DAMAGED STARCH. QUANTITATIVE DETERMINATION IN FLOUR[1]

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ABSTRACT

Damaged starch is readily and rapidly digested by the amylases, whereas the native undamaged starch is markedly more resistant to digestion. Based on these differences, a method is proposed for the quantitative determination of damaged starch in flour. The digestion of undamaged starch is appreciable and is not constant from flour to flour. Accordingly, the proposed method eliminates this digestion as a factor by determinations after 1-hour and 2-hour periods of digestion with extrapolation back to 0 time for the percent damaged starch. The slope of the curve is an indication of the susceptibility of the undamaged starch to digestion.

Starch granules are easily damaged by pressure, shear, or strain such as that applied by grinding procedures (1,8,9). The damage is particularly notable during flour milling and accordingly is of concern to the wheat milling and baking industries (4,8,16).

Many properties of doughs and of baked bread are materially affected by the quantity of damaged starch, e.g., water absorption, gassing power, handling properties of doughs, and loaf volume and tenderness of crumb in the bread (6,8,15).

Studies concerning the relation of damaged starch to these and other baking properties and studies on the milling factors responsible for starch damage have been limited by the lack of a quantitative method for its determination. The purpose of this paper is to present a method for the quantitative determination of damaged starch in flour.

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Properties of Damaged Starch As a Basis for Its Determination. Damaged starch differs from normal starch in a number of respects: water absorption (swelling in water), solubility, susceptibility to staining with iodine and certain dyes, and digestibility by amylases (5,8,9,11,13).

Damage, as evidenced by swelling and staining with dilute iodine (0.0001*N*) or Congo red does not generally extend to all parts of the granule (Fig. 1). It may be a limited local injury or it may extend throughout the granule with only small segments remaining uninjured.

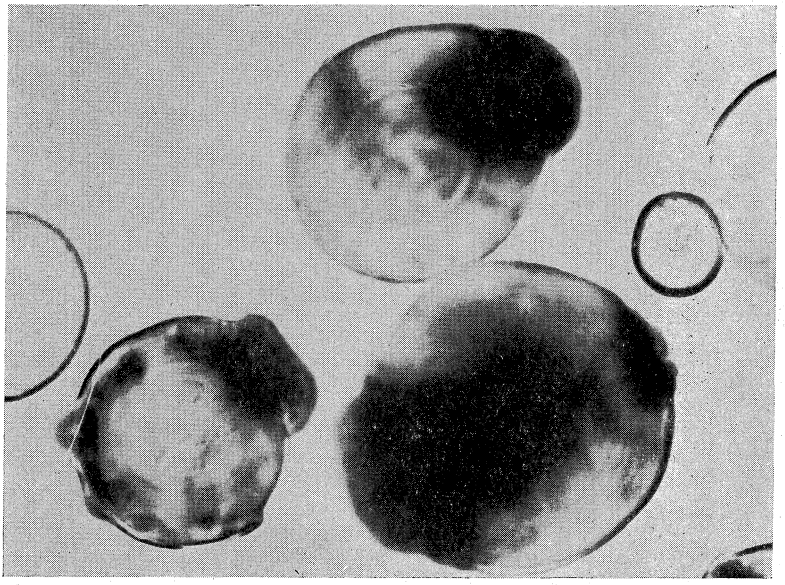

Fig. 1. Damage to starch as evidenced by swelling and staining with dilute iodine.

The variation in intensity of damage is evidenced by the amount of swelling and by the intensity of the staining with iodine in the granules of Fig. 1. Damage is not an all-or-none situation. It apparently covers the entire range from the rupture of a few intermolecular bonds, which would imperceptibly increase swelling and rate of digestion, to extensive rupture of enough bonds to make the starch readily available to amylase action. The differences in properties of damaged and undamaged starch depend both on the extent and on the degree of damage.

As a basis for the quantitative determination of damage in a flour, any of the above mentioned differences could conceivably be used. However, only the increased solubility and increased digestibility ap-

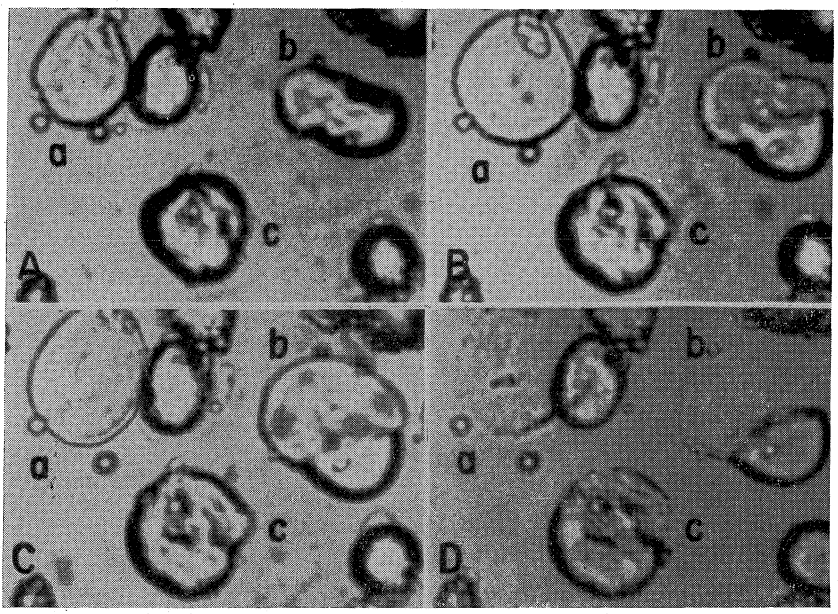

Fig. 2. Swelling and subsequent digestion of mill-damaged starch flooded with a 1:5 malt extract (16 SKB units of alpha-amylase per ml.): A, immediately after wetting (about 1/64 second), before swelling was notable; B, 0.1 second after wetting, swelling incomplete; C, one second after wetting, swelling complete; D, 30 minutes after wetting, digestion of damaged sections apparently complete. These are enlargements of selected frames from a motion picture film (13).

pear to be practical. Methods based on solubility (7) and on digestibility with beta-amylase after inactivation of the enzymes of the flour (5,6) have been proposed.

A normal starch granule is resistant to the action of digestive enzymes. Damaged starch has lost much of this resistance.

Figure 2 (13) shows the swelling and digestion of wheat flour starch after wetting with a 1:5 wheat malt extract. The observable swelling in the damaged starch was complete in less than one second. The slower subsequent digestion of the damaged starch was essentially complete in 30 minutes. At the end of this time there was no apparent action on the undamaged portions of the granules.

Rate of digestion is dependent on the accessibility of the starch molecules to the enzyme. Accordingly, slightly damaged starch may digest almost as slowly as the undamaged. The complete differentiation of damaged and undamaged starch would seem to be impractical, if not impossible. The objective in a method for the determination of damage is to determine that damage which affects the baking properties of flour; this is that degree of damage that allows appreciable

swelling and an appreciably increased digestion. The selection of conditions for such a determination is empirical and is based on the differential rate of digestion of damaged and undamaged starch.

Development of the Method

With some modifications, the ferricyanide method for the determination of maltose value (2,3,10) may be used for this determination. The use of this method for the determination of damaged starch has many advantages, since it is in general use in cereal laboratories and thus the chemicals and equipment are available and the techniques are well known. The only necessary changes in the method are the use of a relatively large quantity of a malt enzyme preparation, 1-g. samples of flour instead of 5-g., and determinations after 1- and 2-hour periods of digestion.

Quantity of Enzyme Required. To measure the damaged starch it is necessary to add enough enzyme to digest completely the readily digestible starch in a reasonably short period of time. For a 1-hour digestion this is approximately enough malt extract to supply 160 SKB units (16) of alpha-amylase. This quantity also is insurance that the relatively small quantities of enzyme naturally occurring or added to the flour during milling will be of little consequence and will cause no appreciable error.

Size of Flour Sample. The amount of maltose produced from the flour must be within the range that can be determined by the 0.1*N* ferricyanide under the conditions specified. The maltose obtained from 1-g. samples of flour by a malt extract containing 160 units of alpha-amylase were found to be within the determinable range.

Specifications for the Enzyme Preparation. Normally a malt sample carrying 160 units of alpha-amylase contains enough maltose to use up approximately half of the 0.1*N* ferricyanide. The essential elimination of this enzyme blank value may be accomplished by a thorough dialysis. Lyophilization is desirable and an alpha-amylase assay is necessary. Enough beta-amylase must be present to convert the alpha-amylase limit dextrins to maltose.[3]

Digestion-Rate Curves of a Series of Starches with Progressively Increasing Amounts of Damage. Mixtures were made of a soft wheat starch (a starch with minimum damage) with increments of a hard wheat starch which had been severely damaged by eight hours of ball-milling. The series contained 0, 10, 20, 30, 40, and 100% of the

[3] Since the lyophilization and enzyme assays are time-consuming operations, it is hoped that some commercial organization interested in enzyme preparations and in this particular work will make available a dry malt enzyme preparation standardized as to alpha-amylase value and containing only a minimum of maltose.

ball-milled starch. The digestion curves obtained from digestion of 0.75-g. samples of these starches (corresponding roughly to the starch in 1 g. of flour), with malt extract containing 160 units of alpha-amylase are shown in Fig. 3.

After 1 hour of digestion the curves are essentially parallel and approximately the same distance apart for each 10% increment of ball-milled starch; indicating that the damaged starch was digested during the first hour and that the portion of the curves, from 1 to 4 hours, represents the rate of digestion of the normal undamaged starch. The distance between curves represents the increment in quantity of damaged starch. Obviously the enzyme added was not enough for the 100% ball-milled sample, as the digestion of damaged starch was not complete in 1 hour.

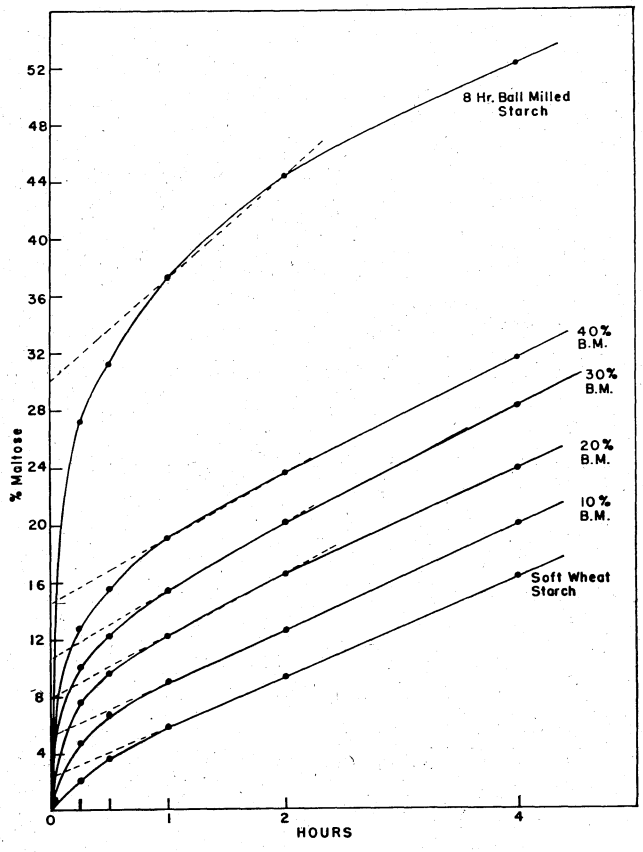

Fig. 3. Digestion-rate curves of a series of starches with progressively increasing amounts of damage.

The portion of the curves below the 1-hour point represents a combination of the rates of digestion of the damaged and the undamaged starch. The rate of digestion of the undamaged starch during this first hour is assumed to be the same as the rate from 1 to 2 hours. If the curves representing the normal starch digestion during the 1- to 2-hour period are extrapolated back to the vertical axis, the intercept at 0 time should represent the amount of maltose produced from the damaged starch.

Using malt as the enzyme source for the digestion of raw starch, the factor for conversion of maltose to starch is practically 1.0[4] (14). Accordingly, this figure for maltose from damaged starch also represents damaged starch. By this procedure the damaged starch in this series of starches was 2.4, 5.2, 8.0, 10.7, and 14.0%. The increase with each 10% increment of ball-milled starch was 2.8, 2.8, 2.7, and 3.3%. These figures make the method look feasible.

Application of the Method to Flour

The curves obtained from flours varying widely in damaged starch (3 to 18.4%) are shown in Fig. 4. The soft wheat flours show the lowest damage, whereas the damage in the hard wheat flours varies with the hardness and with milling conditions (6,8,11).

It has been found that the enzyme system of *Aspergillus oryzae* shows a much greater difference between the rates of digestion of boiled starch and that of raw starch than do the enzyme systems from other sources, including the enzymes from malt (14). This suggests the possibility that the enzyme system of *Aspergillus oryzae* would be better than the malt enzyme system for differentiation between damaged and undamaged starch.

This possibility was investigated and found not to be as expected. The curve "C-fungal" of Fig. 4 shows the type of curve and the damaged starch value obtained when the fungal enzyme system was substituted for the malt system on flour C. Flours similar to E and F with 8 to 9% damaged starch showed only 4% with the fungal enzyme.

Damaged starch has properties intermediate between those of boiled starch and those of undamaged starch. Accordingly, the action of this fungal enzyme system was much too slow on the damaged starch; it gave exceedingly low values.

Variation in Digestibility of Undamaged Starch. As before stated, the slope of the curve represents the rate of digestion of the undamaged starch of the flour. Differences in slopes of the curve also might

[4] The theoretical factor for converting maltose to starch is 0.95. Under the conditions used, the factor for conversion to damaged starch varies from 0.95 to 1.0.

indicate differences in quantity of starch that was only slightly damaged and accordingly was digested slowly. However, it would be expected that the slightly damaged starch, in general, would be in proportion to the highly damaged starch. The data of Fig. 4 indicate that this was not true for these flours. Also, if the slope of the curve were dependent on the quantity of slightly damaged starch, the curves shown in Fig. 3 (slope of curve for 1 to 4 hours) should not be parallel but should become steeper with increasing quantities of damaged starch. The wide range in slope (in digestibility) of undamaged starch from flour to flour was to be expected; it has been shown by photomicrographic methods that there are exceedingly wide variations in

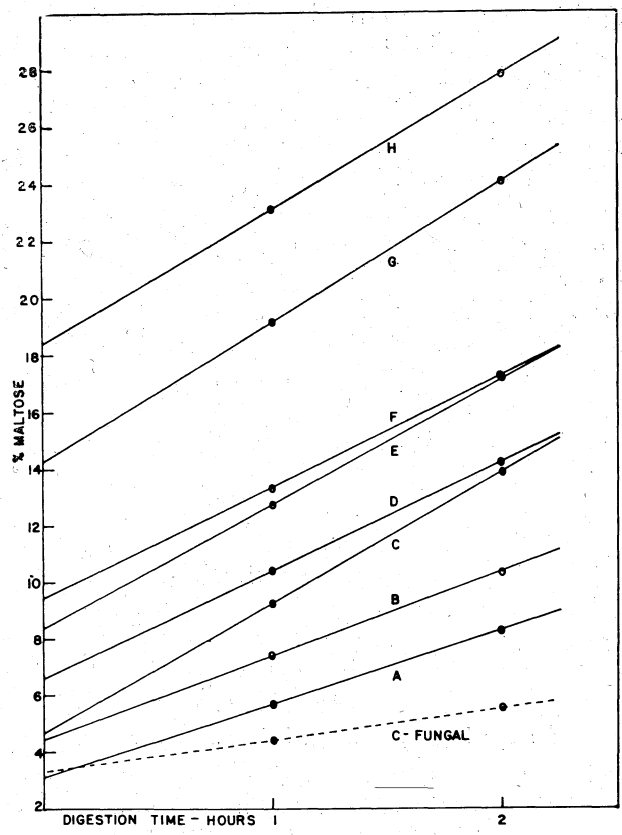

Fig. 4. Application of the method to the determination of damaged starch in flour. A, soft white wheat flour; B, soft red winter flour; C, commercial hard winter wheat flour; D, commercial spring wheat flour; E, commercial spring wheat flour; F, commercial flour; G, durum wheat flour; H, commercial air-classified flour; C-fungal, flour C digested with an enzyme system of *Aspergillus oryzae*.

rates of digestion between individual wheat starch granules (11).

For convenience the slope of the curve may be represented by one figure; the difference between the 1-hour and 2-hour determinations. It would seem that under standard conditions this figure could be used to indicate the relative digestibility of the undamaged starch. Applied to this particular group of flours: A, a soft white wheat flour, had the least digestible starch (value 2.5), and G, a durum, the most (value 5.0); whereas within classes, the two hard springs (E and D) had values of 4.4 and 3.0 and the two hard winters (C and F) had values of 4.6 and 3.4.

Use of Single Determinations vs. 1- and 2-Hour Curves. If the undamaged starch in all flours (or even in all flours of one class) were equally digestible, a single determination after 1 hour's digestion with a correction factor for the digestion of the undamaged starch, could be a measure of the damaged starch. However, the wide variation in slopes of the curves of Fig. 4 show that single values could be misleading. Flours B and C have practically the same damaged starch value but the 1-hour reading alone would give flour B a value of 7.4 and flour C a value of 9.2. The curves for the two hard spring flours D and E, as well as for the two hard winters C and F, have decidedly different slopes.

Effect of Varying the Enzyme Concentration. As is to be anticipated, variations in enzyme concentration cause variations in the rate of digestion of the undamaged starch, i. e., they change the slope of the curve. The data of Fig. 5 indicate that, in so far as the determination of damaged starch is concerned, the slope of the curve is of no consequence.

The variation from 130 units of alpha-amylase to 190 units caused no error in the damaged starch figure on a sample with a low percent of damage. The value for durum flour with 13% damage varied 0.6%. However, durum flour gives erratic results, probably because the flour is largely made up of endosperm cells and fragments of cells (12) which do not readily disintegrate under the conditions used. The 0.6% variation may indicate that the quantity of alpha-amylase should be increased for such highly damaged flours or for durum flours.

Using one enzyme preparation and the method as proposed, the standard deviation obtained from seven determinations on a soft wheat flour with 3.6% damaged starch was 0.17; from seven determinations on a durum wheat flour with 13.5% damaged starch the standard deviation was 0.20.

Continuous Shaking during Digestion. It has been suggested that

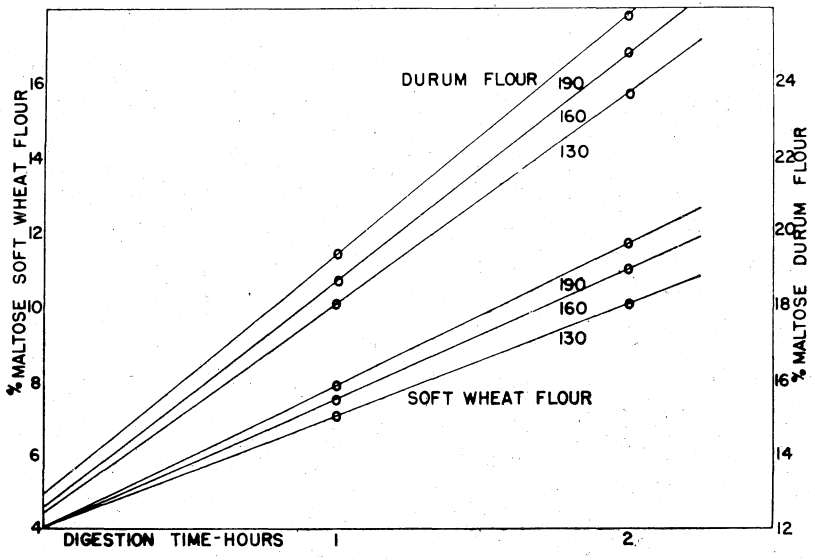

Fig. 5. The effects of varying the enzyme concentration on the amount of damaged starch found and on the slopes of the curves. Numerals 130, 160, 190 indicate the units of alpha-amylase used for the digestions.

shaking the samples during the digestion is unnecessary. Limited experience in this laboratory indicates that this may be true when using flours with relatively low amounts of damage or with soft wheat flours; on the other hand, a durum flour gave results 3 to 4% lower with no shaking than with continuous shaking. The amount of shaking also had pronounced effects on the slopes of the digestion curves. The granular structure of the hard wheat flour makes continuous shaking necessary.

Specifications for the Proposed Procedure[5]

Apparatus: (1) Thermostatically controlled water bath with a shaker.
(2) Filter paper, No. 4 Whatman or equivalent.
(3) Microburet, 10-ml. capacity.

Reagents: (1) Buffer solution. Dissolve 3 ml. glacial acetic acid and 4.1 g. anhydrous sodium acetate and make up to 1 liter with water. The pH of this solution is 4.6 to 4.8.
(2) Sulfuric acid, 3.58*N* (± 0.05). Dilute 10 ml. concentrated H_2SO_4 (sp. gr. 1.84) to 100 ml. and adjust concentration if necessary.
(3) Sodium tungstate solution, 12%. Dissolve 12.0 g. $Na_2WO_4 \cdot 2H_2O$ and dilute to 100 ml.
(4)Alkaline ferricyanide reagent, 0.1*N*. Dissolve 33 g. pure dry $K_3Fe(CN)_6$ and 44 g. anhydrous Na_2CO_3 and dilute to 1 liter. To standardize, add to 10 ml. of this solution 25 ml. acetic acid-salt solution and 1 ml. soluble starch-KI solution, and titrate with 0.1*N* thiosulfate. Exactly 10 ml. should be required to discharge the blue color completely.

[5] See reference 2.

(5) Acetic acid-salt solution. Dissolve completely 70 g. KCl and 40 g. $ZnSO_4 \cdot 7H_2O$ in 750 ml. water, add slowly 200 ml. glacial acetic acid, and dilute to 1 liter with water.
(6) Soluble starch-potassium iodide solution. Suspend 2 g. soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool thoroughly (or the resulting mixture will be dark-colored), add 50 g. KI, dilute to 100 ml., and add 1 drop of saturated NaOH solution.
(7) Thiosulfate solution, 0.1*N*. Dissolve 24.82 g. Reagent quality $Na_2S_2O_3 \cdot 5H_2O$ and 3.8 g. borax and make to 1 liter.
(8) Malt enzyme preparation. A 1:4 malted wheat flour extract thoroughly dialyzed against distilled water (containing 20 mg. $CaCl_2$ per 100 ml.) to remove reducing sugars. The dialyzed extract may be: 1) assayed for alpha-amylase value (16) and stored at approximately 4°C. with an excess of chloroform (alpha-amylase solutions are subject to surface inactivation and accordingly they should not be vigorously shaken with the excess chloroform); 2) lyophilized and the dry powder assayed for alpha-amylase (16).
(9) Malt solution. Dissolve the equivalent of 160 SKB units per 6 ml. water.

Determination: Introduce duplicate 1-g. samples of flour and ½ teaspoonful of ignited quartz sand into 100- or 125-ml. Erlenmeyer flasks, and mix by rotating flask. Add 40 ml. buffer solution at 30°C. and 6 ml. of enzyme solution, and mix by rotating the flask. (Flask and all ingredients should be *individually* brought to 30°C. before being mixed together.) Place in a thermostatically controlled water bath at 30°C. and maintain at this temperature

TABLE I

FERRICYANIDE-MALTOSE (DAMAGED STARCH) CONVERSION

0.1*N* Ferricyanide Reduced	Maltose	0.1*N* Ferricyanide Reduced	Maltose	0.1*N* Ferricyanide Reduced	Maltose
ml	*% of flour*	*ml*	*% of flour*	*ml*	*% of flour*
0.10	0.25	3.10	7.80	6.10	17.1
0.20	0.50	3.20	8.05	6.20	17.4
0.30	0.75	3.30	8.30	6.30	17.7
0.40	1.00	3.40	8.55	6.40	18.1
0.50	1.25	3.50	8.80	6.50	18.4
0.60	1.50	3.60	9.10	6.60	18.7
0.70	1.75	3.70	9.40	6.70	19.0
0.80	2.00	3.80	9.70	6.80	19.3
0.90	2.25	3.90	10.1	6.90	19.6
1.00	2.50	4.00	10.4	7.00	19.9
1.10	2.75	4.10	10.7	7.10	20.3
1.20	3.00	4.20	11.0	7.20	20.6
1.30	3.25	4.30	11.3	7.30	21.0
1.40	3.50	4.40	11.6	7.40	21.3
1.50	3.75	4.50	11.9	7.50	21.6
1.60	4.00	4.60	12.2	7.60	22.0
1.70	4.25	4.70	12.6	7.70	22.3
1.80	4.50	4.80	12.9	7.80	22.6
1.90	4.75	4.90	13.2	7.90	23.0
2.00	5.00	5.00	13.5	8.00	23.3
2.10	5.25	5.10	13.8	8.10	23.6
2.20	5.50	5.20	14.1	8.20	24.0
2.30	5.75	5.30	14.4	8.30	24.3
2.40	6.00	5.40	14.8	8.40	24.6
2.50	6.25	5.50	15.2	8.50	25.0
2.60	6.50	5.60	15.5	8.60	25.3
2.70	6.75	5.70	15.8	8.70	25.7
2.80	7.00	5.80	16.2	8.80	26.0
2.90	7.25	5.90	16.5		
3.00	7.55	6.00	16.8		

for exactly 1 hour for the first sample and 2 hours for the second, shaking the flasks continuously. The shaking should only be enough to keep the flour in suspension. At the end of the 1-hour and 2-hour periods add 2 ml. 3.58*N* H_2SO_4 to the respective flasks and mix thoroughly. Add immediately 2 ml. sodium tungstate solution and again mix thoroughly. Let stand 2 minutes and filter (No. 4 Whatman or equivalent), discarding the first 8 to 10 drops of filtrate.

Pipet a 5-ml. aliquot of the filtrate into a test tube of approximately 50-ml. capacity (length 20 cm., diameter 2 cm.). Add with a pipet exactly 10 ml. alkaline ferricyanide reagent, mix, and immerse the test tube in a vigorously boiling water bath. The surface of the liquid in the test tube should be 3 to 4 cm. below the surface of the boiling water. (The delay between the filtering of the extract and the treatment in the boiling water bath should not exceed 15 to 20 minutes. Further delay may cause error due to fructan hydrolysis in the acid solution.) Allow the test tube to remain in the boiling water bath exactly 20 minutes. Cool the test tube and contents under running water and pour at once into a 100- or 125-ml. Erlenmeyer flask. Rinse out the test tube with 25 ml. acetic acid-salt solution reagent, adding rinsings to the solution in the Erlenmeyer flask. Mix, add 1 ml. of soluble starch-KI solution, mix thoroughly and titrate with 0.1*N* thiosulfate to the complete disappearance of the blue color. (A 10-ml. microburet is recommended for this titration.) Calculate ml. ferricyanide reduced by subtracting ml. thiosulfate required for the determination from the thiosulfate used for the enzyme-reagent blank. Convert to maltose by means of the Ferricyanide-Maltose (Damaged Starch) Conversion Table (Table I). Plot percent maltose against digestion time, drawing a straight line from the vertical axis at 0 time through the 1-hour and 2-hour points. The intercept with the vertical axis at 0 time is the percent maltose produced from damaged starch or percent damaged starch.

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