Amino Acid Compostion and Malting and Brewing Performance of High-Amylose and Hiproly Barleys[1]

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ABSTRACT

Functional properties of two isogenic lines of Glacier barley (regular and high-amylose) and of two isogenic high-protein lines of naked barley (regular, and high-lysine Hiproly) were evaluated in experimental malting and brewing. The barleys modified poorly in malting; were low in malt extract, wort:malt N ratios, and α-amylase; and were high in fine-coarse grind difference. Ratios of β- to α-amylases were higher in the two high-protein lines and lower in the two Glacier lines than in two accepted malting cultivars. Starch in high-amylose barley was not hydrolyzed satisfactorily during malting and mashing. Brew yields and degree of fermentation of the experimental selections were low. In low-protein samples, high-amylose Glacier contained less protein than regular Glacier. Proteins in three samples of high-amylose Glacier contained more lysine, aspartic acid, glycine, and alanine, and less glutamic acid and proline, than proteins of three samples of regular Glacier. Differences in amino acid composition between the two Glacier lines resembled differences between the two high-protein lines.

Two interesting recent developments in barley breeding have been the discovery of a high-protein, high-lysine (Hiproly) barley line with improved nutritional value, and the description of a barley which is characterized by abnormal starch granules and a high amylose content.

The Hiproly barley, CI 3947, is of Ethiopian origin; it is of an erectoid type with naked, slightly shrivelled seeds, and requires a long photoperiod. A sister-line to Hiproly, CI 4362, has similar habit but smooth and heavier seeds (1,2). Both lines are high in protein, but CI 3947 has substantially more lysine in the protein and a higher nutritional value than CI 4362.

In 1967, Merritt (3) described a barley with an apparent amylose content of about 44% of the total starch; in normal barley, the linear starch component comprises about 24% of the total starch. The high-amylose barley was named Glacier Ac38, since it was shown to be a mutant of the North American six-rowed variety Glacier (CI 9676). The original discovery was followed by several investigations which described the biosynthesis, genetic control, and structure of the high-amylose starch (4,5,6).

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[1]Cooperative investigations of the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Agricultural Experiment Station, University of Wisconsin, Madison 53706, and Agricultural Experiment Station, Montana State University, Bozeman 59715. Approved for publication by the Director of the Wisconsin Agriculture Experiment Station. Published with approval of the Director of the Montana Agriculture Experiment Station as Paper No. 345, Journal series. The work of the Barley and Malt Laboratory is supported, in part, by an Industrial Research Grant from Malting Barley Improvement Association.

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This report compares the amino acid composition and functional properties (in malting and brewing) of the two Glacier and of the two high-protein lines.

MATERIALS AND METHODS

Barleys

Three pairs of Glacier (CI 9676) and high-amylose Glacier (Ac38) and three pairs of Hiproly (CI 3947) and its sister l ine (CI 4362) are described in Table I; the table also includes data on two samples of malting barleys used as comparison checks in our laboratory (two-rowed Piroline and six-rowed Larker). All experimental barleys were grown on irrigated land. In 1970, one set of samples was grown on fallow and the other on fall-plowed alfalfa, to provide a range in protein content. There was relatively little variation in the kernel weights and protein contents among the three samples of CI 3947 and CI 4362. Average kernel weights and protein contents were 46.7 mg. and 19.3% in the three CI 4362 samples, and 35.0 mg. and 20.1% in the three CI 3947 samples. There were rather large variations in kernel weights and protein contents among the samples of CI 9676 and high-amylose barleys. Whereas there were only small differences in protein contents between the pairs grown at Bozeman, Mont., there was a large difference between the pairs grown at Mesa, Ariz. (10.4 vs. 8.4%). This marked difference in protein between normal and high-amylose Glacier samples was also reported by Merritt and Walker (5) and was confirmed in low-protein barleys grown at Huntley, Mont., in 1971. Average kernel weights and protein contents were 42.1 mg. and 13.6% in the three CI 9676's and 42.0 mg. and 13.0% in the three high-amylose samples.

Analytical Determinations

The barleys, malts, worts, and beers were analyzed for moisture, Kjeldahl-N, and malting and brewing parameters, according to the methods of analysis of the American Society of Brewing Chemists (7).

TABLE I. DESCRIPTION OF BARLEY SAMPLES

Cultivar or Line	Crop Year	Location	Kernel Weight mg.	Protein (N X 6.25) %	Remarks
Larker	1969	Fargo, N. Dak.	30.7	12.8	Commercial
Piroline	1968	Burley, Idaho	37.7	12.3	Commercial
Hiproly[a]	1969	Bozeman, Mont.	35.1	20.6	Fallow
Hiproly	1970	Bozeman, Mont.	35.2	19.9	Fallow
Hiproly	1970	Bozeman, Mont.	34.6	19.9	Alfalfa stubble
CI 4362	1969	Bozeman, Mont.	46.7	19.4	Fallow
CI 4362	1970	Bozeman, Mont.	47.5	19.9	Fallow
CI 4362	1970	Bozeman, Mont.	45.8	18.6	Alfalfa stubble
Glacier[b]	1969/70	Mesa, Ariz.	48.1	10.4	...
Glacier	1970	Bozeman, Mont.	39.7	13.8	Fallow
Glacier	1970	Bozeman, Mont.	38.6	16.6	Alfalfa stubble
High-amylose Glacier	1969/70	Mesa, Ariz.	47.5	8.4	...
High-amylose Glacier	1970	Bozeman, Mont.	41.2	14.0	Fallow
High-amylose Glacier	1970	Bozeman, Mont.	37.3	16.5	Alfalfa stubble

[a]CI 3947.

[b]CI 9676.

Malting

Duplicate 170-g. lots of each sample were malted under uniform conditions and were composited (8,9). All were steeped to 45% moisture (about 30 hr.) at 16°C. and germinated in malting chambers at 16°C. ±0.5 for 5 days. Final kiln temperature was 85°C. for 2 hr.

Mashing and Brewing

Mashing and brewing were done in the micro-brewing apparatus described by Burkhart et al. (10). For each brew, 230 g. of coarsely ground malt was mashed with 98 g. adjunct, commercial brewer's corn grits. The mashes were "filtered" through cheesecloth in a large Büchner funnel with light suction. The spent grains were sparged with a uniform volume of water for extract recovery. Brew-kettle operation was the same for all brews. Wort plato at pitching averaged 12.0. The worts were pitched with 5 g. of yeast from a suction filter cake and fermented 8 days at 12°C. ±0.5. At the end of fermentation the beer was removed from the settled yeast, a chill-proofing agent was added, and the beers were stored for 2 weeks under 0.84 kg. per cm.^{2} (12 lb. per in.^{2}) CO_2 pressure at 1°to 2°C. The beers were then filtered, bottled, and pasteurized. Crude protein data are given in this report as Kjeldahl-N X 6.25, on a moisture-free basis.

Amino Acids

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 given elsewhere (11). Results of amino acid assays are expressed in g. amino acid per 100 g. amino acid recovered.

TABLE II. THE EVALUATION OF BARLEY AND MALT QUALITY

Cultivar or Line	Barley			Malt Extract (% dry basis)			Wort	
	Kernel weight mg.	Plumpness %	Color score Agtron	Fine grind	Coarse grind	Difference	Color[a]	Clarity
Larker	30.7	75.6	26	75.9	74.7	1.2	2.0	Slightly hazy
Hiproly	34.8	6.7	18	73.2	68.1	5.1	1.9	Clear
CI 4362	47.6	87.6	22	74.8	61.7	13.9	1.4	Slightly hazy
High-amylose Glacier	44.5	80.7	62	68.3	58.4	9.9	1.6	Slightly hazy
Glacier	42.5	84.5	70	74.9	65.9	9.0	1.6	Slightly hazy
Piroline	37.7	84.5	96	77.5	73.9	3.6	1.7	Slightly hazy

Cultivar or Line	Nitrogen (% dry)			Ratio Wort: Malt	Amylase			
	Barley	Malt	Wort		Diastatic Power deg.	Beta (Maltose Equivalent)	Alpha (20° unit)	Ratio Beta: Alpha
Larker	2.05	2.09	0.789	37.8	139	438	35.8	12.2
Hiproly	3.27	3.36	0.806	24.0	223	830	18.4	45.1
CI 4362	3.09	3.18	0.762	24.0	130	464	16.9	27.5
High-amylose Glacier	2.10	2.17	0.677	31.2	38	82	20.5	4.0
Glacier	2.21	2.29	0.663	29.0	48	134	17.4	7.7
Piroline	1.97	2.00	0.686	34.3	94	293	24.0	12.2

[a] In degrees SRM (Standard Reference Method of the American Society of Brewing Chemists, constructed to approach in magnitude Lovibond degrees).

TABLE III. EVALUATION OF WORT

Cultivar or Line	Wort Color	Wort pH	Total N %	Formol N %	Maltose %	Dextrin %	Brew Yield %
Larker	2.5	5.41	0.081	0.018	7.19	2.76	80.4
Hiproly	2.4	5.32	0.099	0.020	6.90	2.98	70.7
CI 4362	1.8	5.40	0.084	0.016	6.57	3.51	71.1
Glacier	1.7	5.41	0.071	0.014	8.05	2.43	73.3
Piroline	1.9	5.46	0.075	0.017	7.81	2.44	79.3

RESULTS AND DISCUSSION

Malting and brewing characteristics of the barleys are summarized in Tables II through IV. We report results of composites which were prepared from three samples (each) of CI 3947, CI 4362, CI 9676, and high-amylose Glacier. In each table the experimental lines are compared with the two check samples of malting barley. We prepared the composites because the individual barley samples were too small for separate brewing tests. Results from the high-amylose barley are given in Table II but not in Tables III and IV, since the starch was not hydrolyzed under the experimental conditions employed. It is known that high-amylose starches resist gelatinization and require either addition of chemicals or heating at high temperatures under pressure (12) for hot-pasting. Neither of those treatments could be used in the mashing process because they would inactivate malt enzymes. On the other hand, unless the starch has been gelatinized, its susceptibility to degradation by amylolytic enzymes is low.

All experimental lines were inferior to the check samples; they were lower in malt extract and higher in fine-coarse grind differences (Table II). High-amylose Glacier was lowest in malt extract. This is to be expected because the high amylose level adversely affects susceptibility to enzymatic action under the test conditions. The experimental lines were also low in wort:malt N ratios. CI 3947 and CI 4362 were much lower in this respect than the Glacier lines, probably because the former were high in protein. Generally, as the level of protein increases, solubility of the additional protein decreases (13).

All experimental malts were low in α-amylase, the most important amylolytic enzyme formed during malting. It is interesting to note that β:α ratios were much higher in the two high-protein selections and much lower in the two Glacier selections than in the two check samples.

TABLE IV. EVALUATION OF BEER

Cultivar or Line	Beer Color	Beer pH	Real Extract %	Alcohol %[a]	Degree of Fermentation %	Total N %	Formol N %	Gas Stability[b]	Clarity Stability[b]
Larker	2.2	4.10	4.86	3.63	58.9	0.054	0.005	S	Q
Hiproly	2.1	4.32	5.64	3.36	53.4	0.070	0.007	Q-S	Q-U
CI 4362	1.6	4.23	6.15	3.10	49.2	0.059	0.005	Q-S	Q
Glacier	1.5	4.18	6.15	3.15	50.0	0.046	0.004	Q-S	Q-U
Piroline	1.5	4.22	5.54	3.38	54.0	0.049	0.005	S	Q

[a]As w./w.

[b]S = satisfactory, Q = questionable, U = unsatisfactory.

TABLE V. KERNEL WEIGHTS, PROTEIN CONTENT, AND AMINO ACID COMPOSITION[a] OF BARLEYS[b]

Parameter	CI 4362	Hiproly	Glacier	High-Amylose Glacier
Kernel weight, mg.	46.7	35.0**	42.1	42.0
Protein, N X 6.25, %	19.3	20.1	13.6	13.0
Lysine	3.0	4.3**	3.4	3.9
Histidine	2.0	2.2	2.1	2.2
Ammonia	3.2	2.9	3.1	2.8
Arginine	4.0	4.8	4.7	5.1
Aspartic acid	5.4	7.4**	6.1	6.9
Threonine	2.7	3.2**	3.1	3.3
Serine	3.4	3.7**	3.6	3.8
Glutamic acid	29.5	25.5	27.2	25.3
Proline	15.3	11.9	12.9	11.9
Half-Cystine	1.1	0.8	1.1	1.1
Glycine	3.3	3.8**	3.6	4.1
Alanine	3.5	4.7**	4.2	5.1
Valine	4.3	5.1**	4.9	5.1
Methionine	1.9	2.1	2.1	2.1
Isoleucine	3.3	3.6**	3.4	3.5
Leucine	6.2	6.5**	6.5	6.5
Tyrosine	2.3	2.3	2.6	2.4
Phenylalanine	5.7	5.4	5.1	4.8
Recovery, N basis, %	88.5	87.2	88.9	87.5

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

[b]Means of three samples (replicates), each run in duplicate.

**Significant difference (at 0.01 level) from CI 4362.

As indicated before, we did not brew the high-amylose Glacier because it modified poorly and the starch was not degraded satisfactorily. Worts of selections CI 3947 and CI 4362 were low in maltose and high in dextrin; their brew yields were poor. The normal Glacier was also low in brew yield (Tables III and IV). The three experimental selections were relatively high in real extract and low in degree of fermentation.

Published information on the experimental lines indicated that they may have desirable characteristics in malting and brewing. The high level of amylose would be expected to enhance degradation to fermentable carbohydrates (6). This increase did not materialize, however, because of the reduced availability (to amylolytic action) of the tightly packed and bonded amylose fraction in high-amylose barley. Munck et al. (2) reported large differences in types of proteins in CI 3947 and CI 4362. The reported differences would be expected to result in more soluble proteins in Hiproly than in CI 4362. The potential differences, however, were absent in the malted samples. The additional protein in the high-protein samples reduced extract and brew yield and provided no significant additional amounts of available nitrogenous compounds for yeast nutrition. It should be pointed out, however, that the poor hydrolysis of high-molecular-weight compounds in the experimental lines also could have resulted from low enzymatic activities (Table II). Whatever the deficiency, the experimental selections were unsatisfactory as barleys for malting.

Amino acid composition of barley (as of most of the other cereal grains) is affected by genetic factors, by environmental conditions, and by cultural practices.

Generally, as the protein content of a cultivar is increased, the concentration of the storage proteins (hordein and glutelin, which are rich in glutamic acid and proline) increases; and concentration of the soluble proteins (albumins and globulins, which are rich in the nutritionally limiting amino acid lysine) decreases.

Amino acid compositions of the experimental lines are compared in Table V. Please note that whereas the data in Tables II to IV were obtained from analyses on composites, the data in Table V are averages of analyzing (in duplicate) three samples of each line. There were relatively small ranges in protein content for Hiproly and CI 4362, and the resulting intravarietal differences in amino acid composition were insignificant. As expected from reports of the Swedish plant breeders (1,2), proteins in Hiproly (CI 3947) contained substantially less glutamic acid and proline and substantially more lysine than proteins in CI 4362. Additional amino acids which varied between the two isogenic lines were aspartic acid, threonine, serine, glycine, alanine, valine, isoleucine, and leucine. The protein level in the small and shrunken CI 3947 was only slightly higher than in CI 4362. The results indicate that high-protein and high-lysine characters are separate in the two lines, i.e., not pleotropic or linked.

The two Glacier lines had almost identical kernel weights and similar average protein contents. Comparison of amino acid compositions shows interesting differences. Basically, the differences are qualitatively identical to (though quantitatively smaller than) those between CI 4362 and 3947; the high-amylose barley resembles Hiproly in amino acid composition. This is borne out by comparing (Fig. 1) the differences between specific amino acids in Hiproly and CI 4362 with the differences between those amino acids in high-amylose Glacier and CI 9676. Whereas none of the differences between the two Glacier lines was significant at the 0.05 level, the correlation coefficient between the differences in the two pairs of barley lines was 0.953** and decreased to 0.894** if glutamic acid and proline were excluded from calculations. No explanation can be offered for the difference in amino acid composition between the two lines of Glacier. However, it might be relevant to point out that under conditions conducive to production of low-protein barleys, the high-amylose barley produced kernels lower in protein than CI 9676. In the study of Merritt and Walker (5) there was no difference in rate of

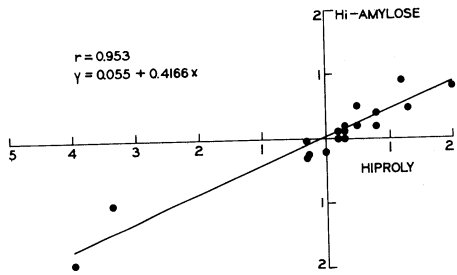

Fig. 1. Plot of regression equation derived from differences between amino acids in Hiproly and CI 4362 (X-axis designated Hiproly) vs. differences between amino acids in high-amylose Glacier and Glacier (Y-axis designated high-amylose). Individual points represent differences for specific amino acids in the proteins of the two pairs of barley lines.

protein synthesis up to 40 days after anthesis; afterwards, protein synthesis in high-amylose barley leveled off, but continued in CI 9676. It would be interesting to confirm the differences in amino acid compositions and patterns of protein synthesis by determining (i.e., by fractionation on gel-filtration columns and by gel electrophoresis) the types of proteins that are synthesized during ripening of the two Glacier lines.

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[Received March 6, 1972. Accepted April 18, 1972]