VISUAL OBSERVATION OF WHEAT-STARCH GELATINIZATION IN LIMITED WATER SYSTEMS[1]

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ABSTRACT

Direct microscopic observation of starch in limited water systems was employed in the development of a visual reference series of gelatinized starch granule structures; this series can be used as an aid in relating these structures to the quality of the final baked or cooked food products. In these products the swelling of starch is controlled not only by temperature but also by the amount of moisture available to the starch granules. The availability of water is determined by the formula or recipe used and by the presence of ingredients or components such as proteins, pentosans, and sugars which compete with starch for water. The amount of moisture which is available for starch gelatinization is also determined by the degree of protection against water absorption which fat provides to the starch particles. Data obtained in this study suggest that the recognized value of pin-milling cake flour may be owing to the release of starch granules from the protection of the protein matrix and, consequently, to greater contact and reaction between starch and water.

Most studies concerning gelatinization of starch employ a large excess of water where changes in starch, as reflected by viscosity and visual structure, are a function of only one variable—temperature. However, in evaluating the continuously changing structure of starch in most baked, cooked, or processed food products, there is no excess of water. Indeed, under practical, working conditions the changes in starch are a function of both temperature and amount of available water. If the temperature is maintained at 100°C, changes in starch depend principally on the amount of available water.

The extent of the effect of starch on physical characteristics of various foods is determined by the degree of structural change of the starch granule as controlled by the amount and availability of water and by the heat treatment to which these foods are subjected. We believe that visual monitoring of physical changes which starch undergoes during preparation of foods is useful in determining the extent of starch gelatinization. Such monitoring will aid in studying the effect of formula and baking or cooking conditions on eating quality and other physical characteristics of various foods.

The present study was designed to demonstrate in pictorial form how gelatinization of starch proceeds in heated systems in which the water available for starch gelatinization is limited. Starch structures in several food products are shown.

MATERIALS

A commercial cake flour and Aytex (R) wheat starch (supplied by General Mills, Inc.) were used in this study. The protein contents of the flour and starch were 8.3 and 0.6%, respectively, and the ash contents were 0.31 and 0.21%,

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respectively; all analytical results were expressed on a 14% moisture basis.

METHODS

Baking Methods

The formula and procedure employed in the preparation of white layer cakes were those previously outlined by Miller and Trimbo (1). A hot farinograph technique (1) was used to obtain samples of cake crumb at different stages of baking under controlled experimental conditions. Bread, prepared by the straight-dough method, incorporated 2.5% yeast, 5% sucrose, 2% salt, 3% nonfat dry milk, 3% shortening, 0.5% yeast food, and 66% water (as per cent of flour content). Bread was baked for 26 min at 460°F. Russian teacake cookies, pie crust, biscuits, and gravy were prepared using the formulas and procedures described in Betty Crocker's Cookbook (2).

Extraction of Starch from Baked Products

Samples of about 3 g were taken from the interior of baked products. One-inch cubes of cake or bread were taken at a distance of at least 0.25 in. from the closest exterior surface. For products baked for short periods of time, samples were magnetically stirred for 30 sec in 50 ml of water. After passing the slurry through 12XX bolting silk (75 μ openings), the dilute suspension was centrifuged and the starch was resuspended in a volume of water sufficient to give a sample suitable for photomicrography. Granule separation was not necessary for microscopic examination. For cake and bread baked for 17 min or longer, it was necessary to effect release of starch through digestion of the protein with ficin. After reacting a cake suspension with the enzyme (5 mg of enzyme per 20 ml of suspension) for 1 hr at room temperature, starch was separated by passing the slurry through bolting silk. The starch-containing portion was then centrifuged and the starch was resuspended in an appropriate volume of water for photomicrography. There was no noticeable effect of ficin on the starch during the period required for visual examination and photographing of the sample.

Photomicrography

Starch granules, isolated from both starch-water systems and from baked products, were photographed at 200× magnification. To enhance contrast and surface detail, oblique substage illumination was used to provide optical shadowing of the starch granules. A high-grade, seven-element condenser, equipped with a translatable iris diaphragm, was used to control the transmitted illumination. Except when oil immersion or very high dry objectives were required, the condenser was used without the top element. A similar technique was reported by Hlinka and Sanders (3) using the rotatable turret iris of Zeiss phase contrast optics.

Preparation of a Starch Structure Index

Starch in Water. A batch method was used in the preparation of a series of visual reference micrographs representing starch granules heated under limited moisture conditions. The starch-water mixture used to prepare each sample was sealed in the lower 2-in. section of a 4 × 9-in. low-density polyethylene "Whirl-Pak" pouch. After tempering and intermittent kneading for 15 min, each sample was submerged in a beaker of boiling water for 10 min while being subjected to occasional kneading. Additional heating resulted in no observable changes in the starch granules. The samples in each series differed only in the amount of Aytex starch used. All samples contained 2.5 ml of water while their starch content varied from 1.7 to 5.4 g. Structural changes in the granule were controlled, therefore, by the starch:water ratio. The starch suspension in each pouch was allowed to cool at room temperature, and the starch was then resuspended in added[3] water, placed on a microscope slide, and photographed. For those samples which formed gels as a result of higher moisture levels, it was possible to separate the starch granules through gentle kneading. This method exhibited excellent reproducibility; starch from all parts of a treated sample gave similar results.

[3]Sufficient water was added to a small portion of the sample to render it suitable for microscopy.

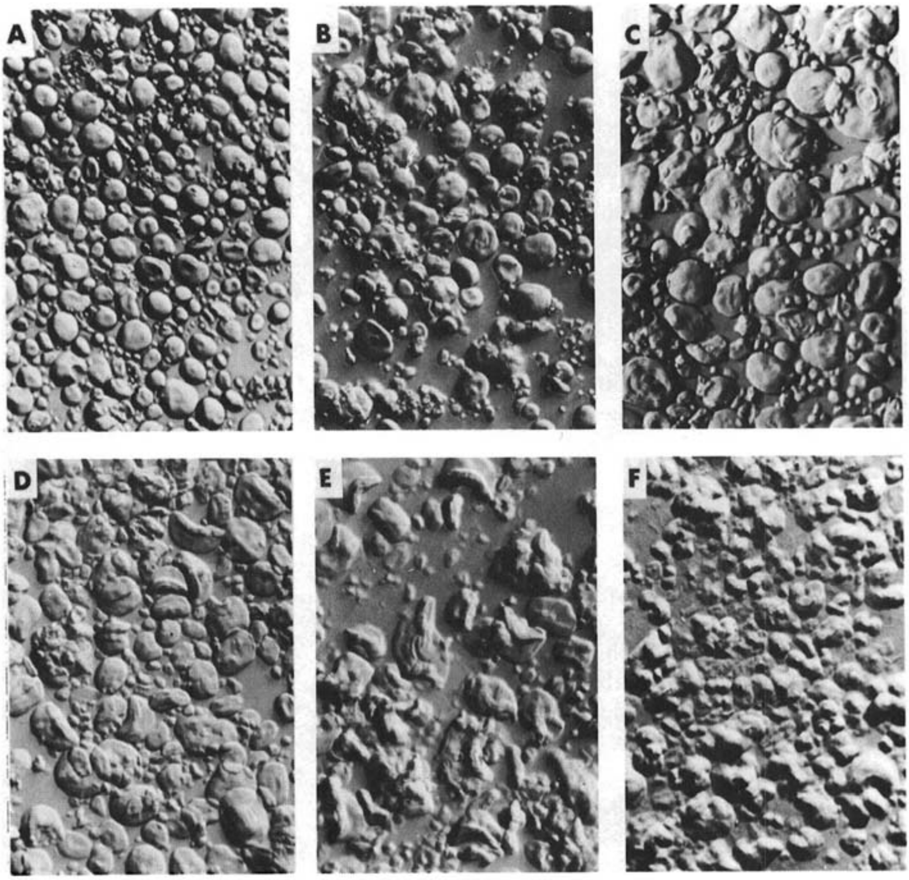

Fig. 1. Starch granules heated to 100°C under various limited moisture conditions. A) Control at 32% moisture; B) 33% moisture, 50% birefringent; C) 39% moisture, major swelling; D) 43% moisture, initial folding; E) 56% moisture, strong folding; and F) 60% moisture, fully gelatinized.

Starch and Sucrose in Water

A second series of visual reference micrographs was obtained using sucrose to limit the amount of water available for gelatinization of starch. In this series the same amount of sucrose was used in all samples, but the amount of water was varied to give different sugar concentrations. The same type of plastic pouch as described above was used. Before adding starch, each pouch was sealed and heated to 80°−100°F to dissolve all added sugar. After the sugar solution had cooled to room temperature, 1 g of starch was incorporated; the pouch was then re-sealed and re-heated in boiling water for 10 min. Following a second cooling period, some of the starch obtained from each sample was resuspended in added[3] water and the suspension was placed on a microscope slide and photographed. Since this method permitted suspension of starch in relatively large volumes of sugar solutions, no gel formed; thus, less difficulty was experienced in photographing the starch suspension.

RESULTS AND DISCUSSION

Visual Index of Starch Gelatinization in Limited Water

Visual observation of starch consists mainly in determining from its structural appearance the extent of granule gelatinization. Visual comparison using a microscopic technique and a visual reference series is especially useful in monitoring the degree of cooking of starch in any given food product. Using this method of analysis, starch granule disintegration—a condition which is usually undesirable—can be quickly determined and corrected. The entire range of the gelatinization reaction, as reflected by its effect on the granule, can be related to the desired properties of a product.

The amount or degree of gelatinization is commonly used as a measure of starch function; however, it has limited meaning when based on a change in

TABLE I
Starch Gelatinization at 100°C in a Limited Amount of Water

Photo	Starch[a]/Water Ratio	Birefringence %	Water %	Granule Structure
A	54:25	90	32	Control[b]
B	52:25	50	33	First-stage swelling[c]
C	39:25	2	39	Approximate second-stage swelling
D	33:25	2	43	Initial folding (third stage)
D	20:25	0	56	Strong folding
F	17:25	0	60	Fully gelatinized (as in gravy)

[a]On dry basis.
[b]At threshold of swelling.
[c]50% loss of birefringence.

birefringence or on some other undefined point further along in the gelatinization reaction. Observations based on loss of birefringence relate to a very restricted *initial* event in the total gelatinization process.

If microscopic techniques are used to determine starch structure changes in various food products, it is necessary to establish the effect of variations in

Fig. 2. Wheat starch heated at 100°C in a sugar solution.

temperature and water availability and amount on observed changes in starch granule structure. It was, therefore, convenient to prepare a series of micrographs for starch which had been cooked under two sets of conditions known to limit the availability of water in foods. Under the first set of conditions, the amount of water available for gelatinization was varied. Since it has been shown that the interior of a cake during baking reaches a temperature of 100°C (4), this temperature was chosen for preparation of the first visual reference series. In this system, the gelatinization reaction proceeds until the reaction is terminated by lack of water and no further significant structural change in the starch takes place.

The reference series, prepared by gelatinizing starch at 100°C under various limiting moisture conditions, is shown in Fig. 1. The six main structural changes which occur in the wheat-starch granule during gelatinization under different limited water conditions are shown in A–F. The control (Fig. 1A) represents heated starch containing 32% water—an amount which is insufficient to permit swelling or loss of birefringence at 100°C. The first observable change in structure is shown in Fig. 1B where a 50% loss of birefringence occurred. This is considered the first "stage" of gelatinization and is commonly referred to in the literature as the "point of gelatinization" (5). This point can be established fairly accurately by the use of polarized light. Figure 1C illustrates the appearance of gelatinized starch as it occurs in a properly baked cake in which there is near-maximum swelling of the starch granules and in which is present a small percentage of folded granules. In a system containing excess water, this structure would occur during the first plateau (or stage of gelatinization) based on an amylograph curve for starch heated in the presence of carboxymethyl cellulose (6).

The folding structure which develops when more water is added is shown in

TABLE II
Starch Gelatinization at 100°C in Water Limited by Sugar Concentration

Sugar g	Water g	Starch[a] g	Moisture in Starch after Heating (Calculated) %	Structure
16.85	7.43	1	32[b]	Control[c]
16.85	8.55	1	61	50% Birefringence, first-stage swelling
16.85	9.67	1	73	Second-stage swelling
16.85	10.8	1	80	Third stage (folding)
16.85	25.4	1	95	Strong folding
16.85	50.5	1	98	Fully gelatinized (as in gravy)

[a]Dry basis.
[b]This value was determined from the ratio of reacting starch and water (Table I).
[c]Control at threshold of swelling.

Fig. 1D. Additional water causes strong folding and collapse of the granules (Fig. 1E) until the gelatinization reaction is virtually complete. Beyond this point the granules collapse and start to disintegrate and the structure resembles one which develops in gravy (Fig. 1F). Data relating to the amounts of water we found necessary to produce the different structures are presented in Table I. The structures shown in photomicrographs D, E, and F (Fig. 1) probably develop during the second stage of gelatinization (6,7) where, in the presence of additional increments of water, viscosity increases rapidly and there is structural collapse of the granules (8).

Since sugar is incorporated in many baked goods, a second method of observing starch gelatinization was explored in which water availability was controlled by varying the concentration of sugar. By this method, a series of gelatinization structures (not shown) was obtained. The structures were similar to those shown in Fig. 1, but two differences were apparent: 1) The granules were greatly disturbed in the hylum area during the swelling process. This effect was made more visible (Fig. 2) through the use of strong optical shadowing. During the third or folding stage, this structure largely disappeared. 2) When sugar was used as a means of controlling moisture availability, relatively more water (Table II) was required to produce structures which were similar to those illustrated in Fig. 1.

To determine how much water was available to the starch in a sugar solution, we found that 1 g of starch heated to 100°C in a solution of 16.85 g of sugar and 7.43 g of water (Table II) resulted in the starch being at its swelling threshold with no loss in birefringence. Since it was not possible to determine directly the moisture content of the starch in the sugar solution, we assumed that the starch contained 32% moisture which was the moisture content for its swelling threshold value shown in Table I. To attain 32% moisture, 1 g of starch was calculated to absorb 0.47 g of water in order to yield a wet weight of 1.47 g ($1.47 \times 0.32 = 0.47$). The remaining 6.96 g of water in the system was unavailable to the starch because of the dissolved sugar.

In Table II, calculation of the amounts of water absorbed by starch in the

TABLE III
Moisture Content of Baked or Cooked Products before and after Heating

Baked or Cooked Product	Moisture Content: Before baking or cooking %	Moisture Content: After baking or cooking %
Russian teacake cookies	11	6.6
Pie crust	19	1.3
Cake	37	30.0
Biscuits	41	30.1
Bread	43	37.7
Gravy	83	83.0

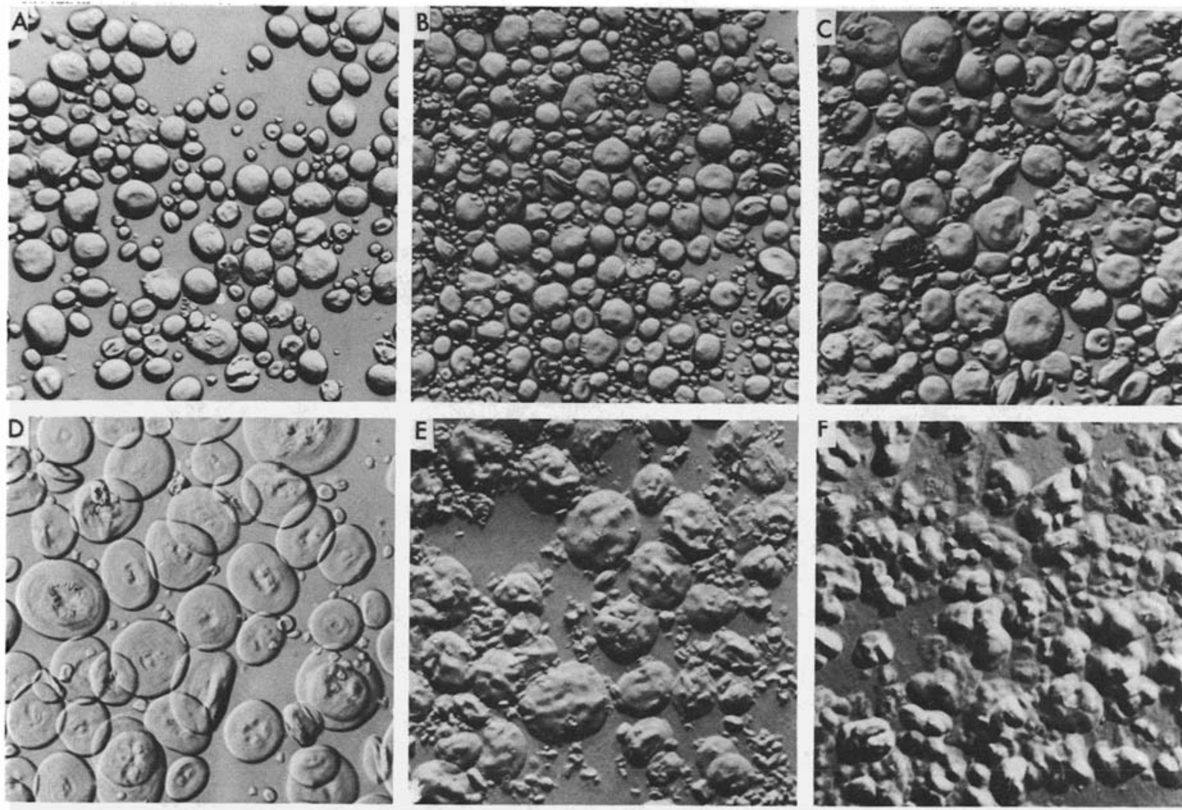

Fig. 3. Photomicrographs of starch taken from several baked or cooked products: A) Control (unheated); B) Russian teacake cookies; C) pie crust; D) cake; E) biscuits or bread; and F) gravy.

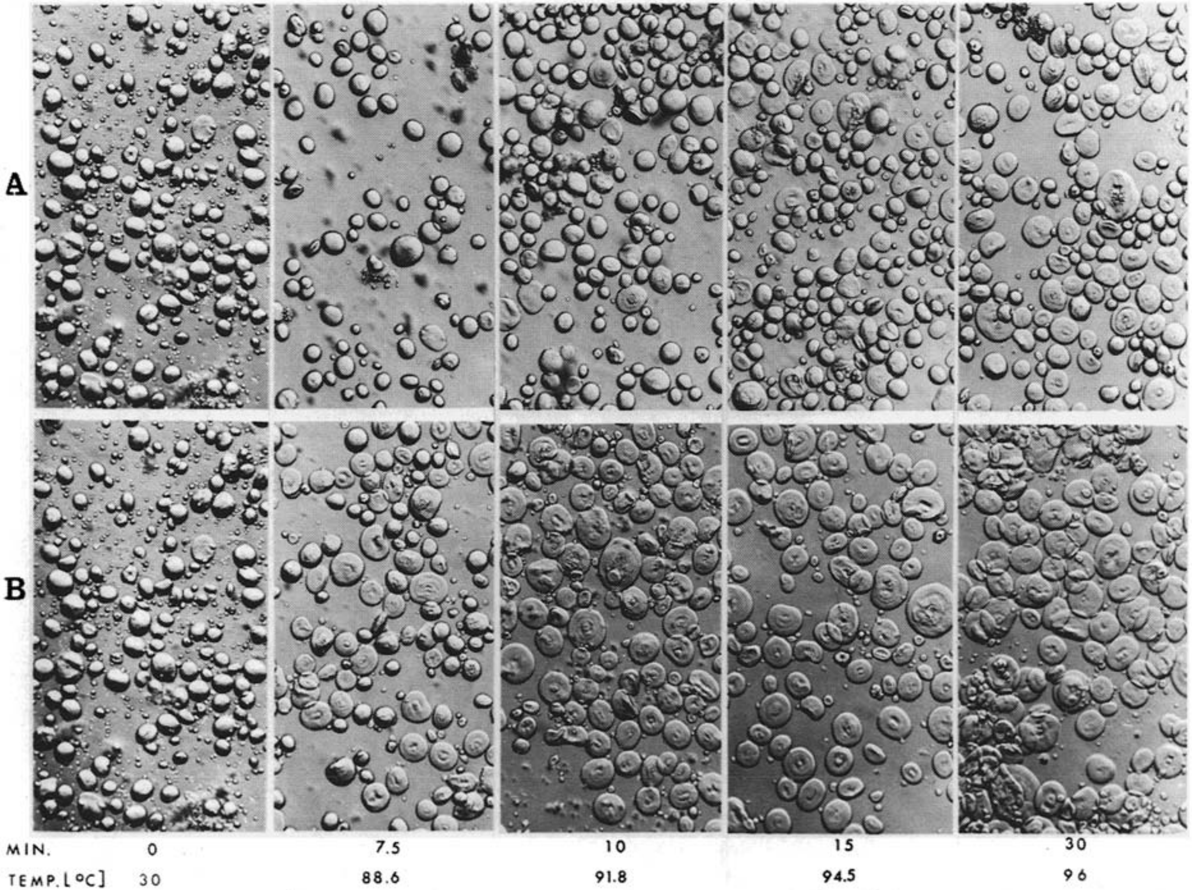

Fig. 4. Photomicrographs of starch taken from cake batters after heating to 96°C in a farinograph (1). A) Commercial white layer cake batter containing 80% liquid (based on flour weight), and B) commercial white layer cake batter containing 120% liquid (based on flour weight).

presence of different concentrations of sugar is based on the assumption that all water in excess of the control amount is available for reaction with starch. This excess water (above the control amount) is required in greater amounts at different stages of starch gelatinization when sugar is present than when it is absent (Table I). Bean and Yamazaki (7) also observed that when heated in concentrated sugar solutions, starch granules attain a higher maximum diameter and display a more rapid rate of increase in diameter once swelling begins than starch granules heated in excess water in the absence of sugar.

Starch Granule Structure in Baked Products

As indicated in Table III, the moisture content of ingredients in various baked and cooked products after mixing, but prior to baking, range from very low (11%) to very high (83%). The structure of the starch granules in these products after baking or cooking is illustrated in Fig. 3. Structural differences reflect differences in the availability of water in the various products. Figure 3A represents the unheated control starch.

The structure of starch granules obtained from Russian teacake cookies is shown in Fig. 3B. This structure is similar to that of the reference control (Fig. 3A) in that practically no loss of birefringence was sustained. Starch obtained from pie crust (Fig. 3C) was also more than 50% birefringent, indicating only a slight change in the starch. Starch gelatinization progresses further in cake and bread (Figs. 3D and and 3E, respectively) in which more water is used. The granules from bread, which contained more moisture than the cake investigated, are shown at the threshold of the folding stage. Fully gelatinized starch, as is observed in gravy, is shown in Fig. 3F.

Differences in the degree of starch gelatinization as affected by moisture content will also affect product volume. The photomicrographs in Fig. 4 show substantial size differences between starch granules obtained from commercial white layer cake batters prepared with 80 (Fig. 4A) and 120% (Fig. 4B) of normal water level. These batters were heated to 96° C in a farinograph using a technique described by Miller and Trimbo (1). Similar differences in the size of starch granules were found in starch samples obtained from actual cakes which were baked for different lengths of time. The volume of cakes prepared with 80 and 120% of normal water level was 600 and 1000 cc, respectively.

Differences in granule structure of starch obtained from two types of commercial precooked noodles are shown in Fig. 5. These products varied widely in eating quality and other physical characteristics. Figure 5A represents starch obtained from oriental fried noodles whereas Fig. 5B shows starch obtained from conventional precooked noodles. The starch samples were extracted after the noodles were steeped for 3 min in hot water following package directions. Before steeping, the appearance of starch from both noodle products was similar to that shown in Fig. 5A. It is apparent, therefore, that the oil used in fried noodles provides some protection against further gelatinization during the steeping process. By contrast, the starch in conventional noodles develops a strongly folded structure during steeping. These differences in the degree of starch gelatinization, as affected by the presence of oil, account for at least some of the differences in eating quality between these two commercial types of noodles.

Comparative Swelling of Free and Endosperm-Embedded Starch

Most of the starch in soft wheat flour (unless the flour is pin-milled) is

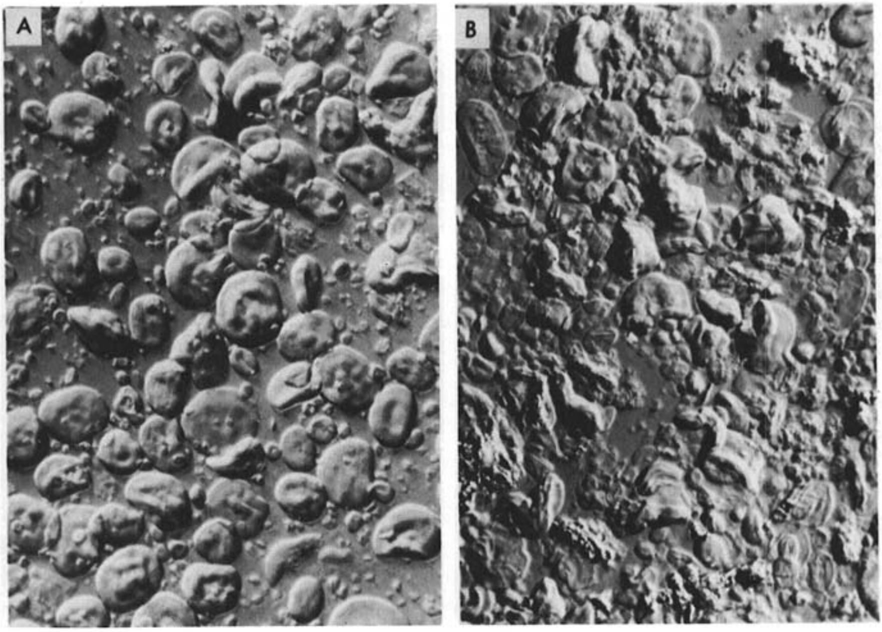

Fig. 5. Starch from precooked noodles after steeping. A) Oriental fried noodles, and B) conventional precooked noodles.

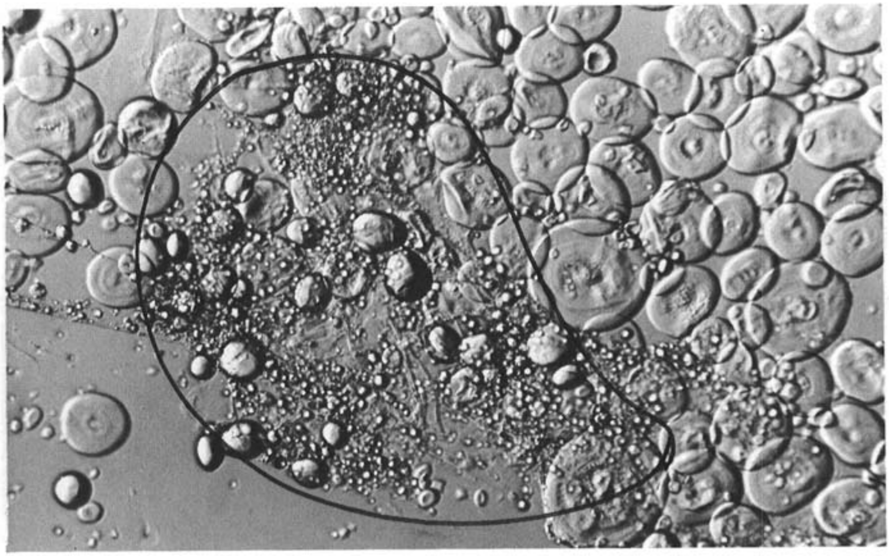

Fig. 6. Photomicrographs of starch and intact endosperm material taken from a cake batter after heating to 96°C in a farinograph.

embedded in the protein matrix. Flour protein minimizes contact and reaction between starch and water and, hence, there is (as shown in the encircled area in Fig. 6) little swelling of the embedded starch.

Miller *et al.* (9) reported that a limited degree of pin-milling will improve the baking quality of cake flour. If the degree of starch damage resulting from pin-milling is greater than about 5.7%, the quality of the flour is adversely affected. As indicated in Fig. 6, the beneficial effect on flour performance of a limited amount of pin-milling appears to be owing to the release of starch from the protein matrix which protects it against gelatinization. Howard *et al.* (10) have demonstrated the essentiality of intact starch granules during the thermal-setting process of cake-making.

Acknowledgment

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