

Starch Damage in Wheat Flours: A Comparison of Enzymatic, Iodometric, and Near-Infrared Reflectance Techniques¹

J. E. MORGAN and P. C. WILLIAMS

ABSTRACT

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Thirty-two flour samples were used to evaluate three methods for determining starch damage: an iodometric method presented by the Tripette/Renaud (Chopin) Company using their SD4 instrument; an enzymatic method marketed by the MegaZyme Company; and a rapid colorimetric method. These methods were compared to the approved AACC method 76-30A and the Farrand method as to their ease of use, precision, and accuracy. For all except the Chopin method, comparative coefficients of determinations (r^2) greater than 0.90 were obtained. For the Chopin method, the r^2 value ranged from 0.78 to 0.82, depending on the method

used for the comparison. When data for soft wheats were excluded, the Chopin method also had r^2 values of greater than 0.90. In a separate study, damaged starch values were determined on 187 flour samples (covering two crop years) by the approved AACC and Farrand methods, giving a coefficient of determination of 0.95. The regression equation generated was used to evaluate near-infrared spectroscopy as another method for starch damage determination, and resulted in a coefficient of determination of 0.90 between the laboratory value and the near-infrared predicted values.

In converting wheat into flour, starch granules are damaged by the pressure and shear forces generated during roller milling (Jones 1940). The extent of damage is directly proportional to the hardness of the kernel, with a correlation coefficient of -0.95 from the particle size index test (Williams et al 1987). Damaged granules differ from sound granules in two important aspects: they are significantly more susceptible to attack by α -amylase and they have an increased ability to bind water (Tipples 1969, Evers and Stevens 1985).

These two altered properties affect dough characteristics and crumb structure of breads and are desirable to a greater or lesser extent, depending on the breadmaking process (Tipples 1969). In flour used for cookies and cakes, where minimal water absorption is required and α -amylase susceptibility is not a factor, minimal starch damage is desirable (Evers and Stevens 1985).

Measurement of damaged starch is of considerable consequence in the milling and baking industries, and there is a need for a rapid and accurate method. Currently accepted methods fall into two categories: enzymatic and iodometric. Enzymatic methods depend on the increased susceptibility of damaged granules to attack by α -amylases, the resultant products being measured volumetrically or spectrophotometrically. Iodometric methods depend

on the increased reactivity of damaged granules with iodine, the reaction being measured amperometrically or colorimetrically.

As new methods for the measurement of damaged starch are developed with the aim of increasing the speed and convenience and decreasing the cost per test, they need to be evaluated against currently accepted methods (Williams and LeSeelleur 1970, Dodds 1971, Tara and Bains 1972). We have compared an enzymatic method (Gibson et al 1992), an amperometric method, a colorimetric method (Williams and Fegol 1969), and near-infrared (NIR) spectroscopy to the AACC (1983) and Farrand (1964) methods.

MATERIALS AND METHODS

Flour samples for the five-method comparative study were from 1991 Western Canadian plant breeder lines and included bread wheat and soft wheat flours. For the regression equation between the Farrand and the AACC method and for NIR evaluation, samples were selected from the 1991 and 1992 harvest surveys and cargo composites, and included wheat from all classes grown on the Canadian prairies. All the samples were milled on the Allis-Chalmers laboratory mill using the GRL sifter flow as described by Black et al (1980).

Analyses

The Farrand (1964) and AACC (1983) methods were used as reference methods against which we evaluated an enzymic method

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marketed as a kit by the MegaZyme Co. (Gibson et al 1992), an amperometric method originally described by Medcalf and Gilles (1965) and incorporated by the Chopin Co. into their SD4 instrument, and a rapid colorimetric method described by Williams and Fogel (1969). The NIR spectroscopy method was evaluated separately as an alternative method of use where large numbers of flours are to be analyzed, or when an instant result is required. Damaged starch determinations were made exactly as described in the literature, except that the malt flour originally used in the Farrand method was replaced by fungal α -amylase (Williams and LeSeelleur 1970). For the Chopin SD4 instrument, data were recorded directly from the electronic signal rather than using the conversion program installed by the manufacturer. The conversion program has little bearing on the precision of the method, but it can bias the results, as was reported by Ranhotra et al (1993). For the NIR determinations, samples were scanned using an NIRSystems 6500 spectrophotometer. Diffuse reflectance spectra were recorded as $\text{Log}_{10}1/R$, at 2-nm intervals from 1,100-2,500 nm. Data were manipulated using the Near Infrared Spectral Analysis Software (NSAS) package (version 3.22) provided by NIRSystems (Silver Springs, MD) and GRAMS, a software system marketed by Galactic Industries (Salem, NH).

RESULTS AND DISCUSSION

Five-Method Comparison

Accuracy. Part of our study involved comparing the AACC and Farrand methods to each other for 187 flours. The range of starch damage was 3.8–13.7 AACC units and realized a regression equation of Farrand units = 5.05 AACC units – 15.36 ($r^2 = 0.95$). These results agree with those of Karkalas et al (1992).

The five wet chemistry methods were compared to each other using a set of 32 samples with starch damage ranging from 3.2–10.25 AACC units. The MegaZyme method and the rapid colorimetric method compared as well with the reference methods as the reference methods did with each other. The Chopin method

had significantly lower r^2 values when compared with the reference methods (Table I and Fig. 1). The data were divided into high (7.0–10.25 AACC units) and low (3.25–5.5 AACC units) starch damage ranges. The Chopin method compared as well as the MegaZyme method against the AACC and Farrand tests in the high range but not in the low range. These observations for the Chopin method agree with those reported by Ranhotra et al (1993). The rapid colorimetric method compared well with the Farrand method in the low range, but had a lower r^2 value compared the reference methods in the high range.

Precision. The repeatability of each method was determined by estimating the standard deviation of a single test, using the same check sample for each method, and then calculating the coefficient of variability. The check sample was analyzed in duplicate with each batch of tests, and testing covered a period of several weeks. The precision of all methods was acceptable (Table II); the MegaZyme method had the highest coefficient of variation at 6.0%. This was attributed to the small sample size (100 mg) used in this test.

Convenience. Drawbacks of the AACC and Farrand methods are: 1) the time required for a single determination (50 and 95 min, respectively, from start to finish, Table II); 2) preparing and standardizing the reagents exerts a significant influence on the absolute results, requiring critical attention.

The Chopin method, an amperometric procedure, is clearly the fastest and most convenient of the five wet-chemistry methods tested for a single determination, requiring only 10 min (Table II) to obtain a result. The reagents for this procedure require ~3 hr to prepare and are stable for one week.

The MegaZyme method calls for a preliminary α -amylase treatment of the damaged granules, as do the AACC and Farrand methods, but the released maltodextrins are enzymatically reduced to glucose, which in turn is measured enzymatically. It takes about 1 hr to get a single result by this method, but the reagents are easily prepared and are stable.

TABLE I
Coefficient of Determination Data Between Five Methods^a

	r^2			
	AACC	Farrand	MegaZyme	Chopin
Range 3.25–10.5 AACC Units $N = 32$ Reference AACC				
Farrand	0.962***			
Evaluated				
MegaZyme	0.980***	0.957***		
Chopin	0.781***	0.810***	0.805***	
Rapid colorimetric	0.954***	0.949***	0.945***	0.707***
Range 3.25–5.5 AACC Units $N = 12$ Reference AACC				
Farrand	0.493***			
Evaluated				
MegaZyme	0.712***	0.815***		
Chopin	0.258*	0.287**	0.373**	
Rapid colorimetric	0.605***	0.851***	0.920***	0.507***
Range 7.0–10.25 AACC Units $N = 20$ Reference AACC				
Farrand	0.917***			
Evaluated				
MegaZyme	0.951***	0.929***		
Chopin	0.916***	0.941***	0.918***	
Rapid colorimetric	0.774***	0.797***	0.731***	0.750***

^a* = Not significant; ** = $P < 0.05$; *** = $P < 0.001$.

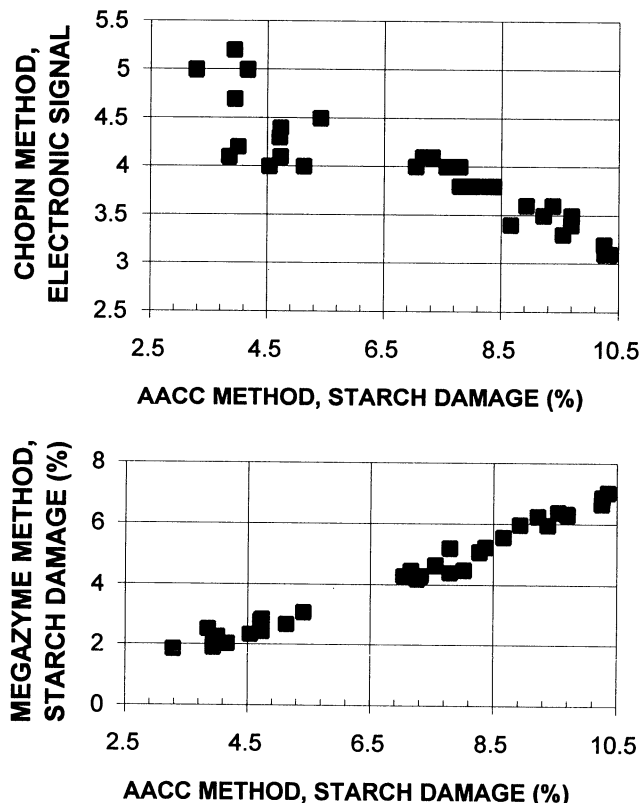


Fig. 1. Relationship between the results obtained using an iodometric method presented by Tripette/Renaud (Chopin) using their SD4 instrument and an enzymatic method marketed by MegaZyme and those obtained using AACC method 76-30A. ($N = 32$)

The rapid colorimetric method is based on the reaction of extracted amylose from damaged granules with iodine to give a violet color which is measured colorimetrically. This test requires extreme precision in the extraction procedure to obtain good reproducibility from day to day. The reagents are easily prepared, and the time required for a single determination would be 0.75 hr.

Where there is a demand for large sample throughput, the operations involved in the MegaZyme method can easily be extended to cover a batch of 12 samples. The iodine reaction of the rapid colorimetric test is readily automated by flow-injection apparatus, making this method suitable for a high volume of tests. Under these circumstances, the Chopin method would be the least convenient of all, as only one test at a time can be performed.

Relationship between the methods. As new methods are developed for the measurement of damaged starch, new units are often introduced. Consequently, when literature values for damaged starch levels are quoted in unfamiliar units, difficulties arise as to the significance of the figures quoted. Until a method becomes established in its own right, its units must be related to those of the more widely accepted methods. To this end, Table III relates the units of the five methods to each other. These results were obtained by means of the appropriate regression equations using the Farrand method as the standard.

NIR Comparison

The NIR method requires that the instrument be calibrated against an external method. Each parameter must first be measured on a set of samples by an independent reference method. In this study, both the AACC and the Farrand methods were used for reference. A sample set ($N = 215$), which had been analyzed for starch damage by the Farrand method, was scanned on the NIRSystems 6500. Using the regression equation generated previously, these results were converted to AACC values. Of these samples, 83 were used to calibrate the instrument, and the remainder were used as a validation set (Fig. 2). These data gave a r^2 of 0.90 for AACC (calc.) vs. AACC (NIR) and $r^2 = 0.92$ for Farrand (lab) vs. Farrand (NIR) with a standard error of prediction (SEP) of 0.615 and 3.00 respectively (Table IV). Using a smaller data set ($N = 52$), which had been analyzed by both the Farrand and the AACC methods, 36 samples were used in a calibration set, and the AACC values predicted on the remainder. Using laboratory-determined AACC values for the validation,

TABLE II
Comparison of Starch Damage Determination Methods
(Analysis Time and Precision)

Procedure	Principle	Analysis Time (min)		Coefficient of Variability (%)
		Single	Batch (12)	
Reference				
AACC	Enzyme/volumetric	50	105	3.41
Farrand	Enzyme/volumetric	95	150	2.4
Evaluated				
MegaZyme	Enzyme/colorimetric	50	85	6
Rapid colorimetric	Iodine/colorimetric	45	65	5.3
Chopin	Amperometric	10	120	2.9
Near-infrared	Spectrographic	0.75	15	1.63

TABLE III
Comparison of Units Used to Express Damaged Starch
by Five Wet-Chemistry Methods

Farrand	Chopin	AACC	Rapid Colorimetric	MegaZyme
5	4.68	4.25	0.09	1.86
10	4.46	5.19	0.15	2.60
20	4.03	7.07	0.27	4.09
30	3.60	8.95	0.39	5.58
40	3.17	10.83	0.51	7.07
50	2.74	12.71	0.63	8.56

we found $r^2 = 0.95$ and $SEP = 0.341$, which is equivalent to about 1.8 Farrand units. When the NIR method for the prediction of AACC starch damage was evaluated using the cross-validation procedure for all 52 samples, $r^2 = 0.85$ and $SEP = 0.533$ AACC units (~2.8 Farrand units) (Table IV). The average standard deviation of a single determination for the AACC method in our laboratory is 0.371 units (about 1.9 Farrand units) for wheats of all classes (CWRS, CPSW, CPSR). NIR precision with a coefficient of variation of 1.63% was better than that of the reference method (Table II), and the NIR precision of hard wheat fours (1.33%) was slightly better than for soft wheat fours (1.93%). These results are in accordance with those of Osborne and Douglas (1981), who reported $r^2 = 0.90$ and $SEP = 4.2$ Farrand units on Buhler-milled flours. They observed the SEP to be approximately twice the standard error of the Farrand method.

Analysis involving several mathematical treatments revealed that the first derivative of $\log 1/R$ gave the best correlations with optimum segment of 10 wavelength points and gap sizes from 4 to 20 points (Table IV). Our cross-validation procedure was done by two different methods with identical results. One method used the first derivative of $\log 1/R$ signal with segment 4 and a gap of 10 and was done manually using the Autocal program in the NSAS package. The other procedure used first derivative with segment 10 and a gap of 20 and used the PRESS function offered in the Partial Least Squares package of GRAMS/386 software.

One of the features of some NIR instruments is calibration drift, which is a gradual change in the accuracy of a calibration, caused by aging of components, dust accumulation, or other

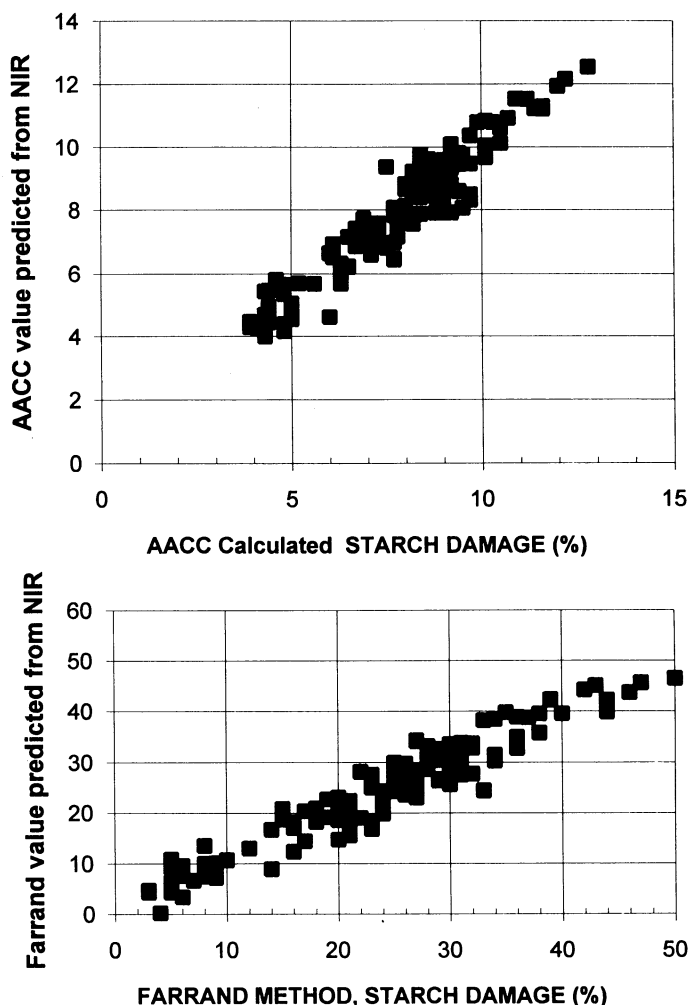


Fig. 2. Relationship between the data for the near-infrared spectroscopic method and the two most widely used methods for measuring starch damage (AACC method 76-30A and the Farrand method).

TABLE IV
Statistical Relationship Between Near-Infrared
and Wet-Chemistry Methods

	Wavelengths Used	r^2	Standard Error of Prediction	d
Data Set 1 ($N = 132$) ^a				
AACC ^b	2,100/2,060	0.9	0.615	0.018
Farrand ^c	2,100/2,060	0.916	3	-0.82
Data Set 2 ($N = 52$) ^d				
AACC MLR ^e	2,120/2,140	0.951	0.341	-0.034
Cross-validation ^f	...	0.852	0.533	0.018
Farrand MLR ^g	1,600/1,420	0.798	1.81	-0.46
Cross-validation ^h	...	0.76	2.38	0.01

^aData set 1: Multiple linear regression on first derivative.

^bSegment 10, gap 4.

^cSegment 10, gap 20.

^dData set 2: Multiple linear regression on first derivative in each case except h.

^eSegment 10, gap 4.

^fManual procedure.

^gSegment 10, gap 20.

^hPRESS partial least squares on first derivative; 5 factors used.

factors. To a great degree, this has been overcome by improvements in instrument electronics and software. It can be controlled by means of periodic check sample analysis, coupled with careful maintenance.

Another characteristic of NIR analysis is the inability of a single calibration set to accommodate samples from a different wheat class, a different crop year, or any circumstance that can affect the sample particle size or shape. Careful monitoring of the system by the established wet-chemistry method will reveal the extent of the condition. It is corrected by introducing samples from the new sample pool into the calibration set. This makes the calibration more robust in that it can now account for variations in the data set that the instrument had not seen before.

CONCLUSION

In our evaluation of the most commonly used methods of testing for damaged starch granules, the enzymatic/colorimetric method marketed by the MegaZyme Company gave the best overall r^2 values when compared to the established reference methods used in the industry today. It lends itself well to either a small or large sample throughput and gives reliable results for both hard and soft wheats. The amperometric method of the Tripette/Renaud (Chopin) Company is suitable for individual samples of hard wheat. For a large number of samples, the method is too slow and inconvenient, and for wheats other than hard wheats, we concluded that the results were unacceptable. The rapid colorimetric method is lacking in accuracy in the high starch damage range compared to the other two methods and offers no advantage in convenience or speed.

The advantages of NIR technology are its speed and convenience, as well as the ability to predict several parameters simultaneously. On the basis of the SEP and standard error of a single test (repeatability), our data showed that the results for starch damage as predicted by NIR can be viewed with as much confidence as the more traditional wet-chemistry methods.

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