Comparison of Methods to Determine Starch Gelatinization in Bakery Foods[1]

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ABSTRACT

Data from viscosity, enzymatic (using glucoamylase), and crystallographic methods for determining starch gelatinization and swelling in bakery foods were compared. Results from x-ray diffraction, polarization microscopy, and enzymatic methods complemented each other. However, crystallographic methods were not as sensitive to small changes in the degree of swelling as was the enzymatic method. In fact, for low moisture products, eg, sugar cookies, at least 11% gelatinization (from glucoamylase) could be present without substantially altering the x-ray pattern or intensity. The amylographic method for determining starch swelling in bakery foods was the least reliable.

The quality of both fresh and stored bakery foods has been related to the starch component. For this reason, many methods have been used to estimate the degree of starch gelatinization in bakery foods.

Yasunaga et al (1968), who used the amylograph to study the pasting characteristics of starch in bread, found that within a loaf of bread the outer 1-cm and 2-cm portions of the crumb produced a lower peak viscosity than did the center portion. Based on those data, they suggested that starch granules in the center of the crumb were less gelatinized than those in the crumb exterior.

On the other hand, light microscopy studies by Moss (1975) showed that the bread crust (a layer extending inward 0.5 mm) contained mainly unswollen granules. The starch granules just below this crust were all damaged, with maximum gelatinization (staining) occurring 0.6 mm from the top surface of the loaf. Below this layer, essentially all starch granules were gelatinized to the same degree.

Other light microscope studies (Flint et al 1970) indicate a gradient of gelatinized starch within a single baked product. Flint et al (1970) found that the top and bottom surfaces of English biscuits contained considerably less damaged starch than did the center portions.

The degree of starch gelatinization in bakery products also varies with the type of product (Derby et al 1975, Hoseney et al 1977, Lineback and Wongsrikasem 1980). Francis and Groves (1962) reported that starch granules in English biscuits were not gelatinized but those in high ratio cakes were completely gelatinized. In contrast, Shepherd and Yoell (1976) found that portions of the crumb of madeira cakes contained ungelatinized starch granules.

Many contradictions concerning the extent of starch gelatinization in bakery foods can be found in the literature, in part because of differences in products, product formulations, and processes. However, we believe that some discrepancies in results may be because of differences in methods used to study starch gelatinization and lack of consensus among researchers on the definition of starch gelatinization. The term has been used to denote: 1) loss of starch granule birefringence during heating, 2) increased susceptibility of starch to enzyme attack, 3) increased dye binding ability of starch, and 4) alterations in starch crystalline organization as measured by x-ray diffraction. The objective of this investigation was to study starch gelatinization in bakery products using the amylograph, enzymes, polarization microscopy, and x-ray diffraction. Comparison of data obtained by those methods may help to determine the extent to which methodology contributes to the discrepancies in published reports.

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MATERIALS AND METHODS

Baking Procedures

The product formulas for white bread and yellow and chocolate cakes are summarized in Table I.

Bread was made by a sponge and dough procedure, using a commercial bread flour (11.3% protein, 0.43% ash) that had been bleached, matured, and malted (470 BU on the amylograph). The flour had a farinograph peak time of 9.0 min and absorption of 65.2%, as determined by AACC method 54-21 (1976). The sponge was fermented 4 hr at 30°C, 86% rh. Sponge and dough were mixed on a Hobart A-200 mixer equipped with a McDuffee bowl and hook. Floor time was 30 min. Two 539-g portions of dough were divided, punched (National sheeting rolls), and given a 10-min intermediate proof. Molding was done on a Moline 100 molder, and the loaves were proofed to 1.5 cm above the pans at 41°C, 92% rh. The loaves were baked at 218°C for 20 min.

Cakes, made with a commercial chlorinated cake flour (8.2% protein, 0.3% ash, 4.8 pH), were mixed in a 5-qt bowl on a Hobart N-50 mixer. Single-stage mixing procedures were used for both yellow and chocolate cakes. Batter specific gravity for the cakes was about 0.90 and 0.95, respectively. Cakes were baked in 8-in. layers (425 g of batter) at 191°C for 25 min.

The sugar cookies were prepared according to AACC method 10-50D (1976). Cake doughnuts were made with a commercial mix (Pillsbury Cake Donut Mix 6060) and fried on a Belshaw Donut Robot 42 doughnut fryer.

All products were cooled 1 hr before being placed in polyethylene bags and frozen. Moisture was determined according to AACC method 44-15A (1976).

TABLE I
Product Formulas for White Pan Bread, Yellow Layer Cake, and Chocolate Layer Cake

Ingredients[a]	White Pan Bread		Yellow Layer Cake	Chocolate Layer Cake
	Sponge	Dough		
Flour	70	30	100	100
Water	42	28	130	100
Yeast	2.5	...	...	...
Salt	...	2.0	3.0	2.0
Sugar	...	7.0	120	113
Shortening	...	3.0	20	7.8
Nonfat dry milk	...	2.0	8.0	12.5
Whole eggs	...	...	...	50
Yeast food	0.18	...	...	...
Whole egg solids	...	...	8.0	50
Baking powder	...	...	6.0	4.2
Baking soda	...	...	...	1.0
Yellow color	...	...	3.0	...
Emulsifier	...	...	1.3	...
Cocoa, natural	...	...	...	3.5
Vanilla	...	...	...	2.1

[a]Ingredients reported in baker's percent.

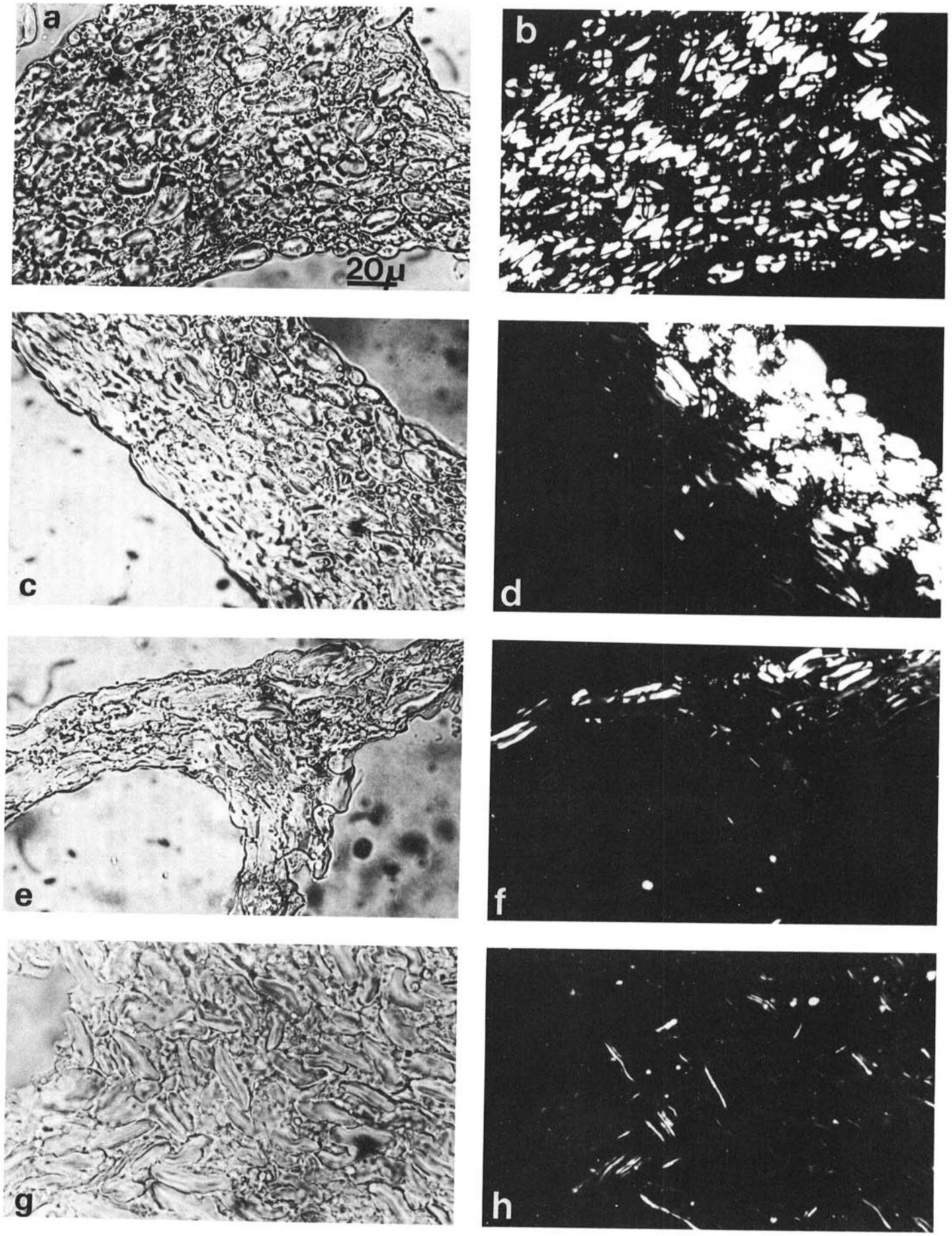

Fig. 1. Light photomicrographs of thin sections of bread, showing the same fields under normal and polarized light, all at the same magnification. **a,b,** bread crust; **c,d,** junction of crust and crumb; **e,f,** gas cells in outer 2-cm section of crumb; **g,h,** bread crumb center.

Amylograph

The frozen bread was divided into layers, and crumb slurries were prepared according to the procedure of Yasunaga et al (1968); the top crust was also studied. Sample moisture content determined the sample size; ie, solids were held constant (60 g). Amylograms were also run on cake crust and crumb by using 90 g of solids.

Frozen bakery samples were placed directly into the distilled water soaking solution and held at 30°C for 1 hr before being dispersed in a Waring Blendor. The amylograms were determined by using a 700-cmg^{-1} cartridge and a normal heating cycle (30–95° C).

TABLE II
Moisture Distribution in Bakery Products[a]

Product	Moisture (%)
White pan bread	
Crust	13.7
1 cm from the crust	33.6
2 cm from the crust	43.0
Center of loaf	42.7
Yellow layer cake	
Crust	19.3
Center	31.8
Chocolate layer cake	
Crust	20.3
Center	28.6
Cake doughnuts	
Crust	13.3
Center	29.1
Cookies[b]	3.1

[a] Products had been allowed to cool 1 hr.
[b] AACC (1976) formulation.

Starch Susceptibility to Enzyme Attack

Chiang and Johnson's method (1977) was used to determine the degree of gelatinization in starch washed from bakery products.

Starch was washed from fresh bakery products by making a slurry of the product with excess distilled water, gently stirring with a magnetic stirrer for 30 min, and then passing the slurry through 116-μm bolting cloth. The filtrate was centrifuged at 2,000 rpm for 10 min, and the supernatant was discarded. The starch sediment was resuspended in distilled water, centrifuged again, and then freeze-dried.

X-Ray Diffraction

X-ray diffraction studies were conducted on starch washed from fresh bakery products. X-ray diffraction patterns of starch (equilibrated to 96% rh) were examined in an x-ray diffractometer (Philips). X-rays were nickel-filtered copper radiation; operation was at 35 KV and 18 MA. Relative crystallinity was determined by Herman's method as described by Nara et al (1978).

Polarization Microscopy

Portions (1 cm^3) of fresh (1-hr) bakery products were frozen and sectioned (12 μm) on an American Optical Cryotome operated at −20° C. Sections were collected on albumin-coated glass slides, air-dried (5 min), and mounted with Permount. Pictures were taken on a Reichert (Austria) light microscope operating in bright-field and polarization modes.

RESULTS AND DISCUSSION

Moisture Distribution

Moisture distributions in bakery products are shown in Table II. The crust portions had consistently lower moisture contents than

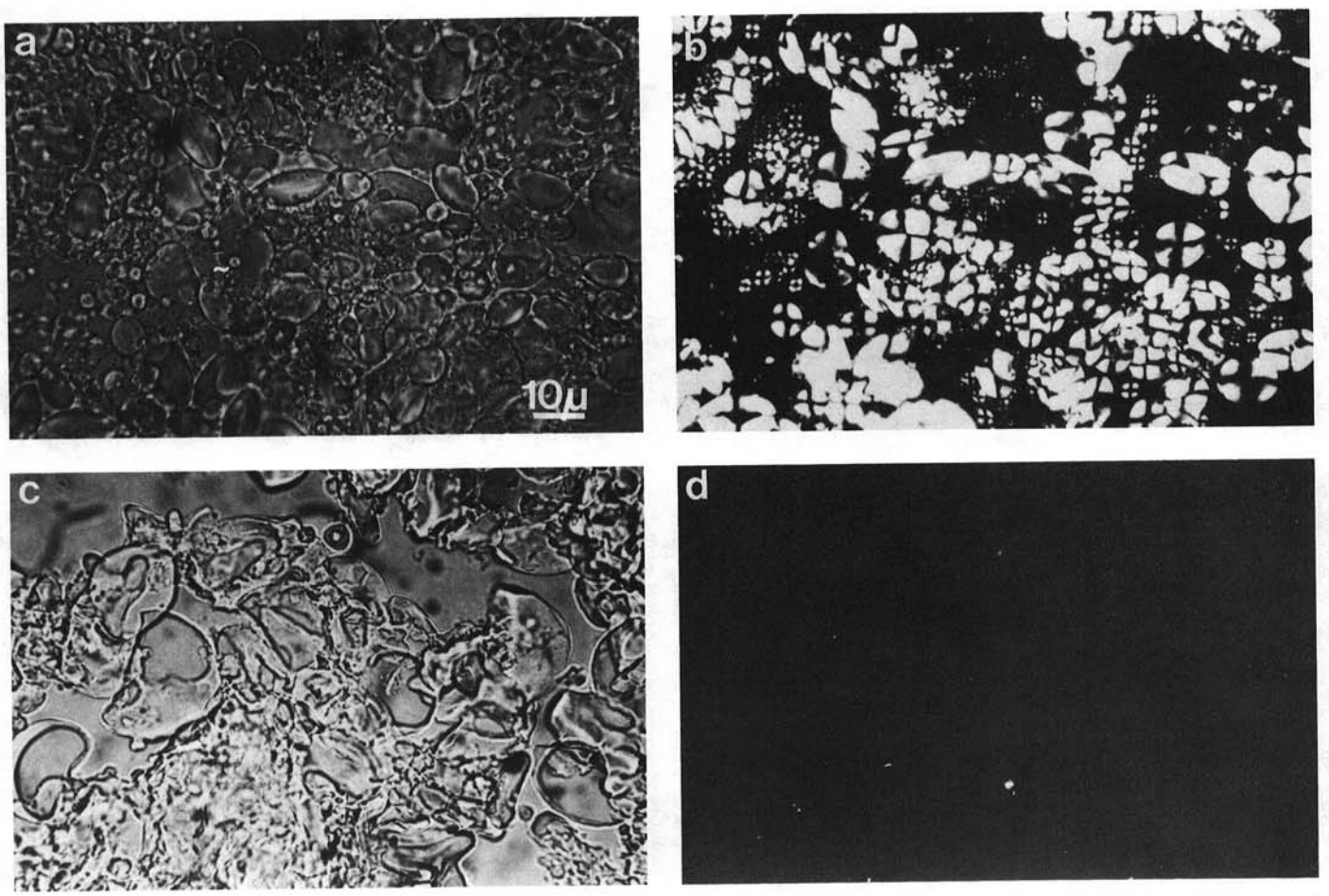

Fig. 2. Light photomicrographs of cake portions, showing the same fields under normal and polarized light, all at the same magnification. **a,b,** cake crust; **c,d,** center crumb.

did the interior portions of the products. Because moisture is continually being lost by evaporation from the surface of the baked products, these results were expected. Audidier (1968) concluded from thermal kenetic studies of bakery products that the free moisture content, rather than the temperature, was the most important factor determining starch gelatinization and swelling. Therefore, based on the moisture data presented in Table II, the peripheral portions of baked products should contain starch granules whose crystalline structure has been preserved to a larger extent than has that of the starch granules in the interior of the product.

Polarization Microscopy

Polarization microscopy of thin sections of bread (Fig. 1) showed that the crust contained many unswollen starch granules (Figs. 1a and b), as evidenced by the presence of maltese crosses. Many starch granules just beneath the crust (Figs. 1c and d) did not exhibit maltese crosses but some maintained slight birefringence, suggesting that limited swelling had occurred. In fact, some birefringent starch granules could be found throughout the crumb, particularly adjacent to gas cells (Figs. 1e and f). Figures 1e and f also illustrate that starch granules can be slightly elongated (deformed) and still maintain birefringence. Starch granules in the crumb center (Figs. 1g and h) were not extensively swollen but were deformed and in many cases exhibited slight birefringence. No differences in degree of starch swelling between outer 2-cm and center portions of the crumb were observed with light microscopy.

Retrogradation was probably not responsible for the birefringent starch granules observed in fresh bread crumb because bread samples were frozen immediately after cooling 1 hr, sectioned while frozen, and photographed within 2 hr after sectioning. Air drying the sections for light microscopy is also unlikely to have produced birefringent starch granules because many starch granules in hydrated (not air-dried) sections from the same sample also exhibited slight birefringence. We think that bread formulation and process are extremely important to these observations. Breads made by the continuous process consistently showed fewer birefringent granules in the fresh crumb than did breads made by the sponge and dough process.

Thin sections of cake (Fig. 2) also showed that the starch granules in the crust (Figs. 2a and b) were unswollen. Those in the cake center (Figs. 2c and d) were considerably more swollen but less deformed than those in the center of the bread crumb (Figs. 1g and h). Few birefringent starch granules were observed in the cake center.

Less than 50% of the starch granules in cake doughnut crust maintained their maltese crosses during the frying treatment (Figs. 3a and b). The granules in the center of the doughnut showed a wide range of swelling patterns, from extensive swelling and folding to limited swelling. Minimal birefringence was observed in the center portion.

Starch granules in sugar cookies (Fig. 4) maintained their maltese crosses at the cookie surface and in the center. The limited water content as well as high oven temperature and short baking time prevented any substantial starch swelling.

Enzymatic Determination of Starch Gelatinization

Data on gelatinized starch in bakery products, measured with glucoamylase and *o*-toluidine reagent (Chiang and Johnson 1977), are presented in Table III. The extent of starch gelatinization varied within each product. Starch gelatinization in bread crumb progressively increased from the exterior to the centermost portion. In addition, starch washed from the crust portions of bakery products was always less gelatinized than that washed from

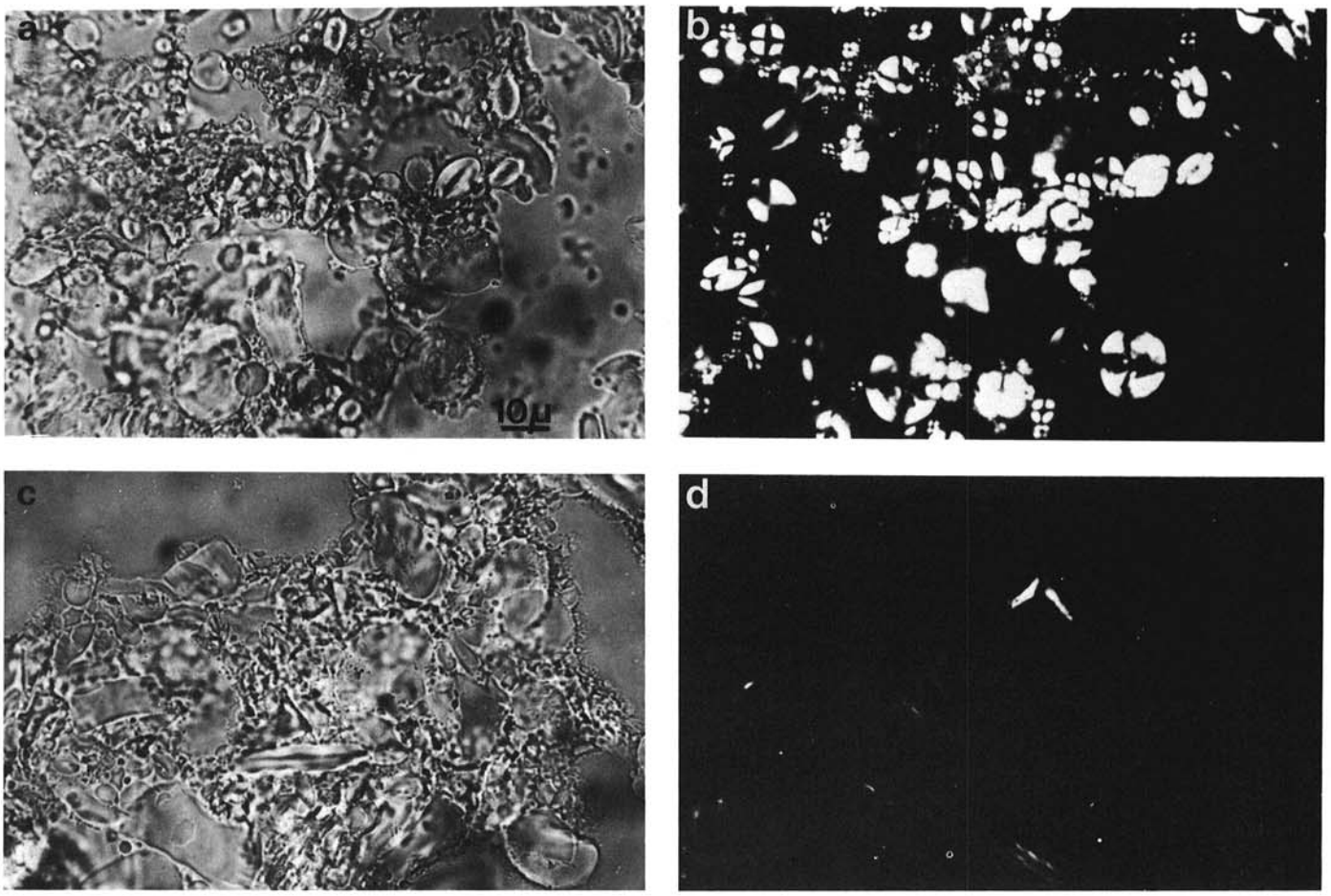

Fig. 3. Light photomicrographs of doughnut portions, showing the same fields under normal and polarized light, all at the same magnification. **a,b,** doughnut crust; **c,d,** center crumb.

the crumb portions, which agrees with the findings obtained with the polarization microscope.

The glucoamylase determination of starch gelatinization also indicated that the degree of starch gelatinization varied among products. The greatest starch gelatinization was found in cake doughnut centers, the least in cookies. Starch washed from cake centers was more gelatinized than was that washed from bread centers.

Determination of Gelatinization with the Amylograph

Amylograms for bread crust and portions of the crumb are shown in Fig. 5. Samples from the center of the loaf gave higher viscosities than did samples from any other portion, which agrees with results obtained by Yasunaga et al (1968). They concluded that viscosities differed because the starch granules in the outer portions of the loaf of bread were more gelatinized than those in the center portions. Their explanation of the amylograph results, however, cannot be reconciled with our microscope and glucoamylase observations. The browning reaction pigments formed in the crust may have inhibited starch swelling.

The viscosity of a bakery product suspension depends on factors other than degree of starch gelatinization or swelling. Because viscosity depends primarily on the effective volume occupied by the macromolecules, randomly oriented molecules should produce greater viscosities than should molecules with a more compact, ordered structure. Hence, differences in viscosities observed for bread crumb portions may be caused by differences in the orientation of starch molecules in the suspension as well as by differences in the degree of starch swelling. The effects of those two factors would be impossible to differentiate with the amylograph. In addition, the physical condition and behavior of other nonstarch polymers in solution would also affect the viscosity. Therefore, we believe that the amylograph method does not accurately indicate starch swelling in bakery products. Instead, the amylogram shows the sum of the contributions of all macromolecules to the viscosity of the crust or crumb slurry.

Data from studies on cake crust and crumb paralleled those obtained with the comparable bread portions (Fig. 6). Cake center portions produced a higher peak viscosity than did the cake crust.

TABLE III
Percent Starch Gelatinization in Bakery Products as Determined by Glucoamylase and *o*-Toluidine Reagent.

Starch Source	Gelatinized Starch[a] (%)
White pan bread	
Hard red winter flour	9.1
Crust	33.4
1 cm from crust	43.9
2 cm from crust	56.6
Center of crumb	70.1
Yellow layer cake	
Cake flour	3.7
Crust	10.5
Center	75.0
Chocolate layer cake	
Crust	9.8
Center	73.9
Cake doughnut	
Crust	61.6
Center	82.3
Sugar cookie [b]	
Cookie flour	4.3
Cookie	11.1

[a] Determinations were done on starch washed from the samples. Least significiant difference = 2.4 ($P < 0.05$).
[b] AACC (1976) formulation.

X-ray Diffraction

X-ray diffraction patterns of starch are altered when starch gelatinizes. Therefore, this method can be used to determine the molecular organization of starch in bakery products. Futhermore, information can be obtained from x-ray patterns by measuring diffraction intensities, which provide an indication of relative starch crystallinity.

X-ray diffraction patterns of starch washed from bread crust and crumb are presented in Fig. 7. Based on d-spacings and diffraction intensities reported by Zobel (1964) and Hellman et al (1954), the x-ray pattern of starch granules in the crust (Fig. 7b) was determined to be predominantly an A pattern superimposed with a V pattern (fat-amylose complex). The strong 4.4Å spacing (line 5) is typical of the fat-amylose complex, whereas the intensities of lines 3, 4, and 6 are indicative of the A pattern. The predominance of the A pattern in the starch from crust portions was not surprising, considering the results obtained with polarization microscopy (Fig. 1b). The high temperature at the surface of the loaf of bread resulted in the continual loss of moisture by evaporation from the crust and thus lessened the extent of starch gelatinization.

Not only did the pattern of starch from the crust differ from the pattern of the unheated starch (Fig. 7a), but the relative starch crystallinity was substantially reduced (Table IV), which indicated that an increase in starch crystallinity in the crust during baking was not responsible for the low amylograph viscosities obtained with crust portions of bakery foods.

Starch from bread crumb portions (Figs. 7c–e) showed only the fat-amylose complex characterized by the strong 4.4Å line (line 5). The intensity of that pattern varied with the location in the crumb (Table IV). Starch from the outer 2-cm portion was more crystalline than was starch from outer 1-cm or center portions. Variations in moisture distribution in the dough during baking probably contributed to variations in pattern intensities. Hellman et al (1954) have shown that for starch pastes with water contents above 43%, the intensity of the fat-amylose complex pattern is

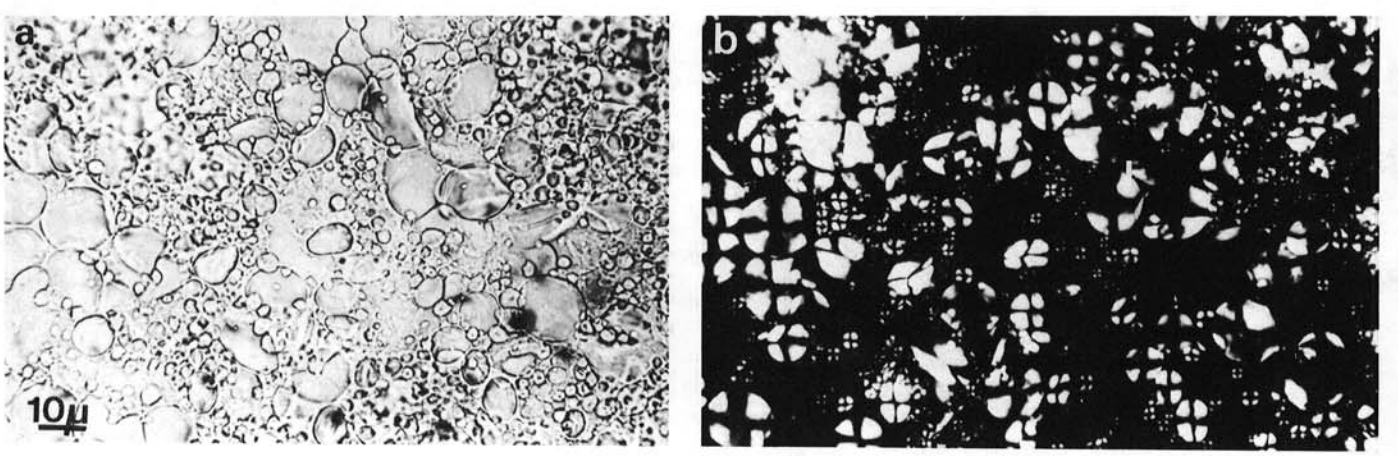

Fig. 4. Light photomicrographs of sugar cookie, showing the same fields under normal (**a**) and polarized (**b**) light.

greatly reduced.

Starch from cake crust (Fig. 8b) had an A pattern; that from the crumb exhibited an intense fat-amylose complex pattern (Fig. 8c). Both patterns were more intense than those observed for starches from bread crust and crumb (Table IV). Microscopy suggested that starch granules in the cake crumb (Fig. 2c) were more swollen but less deformed than starch in bread crumb (Fig. 1g). Those data, coupled with the presence of the intense V pattern of starch from cake crumb, illustrate that starch granules can be relatively swollen and still maintain a high degree of crystallinity. The crystalline arrangement, however, is different from that of the unheated starch (Fig. 8a).

The x-ray patterns of starch granules washed from doughnut crust and crumb are shown in Fig. 9. Starch in the crust (Fig. 9a) exhibited an A pattern superimposed with a V pattern. The intensity was substantially less than in bread or cake (Table IV).

The x-ray pattern of starch from the doughnut center (Fig. 9b) showed a more intense V pattern than that of the crust and considerably less of the A pattern. Relative crystallinity of that starch was equivalent to that obtained from the outer 2-cm bread portion (Table IV).

Starch washed from sugar cookies had an A pattern essentially unaltered from that of unheated starch (Fig. 10), indicating minimal starch gelatinization during baking. Those results agree with other methods used in this study to determine degree of starch gelatinization. Furthermore, data from the enzymatic method (Table III) suggest that in this low moisture system at least 11% of the starch may be gelatinized without substantially changing the x-ray pattern or intensity.

TABLE IV
Relative Crystallinity (RC) of Starches Extracted from Bakery Products[a]

Source of Starch	RC
HRW flour	1.00
White pan bread	
Crust	0.63
1 cm from crust	0.39
2 cm from crust	0.48
Center	0.34
Yellow layer cake	
Crust	0.71
Center	0.57
Cake doughnut	
Crust	0.52
Center	0.47
Sugar cookie	1.01

[a]Determined from x-ray diffraction patterns according to Herman's method as described by Nara et al (1978). $RC = a_c / A_c$, where a_c is the area of the crystalline fraction of the treated sample and A_c is the area of the crystalline fraction of control unheated starch.

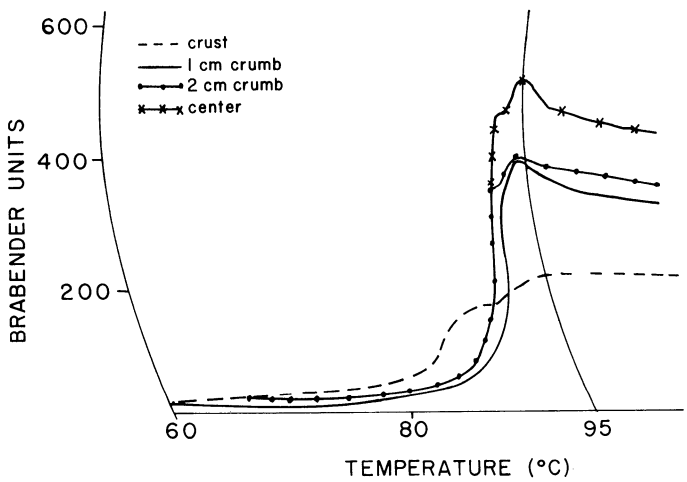

Fig. 5. Amylograms of bread crust and crumb slurries.

SUMMARY AND CONCLUSIONS

Moisture gradients in bakery foods undoubtedly affect the degree of starch gelatinization and swelling. However, information in this area is practically nonexistant. If we are to learn more about the condition of starch in bakery foods, we must know more about the variability in water content and availability within the product during baking and storage.

Our data showed a gradient of moisture distribution within baked products and indicated that, within any one product, the degree of starch gelatinization and swelling paralleled moisture content, ie, the higher the moisture content, the greater the starch swelling. Although the free moisture content probably is the most important factor governing starch swelling (Audidier 1968), the kenetics of the reaction are also dependent on temperature and the heating time at a particular temperature.

To complicate matters further, an equally important problem in understanding the condition of starch in bakery foods is the variability in methods used to determine starch gelatinization and

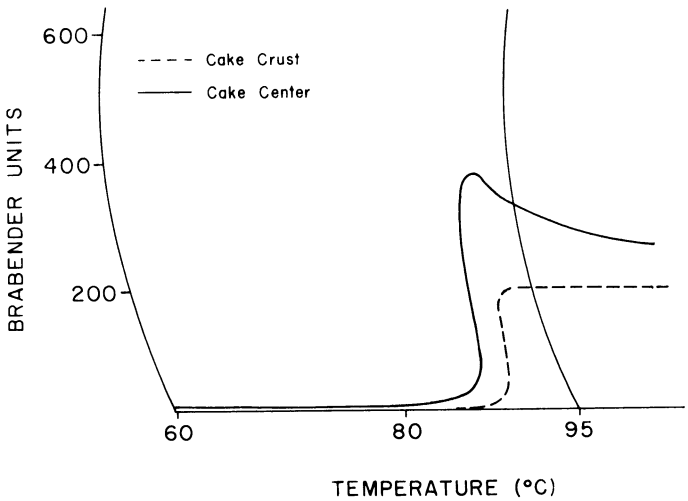

Fig. 6. Amylograms of cake crust and crumb slurries.

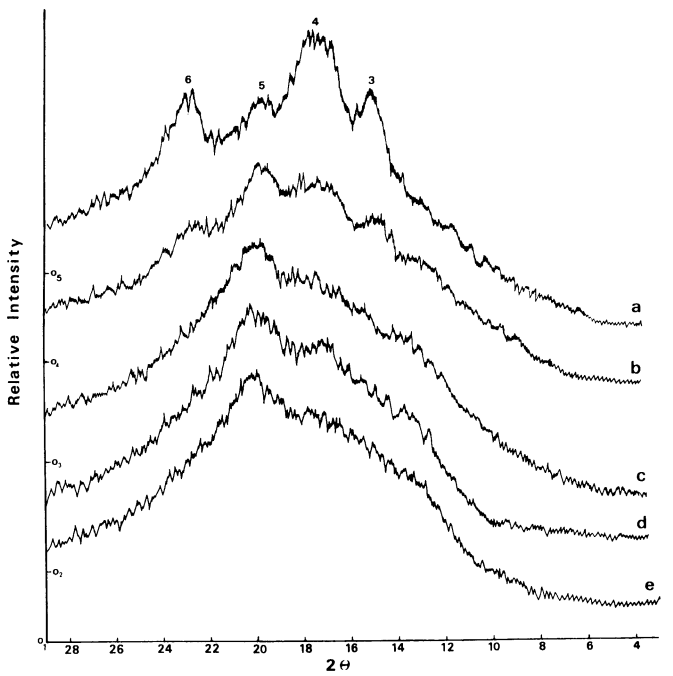

Fig. 7. X-ray diffraction patterns (CuKα) of starch from: **a,** flour; **b,** bread crust portion; **c,** outer 1-cm crumb portion; **d,** outer 2-cm crumb portion; **e,** center crumb.

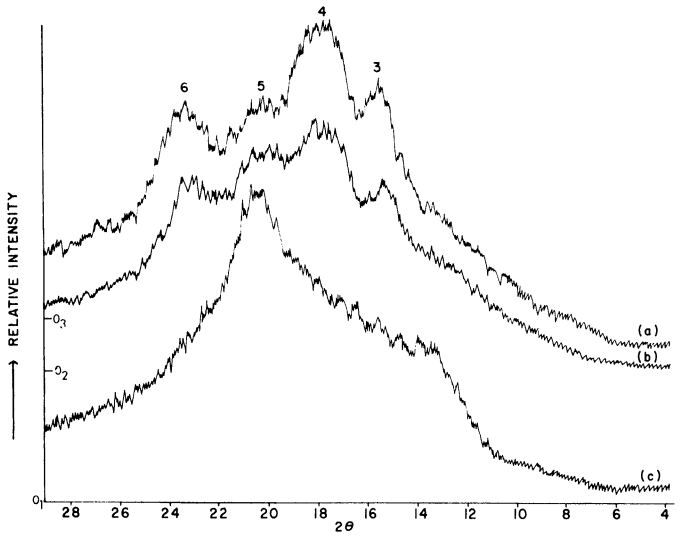

Fig. 8. X-ray diffraction patterns (CuKα) of starch from: **a,** cake flour; **b,** cake crust; **c,** cake center crumb.

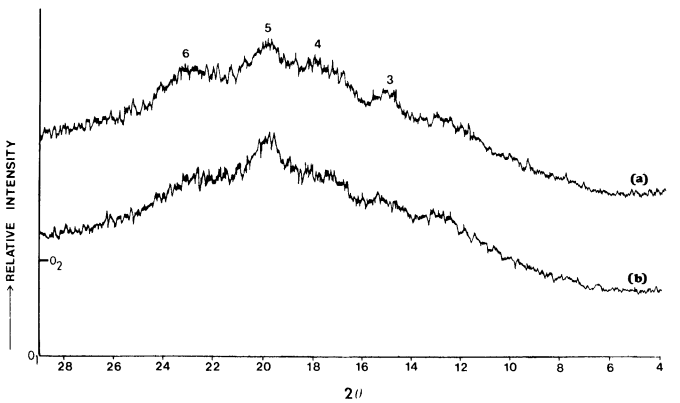

Fig. 9. X-ray diffraction patterns (CuKα) of starch from: **a,** doughnut crust; **b,** doughnut crumb.

swelling. Polarization microscopy, x-ray diffraction, and enzymatic methods paralleled one another and indicated that starch granules in the exterior portions of bakery products were less swollen than were those in the centermost portions. However, amylograph viscosities of crust and crumb slurries suggested the opposite which indicates that the viscosity method might not be reliable for determining the condition of starch granules in bakery foods. In addition, quantitation of differences in degree of starch swelling across various products is almost impossible with the amylograph.

A combination of polarization and bright-field microscopy of frozen thin sections of bakery foods provides a simple means to determine the characteristics of starch granules in situ. Our microscope studies demonstrated that starch granules may be elongated or deformed and still maintain some birefringence. Those data suggest that deformation of starch granules within bakery products cannot be used with confidence as a measure of the extent of starch swelling.

X-ray diffraction patterns of starch washed from bakery foods provide useful information on alterations in molecular organization of starch as well as on changes in starch crystallinity. Although they do not provide quantitative values for starch gelatinization or swelling, they do show that starch granules (eg, in cake crumb) can exhibit extensive swelling and still maintain a high degree of crystallinity.

Enzymatic determinations of starch swelling, using glucoamylase, provide a quantitation of starch swelling within and among

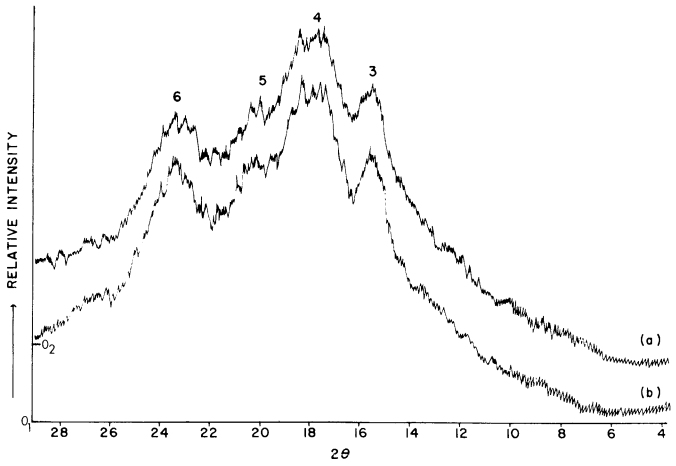

Fig. 10. X-ray diffraction patterns (CuKα) of starch from: **a,** cookie flour; **b,** cookie.

various products. To obtain reproducible results, however, starch must be washed from the bakery foods. Yet washing procedures may only remove the least swollen granules and/or may cause additional swelling or fracturing of already extensively swollen starch granules; hence, this method may provide inaccurate indications of the actual condition of starch in bakery foods.

Obviously, new quantitative methods need to be developed to determine the condition of starch in bakery foods in situ. Until that time, a combination of crystallographic (polarized light, x-ray diffraction) and enzymatic methods can provide a good indication of the extent of starch gelatinization and swelling in bakery foods.

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