

ZONE ELECTROPHORESIS PATTERN OF FREE AMINO ACIDS AS AN INDEX OF STORAGE CONDITION OF WHEAT¹

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ABSTRACT

Paper electrophoresis of free amino acids provided a rapid, simple, and convenient method to detect changes in the free amino acids of wheat during storage. Using 0.025M potassium-hydrogen-phthalate solution (pH 4.0) as buffer, a good resolution of aspartic, glutamic, gamma-aminobutyric, and neutral amino acids (as a group), as well as arginine and lysine, was obtained with 400 volts (14.0 V/cm.) during 90 minutes. For practical purposes 500 volts (18.5 V/cm.) and 30 minutes gave a satisfactory separation, yet no cooling was necessary.

The electrophoresis patterns of free amino acids of wheats of good quality and high viability were characterized by a high glutamic acid peak, an average ratio of glutamic acid/aspartic acid peak height over 2, and a negligible gamma-aminobutyric acid peak. Glutamic acid peak and glutamic acid/aspartic acid peak height ratio decreased as deterioration increased. A marked gamma-aminobutyric acid peak was present without exception in electrophoresis patterns of wheats stored at unfavorable conditions. Dead wheats of extensive deterioration exhibited an increase in most of the free amino acids, including glutamic acid, largely due to protein breakdown.

Glutamic acid peak increased markedly in germinated wheats, with moderate increases in aspartic and basic amino acids and little increase in gamma-aminobutyric acid.

Although the total quantity of free amino acids in stored wheat has been reported to be affected little during the early stages of deterioration (10), Jones and Gersdorff (4) observed that prolonged storage of wheat and milled wheat products decreases protein nitrogen and increases free amino acid nitrogen. DeVay (3) observed changes in the concentrations of certain individual free amino acids in hard red spring wheat (Lee variety) during storage at 19.5% moisture. The most notable change was the appearance of gamma-aminobutyric acid in moldy moist wheat. Linko and Milner (7) showed recently that a considerable change in the composition of the free amino acids of wheat may take place immediately following wetting. Linko (5) studied the effect of storage at 16.2% moisture at 37°C. on the individual free amino acids of Seneca wheat, analyzing the embryo and the endosperm end separately. The most significant change during the first two days of storage was the almost total and irreversible loss of free glutamic acid of the embryo, accompanied

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by as striking an increase in free gamma-aminobutyric acid, evidently due to the activation of glutamic acid decarboxylase by the increased moisture content (1,8). Except for arginine, glutamic acid, and the amides, free amino acids in the endosperm end of the kernel generally increased as a result of proteolysis.

Hence, though the total quantity of free amino acids exhibits a significant increase only at advanced stages of deterioration, it was thought that methods to detect changes in individual amino acids could prove valuable in estimating the degree of deterioration. Paper chromatography of free amino acids showed some promise in this respect (6), but it is relatively time-consuming. Paper electrophoresis with high voltage gradients would not have that disadvantage. Hence it appeared to provide a simple technique to estimate the storage condition of wheat.

Materials and Methods

Fifty-two wheat samples, including several varieties of high viability from 1956 to 1959 crops and some commercial wheats at various stages of deterioration, were investigated. All were stored in moisture-proof containers at 4°C. until the analyses were made. In addition, samples of Pawnee wheat were conditioned to various moisture levels and stored 1 year at 25°C. To compare the changes in free amino acids during storage with those taking place during the early stages of germination, such as may be encountered in sprout-damaged wheats, Seneca wheat was steeped 16 hours at 25°C., followed by 40 hours of germination between filter papers. Samples were taken at suitable time intervals and dried in a forced-air oven at 38°C. for 24 hours.

Extraction of Free Amino Acids. Wheat was ground 2 minutes with a Waring Blendor and mixed well. Five grams (dry weight) were extracted in a mortar with approximately 10 ml. of 70% (w/v) ethanol, followed by centrifugation. This procedure was repeated five times, after which the combined ethanol extracts were passed through an Amberlite IR-120 (H⁺) resin column (1 ml. per minute; column 6 × 100 mm.; resin 16 to 50 mesh, U.S. Standard screen). The column was washed with 25 ml. of 70% (w/v) ethanol, after which the amino acids were displaced by 25 ml. of 1*N* ammonium hydroxide. The eluate was vacuum-distilled to 1 ml. (bath temperature 60°C.).

Electrophoresis. A Durum type Spinco paper electrophoresis apparatus, Model R, Series B, was used. A 20- μ l. aliquot, corresponding to 100 mg. (dry weight) of wheat, was pipetted as a narrow streak

(c. 2×25 mm.) on the center of the 30-mm.-wide filter paper strip (precut Spinco filter paper, No. 300-028). From several buffers tried, 0.025*M* potassium-hydrogen-phthalate (pH 4.0) proved most suitable. Electrophoresis was continued for 90 minutes at 400 V (14.0 V per cm.). The strips were dried horizontally in a forced-air oven at 60°C., dipped in 0.25% ninhydrin in acetone, heated 2 minutes at 80°C., left standing at room temperature overnight, and finally dipped for a moment in a cupric nitrate solution (2 ml. of saturated cupric nitrate in water and 0.2 ml. of 10% nitric acid, filled to 100 ml. with acetone). After drying for about 20 minutes at room temperature the strips were automatically scanned with the Spinco Analytrol using 1.5-mm. slit and 500- $m\mu$ filter.

Results and Discussion

Figure 1 shows that, using 0.025*M* potassium-hydrogen-phthalate (pH 4.0) as buffer, aspartic acid, glutamic acid, alanine (and other neutral amino acids as a group), gamma-aminobutyric acid, arginine, and lysine were separated in 90 minutes with 400 V (14.0 V per cm.). Beta-alanine would appear between the neutral amino acids and

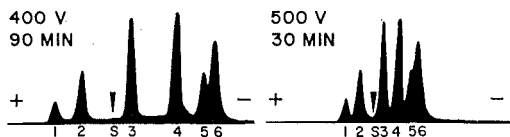


Fig. 1. Paper electrophoresis patterns of authentic amino acids in 0.025*M* potassium-hydrogen-phthalate (pH 4.0). S, point of application; 1, aspartic acid; 2, glutamic acid; 3, alanine + glycine (representing the neutral group); 4, gamma-aminobutyric acid and 5, arginine; 6, lysine.

gamma-aminobutyric acid, but it was omitted from the figure because it was found only in trace amounts in wheat. Figure 1 shows, in addition, that for most practical purposes 30 minutes' electrophoresis with 500 V (18.5 V per cm.) satisfactorily resolves these amino acids. The partial overlapping of arginine and lysine peaks is not of great importance because of the low lysine concentration in wheat. Phosphate buffer (0.067*M*, pH 7.0; 300 V and 6 hours) was not satisfactory, because it did not resolve aspartic acid from glutamic acid, nor gamma-aminobutyric acid from the group of neutral amino acids. Furthermore, evaporation due to heating was undesirably great. Hence 0.025*M* phthalate buffer, 400 V, and 90 minutes were adopted as standard conditions because of a good resolution in a relatively short time, without requiring cooling.

Figure 2 shows examples of electrophoresis patterns for Pawnee wheat stored at various moisture levels at 25°C. for 1 year. Table I shows the viability and mold count (2) of these samples after storage. The shoot length of germinated (for 4 days) kernels stored at 14.5% original moisture content was slightly less than one-half of that of wheat stored at 11.0% original moisture level. All samples stored at

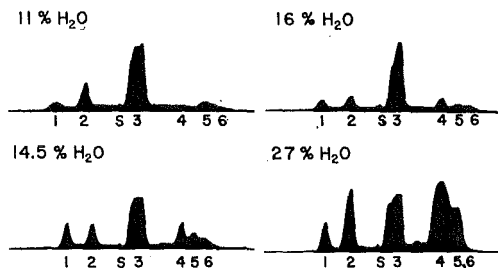


Fig. 2. Paper electrophoresis patterns of free amino acids of Pawnee wheat stored at various moisture levels at 25°C. for 1 year. See Fig. 1 for key to abbreviations.

TABLE I
VIABILITY AND MOLD COUNT OF PAWNEE WHEAT STORED AT VARIOUS
MOISTURE LEVELS AT 25°C. FOR ONE YEAR

MOISTURE		GERMINATION	MOLD COUNT
Before Storage	After Storage		
%	%	%	colonies/g
11.0	9.2	96	7,000
14.5	11.7	41	9,700
16.0	12.4	0	200,000
18.9	24.2	0	>10,000,000
27.0	33.2	0	52,000
36.0	40.0	0	10,500

higher moisture levels had totally lost viability. At original moisture levels below 16.0% the mold count remained relatively constant, which agrees well with the findings of Milner, Christensen, and Geddes (9). The sample conditioned to 16.0% moisture molded very slowly, whereas mold growth was excessive from the very beginning in the sample of 18.9% moisture. In the two high-moisture samples vigorous mold growth was observed in a few days. Later, in about a week, it receded and molds vanished owing to excessive carbon dioxide evolution from the grain. No mold growth could be visually detected in these samples during the rest of the experimental period.

Despite the small increase in mold count, gamma-aminobutyric acid peak was clearly noticeable in the sample with 14.5% original moisture. At 16.0% moisture level the glutamic acid peak had mark-

edly decreased in size, owing to the activation of glutamic acid decarboxylase (8). At 27% moisture level extensive proteolysis had taken place resulting in the production of large quantities of glutamic acid, followed by some decarboxylation to gamma-aminobutyric acid. Hence, if wheat has been stored under very unfavorable conditions the glutamic acid peak may be excessive, but it is then always accompanied by a high gamma-aminobutyric acid peak; this distinguishes the pattern from that of wheat of high quality with a high glutamic acid peak but a negligible gamma-aminobutyric acid peak.

Generally, the patterns presented in Fig. 2 seemed to be characteristic for wheats of different variety and of different condition. This was evident from results presented in Figs. 3 and 4. A relatively high glutamic acid peak, with low ones for aspartic and basic amino acids and virtually no detectable gamma-aminobutyric acid peak, is significant for good-quality wheats of high germination percentage (Fig. 3). The peak height ratio glutamic acid/aspartic acid in these wheats averaged 2.8, and it decreased with increasing deterioration

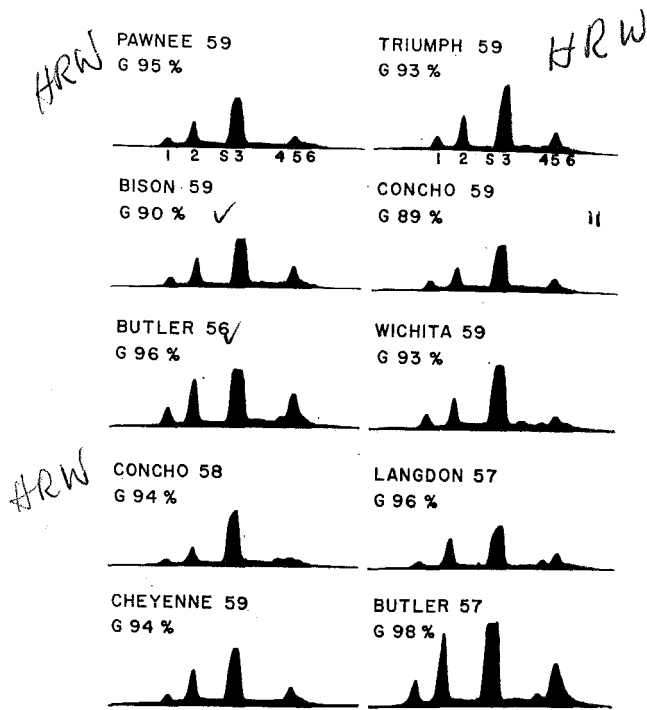


Fig. 3. Paper electrophoresis patterns of free amino acids of wheats of high viability, stored at 4°C. See Fig. 1 for key to abbreviations.

to below 1.0 as can be seen from Fig. 4. Slight variations in the area of the neutral amino acid peak were not significant, since the total quantity of the free amino acids varies somewhat in different wheat samples and since the high total concentration of the neutral amino acids does not permit an accurate determination with the technique. Figure 4 shows electrophoresis patterns of free amino acids obtained from various commercial wheats. When viability decreases, glutamic

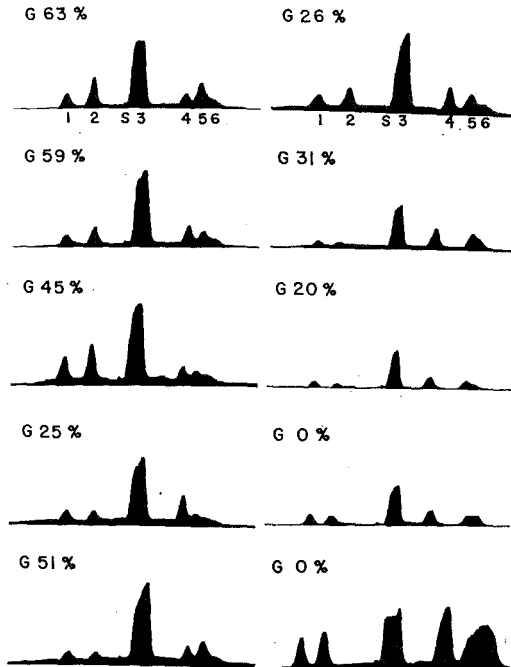


Fig. 4. Paper electrophoresis patterns of free amino acids of different lots of commercial wheats at various stages of deterioration. See Fig. 1 for key to abbreviations.

acid peak generally decreases, followed invariably by a more or less marked gamma-aminobutyric acid peak. It should be noticed, however, that if high glutamic acid peak is accompanied both by high gamma-aminobutyric acid and by high basic amino acid peaks, deterioration is very extensive and wheat has probably been dead a long time under unfavorable storage conditions.

As shown in Fig. 5, the glutamic acid peak also increases markedly during the early stages of germination, followed by a lesser increase in aspartic acid and, later, by increases in basic amino acids. That

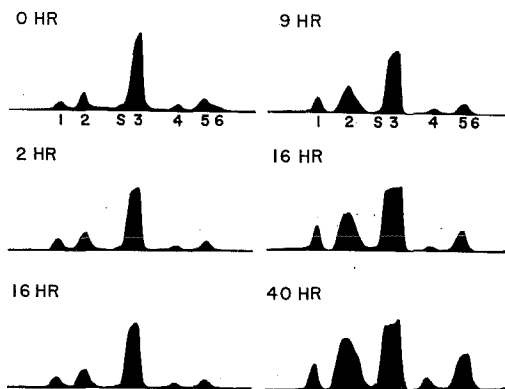


Fig. 5. Paper electrophoresis patterns of free amino acids of Seneca wheat, steeped 0, 2, and 16 hours at 25°C. (left), and steeped 16 hours followed by 9, 16, and 40 hours of germination between filter papers at 25°C. (right). See Fig. 1 for key to abbreviations.

no marked increase in gamma-aminobutyric acid peak could be noticed during the first 2 days of germination distinguished the patterns of sprouted wheats from those of advanced storage deterioration.

Although the method at its present state does not accurately determine the germination percentage in a given sample of wheat, the general pattern of the free amino acids obtained by the technique of paper electrophoresis promises to provide a simple estimation of the average storage condition. The results agree well with the earlier observations of Linko (5) concerning changes in the composition of the free amino acids during storage and germination of wheat. Such patterns as presented in Fig. 3, with a relatively high glutamic acid peak, a glutamic acid/aspartic acid peak height ratio over 2, and a small gamma-aminobutyric acid peak, would indicate that wheat has been stored under good conditions, the germination being most likely between 80 and 100%. Appearance of a gamma-aminobutyric acid peak, accompanied by a relative decrease in the glutamic acid peak, invariably indicates a decrease in quality. This type of pattern would also reveal damage caused by temporarily elevated moisture content during the storage period, regardless of the present moisture level of the grain examined.

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