Determining the Degree of Starch Gelatinization[1]

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ABSTRACT

A method has been devised to measure gelatinization of starch. The procedure involves selective digestion of gelatinized starch with glucoamylase (α-1,4-glucan glucohydrolase, E.C. 3.2.1.3) followed by determining the D-glucose released, using D-glucose oxidase. Glucoamylase also was used to determine total starch. The method, tested on known mixtures of gelatinized and prime starch, accurately measured gelatinization.

Gelatinization initiates important changes in the physical, chemical, and biological properties of starch. A number of procedures (1-4) to measure gelatinization have been devised, based on turbidity, solubility, swelling, chemical reactivity, absorption of dyes, X-ray diffraction, birefringence, or enzymatic digestibility, and more recently, nuclear magnetic resonance spectroscopy (5) and differential scanning calorimetry (6).

The most sensitive and popular methods are based on the loss of birefringence by gelatinized starch or on its increased susceptibility to enzyme attack. In the analysis of foods and feed, birefringence is difficult to apply because starch granules are difficult to count in heterogeneous mixtures and the starch cannot be easily separated from other components in the cooked material. Most investigators, therefore, have used selective digestion of the gelatinized starch by enzymes. The samples are digested with diastase (7-9), β-amylase (10-13), or glucoamylase (α-1,4-glucan glucohydrolase, E. C. 3.2.1.3) (4,14,15), and, in all but one case (9), the resulting fragments are assayed by measuring the increase in reducing power.

Described here is an accurate and sensitive method to determine percent gelatinization of starch using glucoamylase digestion followed by specific enzymatic determination of the D-glucose formed. This method is more accurate and sensitive than methods previously used for three reasons:

1. The dual-enzyme system D-glucose oxidase-peroxidase is a specific, accurate, and sensitive probe (16-18) for the end product of the action of glucoamylase on gelatinized starch. Assays based on reducing-power measurements are less reliable than a specific enzyme method because the former are non-stoichiometric methods that must be performed under rigidly controlled conditions to prevent variations induced by degradation of reducing carbohydrates in the alkaline medium.

2. All enzymatic methods of measuring starch gelatinization are based on the number of carbohydrate molecules released during digestion. Glucoamylase converts gelatinized starch to many more molecules (D-glucose) than α- or β-amylase (predominantly maltose and limit dextrins), so gelatinization is measured by differences between larger numbers when glucoamylase is used.

3. The reducing oligosaccharides released by β-amylase and α-amylase depend on the structure and molecular weight of the amylopectin and branched amylose (19)

fraction of the gelatinized starch, whereas D-glucose released by glucoamylase is independent of this fraction, which is particularly important when starch is cooked at low pH.

In determining gelatinization, we examined the digestibility of intact granular wheat starch by glucoamylase, and have included scanning electron micrographs of granular wheat starch partially digested by a highly purified glucoamylase from *Rhizopus niveus* and by an industrial-grade glucoamylase from *Aspergillus niger.*

MATERIALS AND METHODS

General

Dimethyl sulfoxide (DMSO) (9 volumes) was mixed with water (1 volume) at 25°C. to prepare 90% DMSO. Citrate buffer (0.05M) was prepared by mixing 1 volume of 0.05M aqueous citric acid with approximately 1.2 volumes of 0.05M aqueous sodium citrate. The final adjustment to pH 4.80 was made using a pH meter. *Tris*-HCl buffer was prepared by dissolving *tris*-(hydroxymethyl)-aminomethane (61 g.) in 5M aqueous hydrochloric acid (80 ml.). After water was added to 1 liter, the buffer was warmed to 37°C., and its pH adjusted to 7.00 using a pH meter.

A standard solution of D-glucose was prepared by dissolving the sugar (1 g.) and benzoic acid (2.4 g.) in water (100.0 ml.). This solution is stable indefinitely (18).

Wheat starch was isolated by wet milling (20) from soft red winter wheat and purified according to the procedure of Fellers et al. (21). The starch was freeze-dried to give a product with 4.5% water, 0.7% protein, 0.04% fat, and 0.2% ash.

Gelatinized wheat starch was prepared by autoclaving a 2% aqueous suspension of wheat starch at 120°C. for 1 hr. The material was isolated by solvent exchange in a Waring Blendor with methanol followed by ethyl ether.

Glucoamylase used, unless otherwise stated, was a commercial preparation (Takamine Diazyme, Miles Labs., Elkhart, Ind.) from *A. niger.* Solutions of glucoamylase were prepared by extracting the commercial powder (1.9% nitrogen, Kjeldahl) with 0.05M citrate buffer (pH 4.8). The specific activity of the crude enzyme (protein based on Kjeldahl nitrogen) was determined by measuring (22) the D-glucose released from a large excess of soluble starch; it was found to be 12.0 μmoles glucose (min.)$^{-1}$ (mg. protein)$^{-1}$. In experiments on the digestion of granular wheat starch, glucoamylase from *R. niveus* (Miles Laboratories, Elkhart, Ind.) also was used. The specific activity of the latter, crystalline enzyme was given by the supplier as 20 μmoles glucose (min.)$^{-1}$ (mg. protein)$^{-1}$.[3]

Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.) was prepared by mixing the contents of one vial of "chromogen for "Glucostat X 4" with 4.0 ml. of a 20% ethanolic solution of the surfactant Triton X-100 (Mann Research Laboratories, New York, N.Y.), and dissolving the mixture in 400 ml. of 0.5M *tris*-HCl buffer (pH 7.0) which already contained the contents of one vial of "Glucostat X 4."

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[3] An International Unit (I.U.) of enzyme activity is defined as the amount of enzyme required to release 1 μmole of glucose per min. from a starch substrate at 30°C. under assay conditions specified.

Determination of D-Glucose

D-Glucose was determined either by a modified Worthington procedure (22) or by one of the two procedures described below. Method A was used to determine the total starch content of samples, where digests contained 40 to 120 γ ml.$^{-1}$ of D-glucose. Method B was used to determine D-glucose in the glucoamylase digests of partially gelatinized samples, where the digests contained lower concentrations of D-glucose (6 to 18 γ ml.$^{-1}$) than those assayed by Method A.

Method A. An aliquot (1.0 ml.) of a solution containing 40 to 120 γ ml.$^{-1}$ of D-glucose in absolute ethanol-0.05M citrate buffer-90% DMSO[4] (10:5:1, v./v./v.) was incubated at $37° \pm 0.5°C$. with Glucostat reagent (10.0 ml.). After 1 hr. the reaction was terminated by adding 500 μl. of 4M aqueous hydrochloric acid. The clear solution was cooled to 25°C. and absorbance was measured against a reagent blank at 400 nm. in 10-mm. cells using a Beckman DU Spectrophotometer. Analyses were done in duplicate, and each analytical run included the analysis of a standard solution of D-glucose.

Method B. An aliquot (3.0 ml.) of a solution containing 6 to 18 γ ml.$^{-1}$ of D-glucose in absolute ethanol-0.05M citrate buffer (10:5, v./v.) was incubated at $37° \pm 0.5°C$. with Glucostat reagent (3.0 ml.). After 1 hr. the reaction was terminated by adding 150 μl. of 4M aqueous hydrochloric acid. The remainder of the procedure was identical to Method A.

Determination of Total Starch

Wheat starch (75 to 100 mg.) was weighed accurately into a deciliter volumetric flask containing a magnetic stirring bar of known volume. The flask was placed in a water bath (55°C.) on a magnetic stirring plate and 90% DMSO (preheated to 55°C.) was added to volume with stirring. After its contents were stirred 5 min. at 55°C., the flask was placed in a water bath at $37° \pm 0.5°C$., and the contents allowed to equilibrate to temperature (~10 min.). The volume of the starch solution was adjusted by adding 90% DMSO at 37°C. After the mixture was thoroughly stirred, an aliquot (1.0 ml.) was pipetted quickly into a test tube (18 X 120 mm.) containing glucoamylase (10 I.U.) in 5.0 ml. of 0.05M citrate buffer (pH 4.8). The tube was incubated at 37°C. for 30 min. Ethanol (10.0 ml.) was then added, and the tube was cooled briefly in an ice-water bath and centrifuged. The supernatant was warmed to 37°C. and a 1.0-ml. aliquot was assayed for D-glucose, using Method A.

Determination of Gelatinized Starch

Predetermined amounts of gelatinized wheat starch and/or prime starch were weighed together into 125-ml. Erlenmeyer flasks. Each standard sample contained 100 mg. of starch with 0, 5, 10, 25, 50, 75, or 100% of the total as gelatinized starch. Silica gel (500 mg.; grade, plain; Warner-Chilcot Laboratories, Richmond, Calif.) was added and mixed thoroughly with the starch to facilitate wetting the polysaccharide with water. A solution of glucoamylase (500 I.U.) in 50 ml. of 0.05M citrate buffer (pH 4.8) at 37°C. was added with mixing. After incubation at 37°C. for 30 min. an aliquot (5.0 ml.) was removed from the reaction mixture and added to absolute ethanol (10.0 ml.) in a test tube (18 X 120 mm.). The tube was

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[4]The presence of 5.6% DMSO in the solution of D-glucose being assayed had no effect on the color produced during incubation with Glucostat reagent.

chilled in ice water to facilitate precipitation of protein and undigested starch. After centrifugation, the supernatant solution was warmed to 37°C. and assayed for D-glucose, using Method B. If the supernatant solution contained too high a concentration of glucose, it was diluted with absolute ethanol-citrate buffer (2:1, v./v.).

Glucoamylase Digestion of Granular Wheat Starch

Wheat starch (100 mg.) was treated at 37°C. with 500 I.U. of *A. niger* glucoamylase (Takamine Diazyme) or *R. niveus* glucoamylase in 50 ml. of 0.05M citrate buffer (pH 4.8). Toluene (0.5 ml.) was added to prevent microbial contamination, and each reaction mixture was stirred gently with a magnetic stirring bar. Aliquots (5.0 ml.) were removed periodically and added to 10.0 ml. of absolute ethanol. After centrifugation the supernatant was assayed for D-glucose using Method B. The sediment in the centrifuge tube was washed three times with water, three times with ethanol, then dried in a desiccator over calcium chloride. The sedimented starch samples were mounted on specimen stubs using double-backed Scotch tape, and were coated *in vacuo* with approximately 2 p.p.m. of gold. The coated specimens were viewed in an ETEC auto-scanning electron microscope operating at 10 kv. accelerating potential. Photographs were recorded on Polaroid film (Type 55 P/N, Polaroid Corp., Cambridge, Mass.).

RESULTS AND DISCUSSION

The percentage gelatinization of starch in a food or feed may be calculated by the equation:

$$Y = \frac{(X-B)(100)}{T} \tag{1}$$

where Y is the percentage gelatinization, X is the percentage of starch removed from a sample by glucoamylase digestion, T is the total starch percentage in the sample, and B is a variable correction factor arising from the digestion of intact granular starch by glucoamylase. As the digestion of starch by glucoamylase is to be measured by the amount of D-glucose released into the digest medium, it follows from equation 1 that the accuracy of a glucoamylase-glucose oxidase procedure for determining gelatinization depends on the following: a) reliability of the method used to determine total starch; b) accuracy of the assay procedure for D-glucose; and c) the digestion of intact granular starch in a sample. Each factor was examined individually.

To determine total starch, we chose the method described by Libby (23). This method involves the use of glucoamylase; it is more rapid than the alternate glucoamylase method (24), and easily adoptable to determine starch in whole grain (25). In the Libby procedure starch is dissolved in 90% DMSO and the solution is diluted with buffer (pH 4.8) so that the concentration of DMSO in the glucoamylase digest is 15% by volume. Lineback and Sayeed (26) had previously shown that the rate starch is digested by glucoamylase is unaffected by adding up to 18% by volume of DMSO. The D-glucose released during digestion was subsequently assayed using Glucostat reagent, a commercially available product (16) containing a dye (*o*-dianisidine) and a mixture of two enzymes (glucose

oxidase and peroxidase). The color produced by oxidation of the dye is equivalent to the amount of D-glucose oxidized.

In the early stages of our investigation, we assayed for D-glucose using the Worthington procedures (16) except for substituting *tris*-HCl buffer (17,22) for phosphate buffer (pH 7.0). Incorporating four changes in the method, as suggested by Banks and Greenwood (18), greatly improved the precision of the D-glucose assay procedure. The four changes are as follows: a) *tris*-HCl buffer (pH 7.00) was prepared at 37°C.; b) freshly prepared Glucostat reagent was aged 12 to 24 hr. before use; c) the glucose oxidase-peroxidase catalyzed reaction was allowed to proceed to completion (incubation for 1 hr. at 37°C.); and d) more mineral acid was used to terminate the reaction, which improved clarity of the final solution as well as the stability of its color.

Data in Table I are typical of results obtained using the Worthington procedure

TABLE I. PRECISION OF TWO ASSAY PROCEDURES FOR
D-GLUCOSE USING GLUCOSE OXIDASE-PEROXIDASE

Worthington Procedure with *Tris*-Buffer[a]				Modified Procedure[b]			
			Glucose, γ[c]				
50	75	100	150	40	80	120	160
0.197[d]	0.309	0.428	0.650	0.178	0.360	0.542	0.717
0.200	0.310	0.430	0.640	0.178	0.359	0.542	0.720
0.197	0.320	0.440	0.640	0.178	0.358	0.543	0.720
0.199	0.308	0.430	0.635	0.177	0.360	0.543	0.719

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Mean Absorptivity[e]
4.18
Standard Deviation[f]
0.15
Coefficient of
Variation, %
3.6

Mean Absorptivity
4.488
Standard Deviation
0.028
Coefficient of
Variation, %
0.62

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[a]See ref. 22.
[b]Method A of this work.
[c]D-Glucose in 1.0 ml. of ethanol-citrate buffer – 90% DMSO (10:5:1, v./v./v.) added to Glucostat reagent (10.0 ml.).
[d]Absorbance measured at 400 nm. using a 10-mm cell.
[e]Mean of 16 individual absorptivity values, where absorptivity equals absorbance divided by the product of cell-path length (cm.) and concentration (mg. ml.$^{-1}$).
[f]Standard deviation of mean absorptivity.

TABLE II. REPRODUCIBILITY OF THE MORE SENSITIVE ASSAY
PROCEDURE (METHOD B) FOR D-GLUCOSE[a]

	Average of Duplicate Determinations									
Trial	1	2	3	4	5	6	7	8	9	10
Absorbance	0.296	0.295	0.295	0.294	0.297	0.294	0.296	0.295	0.293	0.298

[a]Mean, 0.295; standard deviation, 0.0015; coefficient of variation, 0.5%. D-Glucose (10 γ) in 1.0 ml. of ethanol-citrate buffer (2:1, v./v.). Analyses were performed over a period of 7 days using a single preparation of Glucostat reagent (stored at 5°C.).

of Method A. When the individual absorbance values in Table I are converted to a common basis, the coefficient of variation of the 16 absorptivity values obtained using the Worthington procedure is five times greater than the coefficient of variation obtained using Method A.

To further demonstrate the improved reliability and sensitivity of Method A, we analyzed a standard solution of D-glucose 10 times over a 5-day period using a single supply of Glucostat reagent. We were able to reproduce the amount of D-glucose measured to ± 0.3% at a concentration of 120 γ ml.$^{-1}$; Banks and Greenwood (18) reported reproducibility of ±0.5% at 20 γ ml.$^{-1}$. The data indicate that only one determination on a D-glucose standard is required to calibrate a new supply of Glucostat reagent, if the reagent is stored at 5 °C. in the dark and used within 5 days.

We also used a method more sensitive than Method A to determine D-glucose. By adjusting reaction volumes, we found Glucostat reagent could be used to assay solutions containing 6 to 18 γ ml.$^{-1}$ of D-glucose (Method B). Ten separate analyses of a standard D-glucose solution (10 γ ml.$^{-1}$) were performed over a 7-day period. Method B (Table II) gave good reproducibility; the coefficient of variation for the 10 γ of D-glucose was 0.5%.

Our results show that the glucose oxidase-peroxidase assay procedure provides a highly accurate, sensitive measure of D-glucose, which is essential to the success of using glucoamylase to measure gelatinization of small amounts of material.

A possible source of error in determining T in equation 1 might arise while dissolving starch in 90% DMSO at 55°C. For unknown reasons, prolonged heating of starch in this solvent decreased its digestibility by glucoamylase. Libby (23) alluded to this phenomenon but presented no data on its magnitude. On the other hand, Leach and Schoch (27) reported solutions of starch in 90% DMSO were stable several months. The curve in Fig. 1 shows that digestibility of starch by glucoamylase (10 I.U. per mg. of starch) decreased linearly as the period of dissolving starch in 90% DMSO at 55° increased from 5 to 30 min.

The amount of glucoamylase used to determine total starch was determined by the amount needed to quantitatively digest the starch in a sample in 30 min. We found 10 or 15 I.U. of glucoamylase gave almost a quantitative (98.0%) yield of D-glucose from 1 mg. of starch in 30 min. at 37°C. Increasing the incubation period to 18 hr. increased the conversion to 98.5% D-glucose. The slightly lower-than-quantitative hydrolysis of starch to D-glucose is not a serious problem because total starch (T) appears in the denominator of equation 1.

The accuracy of our method of determining gelatinization is affected by the selectivity of glucoamylase for gelatinized starch over prime starch. The literature contains conflicting reports concerning the action of glucoamylase on intact starch granules. Macrae and Armstrong (28) reported that a commercial glucoamylase from *A. niger* (Agidex, Glaxo Laboratories, Greenford, Middlesex, England) did not attack raw starch, and Manners (29), that glucoamylase had only limited action on starch granules. On the other hand, Evers et al. (30) presented scanning electron microscopic evidence of attack on starch granules by Agidex. However, it is possible the attack observed by Evers resulted from α-amylase (30–32) in commercial preparations of glucoamylase from *A. niger*. It is well established that α-amylase attacks granular starch (33).

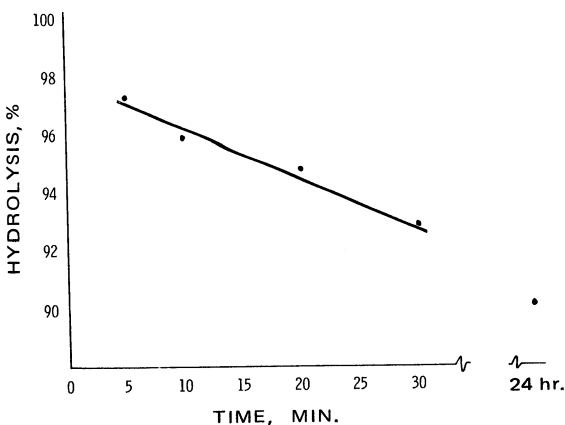Japanese workers (4) reported that a commercial glucoamylase from an

Fig. 1. Digestibility of starch after dissolution in DMSO-water (9:1) at 55°C. The abscissa values indicate the time (in minutes) that starch was heated to obtain dissolution.

Endomyces species gave 6.1 and 1.5% digestion of native corn starch and potato starch, respectively, when 25 mg. of starch was treated with 6 I.U. of enzyme for 1 hr. at 37°C. Again, other workers (34) had previously shown this commercial enzyme preparation contained an α-amylase, which was extremely difficult to separate from the glucoamylase. Leach and Schoch (35) also used an impure glucoamylase to partially digest granular corn starch.

We, therefore, examined the digestibility of intact wheat starch not only with Takamine Diazyme, which we used to determine gelatinization, but also with R. niveus glucoamylase, a crystalline enzyme that contains no α-amylase (31,32,36).

We found[5] both glucoamylases extensively digested wheat starch; therefore pure glucoamylase can indeed attack native starch (Table III). The data in Table III show the initial attack ($\leqslant 2$ hr.) by either enzyme is approximately the same, but at longer digestion times, attack by Takamine Diazyme is more extensive than by R. niveus glucoamylase. After 64 hr., Takamine Diazyme effected 84% conversion of starch to D-glucose, while R. niveus glucoamylase gave only 32% conversion.

Scanning electron microscopy of the partially digested, large wheat starch granules revealed different morphological modes of attack by the two enzymes. During the first 30 min. of digestion, both enzymes exposed equatorial grooves on many starch granules (Fig. 2, B and E); the equatorial groove was not seen in the control starch (Fig. 2, A). At longer digestion times, R. niveus attacked principally the surface of the large granules, giving their exterior a spongy appearance, which was occasionally pierced by a sharply defined, small, cylindrical hole (Fig. 2, C and D). On the other hand, digestion by Takamine Diazyme appeared to be confined less to the surface of the granules. Its attack was characterized by deeper and wider

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[5]Evers et al.(30) treated 5 g. wheat starch with 3,000 Glaxo units of Agidex for 5 days at 50° C. Assuming reaction rate doubled with every 10°C. increase, they used approximately 0.09 I.U. per mg. of starch. We used 5 I.U. per mg. of starch at 37° C.

TABLE III. CONVERSION[a] OF WHEAT STARCH
TO D-GLUCOSE BY GLUCOAMYLASE, PERCENT

Glucoamylase Source	Incubation Period, hr.							
	0.5	1	2	4	8	16	32	64
A. niger[b]	2.6	4.2	7.1	13.2	22.0	38.5	53.2	83.9
R. niveus	3.5	4.7	7.1	9.5	11.0	17.8	26.3	31.9

[a]Starch (100 mg.) with glucoamylase (500 I.U.) in 50 ml. of 0.05M citrate buffer (pH 4.8) at 37°C.

[b]Commercial preparation (Takamine Diazyme).

penetration into a granule's interior, which created scoop-shaped depressions on its surface (Fig. 2, F and G). In addition, tunnels, beginning chiefly at the equatorial groove and widening as they penetrated, were often observed. Such tunnels were not seen in granules exposed to *R. niveus* glucoamylase. The tunneling and higher degree of digestion by Takamine Diazyme could be from α-amylase in this commercial enzyme. Other investigators (37,38) have demonstrated tunneling and extensive internal digestion of large wheat starch granules by α-amylase.

It is apparent then that, in determining gelatinization using glucoamylase, a correction is needed because of digestion of granular starch by glucoamylase. The correction factor can be determined only after the glucoamylase concentration for digestion of partially gelatinized starch is chosen.

We determined empirically the amount of glucoamylase used to digest partially gelatinized starch samples (to obtain the value of X in equation 1). We wished to convert a 100-mg. sample of starch in an *aqueous* medium completely to D-glucose in 30 min., even when the sample contained 100% gelatinized starch. We found approximately 500 I.U. of Takamine Diazyme was needed for the conversion under our experimental conditions.

To find the variable B in equation 1, needed to correct for attack of glucoamylase on granular (ungelatinized) starch, we digested various amounts (25 to 100 mg.) of prime wheat starch with 500 I.U. glucoamylase for 0.5 hr. at 37°C. and measured the amount of D-glucose released (Table IV). The data in Table IV show a linear correlation between the D-glucose released during digestion and the quantity of granular starch available to glucoamylase; that is, B = 0.026(100 − Y). Substitution of the latter expression for B in equation 1 and solving for the degree of gelatinization (Y) yields equation 2, which shows that correction for the digestion of intact starch can be applied without prior knowledge of a sample's degree of gelatinization.

$$Y = \frac{(X - 2.6)(100)}{T - 2.6} \qquad (2)$$

The correction factor (B) depends on the botanical source of starch since the rate of digestibility of different starches by glucoamylase varies.[6]   When testing three starches, the order of decreasing rates of digestibility (5 I.U. of glucoamylase, 1 mg.

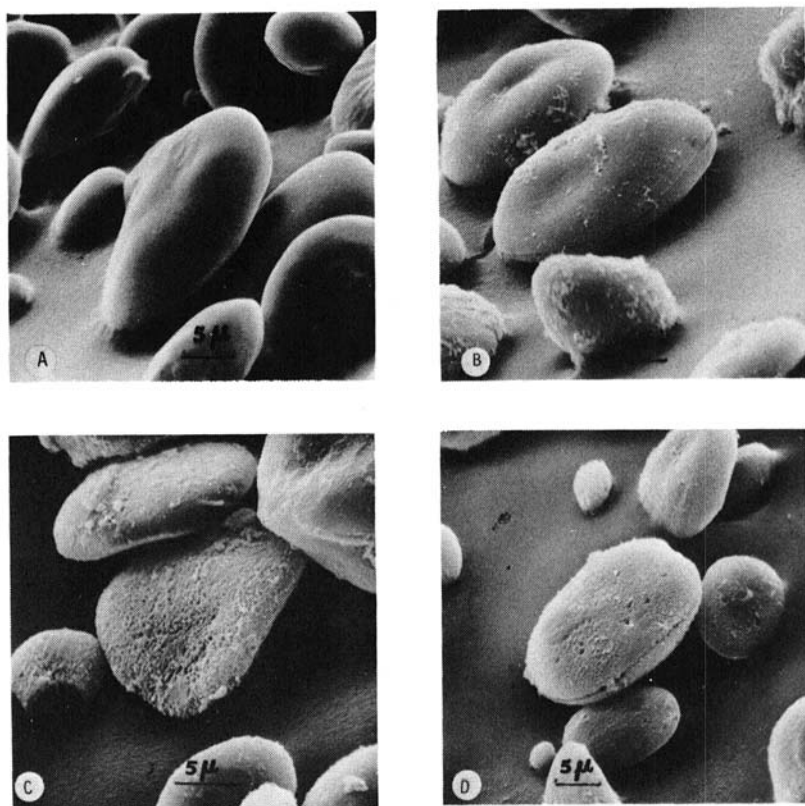[6]Chiang, B. Y., and Johnson, J. A., personal communication.

Fig. 2. (above and facing page). Attack of glucoamylases on wheat starch (see also Table III): A, control; B through D, *R. niveus*; E through G, Takamine Diazyme. Digestion periods: B and E, 0.5 hr.; C and F, 8 hr.; D and G, 32 hr.

TABLE IV. HYDROLYSIS OF GRANULAR STARCH
DURING 30 MIN. DIGESTION[a]

Granular Starch Digested mg.	Glucose Released mg.	Hydrolysis of Starch %
100	2.63	2.52
100	2.70	2.60
100	2.73	2.62
100	2.78	2.66
100	2.67	2.56
75	2.04	2.61
50	1.37	2.62
25	0.69	2.64

[a]Wheat starch (25 to 100 mg.) treated with Takamine Diazyme (500 I.U.) in 50 ml. of 0.05M citrate buffer, pH 4.8.
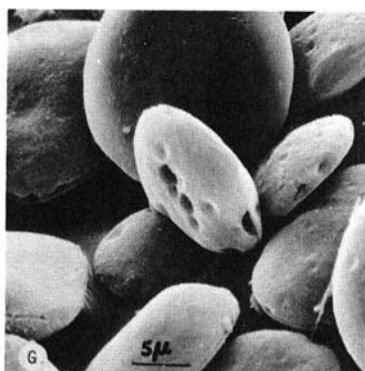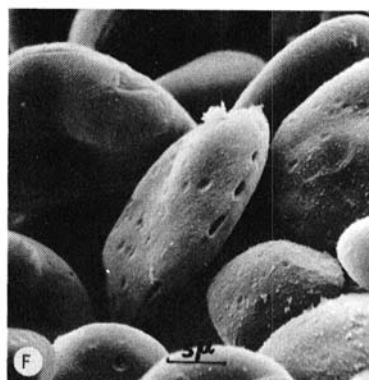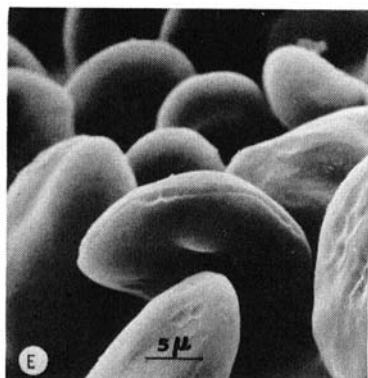
TABLE V. KNOWN AND EXPERIMENTALLY DETERMINED
DEGREES OF GELATINIZATION

Standard Sample, % Gelatinized	Digestibility[a] (X) %	Gelatinized[b] (Y) %
0	2.6	0
5	6.7	4.3
10	11.3	9.1
25	26.3	24.8
50	48.9	48.5
75	73.8	74.6
100	96.5	98.4

[a] Experimentally determined digestibility of a standard sample (total starch = 100 mg.) in 50 ml. of 0.05M citrate buffer for 30 min. at 37°C. with Takamine Diazyme (500 I.U.). Percentages were based on the glucan content of starch as determined (by difference) using the proximate analysis of the starch.

[b] Calculated by equation 2 in which 98% was used for the value of T, since total starch analysis on three standard samples gave 98.0% theoretical yield of D-glucose based on the glucan content of the starch (determined by proximate analysis).

of starch) was found to be corn>wheat>potato. The same order of reactivity had been previously observed with digestion using α-amylase (34). Plots of glucoamylase digestibility (D-glucose released) versus time for the three starches were linear to at least 2 hr. of digestion. The slopes of the lines indicated the relative rates of

digestion of granular corn, wheat, and potato starches were 8:2.5:1, respectively. These linear plots also gave information concerning starch damage in the granular starches. Extrapolation of the digestibility curves gave, at zero time, no digestibility, which indicates no starch damage in the wet-milled starches.

To determine the accuracy of the proposed glucoamylase method of determining degree of gelatinization, we prepared a series of starch standards containing 0 to 100% gelatinized starch (Table V). Total starch determination (using dissolution in 90% DMSO) on three of the samples gave an average of 98.0% theoretical conversion to D-glucose; thus T in equation 2 was 98.0%. To determine the value of X, each standard mixture was digested in 0.05M citrate buffer (pH 4.8) at 37°C. for 0.5 hr. using 500 I.U. of Takamine Diazyme. The experimentally determined degree of gelatinization (Y) was then calculated using equation 2. Agreement between the calculated values and the theoretical values (Table V) was excellent. The largest error observed was at the 5.0% gelatinization level, where our analytical procedure gave 4.3% gelatinization.

Acknowledgment

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