Ghost Microstructures of Starch from Different Botanical Sources[1]

MOHAMED OBANNI and JAMES N. BeMILLER[2]

Cereal Chem. 73(3):333–337

ABSTRACT

A technique applied to characterize the cooking and paste properties of various maize endosperm mutant starches was applied to potato, mung bean, and other cereal starches. This technique, which is based on cooking very dilute starch suspensions without shear and examination of the particulate products by light microscopy, confirmed that the pasting behavior of each starch and the nature of the discontinuous phase of the pastes formed under the conditions employed in the method are unique. Some groupings based on similar appearances of granule remnants were found to parallel the genetic background of the source plant. These groupings (wheat and barley; oats; rice; waxy rice; common maize, sorghum, popcorn, and millet; waxy maize and waxy sorghum), with respect to the cereal starches, are similar to those based on granule size and shape and the presence or absence of surface pores visible by scanning electron microscopy.

Some physicochemical properties and functional characteristics that are imparted to aqueous systems by starches and that determine their uniquenesses in application vary with the biological origin of the starch (Svegmark and Hermansson 1993). Pasting behavior is one of several factors dictating the choice of starch for use in a food product. The source of native, and some modified, granules of most common commercial starches can be determined by light microscopy (Maywald Snyder 1984). There are a few exceptions: native sorghum and maize starch granules cannot be distinguished in this way, and the waxy mutations of normal endosperm starches from cereals can be distinguished from their normal counterparts only after iodine staining. Scanning electron microscopy can also discern some differences among starches from different botanical sources (Fannon et al 1992, Jane et al 1994).

Starch granules undergo considerable changes when heated to temperatures above the gelatinization temperatures, and microstructural differences in the resulting pastes can be determined (Williams and Bowler 1982). We have recently shown that granule ghost (remnant) microstructure is a powerful tool for identifying and distinguishing between 17 genetically modified maize starches (Obanni and BeMiller 1995). Each of the 17 starches examined exhibited unique no-shear cooking behaviors and resulting paste compositions. The purpose of this investigation was to apply the method to starches from different botanical sources to determine whether starches with known differences in cooking and paste characteristics would also give unique photomicrographs that could form part of a library to correlate properties and appearances using this technique. Such a library could then be used to identify valuable new cultivars.

MATERIALS AND METHODS

Starches

Commercial wheat starch and potato starches were provided by Midwest Grain Products, Inc. (Atchison, KS) and Penwest Foods Co. (Englewood, CO), respectively. Starches from millet, normal and waxy sorghum, barley, oat, and popcorn were extracted in the laboratory by the method of Obanni and BeMiller (1995). Normal and waxy rice starches were obtained from B. O. Juliano (International Rice Research Institute, The Philippines) and from California Natural Products (Lathrop, CA). Starches from the latter source are designated Starchplus SPR (normal rice starch) and Starchplus SPW (waxy rice starch).

Sample Preparation for Light Microscopy

Starch (500 mg) was cooked in 250 ml of distilled deionized water by heating for 45 min in an autoclave (121°C, gauge pressure 20 psi [140 MPa]) under static conditions. Hot pastes were centrifuged (1,000 × g) for 2 min. The supernatant was decanted, and the pellet was washed four times with 200 ml of 95–100°C distilled, deionized water to remove any remaining solubilized amylose or amylopectin. A 100-µl suspension of the pellet was then stained with 10 µl of an iodine solution (a solution [filtered through Whatman No. 3 paper] of 5 g of I_2 and 5 g of KI dissolved in 100 ml of water). The samples were viewed under bright field with a Vanox S light microscope (Olympus Optical Co., Ltd., Tokyo) and photographed with Kodak Ektachrome 160T film.

Effect of Na^+ and Ca^{2+} Ions on Potato Starch Ghosts

To examine the effect of sodium and calcium ions on starch ghosts, potato starch was cooked as previously described in 0.1% NaCl solution and in an equimolar concentration (17 m*M*) of $CaCl_2$.

RESULTS AND DISCUSSION

Data from this investigation support the contention that the previously described method (Obanni and BeMiller 1995) can be valuable for selection of cultivars of starch-producing plants that biosynthesize starch with more desirable paste characteristics. All micrographs in Figures 1 and 2, except 2E and 2F, are of materials obtained by centrifugation of a hot paste. All collected material was washed four times with hot water. Iodine staining of all supernatants except those of waxy rice starch (Fig. 1D) and waxy sorghum starch (Fig. 2B) revealed amylose-containing particles and solution. Most ghost structures from amylose-containing starches appeared more reddish to the eye under the microscope than they do in the reproduced photographs. Since experience suggests that a small amount of blue-staining amylose can obscure the reddish brown stain of a much larger amount of amylopectin, we can conclude, as others have done (Prentice et al 1992), that these ghost structures are composed, at least primarily, of amylopectin.

Small-Grain Cereal (Wheat, Barley, Rice, and Oat) Starches

Both large and small granules of wheat starch formed granule remnants or ghosts, which appeared to be made up mostly of the

[1]Journal paper 14,700 of the Purdue University Agricultural Experiment Station.

[2]Whistler Center for Carbohydrate Research, Dept. of Food Science, Purdue Univ., West Lafayette, IN. Corresponding author. E-mail: bemiller@foodsci.purdue.edu

Publication no. C-1996-0402-07R.

© 1996 American Association of Cereal Chemists, Inc.

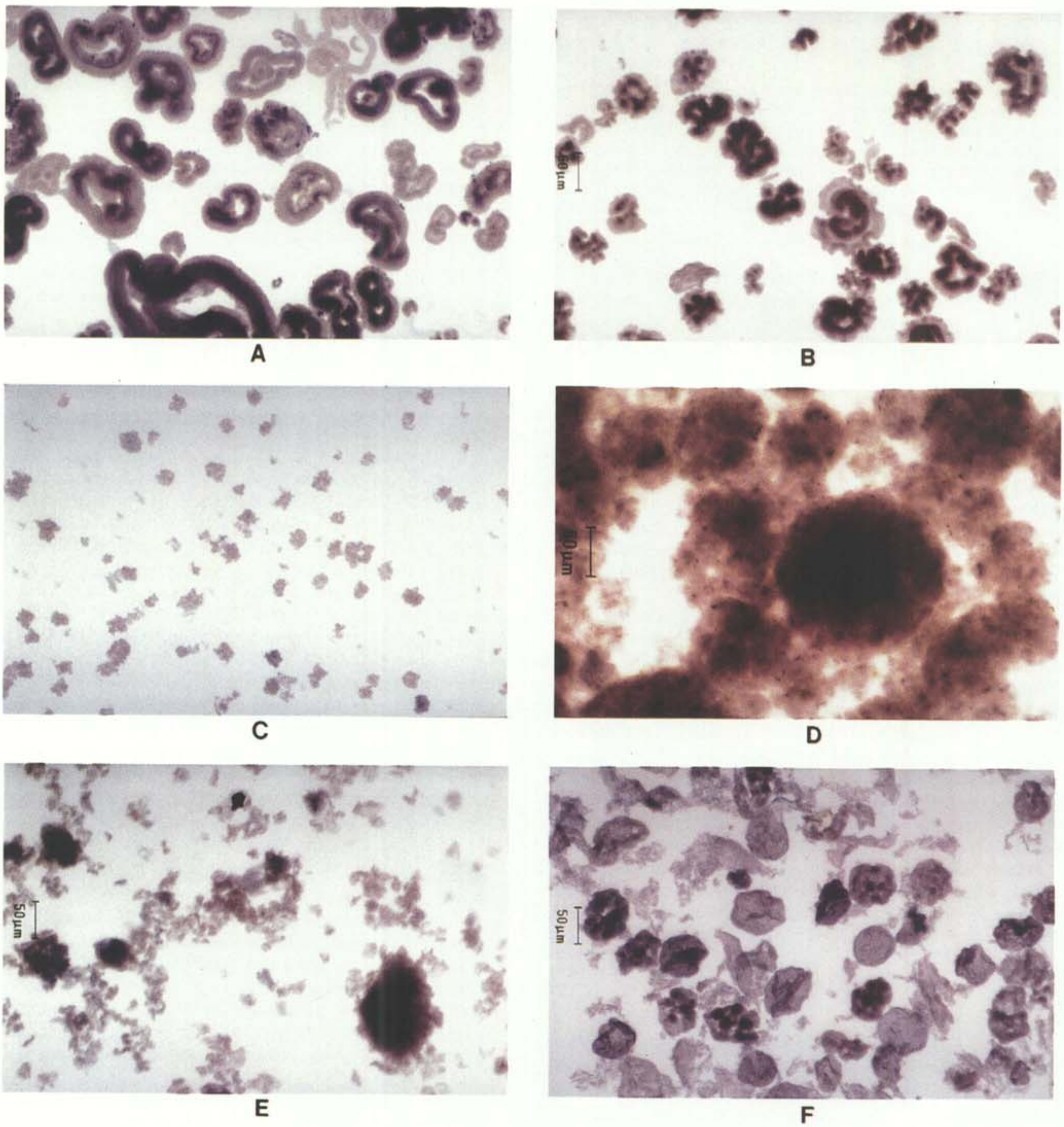

Fig. 1. Micrographs of pellets from centrifuged starch pastes prepared under static conditions (121°C, 45 min) at a concentration of 0.02% and stained with an iodine solution (magnification ×50 unless otherwise noted): **A,** wheat; **B,** barley; **C,** normal rice; **D,** waxy rice (×66); **E,** oat; **F,** popcorn.

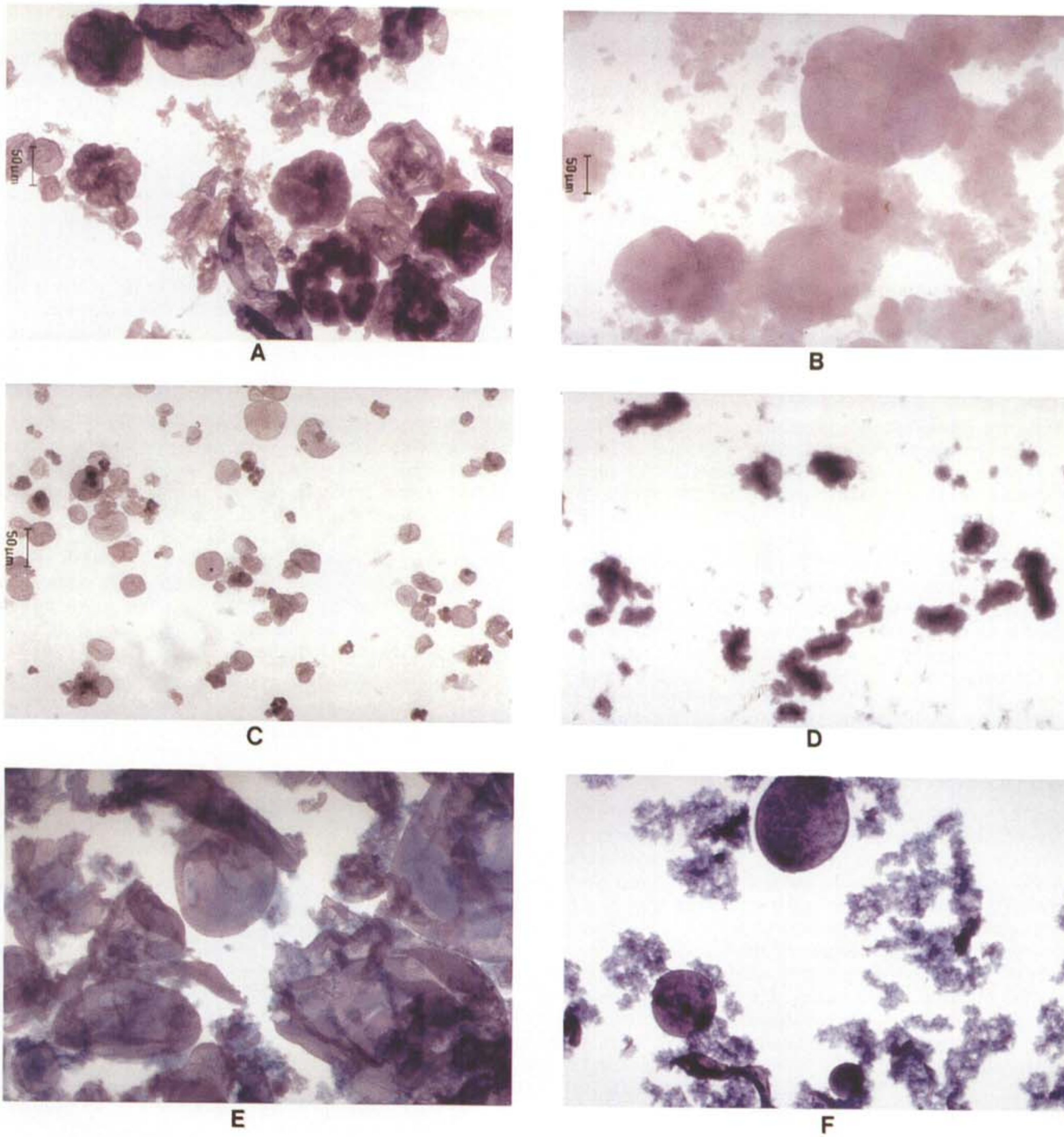

Fig. 2. Micrograph of a pellet from centrifuged starch pastes (unless otherwise noted) prepared under static conditions (121°C, 45 min) at a concentration of 0.02% and stained with an iodine solution (magnification ×50): **A,** sorghum; **B,** waxy sorghum; **C,** millet; **D,** mung bean; **E,** potato (supernatant); **F,** potato in 17 m*M* CaCl (uncentrifuged paste).

outer layer of the granule (Fig. 1A). Similar structures of wheat starch ghosts can be seen in other published micrographs, although more concentrated pastes and different cooking conditions were used (Langton and Hermansson 1989).

Barley starch ghosts (Fig. 1B) resembled those of wheat except that they appeared denser and the remaining shell was highly indented. The barley starch granule remnants in our micrograph resemble those reported by Williams and Bowler (1982), who used scanning electron microscopy to examine patterns of gelatinization of starch granules from the cereals of the Triticeae tribe. With our method, both wheat and barley starch ghosts stained reddish brown.

Although ghosts from rice starches (normal and waxy) were not easily distinguishable, they were clearly different from those of other small grains. This is not surprising because native granules of rice starches are much smaller than those of other cereals and tend to associate, making it difficult to distinguish whether the granule remnants were from a single granule or a cluster of granules. The granule remnants of rice starches stained reddish brown. This is most evident in Figure 1D, a photomicrograph of the paste of a waxy rice starch, which, according to the supplier (California Natural Products), had been prepared by a mechanical, rather than the traditional alkali, milling method. When the starch powder was viewed by scanning electron microscopy (not shown), it was found to be composed of spherical, multigranule aggregates. These spherical aggregates remained after cooking, although they were then composed of highly swollen granules or granule ghosts. (The aggregated ghosts of a normal rice starch from the same supplier, which stained blue before cooking, looked identical to those of the waxy rice starch in aggregate size and color.)

Oat starch granules retained less structure under the conditions of our normal cook than did barley or wheat starch granules (Fig. 1E). Like most starch ghosts, those few from oat starch granules that did not disintegrate stained reddish brown. Others have also reported differences in cooking behaviors of oat starch as compared to other cereal starches (Doublier et al 1987, Paton 1987, Autio 1990, Shamekh et al 1994). Williams and Bowler (1982) also reported that the swelling patterns of oat and maize starches were different from those of wheat, rye, and barley starches. Paton (1979) described oat starch as having high swelling power and solubility.

Other Cereal (Popcorn, Sorghum, and Millet) Starches

Popcorn starch ghosts resembled those of common maize starch, except for being more sensitive to the cooking conditions employed; more fragmented ghosts could be seen in popcorn starch paste (Fig. 1F) than in the common maize starch paste (as previously reported by Obanni and BeMiller [1995]). Again, the color of the ghosts was reddish brown, but it was more uniform than that of common maize starch. The clusters, characteristic of a common maize starch cook (Obanni and BeMiller 1995), seemed to more often be contained in a saclike structure in the popcorn starch cook. The sizes of the native starch granules and of the ghosts were similar to those of common dent corn starch.

Sorghum starch ghosts were bigger than their maize counterparts, as are native sorghum starch granules as compared to maize starch granules (Maywald Snyder 1984). As in common corn starch pastes, the paste from normal sorghum endosperm starch contained clusters (Fig. 2A), although to a lesser extent. Waxy sorghum starch, however, had no clusters (Fig. 2B), which made it similar to waxy maize starch (Obanni and BeMiller 1995). Compared to the ghosts from normal sorghum starch, the ghosts of waxy sorghum starch were more sensitive to breakage, as evidenced by more debris in the dispersed pellet.

Millet starch ghosts also resembled those from normal maize, except that those from millet starch were smaller. Millet starch pastes contained the clusters seen in normal maize starch cooks, and the millet ghosts stained reddish brown (Fig. 2C).

The starch granules of barley, wheat, and rye, all of which belong to the tribe Triticeae, have surface pores (Fannon et al 1992) and formed granule ghosts. Starch granules from maize, sorghum (tribe Andropogoneae), and millet (tribe Paniceae), all of which are in the Panicoideae subfamily, also have surface pores (Fannon et al 1992) and formed ghosts. Starch granules of oat and rice (tribes Aveneae and Oryzeae, respectively) neither have surface pores (Fannon et al 1992) nor formed ghosts (at least in the case of oats) although they are in the same subfamily as wheat, rye, and barley. These relationships in the morphology and behavior of granules of closely related species suggest that these characteristics are under genetic control.

Mung Bean and Potato Starches

The granule remnants from mung bean (a legume) starch were sensitive to breakage, and mostly fragments of ghosts were present in the resuspended pellet (Fig. 2D).

When potato starch was examined in the same manner as other starches, very few ghosts were found in the resuspended pellet. Instead, almost all ghosts remained in the supernatant (Fig. 2E). This phenomenon is more pronounced in potato starch cooks than in cooks of the other starches studied and may be due to lower density of the potato starch ghosts or to higher viscosity at low shear rates (i.e., better suspension-stabilizing property) of the potato starch paste. Potato starch granule ghosts stained reddish brown with iodine. It is well known among starch users that potato starch is sensitive to low concentrations of electrolytes. Thus, potato starch is reported to produce higher viscosity in distilled water than in a sodium chloride solution (Mitch 1984), and the viscosity obtained is considerably reduced by cooking in hard water. This phenomenon has been assumed to be due to the phosphate ester groups of potato starch. When potato starch was cooked in 17 m*M* NaCl (0.1%) or $CaCl_2$, the granules swelled and some amylose leached from the swollen granules and retrograded, but extensive ghost formation was not evident (Fig. 2F).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of C. E. Bracker and D. Sherman of the Electron Microscope Center in Agriculture at Purdue University and thank them for the use of the facility and its equipment.

LITERATURE CITED

AUTIO, K. 1990. Rheological and microstructural changes of oat and barley starches during heating and cooling. Food Struct. 9:297-304.

DOUBLIER, J.-L., PATON, D., and LLAMAS, G. 1987. A rheological investigation of oat starch pastes. Cereal Chem. 64:21-26.

FANNON, J. E., HAUBER, R. J., and BeMILLER, J. N. 1992. Use of low-temperature scanning electron microscopy to examine starch granule structure and behavior. Pages 1-23 in: Frontiers in Carbohydrate Research, 2. R. Chandrasekaran, ed. Elsevier Applied Sci.: London.

JANE, J.-L., KASEMSUWAN, T., LEAS, S., ZOBEL, H., and ROBYT, J. F. 1994. Anthology of starch granule morphology by scanning electron microscopy. Starch/Stärke 46:121-129.

LANGTON, M., and HERMANSSON, A.-M. 1989. Microstructural changes in wheat starch dispersions during heating and cooling. Food Microstruct. 8:29-39.

MAYWALD SNYDER, E. 1984. Industrial microscopy of starches. Pages 661-673 in: Starch Chemistry and Technology, 2nd ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

MITCH, E. L. 1984. Potato starch: Production and uses. Pages 479-490 in: Starch Chemistry and Technology, 2nd ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

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

PATON, D. 1979. Oat starch: Some recent developments. Starch/Stärke 31:184-187.

PATON, D. 1987. Differential scanning calorimetry of oat starch pastes.

Cereal Chem. 64:394-399.
PRENTICE, R. D. M., STARK, J. R., and GIDLEY, M. J. 1992. Granule residues and "ghosts" remaining after heating A-type barley-starch granules in water. Carbohydr. Res. 227:121-130.
SHAMEKH, S., FORSSELL, P., and POUTANEN, K. 1994. Solubility pattern and recrystallization behavior of oat starch. Starch/Stärke 46:129-133.
SVEGMARK, K., and HERMANSSON, A.-M. 1993. Microstructure and rheological properties of composites of potato starch granules and amylose: A comparison of observed and predicted structures. Food Struct. 12:181-193.
WILLIAMS, M. R., and BOWLER, P. 1982. Starch gelatinization: A morphological study of Triticeae and other starches. Starch/Stärke 34:221-223.

[Received July 17, 1995. Accepted January 23, 1996.]