Amino Acid Composition of Maturing Barley[1]

Y. POMERANZ and G. S. ROBBINS[2], National Barley and Malt Laboratory, U.S. Department of Agriculture, Madison, Wisconsin 53705

ABSTRACT

Three barleys were harvested in 1969 and in 1970 between 6 to 39 days after heading; freeze-dried; hand-threshed; and analyzed for amino acid composition. No significant differences in amino acid composition were found between cultivars harvested at comparable stages of maturity. During maturation, increases were largest in glutamic acid, proline, and cystine; and decreases were largest in alanine, lysine, aspartic acid, and threonine. A review of literature indicated similar changes in maturing oats and wheat; during malting of barley, the main changes were increases in concentrations of lysine and aspartic acid, and decrease in glutamic acid.

Gross changes in proteins of maturing barley have been the subject of several investigations (1,2,3). We have reported recently on changes that take place in malting and brewing characteristics (4) and in peptidases (5) of developing barleys. This report concerns the changes in total amino acids of barleys harvested at various stages of maturity in 1969 and 1970.

MATERIALS AND METHODS

Three leading six-row malting barleys (*Hordeum vulgare* L.) were grown in Madison, Wis., in 1969 and 1970. They were the white-aleurone cultivars 'Larker' and 'Dickson', and the blue-aleurone cultivar 'Conquest'. Eleven samples of Larker and Dickson, and ten samples of Conquest were obtained in 1969 during the period

[1]Cooperative investigations between the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, University of Wisconsin, Madison. The work of the Barley and Malt Laboratory is supported in part by an Industrial Research Grant from the Malting Barley Improvement Association.

Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty 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]Research Chemists, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture.

from 7 to 37 days after heading. Five samples were obtained from each cultivar in 1970; they included two samples at early stages of development, and one sample (for each cultivar) harvested at physiological maturity, combine-ripe, and about 1 week after combine-ripe. Primary spikes were hand-cut from the field, freeze-dried to about 12% moisture, and deawned. Moisture contents at the time of harvest, kernel weights of freeze-dried kernels, and protein contents of samples from various stages of maturity in 1969 and 1970 are given in Fig. 1 and Table I, respectively. While averages given in Fig. 1 reflect trends and changes in samples from the three cultivars from 1969, some consistent varietal differences were noted. Thus, for

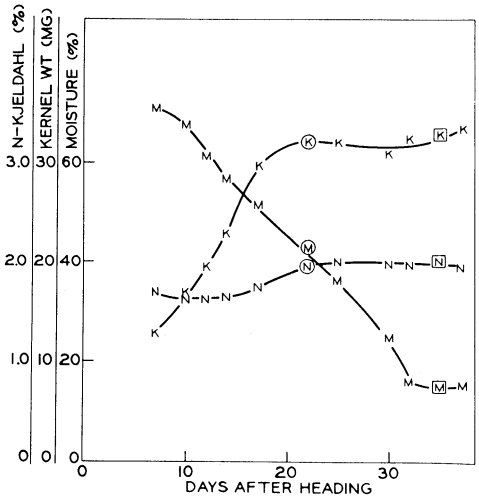


Fig. 1. Some physical and chemical characteristics of barleys harvested at various stages of development in 1969 (averages for three varieties; M = moisture, %, K = weight in mg. of kernels dried to a moisture of about 12%, and N = Kjeldahl-N, %, on a moisture-free basis). Circled figures denote samples at physiological maturity; squared figures, combine-ripe samples.

TABLE I. SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF BARLEYS HARVESTED AT VARIOUS STAGES OF DEVELOPMENT IN 1970

Days After Heading	Larker			Dickson			Conquest		
	Kernel weight[a] mg.	Moisture %	N[b] %	Kernel weight[a] mg.	Moisture %	N[b] %	Kernel weight[a] mg.	Moisture %	N[b] %
6–7	12.5	79.4	1.51	10.3	78.1	1.48	9.0	82.7	1.34
13–14	24.1	60.2	1.33	22.1	66.8	1.29	19.7	70.4	1.31
23[c]	32.1	35.8	1.73	31.9	36.1	1.50	28.4	29.2	1.64
32[d]	33.3	16.4	1.63	31.4	14.9	1.52	28.6	15.1	1.69
39	33.4	14.8	1.52	32.0	15.0	1.53	27.8	15.0	1.71

[a]In kernels dried to about 12% moisture.

[b]On a moisture-free basis.

[c]Physiologically mature.

[d]Combine-ripe.

instance, Conquest kernels were consistently smaller than kernels of Larker and Dickson barleys (about 30, 33, and 34 mg. in mature Conquest, Larker, and Dickson, respectively). Mature Conquest kernels contained more Kjeldahl-N (2.1%) than mature Larker (2.0%) and Dickson (1.9%) kernels. Conquest barley matured 2 to 3 days earlier, and the decrease of moisture was more rapid than in Larker or Dickson. Growing conditions in 1969 and 1970 varied widely. Average temperatures were lower, precipitation was higher, and the growth period from heading to maturity was longer by about 1 week in 1969 than in 1970. However, the barley plants in 1969 lodged considerably in the field. Consequently, the kernel size of the barley harvested in 1969 was relatively small and kernel protein was rather high considering environmental conditions during plant development.

Moisture and protein were determined according to the American Society of Brewing Chemists' methods of analysis (6). Crude-protein data are given in this report as Kjeldahl-N on a moisture-free basis.

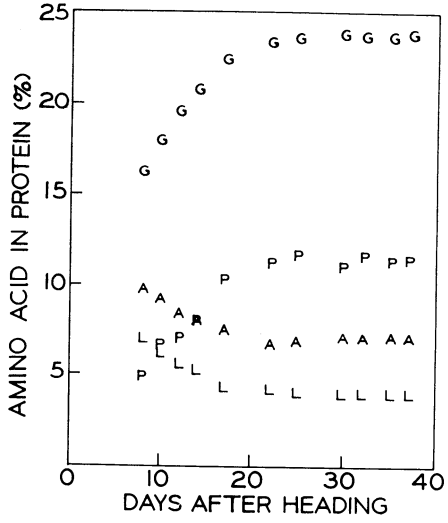
Amino acid analyses were performed on a Beckman 121 automatic amino acid analyzer. After 4 ml. of 6N HCl was added to about 40-mg. samples in 16-mm. Pyrex test tubes, the mixture was frozen in an acetone bath and the test tubes were evacuated to less than 50 μ. The contents were then allowed to melt so that any entrapped air bubbles could escape; and after repeated evacuation, the test tubes were sealed. Hydrolysis was carried out at 110°C. ±1°C. for 22 hr. in a forced-draft oven. After removal from the oven and being cooled to room temperature, the tubes were opened, and the hydrolyzed material was evaporated three times under reduced pressure and diluted to 10 ml. with citrate buffer 0.20N Na^+, pH 2.2. The insoluble humin in the resulting solution was removed by filtration through glass wool. Aliquots of 250 μliters of the hydrolysate were placed automatically on the short and long columns of the instrument for separation of basic, and acidic and neutral amino acids, respectively. The accelerated amino acid analysis at 53.7°C. required a total of 133 min. for separation on both columns.

Data processing was made by electronic integration and conversion of perforated tape to punched cards, which facilitated checking the data. A computer program was used to calculate original output in nine forms; values are expressed in this report in g. amino acid per 100 g. amino acid recovered. Average recovery (based on Kjeldahl-N contents) was 90.3%. Generally, a negligible amount of cysteic acid and an appreciable amount of methionine sulfone were found as a result of hydrolysis, and were added automatically to their precursors. Results of amino acid composition given in this report are rounded off to the first decimal place for easier comparison. Average coefficient of variation was 5.5%, in agreement with the results of Riley and Ewart (7). The coefficient was somewhat larger in sulfur-containing amino acids and considerably smaller in other amino acids, especially glutamic acid (1.5%).

RESULTS AND DISCUSSION

Protein content, on a moisture-free basis, decreased in both crop years during the first 2 weeks after heading, increased during the third week, and remained constant after 23 days (Fig. 1 and Table I). Full kernel weight was attained after about 3 weeks.

Changes in average concentrations (in the proteins of barley from the 1969 crop) of seven amino acids and of ammonia are given in Figs. 2 and 3. Only the average



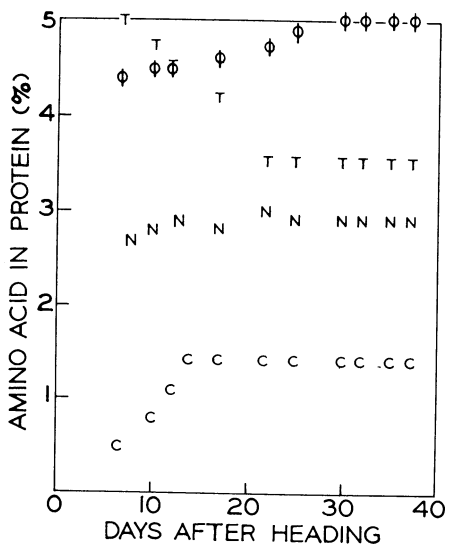


Fig. 2 (left). Aspartic acid (A), glutamic acid (G), lysine (L), and proline (P) concentrations in proteins of maturing barley (averages for three varieties from the 1969 crop).
Fig. 3 (right). Ammonia (N), cystine (C), phenylalanine (Φ), and threonine (T) concentrations in proteins of maturing barley (averages for three varieties from the 1969 crop).

(for the three cultivars) concentrations of amino acids are given to simplify presentation. This simplification is justified, as no significant differences among cultivars analyzed at comparable stages of maturity were recorded. Practically in all cases, illustrated in Figs. 2 and 3, changes which took place in amino acid composition were completed within the first 3 weeks; this coincides with the time at which full dry-kernel weight was attained. The largest average increases during maturation were in glutamic acid (from 16.1 to 24.0%), proline (4.9 to 11.6%), and cystine (0.5 to 1.5%); the largest decreases were in alanine (not shown here, from 8.3 to 4.3%), lysine (6.9 to 3.9%), and aspartic acid (9.8 to 6.9%). Concentration of threonine decreased from 5.1 to 3.5%, phenylalanine increased from 4.3 to 5.1%, and ammonia increased from 2.7 to 2.9%. Additional decreases in concentrations in the proteins were in serine (5.6 to 4.0%), glycine (5.4 to 4.2%), valine (6.2 to 5.3%), isoleucine (4.3 to 3.7%), and leucine (7.7 to 6.7%). Concentrations of other amino acids remained fairly uniform or varied in an irregular manner: histidine, 2.2 to 2.5%; arginine, 5.1 to 5.9%; methionine, 2.1 to 2.5%; and tyrosine, 2.3 to 3.0%.

Decrease in aspartic acid was smaller than increase in glutamic acid; consequently, increase during maturation in the sum of glutamic and aspartic acids was from 25.9 to 30.9%. Assuming that most of the ammonia was from glutamine and asparagine, about 89.0, 83.0, and 79.3% of the two dicarboxylic acids were in the form of amides 7, 14, and 37 days after heading, respectively. However, those values might have been affected significantly by the formation during hydrolysis of high levels of ammonia in samples from early stages of maturity.

Comparison of samples harvested in 1970 (Table II) with samples from 1969 (Figs. 2 and 3) indicates that despite widely differing growth conditions, the patterns of amino acid distribution in the two crop years were very similar. Again, no significant differences were found in the three cultivars when samples were tested at

TABLE II. AVERAGE AMINO ACID COMPOSITION[a] OF THREE BARLEYS HARVESTED AT VARIOUS STAGES OF DEVELOPMENT IN 1970

Amino Acid	Days After Heading				
	6 or 7	13 or 14	23	32	39
Lysine	6.8	5.2	4.1	4.3	4.2
Histidine	2.4	2.3	2.4	2.5	2.4
Ammonia	2.8	3.0	2.9	3.0	3.0
Arginine	5.6	5.4	5.8	6.2	5.5
Aspartic acid	9.6	8.1	7.4	7.6	7.8
Threonine	5.2	4.3	3.7	3.7	3.8
Serine	5.1	4.6	4.1	4.1	4.2
Glutamic acid	14.0	18.7	22.4	22.0	22.9
Proline	5.5	7.7	9.8	9.6	10.0
Cystine/2	0.5	1.2	1.5	1.4	1.4
Glycine	5.6	5.0	4.6	4.6	4.7
Alanine	8.5	6.9	4.7	4.5	4.7
Valine	6.0	5.8	5.5	5.4	5.5
Methionine	3.0	3.0	2.7	2.7	2.5
Isoleucine	4.5	4.1	3.8	3.8	3.9
Leucine	7.5	7.5	7.0	7.0	7.3
Tyrosine	2.9	2.7	2.9	2.9	2.8
Phenylalanine	4.5	4.5	5.0	5.0	4.9

[a]g. amino acid per 100 g. amino acid recovered.

TABLE III. AMINO ACID COMPOSITION[a] OF IMMATURE AND MATURE OATS, WHEAT, AND BARLEY; AND MALTED BARLEY

Amino Acid	Oats[b]		Wheat[c]		Barley[d]		Barley[e]	
	5 days after flowering	Mature	Days preripe 23	0	Days preripe 25	0	Malted for 2 days	11 days
Lysine	7.6	4.2	3.8	2.6	6.9	4.1	3.7	4.6
Histidine	2.1	1.9	2.4	2.3	2.4	2.4	2.4	2.7
Ammonia	3.7	3.0	3.5	3.7	2.8	3.0	3.2	3.0
Arginine	5.0	6.5	4.8	4.1	5.7	5.5	5.1	5.7
Aspartic acid	12.0	9.1	7.1	5.0	9.7	7.4	7.0	8.7
Threonine	4.9	3.3	3.6	3.1	5.1	3.7	3.5	3.8
Serine	4.8	4.7	4.7	4.6	5.3	4.1	4.2	4.1
Glutamic acid	16.2	22.0	27.9	33.8	15.1	23.4	24.6	20.3
Proline	4.7	6.1	8.2	10.0	5.2	10.7	11.3	11.9
Glycine	5.3	4.8	5.3	4.2	5.5	4.5	4.1	4.3
Alanine	7.3	5.0	5.2	3.6	8.4	4.6	4.5	,4.5
Cystine/2	0.2	1.3	...	...	0.5	1.4	1.2	1.3
Valine	6.4	6.0	5.2	3.9	6.0	5.5	5.2	5.3
Methionine	1.1	1.5	...	...	2.7	2.4	2.3	2.3
Isoleucine	4.6	4.2	4.1	3.9	4.4	3.8	3.7	3.8
Leucine	7.5	8.0	7.3	6.9	7.5	7.1	6.6	6.7
Tyrosine	3.2	3.7	2.0	2.2	2.8	2.8	2.6	2.4
Phenylalanine	4.0	5.2	4.7	4.8	4.4	5.0	5.1	5.1

[a]g. amino acid per 100 g. amino acid recovered.

[b]Average of primary and secondary groats, except for cystine. Calculated from reference 8.

c and [e]Averages of two cultivars. Calculated from references 9 and 10, respectively.

[d]Average of three cultivars, each, from the 1969 and 1970 crops.

comparable stages of maturity. Consequently, the results in Table II are averages for the three cultivars; the range in harvest dates (days after heading) resulted from varietal differences in development rates. Concentrations at the earliest stages differed somewhat in 1969 from 1970, as the first samples in 1970 were harvested somewhat earlier than in 1969. However, the mature grains in both years contained comparable concentrations of amino acids. Increases were largest in glutamic acid, proline, and cystine; and decreases were largest in alanine, lysine, aspartic acid, and threonine. Changes in concentration of other amino acids in both crop years were similar.

It is interesting to compare changes in distribution of amino acids in barley with literature data on amino acids in other maturing cereals. Such a comparison with oats (8) and wheat (9), and with barley malted for 2 and 11 days (10), is given in Table III. The comparison in Table III is an oversimplification, as only two extreme stages in development (or malting) are recorded. During maturation of oats, wheat, and barley, concentrations of lysine, aspartic acid, threonine, serine, glycine, alanine, valine, and isoleucine decreased; and concentrations of glutamic acid, proline, cystine, tyrosine, and phenylalanine increased. Changes in concentrations of histidine, ammonia, arginine, and methionine were either small or inconsistent. Increasing length of malting caused relatively smaller changes than maturation. Some of the changes during malting were a reversal of those occurring during maturation. During malting, concentrations of lysine and aspartic acid increased, and glutamic acid decreased.

Acknowledgments

H. L. Shands (Dept. of Agronomy, University of Wisconsin, Madison) provided facilities and guidance in growing the barleys; J. T. Gilbertson is thanked for technical assistance with the amino acid analyses.

Literature Cited

1. BISHOP, L. R. The composition and quantitative estimation of the barley proteins. IV. The proteins of barley during development and storage and in the mature grain. J. Inst. Brew. (London) 36: 336 (1930).
2. HARRIS, G., and MacWILLIAMS, I. C. Carbohydrates in malting and brewing. VI. Changes in the carbohydrate composition of barley on ripening and corresponding variations in nitrogenous constituents. J. Inst. Brew. (London) 63: 210 (1957).
3. KING, H. Uber Hordein und Gliadin im unreifen und reifen Samenkorn. 17 Mitteilung von E. Waldschmidt-Leitz und Mitarbeitern. Brauwissenschaft 21(5): 182 (1968).
4. POMERANZ, Y., STANDRIDGE, N. N., and SHANDS, H. L. Functional and biochemical changes in maturing barley. I. Changes in malting and brewing characteristics. Crop Sci. 11: 85 (1971).
5. PRENTICE, N., MOELLER, MARY, and POMERANZ, Y. A note on changes in peptide hydrolase, esterase, and amidase of maturing barley. Cereal Chem. 48: 714 (1971).
6. AMERICAN SOCIETY OF BREWING CHEMISTS. Methods of analysis. The Society: Madison, Wis. (1958).
7. RILEY, R., and EWART, J. A. D. The effect of individual rye chromosomes on the amino acid content of wheat grains. Genet. Res. (Cambridge) 15: 209 (1970).
8. BROWN, C. M., WEBER, E. J., and WILSON, C. M. Lipid and amino acid composition of developing oats (*Avena sativa* L. cultivar 'Brave'). Crop Sci. 10: 488 (1970).
9. POMERANZ, Y., FINNEY, K. F., and HOSENEY, R. C. Amino acid composition of maturing wheat. J. Sci. Food Agr. 17: 485 (1966).
10. ROBBINS, G. S., and POMERANZ, Y. Amino acid composition of malted cereals and malt sprouts. Amer. Soc. Brew. Chem. Proc. 1971, p. 15.

[Received July 12, 1971. Accepted February 28, 1972]