NOTE

Aberrant Falling Numbers of Waxy Wheats Independent of α-Amylase Activity[1]

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Waxy wheat (*Triticum aestivum* L.) is characterized by the production of amylose-free endosperm starch. Waxy wheats were first developed through classical breeding techniques (Nakamura et al 1995) to pyramid, in common genetic backgrounds, null (nonfunctional) alleles at the three *wx* loci. The *wx* loci encode isoforms of the granule-bound starch synthase (GBSS), the enzyme responsible for amylose synthesis. When the three null alleles are present in a common background, no functional GBSS, and hence, no amylose is produced in endosperm cells.

The falling number device often is cited (Halverson and Zeleny 1988, Mailhot and Patton 1988) as a means of assessing α-amylase activity in grain and flour samples. Preharvest sprouting due to late season rains is accompanied by an increase in wheat grain α-amylase activity, with a concomitant loss of processing quality due to partial digestion of starch. Hence, minimum falling number values often are included in specifications for grain and flour shipments in commercial channels. More correctly, falling number is a measure of starch hot paste viscosity and not a direct measure of α-amylase activity. The falling number device actually measures partial degradation of starch by a decrease in starch viscosity while heating to 95°C.

Starch pasting properties of waxy wheats have been assessed by use of the Rapid Visco Analyser (Hayakawa et al 1997, Kiribuchi-Otobe et al 1997). In both investigations, peak viscosities of waxy wheat samples were attained at temperatures ≈10–15°C cooler than wild-type or partial waxy (reduced amylose) wheats. Peak viscosities of the waxy wheat samples varied. Kiribuchi-Otobe et al (1999) demonstrated increased peak viscosity in waxy wheat samples, while Hayakawa et al (1997) found peak viscosities to be lower in waxy wheat samples than in wild-type wheat samples. Despite the discrepancies, it is clear that the loss of amylose from waxy wheats results in a starch with markedly different starch pasting properties. The present investigation was undertaken to determine what effect elimination of starch amylose would have on falling numbers of waxy wheats and to what extent, if any, waxy wheat falling numbers were dependent on α-amylase activities.

MATERIALS AND METHODS

Forty waxy experimental wheat lines derived from the cross BaiHuo/Kanto107 were grown in southern California, south of the town of Brawley, in 1998. These waxy wheat samples were compared to three additional sets of wheat: 1) 10 wild-type hard red spring wheats grown at the same location in southern California; 2) a set of 19 hard red and hard white winter wheats grown in 1998 at Lincoln, Nebraska; and 3) 10 sprout-damaged soft white wheats grown in Ohio in 1998. Grain samples were tempered to 15.2% moisture and milled to flour on a laboratory mill (Quadraplex, Brabender, Hackensack, NJ). Falling numbers were measured on a falling number device (model 1800, Perten Instruments, Reno, NV). Starch pasting properties (time to peak viscosity, peak viscosity, breakdown viscosity, setback viscosity, and final viscosity) were measured using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia). Starch pasting variables were determined using manufacturer supplied software. Experimental conditions in the RVA were: hold 1 min at 50°C, heat to 95°C over a 3-min period, hold at 95°C for 2 min, cool to 50°C over a 5-min period, and hold at 50°C for 1 min. An α-amylase kit (Megazyme, Bray, Ireland) was used to measure α-amylase activities (units/g of flour) in the flours.

Statistical programs and procedures (SAS Institute, Cary, NC) were used for analysis of variance using a completely random design. Mean values for each sample group were computed for each variable and compared using least significant differences ($P = 0.05$).

RESULTS AND DISCUSSION

Average falling number for the 40 waxy wheat samples was 71.2 sec (Table I) with a standard deviation of <2.0 sec. In contrast, the wild-type hard red spring wheats grown in the same California location had an average falling number of 509.1 sec. Mean falling number of the waxy set also was significantly lower than the hard winter wheat set from Lincoln (411.7 sec) and the sprout damaged samples from Ohio (210.4 sec). In contrast, mean α-amylase level of the waxy set was not significantly different from the California-grown hard red spring wheats nor from the hard winter Lincoln-grown wheats. Enzyme levels of these three sample sets were negligible. Mean α-amylase for the sprout-damaged set was significantly (six times) higher than the waxy and sound wheat sample sets.

Starch pasting properties (Table I) of the waxy wheat samples were significantly different from those of both the sound wheat sets and the sprout-damaged group. Peak viscosity was attained at 3.4 min and ≈80°C. In contrast, the remaining sets achieved peak viscosities 5–6 min and 95°C. Mean peak viscosities of the waxy samples did not differ significantly from those of the sound hard red spring wheats from the same California location. Breakdown of the waxy samples was significantly higher than those of the California and Lincoln sound wheats, while both setback and final viscosities were significantly lower than those of the wild-type wheats from California. Waxy wheats had higher mean paste viscosities, greater breakdown, and higher final viscosities when compared to the sprout-damaged set. Setback of waxy and sprout-damaged samples did not differ. Mean peak time of the sprout-damaged set was 5.0 min at 95°C, which was more similar to the sound wild-type wheats than to the waxy wheat samples.

Examination of pasting curves (based on mean values) (Fig. 1) of the four sample sets explains the aberrant falling numbers of waxy wheats. The falling number test subjects a hydrated flour or starch sample to high shear at 95°C and then records the time nec-

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TABLE I
Characteristics of Wheat Flour Samples

Group[a]	*n*	Falling Number (sec)		Peak Time (min)		Peak Viscosity (RVU)		Breakdown (RUV)		Setback (RVU)		Final Viscosity (RVU)		α-Amylase (units/g)	
		Mean	StD	Mean	StD	Mean	StD	Mean	StD	Mean	StD	Mean	StD	Mean	StD
Waxy	40	71.2d[b]	1.9	3.4c	0.2	228.8a	17.9	139.4a	14.6	28.7b	3.8	118.1b	7.3	0.044b	0.01
CAS	10	509.1a	37.2	6.0a	0.03	235.0a	15.4	90.2b	12.6	111.3a	5.5	256.1a	12.3	0.054b	0.01
LNK	19	411.7b	41.0	5.9a	0.2	212.2b	41.0	81.3b	25.8	114.7a	18.1	245.6a	44.8	0.066b	0.02
SD	10	210.4c	49.7	5.0b	0.5	95.0c	42.4	54.9c	22.9	37.0b	23.8	77.0c	45.2	0.330a	0.23

[a] Waxy = waxy wheats, CAS = sound nonwaxy wheats from Brawley, CA; LNK = sound nonwaxy wheats from Lincoln, NE; SD =sprout-damaged wheats from Ohio.
[b] Mean values followed by the same letter did not differ significantly at *P* = 0.05. StD = standard deviation. RVU = Rapid Visco Analyser units.

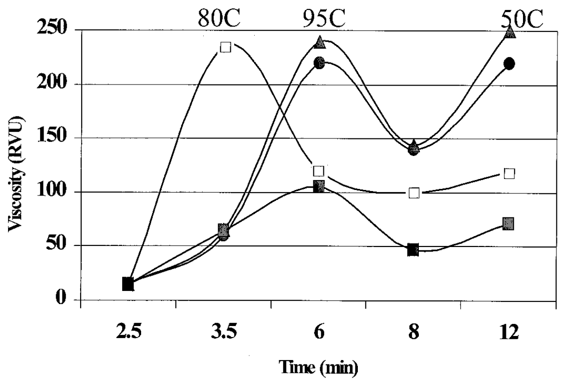

Fig. 1. Rapid Visco Analyser pasting curves (mean values). □ = Waxy wheats from Brawley, CA; ● = LNK nonwaxy wheats from Lincoln, NE; ▲ = CAS nonwaxy wheats from Braweley, CA; ■ = SD sprout-damaged wheats from Ohio.

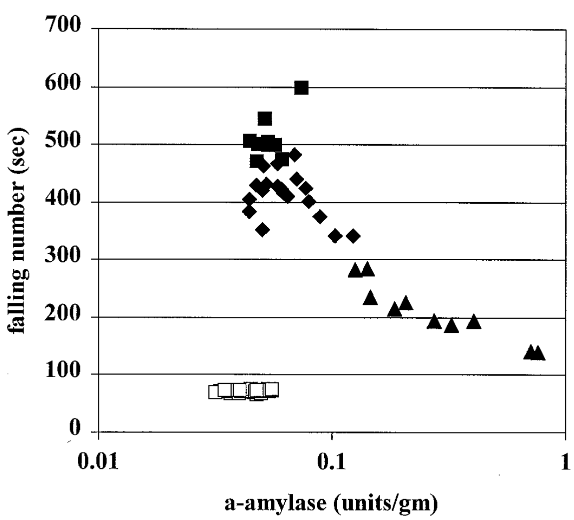

Fig. 2. Relationship between falling number and α-amylase activities. □ = Waxy wheats from Brawley, CA; ◆ = LNK nonwaxy wheats from Lincoln, NE; ■ = CAS nonwaxy wheats from Braweley, CA; ▲ = SD sprout-damaged wheats from Ohio.

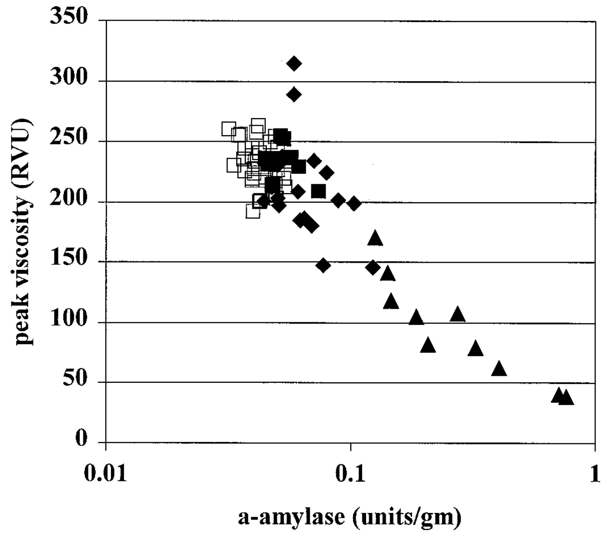

Fig. 3. Relationship between Rapid Visco Analyser peak viscosity and α-amylase activity. □ = Waxy wheats from Brawley, CA; ◆ = LNK nonwaxy wheats from Lincoln, NE; ■ = CAS nonwaxy wheats from Braweley, CA; ▲ = SD sprout-damaged wheats from Ohio.

essary for a metal probe to fall through the starch slurry. In the RVA, waxy wheats under shear attained peak viscosity at 80°C. At 95°C, waxy wheat samples had reached their lowest (breakdown) viscosities, a point at which samples were least resistant to both RVA shear and to the fall of the metal probe in the falling number test. The pasting properties of waxy wheat, therefore, result in extremely low falling numbers despite a lack of significant α-amylase activity.

Sound wheats with low α-amylase levels generally produce high falling numbers. Sprout damage results in increased α-amylase levels and low falling numbers. This relationship holds true for the wheat samples examined in this study (Fig. 2) with the exception of the waxy samples. α-Amylase levels of waxy wheats were identical to sound wheats yet the falling numbers were markedly and uniformly reduced. When sample sets include waxy wheats, RVA peak viscosity (Fig. 3) provides a more accurate assessment of α-amylase levels.

In summary, waxy wheat flours had low falling numbers that were independent of α-amylase levels. This occurred because waxy wheat samples attained peak viscosities at a lower temperature (80°C) than that of typical wheat samples (95°C) and were more susceptible to breakdown under conditions of high temperature and mechanical shear. Should waxy wheat become commercially viable, strict identity preservation after harvest will be required. Otherwise, blending of waxy with nonwaxy flour shipments would diminish the effectiveness of the falling number assay as a means of assessing sprout damage in shipments. Alternatively, the effect of waxy wheat on falling number may actually be beneficial in marketing channels.

Preliminary observations (D. R. Shelton and R. A. Graybosch, *unpublished*) showed waxy wheats have negative effects on bread produced by a 100-g straight-dough procedure. Thus, blending of waxy and nonwaxy wheats must be avoided. In the future, perhaps falling number will not be viewed as an indirect measure of α-amylase level but rather as one indication that a wheat sample has poor potential as a breadmaking flour. This poor potential would be either a consequence of elevated α-amylase levels or the inclusion of too much waxy wheat. If waxy wheats find commercial application, falling number actually may serve as an easy means of enforcing their market segregation.

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