

Retrogradation of Starches from Different Botanical Sources¹

MARK R. JACOBSON,^{2,3} MOHAMED OBANNI,^{2,4} and JAMES N. BEMILLER^{2,5}

ABSTRACT

Cereal Chem. 74(5):511–518

Retrogradation in 2% pastes prepared from unmodified commercial starches by cooking at 98–100°C under low shear, then held at 4°C for 56 days, was examined by turbidometric analysis and light microscopy. Turbidometric analysis revealed that retrogradation rates followed the order of wheat, common corn > rice, tapioca, potato >> waxy maize. Microstructures of stored pastes were examined both before and after centrifugation. Granule remnant morphologies and fresh and stored paste microstructures were unique to each starch examined. Fresh pastes from

amylose-containing starches were dominated by networked amylose that condensed into higher density aggregates upon storage. Unique phenomena seen in some stored pastes included interactions of granular remnants with aggregated amylose, composite networks of co-associated amylopectin and amylose, and slight birefringence regained by granule remnants. Microstructural changes in stored pastes could be related to changes in turbidity and to the results of other methods used to quantitate retrogradation.

Retrogradation of gelatinized starch is a reorganization process involving both amylose and amylopectin, with amylose undergoing retrogradation at a more rapid rate than amylopectin. The rate of retrogradation depends on a number variables, including the structures of amylose and amylopectin, ratio of amylose to amylopectin, temperature, starch concentration, botanical source of the starch, and presence and concentration of other ingredients.

A variety of methods have been used to quantitate retrogradation in low-concentration pastes of starches from various sources. An early and often referenced study is that of Whistler (1954), who evaluated retrogradation in 2% pastes (probably prepared with shear and previously centrifuged while hot) of a variety of starches by measuring the amount of precipitate formed with time of storage (Whistler and Johnson 1948). Rates of retrogradation can also be determined using light-scattering equipment (Paschall and Foster 1952, Foster and Serman 1956) or a spectrophotometer (reduction in transmitted light) (Miles et al 1985, Ring et al 1987, Gidley and Bulpin 1989). Relatively few qualitative evaluations have been performed, resulting in a lack of information on the fundamental changes in starch pastes and paste microstructures over time. An early investigation used phase contrast light microscopy to evaluate starch pastes subjected to freezing (Woodruff and McMasters 1938), but slow freezing changes paste microstructure and accelerates retrogradation (Jacobson 1994). Effects of storage on the microstructure of potato starch pastes (Langton and Hermansson 1989; Svegmärk and Hermansson 1991, 1992, 1993) and oat and barley starch pastes (Autio 1990, Autio et al 1992, Virtanen et al 1993) have been evaluated using light microscopy of iodine-stained pastes. The size of swollen starch granules (determined microscopically and by swelling power) has been correlated to the melting enthalpy of crystalline amylopectin (Ellis et al 1988).

The objectives of this research were to quantitate retrogradation in low-concentration (2%) pastes of various starches turbidometrically, determine microstructural changes in the retrograding pastes using a simple microscopic method (Obanni and BeMiller 1995), and relate turbidometric and microstructural changes resulting from retrogradation.

MATERIALS AND METHODS

Materials

Waxy maize (WM) starch (11.7% moisture) and common corn (CC) starch (10.8% moisture) were obtained from A.E. Staley Manufacturing Co. (Decatur, IL), potato starch (12.1% moisture) from Penwest Foods Co. (Englewood, CO), rice starch (11.2% moisture) from Sigma Chemical Company (St. Louis, MO), wheat starch (9.9% moisture) from Midwest Grain Products, Inc. (Atchison, KS), tapioca (manioc, cassava) starch (12.0% moisture) from National Starch and Chemical Co. (Bridgewater, NJ), and potassium iodide and iodine from Mallinckrodt, Inc. (St. Louis, MO). AAC Method 44-40 (AACC 1983) was used to determine moisture content.

Paste Preparation

Pastes (2.0%, w/w, concentration) were prepared by atmospheric cooking (98–100°C) with only mild shear forces.

Turbidometric Analysis of Retrogradation

Common corn, wheat, rice, potato, tapioca, and waxy maize starch slurries (2.0%, w/w, dwb, 200 mL) were prepared by combining the appropriate amounts of starch and water in 250-mL plastic screw-capped bottles. Bottles were sealed and immersed for 1 hr in a boiling water bath with continuous gentle stirring, then cooled for 20 min in a 25 ± 2°C water bath with continuous stirring. Triplicate paste samples were placed in disposable cuvettes, which were then placed briefly under slight vacuum to remove air bubbles. Initial turbidity was determined by absorbance at 640 nm (Miles et al 1985) using a Varian DMS 80 UV/visible spectrophotometer equipped with a programmable cell changer and Citizen MSP-10 printer. The bottles containing the remaining starch pastes were stored in a refrigerator at 4°C.

Starting at 28 days, pastes were monitored for microbial growth using a Leitz Laborlux 12POL light microscope; only after 56 days were pastes found to contain microbial growth. (A bacteriostatic agent was not used due to a potential effect on starch gelatinization and retrogradation.) After 1, 3, 5, 7, 10, 14, 21, 28, and 56 days at 4°C, bottles were agitated briefly by a wrist-arm shaker and triplicate paste samples were placed in disposable cuvettes. The cuvettes were subjected briefly to slight vacuum (to remove air bubbles) and placed for 5 min in a 25 ± 2°C water bath. A_{640} was then determined. Normalized absorbance was calculated as $A_X - A_0/A_{56} - A_0$, where A_0 , A_X , and A_{56} are absorbances of the fresh paste, paste after X days, and paste after 56 days, respectively.

¹Journal paper 15345 of Agricultural Research Programs, Purdue University.

²Whistler Center for Carbohydrate Research, 1160 Smith Hall, Purdue University, West Lafayette, IN 47907-1160.

³Present address: Nestlé Research and Development Center-Connecticut, 201 Housatonic Ave., New Milford, CT 06776-5528.

⁴Present address: Research and Development, California Natural Products, P.O. Box 1219, Lathrop, CA 95330.

⁵Corresponding author. E-mail: bemiller@foodsci.purdue.edu.

Microscopy

Two microcentrifuge tubes were filled with paste samples used for turbidometric analysis (0, 7, 14, 28, and 56 days at 4°C) and centrifuged for 3 min at 13,600 × *g*. Samples (100 μL) of resuspended pellets, supernatants, and original pastes were mixed with 10 μL of an iodine solution (3% I₂ and 3% KI) using gentle agitation. Stained samples were viewed and photographed using an Olympus Vanox photomicroscope (Olympus Optical, Tokyo, Japan).

RESULTS AND DISCUSSION

Turbidometric Analysis

Large differences in initial turbidities of the various starch pastes were observed (Fig. 1). Three groupings based on initial turbidity were evident: A) those from potato and tapioca starches having low initial turbidity, B) that from WM starch having intermediate initial turbidity, and C) those from rice, wheat, and CC starches having high initial turbidity. These results are similar to those of Craig et al (1989), yet there were differences in the order of individual turbidities (reversal of CC and wheat starches) and in magnitudes of the turbidities. The differences may be related to the nature of the starches (cultivars), concentration, or the cooking and storage conditions (amount of shear during heating, rates of heating and cooling, and cooking times) used.

Results of the effects of storage of these pastes for 56 days are displayed in two ways: as absolute changes in absorbance (Fig. 1) and as normalized absorbance, i.e., as the relative rates of turbidity development between pastes over the 56-day period of analysis (Fig. 2). Both the absolute absorbance differences and the relative retrogradation rates varied considerably. Based on the rates of absorbance changes and ultimate turbidity development (Fig. 1), the starches could again be grouped into three categories: A) amylose-containing cereal (rice, wheat, and CC) starches, B) root and tuber (potato and tapioca) starches, and C) WM starch. When absorbances were normalized (Fig. 2), the groupings were similar, except that the rice starch paste behaved more like the potato and tapioca starch pastes.

Wheat and CC starch pastes, which had the highest initial turbidities, showed the highest initial rates of turbidity development, with turbidity plateauing after only a few days. Pastes of tapioca and potato starches, which initially were the least turbid, developed turbidity fairly rapidly for the first seven days, followed by continued slow turbidity development throughout the following

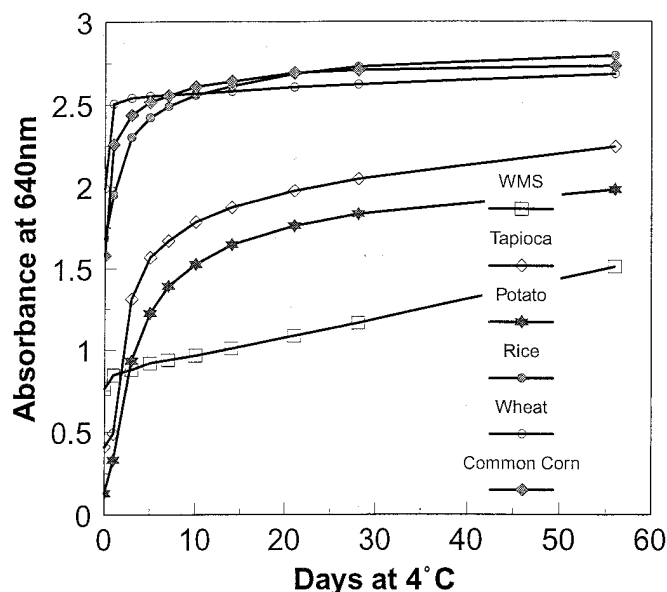


Fig. 1. Actual turbidities of 2% pastes of starches from various sources as a function of days of storage at 4°C.

49-day period. Rice starch paste, while initially having a high absorbance like pastes of the other amylose-containing cereal starches, had relatively slow initial rates of turbidity development, similar to those of potato and tapioca starches (Fig. 2). WM starch paste, which had an intermediate initial turbidity, had a small, but relatively rapid, increase in turbidity during the first day of storage, followed by slow and constant retrogradation over the following 55 days. This rapid, but brief, initial turbidity development in the WM starch paste may be due to the small amount (generally at least 3%) of CC starch contamination present and quite evident (Obanni and BeMiller 1995) in commercial waxy maize starch, as is the similar contamination of commercial CC starch by WM starch.

These results are, in general, congruent with those reported by Whistler (1954) for some of the same starches at the same concentration. They are incongruent with reports of others who used different methods and concentrations. Orford et al (1987) reported the following orders of short-time (minutes) rates of retrogradation: CC > wheat > potato (development of shear modulus in 10–40%, w/w, gels); the following long-term (days) rates of retrogradation: potato > CC > wheat (development of shear modulus in 30%, w/w, gels); and the following extents of retrogradation: potato > CC > wheat (enthalpy by DSC in 30%, w/w, gels). Roulet et al (1990) reported the following orders of rates of retrogradation in 40% db gels: potato > rice > tapioca, wheat (compression modulus) and potato > rice, wheat > tapioca (enthalpy by DSC), and the following extents of retrogradation: rice, tapioca > potato > wheat, (enthalpy by DSC). Here we report the following relative rates of retrogradation in 2% pastes stored at 4°C: wheat, CC > rice, tapioca, potato >> WM and the following relative extents of retrogradation after 30 days at 4°C: CC, rice > wheat >> tapioca > potato >> WM and, when the data were normalized: CC > rice > potato, wheat > tapioca >> WM.

Microstructural Analysis

Light microscopy after iodine staining provided considerable information about the effects of retrogradation on the composite nature of low-concentration fresh and stored starch pastes. Evaluation of the pellets and supernatants of centrifuged fresh and stored pastes was valuable in determining not only the nature of the precipitated material but also changes in the composition and relative density of polymer networks in and components of the paste.

The composite nature of fresh starch pastes, which consisted of granule remnants bound together by a network of interstitial amylose, could be seen clearly in the fresh wheat starch paste (Fig. 3A). Granule remnants, which were the characteristic brick-red color of iodine-stained amylopectin, were folded, as is typical of gelatinized wheat starch (Bowler et al 1980). These remnants appeared to be bound together with characteristically blue-staining amylose, creating a network of granule remnants. Free-standing remnants without bound amylose were not observed. Wheat amylose was present in two forms—as sheets covering granule remnants and as aggregates associated with surfaces of granule remnants. The amylose sheets, which did not appear to be homogeneous, were much larger than the individual granules. Small (approximately 1 μm diameter), intensely stained amylose aggregates associated with granule surfaces could be seen.

The pellet from a centrifuged fresh wheat starch paste (Fig. 3B) consisted mainly of granule remnants of a fairly uniform size that were similar in appearance to those in the whole paste. Remnant color varied from brick red to purple. Small amylose aggregates could be seen in intergranular spaces. The presence of large amylose sheets in the supernatant (Fig. 3C) indicates that the density of the amylose network was less than that of the granule remnants and that the network was not bound well enough to granule remnants to be pelleted with them. Some regions of faint red-purple could be seen, indicating the presence of some amylopectin.

Appearance of granule remnants did not change upon continued storage of the wheat starch paste at 4°C from seven days (Fig. 3D) to 56 days, but the appearance of the amylose changed considerably. Sheets seen in the fresh paste were absent, and the amount of interstitial aggregated amylose bound to granule remnant surfaces appeared to increase. This indicated that during storage at 4°C, amylose in wheat starch pastes underwent a transition in state from sheets to small aggregates associated with surfaces of granule remnants. This confirms the suggestion by Langton and Hermansson (1989) that amylose was deposited on amylopectin fragments in wheat starch pastes. The pellet from wheat starch pastes stored at 4°C for seven days or longer contained intensely blue-staining interstitial amylose aggregates that bound granule remnants into large assemblies similar to those seen in the upper right corner of Figure 3D. Supernatants of the stored pastes contained no stained material in any form. In aged paste, amylose was associated only with granule remnants, but it is not known if, in retrograding wheat starch, swollen granules nucleate amylose crystallization or if preformed amylose aggregates associate with granule remnants.

We have concluded that the rapid initial rate of turbidity development in stored wheat starch pastes (Figs. 1 and 2) and the rapid initial rate of retrogradation reported by Whistler (1954) is related to the loss of networked amylose, the development of amylose aggregates, and binding of granule remnants into assemblies by amylose and amylose aggregates. The plateaued rates of retrogradation after seven to ten days observed by turbidometric analysis (Figs. 1 and 2) and as determined by collection of precipitate (Whistler 1954) corresponds to an increase in the amount of aggregated amylose associated with granule remnants.

Examination of the microstructure of a fresh CC starch paste (Fig. 3E) also revealed a composite nature, i.e., granule remnants (20–100 µm diameter) connected by a matrix of interstitial amylose. However, appearance of the stained granule remnants was quite different from that seen in the wheat starch paste. CC starch paste contained light-staining, somewhat spherical remnants, as compared to the highly folded, intensely stained remnants found in wheat starch pastes. In addition, some granule remnants appeared to be clusters, with three or more units (5–25 µm diameter) per cluster (Obanni and BeMiller 1995). Color of the granule remnants varied from brick red to purple. As seen in wheat starch pastes, amylose was attached to granule remnants, and sheets of networked amylose were present, although the sheets were not as large nor as prevalent as those in the fresh wheat starch paste (Fig. 3A). Some light-staining interstitial amylose not associated with the blue-staining sheets was also present.

Extrgranular amylose was not present in a fresh CC starch paste pellet (Fig. 3F), indicating that no amylose, in any structured form that could be seen, was bound as tightly to granule remnants as it was in wheat starch pastes (Fig. 3B). A diversity of size, shape, color, and nature of granule remnants was observed, along with clustered remnants, each unit of which had a dense center (Fig. 3E). Intragranular amylose, the location of which was verified by changing the focus, could be seen in some of the remnants. It could not be determined whether the amylose had been entrapped within the swollen granule or was actually part of the network comprising the granule remnant. Fresh CC starch paste supernatant consisted of amylose sheets very similar in size and color density to those seen in the fresh wheat starch supernatant (Fig. 3C).

Storage of CC starch paste for seven days at 4°C resulted in aggregation of some interstitial amylose, which appeared to be associated with granule remnant surfaces, similar to what was observed in wheat starch pastes. However, some amylose remained associated with remnant surfaces after 14 days, but not after 28 days, in contrast to the behavior of the components of wheat starch pastes. Intragranular aggregates of amylose could also be seen. Aggregation of amylose in CC starch pastes appeared to be a slower process than it was in wheat starch pastes, a conclusion

supported by the presence of remnants of amylose networks in the supernatant of the centrifuged paste after seven days at 4°C (Fig. 3G), although no visible structures were present after this time. In contrast, no visible structures, amylose or otherwise, were present in stored (4°C) wheat starch paste supernatants.

Pellets resulting from centrifugation of CC starch pastes stored at 4°C were similar in appearance to stored whole pastes prior to centrifugation. In pellets from pastes stored seven days, amylose was present in two states: as a few large sheets of aggregated amylose and as aggregates bound to granule remnants. Since aggregated amylose was present in the pellets of pastes stored seven days, either the density of the aggregates increased enough so that they pelleted or they were bound to granule remnants tightly enough to be pelleted with the remnants. Upon further storage, the amount of sheeted amylose decreased, while the amount of aggregated amylose bound to remnants increased. Interstitial amylose aggregates appeared to bind granule remnants together, since no individual (unbound) remnants were present after seven days. In relation to the results of the turbidometric analysis (Figs. 1 and 2) and those of Whistler (1954), the microstructures of CC starch pastes seemed to change in a manner similar to those of wheat starch pastes with regard to retrogradation, i.e., the rapid initial rise in retrogradation corresponded with an aggregation of amylose and the disappearance of amylose sheets and was followed by a reduced retrogradation rate that corresponded to a loss of amylose sheets and a subsequent increase in the number of interstitial amylose aggregates.

Granule remnants in fresh rice starch pastes (Fig. 3H) were much smaller (5–20 µm diameter) than those in CC and wheat starch pastes and had colors that ranged from brick red to blue. Granule remnants had non-distinct shapes and appearances, as compared to those in CC starch and wheat starch pastes. As in the fresh CC starch paste, amylose was present in two states: as sheets of semi-aggregated material that covered regions of granule remnants and as material that appeared to bind granule remnants together. In the fresh paste pellet, granule remnants and remnant-binding amylose were similar in appearance to those in the fresh paste (Fig. 3I), yet many of the amylose sheets were absent. This indicates that the amylose sheets had a lower density than and were not bound tightly to denser granule remnants. The supernatant of the centrifuged fresh paste was similar in appearance to that of fresh CC starch paste supernatant, except that larger regions of sheets were purple in color, indicating the presence of amylopectin.

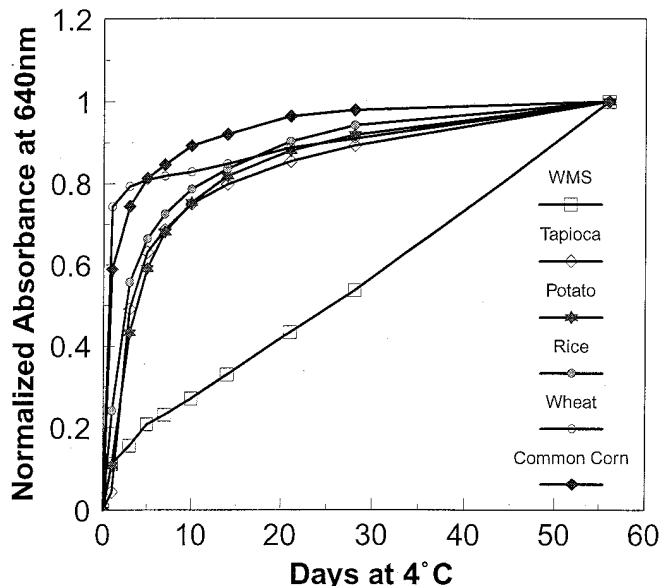


Fig. 2. Normalized turbidities of 2% pastes of starches from various sources as a function of days of storage at 4°C.

Upon storage of the rice starch paste at 4°C for seven days, small amylose aggregates (like those seen in pastes of other cereal grain starches on storage) were present in interstitial amylose networks, and amylose sheets were absent. Interstitial amylose networks again seemed to bind granule remnants together, as indicated by a lack of any individual granule remnants. Following initial changes after seven days, paste microstructure appeared unchanged up to 56 days. The pellet of the stored paste consisted mainly of granule remnants with aggregated intergranular networks, although some sheets of aggregated amylose were present even after 28 days at 4°C. As in the paste, most of the changes in

the pellet occurred in the first seven days, with little change up to 56 days. The supernatant of stored rice starch paste, on the other hand, changed substantially over the first 28 days. After seven days at 4°C, the blue sheets in the fresh paste supernatant had become reddish purple in color, indicating the presence of significant amounts of amylopectin and the loss of amylose (Fig. 3J). This suggests that sheets seen in the fresh paste contained both amylose and amylopectin with the blue-stained amylose obscuring the amylopectin, that amylopectin was in at least two nonsoluble states after gelatinization (networked with amylose and in granule remnants), and that the amylopectin portion of the network was

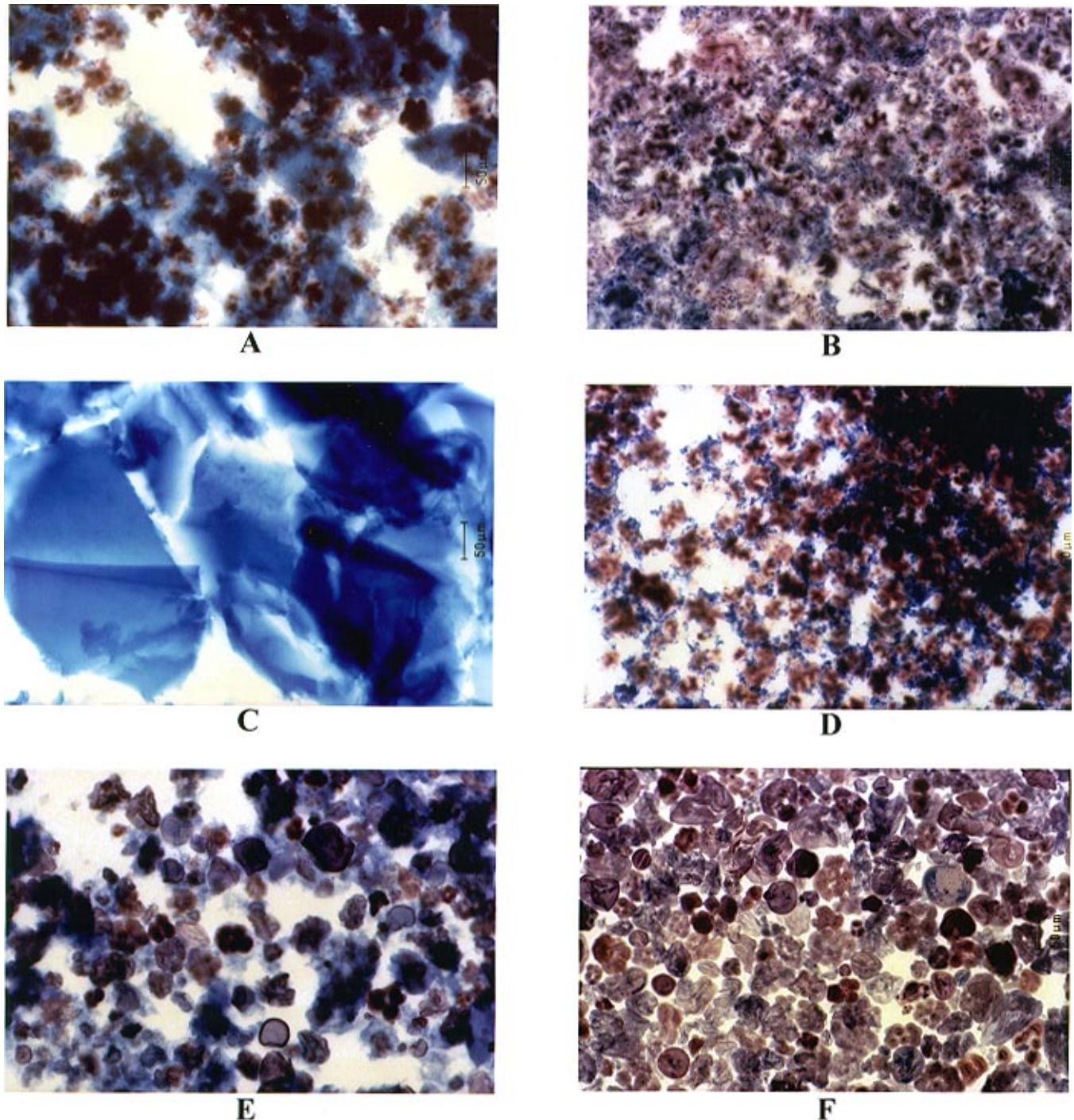
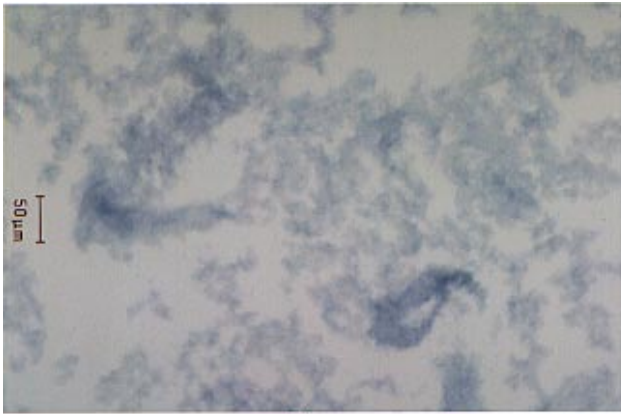
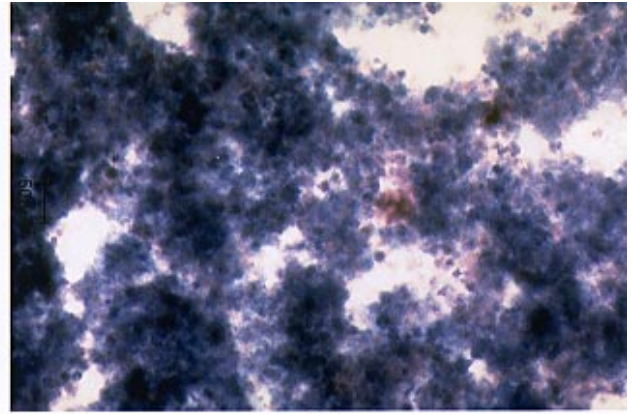


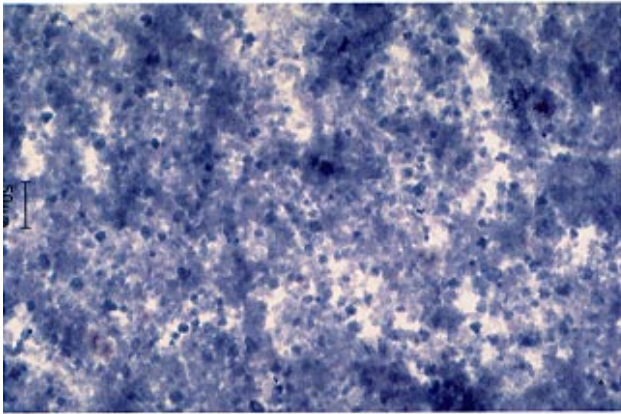
Fig. 3. Micrographs (50×) of original 2% starch pastes, supernatants (from centrifugation), and resuspended pellets, each stained with an iodine solution. Pastes were prepared under gentle cooking conditions (100°C, 1 h, gentle stirring), then stored at 4°C. Bars are 50 μm indicators. Descriptors denote starch type, paste fraction, and days of storage at 4°C. **A**, wheat, whole, 0; **B**, wheat, pellet, 0; **C**, wheat, supernatant, 0; **D**, wheat, whole, 7; **E**, common corn, whole, 0; **F**, common corn, pellet, 0 ("clusters" characteristic of CC starch [Obanni and BeMiller 1995] can be seen); **G**, common corn, supernatant, 7; **H**, rice, whole, 0; **I**, rice, pellet, 0; **J**, rice, supernatant, 7; **K**, potato, whole, 14; **L**, tapioca, pellet, 0; **M**, tapioca, whole, 14; **N**, waxy maize, whole, 14 (characteristic contamination with CC starch found in commercial preparations).



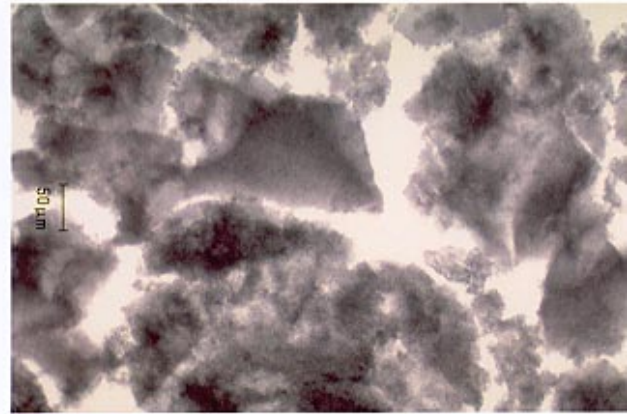
G



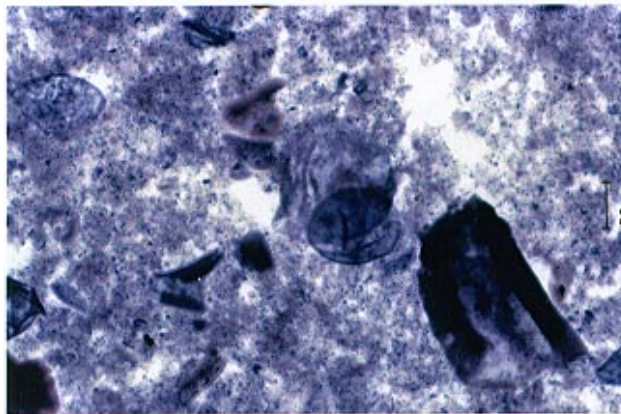
H



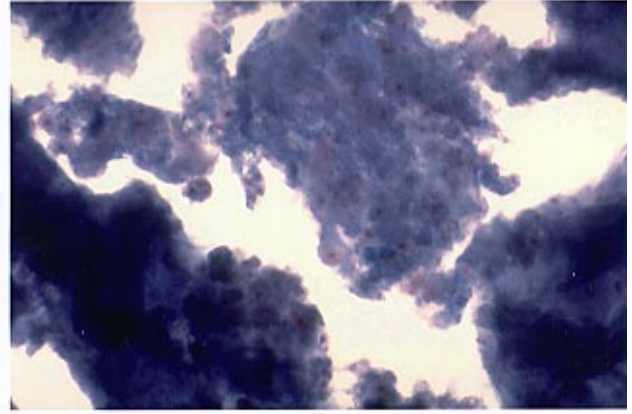
I



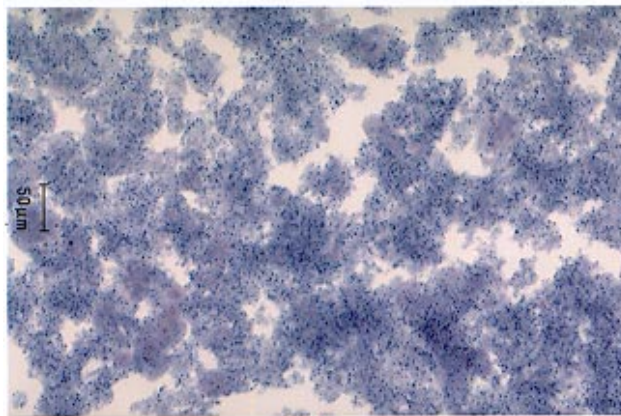
J



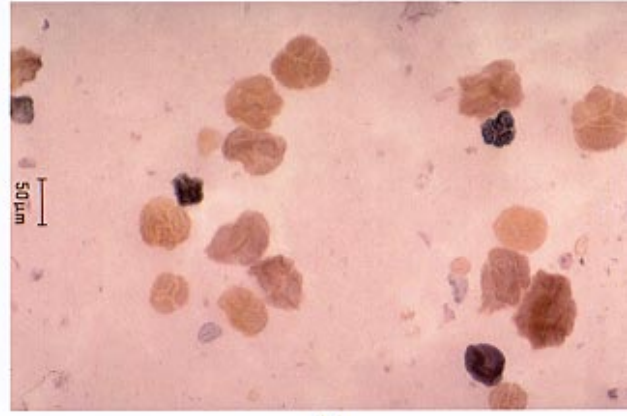
K



L



M



N

sufficiently strong to remain after condensed retrograded amylose was removed by centrifugation. After 14 days, the sheets of amylopectin in the supernatant appeared to lose color density and to be deteriorating; after 28 days, these sheets were completely absent in the supernatant. Absence of amylopectin sheets in the supernatant and pellet suggests that it too changed to a more dense state that was removed by centrifugation.

The fairly rapid rise in turbidity of stored rice starch pastes (Figs. 1 and 2) corresponded to aggregation of amylose and a significant reduction in the blue color of the sheets present in the supernatant. The slower secondary rise in turbidity corresponded with a loss of integrity of the sheets of amylopectin present in the supernatant after seven days. Of the three amylose-containing cereal grain starch pastes analyzed, rice was the only one in which microstructural changes in amylopectin aggregates could be related to turbidity changes.

Fresh potato starch paste, supernatant, and pellet consisted mainly of extremely large, blue-stained sheets of amylose, similar to those seen in the wheat starch paste supernatant (Fig. 3C). Darker staining regions (likely granule remnants), varying in size and shape, could be seen within the sheets of the supernatant and more so in the whole paste. The pellet contained a number of dark, blue-staining, spherical granule remnants (15–75 μm diameter) that were likely uncooked or partially swollen granules (based on size). These granules were dense enough to be removed by centrifugation. The reason for their presence is not known.

During seven days at 4°C, the microstructure of the potato starch paste underwent several changes, the most notable being that the large amylose sheets present in the fresh paste had deteriorated considerably, leaving networks of intensely stained amylose aggregates with only a little sheet-like character (Fig. 3K). The aggregates that were present in the network were somewhat larger (1–3 μm diameter) than those seen in cereal starch pastes. The color of the network changed from blue to purple, probably as a result of aggregation of amylose, allowing the red color of amylopectin to be discerned. Granule remnants and fragments were observed as defined darker regions (50–250 μm diameter) within the blue sheets and became more visible as the network deteriorated. The delicate nature of cooked potato starch granules, as compared to those of cereal starches, was evidenced by the relatively few granule remnants that resembled swollen granules and the presence of a large number of granule fragments. After 14 days at 4°C, well-defined, blue-staining granule remnants could be seen as a result of continued deterioration of the interstitial matrix. Some brick red colored fragments were also observed. In addition, the color of the interstitial matrix continued to change gradually from blue to red as the number of small amylose aggregates increased. These trends continued during storage up to 56 days.

The potato starch paste pellet also indicated a deterioration of amylose sheets after seven days, yet there was still some network matrix present. Within this matrix were small intensely stained amylose aggregates similar to those seen in the paste after seven days. This indicates that the small aggregates were either not dense enough or bound too tightly to the matrix to be pelleted individually. Appearance of the pellet obtained after centrifugation of a stored paste suggested a continual breakdown of the paste matrix. The original sheets gradually became less defined, while the number of blue-stained granule remnants and fragments in the pellet appeared to increase, and additional brick-red-staining regions could be discerned. Whether these red-staining regions were granule remnants or resulted from amylopectin aggregation, it was apparent that both amylose and amylopectin were present in interstitial networks of the fresh paste. These changes continued progressively over the entire time (56 days) paste microstructures were examined.

After seven days of storage, the large sheets of blue-stained amylose seen in the fresh potato starch paste had disappeared from the supernatant, but poorly defined regions of networked

amylose containing small amylose aggregates could be seen. Within these amylose-rich regions, smaller red regions were present, possibly a result of the presence of granule remnants and fragments. In addition, fewer large, blue-staining granule remnants were present in the supernatant. After 14 days of storage, the matrix in the supernatant showed continued deterioration to the point that distinct structural features could not be discerned. The existing matrix continued to become more red in color, and the number of small amylose aggregates continued to increase. These trends continued over the entire period (56 days) in which the stored paste microstructure was observed. One important feature of potato starch paste, pellet, and supernatant that should be noted was that, although the number of small amylose aggregates increased during storage, the size and number in the pellet varied little, indicating that either these aggregates were not dense enough or the matrix was too strong to allow them to be pelleted by centrifugation.

The initial fairly rapid retrogradation in potato starch paste revealed by turbidometric analysis (Figs. 1 and 2) and the results of Whistler (1954) corresponded to a loss of the network formed from amylose sheets and an increase in the number of small amylose aggregates present. Quantitative and qualitative results could be related, for as the amylose (which initially appears as sheets) aggregated into more dense particles, scattering of light increased. The initial fairly rapid increase in turbidity was followed by a slower increase that corresponded to the progressive appearance of greater numbers of small amylose aggregates, resulting in more scattering.

Fresh tapioca starch paste and its supernatant microstructure consisted primarily of very large, blue-stained amylose sheets (>250 μm in length) similar to those seen in wheat starch paste supernatants (Fig. 3C). Some non-distinct, purple regions were present in the sheets. The fresh paste pellet (Fig. 3L) also contained large amylose sheets, but within the sheets were non-distinct, red-staining clusters (5–20 μm diameter). The clusters were likely composed of amylopectin and had distinct dark-staining centers, making them similar in appearance to those seen in CC starch pastes. They appeared to be granule remnants but might have resulted from phase separation and amylopectin aggregation during storage.

Although remnants of the large amylose sheets were still discernible in the tapioca starch paste after storage at 4°C for seven days, they had deteriorated, leaving a light-staining network of amylose between non-distinct regions of amylopectin (that appeared to be the clusters seen in the fresh paste pellet based on similarity in size). Within the amylose networks, small (1–3 μm diameter) intensely blue-staining amylose aggregates, like those present in stored potato starch paste, were present. Aggregates and amylopectin regions appeared to be connected in the amylose network. Upon further storage for 14 days (Fig. 3M) and up to 56 days, remnants of the initial sheets seen in the fresh paste remained, but the networks continued to deteriorate, leaving less amylose in the network between amylopectin regions and resulting in more small amylose aggregates.

Appearance of the pellet from the stored tapioca starch paste was similar to that of the whole paste prior to centrifugation, but there was a progressive increase in the number of small, intensely staining amylose aggregates with time. After 28 days, pellet microstructure was dominated by amylose aggregates and non-distinct regions of amylopectin. Aggregates always appeared bound to networked amylose. The large blue-staining sheets present in the supernatant of the fresh paste changed color (from blue to reddish purple) after seven days at 4°C, indicating the presence of networked amylopectin in the sheets present in the fresh paste, which became apparent only after amylose had condensed into small aggregates. After seven days, microstructure of the tapioca starch paste supernatant appeared to be very similar to that of the supernatant of rice starch paste stored seven days (Fig. 3J). Some of the edges of the large sheets no longer appeared

sharp and defined, and some sheets had lost their apparent homogeneous nature.

In comparison to the microstructure of the whole paste after similar storage time, there was a noticeable absence of small, intensely staining aggregates. Closer inspection of the sheet microstructure revealed the presence of very small aggregates that may have been precursors to the small amylose aggregates seen in the paste and supernatant. Upon further storage of tapioca starch paste, the reddish purple sheets in the supernatant became progressively more red in color and continued to deteriorate in a manner similar to that which occurred during the first seven days. At no time were the small, intensely blue-staining aggregates seen in the paste and pellet present in the supernatant, which indicates that the blue aggregates present in the paste after storage were either very dense relative to other paste components or aggregates were not bound tightly to the paste matrix, thus allowing the aggregates to be pelleted upon centrifugation.

These results suggest that amylopectin was in, at least, two states after gelatinization: integrated with amylose to form the large sheets (seen in the fresh paste supernatant) and as clusters of non-distinct amylopectin (seen in the fresh paste pellet). Results also indicated that the amylopectin present in the sheets formed independent networks that remained after amylose was removed by retrogradation and centrifugation.

The initial fairly rapid rise in retrogradation in tapioca starch pastes as determined by turbidometric analysis (Figs. 1 and 2) and by Whistler (1954) corresponded to a loss of networked amylose in the paste supernatant and an increase in the number of small amylose aggregates. After the initial rapid rise in turbidity, slow retrogradation continued, a result that corresponded with an increase in the number of small amylose aggregates during the storage period. The results of Whistler (1954) indicated very little precipitation of tapioca starch from 5 to 60 days, while in this work, progressive microstructural changes and increases in turbidity were observed during this period.

Storage of WM starch paste had little effect on its microstructure, i.e., the microstructure of a paste stored 14 days at 4°C (Fig. 3N) was found to be indistinguishable from that of the fresh paste. Pastes consisted of granule remnants (40–100 µm diameter) and smaller fragments varying in brick-red color intensity in a matrix of very lightly red-stained amylopectin (Obanni and BeMiller 1995).

The commercial WM starch used to make the paste appeared to have been contaminated with CC starch (based on the presence of dark blue-purple-staining granule remnants), a general and unavoidable occurrence in commercial preparations arising from incomplete segregation during pollination, transport, storage, and factory processing. The observed ratio of CC starch granules to WM starch granules is likely exaggerated due to differences in gelatinized granule integrity, i.e., swollen WM starch granules break down more readily than do swollen CC starch granules.

Microstructure of fresh and stored WM starch paste pellets consisted almost entirely of granule remnants and fragments. In the little interstitial space present, lightly stained amylopectin could be seen. No granule remnants or fragments were present in supernatants from fresh and stored WM starch pastes. Only lightly stained amylopectin with little fine structure could be seen. Pellet and supernatant microstructures underwent no discernible changes upon storage at 4°C for up to 56 days. Lack of granule remnants in the supernatant indicated that the density of the granule remnants was sufficient that the amylopectin network formed from gelatinized WM starch could not keep them suspended on centrifugation. These microstructural changes are congruent with the results of Whistler (1954), which showed very little change in WM starch paste on storage. The gradual increase in WM starch paste turbidity (Figs. 1 and 2) could not be related to any visible microstructural changes using iodine staining and light microscopy.

Presence of substantial amounts of amylopectin in the extragranular network of rice, potato, and tapioca starch pastes and its absence in WM starch pastes might indicate a requirement for co-association of amylose and amylopectin. This idea is supported by the observation that, in wheat and CC starch pastes, amylose is always found associated with swollen granules and granule ghosts, which contain mostly amylopectin and which may provide nuclei for amylose crystallization. However, another possible reason for these phenomena is that there is something about the fine structure of the amylopectin or something about the granule characteristics of rice, potato, and tapioca starches, as opposed to CC and WM starches, that allows more amylopectin to leach from gelatinized granules of the former starches.

With regard to amylopectin association, Callaghan and Lelievre (1985, 1986) determined from self-diffusion coefficients obtained using pulsed-field gradient NMR that wheat starch amylopectin molecules in water aggregate to form structures with molecular volumes 400 times greater than the molecular volumes found when DMSO is the solvent. They reported that, in DMSO, wheat starch amylopectin takes the shape of a highly planar, oblate ellipsoid and concluded "that the planar shape of the amylopectin molecule is conducive to aggregation in poor solvents," viz, water, in which more spherical aggregates occur. Sedimentation-coefficient values obtained by analytical ultracentrifugation confirmed the flat-sheet, highly planar nature of wheat starch amylopectin (Lelievre et al 1986). Lelievre et al (1986) determined that, in DMSO, the flat-sheet structure of wheat starch amylopectin had a semi-major to semi-minor axis ratio of 37.5. Callaghan and Lelievre (1985) determined the ratio to be 17.5. In light of this information, aggregation of solubilized amylopectin into networks, either with or without amylose, does not seem surprising.

Swinkels (1985) reported the percent by weight of starch that precipitated from 2% solutions after 30 days of storage at 0–2°C as: WM, 1; tapioca, 13; potato, 20; wheat, 52; CC, 62. For WM, tapioca, and potato starches, the amount of precipitate was roughly proportional to the amylose content. However, considerable amylopectin must have coprecipitated with amylose from common corn and wheat starch pastes. Without mentioning that the precipitate must have contained approximately 50% amylopectin, Swinkels (1985) speculated that the large amount of precipitate might be related to the relatively small molecular size of corn and wheat starch amylose and/or to the relatively high lipid content of cereal starches.

When pastes were inspected for microbial growth (after 28 days) with plane-polarized light using non-stained paste samples, it was discovered that some starch granule remnants had become birefringent. The birefringence lacked any pattern and occurred near the outer edges of intact swollen granules (not in ghosts, clusters, or fragments). The phenomenon was seen frequently in potato and wheat starch pastes, to a lesser extent in CC starch and WM starch pastes, and was not observed in either rice or tapioca starch pastes.

To determine if this occurrence was due to an incomplete loss of birefringence upon cooking, potato starch was gelatinized on a hot stage microscope by heating to a temperature just past the temperature at which all granules lost birefringence. After storage at 4°C for seven days, many of the granules regained birefringence when viewed with plane-polarized light. Other starches were then gelatinized by the same procedure to determine if they too would regain birefringence when gelatinized under static conditions, then held at 4°C. The same starches that regained birefringence in the 2% pastes regained birefringence after static cooking on the hot stage. Since this phenomena occurred with WM starch, organization of amylopectin in the swollen granules must have been the cause of the anisotropy.

These results indicate that A) during gelatinization at a minimal temperature and without shear, the amylopectin in potato, wheat, CC, and WM remained in a somewhat ordered, but isotropic, state

and B) amylopectin in granules swollen under gentle cooking conditions was able to form organized anisotropic regions, which may be related to the order in native granules and may have been the result of amylopectin recrystallization in swollen granules. Interestingly, the two starches that did not regain birefringence (tapioca and rice) both produced pastes that, on aging, showed the presence of networked amylopectin. This may indicate that the amylopectin in these two starches may be more readily leached from swollen granules during gelatinization, resulting in the inability of gelatinized granules to regain birefringence.

CONCLUSIONS

Retrogradation in stored (4°C for 56 days) 2% pastes prepared by atmospheric cooking under mild shear conditions of starches from various botanical sources as determined quantitatively by turbidometric analysis revealed that retrogradation rates followed the order wheat, common corn > rice, tapioca, potato >> waxy maize. Fresh and stored paste microstructures and granule remnant morphologies were unique to each starch evaluated, reinforcing the conclusion that each starch is unique in its behaviors. Fresh pastes from amylose-containing starches were dominated by networked amylose. Upon storage, the networked amylose underwent the most obvious changes, often transforming to a dense, aggregated state. Amylopectin was present in fresh pastes in a variety of states that changed very little during storage. Overall, in amylose-containing starches, solubilized amylose generally co-crystallized or precipitated with amylopectin and/or crystallized or precipitated onto amylopectin-rich granule remnants. The latter interaction appeared to be generally weak, as low-g centrifugation usually resulted in at least some separation of the phases. It was also indicated that, in fresh pastes of some starches, solubilized amylopectin and amylose interacted to form networks. Birefringence could be regained in a portion of the granule remnants of some starches, indicating that amylopectin present in granule remnants of these starches has the ability to become anisotropic, possibly reflecting the original molecular order of the native granule. Microstructural changes in stored pastes were related to turbidometric results. Curves reflecting changes in turbidity and microscopic observations both reinforce the concept that retrogradation in pastes is a complex process involving amylose and amylopectin in different states, individually and together.

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 use of the facility and the Olympus Vanox photomicroscope.

LITERATURE CITED

American Association of Cereal Chemists. 1983. Approved Methods of the AACC, 8th ed. Method 44-40, approved April 1961, reviewed October 1976 and October 1982. The Association: St. Paul, MN.

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

Autio, K., Poutanen, K., Suortti, T., and Pessa, E. 1992. Heat-induced structural changes in acid-modified barley starch dispersions. *Food Struct.* 11:315-322.

Bowler, P., Williams, M. R., and Angold, R. E. 1980. A hypothesis for the morphological changes which occur on heating lenticular wheat

starch in water. *Starch/Stärke* 32:186-189.

Callaghan, P. T., and Lelievre, J. 1985. The size and shape of amylopectin: a study using pulsed-field gradient nuclear magnetic resonance. *Biopolym.* 24:441-460.

Callaghan, P. T., and Lelievre, J. 1986. The influence of polymer size and shape on self-diffusion of polysaccharides and solvents. *Anal. Chim. Acta* 189:145-166.

Craig, S. A. S., Maningat, C. C., Seib, P. A., and Hosney, R. C. 1989. Starch paste clarity. *Cereal Chem.* 66:173-182.

Ellis, H. S., Ring, S. G., and Whittam, M. A. 1988. Time-dependent changes in the size and volume of gelatinized starch granules on storage. *Food Hydrocoll.* 2:321-328.

Foster, J. F., and Serman, M. D. 1956. A light scattering investigation of the retrogradation of amylose. *J. Polym. Sci.* 21:91-101.

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

Jacobson, M. R. 1994. Starch retrogradation: acceleration, inhibition and microstructure. Ph.D. Thesis, Purdue University.

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

Lelievre, J., Lewis, J. A., and Marsden, K. 1986. The size and shape of amylopectin: a study using analytical ultracentrifugation. *Carbohydr. Res.* 153:195-203.

Miles, M. J., Morris, V. J., and RING, S. G. 1985. Gelation of amylose. *Carbohydr. Res.* 136:257-269.

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

Orford, P. D., Ring, S. G., Carroll, V., Miles, M. J., and Morris, V. J. 1987. The effect of concentration and botanical source on the gelation and retrogradation of starch. *J. Sci. Food Agric.* 39:169-177.

Paschall, E. F., and Foster, J. F. 1952. An investigation by light scattering of the state of aggregation of amylose in aqueous solutions. *J. Polym. Sci.* 9:73-84.

Ring, S. R., Colonna, P., l'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., and Orford, P. D. 1987. The gelation and crystallization of amylopectin. *Carbohydr. Res.* 162:277-293.

Roulet, P., MacInnes, W. M., Gumy, D., and Würsch, P. 1990. Retrogradation kinetics of eight starches. *Starch/Stärke* 42:99-101.

Svegmark, K., and Hermansson, A.-M. 1991. Distribution of amylose and amylopectin in potato starch pastes: effects of heating and shearing. *Food Struct.* 10:117-129.

Svegmark, K., and Hermansson, A.-M. 1992. Microstructure and viscoelastic behavior of potato starch pastes. Pages 93-99 in: *Gums and Stabilizers for the Food Industry*, Vol. 6. G.O. Phillips, D.J. Wedlock, and P.A. Williams, eds. Oxford University Press: Oxford.

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.

Swinkels, J. J. M. 1985. Composition and properties of commercial native starches. *Starch/Stärke* 37:1-5.

Virtanen, T., Autio, K., Suortti, T., and Poutanen, K. 1993. Heat-induced changes in native and acid-modified oat starch pastes. *J. Cereal Chem.* 17:137-145.

Whistler, R. L. 1954. Starch retrogradation. Pages 213-228 in: *Starch and Its Derivatives*, 3rd Ed. J.A. Radley, ed. John Wiley & Sons: New York.

Whistler, R. L., and Johnson, C. 1948. Effect of acid hydrolysis on the retrogradation of amylose. *Cereal Chem.* 25:418-424.

Woodruff, S., and McMasters, M. M. 1938. Gelatinization and retrogradation changes in corn and wheat starches shown by photomicrographs. Pages 1-43 in: *University of Illinois Agricultural Experimental Station Bulletin* 445.

[Received February 2, 1997. Accepted June 20, 1997.]