Polyacrylamide Gel Electrophoresis of Wheat Gliadins: the Effect of Environment and Germination[1]

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ABSTRACT

The effect on the physical and functional properties of wheat seed from plants grown under environmentally stressed and nonstressed conditions were compared to their respective gliadin electrophoregrams. Late spring frosts, which severely affected maturation, caused large changes in the physical and functional properties of the wheat seed and flour. Gliadin electrophoregrams of a given cultivar were identical for severely frosted and nonfrosted samples. The effects of germinating wheat seed for up to 44 hr on the physical and functional properties of wheat seed and flour were also studied. Those effects were noticeable after 20 hr of germination, but gliadin electrophoregrams of those samples were unchanged through the maximum germination time of this study, 44 hr.

The functional properties of wheat proteins have often been used as a measure of protein quality (Finney and Barmore 1945a, b). Gliadin, the major storage protein in wheat, has been studied by polyacrylamide gel electrophoresis (PAGE), but the relationship between reduced protein quality and PAGE patterns has not been reported. The gliadin PAGE patterns (electrophoregrams) have been shown to be independent of the location of growth and nitrogen fertilization level (Zillman and Bushuk 1979). The electrophoregrams depended only on the wheat genotype (Lee and Ronalds 1967; Wrigley 1970, Zillman and Bushuk 1979). The electrophoretic differences found between wheat genotypes have been used to identify wheat cultivars by PAGE of their gliadin proteins (Bushuk and Zillman 1978, Lookhart et al 1982, Wrigley et al 1982).

This study extends published information on wheat proteins by describing the changes found in the physical and functional properties of environmentally stressed wheat samples and compares those properties to their respective electrophoregrams.

MATERIALS AND METHODS

Wheat Cultivar Samples

The wheat cultivars, Vona and Newton (frosted and nonfrosted samples), were obtained from the Kansas State University farms; the Marquis standard, from Dr. W. Bushuk at the University of Manitoba. Protein, moisture, and ash were determined by AACC Approved Methods 46-11, 44-15A, and 08-01, respectively (AACC 1976). The various wheats (100 g) were milled to straight-grade flours using a modified Brabender Senior micromilling system (Finney 1964).

Germination

Four 100-g samples of Newton wheat were germinated at 25°C with distilled water for 0, 20, 34, and 44 hr. The samples were placed in aluminum pans and saturated with an excess of water. The water on the samples was changed every 4 hr for the first 12 hr and every 12 hr thereafter. After germination, the samples were air-dried via a large fan.

The 20-hr germinated material had radicle growth just covering the germ. The 34-hr germinated material showed 1-mm coleoptiles and 2–3-mm radicles. The 44-hr material had 3-mm coleoptiles and 5–6-mm radicles.

Frosted Seed Separation

The frosted Vona sample was separated by visual discrimination into sound (64.2%) and shriveled (35.8%) fractions. The shriveled fraction was further segregated with a Clipper Super 298 D into three portions, scalp (no. 11 screen), screen (3.2 × 12.7 mm), and air blast. The frosted Newton sample was also separated by visual discrimination into sound and shriveled seed fractions.

Mixograph Preparation

Mixograms were determined on flours from various wheat cultivars according to the procedure of Finney and Shogren (1972).

Breadbaking

The breadmaking (straight dough) method included mixing to minimum mobility (optimum) and using 6.05 ml of water (optimum), 50 ppm of ascorbic acid (an excess in the absence of nonfat dry milk), 10 g of flour (14% mb), 0.6 g of sugar, 0.15 g of salt, 0.3 g of shortening, 0.53 ± 0.2 g of compressed yeast, and 0.025 g of barley malt (52 dextrinizing units per gram). Compressed yeast was a 50:50 blend of fresh, weekly shipments from Anheuser-Busch, Inc., and Standard Brands, Inc. Ascorbic acid was its own buffer against over-oxidation (Shogren and Finney 1974). Straight doughs were fermented 52 min to first punch, 77 min to second punch, and 90 min to pan and were proofed 34 ± 2 min (the time required to proof controls to 4.8 cm) at 30°C. They were baked 13 min at 235°C. Loaf volume was determined 2 hr after baking by dwarf rapeseed displacement, and loaf volumes that differed by 3 cc were statistically significant at $P = 0.05$. Additional related details are given by Finney (1945), Finney and Barmore (1943, 1945a,b), and Finney et al (1976).

Electrophoregram Preparation

Wheat samples were ground and extracted according to the procedure of Lookhart et al (1982). The extracts were analyzed with a temperature modification of that procedure: electrophoresis for 7 hr at 10°C, instead of for 5-¼ hr at 20°C. The resulting gels were stained to produce visible bands, which were photographed for hard copy storage (Lookhart el al 1982).

RESULTS AND DISCUSSION

Germination

The rheological and physical properties of the hard red winter wheat, Newton, were affected by germination (Tables I and II). Ungerminated (control) and 20-hr germinated samples showed similar test weight, flour yield, and ash and protein content. Therefore, germination of Newton wheat for up to 20 hr did not deleteriously affect its rheological and physical properties. However, wheat germinated for 34 and 44 hr had reduced rheological and functional properties, which suggests that biochemical and enzymatic changes had occurred in the kernel.

All of the baking quality parameters except loaf volume decreased with increasing germination times (Table II). The loaf volume was larger for the wheat germinated for 20 hr (85 cc) than

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for the control (81.5 cc). That may have been due to the limited proteolysis of a medium strong flour, Newton, making the protein material more easily extendable (mellow) and thereby, increasing loaf volume. However, increased proteolysis (beyond 20 hr) would give peptide fragments and reduce elasticity and loaf volume. The doughs from wheats germinated for 34 and 44 hr had impaired crumb grain and reduced loaf volumes, mixing times, and water absorption. These decreases in the breadbaking parameters are a measure of the detrimental effects of germination on baking quality and are consistent with the effects of germination on physical properties (Table I).

Newton wheat, grown at Manhattan, KS, was germinated for 0, 20, 34, and 44 hr. Mixograms of those germinated samples (Fig. 1) indicated that the mixing time and tolerance to mixing were reduced by increased germination time. The mixing time and tolerance for the 0- and 20-hr germinated samples were within the usable range for baking bread, but the wheat samples germinated for 34 and 44 hr had appreciably reduced mixing times and tolerances. Those mixograph data corroborate the data found and discussed previously for the physical, rheological, and functional properties.

Electrophoregrams of the same set of germinated samples (0, 20, 34, and 44 hr) are shown in Figure 2, with the Marquis standard in slots 1, 4, and 8. Gliadin extracts from two separate ungerminated Newton samples were placed in slots 2 and 3; slots 5, 6, and 7 contained extracts of the 20-, 34-, and 44-hr germinated Newton samples, respectively. The identical band patterns in slots 2, 3, 5, 6, and 7 indicate that gliadin PAGE was not affected by germinating wheat for up to 44 hr. Cultivar identification via gliadin electrophoresis is not affected by up to 44 hr of germination, which is equivalent to severe sprout damage.

Effects of Climatic Changes

Many wheat plants in the Great Plains area were in the bloom stage and adversely affected by a late spring frost May 11, 1981. The official low temperature at Manhattan, KS, that day was 0°C; however, temperature fluctuations are expected from towns to fields and from valleys to hilltops. Frost was reported at the St. John's and Manhattan Agricultural Experiment Stations that morning, and samples of grain from those fields were studied. The

TABLE I
Effects of Germination Time on Physical Properties of Newton Wheat

Germination Time (hr)	Test Weight		Flour		
	(kg/hl)	(lb/bu)	Yield (%)	Ash (%)	Protein (%)
0	77.7	60.4	71.3	0.4	12
20	70.9	55.1	72.7	0.4	12
34	63.3	49.2	69	...[a]	11.6
44	48.9	38	67.2	...[a]	11.4

[a] Less than 0.40%.

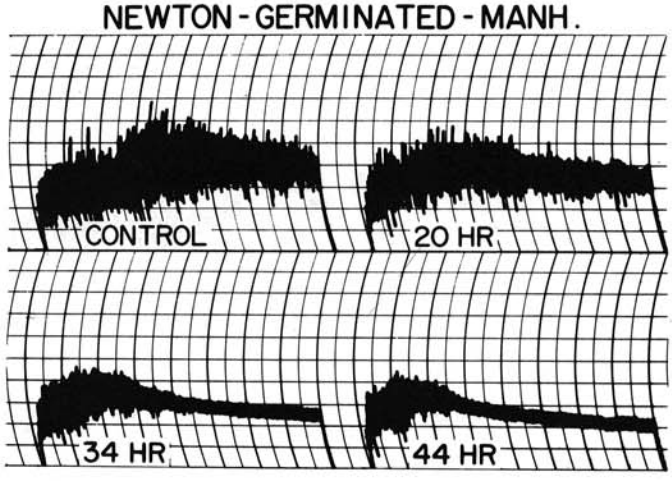

Fig. 1. Mixograms of Newton wheat (harvested at Manhattan, KS) germinated for 0, 20, 34, and 44 hr.

TABLE II
Effects of Germination Time on Functional Properties of Newton Wheat Flour

Germination Time (hr)	Baking		Loaf	
	Absorption (%)	Mix Time (min)	Volume (cc)[a]	Crumb Grain[b]
0	59.5	4.13	81.5	S
20	57.7	2.63	85	S
34	55.1	1.63	76	U
44	52.9	1.25	67.8	U

[a] Average of duplicate measurements.
[b] S = satisfactory, U = unsatisfactory.

TABLE III
Effects of Late Spring Frost on Physical Properties of Vona and Newton Wheats Grown at Manhattan, KS, and St. John, KS, Respectively, in 1981

Frosted Wheat Fractions	Test Weight		Flour		
	(kg/hl)	(lb/bu)	Yield (%)	Ash (%)	Protein (%)
Vona[a]					
Good seed	78.6	61.1	70.5	0.42	14.6
Scalp	58	45.1	35.5	0.69	15.3
Screen	68.6	53.3	47.7	0.51	13.7
Air-blast	61.6	47.9	34.6	0.67	15.4
Newton[b]					
Good seed	76.4	59.4	69.7	0.46	13.5
Poor seed	61.5	47.8	47.8	0.63	15.1

[a] From Manhattan.
[b] From St. John.

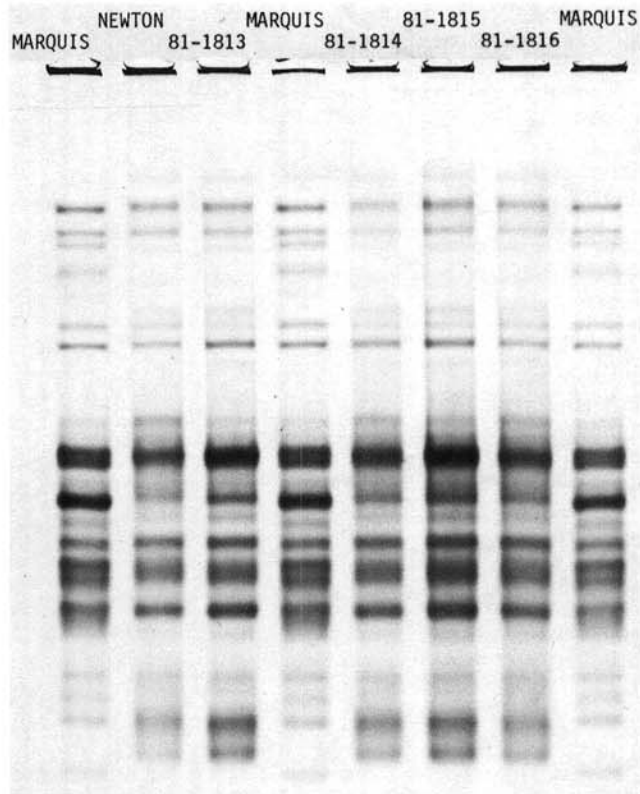

Fig. 2. Electrophoregrams of gliadin extracts of a Newton standard and of Newton samples germinated for 0, 20, 34, and 44 hr are in slots 2, 3, 5, 6, and 7, respectively, and extracts of standard Marquis are in slots 1, 4, and 8.

physical, rheological, and functional properties of the kernels and their flours from the affected wheat plants were drastically changed (Tables III and IV). The physical properties, test weight, flour yield, and ash values, were completely unacceptable for the poor seed fractions but were very acceptable for the good seed fractions. The flour yield data followed the test weight as expected. Those changes in physical properties were due to substantial changes in the overall protein and grain characteristics. The baking functional properties confirmed effects of the late frost. The poor seed of both varieties had increased water absorption and decreased mixing time and loaf volume. Those decreases indicated overall changes in protein functionality. The good seed of both varieties had acceptable functional properties, which indicated that the protein had not been adversely affected.

Mixograms of the good and poor seed fractions of Vona and Newton (Fig. 3) corroborated the physical and functional property changes. Reduced protein quality was found in the poor seed, as shown by the shorter mixing time and less tolerance to overmixing than that of the good seed. A comparison of the mixograms of good seed from Newton in Figure 3 with the mixograms of the 0- and 20-hr germinated Newton samples in Figure 1 indicated that some

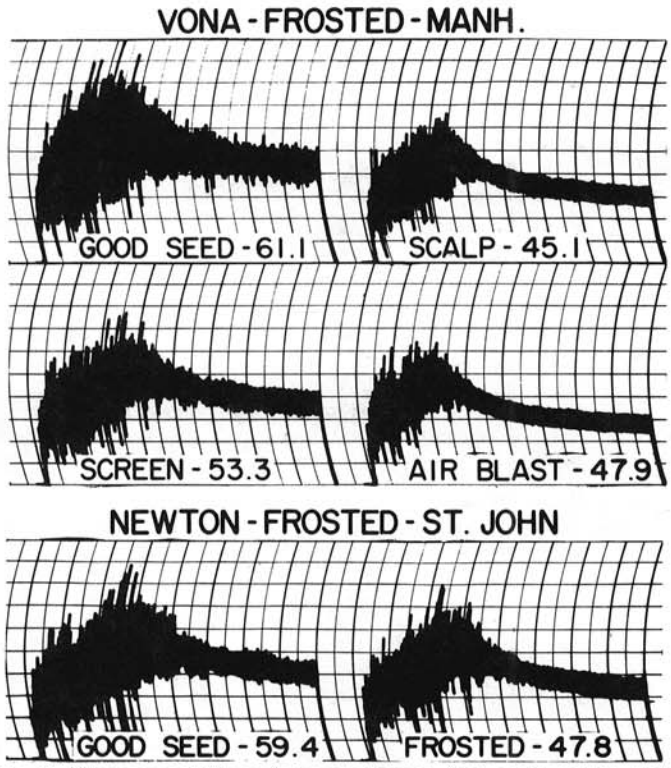

Fig. 3. Mixograms of flours from good Vona, from scalp, screen, and air-blast seed fractions of frosted Vona, and from good Newton and frosted Newton. Vona and Newton were harvested at Manhattan, KS, and St. John, KS, respectively. Values under curves are test weights.

TABLE IV
Effects of Late Spring Frost on Functional Properties of Wheat Flour from Vona and Newton Varieties[a]

Frosted Wheat Fractions	Baking		Loaf	
	Absorption (%)	Mix Time (min)	Volume (cc)[b]	Crumb Grain[c]
Vona-M				
Good seed	63.4	2.88	90	Q-S
Scalp	67	2.25	56	U
Screen	64	2.63	75	Q-U
Air-blast	66.2	2.25	53.8	U
Newton-SJ				
Good seed	63.1	3.38	84.3	S
Poor seed	66.3	2.88	73.5	U

[a] Grown at Manhattan and St. John, KS, respectively, in 1981.
[b] Average of duplicate measurements.
[c] S = satisfactory; Q = questionable; U = unsatisfactory.

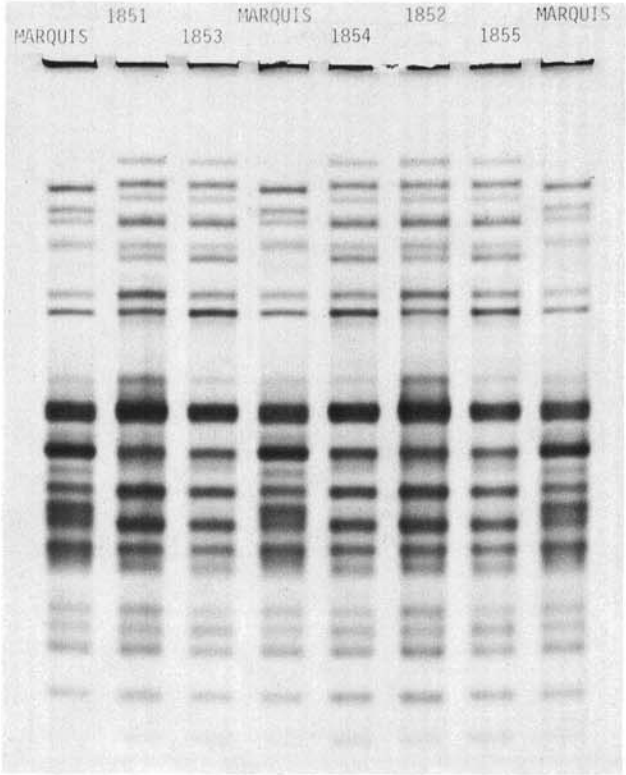

Fig. 4. Electrophoregrams of gliadin extracts of the original Vona, Vona scalp, screen, good seed, and air-blast fractions are in slots 2, 3, 5, 6, and 7, respectively. Slots 1, 4, and 8 contain extracts of the Marquis standard.

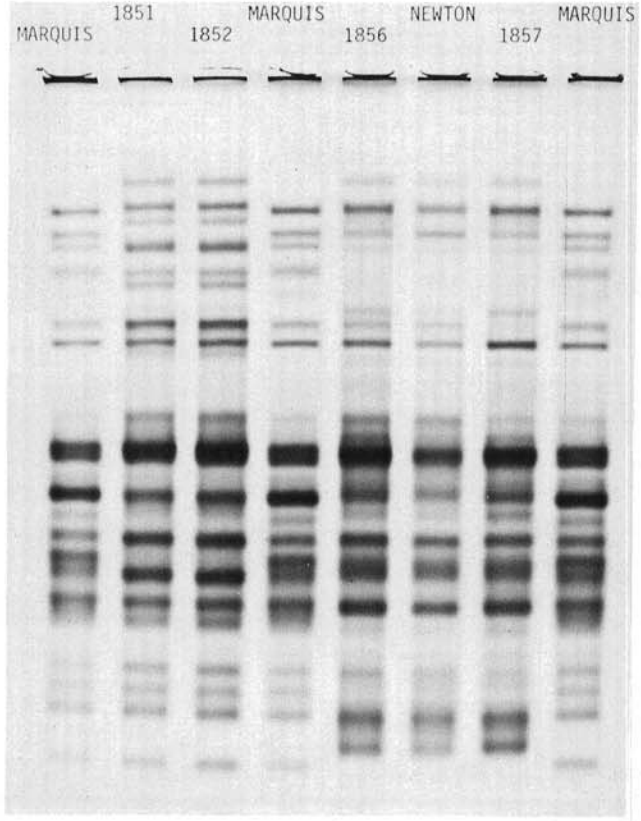

Fig. 5. Electrophoregrams of gliadin extracts of Marquis are in slots 1, 4, and 8, and original Vona, good Vona, good Newton, a standard Newton, and poor Newton seed fractions are in slots 2, 3, 5, 6, and 7, respectively.

protein changes had occurred to the good seed of Figure 3, because the mixing time and tolerance were more similar to those of the 20-hr than the 0-hr germinated material of Figure 1. Therefore, visual discrimination cannot be used as an absolute measure of damage.

Good and poor seed fractions from Vona and Newton wheats were subjected to PAGE, and the results are shown in Figures 4 and 5, respectively. Slots 1, 4, and 8 contained the Marquis standard. Slots 2, 3, 5, 6, and 7 (Fig. 4) contained gliadin extracts of the original Vona sample, the scalp, screen, good seed, and air blast fractions, respectively. Electrophoregrams of all fractions were identical. Samples with identical patterns have the same genetic background or are very closely related (Jones et al 1982). The identical nature of the patterns, therefore, nearly preclude the possibility of a mixture giving rise to the fractions. The adverse weather that caused the differences in kernel shapes did not alter the gliadin electrophoregrams. In Figure 5, slots 2, 3, 5, 6, and 7 contained gliadin extracts of the original Vona, good Vona seed, good Newton seed, a Newton standard, and the poor Newton seed, respectively. The Vona seed extracts were identical electrophoretically, as were the Newton extracts. The electrophoregrams depended on genetic background but were independent of adverse weather conditions that had caused substantial protein changes as shown by mixograph studies and physical, rheological, and baking functional properties. Thus, wheat quality is not related to the gliadin electrophoregrams, except as the patterns are used to identify a cultivar.

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