AMINO ACID COMPOSITION OF BARLEY KERNELS FROM DIFFERENT PARTS OF THE SPIKE[1]

Y. POMERANZ[2], D. M. WESENBERG[3], R. T. SMITH[4], G. S. ROBBINS[5], and J. T. GILBERTSON[5], Barley and Malt Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Madison, WI 53705

Cereal Chemistry 53(6): 839-845

ABSTRACT

Kernel weight, protein contents, and amino acid composition were determined in five widely different barley cultivars; in kernels from top, middle, and bottom portions of spikes of the five cultivars; in lateral and median kernels from six-rowed selections; and in corresponding two-rowed isogenic lines. Kernels from the middle of the barley spike were largest and from the top smallest. Position on the spike had no significant effect on protein content or amino acid composition. Kernels from two-rowed isogenics were larger and higher in protein than kernels from six-rowed isogenics. Proteins in two-rowed isogenics contained more glutamic acid, proline, and phenylalanine, and less of most of the other amino acids than proteins of six-rowed isogenics. Similarly, median kernels were larger and higher in protein than lateral kernels; proteins in median kernels contained more glutamic acid, proline, and phenylalanine. Lysine was significantly associated with arginine and valine for all spike positions and with histidine for all isogenic lines.

We have previously reported results of several studies on amino acids in proteins of barley. The studies concerned the distribution of amino acids in barley tissues and milled products (1), changes which occur during maturation

[1]Cooperative investigations of the Barley and Malt Laboratory, Agricultural Research Service, North Central Region, USDA, Madison, Wis.; Agricultural Research Service, Western Region, USDA, Aberdeen, Idaho; the Wisconsin Agricultural Experiment Station, Madison; and the Idaho Agricultural Experiment Station, Moscow. Published with approval of the Directors of the Wisconsin Agricultural Experiment Station and the Idaho Agricultural Experiment Station.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2]Chemist in Charge, Barley and Malt Laboratory, USDA, and Professor, Agronomy Department, University of Wisconsin, Madison. Present address: Director, Grain Marketing Research Center, ARS, USDA, Manhattan, Kansas.

[3]Research Agronomist, Aberdeen Research and Extension Center, ARS, USDA, Aberdeen, Idaho.

[4]Research Agronomist, Field Crops Laboratory, ARS, USDA, Beltsville, Md.

[5]Respectively: Research Chemist and Research Assistant, Barley and Malt Laboratory, ARS, USDA, Madison, Wis.

(2,3), amino acid composition of high-amylose and Hiproly barleys (4), and amino acids in proteins of two-rowed and six-rowed barley cultivars (5).

Fischbeck (6) reported that, in 11 varieties of two-rowed spring barleys, kernels from the top third of the spike were the lightest, those from the middle third had the highest kernel weight, and those from the bottom third were slightly lighter than those from the middle. The protein content of the kernels decreased from the top to the bottom of the spike.

McNeal and Davis (7) found that kernels from the top of the spike, in three wheat cultivars, were significantly lower in protein than those from the bottom and middle portions. The authors concluded that the kernels formed early on a spike contain the highest protein content and that this highest protein content requires availability and adequate translocation of nitrogenous compounds from the stems and leaves to grain late in the growing season. No significant differences were found in the protein content of median and lateral kernels (7).

To our knowledge, no investigation has been published on the amino acid composition of proteins in barley kernels from various positions on the spike. In this study, we analyzed kernels from the top, middle, and bottom portions of spikes of five widely different barley cultivars; lateral and median kernels from the six-rowed members of 10 six-rowed/two-rowed barley isogenic pairs; and kernels from the corresponding two-rowed members of the 10 isogenic pairs.

MATERIALS AND METHODS

All barleys were grown under irrigation at Aberdeen, Idaho, in 1972. The five cultivars used in the analyses of kernels from the top, middle, and bottom portions of the spike were Larker, Conquest, Firlbecks III, Hiproly, and CI 4362. Larker and Conquest are hulled, six-rowed cultivars; Firlbecks III is a hulled, two-rowed cultivar; and Hiproly and CI 4362 are hull-less, two-rowed cultivars. Hiproly and CI 4362, which are morphologically similar and often considered as an isogenic pair are rich in protein, but Hiproly has shriveled seed, lower yield, and substantially more lysine in the protein compared with CI 4362. Fifteen random spikes of each cultivar were obtained from the center rows of each of two four-rowed plots. The top, bottom, and middle samples by replicate for each cultivar included, respectively, the top six spikelets of each of 15 spikes, the bottom six spikelets of each of the same 15 spikes, and the remainder. By weight, this separation resulted in average top, middle, and bottom samples of 5.6, 7.0, and 7.5 g, respectively.

For analyses of median and lateral kernels, 10 pairs of six-rowed/two-rowed isogenic lines developed by G. A. Wiebe were grown. A pair of isogenic lines is assumed to differ by a single gene although, more accurately, pairs differ by small gene blocks identified by a marker gene —in this study VV (two-rowed) and vv (six-rowed). Fifteen spikes from each of the six-rowed isogenic lines were separated into median and lateral kernels. The kernels from 15 spikes of each of the two-rowed isogenics constituted the corresponding two-rowed samples.

Kernel weights (moisture-free basis), moisture, and protein (Kjeldahl-N $\times$ 6.25, moisture-free basis) were determined according to the methods of the American Society of Brewing Chemists (8).

Amino acid analyses were performed on a Beckman 121 automatic amino acid analyzer. Detailed descriptions of the acid hydrolysis, the assay, and the

computations have been reported (2). Results of amino acid assays are expressed in g amino acid/100 g amino acid recovered.

In the analyses of variance for kernels from various positions on the spike (top, middle, and bottom) the following model was used:

Source of variation	*Degrees of freedom*
Replication	1
Cultivar	4
Replication × cultivar = error a	4
Kernel position	2
Kernel position × cultivar	8
Residual = error b	10
Corrected total	29
Mean	1

Error a was used to challenge cultivar and error b was used to challenge kernel position and the interaction between cultivar and kernel position.

In the analyses of variance for the kernels from isogenic lines, the isogenic lines were considered as replicates and their variance mean squares were accounted for in the model. The interactions between isogenic lines and kernel types were calculated according to:

Source of variation	*Degrees of freedom*
Isogenic lines (I)	9
Two-rowed *vs.* six-rowed (R)	1
Lateral *vs.* median (L)	1
I × R	9
I × L	9
Corrected total	29
Mean	1

Isogenic lines were challenged by pooling the mean squares for I × R and I × L. The two-rowed *vs.* six-rowed source of variation was challenged by the I × R interaction and lateral *vs.* median by the I × L interaction.

RESULTS AND DISCUSSION

Means and statistical analyses of metameters for the five barley cultivars are summarized in Table I. There were highly significant differences among the five cultivars in most parameters except kernel weight, histidine, NH_3, serine, cystine, methionine, isoleucine, leucine, and tyrosine. CI 4362 produced the largest kernels and the two naked barley cultivars (Hiproly and CI 4362) were higher in protein than the three hulled cultivars. Protein of Hiproly had substantially more lysine than any of the other cultivars. The lower NH_3 content of Hiproly reflected, in part at least, the low glutamine content of this cultivar. Hiproly was also the richest in arginine, aspartic acid, glycine, alanine, valine, isoleucine, and tyrosine. The large-kerneled, protein-rich CI 4362 contained the highest levels of

glutamic acid and proline (major components of storage proteins in cereals) and phenylalanine. The high concentrations of glutamic acid and proline in proteins of CI 4362 lowered concentrations of most of the other amino acids. Hiproly was lowest in glutamic acid, proline, and cystine, reflecting the shrunken kernels of this cultivar and the relatively low content of starchy endosperm.

Statistical analyses (data not shown here) indicated high coefficients of variability (11.9–13.0%) for kernel weight, cystine, methionine, and tyrosine. None of the spike position × cultivar interactions was statistically significant, except for glycine and alanine.

Position on the spike affected kernel weight, the middle kernels being the largest and the top kernels the smallest (Table II); this agrees with the data reported by Fischbeck (6). There were no significant differences in protein content. We could confirm neither the results of Fischbeck (6) who reported a decrease in protein content from the top to the bottom of the barley spike, nor the results of McNeal and Davis (7) who reported that top kernels were lower in protein than bottom and middle kernels in the wheat spike. Among the amino acids, only leucine and phenylalanine were affected by the position of the kernel on the spike and those effects were rather small. This uniformity in amino acid composition might reflect the uniform protein content. This finding agrees with a previous observation that the amino acid composition in proteins of two-rowed and six-rowed barleys is governed by the total protein content rather than type of barley (5).

TABLE I
Means and Statistical Analyses of Parameters[a] for Five Barley Cultivars

Parameter	Cultivar Mean					Least Significant Difference (0.05)	P > F
	Larker	Conquest	Firlbecks III	Hiproly	CI 4362		
Kernel wt., mg	41.30	40.78	48.91	41.93	58.21	12.74	0.0690
Protein, %	15.75	15.68	16.45	18.53	19.25	1.33	0.0069*
Lysine	3.60	3.85	3.46	4.53	3.26	0.32	0.0038*
Histidine	2.21	2.33	2.20	2.31	2.16	0.22	0.3009
NH_3	3.18	3.20	3.21	3.01	3.35	0.32	0.2327
Arginine	5.03	5.00	4.93	5.46	4.20	0.50	0.0175*
Aspartic acid	6.20	6.38	5.91	7.15	5.43	0.24	0.0012*
Threonine	2.99	3.15	2.93	3.05	2.70	0.26	0.0490*
Serine	3.58	3.71	3.63	3.51	3.26	0.47	0.2566
Glutamic acid	27.63	27.40	28.35	25.31	29.88	1.05	0.0036*
Proline	11.31	10.61	11.41	9.90	12.83	1.60	0.0434*
Cystine	1.23	1.11	1.23	0.96	1.20	0.20	0.0730
Glycine	3.58	3.78	3.55	3.80	3.36	0.20	0.0191*
Alanine	4.06	4.20	4.05	4.60	3.60	0.22	0.0033*
Valine	4.98	4.91	4.09	5.11	4.46	0.19	0.0061*
Methionine	2.31	2.26	2.10	2.58	2.30	0.62	0.4307
Isoleucine	3.56	3.55	3.50	3.60	3.50	0.16	0.1261
Leucine	6.63	6.70	6.53	6.58	6.45	0.17	0.0783
Tyrosine	2.28	2.15	2.33	2.80	2.10	0.58	0.1278
Phenylalanine	5.55	5.66	5.70	5.66	6.08	0.08	0.0011*

[a]Amino acids are expressed as g/100 g amino acids recovered in analyses.

TABLE II
Means and Statistical Analyses of Parameters[a] for Barley Kernels (Five Cultivars) from Three Spike Positions

Parameter	Spike Position			Least Significant Difference (0.05)	P > F
	Top	Middle	Bottom		
Kernel weight, mg	42.36	50.45	45.88	4.11	0.0049*
Protein, %	17.06	17.12	17.22	0.71	0.8792
Lysine	3.80	3.76	3.67	0.23	0.5354
Histidine	2.28	2.24	2.22	0.12	0.5228
NH_3	3.21	3.18	3.19	0.13	0.8684
Arginine	4.87	4.98	4.93	0.35	0.7927
Aspartic acid	6.14	6.25	6.26	0.17	0.2737
Threonine	2.94	2.95	2.99	0.68	0.2690
Serine	3.56	3.55	3.52	0.13	0.7923
Glutamic acid	27.87	27.45	27.83	0.64	0.3136
Proline	10.94	11.32	11.39	0.93	0.5336
Cystine	1.10	1.18	1.17	0.15	0.5514
Glycine	3.60	3.64	3.61	0.08	0.5128
Alanine	4.16	4.12	4.03	0.18	0.3109
Valine	4.94	4.87	4.82	0.12	0.1177
Methionine	2.30	2.30	2.34	0.22	0.8933
Isoleucine	3.52	3.53	3.52	0.05	0.8684
Leucine	6.66	6.55	6.53	0.08	0.0095*
Tyrosine	2.29	2.40	2.31	0.28	0.6544
Phenylalanine	5.82	5.71	5.67	0.11	0.0369*

[a]Amino acids are expressed as g/100 g amino acids recovered in analyses.

TABLE III
Means and Statistical Analyses of Parameters[a] for Barley Kernels from Two-Rowed (Medians) and Six-Rowed (Laterals and Medians) Pairs of Isogenic Lines

Parameter	Means		P > F for Two-Rowed *vs.* Six-Rowed	Means		P > F for Laterals *vs.* Medians
	Two-Rowed	Six-Rowed		Laterals	Medians	
Kernel wt., mg	49.25	36.92	0.0001*	31.75	45.67	0.0001*
Protein, %	17.16	14.04	0.0001*	13.95	15.65	0.0001*
Lysine	3.47	3.85	0.0008*	3.89	3.64	0.0002*
Histidine	2.23	2.39	0.0003*	2.39	2.31	0.0084*
NH_3	3.22	3.14	0.0301*	3.11	3.19	0.1618
Arginine	4.67	5.19	0.0001*	5.22	4.91	0.0093*
Aspartic acid	5.78	6.14	0.2104	5.88	6.09	0.7294
Threonine	2.83	3.01	0.0026*	3.02	2.91	0.0078*
Serine	3.57	3.73	0.0185*	3.78	3.63	0.0009*
Glutamic acid	28.18	26.47	0.0009*	26.36	27.38	0.0021*
Proline	12.46	11.17	0.0004*	11.06	11.87	0.0012*
Cystine	1.14	1.09	0.2509	1.08	1.12	0.1804
Glycine	3.47	3.85	0.0003*	3.89	3.64	0.0002*
Alanine	3.95	4.24	0.0040*	4.30	4.07	0.0015*
Valine	4.86	5.11	0.0020*	5.31	4.97	0.0014*
Methionine	2.09	2.14	0.5624	2.15	2.11	0.5705
Isoleucine	3.56	3.61	0.0307*	3.63	3.58	0.0065*
Leucine	6.61	6.76	0.0096*	6.78	6.67	0.0014*
Tyrosine	2.24	2.39	0.0728	2.30	2.36	0.6301
Phenylalanine	5.66	5.48	0.0257*	5.45	5.58	0.0418*

[a]Amino acids are expressed as g/100 g amino acids recovered in analyses.

TABLE IV
Correlation Coefficients[a] with Lysine and Other Essential Amino Acids and Kernel Weight for Spike Position and Kernel Origin from Isogenic Lines

Spike Position	Lysine *vs.*: Kernel weight, mg	Histidine	Arginine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Total[b]
					From Five Cultivars					
Top	−0.387	0.851*	0.830*	0.254	0.800*	0.344	0.577	0.409	−0.557	19
Middle	−0.347	0.411	0.641*	0.521	0.749*	0.287	0.363	−0.249	−0.449	9
Bottom	−0.553	0.656*	0.688*	0.772*	0.719*	0.539	0.760*	0.600	−0.291	25
					From Isogenic Pairs					
Six-rowed medians	0.203	0.820*	0.104	0.307	0.583	0.343	0.479	0.648*	0.556	6
Six-rowed laterals	0.153	0.639*	−0.160	0.375	0.571	0.465	0.420	0.707*	0.175	5
Two-rowed medians	−0.525	0.839*	−0.059	0.040	0.380	−0.222	−0.109	0.465	0.131	5

[a]Asterisks indicate significance at ≤ 0.05 probability with 8 degrees of freedom.
[b]Number of significant correlation coefficients ($P \leq 0.05$) of all possible 45 correlations.

Two-rowed and six-rowed isogenic lines and lateral and median kernels are compared in Table III. Kernels from the two-rowed isogenic lines were larger and higher in protein percentage than kernels from the six-rowed lines. The proteins in two-rowed isogenics contained more glutamic acid, proline, and phenylalanine and less, if significantly different, of most of the other amino acids than the proteins of the six-rowed isogenics. Median kernels were higher than laterals in kernel weight, protein, glutamic acid, proline, and phenylalanine; proteins in laterals contained higher concentrations of all the other amino acids except aspartic acid, cystine, methionine, and tyrosine. Concentrations of the latter four amino acids in medians and laterals were not significantly different.

Correlation coefficients for lysine, the first limiting amino acid in barley, *vs.* essential amino acids (except tryptophan) and kernel weight are shown in Table IV. Lysine was highly correlated with arginine and valine for kernels from top, middle, and bottom of the spike, to histidine (top and bottom), and threonine and isoleucine (bottom only). When all possible 45 correlations among amino acids and kernel weight were considered for each position, a preponderance of significant ($P \leq 0.05$) correlations were found for the extremities of the spike. Since the barley spike matures from the middle toward the ends, it is suggested that the preponderance of significant paired relationships, at least for the essential amino acids, may be related to developmental stages of the kernels rather than to kernel size.

Lysine was significantly associated with histidine for the six-rowed and two-rowed isogenic lines and with leucine for the six-rowed isogenic lines (Table IV). The data suggest that significant relationships among essential amino acids occur vertically on the spike, as evidenced by the large total number of significant correlations for kernels from the top and bottom of the spike. Significant chi-square values from tests of homogeneity of correlation coefficients between and among major groups limited statistical inferences from pooled data.

Acknowledgment

E. J. Koch was consulted regarding statistical models and analyses.

Literature Cited

1. ROBBINS, G. S., and POMERANZ, Y. Composition and utilization of milled barley products. III. Amino acid composition. Cereal Chem. 49: 240 (1972).
2. POMERANZ, Y., and ROBBINS, G. S. Amino acid composition of maturing barley. Cereal Chem. 49: 560 (1972).
3. POMERANZ, Y., WESENBERG, D. M., ROBBINS, G. S., and GILBERTSON, J. T. Changes in amino acid composition of maturing Hiproly barley. Cereal Chem. 51: 635 (1974).
4. POMERANZ, Y., ESLICK, R. F., and ROBBINS, G. S. Amino acid composition and malting and brewing performance of high-amylose and Hiproly barleys. Cereal Chem. 49: 629 (1972).
5. POMERANZ, Y., ROBBINS, G. S., WESENBERG, D. M., HOCKETT, E. A., and GILBERTSON, J. T. Amino acid composition of two-rowed and six-rowed barleys. J. Agr. Food Chem. 21: 218 (1973).
6. FISCHBECK, G. Properties of different corns from the same ear of barley. Brauwissenschaft 18: 1 (1965).
7. McNEAL, F. H., and DAVIS, D. J. Protein content of wheat kernels from different parts of the spike. Agron. J. 58: 635 (1966).
8. AMERICAN SOCIETY OF BREWING CHEMISTS. Methods of analysis. The Society: St. Paul, Minn. (1958).

[Received November 24, 1975. Accepted December 23, 1975]