Amino Acid Composition of Selected High-Protein Wheats[1]

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ABSTRACT

We evaluated 15 productive high-protein winter wheat lines from the cross Atlas 66 × Comanche and two lines from the cross Atlas 66 × Wichita for amino acid content of their seed. The average protein level of the grain of Comanche-derived high-protein lines was 18.9% higher than that of Comanche in 1965 and 15.4% higher in 1966. The Wichita-derived lines averaged respectively 16.0 and 24.5% more protein in their grain than Wichita in 1965 and 1966. The quantitative relationships of individual amino acids tended to be similar in the parents and their progeny lines. Therefore, the high-protein lines in which the quantitative balance of amino acids was unchanged could be considered nutritionally superior to the lower-protein parent varieties. Some high-protein lines exhibited enough variation in the amount of individual amino acids to suggest that there could be effective selection for improved amino acid balance.

The quantitative aspects of protein in wheat (*Triticum aestivum* L.) have been under co-operative study by the Nebraska Agricultural Experiment Station and the Agricultural Research Service, U.S. Department of Agriculture, since 1956 (1,2,3,4). From crosses of Atlas 66, a high-protein soft wheat developed by the North Carolina Experiment Station (5,6), with the hard winter varieties Comanche and Wichita, we selected productive lines that were significantly higher in grain protein than the hard wheat parents (7). The magnitude of the protein increase was from 2 to 3% actual protein. The higher level of grain protein was attainable without sacrifice of wheat yield. An array of quality types was recovered among the selected high-protein lines. Some lines approached the Comanche and Wichita varieties (hard wheat parents) in over-all bread wheat milling and baking characteristics.

In many areas of the world, wheat is the major source of protein in the diet. The nutritional adequacy of wheat should be of major importance in the characterization of its quality in these areas. Higher protein through breeding can be achieved in wheat without drastic alteration of accepted quality standards (7). The nutritional value of higher-protein wheat would be enhanced on the basis of protein quantity alone, provided that the additional protein was not associated with undesirable shifts in the balance of essential amino acids.

This study was undertaken to determine the effect of high protein in wheat on the amino acid composition of the protein. We assayed the

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amino acids in selected high-protein lines and in the parental varieties Atlas 66, Wichita, and Comanche. Grain produced in 1965 was analyzed by microbiological methods for lysine, methionine, and threonine, all of which usually occur in limited quantity in wheat. Grain of the same lines grown in 1966 was again analyzed for lysine, methionine, and threonine and for several additional amino acids by ion-exchange chromatography.

MATERIALS AND METHODS

Atlas 66 is not sufficiently winter-hardy to be grown in Nebraska. Seed of Atlas 66 used in this study was produced in North Carolina in 1963. Comanche and Wichita, the other parental varieties, and the selected experimental lines were grown at Lincoln, Nebraska, in 1965 and 1966.

Grinding

Wheat samples produced in 1965 were ground with an intermediate Wiley mill through a 40-mesh screen. The 1966 samples were ground with the Udy Cyclone hammer mill through a 0.024-in. screen.

Hydrolysis for Microbiological Determinations

Ground wheat samples (200 mg.) for microbiological studies were dispersed in 5 ml. of 6*N* hydrochloric acid in a 20-ml. Pyrex tube. The contents were frozen and the tubes were evacuated and sealed. Hydrolysis was carried out in an oven at 110° ± 2°C. for 20 hr. After hydrolysis, the contents were filtered through Whatman No. 42 filter paper, washed carefully with 100 ml. of water, and evaporated almost to dryness in a rotary evaporator at 55°–60°C. under vacuum. Four separate additions of 25 ml. of water were made, and the evaporation process was repeated after each addition to remove all traces of the hydrolyzing acid. The final residue was washed into a beaker and diluted to 20 ml. with distilled water. The pH of the hydrolysate was approximately 2.0 ± 0.1. Following the procedure of Horn *et al.* (8), we precipitated interfering substances by adjusting the hydrolysate to pH 4 with 1*N* potassium hydroxide. The hydrolysates were brought to 25-ml. volume and filtered through Whatman No. 42 filter paper. A 20-ml. aliquot was adjusted to a final pH of 6.8, increased to 25-ml. volume with distilled water, and stored under toluene at 5°C. Following the recommendation of Chitre *et al.* (9), we maintained sodium content at a low level by using potassium hydroxide in place of sodium hydroxide in all adjustments of pH.

The test organisms were *Leuconostoc mesenteroides* P-60 for lysine and methionine assay and *Streptococcus faecalis* for threonine. They were maintained by subculture every 2 weeks in a Bacto Micro-Inoculum Broth followed by a stab in agar media.

The assay procedures were those described in the Difco Manual (10) with certain modifications. We used Difco dehydrated assay media for the three amino acids. The cells were washed twice with sterile saline solution (0.9%) and suspended in 100 ml. of the same sterile saline solution. We used 1 drop of this suspension to inoculate each of the assay tubes. These extra washings of the cells were necessary for low blank (11).

Standard curves were established for each set of assays with triplicate

tubes containing 10 levels of amino acids. The L-form of the amino acids was used for preparation of standard solutions. L-lysine hydrochloride was used in the range 0–50 mg./tube, L-methionine was used in the range 0–20 mg./tube, and L-threonine was used in the range 0–25 mg./tube. Total tube volumes of 2 ml. were used for all assays. After incubation for 66–72 hr. at 37°C., the lactic acid produced was titrated electrometrically with 0.05*N* sodium hydroxide.

Hydrolysis for Ion-Exchange Amino Acid Analysis

Ground wheat samples (100 mg.) for the amino acid analyzer were dispersed in 10 ml. of 6*N* HCl in a 25-ml. Pyrex test tube. The contents were frozen, and tubes were evacuated and sealed. Hydrolysis was carried out in an oven held at 110° ± 2° for 24 hr. Tube contents were filtered, evaporated to dryness in a rotary evaporator, and placed in a vacuum desiccator over NaOH for 24 hr. Hydrolysates were dissolved in 10 ml. of pH 2.2 citrate buffer and refiltered. Aliquots were analyzed for their amino acid content (12) with a Beckman-Spinco Model 120C amino acid analyzer.

The amino acid values were not corrected for losses due to instability or slow release during acid hydrolysis. However, samples were processed uniformly and the values obtained were relative and, therefore, valid for purposes of comparison.

Protein

Nitrogen was determined by the Gunning Kjeldahl method. Total nitrogen × 5.7 was used to convert nitrogen to protein values.

RESULTS AND DISCUSSION

We made preliminary studies on hydrolysates prepared in triplicate to test the precision of the microbiological method employed on 1965-grown material. To test the accuracy of the method, we studied known levels of amino acids with three levels of hydrolysate. Recovery of the amino acids approached 100%. Similar recovery of the amino acids at different levels of hydrolysate indicated a valid assay and absence of any inhibitory factor in the hydrolysate.

Protein, Lysine, Methionine, and Threonine Comparisons

Data from the analyses of wheat samples produced in 1965 by microbiological methods, along with data on the same samples grown in 1966 and analyzed by ion-exchange chromatography, are given in Table I. Protein values for Wichita and Comanche produced in 1965 were 14.0 and 14.3%, respectively. Their high-protein selections ranged from 15.3 to 18.7% protein. By comparison, the protein content of Atlas 66 was 18.3%.

The Wichita and Comanche varieties grown in 1966 produced seed with protein contents of 14.1 and 15.0%, respectively. The high-protein progeny ranged from 16.2 to 18.3%. The superiority in protein content of the high-protein lines over the low-protein parents was typical of observations made at Lincoln in other years of study (1,2,3,4).

The values for lysine and methionine obtained for samples grown in 1965 and analyzed by microbiological methods agree reasonably well with values for these amino acids in the literature (13,14,15,16). However,

TABLE I

LEVELS OF TOTAL PROTEIN, LYSINE, METHIONINE, AND THREONINE IN THE GRAIN OF HIGH-PROTEIN SELECTIONS GROWN IN TWO DIFFERENT YEARS AT LINCOLN, NEBRASKA[a]

	SAMPLE		C.I. OR SELECTION NO.	PROTEIN		LYSINE		METHIONINE		THREONINE	
	1965	1966		1965	1966	1965	1966	1965	1966	1965	1966
				%	%	*g./100 g. protein*	*g./100 g. protein*	*g./100 g. protein*	*g./100 g. protein*	*g./100 g. protein*	*g./100 g. protein*
Atlas 66			12561	18.3[b]	....	3.3	3.3	1.8	1.1	3.3	3.4
Wichita	1555	2497	11952	14.0	14.1	3.3	3.2	1.8	1.6	4.5	3.4
Comanche	1556	2498	11673	14.3	15.0	3.4	3.2	1.9	1.7	4.5	3.5
Atl. 66 × Cmn	1557	2499	631005	17.8	18.2	3.5	3.3	1.8	1.7	4.3	3.1
	1558	2500	631041	16.6	18.3	3.4	3.2	1.7	1.7	4.5	3.2
	1559	2501	631068	18.1	16.2	3.4	3.2	1.8	1.9	4.4	3.2
	1560	2502	631099	18.7	17.7	3.2	3.0	1.7	1.5	4.2	3.2
	1561	2503	631250	17.2	16.4	3.3	3.3	1.8	1.2	4.5	3.4
	1562	2504	631260	18.4	17.9	3.2	3.4	1.6	1.1	4.3	3.7
	1565	2507	631273	16.9	17.2	3.5	3.7	1.7	1.7	4.5	2.6
	1566	2508	631281	16.2	16.8	3.4	3.7	2.1	1.8	4.8	3.2
	1567	2509	631284	16.6	18.3	3.6	3.5	2.0	1.8	4.8	3.3
	1568	2510	631343	15.9	16.5	3.7	3.4	2.1	1.7	4.8	3.2
	1569	2511	631388	18.0	17.8	3.5	3.5	2.0	1.9	4.7	3.3
	1570	2512	631413	16.4	17.0	3.7	3.2	1.9	1.7	4.3	3.4
	1571	2513	631417	15.3	17.4	3.5	3.4	2.0	1.7	4.8	3.2
	1572	2514	631423	16.3	17.1	3.7	3.4	1.7	1.9	4.3	3.3
	1573	2515	631370	15.6	17.5	3.4	3.4	1.9	1.7	4.5	3.1
Mean				16.9	17.4	3.5	3.4	1.9	1.7	4.5	3.2
Atl. 66 × Wi	1574	2516	631168	16.8	17.2	3.4	3.5	2.0	1.4	4.3	3.1
	1576	2517	631206	15.7	17.8	3.9	3.4	2.1	1.8	4.6	3.2
Mean				16.3	17.5	3.7	3.5	2.1	1.6	4.5	3.2
Mean of all HP selections				16.8	17.4	3.5	3.4	1.9	1.7	4.5	3.2

[a] Dry weight basis.
[b] Grown in North Carolina, 1963.

threonine contents of parental varieties and progeny of the 1965 samples were 25 to 30% higher than literature values. Duplicate analyses for threonine with newly prepared standards still gave high values.

Values obtained from samples grown in 1966 and analyzed by ion-exchange chromatography agree well with literature values (13,14,15,16) for all three of the amino acids. Although there was general similarity in amino acid content of the low-protein parents and their high-protein progeny, some selections were higher than Wichita and Comanche in both lysine and methionine but somewhat lower in threonine.

Percentage Differences

The percentage differences between the amino acid content of the protein for the low-protein parents and the high-protein lines grown in 1966 are given in Table II. Only two lines were lower in lysine than their low-

TABLE II

PERCENT DIFFERENCE IN AMINO ACID CONTENT IN PROTEIN BETWEEN HIGH-PROTEIN LINES AND THEIR LOW-PROTEIN PARENT (1966 CROP)

SAMPLE	DIFFERENCE			SAMPLE	DIFFERENCE		
	Lysine	Methionine	Threonine		Lysine	Methionine	Threonine
	%	%	%		%	%	%
Atl 66 × Cmn				Atl 66 × Cmn			
2499	1.9	0.6	−12.4	2511	8.7	12.0	−6.5
2500	0.9	−1.2	−10.7	2512	−1.9	−1.2	−4.8
2501	0.0	12.0	−10.7	2513	6.5	1.8	−9.0
2502	−6.5	−12.6	−10.2	2514	5.0	13.8	−7.9
2503	2.8	−28.1	−4.2	2515	6.2	0.6	−13.0
2504	4.6	−31.7	4.2				
2507	15.2	4.2	−26.0	Atl 66 × Wi			
2508	13.0	10.2	−10.7	2516	11.7	−13.8	−9.6
2509	6.8	9.6	−6.2	2517	6.6	10.6	−8.1
2510	4.3	0.0	−9.0	Mean	5.0	−0.8	−9.1

protein parent. Six of seventeen lines were lower in methionine and all but one line were lower in threonine. These percentages, which range from +15.2 to −31.7%, tend to be magnified because of the relatively low levels of amino acids.

Lysine values of the high-protein lines range from 6.5% lower to 15.2% higher than the low-protein parent, with a mean advantage of +5.0%. Methionine values are less stable, and high-protein lines range from 31.7% lower to 13.8% higher than the low-protein parent. The mean value for methionine was 0.8% lower than the parent value. Threonine values are almost all lower than those of the low-protein parent, ranging from −26.0% to +4.2%, with a mean difference of −9.1%.

Percentage Increases Based on Grain Weight

The most important nutritional consideration is the total amount of an essential amino acid in a given weight of grain. Such values, for lysine, methionine, and threonine expressed as percentage increases over the low-protein parent, appear in Table III.

Protein content of the highest-protein line of Atlas 66 × Comanche was 22.0% higher than Comanche in 1966. The best Atlas 66 × Wichita

TABLE III

PERCENTAGE INCREASE IN TOTAL PROTEIN, LYSINE, METHIONINE, AND THREONINE IN HIGH-PROTEIN LINES OVER THE LOW-PROTEIN PARENT IN A GIVEN WEIGHT OF WHEAT (1966 CROP)

SAMPLE	INCREASE				SAMPLE	INCREASE			
	Protein	Lysine	Methionine	Threonine		Protein	Lysine	Methionine	Threonine
	%	%	%	%		%	%	%	%
Atl 66 × Cmn					Atl 66 × Cmn				
2499	21.3	23.1	21.6	5.8	2511	18.7	28.9	32.8	10.9
2500	22.0	20.5	20.2	8.6	2512	13.3	11.0	11.8	7.7
2501	8.0	8.0	20.5	−3.9	2513	16.0	23.2	17.8	5.2
2502	18.0	10.2	3.0	5.9	2514	14.0	19.2	29.2	4.6
2503	9.3	12.0	−21.5	4.7	2515	16.7	23.7	17.1	1.3
2504	19.3	24.6	−18.7	24.4					
2507	14.7	31.6	19.0	−15.5	Atl 66 × Wi				
2508	12.0	26.6	23.4	0.0	2516	22.0	36.6	5.6	10.7
2509	22.0	30.1	33.4	14.2	2517	26.2	34.9	40.0	16.2
2510	10.0	14.8	10.0	0.0	Mean	16.7	22.3	15.6	5.9

line had 26.2% more protein in its grain than Wichita. Although not shown in Table III, protein increases of comparable magnitude were recorded in 1965. The largest increase for Atlas 66 × Comanche lines was 30.7% in 1965 and for Atlas 66 × Wichita lines, 19.9%.

The magnitude of amino acid increases was similar to the protein increases. The amounts of lysine in a given weight of grain of the high-protein lines average 22.3% more than the amount in their low-protein parent, with a range of 8.0 to 36.6%. Methionine ranges from −21.5 to +40.0%, and averages 15.6% more than the low-protein parent. Actual percentage levels of threonine (Table II) were lower than that of the low-protein parent. However, the low values tend to disappear when amounts of threonine in a given weight of high-protein wheat are compared to the parental variety (Table III). Increases for threonine average 5.9% and range from −15.5 to +24.4%. These findings are in agreement with those of Hepburn and Bradley (17), who state that the total amino acid contribution of any sample of wheat is determined primarily by amount of protein.

Comparisons of 17 Amino Acids

Table IV lists analyses for 17 amino acids in high-protein wheats and their parents. There is no evidence that lysine values decrease with higher protein levels. The percent of lysine in Wichita and Comanche was 3.2 and 3.2%, respectively. These values are less than the average of 3.4% for all high-protein samples. Lawrence *et al.* (18) reported that lysine decreased significantly with increasing protein contents up to 13.5% protein. The high-protein wheats in this study exceeded the 13.5% protein level.

Methionine values of 1.1, 1.2, and 1.1 g. per 100 g. of protein for Atlas 66 and selections 2503 and 2504, respectively, are below the average value of 1.7 for all samples. The average percentage of threonine, 3.2%, was lower than Atlas 66, Wichita, or Comanche (3.4, 3.4, and 3.5%, respectively). However, selection 2504 had a threonine value of 3.7%, which was higher than any of the three varieties.

TABLE IV

AMINO ACID COMPOSITION OF SELECTED HIGH-PROTEIN WHEATS AND THEIR PARENT VARIETIES GROWN IN 1966[a]

AMINO ACID	ATL 66	WI	CMN	2499	2500	2501	2502	2503	2504	2507
Lys	3.3	3.2	3.2	3.3	3.2	3.2	3.0	3.3	3.4	3.7
Hist	2.9	2.7	2.8	2.9	2.9	2.9	2.7	2.8	3.0	3.0
NH_3	4.6	4.1	4.5	4.2	4.3	4.1	4.1	4.5	4.6	4.2
Arg	5.6	5.5	5.5	5.5	5.4	5.5	5.2	5.5	5.8	5.6
Asp	5.7	5.6	6.3	5.7	5.5	6.0	5.4	5.8	6.5	6.0
Thre	3.4	3.4	3.5	3.1	3.2	3.2	3.2	3.4	3.7	2.6
Ser	5.6	5.0	5.7	4.9	5.0	4.8	4.9	5.0	6.1	4.6
Glut	36.8	34.2	36.1	35.2	36.2	36.3	35.3	35.8	35.7	32.8
Prol	12.7	12.1	12.6	13.0	12.2	12.7	12.2	12.0	12.7	12.4
Glyc	4.7	4.4	4.6	4.3	4.4	4.5	4.4	4.4	4.9	4.5
Alan	3.9	3.7	3.7	3.7	3.6	3.7	3.6	3.7	4.4	3.8
½ cys	1.8	2.0	1.9	2.0	2.0	2.2	1.8	1.7	2.0	2.0
Val	4.6	4.3	4.6	4.6	4.5	4.7	4.5	4.7	5.4	4.7
Meth	1.1	1.6	1.7	1.6	1.7	1.9	1.5	1.2	1.1	1.7
iLeu	3.8	3.9	3.9	3.8	3.8	3.9	4.0	3.8	4.4	4.0
Leu	7.6	7.8	7.5	6.8	7.4	7.6	7.4	7.1	8.2	7.5
Tyr	3.8	3.7	3.9	4.0	3.8	4.0	3.1	3.7	4.2	3.8
Phen	5.3	5.5	5.6	5.9	5.4	5.6	5.3	5.0	6.0	5.3
Prot. % d.w.	18.0	14.1	15.0	18.2	18.3	16.2	17.7	16.4	17.9	17.2

	2508	2509	2510	2511	2512	2513	2514	2515	2516	2517
Lys	3.7	3.5	3.4	3.5	3.2	3.4	3.4	3.4	3.5	3.4
Hist	3.0	3.1	2.9	3.1	2.7	3.0	2.8	3.0	3.0	3.1
NH_3	4.3	4.6	4.0	4.3	4.0	4.1	4.1	4.2	4.1	4.3
Arg	5.6	5.6	5.6	5.7	5.4	5.5	5.7	5.6	5.8	5.6
Asp	5.2	6.2	6.2	6.2	5.6	6.2	6.5	6.2	6.7	6.2
Thre	3.2	3.3	3.2	3.3	3.4	3.2	3.3	3.1	3.1	3.2
Ser	4.6	4.7	4.7	4.7	5.0	4.7	4.1	4.5	4.2	4.6
Glut	35.5	36.1	35.6	35.6	36.2	35.5	35.8	36.1	34.5	36.7
Prol	12.5	12.4	12.2	12.5	12.7	12.6	12.6	12.6	11.9	12.7
Glyc	4.6	4.7	4.5	5.6	4.4	4.6	4.7	4.4	4.5	4.4
Alan	3.7	3.9	3.7	3.9	3.7	3.8	4.0	3.7	4.0	3.7
½ cys	2.1	2.5	2.2	2.1	2.1	2.2	2.1	2.1	2.3	2.1
Val	4.7	4.7	4.6	4.7	4.6	4.7	4.8	4.6	4.8	4.7
Meth	1.8	1.8	1.7	1.9	1.7	1.7	1.9	1.7	1.4	1.8
iLeu	4.0	3.9	4.0	4.0	3.9	3.8	4.0	3.9	3.9	4.0
Leu	7.8	7.7	7.5	7.7	7.4	7.5	8.0	7.5	7.3	7.5
Tyr	4.0	3.9	3.8	4.0	3.9	4.1	4.0	4.0	3.8	3.9
Phen	5.5	5.5	5.6	5.9	5.6	5.5	5.7	5.7	5.5	5.7
Prot. % d.w.	16.8	18.3	16.5	17.8	17.0	17.4	17.1	17.5	17.2	17.8

[a]Data expressed as g. of amino acid per 100 g. protein, dry weight basis. Abbreviations over first three columns: Atlas 66; Wichita; Comanche.

Selection 2509 exhibits the best balance of all three amino acids. Our data indicate that high protein in wheat need not be associated with an altered, less favorable, amino acid balance, and that selection for improved amino acid balance might be effective in high-protein materials.

Protein levels in wheat can be improved genetically. Wheats with higher protein contents derived from Atlas 66 are similar in amino acid composition to conventional wheats. There has been no nutritional penalty in the high-protein lines for the amino acids lysine, methionine, and threonine.

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