INVERTASE ACTIVITY AS A MEASURE OF MALTING QUALITY OF BARLEY[1]

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ABSTRACT

Soluble and insoluble invertase activities were measured on several six-row and two-row barleys after they had germinated. The activity of the soluble invertase was related to malting quality as indicated by conventional analyses, but the insoluble enzyme had no consistent relation to malt quality.

The utilization of sucrose in embryonic tissues of barley appears to be essential for efficient germination. During germination, gibberellin is secreted from the embryo into the aleurone layer where hydrolases are synthesized. Some of these hydrolases degrade storage polysaccharides which diffuse into the scutellum (1,2) and aleurone cells (3) and are converted into sucrose. The sucrose in the scutellum is transported to the growing root and shoot where it is hydrolyzed by invertase and further metabolized. If an abnormally high concentration of sucrose occurs in the scutellum, gibberellin secretion is inhibited and the rate of germination decreases (2). It, therefore, seems likely that invertases play a significant role in sucrose utilization and in regulation of growth rate.

In germinated barley, invertases are found in the roots, shoot, scutellum, and embryonic axis (4). There are two soluble enzymes in the shoot, and another soluble invertase in all other embryonic tissues. In addition, all these tissues have invertase activity that cannot be solubilized.

In previous work, a malting barley and two feed barleys were examined for soluble and insoluble invertases throughout a 5-day growth period (5). At the completion of this period the malting barley showed a higher level of soluble invertases than did the feed barleys, although the activities of the insoluble enzymes did not differ. The findings suggested that the level of soluble invertase activity may be a useful measure of malting quality. This possibility has been explored by measuring invertase activity after germination in six-row and two-row types of malting barleys that display a range in malting qualities.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* **L.**)

The six-row barleys—Conquest, Manker, Bonanza, Dickson, Beacon, Manchuria, and Barbless—were grown in 1972 at Crookston, Minn., and East

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Lansing, Mich. They are varieties and selections from the Mississippi Valley Uniform Nursery.

The two-row barleys—ID 601810, ID 67505, Zephyr, and Shabet—were grown in 1972 at Aberdeen, Idaho, and Fort Collins, Colo. They are varieties and selections from the Western Two-Row Nursery.

Germination Procedure

Of the cleaned barleys, 170 g (dry basis) were steeped to 45% moisture at 16° C and grown at this temperature and moisture content in the dark for 5 days in a modified drum-type germination chamber where 36 perforated cans containing

TABLE I
Analyses of Malts and Germinated Barleys

Location[a] and Variety		Malt Extract %	Extract Fine-Coarse Diff. %	Wort N %	Sol N % of Malt N	α-Amylase 20° U/g[b]	Sol Invertase U/100 kernels[c]	Insol Invertase U/100 kernels[c]
Six Row								
Conquest	C	74.8	1.6	0.713	33.2	49.5	69	112
	EL	76.5	1.2	0.845	37.9	57.8	70	83
Manker	C	74.9	1.4	0.946	42.2	48.6	69	115
	EL	76.6	1.1	0.966	46.9	49.2	80	127
Bonanza	C	76.2	1.6	0.697	34.3	48.4	58	114
	EL	77.2	2.0	0.835	40.3	61.3	105	138
Dickson	C	74.7	2.5	0.676	33.0	42.2	62	112
	EL	75.8	2.1	0.768	37.1	41.3	84	97
Beacon	C	76.6	2.0	0.702	32.4	36.3	50	125
	EL	77.3	2.7	0.775	35.7	38.8	72	136
Manchuria	C	73.5	2.8	0.705	31.9	35.0	47	110
	EL	75.4	4.6	0.815	35.3	39.7	62	107
Barbless	C	70.8	5.7	0.552	23.9	18.2	38	98
	EL	71.7	4.0	0.695	29.3	29.7	69	86
Two Row								
ID 601810	A	78.1	2.7	0.755	33.4	45.2	84	165
	FC	81.2	2.3	0.752	38.4	47.3	70	154
Zephyr	A	76.6	2.8	0.518	24.7	19.6	46	130
	FC	79.2	2.3	0.559	30.5	26.0	63	140
Shabet	A	74.8	3.6	0.553	25.7	22.6	75	172
	FC	79.6	5.7	0.599	31.5	25.7	59	137
Betzes	A	75.2	3.8	0.612	25.0	21.9	55	121
	FC	79.7	4.6	0.618	31.5	26.8	61	136

[a]C = Crookston, Minn.; EL = East Lansing, Mich.; A = Aberdeen, Idaho; FC = Fort Collins, Colo.
[b]U = Units.
[c]Mean of two determinations — error ± 5%.
[d]Mean of two determinations — error ± 7%.

the samples were slowly rotated. Moisture-conditioned air was circulated through the chamber and samples during the germination period. The germinated barleys were lyophilized to less than 14% moisture and stored in a freezer. Roots were not removed.

Malting and Kilning

Malt was produced as described previously (5) from 170-g (dry basis) portions of the barleys.

Malt Analysis

Standard methods (6) were used to determine fine grind extract, coarse grind extract, malt and wort nitrogen, α-amylase, and moisture. Rootlet loss was measured by removing and weighing the brittle roots after kilning.

Extraction of Invertase and Assay for Activity

Of the lyophilized germinated barleys, 2-g samples were extracted and assayed for soluble and insoluble invertase as described previously (5). Since essentially all of the invertase is in the embryonic tissues (4), the activities were calculated on a per kernel basis. Invertase was measured with sucrose substrate as described previously (4) at 25° C and pH 4.35. A unit of activity is the production of 1 μg of reducing sugar, as glucose, per min.

RESULTS AND DISCUSSION

During germination (malting) of barley, enzymatic hydrolysis of high-molecular-weight constituents, primarily proteins and carbohydrates, occurs, and the water solubility of the ground malt is increased. The array of changes that occurs during malting is called "modification." Thus, for a well-modified malt, the amount of water-soluble material in the wort is not affected greatly by the particle size of the ground malt used to prepare it. However, the quantity of material leached from a poorly modified malt during mashing depends primarily on the particle size of the ground malt.

In Table I the "Malt Extract" value is the per cent by weight of finely ground malt which is water-soluble. The difference in this value and that for coarsely ground material is the value given as "Extract, Fine Minus Coarse Grinds." Adequate modification is indicated by high protease activity as reflected by wort nitrogen values and by a large solubilization of the total malt nitrogen, and by high amylase activity.

Six-Row Varieties

On the basis of the data (Table I) for malt extract, fine-coarse extract, per cent of nitrogen as soluble nitrogen, and α-amylase the samples of Manchuria, Barbless, and possibly Beacon (because of low α-amylase) would not be suitable for the usual commercial malting. This is particularly true for those varieties grown at Crookston which showed consistently low values for extract, α-amylase, and proportion of soluble nitrogen.

Table II shows the means for both locations and the ranking according to magnitude of enzymatic activity. Except for the insoluble invertase, Barbless and Manchuria are lowest in activity and are ranked sixth and seventh, but not

TABLE II
Means of Both Locations[a]

Variety	Sol. Invertase Units/100 kernels	Insol. Invertase Units/100 kernels	α-Amylase 20° Units/g	Sol. N % of Malt N
Six Row				
Conquest	70 (4)	97 (6)	53 (2)	35 (3)
Bonanza	81 (1)	126 (2)	54 (1)	37 (2)
Manker	75 (2)	121 (3)	49 (3)	44 (1)
Dickson	73 (3)	104 (5)	42 (4)	35 (4)
Beacon	61 (5)	130 (1)	37 (5)	34 (5)
Barbless	54 (6)	108 (4)	23 (7)	26 (7)
Manchuria	53 (7)	92 (7)	37 (6)	33 (6)
Two Row				
ID 601810	77 (2)	160 (2)	46 (2)	35 (2)
ID 67505	95 (1)	172 (1)	48 (1)	37 (1)
Zephyr	54 (5)	135 (4)	23 (5)	28 (5)
Shabet	67 (3)	154 (3)	24 (3)	29 (3)
Betzes	58 (4)	128 (5)	24 (4)	29 (4)

[a]Values in parentheses are rankings of varieties according to enzymatic activities.

TABLE III
Correlation Coefficients[a,b]

Barley Class	Variable	Malt Extract	Extract Fine-Coarse Grinds	Wort N	Sol N % of Malt N	α-Amylase
	Extract Fine-Coarse Grinds	−0.15				
6 Row plus 2 Row	Wort N	0.10	−0.58**			
	Sol N % of Malt N	0.40*	−0.60**	0.91**		
	α-Amylase	0.28	−0.71**	0.83**	0.84**	
	Sol Invertase	0.43*	−0.39	0.54**	0.58**	0.65**
	Insol Invertase	0.67**	−0.002	−0.12	0.04	0.02
	Extract Fine-Coarse Grinds	−0.71**				
	Wort N	0.60*	−0.60*			
6 Row	Sol N % of Malt N	0.72**	−0.73**	0.95**		
	α-Amylase	0.75**	−0.80**	0.68**	0.77**	
	Sol Invertase	0.56*	−0.48*	0.62*	0.69**	0.72**
	Insol Invertase	0.59*	−0.31	0.29	0.42	0.30
	Extract Fine-Coarse Grinds	−0.12				
2 Row	Wort N	0.63*	−0.37			
	Sol N % of Malt N	0.91**	−0.26	0.85**		
	α-Amylase	0.69*	−0.49	0.97**	0.89**	
	Sol Invertase	0.35	−0.41	0.77**	0.50*	0.81**
	Insol Invertase	0.19	−0.42	0.57	0.44	0.66*

[a]Significance: 6 row plus 2 row = 1% 0.50; 5% 0.40; 6 row = 1% 0.64; 5% 0.51; 2 row = 1% 0.73; 5% 0.60.
[b]**Significant at 1% leve; * significant at 5% level.

consistently in that order for each property. Beacon is somewhat higher than Barbless, but not Manchuria except for soluble invertase. Bonanza, Manker, Dickson, and Conquest are the top four, but not always in the same order. Manker is a variety that is characteristically high in protease activity.

Two-Row Varieties

The data of Tables I and II show that on the basis of wort nitrogen, proportion of soluble nitrogen, and α-amylase the varieties can be divided into two groups: those which modify well (ID 601810 and ID 67505), as reflected by high values, and those which do not (Shabet, Zephyr, and Betzes) as shown by low α-amylase and proportion of soluble nitrogen. These malt properties are consistently higher for barleys from Fort Collins. Based on the soluble invertase the same grouping can be made, although the Fort Collins values are not always the higher of the two locations. The insoluble invertase values tend to support the grouping except for Shabet, ID 601810, and ID 67505 from Aberdeen which have about the same value.

Variation of malt characteristics for a barley grown in different locations is, of course, to be expected. However, the soluble invertase for all barleys in Table I appears to vary more in this respect than do the other enzymatic activities.

Table III shows the correlation coefficients for invertase activities and the various malt quality factors. The soluble invertase is significantly correlated with all the usual malt quality factors except for fine-coarse extracts for the 6-row plus 2-row barley class and malt extract for the two-row class. The insoluble invertase activity does not show any such consistent correlation. Thus, it appears that soluble invertase is a measure of malt quality. However, since an appreciable amount of the soluble invertase is in the roots (4), and since the roots represent a major site of sucrose utilization during malting, the roots must be included in the analyzed material, and assays should be done before kilning. The usual malt analyses are done on the kilned malt after the roots are removed. As far as the maltster and brewer are concerned, the invertases have performed their function during the germination period when protease, carbohydrase, and other enzyme syntheses occur. Starch and protein are the substrates of importance during mashing, and maltose, glucose, and amino acids during fermentation of wort.

The solubility of invertases in plant tissue is not clearly defined: in some tissues, including barley malt, the solubility depends to a degree on the method of extraction (4,7–10). Thus, the *in vivo* distribution of these enzymes is highly speculative. The insoluble enzyme may be associated to a large extent with cell walls where it is involved in the synthesis of this structure (9). In germinating barley, insoluble invertase is present in all tissues of the embryo, but not elsewhere in the kernel (4). The proportion of insoluble to soluble enzymes is highest in the root.

Present ignorance of the significance of invertase solubility need not preclude use of the soluble invertase as a measure of malt quality that can supplement the conventional analyses. Unfortunately, the assay procedure for invertase requires more time than for the conventional malt assays.

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