DETERMINATION OF STARCH DAMAGE BY RATE OF IODINE ABSORPTION[1]

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ABSTRACT

The rate of iodine absorption by a starch suspension depends on the characteristics of the granules. Damaged granules absorb iodine at a faster rate than do undamaged ones. A convenient means to measure this absorption is by amperometric titration. A method is proposed to use this technique for quantitative determination of damaged starch in flour. Reagents and equipment requirements are simple, only 0.2-g. samples are needed, and the time for each determination is approximately 15 min. A linear response was obtained for starch damages ranging from very high to very low. Starch damage determinations were performed on a series of flour samples using the proposed amperometric method, two enzymatic methods, and a colorimetric method. The proposed technique gave results comparable to these published procedures for starch damage values below 10%. Above 10% starch damage, the amperometric method gave higher values. It is suggested that the amperometric method may give a more accurate indication of starch damage when the amount of damage is relatively large, such as in air-classified fractions.

The problem of overgrinding flour and the resulting damage to the starch granules is well known (1,2,3). An excessive amount of damaged starch is undesirable, although some damage is necessary for proper enzymatic action on the starch.

It is relatively simple to show qualitatively that some damage to starch granules occurs during milling. The quantitative determination of this damage is more difficult. Several methods have been devised for this purpose. Early procedures involved staining of the starch granules, followed by microscopic examination (4,5). Hampel (6) developed a colorimetric method based on the reaction of starch with iodine. Recently, several enzymatic procedures have been developed (7–11).

Enzymatic methods have proved to be the most useful for the quantitative determination of damaged starch in flour. However, several problems exist. All are relatively time-consuming for small numbers of determinations. In addition, their response is not linear over a wide range of starch damage. More enzyme and/or smaller samples must be used when very large amounts of damage are present.

Coton *et al.* (12) modified the amperometric titration method of Larson *et al.* (13) and suggested that the iodine absorption curves of starches could be used to detect overgrinding in flour. Apparently, they did not pursue this idea further.

The purpose of the present work was to devise a method for the quantitative determination of starch damage in flour which would give linear results over a wide range of damage and which would require a minimum amount of time for each determination. This paper describes such a method, based on the rate of iodine absorption of flours determined by amperometric titration.

Apparatus and Materials

Description of Apparatus. The electrical circuit was similar to that described by Coton *et al.* (12). It consisted of a standard 1.5-v. dry cell, two variable resistance boxes, a galvanometer whose sensitivity could be altered by use of a variable resistance shunt, and the platinum electrodes.

The titration assembly was a 150-ml. graduated beaker fitted with a No. 12 rubber stopper which contained three openings. The electrodes were sealed into one opening and a microburet tip and a stirring rod were inserted into the other two. When in use, the assembly was placed in a water bath at 25°C. and stirred at a constant rate[3] by means of an electric motor.

The electrodes were thin platinum wires sealed into glass tubing. When inserted through the rubber stopper the electrodes were approximately 1 mm. apart, and 5 cm. of wire extended below the stopper.

Starch and Flour Samples. Starch was isolated in the laboratory from hard red spring (HRS) wheat flour. A portion was ball-milled for 12 hr. and then mixed with the original material to give the various concentrations of ball-milled starch.

Flour samples consisted of both commercial and laboratory-milled material. Samples of commercial HRS wheat flour were ball-milled for various times using both a 1-qt. (I) and a 5-qt. jar (II). All starch-damage results are reported on "as-is" basis. That is, no corrections were made for differences in amount of starch present in the various flours.

Samples of commercial HRS wheat flour also were treated with various flour-bleaching and improving reagents. These included chlorine gas (320 and 950 p.p.m.), benzoyl peroxide (150 and 300 p.p.m.), azodicarbonamide (20 and 50 p.p.m.), and potassium bromate

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[3] For the system used in this work, the stirring rate was 1,600 r.p.m.

(20 and 50 p.p.m.). Flour was combined with chlorine gas, using a Wallace & Tiernan laboratory bleaching apparatus. Other reagents were combined with the flour by mixing for 5 min. in a MacLellan mixer.

Determination of Iodine Absorption

General Considerations. The procedure used in this work was essentially that of Coton *et al.* (12). Since iodine solutions are relatively unstable, a standard iodate solution was used which reacts with potassium iodide in the acidic reaction medium to produce free iodine *in situ.* To ensure reproducible results, the amount of iodine available to the sample must be constant. To keep it so, the free iodine concentration in the system is kept within ± 10% of some arbitrarily chosen value. The electrical circuit is adjusted so that the galvanometer reads 10 units[4] when the iodine concentration is at this value. When a sample is present, iodate is added until the iodine concentration is 10% above the chosen value (a reading of 11 units on the galvanometer). When the iodine concentration is reduced to 10% below this value (a reading of 9 units on the galvanometer), enough iodate is added to bring the iodine back to 10% excess. In this way fluctuations in the iodine concentration are constant, symmetrical, and always within ± 10% of the chosen value.

Blank Determination. The reagent solution used in this work consisted of 85 ml. water, 10 ml. 1N hydrochloric acid, and 5 ml. 0.4N potassium iodide solution. This solution was added to the titration cell, the electrodes were inserted, and the assembly was placed in the water bath. It was stirred at a rapid and constant rate, and 0.4 ml. of 0.005N potassium iodate solution added by means of a microburet, the tip of which was inserted through the stopper into the cell. This gave a free iodine concentration of 20 μN which was the concentration maintained in the system for this work. The electrical circuit was adjusted so that the galvanometer read 10 units. This served as a blank determination and was repeated periodically.

Rate of Iodine Absorption. The sample (0.2 g., dry basis) was added to a solution containing 70 ml. distilled water, 10 ml. 1N hydrochloric acid, and 5 ml. 0.4N potassium iodide solution. The mixture was agitated briefly in a Waring Blendor (360-ml. bowl) to ensure uniform suspension and transferred to the titration cell (using a stream of water from a wash bottle to ensure quantitative transfer). More water was added to bring the total volume of reagent solution to 100 ml., and the assembly was completed as described above. A

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[4] Any galvanometer reading could be used. The values corresponding to ± 10% of this reading would then be adjusted accordingly.

timer was started and sufficient iodate added to give a galvanometer deflection of 11 units. The amount of iodate added was noted; when the galvanometer fell to 10 units, the total reaction time was recorded. When the galvanometer reached 9 units, enough additional iodate was added to bring the galvanometer reading back to 11, and the total amount of iodate added was noted. As the galvanometer again reached 10, the total reaction time was recorded. This procedure was repeated for the duration of the experiment. The amount of iodine absorbed at a given reaction time was calculated from the ml. of iodate added (12) after taking into consideration the amount of iodate needed to maintain the free iodine concentration at 20 μN. Percent iodine absorbed (g. I/100 g. sample) was plotted against time to give a curve showing the rate of iodine absorption. After each determination the electrodes were cleaned by submersion in 50% sodium hydroxide solution, followed by thorough washing with distilled water.

Iodine Absorption Value (IAV). Experimental results indicated that in all cases the curves showing the rate of iodine absorption became essentially linear after approximately 8–10 min. (Fig. 1). To facilitate and expedite the work for routine determinations, it was considered unnecessary to record the total amount of iodate and the reaction time in the early stages of the determination. The procedure was followed as described above; however, no readings were recorded until 9 min. had elapsed. Then the total reaction time and total ml. of iodate added, which corresponded to the next galvanometer reading of 10, were recorded. No further readings were taken until the reaction had proceeded for a total of 14 min.; again, total reaction time and total ml. of iodate added, which corresponded to the next galvanometer reading of 10, were recorded. The amount of iodine absorbed at these two time intervals was plotted against the reaction time and the line connecting the two points extrapolated to zero time. The point of intercept on the axis for percent iodine absorbed was multiplied by 100 to give the Iodine Absorption Value (IAV) of the sample. Samples were determined in duplicate and the results averaged. Duplicate results always agreed within ± 4 and usually within ± 2 IAV units. The standard deviation obtained from 10 determinations on a starch sample with an IAV of 40 was 1.78. Ten determinations on a flour sample with an IAV of 50 gave a standard deviation of 2.07.

Starch Damage Methods

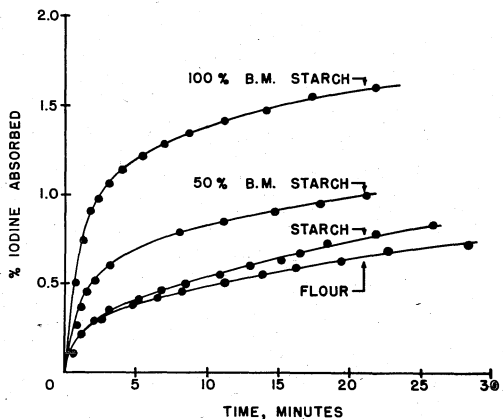Three starch damage methods were selected for comparison with the amperometric procedure.

Fig. 1. Plots of rates of iodine absorption determined amperometrically. B.M. refers to 12-hr. ball-milled.
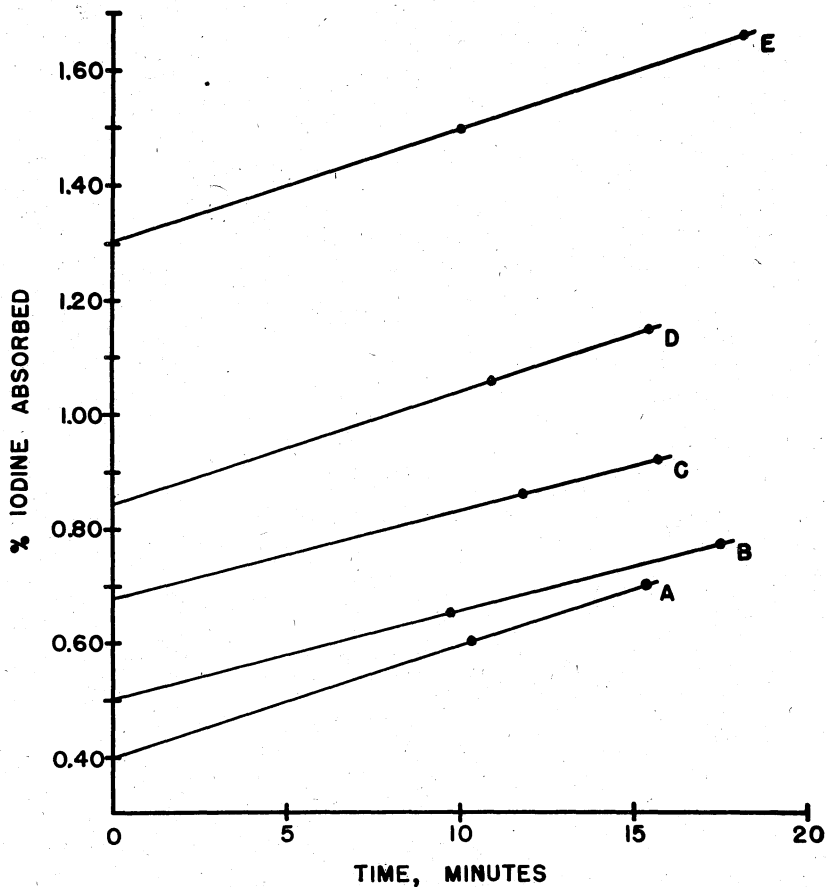

Fig. 2. IAV plots of starch and flour samples: A, starch; B, flour; C, 12-hr. ball-milled flour; D, air-classified flour; E, 12-hr. ball-milled starch.

The enzymatic procedure of Sandstedt and Mattern (7) was used, with slight modification. Flasks were shaken every 5 min. instead of continuously. The enzyme preparation used was "Low Maltose Amylase" obtained from Wallerstein Co., Staten Island, N.Y.

The enzymatic procedure of Donelson and Yamazaki (9) was used without modification.

The colorimetric method for determination of amylose numbers was used as described by Hampel (6), except that the color concentration was measured with a Bausch & Lomb Spectronic 20 colorimeter at 620 mμ.

Results and Discussion

Plots which show the rate of iodine absorption by wheat flour and starch are shown in Fig. 1. As pointed out by Coton et al. (12), flour gave results similar to those of starch.

Typical IAV plots of flour and starch samples are shown in Fig 2. This illustrates the method of curve extrapolation. The percent iodine absorbed which corresponded to the intercept was multiplied by 100 to give IAV.

The IAV's of a series of starch samples which contained various amounts of 12-hr. ball-milled starch were determined. IAV was
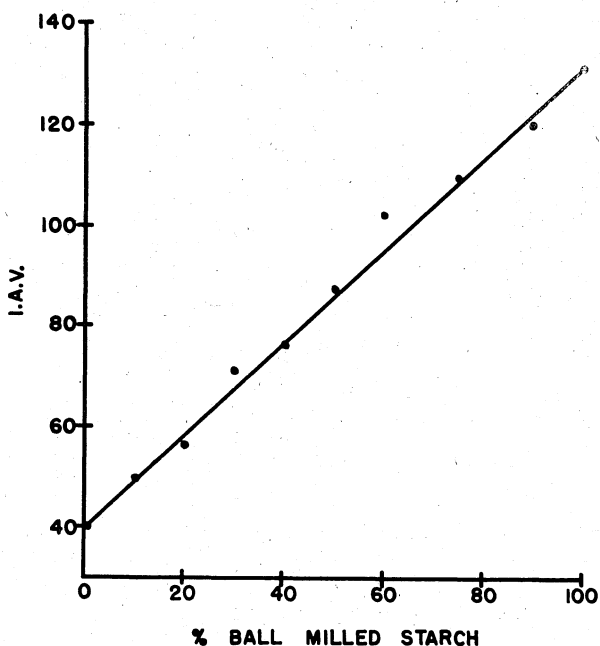

Fig. 3. Effect of various amounts of 12-hr. ball-milled starch on IAV.

linearly related to the amount of ball-milled starch, as shown in Fig. 3.

These results indicated that this method should be applicable to the determination of damaged starch in flour. The IAV of a series of flour samples was compared to the percent starch damage in these samples as determined by two enzymatic methods (7,9), and to their amylose numbers as determined by the Hampel colorimetric procedure (6). Results are shown in Table I.

TABLE I

DATA ON COMPARISON OF STARCH DAMAGE METHODS

FLOUR SAMPLE	IODINE ABSORPTION VALUE	STARCH DAMAGE [a]		AMYLOSE NUMBER
		A	B	
		%	%	
Commercially milled				
Soft wheat	38	3.5	4.1	60
Hard winter wheat	50	7.0	8.1	310
HRS wheat	50	7.6	9.0	288
Ball-milled: HRS I, 1 hr.	53	8.0	9.1	280
HRS I, 3 hr.	58	9.2	10.0	280
HRS I, 5 hr.	62	8.7	10.8	443
HRS I, 8 hr.	64	10.6	11.7	455
HRS I, 12 hr.	66	11.3	12.0	483
HRS II, 4 hr.	63		11.1	
HRS II, 8 hr.	82		13.3	
HRS II, 16 hr.	84		13.6	
Bread flour I	44	6.5	7.3	185
Bread flour II	49	8.0	9.2	235
High-protein flour I	47	6.4	8.2	205
High-protein flour II	45	5.6	8.9	215
Laboratory-milled				
HRS (Buhler)	42	4.4	7.0	153
Durum (Buhler)	54	8.4	11.2	158
HRS (Miag) I	44	4.8	6.3	158
HRS (Miag) II	42		6.5	
Soft wheat (Quadrumat-milled)	34	1.8	2.1	55
Air-classified				
2nd Coarse	46	5.4	6.0	190
1st Fines (high-protein)	82	12.9	14.0	483
2nd Fines (low-protein) I	67	9.8	11.0	420
2nd Fines (low-protein) II	72		11.8	
2nd Fines (low-protein) III	86		13.8	

[a] Values in column A were determined by the Sandstedt and Mattern method (7); those in column B, the Donelson and Yamazaki method (9).

A few more points of comparison were obtained for the Donelson-Yamazaki procedure, since this was the simplest and undoubtedly the most useful of the three methods used for comparison with the amperometric technique. The relationship of IAV's to the other values obtained is indicated by the scattergrams shown in Figs. 4, 5, and 6.

Figure 4 indicates that IAV is essentially linearly related to amylose number for the total range of samples tested.
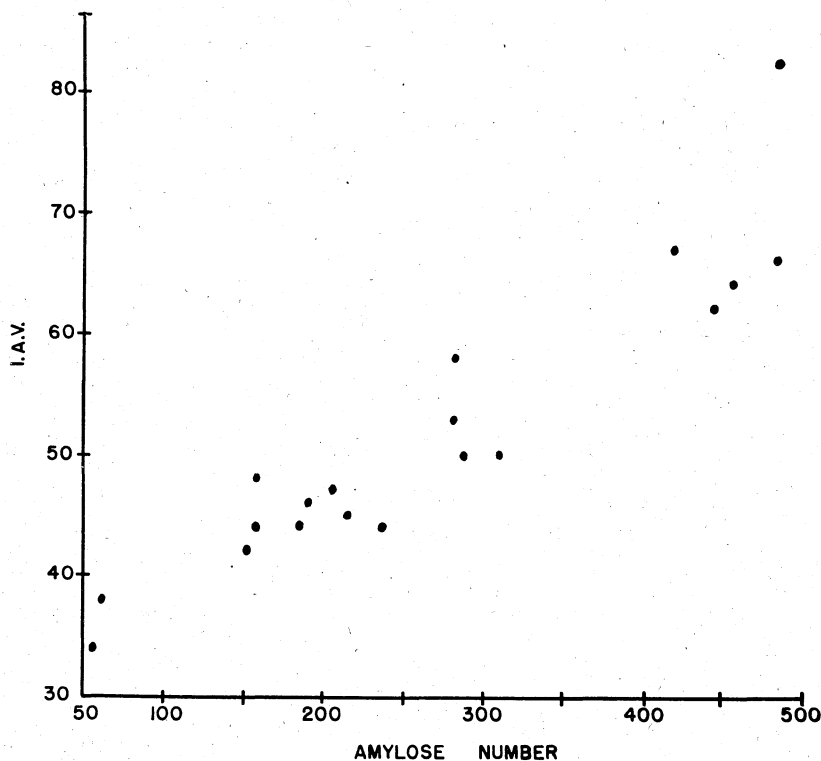
Fig. 4. Scattergram of IAV *vs.* amylose number.

Figures 5 and 6 indicate a more complex relationship between IAV and the values obtained by the two enzymatic procedures. The data fall into two groups, as indicated by the two regression lines (A and B) in each figure. In Fig. 5, line A was calculated from the points representing starch damage values below 10% (determined enzymatically). In Fig. 6, line A was based on points below 8% starch damage (determined enzymatically). Values obtained using the Sandstedt and Mattern procedure are somewhat low, owing to the fact that agitation was not continuous. However, this would not appreciably affect the relative values of the various flours. In both figures, the over-all regression line is indicated by line C. Statistical analysis indicated that the data in both figures are represented significantly better by the regression lines for the grouped data (lines A and B) than by the over-all regression line (line C). The fact that the slope of the regression line increased for the samples with greater starch damage indicated that the enzymatic methods did not respond linearly across the wide range of damage in the samples studied.
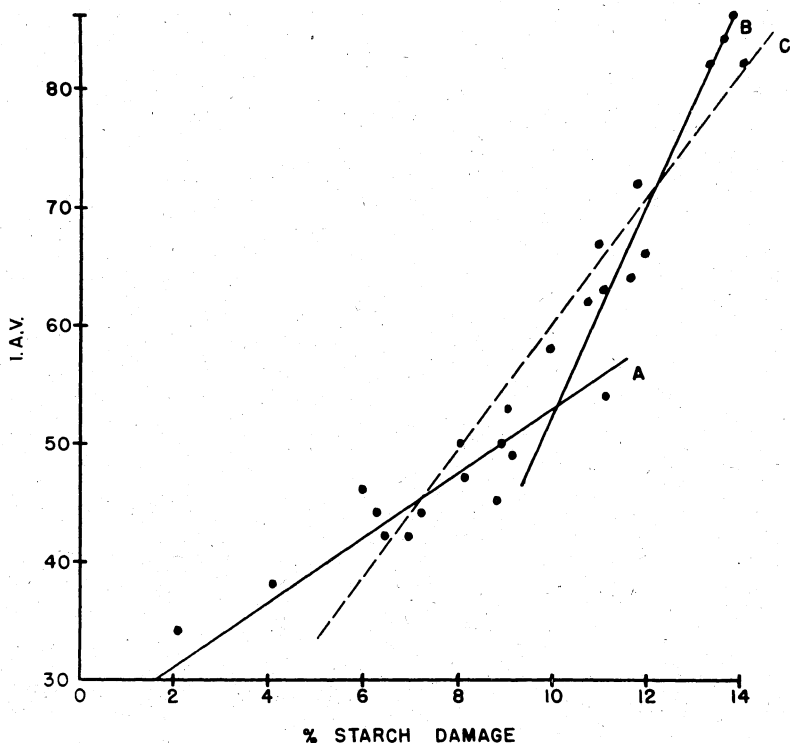
Fig. 5. Scattergram of IAV *vs.* percent starch damage determined by the Donelson-Yamazaki procedure. Line A is the regression line for points below 10% starch damage (enzymatic); line B is the regression line for points above 10% starch damage; line C is the over-all regression line.

Iodine Absorption Values were converted to percent starch damage values, one of the enzymatic methods being used as a standard. The Donelson-Yamazaki procedure was chosen because of its simplicity. Calculation of percent starch damage was based on the following considerations. Using the experimental conditions described, undamaged HRS wheat starch had an average IAV of 40 (14). Assuming flour to contain 70% starch, undamaged flour would have an IAV of 28. A commercial HRS wheat flour was used as a standard. It had 9.0% starch damage (determined by the standard enzymatic method) and an IAV of 50. Since it had already been shown (see above) that IAV's are linearly related to the amount of damaged starch, it followed that 22 IAV units were equal to 9% starch damage. Percent starch damage could then be calculated by the following formula: percent starch damage = 0.41(IAV − 28).
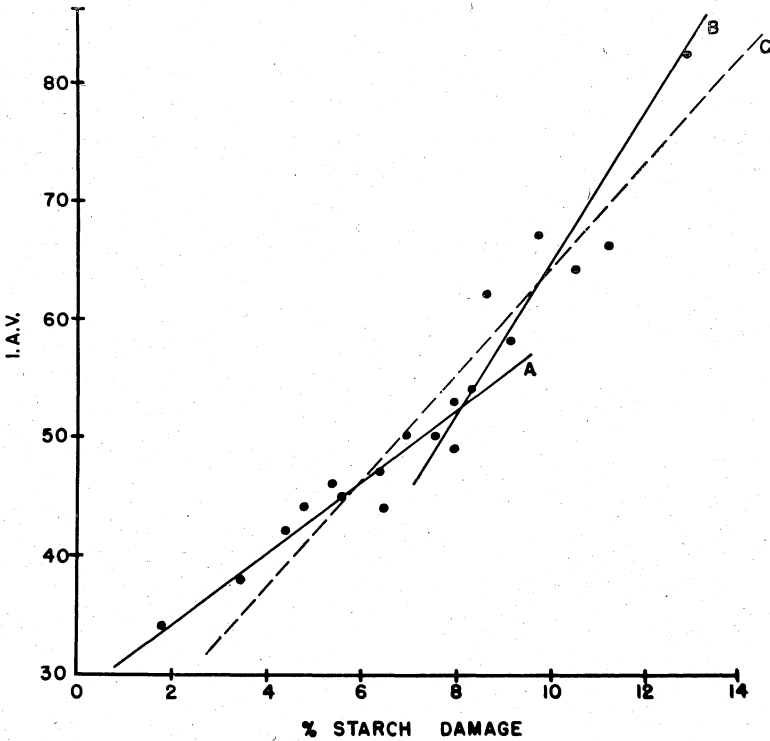
Fig. 6. Scattergram of IAV *vs.* percent starch damage determined by the Sandstedt and Mattern procedure. Line A is the regression line for points below 8% starch damage (enzymatic); line B is the regression line for points above 10% starch damage; line C is the over-all regression line.

The IAV method, like all other starch damage methods, is empirical. Thus, IAV's are not absolute, and depend on the conditions under which they are measured. A different set of electrodes, different stirring speed, etc., would result in a different series of IAV's. However, by use of standard samples of flour and undamaged starch, as well as a standard reference procedure, a formula to convert IAV to percent starch damage can be calculated for each set of conditions. Starch damage values obtained under different conditions should then be directly comparable.

Using the formula described above, IAV's for the flour samples used in this work were converted to percent starch damage. Results are shown in Table II along with the percent starch damage determined by the Donelson and Yamazaki method. As expected, the results agreed reasonably well for values below 10% starch damage (determined enzymatically). This is the range most commonly found

in normal commercial flours. For samples containing more the 10% starch damage, the values obtained by the iodine absorption method are generally higher than the corresponding values obtained by the enzymatic method. Since the IAV method responds linearly over the whole range of starch damages, it was concluded that this method gives a more accurate indication of starch damage when the damage is above 10%. Thus, this method should have particular application where high starch damage is expected, such as in air-classified flours.

TABLE II
COMPARISON OF PERCENT STARCH DAMAGE OBTAINED BY ENZYMATIC AND
IODINE TITRATION TECHNIQUES

FLOUR SAMPLE	PERCENT STARCH DAMAGE	
	From IAV	Enzymatic [a]
Commercially milled		
Soft wheat	4.1	4.1
Hard winter wheat	9.0	8.1
Hard red spring wheat	9.0	9.0
Ball-milled: HRS I, 1 hr.	10.2	9.1
HRS I, 3 hr.	12.3	10.0
HRS I, 5 hr.	13.9	10.8
HRS I, 8 hr.	14.8	11.7
HRS I, 12 hr.	15.6	12.0
HRS II, 4 hr.	14.4	11.1
HRS II, 8 hr.	22.2	13.3
HRS II, 16 hr.	22.9	13.6
Bread flour I	6.6	7.3
Bread flour II	8.6	9.2
High-protein flour I	7.8	8.2
High-protein flour II	7.0	8.9
Laboratory-milled		
HRS (Buhler)	5.8	7.0
Durum (Buhler)	10.7	11.2
HRS (Miag) I	6.6	6.3
HRS (Miag) II	5.7	6.5
Soft wheat (Quadrumat-milled)	2.5	2.1
Air-classified		
2nd Coarse	7.4	6.0
1st Fines (high-protein)	22.2	14.0
2nd Fines (low-protein) I	16.0	11.0
2nd Fines (low-protein) II	18.0	11.8
2nd Fines (low-protein) III	23.8	13.8

[a] Donelson and Yamazaki method (9).

A study was made to determine whether various reagents used to bleach and improve flours would affect the IAV of a commercial HRS flour (IAV = 50). Materials used included two bleaching agents (chlorine gas and benzoyl peroxide), a maturing agent (azodicarbonamide), and potassium bromate. In each case, two concentration levels were used. The higher value generally represented the highest concentration of the reagent which would normally be encountered in com-

mercial practice. In all cases, the IAV of the treated flour was the same as that of the original flour (within the experimental error of the method).

Time required for a single IAV determination is approximately 15 min. The simplest enzymatic procedure requires at least 45 min. before a starch damage value can be obtained. Experience in this laboratory indicates that the IAV method is as fast as the enzymatic procedures, or faster, particularly for a small number of samples. Equipment requirements for the method are modest, and the reagents are simple to prepare and easy to maintain.

Acknowledgment

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