rots caused by bacteria and fungi growing on amino acids and other nutritive substances leaking out of aging cells. N^6-Benzyladenine appears to delay this by maintaining cell vigor. It seems probable that its effect is partially due to a delay in proteolysis. Richmond and Lang (5) showed that kinetin can reduce or prevent the accelerated protein loss that is typical of detached leaves; at the same time, it delays the loss of chlorophyll and extends the life span of the leaf. It is thus not a biocide but acts through its effect on the physiology of the tissue. Use of this senescence inhibitor would not be an alternative to present practices, but would be a supplement to rapid, careful handling, and proper storage conditions.

Literature Cited

(1) Bessey, P. M., *Univ. Arizona Expt. Sta. Rept.* **189**, 5–8 (1960).
(2) Bonner, J., "Plant Biochemistry," Academic Press, New York, 1950.
(3) Chibnall, A. C., "Protein Metabolism in Plants," Yale Univ. Press, New Haven, Conn., 1939.
(4) Michael, G., *Z. Botan.* **29**, 385 (1935).
(5) Richmond, A. E., Lang, A., *Science* **125**, 650-1 (1957).
(6) van Overbeek, J., Shell Development Co., personal correspondence.

WHEAT GLUTEN PROTEINS

Amino Acid Composition of Proteins in Wheat Gluten

J. H. WOYCHIK, J. A. BOUNDY, and R. J. DIMLER

Northern Regional Research Laboratory, Peoria, Ill.

The recent chromatographic isolation of purified protein components from wheat gluten resulted in a program of basic studies on the structure of these proteins as related to the unique physicochemical properties of gluten. The first step was determination of the amino acid composition of six gluten components or fractions. While their compositions were similar, significant differences were observed which indicate the individuality of these components. Glutamic acid and proline together accounted for one half or more of the peptide-bound amino acids, and therefore probably are of particular structural importance in the gluten proteins. The composition of a water-soluble fraction isolated from gluten preparations indicated that it corresponds to albumins and/or globulins wheat.

THE UNIQUE physicochemical properties exhibited by hydrated wheat gluten proteins have prompted numerous investigations designed to explain the chemical and physical structures responsible. Particular emphasis has been placed on the role of sulfhydryl and disulfide bonding in gluten proteins (2, 4, 11). Although much information has been obtained from these and related studies, the ultimate explanation of gluten behavior will require broader fundamental investigations of gluten protein structure.

The recent development in this laboratory of a chromatographic technique for separating and isolating purified gluten proteins on carboxymethylcellulose columns (12) has permitted the initiation of a program of basic studies on gluten protein structure.

The first step was to determine the amino acid composition of purified gluten components using the ion exchange procedure of Moore, Spackman, and Stein (5). Six gluten components or fractions were analyzed.

Experimental

Preparation of Gluten Proteins. Proteins corresponding to the electrophoretic components demonstrated in Ponca hard red winter wheat gluten by Jones, Taylor, and Senti (3) were studied in this investigation. These components, with the exception of alpha-1, were isolated by chromatographic fractionation on carboxymethylcellulose (12). The alpha-1 component was obtained by pH precipitation (3).

Amino Acid Analysis. Protein samples (25 mg.) were hydrolyzed with constant boiling hydrochloric acid distilled from an all-glass apparatus. Each sample was dissolved in 12 ml. of acid contained in a 25-ml. borosilicate glass tube. The tubes were sealed and the hydrolysis was carried out in an autoclave held at $110° \pm 2°$ C. for 24 hours. The hydrolyzates were light tan in color and contained very small amounts of humin. After centrifugation, the humin was removed and analyzed for nitrogen; no significant amount of humin nitrogen was found in any hydrolyzate. Hydrochloric acid was removed by repeated evacuation on a rotary evaporator at 40° C. Protein samples for cystine analyses were oxidized with performic acid by the method of Schram and Bigwood (9) prior to hydrolysis. Tryptophan content was estimated by the colorimetric procedure of Spies and Chambers (10). The nitrogen content of 2-ml. aliquots was determined by duplicate micro-Kjeldahl analyses.

The preparation and use of Amberlite IR-120 columns were as described by Moore, Spackman, and Stein (5), except that the basic column was increased in length to 25 cm. and eluted at pH 5.28 with $0.33N$ instead of $0.35N$ buffer. These modifications were necessary to prevent overlapping of histidine and ammonia peaks as a result of the high level of amide ammonia in the hydrolyzates. Effluent fractions of 2 ml. were collected by drop count with a Technicon automatic fraction collector. Amino

Table I. Amino Acid Composition of Gluten

Amino Acid Grams/16 Grams Total Nitrogen

Amino Acid	Present report[a]	Pence et al. (8)	Block-Weiss (1), av.
Alanine	2.4	2.0	2.1
Arginine	2.4	4.3	3.6
Aspartic acid	2.9	3.4	4.8
Cystine	2.1	1.7	2.0
Glutamic acid	37.3	32.5	30.7
Glycine	3.1	3.2	4.0
Histidine	2.2	2.1	1.9
Isoleucine	4.0	4.2	4.2
Leucine	6.8	7.0	6.8
Lysine	1.2	1.6	1.6
Methionine	1.8	1.7	1.6
Phenylalanine	4.9	4.9	5.1
Proline	13.7	11.6	11.1
Serine	5.2	4.3	4.3
Threonine	2.5	2.4	2.5
Tryptophan	1.0	1.0	0.9
Tyrosine	3.8	2.8	3.1
Valine	4.1	4.3	4.3
Ammonia	5.1	..	..

[a] Average of at least four determinations.

acids present in the effluent were determined quantitatively by a colorimetric ninhydrin method (6). The ninhydrin reagent was prepared fresh for each chromatographic run and its color yield was determined by analysis of a standard leucine solution during color development of the effluent fractions. Absorbance was measured with a Beckman Model B spectrophotometer. Amino acid values are reported on the basis of 16 grams of total nitrogen.

Results and Discussion

The hydrolysis period required for complete cleavage of the peptide bonds of gluten was established by comparing the results of amino acid analysis following 24- and 72-hour hydrolysis.

Except for the decreased values in threonine and serine contents, no significant change in the content of any other amino acid was observed with longer hydrolysis. No corrections were applied for hydrolytic loss of threonine or serine.

Recoveries following analysis of a synthetic amino acid mixture indicated an accuracy of ±5%. Amino acid data presented for gluten proteins are the average of at least four determinations. Nitrogen recovery following chromatography of the hydrolyzates was 100 ± 5%.

A summary of the analysis of whole gluten prepared (3) from Ponca hard red winter wheat flour is presented in Table I, together with values reported by Pence et al. (8) and an average of literature values as published by Block (1). The data indicate general agreement in relative amino acid composition of gluten proteins obtained from different sources. Individual variations among amino acid values can perhaps be ascribed to differences in methods of gluten preparation. Entrainment of variable amounts of soluble protein could logically lead to differences, particularly between arginine, aspartic acid, glutamic acid, and proline content. Varietal differences and differences in the accuracy and precision of the analytical methods used also could account for some variation.

The amino acid composition of the isolated gluten components is presented in Table II and a bar graph of these data for easier visual comparison is shown in Figure 1. A summation of the amino acid values for individual gluten components, weighted according to their approximate percentage composition in whole gluten, compared favorably with data obtained in Table I.

Water-Soluble Fraction. A water-soluble protein fraction comprising 6% of the total gluten protein was present in the particular preparations analyzed. This fraction is soluble in dilute salt solutions and lacks the typical gluten properties of elasticity and cohesiveness. The electrophoretic mobility of this protein corresponds to that of proteins isolated from a water extract of wheat flour; on this basis, the fraction has been tentatively identified as albumin or globulin. These proteins are believed to be present in the gluten preparation as a result of physical entrapment during the gluten dough-ball washing procedure (3).

The nongluten nature of this fraction is further emphasized by marked differences in amino acid composition when compared with the other components present in wheat gluten (Figure 1). Particularly significant differences are observed in the content of basic and dicarboxylic acids; further differences are present among alanine, cystine, phenylalanine, and tryptophan. The basic amino acids—arginine, histidine, and lysine—account for about 21% of total nitrogen in this fraction; whereas they account for only 9% of nitrogen in the other gluten components. Although the water-soluble fraction contains two to three times the aspartic acid content of the gluten components, the total dicarboxylic content is about one third less.

The over-all amino acid content of water-soluble proteins isolated from the gluten preparation is in general agreement with the values obtained for wheat albumins and globulins by Pence (7). However, the ammonia value of 18% obtained in this analysis tends to reflect an amide content similar to that of gluten proteins, rather than an amide content (less than 10%) typical of albumins and globulins. The high

Table II. Amino Acid Composition of Gluten Components[a]

Amino Acid	Grams/16 Grams Total Nitrogen						Millimoles/16 Grams Total Nitrogen					
	Alpha-1	Alpha-2	Beta	Gamma	Omega	Water soluble	Alpha-1	Alpha-2	Beta	Gamma	Omega	Water soluble
Alanine	2.3	2.1	2.3	2.2	1.4	3.8	25.8	23.6	25.8	24.7	15.8	51.0
Arginine	2.9	3.1	2.0	1.8	1.2	6.1	16.6	17.8	11.5	9.8	12.2	34.9
Aspartic acid	2.5	1.8	2.5	1.8	1.2	6.2	18.8	13.5	18.8	13.5	9.0	47.0
Cystine	1.4	1.8	1.9	2.2	1.3	4.0	11.6	14.9	15.7	18.2	11.0	42.8
Glutamic acid	37.6	40.6	42.8	43.4	44.2	20.3	255.6	275.9	290.9	295.0	300.4	143.9
Glycine	5.0	4.3	1.5	1.9	2.0	3.9	66.6	57.3	20.0	25.3	27.2	54.2
Histidine	1.7	2.6	1.6	1.6	0.7	3.6	11.0	16.8	10.3	10.3	4.6	22.9
Isoleucine	3.1	4.3	4.5	4.3	2.2	3.8	23.6	32.8	34.3	32.8	16.5	37.1
Leucine	6.2	7.3	7.4	6.4	4.4	7.1	47.3	55.7	56.4	48.8	33.9	58.3
Lysine	1.3	0.8	0.7	0.7	0.7	4.1	8.9	5.5	4.8	4.8	5.1	27.9
Methionine	1.2	1.6	0.8	1.2	0.8	1.9	8.0	10.7	5.4	8.0	5.2	12.9
Phenylalanine	4.4	4.6	4.9	6.6	10.6	3.9	26.6	27.8	29.7	40.0	64.4	15.1
Proline	13.5	17.3	18.8	19.3	23.1	10.8	103.0	131.9	143.4	147.2	176.4	70.2
Serine	5.1	4.8	4.3	3.8	4.0	5.1	48.5	45.7	40.9	36.2	38.0	48.1
Threonine	2.8	2.6	2.7	2.1	2.4	2.6	23.7	22.0	22.9	17.8	20.0	33.1
Tryptophan	1.7	1.2	0.6	0.9	0.5	2.8	8.3	5.9	2.9	4.4	2.7	13.7
Tyrosine	4.1	2.8	2.8	1.7	2.5	3.9	22.6	15.5	15.5	9.4	14.0	18.6
Valine	3.6	4.2	4.6	3.7	2.5	6.1	30.7	35.9	39.3	31.6	21.3	50.8
Ammonia	4.6	5.2	4.6	5.2	4.6	3.5	270.6	305.9	270.6	305.9	269.0	208.5

[a] Average of at least four determinations.

tryptophan values therefore might provide a better index of the identity of the soluble protein fraction. The molar amounts of ammonia and dicarboxylic acids in the water-soluble proteins are essentially equal. It is unlikely that amino acid destruction during hydrolysis could account for the higher ammonia values. Although water-soluble proteins comprise only a small portion of the gluten preparation, substantial differences in amino acid composition can influence the analytical values obtained for whole gluten.

Gluten Components. The high content of glutamic acid and proline characterizes in part the amino acid composition of gluten proteins. These acids were present in the largest amounts in the omega component, with 44 and 23 grams per 16 grams of nitrogen, respectively. The alpha-1 component contained approximately 15% less glutamic acid and 30% less proline than the average of the remaining components.

The basic amino acid content varied among the protein components. Both alpha proteins contained approximately 50% more arginine than the other gluten components. The alpha-2 component was distinguished from alpha-1 by higher histidine content. The lysine content was essentially constant among the proteins, except that it was higher in the alpha-1 component. Good agreement was obtained between the observed electrophoretic mobilities of these components in acidic buffers and their relative basic amino acid content, which for the most part accounts for the positive charge in acidic media.

The cystine content varied among the components with a range of 11.0 to 18.2 mmoles per 16 grams of nitrogen. No relationship was observed between the content of cystine and methionine.

Serine, tryptophan, and tyrosine decreased progressively from the alpha-1 to omega components, whereas phenylalanine increased in this order. The high content of phenylalanine serves to distinguish the omega fraction from other components.

Isoleucine and leucine appeared somewhat related in that approximately 2 mmoles of leucine were present for each millimole of isoleucine.

Glycine accounts for the largest single difference among the gluten proteins, characterizing the alpha-1 and alpha-2 components by their high glycine content as compared with the other gluten components (66 and 57 vs. a maximum of only 27 mmoles).

No attempt was made to determine what portion of the dicarboxylic acids were present as their amides. However, a comparison of the molar amounts of ammonia (uncorrected for amino acid destruction) and of dicarboxylic acids (Table III) reveals that sufficient ammonia was present to account for over

90% of the dicarboxylic acids as their amides.

Probable structural importance of glutamic acid and proline is suggested by the amino acid composition determined for gluten components. Glutamic acid accounts for approximately 35 to 40% of total amino acid residues (Table III). Similarly, proline accounts for another 13 to 23% of total residues. Together, these amino acids constitute one half or more of the total peptide-bound amino acid in these proteins, with the exception of the water-soluble fraction. Further studies may reveal a unique structural sequence of glutamic acid and proline which could be directly related to the physicochemical properties of gluten proteins.

In view of marked differences in solubility and viscosity between the classical gluten fractions, "gliadin" (prolamine) and "glutenin" (glutelin), one might also expect rather marked differences in amino acid composition between components comprising these fractions. In a recent report, Jones and coworkers

(3) demonstrated that the alpha-1 component comprises over 90% of glutenin, while gliadin consists of the remaining components. From the amino acid compositions determined, the authors have been unable to demonstrate any major difference in composition between the principal glutenin component, alpha-1, and the remaining components comprising gliadin, which could account for gross property differences present in these fractions. It therefore appears likely that differences in molecular weight or structural variations are responsible for the characteristic differences in solubility of these two fractions.

Acknowledgment

Literature Cited

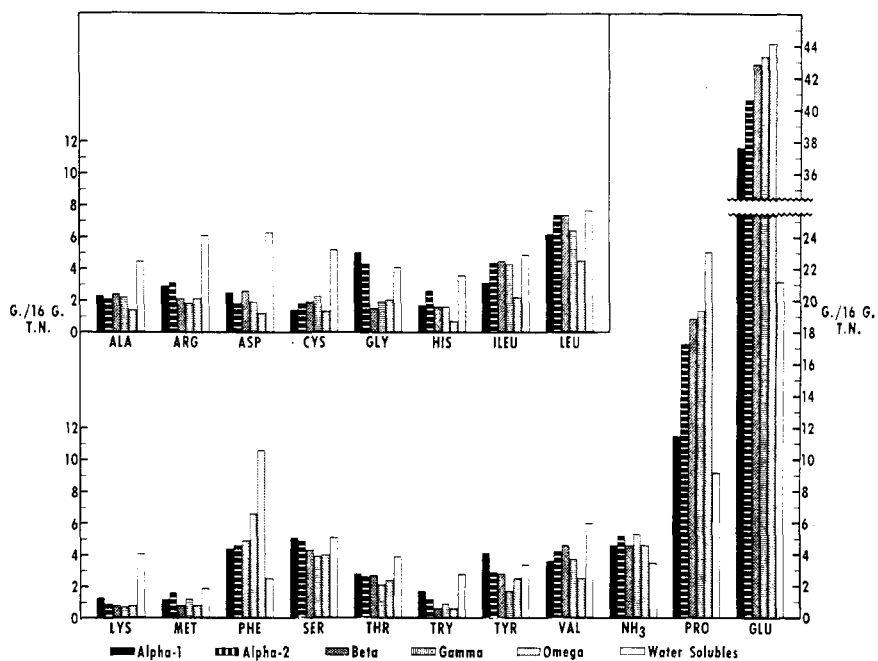(1) Block, R. J., Weiss, K. W., "Amino Acid Handbook," p. 346, Charles C Thomas, Springfield, Ill., 1956.

Figure 1. Amino acid composition of isolated component proteins of wheat gluten, expressed as grams of amino acid per 16 grams of total nitrogen

Table III. Amino Acid Summation[a]

Amino Acid	Alpha-1	Alpha-2	Beta	Gamma	Omega	Water-Soluble
Aspartic acid	19	14	19	14	9	47
Glutamic acid	256	276	291	295	300	144
Ammonia	271	306	271	306	269	208
Proline	103	132	143	147	176	70
Basic amino acids	36	40	26	25	22	86
Neutral acids	343	347	308	297	270	436
Total[b]	757	809	787	778	777	783

[a] Millimole/16 g. nitrogen.
[b] Excludes ammonia.

(2) DeLange, P., Hintzer, H. M. R., Cereal Chem. **32,** 307–13 (1955).
(3) Jones, R. W., Taylor, N. W., Senti, F. R., Arch. Biochem. Biophys. **84,** 363–76 (1959).
(4) Kong, R. W., Mecham, D. K., Pence, J. W., Cereal Chem. **34,** 201–10 (1957).
(5) Moore, S., Spackman, D. H., Stein, W. H., Anal. Chem. **30,** 1185–90 (1958).
(6) Moore, S., Stein, W. H., J. Biol. Chem. **211,** 907–13 (1954).
(7) Pence, J. W., personal communication, October 26, 1959, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Dept. of Agriculture, Albany, Calif.
(8) Pence, J. W., Mecham, D. K., Elder, A. H., Lewis, J. C., Snell, N. S., Olcott, H. S., Cereal Chem. **27,** 335–41 (1950).
(9) Schram, E., Moore, S., Bigwood, E. J., Biochem. J. **37,** 33–37 (1954).
(10) Spies, J. R., Chambers, D. C., Anal. Chem. **21,** 1249–66 (1949).
(11) Sullivan, B., J. Agr. Food Chem. **2,** 1231–34 (1954).
(12) Woychik, J. H., Dimler, R. J., Senti, F. R., Arch. Biochem. Biophys., **91,** 235–9 (1960).

QUALITY OF STORED WHEAT

Simple and Rapid Manometric Method for Determining Glutamic Acid Decarboxylase Activity as Quality Index of Wheat

PEKKA LINKO

Department of Flour and Feed Milling Industries, Kansas State University, Manhattan, Kan.

Recent studies have indicated that glutamic acid decarboxylase activity gives a good estimate of the storage condition of wheat. Hence a simple and rapid manometric method was developed for its determination. Ground wheat was mixed with glutamic acid in phosphate buffer at pH 5.8, and the carbon dioxide evolution due to the decarboxylation of glutamic acid was measured with Sandstedt and Blish pressuremeters using ethyl lactate as manometer liquid. The correlations between germination percentage and the observed pressure increase ($r = +0.841***$) or $\log_{10}$ pressure increase ($r = +0.928***$), respectively, were highly significant. Estimate of the storage condition of wheat was equal to or better than that by fat acidity determination.

THE IMPORTANCE of quick and reliable tests to estimate the quality of stored wheat is generally recognized. Numerous attempts have been made to determine the storage condition of cereal grains (*8*). Fat acidity has been shown to be a good index of the extent of deterioration (*3, 19*) and is widely used by the cereal industry to evaluate wheat. Although relatively simple, the standard method involved is somewhat time-consuming. A rapid method for determining fat acidity has been developed (*2*), but the technique requires prior drying of grain below the 10% moisture level. It was shown recently that characteristic changes in the composition of free amino acids of wheat take place shortly after the beginning of water imbibition, because of the activation of certain enzymes, such as glutamic acid decarboxylase, transaminases, and proteases, at low moisture levels (*8, 12, 13*). These changes continue during storage, particularly at elevated moisture levels and temperatures. Subsequently, tech-

niques based on the study of paper chromatogram (*10*) and, particularly, electrophoresis (*9*) patterns of free amino acids of wheat seemed promising as a convenient means of estimating the degree of deterioration.

On the other hand, enzymes themselves begin to lose activity during storage of grain under unfavorable conditions, and glutamic acid decarboxylase activity decreases during storage of wheat, especially at moisture levels above 15% (*4, 8, 15*). Glutamic acid decarboxylase activity, as determined by Warburg manometric (*14*), colorimetric (*11*), or electrophoretic (*11*) techniques, appears to be a good index of the storage condition of wheat. All these methods, however, are either relatively time-consuming or require elaborate and expensive equipment. The present paper describes a method suitable for rapid and accurate routine determination of the glutamic acid decarboxylase activity as an index of the quality and storage condition of wheat.

Experimental

Materials. The 60 wheat samples investigated included several varieties of relatively high viability from 1956 to 1959 crops, and a number of commercial wheats at various stages of deterioration. Their moisture content varied from 9.9 to 12.9%, but it seemed likely that the moisture content at one time of storage had been much higher in some of the samples. The viability range covered was from 0 to 99% germination, and that of fat acidity from 10.2 to 79.8. Moisture, germination, fat acidity, and Warburg manometric analyses were performed as described earlier (*14*).

Apparatus. Sandstedt and Blish (*16*) pressuremeters were employed to determine the glutamic acid decarboxylase activity. However, instead of being filled with mercury, the manometers were filled to the 300-mm. level with ethyl lactate colored with crystal violet (*18*). This provided the necessary sensitivity for short-term operations.