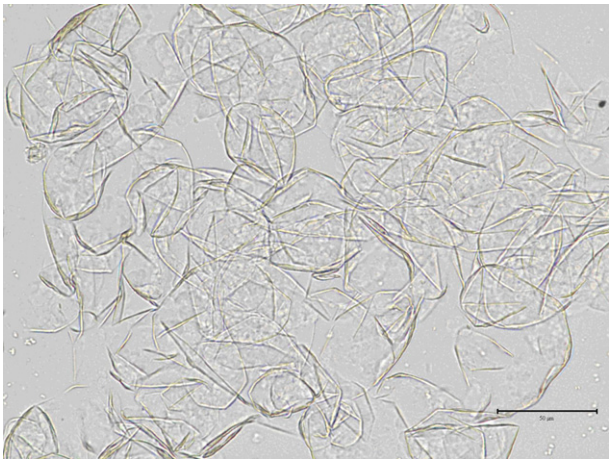

Fig. 7. Brightfield image of waxy starch after heating in the presence of excess LPC. Scale bar represents 50 μm.

No spherulites were observed in the presence of excess LPC. Instead, a fine grainy texture was formed at high amylose concentrations, while a film (or "balloon") morphology was formed at high amylopectin contents (Fig. 6). These two structures also appeared in samples of common maize starch and the relative proportion was roughly equal to the ratio of amylose:amylopectin. A similar 'balloon' morphology was also formed in samples containing granular waxy maize starch (Fig. 7). This 'balloon' morphology resembles what some have referred to as granule "ghosts," but since it formed regardless of whether the starting material was granular or not, it cannot simply be remnants of the original granular structure.

Since the melting point of the spherulitic material was higher than that of the amylose–lipid complex, the preferential crystallization of the latter must be due to the more rapid kinetics of amylose–lipid complexation vis-à-vis double helix formation (we thank the reviewer for this suggestion).

4. Conclusions

The LPC–DSC method for amylose content in starches appears to be sensitive to the degree of branching in the amylose fraction. Consequently, for accurate quantitative analysis an amylose standard of similar structure should be used. This reference material can be obtained by leaching amylose from granular starch of the appropriate botanical origin at temperatures below the gelatinizaton temperature. Spherulitic crystallization on rapid cooling appears to be inhibited by the presence of LPC. Therefore, the mechanism of spheru-

lite formation during rapid cooling appears to be different than that observed on slow cooling in the presence of lipids (Fanta, Felker, & Shogren, 2002).

Acknowledgments

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References

Banks, W., & Greenwood, C. T. (1968). The hydrodynamic behaviour of native amylose in good solvent. *Carbohydrate Research, 7*, 414–420.

Benesi, A. J., & Brant, D. A. (1985). Trends in molecular motion for a series of glucose oilgomers and the corresponding polymer pullulan as measured by ^{13}C NMR relaxation. *Macromolecules, 18*, 1109–1116.

Biliaderis, C. G., Page, C. M., Slade, L., & Sirett, R. R. (1985). Thermal behavior of amylose–lipid complexes. *Carbohydrate Polymers, 5*, 367–389.

Blennow, A., Bay-Smidt, A. M., Olsen, C. E., & Moller, B. L. (2000). The distribution of covalently bound phosphate in the starch granule in relation to starch crystallinity. *International Journal of Biological Macromolecules, 27*, 211–218.

Chowdhury, F., Haigh, J. A., Mandelkern, L., & Alamo, R. G. (1998). The supramolecular structure of ethylene–vinyl acetate copolymers. *Polymer Bulletin, 41*, 463–470.

Everett, W. W., & Foster, J. F. (1959). The conformation of amylose in solution. *Journal of the American Chemical Society, 81*, 3464–3469.

Falk, H., Micura, R., Stanek, M., & Wutka, R. (1996). Structural aspects of native and acid or enzyme degraded amylopectins – a C13 NMR Study. *Starch/Stärke, 48*, 344–346.

Falk, H., & Stanek, M. (1997). Two-dimensional ^{1}H and ^{13}C NMR spectroscopy and the structural aspects of amylose and amylopectin. *Monatshefte fur Chemie, 128*, 777–784.

Fanta, G. F., Felker, F. C., & Shogren, R. L. (2002). Formation of crystalline aggregates in slowly-cooled starch solutions prepared by steam jet cooking. *Carbohydrate Polymers, 48*, 161–170.

Gerard, C., Barron, C., Colonna, P., & Planchot, V. (2001). Amylose determination in genetically modified starches. *Carbohydrate Polymers, 44*, 19–27.

Gidley, M. J., & Bulpin, P. V. (1989). Aggregation of amylose in aqueous systems: the effect of chain length on phase behavior and aggregation kinetics. *Macromolecules, 22*, 341–346.

Godet, M. C., Bouchet, B., Colonna, P., Gallant, D. J., & Buléon, A. (1996). Crystalline amylose-fatty acid complexes: morphology and crystal thickness. *Journal of Food Science, 61*(6), 1196–1201.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers, 45*, 253–267.

Klucinec, J. D., & Thompson, D. B. (2002). Structure of amylopectins from ae-containing maize starches. *Cereal Chemistry, 79*(1), 19–23.

Kugimiya, M., & Donovan, J. W. (1981). Calorimetric determination of the amylose content of starches based on formation and melting of the amylose–lysolecithin complex. *Journal of Food Science, 46*, 765–770.

Mandelkern, L., Go, S., Peiffer, D., & Stein, R. S. (1977). Light scattering studies of mixtures and fractions of linear polyethylene. *Journal of Polymer Science, Polymer Physics Education, 15*, 1189–1198.

Mestres, C., Matencio, F., Pons, B., Yajid, M., & Fliedel, G. (1996). A rapid method for the determination of amylose content by using differential scanning calorimetry. *Starch/Stärke, 48*, 2–6.

Mua, J. P., & Jackson, D. S. (1995). Fractionation of regular corn starch: a comparison of aqueous leaching and aqueous dispersion methods. *Cereal Chemistry, 78*, 508–511.

Nordmark, T. S., & Ziegler, G. R. (2002a). Spherulitic crystallization of gelatinized maize starch and its fractions. *Carbohydrate Polymers, 49*, 439–448.

Nordmark, T. S., & Ziegler, G. R. (2002b). Structural features of non-granular spherulitic maize starch. *Carbohydrate Research, 337*, 1467–1475.

Nuessli, J., Putaux, J. L., LeBail, P., & Buléon, A. (2003). Crystal structure of amylose complexes with small lipids. *International Journal of Biological Macromolecules, 33*, 227–234.

Ring, S. G., l'Anson, K. J., & Morris, V. J. (1985). Static and dynamic light scattering studies of amylose solutions. *Macromolecules, 18*, 182–188.

Sievert, D., & Holm, J. (1993). Determination of amylose by differential scanning calorimetry. *Starch/Stärke, 45*, 136–139.

Ziegler, G. R., Creek, J. A., & Runt, J. (2005). Spherulitic crystallization in starch as a model for starch granule initiation. *Biomacromolecules, 6*, 1547–1554.

Ziegler, G. R., Nordmark, T. S., & Woodling, S. E. (2003). Spherulitic Crystallization of starch: influence of botanical origin and extent of thermal treatment. *Food Hydrocolloids, 17*, 487–494.