Composition and Utilization of Milled Barley Products. III. Amino Acid Composition[1]

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ABSTRACT

Protein content and amino acid composition were determined in acid hydrolysates of covered and hulless barley cultivars and isogenic lines; in lemma, palea, germ, and endosperm fractions dissected by hand; in fines and pearls from tangential abrasion on a barley pearler; and in streams from conventional roller-milling and air classification. No varietal differences in amino acid composition were established. The low-protein hulls (lemma and palea) and the high-protein germ were higher in lysine and aspartic acid and lower in glutamic acid and proline than the proteins in the whole kernel. With increase in pearling time, the protein contents of the fines and pearls decreased and their amino acid composition approached that of the starchy endosperm. Analyses of roller-milled fractions are interpreted on the basis of data from hand-dissection and pearling studies. Air fractionation resulted in significant shifts in protein contents and amino acid composition.

Several investigators have determined the gross composition and amino acid distribution in wheat and corn kernels. Similarly, the amino acid composition of products of wheat and corn milling has been studied to determine the nutritional value of the milled products. The amino acid composition of whole barley kernels was reported in recent years by investigators from several research centers (1-5). There is, however, no published information on distribution of nutrients in the barley kernel and products of barley milling.

A previous paper (6) described the gross composition of roller-milled and air-separated barley fractions; use of the milled products as adjunct in brewing was described elsewhere (7). This report concerns the amino acid composition of barley, barley fractions, and roller-milled and air-classified fractions.

MATERIALS AND METHODS

Barleys and Barley Fractions

Three covered barley cultivars were used in this study. 'Larker', one of the leading six-row malting cultivars from the Red River Valley in the U.S., was grown in 1968 in Fargo, N.D. The two-row malting barley 'Piroline' was from the 1968 crop from Burley, Idaho. 'Atlas', a six-row coast-type barley, was grown in 1965 in the Sacramento Valley, Calif. A sample of "naked" (hulless) 'Himalaya' barley was from the 1963 crop grown at Aberdeen, Idaho.

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In addition to the four barley cultivars, three pairs of isogenic lines (hulled and hulless) grown in 1969 in Aberdeen, Idaho, were used. A pair of isogenic lines is assumed to differ by only a single gene, in this case hulled vs. hulless. We used lines developed by G. A. Wiebe; the pairs were 49-N-26 and 49-n-26, 50-N-26 and 50-n-26, 51-N-24 and 51-n-24. The first number is an accession number; N and n denote hulled and hulless, respectively; the last number refers to the selfed generation in which two homozygous lines were selected.

The roller-milled and air-separated fractions were described in detail previously (6).

The barley kernels were separated into hulls (lemma and palea), germ, and dehulled-degermed caryopses, by hand-dissection of 100 kernels that were previously sonicated for 30 sec. in ice-cold water. The sonication facilitated dissection, and will be described in detail elsewhere. Starchy endosperm was hand-dissected from the cheek of transverse sections of Himalaya barley kernels.

Removal of outer layers of the barley kernel was done by abrading Himalaya barley for 30, 90, and 270 sec. on a Strong-Scott barley pearler.

Analytical Methods

Moisture and crude protein were determined as described in the AACC Approved Methods (8). Crude-protein data are given in this report as Kjeldahl-N X 6.25, on a moisture-free basis.

Amino acid analyses were performed on a Beckman 121 automatic amino acid analyzer. Hydrolysis was carried out by adding 4 ml. of 6N HCl to samples containing about 1 mg. N in 16-mm. Pyrex test tubes. The mixture was frozen in a dry ice-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 cooling to room temperature, the tubes were opened; the hydrolyzed material was evaporated to near dryness under reduced pressure; and, in order to assure removal of HCl, the evaporation was twice repeated after the addition of water. The hydrolysates were then diluted to 10 ml. with citrate buffer 0.2N Na^+, pH 2.2. The insoluble humin in the resulting solution was removed by filtration through glass wool. Aliquots of 250 μliters of 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. on the two ion-exchange columns required a total of 133 min.

Data processing was facilitated by electronic integration and conversion of perforated tape to punched cards. A computer program was used to calculate original output in 9 forms; values are expressed in this report in grams amino acid per 100 g. amino acid recovered. Cystine and methionine values were respectively corrected for the small amount of cysteic acid and the appreciable amount of methionine sulfone. Results on amino acid composition given in this report are rounded-off to the first decimal place for easier comparison; computer print-out sheets gave results with two figures after the decimal point.

Average recoveries (based on Kjeldahl-N contents) in analyses of whole kernels, endosperm, and germ fractions were 88, 89, and 83%, respectively; analyses of hulls and of cellulose-rich abraded fractions from pearling were 69 and 80%, respectively,

indicating high concentrations of nonprotein nitrogenous materials. Tkachuk and Irvine (1) reported recoveries of about 83% in germ, 79% in bran, and 98% in endosperm of wheat products; their analyses included assays of tryptophan.

The average coefficient of variation of amino acid analyses was 3.2%. All separations and assays were made at least in duplicate.

RESULTS AND DISCUSSION

Protein contents and amino acid composition of whole (covered) barleys and barley fractions are given in Table I. The results are averages of analyzing two or four barleys; no consistent varietal differences could be established. The lemma and palea, which comprised slightly over 10% of the kernel, were low in protein; the palea was slightly, but consistently, higher in protein than the lemma. The germ had, as expected, the highest protein content.

There were only small differences in amino acid composition between the lemma and the palea; the largest difference was in proline. The hull fractions resembled the germ fraction in their high lysine and aspartic acid, and low glutamic acid and proline contents. Both hull fractions and the germ differed substantially from the whole kernel in amino acid composition. The main differences were in the concentrations of the major amino acids (glutamic acid, proline, and aspartic acid). Shifts in the major components were accompanied by significant changes in most of the other amino acids.

TABLE I. YIELD, PROTEIN CONTENTS[a], AND AMINO ACID COMPOSITION[b] OF WHOLE (COVERED) BARLEY AND BARLEY FRACTIONS

Assay	Whole Kernel[c]	Lemma[d]	Palea[d]	Germ[c]	Dehulled-Degermed[c]
Yield, %	100.0	7.3	3.1	3.7	85.9
Protein	12.4	1.7	2.0	35.0	12.3
Lysine	3.9	6.0	6.1	7.2	3.6
Histidine	2.2	1.5	1.8	3.1	2.2
Ammonia	3.0	3.2	3.4	2.3	3.1
Arginine	4.4	4.9	5.0	9.5	4.4
Aspartic acid	6.8	11.6	11.7	10.6	6.3
Threonine	3.4	5.5	5.5	4.5	3.5
Serine	3.7	5.9	6.1	4.4	3.7
Glutamic acid	26.1	12.8	13.1	14.6	27.0
Proline	11.4	4.9	3.8	3.9	11.8
Cystine/2	1.0	0.1	0.2	0.7	1.1
Glycine	4.2	7.4	7.5	6.7	4.0
Alanine	4.4	7.7	7.9	7.0	4.1
Valine	5.3	7.1	7.1	6.0	5.2
Methionine	2.6	2.0	1.8	2.3	2.6
Isoleucine	3.8	4.5	4.4	3.7	3.8
Leucine	7.1	8.3	8.1	6.9	7.1
Tyrosine	1.9	2.5	2.3	3.0	2.2
Phenylalanine	5.4	4.7	4.5	4.3	5.4

[a]N X 6.25, %.

[b]Grams amino acid per 100 g. recovered.

[c]Average of two samples: Larker and Piroline.

[d]Average of four samples: Larker, Piroline, and two isogenic lines.

TABLE II. PROTEIN CONTENTS[a] AND AMINO ACID COMPOSITION[b] OF WHOLE KERNEL, GERM, AND ENDOSPERM OF HIMALAYA BARLEY

Assay	Whole Kernel	Germ	Endosperm	Assay	Whole Kernel	Germ	Endosperm
Protein	17.6	33.2	8.3	Proline	11.9	4.9	14.0
				Cystine/2	1.0	0.7	1.2
Lysine	3.2	6.2	2.8	Glycine	3.6	6.3	2.9
Histidine	2.3	3.3	2.0	Alanine	3.8	6.2	3.2
Ammonia	3.5	3.5	3.6	Valine	5.2	5.7	5.0
Arginine	4.3	9.8	3.9	Methionine	2.3	2.1	2.9
Aspartic acid	6.0	10.3	4.8	Isoleucine	3.8	3.5	3.7
Threonine	3.2	4.1	2.8	Leucine	6.7	6.5	6.7
Serine	3.9	4.4	3.2	Tyrosine	1.9	2.7	2.6
Glutamic acid	28.2	15.8	29.5	Phenylalanine	5.3	4.2	5.4

[a]N X 6.25, %.

[b]Grams of amino acid per 100 g. recovered.

TABLE III. YIELD, PROTEIN CONTENTS[a], AND AMINO ACID COMPOSITION[b] OF FRACTIONS OF PEARLED HIMALAYA BARLEY

Assay	Fines after Abrasion for (sec.)			Pearls after Abrasion for (sec.)		
	30	90	270	30	90	270
Yield, %	7.9	20.1	53.0	92.1	79.9	47.0
Protein	25.5	23.9	21.2	15.5	14.5	12.0
Lysine	5.4	4.5	3.5	3.0	2.9	3.0
Histidine	2.7	2.5	2.2	2.1	2.1	2.2
Ammonia	2.7	2.9	3.0	3.3	3.2	3.3
Arginine	7.7	6.4	5.4	4.2	3.8	3.9
Aspartic acid	8.9	7.2	6.2	5.2	5.2	5.1
Threonine	3.8	3.3	3.1	2.9	2.9	2.8
Serine	4.1	3.8	3.6	3.4	3.5	3.3
Glutamic acid	19.3	23.0	26.6	29.2	30.2	29.7
Proline	7.4	9.8	11.6	13.5	13.7	13.1
Cystine/2	1.0	1.1	1.2	1.1	1.1	1.1
Glycine	5.7	4.7	4.0	3.4	3.2	3.1
Alanine	5.7	4.8	4.2	3.6	3.5	3.5
Valine	5.8	5.4	5.3	5.1	5.1	5.2
Methionine	2.4	2.2	2.4	2.5	2.4	2.8
Isoleucine	3.8	3.7	3.7	3.7	3.8	3.8
Leucine	6.8	6.6	6.6	6.6	6.8	6.9
Tyrosine	2.9	2.8	2.8	2.2	1.8	2.1
Phenylalanine	4.7	5.1	5.2	5.4	5.5	5.4

[a]N X 6.25, %.

[b]Grams of amino acid per 100 g. recovered.

As mentioned before, no significant varietal differences could be established in amino acid composition of hulls from covered barleys (Larker, Piroline, or isogenic lines). Similarly, there were no consistent differences in amino acid composition of germ from covered cultivars and from either hulless or hulled isogenic lines. Tables II and III summarize protein contents and amino acid composition of various fractions in hulless Himalaya barley. The fractions in Table II were from hand dissection; in Table III, from abrasion on the Strong-Scott barley pearler. With

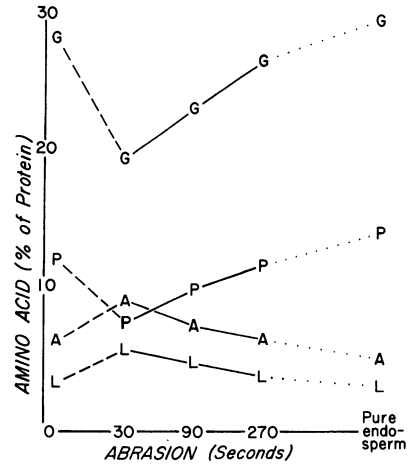

Fig. 1. Glutamic acid (G), proline (P), aspartic acid (A), and lysine (L), in proteins of the whole-kernel (O), fines of Himalaya barley pearled for 30, 90, and 270 sec., and from hand-dissected starchy endosperm.

increasing pearling time (and corresponding increase in amounts of fines and decrease in yield of pearls) there is a consistent decrease in protein concentration in both products. Total protein content, which is the sum of weighted contributions from both products, is constant (within experimental error) and equal to that in the whole kernel.

The accompanying changes in amino acids of fines are illustrated for patterns of four amino acids in Fig. 1. This figure and the data in Tables II and III show that with increase in pearling time, the amino acid composition of the fines and of the pearls approaches that of the hand-dissected starchy endosperm. Note that results for each abrasion time were obtained by pearling the original barley for 30, 90, or 270 sec.

Fines removed after 30 sec. of pearling likely contained pericarp fractions and aleurone layer primarily. The proteins of the pericarp and aleurone seem to be richer than the starchy endosperm in lysine, histidine, arginine, aspartic acid, threonine, serine, glycine, alanine, and valine; and lower in glutamic acid, proline, cystine, and phenylalanine. Thus, the outer layers (namely pericarp) in the hulless caryopsis of Himalaya barley resemble in amino acid composition the hull fractions (palea and lemma) of covered barleys.

Results of fractionation by hand dissection and by pearling can be used to interpret analyses of conventionally-milled fractions of the covered Atlas barley.

Conventional roller-milling yielded four major streams, varying widely in protein content (Table IV). The flour would be expected to contain, primarily, the starchy endosperm; the shorts and tailings flour, a mixture of aleurone and pericarp, with some germ and starchy endosperm; and the bran, hulls and pericarp. The flour of 65% extraction and tailings flour contained more protein than the whole kernel. The relatively high protein content of the 65%-extraction flour resulted from incomplete separation of the starchy endosperm from the rest of the kernel and from the fact that some tailings flour was added to prepare the 65%-extraction

TABLE IV. YIELD, PROTEIN CONTENTS[a], AND AMINO ACID COMPOSITION[b] OF ROLLER-MILLED BARLEY

Assay	Whole Kernel	Flour, 65% Extraction	Tailings Flour	Shorts	Bran
Yield, %	100.0	65.0	17.7	11.9	5.4
Protein	9.3	9.8	11.3	8.8	3.1
Lysine	4.2	4.1	4.1	4.8	5.0
Histidine	2.4	2.4	2.4	2.1	1.4
Ammonia	3.1	3.1	3.0	2.9	3.5
Arginine	5.3	5.5	5.7	5.9	4.6
Aspartic acid	7.4	7.1	7.5	8.2	8.6
Threonine	3.6	3.6	3.6	3.8	4.2
Serine	4.1	4.0	4.1	4.2	4.7
Glutamic acid	22.6	23.3	22.9	21.2	20.6
Proline	11.4	10.1	9.6	9.2	9.9
Cystine/2	1.1	1.4	1.3	1.1	0.3
Glycine	4.5	4.3	4.7	5.1	5.0
Alanine	4.6	4.4	4.7	5.1	5.0
Valine	5.3	5.2	5.3	5.5	6.1
Methionine	2.5	2.7	2.5	2.5	2.3
Isoleucine	3.6	3.7	3.6	3.7	3.7
Leucine	6.8	7.0	6.8	6.9	7.5
Tyrosine	2.7	3.2	3.0	2.9	2.5
Phenylalanine	4.9	5.0	5.2	5.0	5.1

[a]N X 6.25, %.

[b]Grams of amino acid per 100 g. recovered.

TABLE V. YIELD, PROTEIN CONTENTS[a], AND AMINO ACID COMPOSITION[b] OF AIR-FRACTIONATED BARLEY FLOUR

Assay	Flour, 65% Extraction	Air-Classified Fractions				
	A	B	C	D	E	EE
Yield, %	100.0	5.3	18.7	27.9	14.4	33.7
Protein	9.8	23.3	11.9	6.5	7.1	10.5
Lysine	4.1	3.3	3.8	3.9	4.0	4.6
Histidine	2.4	2.2	2.3	2.2	2.3	2.4
Ammonia	3.1	3.2	3.3	3.2	3.2	2.8
Arginine	5.5	4.6	5.3	5.2	5.1	6.0
Aspartic acid	7.1	6.0	6.7	7.0	7.2	8.0
Threonine	3.6	3.3	3.6	3.5	3.7	3.7
Serine	4.0	4.0	4.1	3.8	4.1	3.9
Glutamic acid	23.3	27.2	26.2	23.8	23.2	20.6
Proline	10.1	11.4	7.8	10.5	11.1	9.6
Cystine/2	1.4	1.6	1.3	1.2	1.1	1.3
Glycine	4.3	3.8	4.2	4.1	4.4	4.8
Alanine	4.4	3.8	4.1	4.1	4.4	4.9
Valine	5.2	5.1	5.4	5.5	5.5	5.5
Methionine	2.7	2.2	2.7	3.2	2.5	3.2
Isoleucine	3.7	3.5	3.7	3.7	3.7	3.8
Leucine	7.0	6.8	7.1	7.4	6.8	7.2
Tyrosine	3.2	2.6	3.0	2.7	2.6	3.0
Phenylalanine	5.0	5.4	5.4	5.1	5.1	4.8

[a]N X 6.25, %.

[b]Grams of amino acid per 100 g. recovered.

flour. The bran was particularly low in protein, indicating high hull and pericarp contents. Proteins in the shorts and in the bran contained more lysine, aspartic acid, threonine, serine, glycine, alanine, and valine, and less glutamic acid, than the proteins of the whole kernel, the 65%-extraction flour, or the tailings flour. The bran proteins were particularly deficient in histidine and cystine, amino acids present in lowest concentrations in hull proteins.

Significant shifts are indicated in Table V in protein content and amino acid composition of fractions from air classification of 65%-extraction barley flour. The high-protein fraction B contained almost 2.4 times as much protein as the original flour A, and had about 3.6 times the protein content of fraction D. The protein-rich fraction B was the lowest in lysine, arginine, aspartic acid, threonine, glycine, alanine, valine, methionine, and isoleucine, and highest in glutamic acid, proline, and cystine. Evaluation of the low-protein fractions D and E is rather difficult, as the unfractionated residue EE comprised about one-third of the total air-fractionated flour. Visual examination and gross composition (high protein, ash, and lipid) indicated that the residue EE contained substantial amounts of particles originating from outer kernel layers. Compared to high-protein fractions B and C, low-protein fractions D and E were high in lysine and aspartic acid, and low in glutamic acid and cystine.

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