Colorimetric Determination of Damaged Starch in Flour[1]

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ABSTRACT

A rapid colorimetric test is described for routine determination of damaged starch in wheat flours. The test is based on the color developed on addition of iodine to an extract made by treating flour with a strong solution of sodium sulfate containing 15% formamide and 0.2% sulfosalicylic acid. The results are recorded simply as absorbance, and compare well with damaged-starch estimations made on a series of flours by conventional enzymatic procedures. Precision and reproducibility are equal if not superior to current techniques. A procedure for automation of the colorimetry is described.

The importance of mechanically damaged starch in modern flour technology is based largely on the influence of damaged starch on flour water absorption and diastatic activity. The amount of damaged starch present in a flour has, of recent years, come to be one of the routine analyses made on flour in many mills and other laboratories. Mechanical damage to starch kernels apparently was first demonstrated by Scheffer (1), but it was the more precise and extensive work of C. R. Jones (2) which established that starch damage could be effected by roll pressure in flour mills. Both workers employed the technique of direct observation of differentially stained starch granules, with the use of a microscope.

Microscopic methods do not lend themselves to quantitative estimation of starch damage in a flour; they are laborious and are subject to large sampling errors; and finally, starch granules are invariably damaged to different degrees during their passage through mill rolls. Consequently a number of procedures, enzymatic, colorimetric, and amperometric, have been developed. For quantitative estimation of "damaged starch" in flour, enzymatic procedures are the most common, and all are based on the experimental finding that damaged granules are more susceptible to attack by diastatic enzymes than are sound granules.

If a sound flour is analyzed for diastatic activity by the conventional Blish-Sandstedt method over periods from 5 min. to 5 hr., the amount of maltose produced shows a rapid initial rise for the first 30 - 45 min., after which maltose is produced in regular increments. The initial rapid rise is due to autolytic digestion of the damaged starch present by beta-amylase; the subsequent straight-line increase is due to digestion of sound starch, at a slower but constant rate. Differences in slopes of the lines are due to differences in susceptibility of sound starch to attack by the amylases, and extrapolation of the slope to zero time gives a measure of damaged starch. If, however, flours are milled from wheat high in alpha-amylase activity, considerably higher amounts of maltose are produced initially and the results may not be reliable, since they are now dependent on alpha-amylase levels as well as on damaged starch. The conventional enzymatic methods for measuring damaged starch consequently involve some sort of preliminary control of the amylases present.

The procedures described by Farrand (3), Sandstedt and Mattern (4), Donelson and Yamazaki (5), and Audidier *et al.* (6) all involve addition of excess

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alpha-amylase to digest the damaged granules rapidly during the first few minutes of the test, after which sound starch is digested at a slower and constant rate. The amount of damaged starch present is responsible for determining the level at which sound starch commences to be digested, and an estimation of the amount of damaged starch present may be derived from the final amount of maltose produced, as determined by the Blish-Sandstedt test. The techniques of Dadswell and Gardner (7), Greer and Stewart (8), and Sullivan, Anderson, and Goldstein (9) involved preliminary destruction of all enzyme activity followed by the addition of enzyme extracts or crystalline enzyme preparations, but led to a similar type of final result.

With the exception of the Donelson-Yamazaki and the Audidier methods, which are of limited range, enzymatic procedures require from 2 hr. in the Farrand test to about 5 hr. in the Greer-Stewart procedure. In an effort to reduce the time involved for a single test, Medcalf and Gilles developed an amperometric procedure based on the differential rate of absorption of iodine by flours of different damaged-starch content (10). A colorimetric method of estimation of damaged starch was described by Hampel (11), whose "amylose number" test was based on the observation that the amylose present in mechanically damaged starch granules was more rapidly extracted by saturated ammonium sulfate solution than was that in sound starch granules. A modification of this test has been developed in this laboratory, and forms the subject of this communication.

MATERIALS AND METHODS

Materials

Formamide - Sodium Sulfate Stock Solution. Prepare a 1.41*M* solution of sodium sulfate by dissolving 400 g. anhydrous sodium sulfate in 1,500 ml. of distilled water, and dilute to 2 liters. Dilute 300 ml. of formamide to 2 liters with this sodium sulfate solution. This stock solution is stable for about 1 month.

Extracting Solution. Dissolve 2.0 g. sulfosalicylic acid in 1 liter of formamide - sodium sulfate stock solution. This must be prepared fresh daily. The amount of extracting solution to be prepared will depend on requirements.

Iodine Stock Solution. Dissolve 5.5 g. AR grade iodine crystals and 11.0 g. of AR grade potassium iodide crystals in the minimum amount of water (about 25 ml.) and dilute to 250 ml. in a tinted 250-ml. volumetric flask. This solution must be stored in the dark.

Iodine Reagent. Dilute 10 ml. stock iodine solution to 100 ml. with distilled water. This reagent must be prepared fresh daily.

Gelatin Solution. Dissolve 0.5 g. reagent-grade powdered gelatin in 100 ml. of recently boiled distilled water and filter through glass wool.

Diluting Solution. Dilute 50 ml. of gelatin solution and 2.5 ml. of AR grade 100 vol. (30%) hydrogen peroxide solution to 500 ml. with recently boiled distilled water.

Celite Analytical Filter-Aid. Use Johns-Manville, or suitable equivalent.

Procedure

Extract flour samples (1 g.) with 25 ml. of extracting solution for 15 min. at 50°C. with thorough shaking at 5-min. intervals. (Optimum reproducibility is achieved by timing the addition of the extracting solution to the samples so that each sample is in the water bath for exactly 15 min.) Add about 0.25 g. Celite to the suspensions, shake briefly, allow to stand 1 or 2 min., and filter through

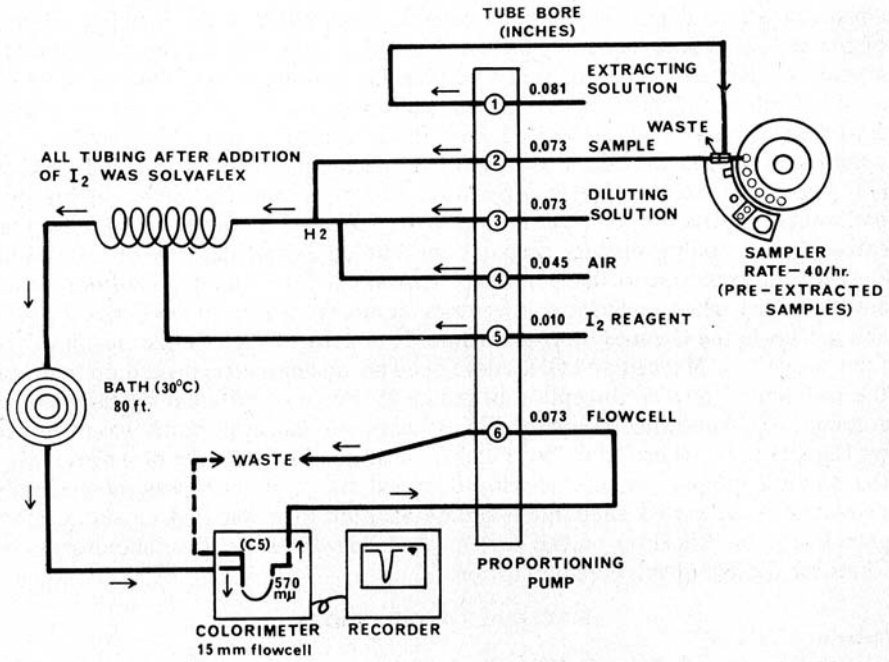

Fig. 1. Technicon Autoanalyzer flow-sheet for colorimetric measurement of damaged starch.

Whatman No. 4 filter paper (or suitable equivalent). Pipet 10-ml. aliquots into test-tubes containing 10 ml. of diluting solution. To each tube add 0.5 ml. of iodine reagent, mix thoroughly, and allow to stand in a water bath at 30°C. for 15 min. Determine absorbance at 555 mμ against a reagent blank.

Automation

The colorimetry was speeded up considerably by means of a Technicon Autoanalyzer (Technicon Corp., Ardsley, New York, N.Y. 10502). The flow sheet is shown in Fig. 1. After extraction and filtration of the samples, the clear filtrates were poured into sample cups on the Autoanalyzer. The samples were mixed with diluting solution, then the iodine solution was added to the stream. The stream was mixed and passed through a water bath at 30°C. before being passed through the colorimeter. Optimum sample rate was 40 per hr. Since the iodine solution was found to attack the tygon tubing, all tubing after the addition of the iodine was replaced by Solvaflex. The automation of the test served to verify that a high degree of reproducibility can be achieved in both extraction and colorimetry.

RESULTS

The test as described was applied to 53 flours representing a very wide range of starch damage, wheat variety, and environment. Commercial and experimentally milled Canadian and Australian flours were included. The flours were also analyzed by the procedures of Farrand (3), Sandstedt and Mattern (4), Greer and Stewart

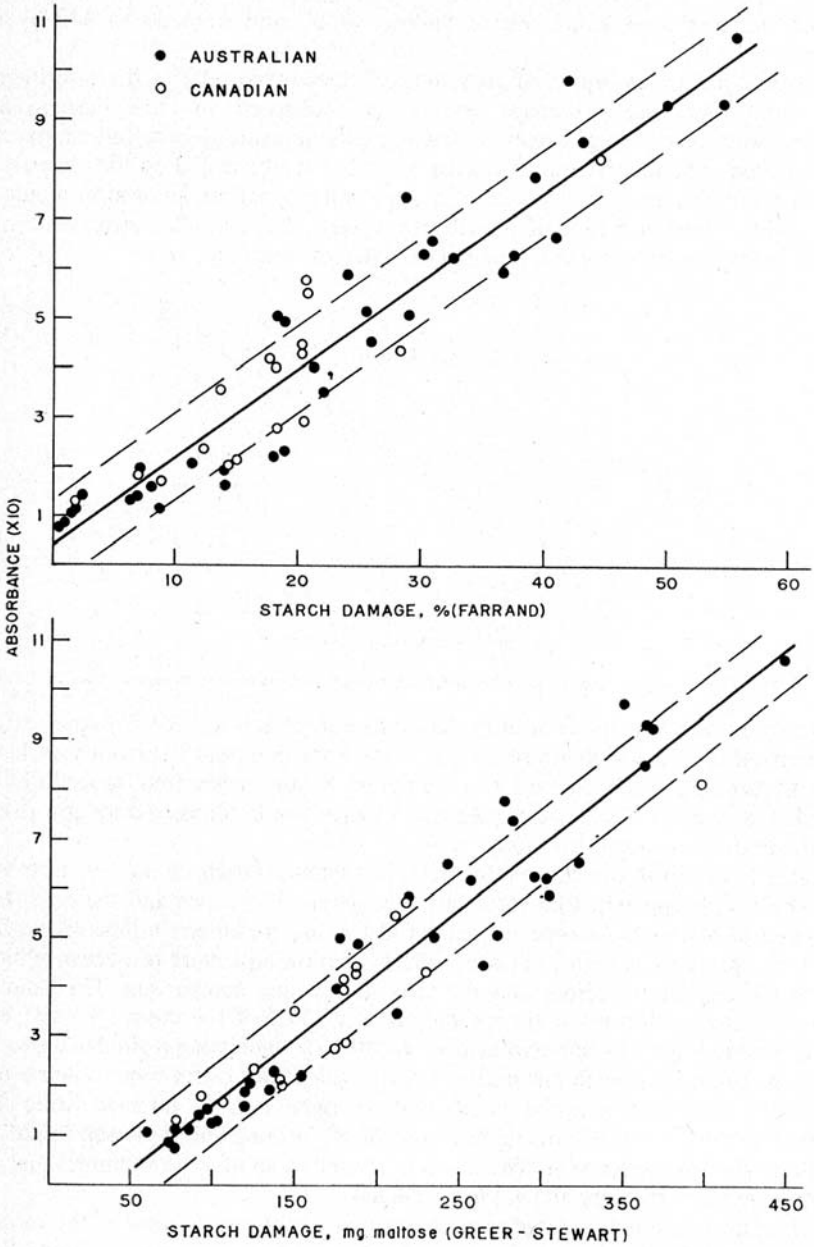

Fig. 2. Relation of two enzymatic methods of measuring damaged-starch content to proposed colorimetric method.

(8), Sullivan *et al.* (9), and Medcalf and Gilles (10). Absorbance ranged from 0.074 to 1.07. Flour damaged starch content ranged from 0.3 to 55.8% by the Farrand procedure, from 2.6 to 14.5% by the Sandstedt-Mattern procedure, from 40 to 367

by the starch-damage index test of Sullivan *et al.*, and from 60 to 449 by the Greer-Stewart test.

Figure 2 illustrates typical scattergrams obtained by correlating the colorimetric test with flour starch damage content as measured by the Farrand and Greer-Stewart procedures, which represent methods using respectively alpha- and beta-amylase. The relatively high degree of scatter is augmented by the diversity of the flour samples used. In material of a more uniform nature, less scatter would be anticipated. Thus in a plot of absorbance against, for example, increments of a ball-milled starch sample, the points fall on a straight line (Fig. 3).

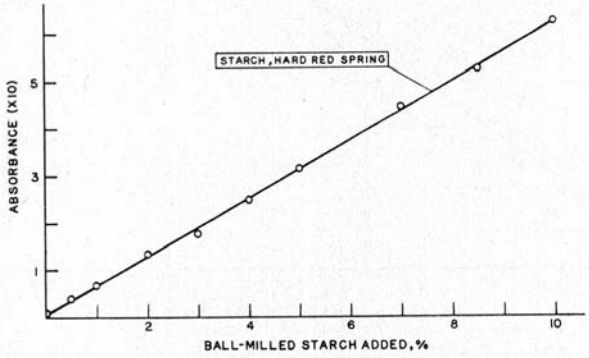

Fig. 3. Increase in absorbance on addition of ball-milled starch to sound starch.

Relations between the four enzymatic procedures and the colorimetric test are summarized in Table I. Regression equations for the computation from absorbance data of damaged-starch content as determined by the other four procedures are included, as well as the errors of estimate for each test in terms of both absorbance and the units of the respective test.

Table II demonstrates the relative relations between each of the five tests with the others. It is apparent that the relation between absorbance and the other tests described is of the same order of magnitude, as are the interrelations of the four enzymatic procedures with each other. Such relationships must of necessity incorporate the cumulative errors of both tests in any one comparison. The standard error of a single colorimetric determination is ±0.17% of the mean (N = 53); that of the Farrand test (in our experience) is ±0.19%, indicating a similar degree of precision. Difficulties were encountered with the Medcalf-Gilles amperometric test. These were associated with the maintenance of rigidity in the rather delicate electrodes, also with speed of stirring and control of frothing during suspension of the sample in the Waring Blendor. The test was applied to all of the 53 samples, but the results were unsatisfactory and are not included.

Calibration of starch-damage tests is somewhat arbitrary in view of the variable degree to which granules are damaged and to which such material affects flour properties. Ball-milled starch is not very reproducible material, "starch damage" values varying from 120 to 180% on the Farrand scale, depending upon the time of ball-milling, number and size of balls used, amount of starch per ball mill, mill speed, etc. We prefer calibration against a series of flours of known wide range of starch damage, followed by computation of a regression equation (see Table I). This

TABLE I. REGRESSION EQUATIONS, STANDARD ERRORS OF ESTIMATE, AND CORRELATION COEFFICIENTS FOR DIFFERENT METHODS FOR STARCH DAMAGE DETERMINATION, AS COMPARED WITH THE COLORIMETRIC TEST

Methods	Regression Equations[a]	Standard Errors of Estimate		Correlation Coefficient
		Units of y[a]	Units of x[a]	
Greer-Stewart	Y=−0.11 + 0.0027X	±0.08		r=0.96
	X= 52.7 +346Y		±27.6	
Farrand	Y= 0.039 + 0.018X	±0.09		r=0.95
	X= 0.286 + 50.3Y		± 4.6	
Sullivan	Y=−0.070 + 0.0029X	±0.10		r=0.93
	X= 44.4 + 298Y		±32.6	
Sandstedt-Mattern	Y=−0.19 + 0.083X	±0.13		r=0.88
	X= 3.39 + 9.38Y		± 1.4	

[a] Y = absorbance units; X = unit of specified method.

TABLE II. CORRELATION COEFFICIENTS FOR INTERRELATIONSHIPS OF FIVE STARCH-DAMAGE TESTS

Starch Damage Test	Farrand	Sullivan *et al.*	Sandstedt-Mattern	Greer-Stewart
Proposed colorimetric	0.95	0.93	0.88	0.96
Farrand	....	0.93	0.90	0.98
Sullivan *et al.*		....	0.95	0.97
Sandstedt-Mattern			....	0.94

technique is applicable to any laboratory having access to a range of flour samples, and the absorbance may be related to the method of starch-damage estimation practiced in that laboratory by means of a suitable regression equation. Calibration of absorbance against cuprammonium solution as proposed by Hampel is of doubtful value, since the color is of a different wave length to the color developed in flour samples.

DISCUSSION

In our experience with the original procedure described by Hampel we found that the measurable range of starch damage was too small to accommodate hard wheat flours, and that the sensitivity left much to be desired. Further difficulties were associated with premature fading of the color and persistent turbidities after addition of iodine. It was found that the first two objections could be corrected by the use of sodium sulfate in place of ammonium sulfate, together with a higher extraction temperature. The procedure was further simplified by adjusting the concentration of the iodine solution and abandoning the boiling water bath. The present method requires approximately 40 min. for a single test or batch of tests. According to the needs of a laboratory, this could be increased or shortened by varying the time of extraction and for color development, provided that the test is calibrated under the revised conditions in the respective laboratory. For example, the extraction time can be lengthened to 30 min., allowing more time for the

organization of the next batch, without materially affecting the results. Also, the absorbance measurements may be made 5 min. after addition of the iodine. Although there is a slight but definite increase in absorbance between 5 and 30 min., this is unimportant as long as the time of reading is fixed. The 30° C. water bath may be abandoned in favor of room temperature color development if desired, provided that this is reasonably stable at (preferably) about 25° C. (77° F.).

However, the concentration of iodine is important in relation to that of potassium iodide. If the ratio of potassium iodide to iodine is above 4:1, turbidities develop which render the test useless. Also, the absorbance for a given sample increases directly as the ratio of potassium iodide to iodine, which may lead to less accuracy at high levels of damaged starch. The formamide and sulfosalicylic acid assist both in the extraction and in the maintenance of clarity in the extract, and are indispensable. Centrifuging at 6,000 r.p.m. for 10 min. can replace filtration, if desired. The Celite not only improves the rate of filtration, but also leads to a perfectly clear extract, after either filtration or centrifugation, which is essential for a colorimetric procedure. The extracts are almost invariably turbid in the absence of Celite. The purpose of the diluting solution is twofold: in addition to lowering the absorbance range it stabilizes the color. In the absence of hydrogen peroxide the color developed by the iodine fades rapidly, whereas the gelatin appears to assist in maintaining absolute clarity after addition of iodine. The present method is admirably suited for routine use, either in laboratories of modest means, or in more sophisticated laboratories where automation may be desired. Advantages of the method included simplicity of execution, rapidity, and the relatively small number of reagents necessary.

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