

# Detection of Wheat Preharvest Sprouting Using a Pregelatinized Starch Substrate and Centrifugation

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## ABSTRACT

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Wheat sprouting in the field before harvest is a serious negative quality attribute. Even low levels of preharvest sprouting affect the economic value of the grain. Unreleased test lines of wheat should be screened for resistance to preharvest sprouting. However, screening large numbers of test lines is relatively time-consuming or expensive, depending on the existing method used. A new screening test for preharvest sprouting was developed and compared with the viscograph and  $\alpha$ -amylase activity (AAA) methods. The new method used the activity of sprout-related elevation in  $\alpha$ -amylase to partially degrade added pregelatinized starch. The hydrolytic products were centrifuged and the weight of the centrifugate was expressed as a percentage of the original weight of the added pregelatinized starch plus the original meal or flour weight. The result reflected the AAA on pregelatinized starch (AAAPGS) as a

measure of the degree of preharvest sprouting. The AAAPGS test had less standard error and was more sensitive at low levels of preharvest sprouting than the AAA method. Three grinders to produce wheat meal were compared for their effect on AAAPGS values. Flours produced slightly lower AAAPGS values than meals, but the coefficients of variation of each were comparable and both were less than that of the AAA method. The lowest levels of sensitivity to preharvest sprouting that could be detected by the AAA and AAAPGS methods were identified as areas of uncertainty, below which very low levels of preharvest sprouting could not be differentiated from sound, unsprouted background values. The new AAAPGS method was equally rapid and will be more economical than the AAA method or the viscograph when used for preharvest sprouting susceptibility of large numbers of samples.

Procedures for estimating preharvest sprouting damage in wheat and flour include the Falling Number method, the Rapid Visco Analyser method, and the Brabender amylograph-viscograph method (AACC 2000). Those methods use instruments designed to heat wheat meal or flour suspended in water to 92–100°C. That procedure heats starch past its gelatinization temperature and causes the suspension to thicken. Relative to the concentration, the liquefying action of amylolytic enzymes present, particularly  $\alpha$ -amylase, on the swollen, gelatinized starch granules, reduces the viscosity of the suspension compared with the potential viscosity if the enzymes were not present. Preharvest sprouting increases amylolytic activity and reduces the viscosity of heated suspensions of flour in water. The process of heating past the gelatinization temperature of starch with continued stirring in a standardized procedure such as the three instruments identified above is starch pasting. Measurements of the apparent viscosity produced during heating and stirring is pasting viscosity.

An important uncontrolled source of variation inherent in the pasting viscosity methods is a large natural range in the pasting viscosity among sound, nonfield-sprouted samples. For instance, granule bound starch synthase alleles and the resulting amylose-to amylopectin ratio have substantial influence on starch pasting viscosity (Kiribuchi-Otobe et al 1997; Zeng et al 1997; Graybosch et al 2000; Sasaki et al 2000).

It is difficult to directly compare the degree of preharvest sprouting between any two samples (Anker and Geddes 1944). A more direct method of determining  $\alpha$ -amylase activity (AAA) is to supply the enzyme with a known, standardized substrate. AACC Approved Methods 22-05 and 22-06 use this principal, successfully using cibacron-blue amylose tablets or the equivalent to predict AAA.

A modification of the amylograph Approved Method 22-12 (Ranum et al 1978; Perten 1984) uses a fungal source of  $\alpha$ -amylase added

to a 70:30 blend of pregelatinized starch and flour to evaluate the relative amylase enzyme activity of flour. Greater fungal enzyme activity is inversely proportional to pasting viscosity.

The success of the fungal  $\alpha$ -amylase method suggested that a rapid and inexpensive method for detecting preharvest sprouting could be developed that is not influenced by inherently variable and time-consuming pasting viscosity evaluation. Preharvest sprout-generated AAA of meals or flours could hydrolyze pregelatinized starch in excess water during an incubation period. Increased amylolytic activity from preharvest sprouting would greatly increase the hydrolysis of starch, which could be poured off after centrifugation, causing a reduction in the weight of the centrifugate. We wished to develop a method for large wheat-screening programs that uses a small amount of sample and has fewer associated expenses, requiring only a buffer, a balance, and a centrifuge and tubes. The procedure is a microtest, requiring only 0.3 g of sample and 0.2 g of pregelatinized starch. In addition, the method is relatively free of many of the problems associated with the measurement of the starch pasting properties of flours and meals.

## MATERIALS AND METHODS

### Wheat and Flour Samples

Wheat used to determine standard errors for meals consisted of 10–15 samples each of 12 soft wheat cultivars ( $n = 167$ ). Cultivars used were Argee, Arthur, Augusta, Blackhawk, Caldwell, Fillmore, Freedom, Geneva, Hillsdale, Pioneer 2555, Pioneer 2550, and McNair 1003. Various flours used to determine standard errors were derived from milling 158 soft red and white wheats and 32 hard red winter wheats retrieved from the sample library of the Soft What Quality Laboratory at The Ohio State University (Wooster, OH). Three wheats were each obtained as sets of sound (0% sprout) and fully sprouted (100% sprout) and were used in blending studies. Samples of 11 soft red winter wheat cultivars (Argee, Arthur, Augusta, Blackhawk, Caldwell, Fillmore, Freedom, Hillsdale, Pioneer 2555, Pioneer 2550, and McNair 1003) and one white winter wheat cultivar (Geneva) were used to compare the effects of three grinders to prepare wheat meals.

### Production of Flours, Meals, and Starch

Straight-grade flours were produced using either Quadrumat Jr. experimental mill Approved Method 26-50 (AACC 2000) or Allis-Chalmers experimental mill Approved Method 26-32. A few samples were hard red winter wheat flour of unknown cultivar

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and milling. Whole kernel meals were produced using a CRC micro-mill (Chemical Rubber Company, Cleveland, OH), except for the grinder type evaluation, which used a Falling Number grinder (Perten Instruments, IL) and a Udy cyclone grinder (Udy Corp., Rome, CT) using 1.0-mm screen size. Pregelatinized starch (PreGel 10) was obtained from Midwest Grain Products, Atchison, KS. Flour, meal, and starch moisture contents were determined using Approved Method 46-16.

**α-Amylase Activity on Pregelatinized Starch Procedure**

Equipment and calculations were those used in Approved Method 56-10 for determining alkaline water retention capacity in flour. The suspension medium was an acetic acid and sodium acetate buffer solution at pH 4.7 ± 0.1. Buffer was prepared by diluting 3 mL of glacial acetic acid and 4.1 g of anhydrous sodium acetate to 1L with deionized water. The method was convenient to assay the number of samples that would fill most typical centrifuge heads (16–24 at a time).

Glass centrifuge tubes (15 mL) and the stopper were weighed. Pregelatinized starch (0.200 g, 14% mb) and flour or meal (0.300 g, 14% mb) were weighed into the tubes. The starch and flour or meal were blended by carefully rotating the tube. Acetic acid and sodium acetate buffer (5 mL) was added and the tube was stoppered and thoroughly shaken to suspend the sample. Tubes were placed in a 30°C incubator for 45 min. Immediately after incubating, the tubes were remixed by inversion and placed into the centrifuge head. They were centrifuged at 1,000 × g for 15 min. The centrifuge was allowed to stop without breaking. Immediately, the supernatant was poured off and the tube was drained at a 45° angle for 5 min. The mouth of the tube was blotted with tissue paper and the tube was inverted to 90° and drained an additional 5 min. Immediately after blotting, the tube, centrifugate, and stopper were weighed. Calculation of AAA on pregelatinized starch (AAAPGS) was

$$\% \text{ AAAPGS} = \left[ \frac{(\text{tube, stopper, gel wt}) - (\text{tube, stopper wt})}{\text{sample wt}} \right] \times \left[ \frac{86}{100 - \text{flour moisture}} \right] - 1 \times 100$$

No chemical incubation stop is employed; therefore, procedural timing steps of the method are important, but timing deviations of less than a minute appear to be tolerable.

**Reference (Colorimetric) AAA**

Approved Method 22-06 (AACC 2000) assays AAA on an amylose substrate bound to a blue dye. The method uses an incubation period of 60°C for 5 min, during which the activity of the α-amylase hydrolyzes the amylose substrate, liberating soluble blue color that is read spectrophotometrically at 620 nm. Absorbance values were used for comparisons with sample values derived from the AAAPGS method. Both methods are relatively free of problems and variance associated with methods used to determine starch pasting viscosity.

**Water Solubles Recovery**

During evaluation of the AAAPGS method, the solubles content of the supernatant was determined. The supernatants were transferred to preweighed 10-mL beakers and were evaporated to dry at 120°C for 2 hr. After cooling, weight of solubles was determined.

**Statistical Analysis**

All data were duplicated and were analyzed by Statistica99 for Windows PC (StatSoft, Tulsa, OK). Regression analysis was accomplished using a simple least squares model. Where required, analysis of variance, pooled standard deviations, and coefficients of variation were determined. Differences among multiple means were tested using Duncan’s multiple range test (*P* < 0.05). Simple linear correlation coefficients were determined when appropriate. Spearman rank order correlations were determined for nonparametric analyses as appropriate to compare ranking abilities of the grinders.

**AAAPGS Method Sensitivity to AAA**

Preliminary studies evaluated starch substrate concentrations of 2, 3, and 4 g; incubation times of 20, 30, 45, and 60 min; and incubation temperatures of 25 and 30°C. After preliminary investigations, two combinations of incubation time and temperature were evaluated further. AAAPGS method one employed a 20-min hydration times at 25°C and method two employed a 45-min hydration time at 30°C. The sensitivities of the two methods were compared using samples that were blended to have various low percentages of 100% sprouted meal. The relative levels of sprouting were also determined using viscograph peak viscosity and AAA (Table I). AAAPGS method one was not sufficiently sensitive to low levels of sprout, whereas AAAPGS method two responded to low levels. For sprouted flour at ≤10%, the correlation coefficient between AAAPGS (method two) and percentage of sprout in the meal blend was -0.99 (*P* < 0.01) and the correlation between AAPGS (method two) and viscograph peak viscosity was -0.94 (*P* < 0.01). AAAPGS (method two) had a 0.97 correlation coefficient with AAA. Results from method one were not statistically correlated with the other parameters for sprouted meal at ≤10%. Method two was selected for subsequent studies. The authors have studied four commercial pregelatinized starches, all of which have been acceptable substrates for the AAAPGS method. However, like other empirical sprout-detection laboratory methods, we recommend use of an internal standard meal or flour and a control pregelatinized starch.

**Sprouted Flour Concentration Series**

Another highly sprouted meal was blended from 0–100% with a nonsprouted meal (Table II). Gravimetrically determined water solubles increased, AAAPGS values decreased, and AAA values increased when only 1% of the sprouted flour was substituted for sound flour. Even though a direct analysis of the amount of water solubles released by α-amylase is a more direct analysis of AAA, the additional steps used by the procedure for the analysis of water solubles is impractical for screening evaluations of many samples. Compared with zero added sprouted meal, the substitution of 1% sprouted meal increased AAA by 2.2%, increased the water solubles content by 4.3%, and decreased the AAAPGS by 4.6%,

**TABLE I**  
Effect of Two Incubation Protocols (Methods One and Two) on Sensitivity of α-Amylase Activity (AAA) on Pregelatinized Starch (AAAPGS) Values Compared with Viscograph Peak Viscosity and AA<sup>a</sup>

Sprout Level (%)	Viscograph Peak Viscosity (BU)	AAA (abs)	AAAPGS (%)	
			Method One	Method Two
0	867	0.067	299	232
1	793	0.071	298	229
3	708	0.089	301	224
5	638	0.091	299	220
10	528	0.117	295	198
100	150	0.675	186	91

<sup>a</sup> Data are means of two wheats. CRC micro-mill grinder.

**TABLE II**  
Water Solubles, Relative Resistance, and α-Amylase Activity (AAA) of Blends of Sprouted and Nonsprouted Meals

Sprouted Wheat (%)	Water Solubles (mg)	AAAPGS <sup>a</sup> (%)	AAA (abs)
0	70	239	0.093
1	73	228	0.095
5	78	208	0.133
10	87	191	0.185
25	107	145	0.256
100	141	97	0.787

<sup>a</sup> α-Amylase activity on pregelatinized starch. CRC micro-mill grinder.

**TABLE III**  
Mean, Standard Deviation, and Coefficient of Variation (CV) for Replicate AAAPGS<sup>a</sup> Results from Three Laboratory Grinders

Source	AAA (abs)	Falling Number Grinder (%)	Udy Cyclone Grinder (%)	CRC Micromill (%)
Mean AAAPGS	0.09	249a	216c	234b
Replicate pooled CV	8.0%	2.2%	3.4%	2.8%

<sup>a</sup> AAAPGS =  $\alpha$ -amylase activity on pregelatinized starch. Values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ).

**TABLE IV**  
Mean  $\alpha$ -Amylase Activity and AAAPGS Values of Meals Produced by Three Grinders<sup>a</sup>

$\alpha$ -Amylase Activity		Falling Number		Udy Cyclone		CRC Micromill	
Cultivar Rank	A <sub>620</sub> Mean	Cultivar Rank	AAAPGS Mean	Cultivar Rank	AAAPGS Mean	Cultivar Rank	AAAPGS Mean
Blackhawk	0.119a	Blackhawk	225a	Blackhawk	196a	Blackhawk	207a
Geneva	0.111ab	Argee	235ab	Argee	199ab	Argee	215ab
Arthur	0.095bc	Caldwell	246bc	Caldwell	203abc	Arthur	222bc
Argee	0.090c	Arthur	247bc	Arthur	211b-d	Geneva	230b-d
Augusta	0.089c	Freedom	249c	Geneva	214c-e	Augusta	230cd
Hillsdale	0.087c	Hillsdale	250c	Freedom	215c-e	McNair 1003	237c-e
Pioneer 2555	0.087c	McNair 1003	250c	McNair 1003	215c-e	Freedom	237c-e
Caldwell	0.087c	Geneva	254c	Fillmore	222d-f	Caldwell	239de
Freedom	0.084c	Fillmore	255c	Hillsdale	223d-f	Pioneer 2555	241de
Pioneer 2550	0.084c	Augusta	255c	Pioneer 2550	226d-f	Pioneer 2550	243de
McNair 1003	0.082c	Pioneer 2555	256c	Pioneer 2555	229ef	Fillmore	246e
Fillmore	0.082c	Pioneer 2550	258c	Augusta	231f	Hillsdale	249e
Mean	0.090	...	249	...	216	...	234
Pooled standard deviation	0.0085	...	5.5	...	6.2	...	6.2
Coefficient of variance	9.5%	...	2.2%	...	2.9%	...	2.7%

<sup>a</sup> AAAPGS =  $\alpha$ -amylase activity on pregelatinized starch. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

which suggests that the AAAPGS procedure may be more sensitive to very low levels of preharvest sprouting.

### Grinder Type and Meal Particle Size

Three common sample grinders were evaluated for their effects on AAAPGS data. Stock from the Falling Number grinder passes directly through the grinder without restriction. Stock from the Udy cyclone grinder must be reduced in particle size until it can exit through a screen. The CRC micromill has a residence chamber in which ground stock does not escape and is variably ground depending on processing time. The Udy cyclone grinder and the CRC micromill feature a substantial degree of regrinding.

Five wheat cultivars (Argee, Augusta, Fillmore, Freedom, and McNair 1003) were ground by the three devices. Those meals were used to produce AAAPGS values as normal, but the buffer was replaced with 0.1N sodium bicarbonate solution, pH 8.5, which is well out of the range of optimum activity for nonstabilized  $\alpha$ -amylase enzyme. AAAPGS mean values were 299, 259, and 280 for the Falling Number grinder, Udy cyclone grinder, and CRC micro-mill, respectively. Regular AAAPGS mean values were 249, 216, and 234 for the Falling Number grinder, Udy cyclone grinder, and CRC micro-mill, respectively (Table III). Coefficients of variation for the three grinders were only about one-third the size of that of the  $\alpha$ -amylase analysis.

Little or no AAA influenced the AAAPGS values at the high pH; therefore, the observed differences in AAAPGS among the three grinders must have resulted from another influence, such as differences in meal particle size. Mean diameter particle size for the three grinders was 323, 102, and 172  $\mu$ m for the Falling Number grinder, Udy cyclone grinder, and CRC micro-mill, respectively. Likely, the larger mean diameter particles produced by the Falling Number grinder entrapped solution after centrifugation, producing higher AAAPGS values.

Employing the Duncan's multiple range test, the three grinders were evaluated for their relative abilities to differentiate among 12 wheat cultivars (Table IV). Echoing lower coefficients of variation (Table III), the AAAPGS of all three grinders better differentiated among the cultivars than did the AAA determinations.

The Udy cyclone grinder and the CRC micromill revealed most differences among the cultivars. Our preference is the CRC micromill, however, any grinder may be useful for preparing samples for AAAPGS analysis if it can produce sufficiently small meal particle size.

### $\alpha$ -Amylase Activity vs. AAAPGS Replicate Error

Replicate pooled standard deviations for AAA were 0.0038 and 0.0050 for 34 flours and meals, respectively, which corresponded to coefficients of variation of 5.3 and 6.1% for flours and meals, respectively. Replicate pooled standard deviations for AAAPGS were 2.56 and 3.68 for flours and meals, respectively, which corresponded to coefficients of variation of 1.2 and 1.7% for flours and meals, respectively.

### AAAPGS Values of Wheat Meals vs. Wheat Flours

Soft wheats ( $n = 43$ ) were evaluated as meals and flours. AAA of meals and flours had a correlation coefficient of 0.91 ( $P < 0.01$ ). The meals and flours had mean AAAPGS values of 231 and 218%, respectively. The AAAPGS of the meals and flours had a correlation coefficient of 0.89 ( $P < 0.01$ ). Between meal AAA and meal AAAPGS the correlation coefficient was  $-0.89$  ( $P < 0.01$ ) and between flour AAA and flour AAAPGS it was also  $-0.89$  ( $P < 0.01$ ).

### AAAPGS Practical Detection Limits vs. $\alpha$ -Amylase Activity

AAAPGS values and AAA log scale values of 190 flours and 167 meals were plotted (data not shown). Cultivars (and flours vs. meals) have slightly different regression slopes and intercepts. That made it impossible to know exactly when a wheat cultivar exceeded its unsprouted, base level for apparent AAA. Those slope and intercept variations may have resulted from natural differences in base level AAA combined with different rate responses to sprout inducing events, but other possible influences include starch structure, lipid content, and total protein. In these studies, AAAPGS values  $>202\%$  and  $\alpha$ -amylase values  $<0.13$  absorbance must be considered to be unsprouted. However, AAAPGS values extended up to 253% and AAA values extended down to 0.07 absorbance, producing an area of uncertainty.

Thus, the area of uncertainty where the AAAPGS and AAA values can fluctuate suggested possible low levels of sprout damage that cannot be measured accurately or suggested possible fluctuations in native base levels of AAA. On the other hand, wheats exhibiting AAAPGS values <202% or  $\alpha$ -amylase values >0.13 absorbance are certainly sprouted. Either of the AAAPGS or the AAA critical detection limits was sufficient for screening test lines of soft wheat for preharvest sprouting resistance because they are practically at the zero level. However, the AAAPGS method appears to be more sensitive at detecting elevated apparent  $\alpha$ -amylase at the lowest detectable levels of activity.

### CONCLUSIONS

Compared to the AAA method, the AAAPGS method produced less replicate error and could detect low levels of preharvest sprouting. Producing meals is quicker than milling and sieving flour; however, the AAAPGS procedure works equally well for both flours and meal. However, the grinder selected for producing the meals should produce a standardized and relatively fine particle size. Both the AAA and the AAAPGS procedures have a threshold of uncertainty at very low levels of sprouting, below which neither method can distinguish AAA from natural base variation in concentrations of the  $\alpha$ -amylase enzyme. For number-intensive evaluation programs, the AAA procedure requires somewhat costly filters and dye-labeled substrate tablets. Depending on the size or number of centrifuges available, our experience is that the AAAPGS procedure is more economical and more reliable than the AAA procedure, and equally rapid.

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