Effect of Phytate, pH, and Acid Treatment on the Falling Number of Sound and Weathered Wheats[1]

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ABSTRACT

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Phytic acid and sodium phytate affected the falling number by altering the slurry pH rather than through chemical interactions. A 25-min pretreatment with 0.14*N* HCl appeared to inactivate the endogenous α-amylases, thereby improving the starch-pasting properties of the weathered samples. However, addition of exogenous α-amylase to the neutralized acid-treated samples reversed this improvement as shown by falling number analysis.

Phytic acid is widely distributed in cereal grains, including wheat, where it exists mainly in the form of calcium-magnesium phytate (Hay 1942). Because of its ability to complex with numerous minerals (Cheryan 1980)—especially calcium, a cofactor required for maximum α-amylase activity—phytic acid has been suspected of being able to inhibit wheat α-amylases (Cawley and Mitchell 1968). However, a study by Sharma et al (1978) indicates that phytic acid's inhibitory property is due to its direct interaction with the enzyme, rather than its complexing with calcium.

The falling number method was developed by Hagberg (1960) and Perten (1964) to evaluate α-amylase activity in cereal flours and wholemeals. Bean et al (1974) examined the effect of various additives on the amylograph viscosities of sprout-damaged wheat used in noodle doughs. Most additives, including sodium phytate, had little effect on the amylograph hot-paste viscosities, as most of the effect was attributable to their effect on pH. In contrast, Westermarck-Rosendahl et al (1980a, 1980b) concluded that the increase in falling number values of sprout-damaged wheat by sodium phytate was not due to pH changes and suggested that phytate might be used as an additive in yeast doughs to improve the bakeability of sprouted wheat.

The present investigation was undertaken to assess the effect of phytic acid and sodium phytate on the falling number of sound and weathered wheats.

MATERIALS AND METHODS

Two hard red spring wheats, Neepawa and Columbus, were used in the study. Columbus possesses good sprouting resistance at harvest. The wheats were field grown at Glenlea, Manitoba, in 1982. Neepawa was harvested at maturity and after several weeks of weathering, whereas Columbus was harvested only at the same weathered stage as Neepawa. After harvest, the wheat heads were air-dried at room temperature (25° C) for 48 hr and threshed, and the grains were milled on a Udy cyclone mill equipped with a 0.1-mm sieve.

Falling numbers were determined on 7.0 g of wholemeal samples and 25.0 ml of distilled water or test solution according to AACC method 56-81B (AACC 1972). Sodium phytate (anhydrous) and phytic acid (40% solution) were purchased from ICN Pharmaceuticals, Inc. Phytate buffers were prepared by mixing 0.2% solutions of sodium phytate and phytic acid to various pH levels. Other buffers were prepared from reagent grade chemicals. A Fisher Accumet pH meter (model 610A) was used on solutions or on slurries immediately before the falling number analysis. All falling number and pH measurements are the mean of two determinations. Standard deviations for falling numbers and pH measurements were ±9.00 and ±0.04, respectively.

Inactivation of α-amylase activity was based on the acidification procedures of Sandstedt et al (1937) and Meredith (1970) with the following modifications. Twelve and one-half milliliters of 0.14*N* HCl solution was added to the 7.0-g wholemeal sample, mixed on a vortex mixer, then incubated for 25 min at 25° C in a shaking waterbath. After the incubation period, 6.25 ml of 0.28*N* NaOH was added to neutralize the acid, followed by the addition of 6.25 ml of distilled water, phytic acid (0.2, 0.4, 0.6%), or sodium phytate (0.6, 1.2%) solutions to bring the total liquid volume to 25.0 ml. The acidified slurries and the added solutions were vigorously shaken by hand before measurement of slurry pH and determination of falling number. α-Amylase (porcine pancreas, type VI-A: approximate activity 18 mg of maltose per 3 min at pH 6.9, at 20° C; from Sigma Chemical Co., St. Louis, MO) was added in powder form either before the acidification step or after the measurement of slurry pH but was mixed thoroughly before the falling number analysis.

RESULTS AND DISCUSSION

pH of Phytic Acid and Sodium Phytate in Solution and Slurries

Table I presents the concentrations and pH values of the phytic acid and sodium phytate solutions and pH values of the corresponding wholemeal slurries for sound Neepawa (S-Np), weathered Neepawa (W-Np), and weathered Columbus (W-Clms). The pH of distilled water was slightly acidic at 5.39, whereas the

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slurries with water ranged from pH 6.01 to 6.25 (Table I). The wholemeal appeared to buffer the sodium phytate solutions to a greater extent than the phytic acid solutions, since the phytate at the same concentration caused a smaller pH shift from the pH value of the water-wholemeal slurry than the acid. The pH of the slurries prepared with the weathered samples and the various test solutions were similar to the values for S-Np but were slightly more acidic at the lower concentrations. The slight acidic nature of the weathered samples may be accounted for by the products of hydrolysis of some grain constituents during weathering.

Effect of Phytic Acid, Sodium Phytate, and Buffer pH on Falling Number

Figure 1 illustrates the changes in falling numbers caused by pH changes with various concentrations of phytic acid and sodium phytate. Falling numbers with distilled water were 507, 165, and 325 for S-Np (D), W-Np (D), and W-Clms (D), respectively. The falling numbers showed a linear decrease from pH 6.25, with decreasing pH for S-Np. With higher sodium phytate, however, the falling number increased to a maximum of 640 at pH 7.07 and then decreased to 435 at pH 8.85.

For W-Np, the falling numbers increased from 165 at pH 6.01 to 365 at pH 3.69 with the addition of phytic acid, then decreased almost linearly with further decrease in pH. This decrease in falling number below pH 4.0 was similar for the sound and weathered

TABLE I
Effect of Phytic Acid and Sodium Phytate Solutions on the Slurry pH for the Three Wheats

	pH			
		Slurry[a]		
Test Solution	Solution	S-Np	W-Np	W-Clms
Distilled water	5.39	6.25	6.01	6.03
Phytic acid (%)[b]				
0.05	2.32	5.24	5.20	5.20
0.1	2.15	4.68	4.55	4.57
0.2	2.03	3.81	3.69	3.68
0.3	1.88	3.06	2.97	2.96
0.4	1.80	2.65	2.59	2.65
0.6	1.66	2.22	2.16	2.17
Sodium phytate (%)[b]				
0.1	10.45	6.80	6.55	6.62
0.2	10.45	7.07	6.99	7.00
0.3	10.46	7.27	7.25	7.21
0.4	10.49	7.69	7.74	7.70
0.5	10.50	7.99	8.08	7.90
0.6	10.52	8.20	8.23	8.22
0.8	10.59	8.85	9.04	8.84

[a]S-Np = sound Neepawa; W-Np = weathered Neepawa; W-Clms = weathered Columbus.
[b]Concentration expressed as percentage of the solution; slurries were prepared with 7.0 g of wholemeal and 25.0 ml of solution.

TABLE II
Effect of Phytate, Tartrate, and Phosphate Buffers at Various pH Levels on the Falling Number for the Three Wheats

		Falling Number[a]		
Buffer pH	Slurry pH[b]	S-Np	W-Np	W-Clms
0.2% phytate				
2.00	3.93	387	369	381
3.00	5.48	484	177	310
5.00	5.87	4.85	179	327
6.00	6.04	543	193	320
9.00	6.76	533	237	378
10.00	7.03	528	257	412
0.1 M tartrate				
2.50	3.07	331	321	310
3.00	3.53	366	370	354
4.00	4.56	428	222	353
5.00	5.56	497	182	327
6.00	5.98	521	185	336
0.1 M phosphate				
6.00	5.96	553	196	353
7.00	6.91	632	333	490
8.00	7.51	498	391	439

[a]S-Np = sound Neepawa; W-Np = weathered Neepawa; W-Clms = weathered Columbus.
[b]Mean pH for the three wheat slurries.

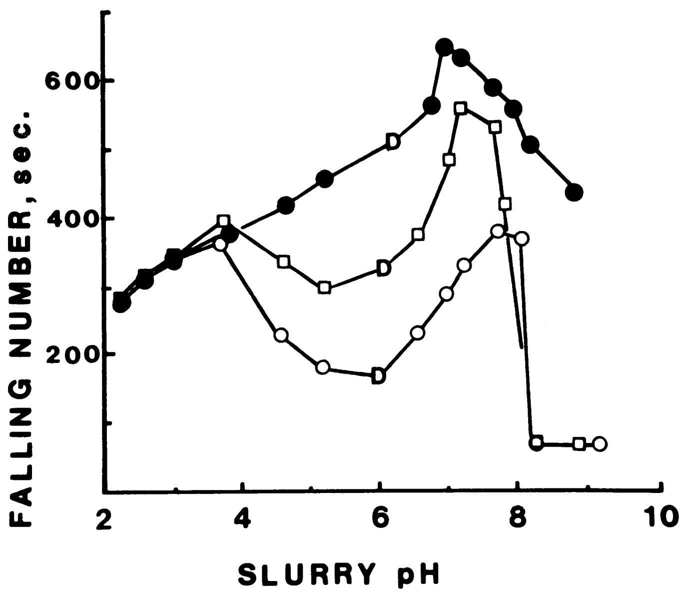

Fig. 1. Falling numbers of wholemeal sound Neepawa (●), weathered Neepawa (O), and weathered Columbus (□) with the various test solutions. Experimental points to the left and right of the distilled water (D) values are in the same sequence as the phytic acid and sodium phytate solutions in Table I, respectively.

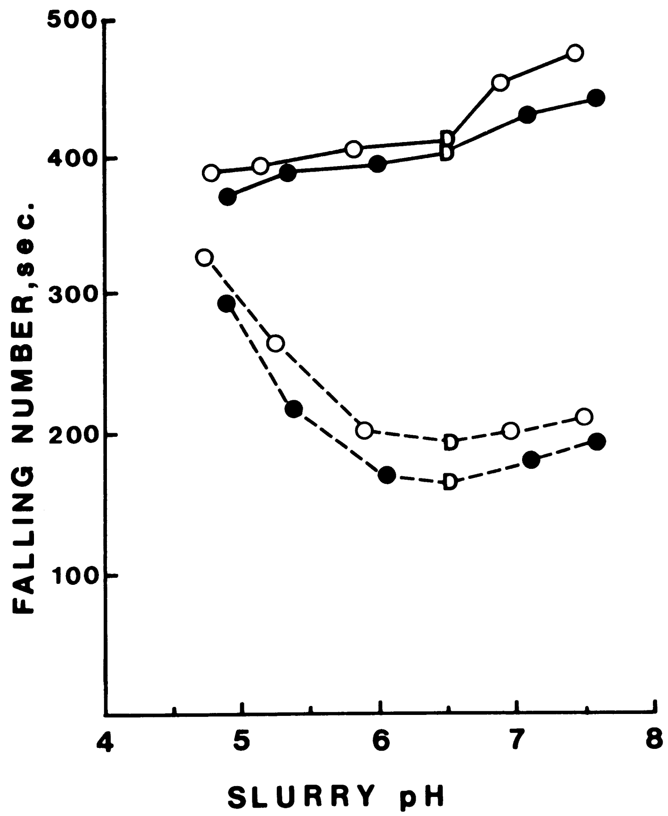

Fig. 2. Effect of pH on the falling number of the neutralized acid-treated sound Neepawa (●) and weathered Neepawa (O) samples with (– – –) and without (—) added α-amylase. D = neutralized acid-treated samples and distilled water.

samples. With addition of sodium phytate, the falling numbers increased with increasing slurry pH up to about pH 8.0 for W-Np, then dramatically decreased to 68 at pH 8.23. The changes in falling number with pH for W-Clms were similar to those for W-Np, but above pH 3.70 the falling number values were between those of S-Np and W-Np.

Table II shows the effect of pH of phytate, tartrate, and phosphate buffers on falling number. With the various buffers, the wholemeals had a buffering character similar to that observed for the phytic acid and sodium phytate solutions. Among the buffers tested, the tartrate and phosphate buffers had falling-number values (at pH 6.0) closest to the values with distilled water. Consequently, these buffers were selected to compare the effects of pH on falling number with the effects observed with the phytate buffers. The effect of pH of the various buffers, including the phytate buffers, on falling number was similar to the effects of phytic acid and sodium phytate solutions.

Neutralized Acid-Treated Samples

For both Neepawa samples the pH of the neutralized slurries was 6.53 (D, Fig. 2), which was somewhat higher than the pH for the untreated samples with water. The higher pH may be due to a lag in the neutralization process since the acid treatment extended over 25 min, whereas the neutralization time with the hydroxide solution was approximately 1 min before the pH measurement.

Falling numbers of the neutralized acid-treated samples exhibited a small but gradual decrease with decreasing slurry pH with addition of phytic acid (Fig. 2). The pattern for falling number vs. slurry pH (Fig. 2) was similar for both neutralized Neepawa samples, although the falling number values for W-Np were slightly higher at all pH values than those for the sound samples. The pattern for W-Clms (not shown) was similar to the pattern for W-Np. Addition of sodium phytate to the neutralized slurries raised the pH and increased the falling number of the three samples. However, the increase in falling number was not as great as that for the untreated samples.

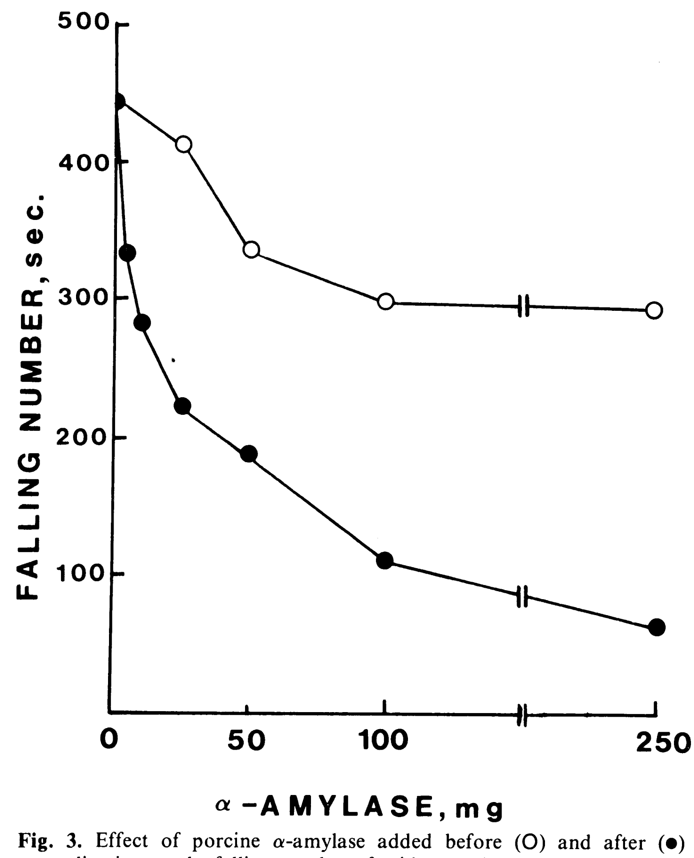

Fig. 3. Effect of porcine α-amylase added before (O) and after (●) neutralization on the falling number of acid-treated weathered Columbus wholemeal.

Neutralized Acid-Treated Samples Plus α-Amylase

Addition of α-amylase to the neutralized samples at various pH levels had a dramatic effect on the falling number (Fig. 2). For example, the falling number of W-Np (D) decreased from 412 to 179 with the addition of 50 mg of powdered porcine α-amylase just before falling number analysis. Similarly, for S-Np (D), the falling number decreased from 406 to 159. Falling number increased dramatically as pH was decreased with phytic acid for the neutralized Neepawa samples with added α-amylase. Addition of sodium phytate increased slurry pH, but the falling numbers increased only somewhat.

The falling number decreased curvilinearly with increasing level of α-amylase added after neutralization (Fig. 3). For example, the falling number decreased from 444 to 181 with addition of 50 mg of the porcine pancreas α-amylase. However, when the same amount of α-amylase was added before acidification (thus exposing the α-amylase to acid), the falling number decreased to only 333.

Effect of Sodium Chloride on Falling Number

Because sodium chloride is a product of the acidification and neutralization steps, the falling numbers of the three untreated samples were determined with 0.07*N* NaCl solutions instead of distilled water. The falling numbers were 524, 173, and 325, respectively, for S-Np and W-Np and W-Clms. Those falling numbers were similar to the values obtained with distilled water (D, Fig. 2). Thus, the sodium chloride had no significant effect on falling number, and this is in agreement with the finding of Meredith (1970).

CONCLUSIONS

Both phytic acid and sodium phytate exert their influence on the starch-pasting properties of wholemeal samples, as determined by the falling number, by altering the slurry pH and not by a chemical action of the phytate molecule. This conclusion is in agreement with the work of Bean et al (1974) on amylograph viscosities of sprout-damaged wheat, but it contradicts the work of Westermarck-Rosendahl et al (1980a), who claim that the effect of phytate was not caused by pH changes. The data also support the complete inactivation of the endogenous α-amylase in the grain by the acid treatment, as reported by Sandstedt et al (1937) and Meredith (1970). In addition, comparison of the falling numbers of the nonacid-treated (D, Fig. 1) with the neutralized acid-treated S-Np (D, Fig. 2) indicates that the acid treatment does alter the pasting properties of wholemeal slurries to some extent.

The acid treatment was able to inhibit porcine α-amylase only partially. Porcine α-amylase, as an additive, has an effect on the falling number similar to the native grain α-amylase; that is, both α-amylases were responsible to a large extent for the lower falling numbers.

It appears that the use of phytate as an α-amylase inhibitor in sprout-damaged wheat is not justified as suggested by Westermarck-Rosendahl et al (1980a, 1980b). The improving effect of phytate on the starch-pasting properties is due to its effect on slurry pH, which may be better achieved through the use of inorganic phosphates (Bean et al 1974, Westermarck-Rosendahl et al 1980b).

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