THE AMINO ACID COMPOSITION OF HARD WHEAT VARIETIES AS A FUNCTION OF NITROGEN CONTENT[1]

FRANK N. HEPBURN AND WILLIAM B. BRADLEY

ABSTRACT

Samples of commercial wheats, representing different types and different amounts of total nitrogen content, were found to differ in the proportion of certain amino acids. Samples of pure varieties of spring and winter hard wheats were selected to permit comparisons of the amino acid composition between 1) different varieties at a single level of total nitrogen and 2) between samples of varieties which differed widely in nitrogen content. The proportions of amino acids were nearly constant for all varieties of wheat in samples of similar nitrogen content, although cystine and methionine tended to be higher in the spring wheats. Samples of the highest and lowest ranges of nitrogen exhibited different proportions of most amino acids, but the differences were small relative to the magnitude of the difference in total nitrogen. Histidine, isoleucine, and tyrosine showed the least variation with nitrogen level. Values for glutamic acid, phenylalanine, and proline tended to be higher in high-protein samples, whereas the remaining amino acids showed the reverse trend to varying degrees.

Results from a number of studies (1–6) indicate that the proportion of certain amino acids in wheat protein may depend upon the total nitrogen content of the wheat. Lysine, in particular, has been found in greater concentration in wheats of low protein content than in high-protein samples. Such variations were not found by this laboratory in a previous study (7). Four commercial blends of hard wheat were shown to have nearly the same amino acid composition when results were expressed as g./16 g. nitrogen. Only values for tryptophan were statistically significant, and these differences were small in magnitude. It was not apparent whether the constancy observed in this study was a consequence of the blending process, by which possible varietal differences might be averaged, or whether in the range of nitrogen of these samples (2.4–3.1%) there is a reduced tendency for the amino acid composition to vary with total protein.

The study of amino acids in commercial wheats has been extended to include samples differing in type and protein content. The results, which are presented here, show obvious differences in the proportions of certain amino acids but do not permit a distinction between the effect of wheat type and that of nitrogen content. The principal purpose of this paper is to present the results of studies of samples of

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individual varieties of wheat which were selected, first for similarity and secondly for diversity of nitrogen content.

Materials and Methods

Sample Description. Commercial wheat samples (Table I) were obtained as cleaned wheat from principal areas of wheat production. The sample of Elmar was grown in California; Baart wheat, in Washington; and the Southwest wheats, in Texas and Oklahoma. Baart

TABLE I
AMINO ACID CONTENT OF COMMERCIAL WHEATS

	Pacific Coast Wheat					Southwest Wheats		Northwest Wheats
	Club, Elmar	White, Baart	Blends of HRW and Baart			Blend, Bakers' Mix	Blend, Family Mix	Blend, HRW and HRS
			A	B	C			
Nitrogen content, % (14% moisture basis)	1.41	1.93	2.02	2.12	2.18	2.13	2.31	2.37
Amino acid content, mg./g. N								
Alanine	227	233	228	221	206	214	202	197
Arginine	308	322	329	308	294	304	289	289
Aspartic acid	313	296	320	301	283	291	280	276
Cystine	111	148	144	149	122	118	119	120
Glutamic acid	1,594	1,656	1,662	1,675	1,756	1,731	1,794	1,794
Glycine	246	235	229	237	248	261	251	231
Histidine	119	115	117	108	118	112	118	121
Isoleucine	235	241	240	239	231	235	234	229
Leucine	386	421	410	397	387	390	388	384
Lysine	191	173	175	168	165	168	162	159
Methionine	102	90	95	99	100	99	102	99
Phenylalanine	239	285	296	274	256	256	266	262
Proline	561	681	694	694	609	590	631	597
Serine	310	328	323	321	309	304	305	314
Threonine	182	176	185	173	173	176	171	169
Tryptophan	82	76	77	72	71	69	66	71
Tyrosine	180	214	209	206	193	192	190	191
Valine	294	278	284	282	276	280	277	281

wheat blends A, B, and C were obtained from different sources but were each described as consisting of 50% of hard red winter wheat. The blend of hard red winter and spring wheats (50% each) was selected to be representative of samples examined in the previous study (7).

Samples of individual hard wheat varieties were obtained from the Crops Research Division of the United States Department of Agriculture, Agricultural Research Division. The samples were from the 1960 crop year and consisted of 16 varieties, each of which had been grown

at 4 to 14 different experiment station locations. From the total number of 130 separate samples, 8 of winter wheat varieties (Table II) and 4 of spring wheat varieties (Table III) were selected for their similar

TABLE II

AMINO ACID CONTENT OF VARIETIES OF HARD RED WINTER WHEAT IN SAMPLES OF SIMILAR NITROGEN CONTENT

	Bison	Comanche	Concho	Kaw	Omaha	Ottawa	Pawnee	Tascosa	Average
Nitrogen content, % (14% moisture basis)	2.37	2.37	2.37	2.37	2.42	2.40	2.40	2.34	
Amino acid content, mg./g. N									
Alanine	201	201	209	216	212	216	209	211	209
Arginine	251	264	286	296	289	287	275	271	277
Aspartic acid	283	283	304	301	306	303	301	299	298
Cystine	109	103	99	111	102	100	99	111	104
Glutamic acid	1,875	1,852	1,758	1,848	1,805	1,798	1,853	1,850	1,830
Glycine	231	241	248	242	241	241	241	256	243
Histidine	113	115	119	126	118	120	116	122	9
Isoleucine	235	232	234	234	234	239	242	228	[illegible]
Leucine	392	396	395	409	401	409	408	398	401
Lysine	155	155	170	172	164	159	151	172	162
Methionine	75	78	78	83	78	79	80	83	79
Phenylalanine	278	284	269	281	279	264	261	254	271
Proline	618	589	588	634	598	584	605	581	600
Serine	328	322	325	323	320	319	313	318	321
Threonine	175	170	164	163	164	169	166	173	168
Tryptophan	62	64	66	65	69	66	64	74	66
Tyrosine	180	190	188	178	191	178	191	174	184
Valine	284	302	300	288	330	311	305	316	304

concentrations of nitrogen. Varieties Bison, Comanche, and Pawnee were grown at Alliance, Nebraska; Concho and Kaw, at Garden City, Kansas; Omaha and Ottawa, at Fort Collins, Colorado; and Tascosa, at Cherokee, Oklahoma. The spring wheats were all grown in North Dakota: Conley and Thatcher, at Fargo; Lee, at Minot; and Selkirk, at Langdon.

For the final study, pairs of five winter wheat varieties were selected which afforded the greatest differences in their nitrogen content. Because only one spring wheat variety was represented at comparable extremes of nitrogen, the sample of highest nitrogen concentration and that of lowest were also included. Of the higher-protein group (Table IV) varieties Thatcher and Canthatch were grown at Williston, North Dakota, and all others at Akron, Colorado. Of the low-protein group (Table V) Canthatch was grown at Fargo, North Dakota; Selkirk at

TABLE III

AMINO ACID CONTENT OF VARIETIES OF HARD RED SPRING WHEAT IN SAMPLES OF SIMILAR NITROGEN CONTENT

	CONLEY	LEE	SELKIRK	THATCHER	AVERAGE
Nitrogen content, % (14% moisture basis)	2.43	2.44	2.42	2.35	
Amino acid content, mg./g. N					
Alanine	212	200	209	208	207
Arginine	291	287	259	272	277
Aspartic acid	307	315	290	305	304
Cystine	121	116	112	110	115
Glutamic acid	1,879	1,891	1,918	1,849	1,884
Glycine	251	234	246	248	245
Histidine	121	122	117	119	120
Isoleucine	231	241	245	247	241
Leucine	390	400	398	400	397
Lysine	176	154	171	166	167
Methionine	91	97	84	84	89
Phenylalanine	265	260	280	258	266
Proline	602	600	618	599	605
Serine	331	327	321	328	327
Threonine	174	167	159	179	170
Tryptophan	79	64	71	76	72
Tyrosine	164	169	184	183	175
Valine	312	300	314	305	308

TABLE IV

AMINO ACID CONTENT OF HARD WHEAT VARIETIES IN SAMPLES OF HIGHER NITROGEN CONTENT

	THATCHER	CANTHATCH	BISON	PAWNEE	COMANCHE	CONCHO	AVERAGE
Nitrogen content, % (14% moisture basis)	3.34	3.13	3.04	3.04	2.98	2.97	
Amino acid content, mg./g. N							
Alanine	208	206	189	201	196	194	199
Arginine	281	298	280	293	296	287	289
Aspartic acid	304	310	278	305	295	302	299
Cystine	122	116	119	111	112	119	116
Glutamic acid	1,694	1,792	1,869	1,854	1,894	1,764	1,811
Glycine	241	256	247	238	256	234	245
Histidine	113	117	106	114	113	110	112
Isoleucine	227	223	224	226	230	226	226
Leucine	379	381	386	379	391	384	383
Lysine	163	169	154	159	168	172	164
Methionine	91	96	85	94	103	89	93
Phenylalanine	293	308	298	305	288	276	295
Proline	664	651	638	627	652	634	644
Serine	268	272	259	255	270	249	262
Threonine	154	153	147	145	159	148	151
Tryptophan	55	58	54	66	56	58	58
Tyrosine	179	172	185	172	182	187	179
Valine	294	269	261	282	276	266	275

TABLE V

AMINO ACID CONTENT OF HARD WHEAT VARIETIES IN SAMPLES OF LOWER NITROGEN CONTENT

	Canthatch	Selkirk	Bison	Pawnee	Comanche	Concho	Average
Nitrogen content, % (14% moisture basis)	2.26	2.21	1.98	1.97	1.92	1.76	
Amino acid content, mg./g. N							
Alanine	204	209	212	218	219	226	215
Arginine	322	304	286	336	319	324	315
Aspartic acid	330	318	322	339	341	353	334
Cystine	122	138	125	135	125	134	130
Glutamic acid	1,691	1,800	1,730	1,758	1,786	1,679	1,741
Glycine	272	267	273	256	278	260	268
Histidine	114	109	108	115	114	112	112
Isoleucine	229	228	228	244	239	249	236
Leucine	406	432	400	421	404	409	412
Lysine	163	163	180	182	188	184	177
Methionine	101	109	98	105	108	109	105
Phenylalanine	264	269	264	262	266	262	264
Proline	610	621	612	583	629	632	614
Serine	288	276	312	294	314	299	297
Threonine	171	170	168	174	164	187	172
Tryptophan	73	65	65	69	75	72	70
Tyrosine	178	178	174	201	177	188	183
Valine	277	292	281	296	291	311	291

Minot, North Dakota; Comanche at Garden City, Kansas; and the remainder at North Platte, Nebraska.

Procedures. All wheat samples were ground in a hammer mill to pass a 0.024-in. screen. Nitrogen was determined by the Kjeldahl-Gunning procedure, and moisture by the conventional air-oven method.

The 18 commonly occurring amino acids were determined in all samples by microbiological analysis using the procedures described previously (7,8). Tryptophan was assayed after hydrolysis with barium hydroxide. Acid hydrolysis was effected by autoclaving at 125°C., using 250 ml. of hydrochloric acid solution per g. of sample. Cystine was determined after hydrolysis with 2*N* HCl for 2 hr. For the determination of the remaining amino acids, the commercial wheat samples were hydrolyzed with 2.5*N* HCl for 8 and 12 hr. and the individual wheat varieties were hydrolyzed with 4*N* HCl for 8 and 24 hr. After hydrolysis the excess HCl was removed by vacuum distillation. The residue was taken up in water, adjusted to pH 4 with concentrated KOH, diluted to the appropriate volume, and filtered through Whatman No. 42 paper. The filtrates were brought to pH 6.8 prior to microbiological assay.

The samples of individual varieties were also analyzed by ion-ex-

change chromatography. The improved system of Moore, Spackman, and Stein (9) was employed, with the fraction collector procedure. As suggested by these authors, buffer flow was controlled with a constant-volume pump to permit the use of the finer-sized resin particles normally employed with the automated procedure. Samples were hydrolyzed for chromatographic analysis by autoclaving for 24 hr. with 4*N* and 6*N* HCl. The subsequent preparatory steps were conducted as with the hydrolysates for microbiological assay, except that after filtration at pH 4 the filtrates were brought to pH 2.2 with concentrated HCl.

Results

As indicated in the tables, all values for nitrogen are expressed on the 14% moisture basis. Amino acid data are expressed as mg. per g. of nitrogen in accordance with the suggestion of the FAO (10). The data of Table I were determined only by microbiological analyses. No differences were found between the 8- and 12-hr. hydrolysates, and the reported values represent the averages of analyses from both preparations.

Similarly, microbiological assays of the individual wheat varieties showed no consistent differences between the 8- and 24-hr. hydrolysates except with serine, for which the longer hydrolysis time resulted in values 10–20% lower than those obtained after 8-hr. hydrolysis of all samples. Data obtained by chromatographic analysis support the conclusion that this difference reflects the destruction of serine during prolonged hydrolysis. Comparably low serine values were found by chromatography of 24-hr. hydrolysates, but a limited number of analyses performed on samples hydrolyzed for 8 hr. with 4*N* HCl produced values for serine approaching those obtained by microbiological assay, even though values for most other amino acids were lower because of incomplete hydrolysis. Serine values presented in Tables II–V are based therefore upon results obtained by microbiological assay of 8-hr. hydrolysates.

In addition to the values for tryptophan (which is destroyed during acid hydrolysis) and serine, those for cystine and methionine in Tables II–V are given as determined only by microbiological assay. Less confidence was placed in chromatographic results for the sulfur amino acids because of erratic variations in replicate determinations. The cause is believed to be related to their partial oxidation and to the difficulty of accurately accounting for the oxidative products, as well as the relatively low concentration of these amino acids in wheat.

Chromatographic analyses showed no differences between the 4*N* and 6*N* HCl hydrolysates of wheats. The results for all other amino

acids were in excellent agreement with those found by microbiological assay, and the values obtained by both methods were averaged for presentation in Tables II–V.

The amino acids account for approximately 75% of the total Kjeldahl nitrogen of all wheats in Tables I–V with a range of 72–77%. Amide nitrogen, the principal source of remaining nitrogen, was not determined specifically. The ammonia content of the 24-hr. acid hydrolysates would be expected to include that arising from the partial decomposition of amino acids, especially serine, as well as from the amides. Ammonia was not measured quantitatively in all sample hydrolysates, but values obtained for 19 of the wheats indicated that an average of 21% of the total nitrogen appeared in this form. If it is assumed that glutamic acid and aspartic acid are present in the wheat entirely as glutamine and asparagine, respectively, it can be calculated that amide nitrogen would supply an average of 20% of the total nitrogen. Under the same assumption, if the respective amide nitrogen contribution is added to the sum of amino acid nitrogen for each wheat in Tables I–V, an average of 95% with a range of 92–98% of the Kjeldahl nitrogen is accounted for.

Commercial Wheats. The values for the commercial wheats (Table I) are remarkably consistent for most of the amino acids despite the range in wheat type and nitrogen content. Trends which appear to correlate best with changes in total nitrogen are evident from the data for glutamic acid, which tend to be lower in samples of lower nitrogen, and from those of lysine and tryptophan, which show the reverse tendency. Other amino acids showing more pronounced variations in their concentrations, such as arginine, cystine, phenylalanine, and proline, are not consistent with differences in total nitrogen and suggest possible differences between wheat types.

Individual Hard Wheat Varieties. In samples of similar nitrogen content, differences in amino acid concentrations among varieties of winter wheat (Table II) and among those of spring wheat (Table III) were very small. Average values from each of the two tables show very close agreement between winter and spring wheats for all amino acids except cystine and methionine. Data for the latter suggest that the spring wheat varieties may contain greater amounts of the sulfur-containing amino acids.

Greater differences are evident between the samples of higher and lower nitrogen content (Tables IV and V). A comparison of the average values from both tables shows that most of the amino acids tended toward greater concentrations in the samples of lower nitrogen content. Tryptophan exhibited an average difference of only 12 mg./g.

of nitrogen but represented the largest percentage change because of its small concentration. Only the amounts of glutamic acid, phenylalanine, and proline tended to vary in the direction of nitrogen difference. The amino acids which showed the least tendency to vary with nitrogen content were histidine, isoleucine, and tyrosine. Differences between the two sets of values are more apparent when the pairs of varieties are compared, because the effect of varietal differences is minimized. For example, the values for methionine in both samples of Bison fall below the averages for each set of data in Tables IV and V and appear to disrupt a trend toward increased amounts at lower nitrogen values; but the difference between these two samples is of the same magnitude and direction as found with the other varieties.

Discussion

The changes in the relative proportions of amino acids as the protein content of wheat varies are undoubtedly explained by changes in the proportions of the various constituent proteins of the wheat. Lawrence *et al.* (6) stated that the proportion of endosperm proteins tended to be lower in wheats of low total protein, and that the endosperm proteins of low-protein wheat tend to have a higher concentration of lysine. Studies with protein fractions of wheat (11,12) reveal that the separated components may differ in their amino acid composition and that the proportions of these proteins vary with the total nitrogen of the sample. This method of approach appears to offer measurements of much greater sensitivity as compared to analyses of the entire-wheat protein.

The results obtained with individual varieties in the present study must be interpreted with caution. The data indicate that differences in amino acid concentration are more influenced by the protein level than by the particular variety. However, the nitrogen variations are more extreme than are likely to be encountered in hard wheat blends intended for the production of bread flour. Furthermore, it cannot be assumed that these changes occur linearly with nitrogen level. Lawrence *et al.* (6) found lysine increased significantly with decreasing protein content when the latter was less than 13.5%, but samples containing greater than this level of protein did not exhibit a significant correlation. A portion of the effect of nitrogen change must also be referred to the change in the percentage of alpha amino nitrogen at different nitrogen levels. This fact may account for the observation that the majority of the amino acids tended to be higher in the samples of lower total nitrogen content.

The trends in the changes of amino acid concentrations with

change of wheat nitrogen are generally in the same directions as noted by Sosulski *et al.* (13), but the extent of variation differs for some amino acids. These workers did not find phenylalanine to be dependent upon wheat protein level, in contrast to the results reported here; but greater effects were found for other amino acids, particularly arginine, lysine, histidine, and glutamic acid. The Thatcher wheat used in their studies was grown in a growth chamber, and variations in protein content were induced by regulating the water supply, nitrogen fertilization, and air temperature. It is possible that one or more of these factors is responsible for the differences noted.

From the standpoint of nutritional interest, the importance of the present data is the relatively small degree of change in amino acid proportions with differences in total nitrogen. Although the nitrogen of the samples in Table V is approximately one-third less for each variety than that for the corresponding samples of Table IV, the greatest changes in amino acid concentrations (except for tryptophan) amount to approximately 10% or less. It is obvious that the total amino acid contribution of any given sample of wheat is determined primarily by the amount of protein it contains; a high-protein wheat would contribute greater total amounts of each amino acid than would a wheat of distinctly lower protein. The constancy of amino acid values between varieties and between types of hard wheat also serves to increase confidence in the reliability of using typical analyses for evaluation of the amino acid contribution of wheat in diets.

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