Invertase Activities during the Germination of Barleys that Differ in Malting Properties[1]

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ABSTRACT

Invertase activities of Larker barley of good malting quality and of Trebi and Feebar barleys of poor malting quality were examined during 5 days of germination. During the first 3 days the levels of soluble and insoluble invertases of the poor malting barleys were at least as high as those of Larker. Amylase and protease activities of Trebi and Feebar were low throughout the entire germination period. After 5 days of germination the levels of the soluble invertases of Trebi and Freebar were low, but the levels of the insoluble invertases were similar to that of Larker. The levels of soluble invertases of Trebi and Feebar were low in all tissues of the embryo (roots, shoot, axis, and scutellum). Sucrose levels in the scutellum were similar for all varieties at the 3- and 5-day stages of germination. The author postulates that if amylase and protease syntheses in the aleurone cells depend upon invertase activity in the embryo, then the soluble invertases are limiting in Feebar and Trebi.

The data of Radley (1,2) have shown that the secretion of gibberellins in the barley kernel occurs in the scutellum during the first 2 days of germination and thereafter from the embryonic axis. Normally these compounds stimulate carbohydrase and other enzyme synthesis in the aleurone cells. Glucose formed from enzymatic hydrolysis of carbohydrate in the endosperm is translocated to the scutellum where sucrose is synthesized (3), and the sucrose is available for the synthesis of new tissue by the developing embryo. According to Radley, high levels of sucrose in the scutellum inhibit the secretion of gibberellin, and lead to reduced gibberellin-stimulated synthesis of hydrolases in the aleurone cells.

Palmer (4) showed that the sucrose concentration was higher than the raffinose concentration in the scutellum and axis of the ungerminated kernel, but the sucrose level rapidly decreased during germination and increased again after root emergence when sugars from the modified endosperm were translocated to the scutellum. As growth continued, the sucrose was used rapidly by the developing roots where invertase activity was high. Jones and Armstrong (5) claim that high concentrations of sugars arising from hydrolysis of endosperm constituents near the aleurone cells inhibit the production of α-amylase by these cells. This increased concentration of low-molecular-weight carbohydrates near the aleurone cells could be caused by inadequate utilization of sucrose in the scutellum.

Apparently, for germination to proceed properly there must be a continuous formation and utilization of sucrose. One of the enzymes obviously associated with sucrose metabolism is invertase (D-fructofuranoside fructohydrolase, E.C.3.2.1.26).

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Early work by Nolte and Kirchdorfer (6) and by Hoffmann and Günzel (7) showed that invertase activity in barley increased during germination and that the magnitude of the increase might be characteristic of the barley variety. In germinated barley there are two soluble invertases in the shoot. The scutellum, roots, and axis contained one soluble invertase that differs from the shoot enzymes. All the tissues of the embryo contain an insoluble invertase. The degermed caryopsis has little or no invertase activity.

This report describes the changes in invertase activities during the germination of barley, and discusses the relation between invertase activity and malting quality.

MATERIALS AND METHODS

Barley

The barleys (*Hordeum vulgare* L.) Larker, Trebi, and Feebar were grown at the same location in South Dakota in 1970.

Germination Procedure

One hundred seventy grams (dry basis) of the cleaned barleys were steeped to 45% moisture at 16°C., and germinated at this temperature in the dark. The germination chamber is a modified drum type that slowly rotates 36 perforated cans containing the samples. Moisture-conditioned air is circulated through the chamber and samples during the germination period. Portions of the barleys were removed after 1, 3, and 5 days of germination and were lyophilized to 4 to 8% moisture. Rootlets were not removed.

Malting

The barleys were treated as in the germination procedure above except that they were germinated for 5 days and then kilned according to the following program: 4 hr. at 35°C., 8 hr. at 45°C., 8 hr. at 55°C., and 4 hr. at 65°C. Rootlets were removed after kilning.

Malt Analysis

The following characteristics of malt were examined by standard methods (8): fine grind extract; coarse grind extract; malt nitrogen; wort nitrogen; diastatic power; α-amylase; and moisture. The growth indices were determined by the method of Shands et al. (9). Rootlet loss was determined by removing and weighing the brittle rootlets after kilning.

Extraction of Invertase and Assay for Activity

These procedures have been described in detail (10). Briefly, 1-g. samples of ungerminated barley or barley germinated 1, 3, or 5 days were ground finely and extracted with 12 ml. 0.2M citrate buffer, pH 5.0, for 1 hr. at 0°C. The suspensions were centrifuged at 55,000 × g and the pellet was washed five times with 6 ml. of the buffer. Each washing was centrifuged as before. The combined extracts and washings, containing the soluble enzymes, were dialyzed against 0.02M citrate buffer, pH 5.0. The residues, containing the insoluble enzymes, were suspended in 20 ml. of the dialysis buffer and dialyzed against this buffer. Similarly, 50 mg. of scutellum tissues and 15 mg. of the other embryo tissues separated from the barleys germinated for 5 days were extracted, with quantities of the buffer reduced appropriately.

The invertase activities of these preparations with sucrose substrate were measured by the copper reduction method described previously (10) and in accordance with the demonstrated temperature and pH requirements of the enzymes. The soluble acidic enzyme was incubated with substrate at pH 5.0 and 25°C.; the soluble basic enzyme and the insoluble enzymes were assayed at pH 4.1 and 30°C.; and mixtures of the soluble acidic and soluble basic enzymes at pH 4.35 and 25°C. A unit of activity is 1 γ reducing sugar, as glucose, produced per minute.

Electrofocusing

The soluble enzyme fractions from 5 g. of Larker and Trebi barleys that had been germinated 5 days were electrofocused. This procedure has been described by Prentice (10). In the present study the ampholyte (LKB Instruments, Inc., Rockville, Md.) of pH range 3 to 10 was used, and the enzymes after separation into acidic and basic fractions were dialyzed against 0.02M citrate buffer, pH 5.0, before assaying for activity.

The fractions containing the basic invertase were pooled, and the volume and activity of the solution determined. Similarly, the total amount of the acidic enzyme was measured.

Separation of Scutella, Roots, Shoots, and Axes

The barleys were germinated for 3 and 5 days and then frozen. A few kernels at a time were thawed and the tissues were excised with a scalpel and frozen immediately. When tissues from 350 to 500 kernels were obtained from each barley at each of the two stages of germination, the material was lyophilized and ground finely in a mortar.

Determination of Moisture in Freeze-Dried Tissue

Weighed samples of ground scutella, roots, axes, and shoots were stored over phosphorous pentoxide for 1 month and then reweighed.

TABLE I. MALT ANALYSES

Barley Variety	Extract Fine Coarse Grinds %	Soluble Nitrogen % of Malt N	α-Amylase 20° units/g.	Diastatic Power degrees/g.	Growth Index	Rootlets % of Malt
			5-Day Germination			
Larker	1.0	42	65.1	285	98	3.4
Trebi	5.6	29	16.9	156	92	3.6
Feebar	8.1	29	39.7	146	96	4.0
			3-Day Germination			
Larker	2.4	38	48.6	245	88	2.4
Trebi	10.1	27	12.2	136	95	2.2
Feebar	11.1	27	27.0	117	94	3.4
			1-Day Germination			
Larker	18.8	23	...	173	28	0.4
Trebi	18.8	19	...	88	28	0.7
Feebar	21.7	19	...	60	25	0.9

TABLE II. AMYLASE AND INVERTASE ACTIVITIES

Barley Variety	α-Amylase 20° Units/ Kernel	Diastatic Power Degrees/Kernel	Invertase Units/Kernel	
			Soluble	Insoluble
		5-Day Germination		
Larker	1.66	7.3	4.1	5.0
Trebi	0.60	5.6	2.8	6.7
Feebar	1.13	4.2	3.0	5.6
		3-Day Germination		
Larker	1.18	7.2	4.1	4.2
Trebi	0.43	4.9	4.2	4.2
Feebar	0.77	3.3	5.3	7.8
		1-Day Germination		
Larker	Trace	4.4	1.0	0.9
Trebi	Trace	3.1	0.6	1.5
Feebar	Trace	1.7	0.9	1.2
		Ungerminated Barley[a]		
Larker	...	...	0.7	2.8
Trebi	...	...	1.0	3.0
Feebar	...	...	0.7	2.1

[a]Not analyzed for α-amylase and diastatic power.

Determination of Sucrose in Scutella

The sugars were extracted from 15-mg. portions of tissue with 200 μl. of boiling 80% ethanol. Sucrose was separated by the usual quantitative paper chromatographic procedures with ethyl acetate:pyridine:water 120:50:40 as the solvent (11) and assayed with the phenol-sulfuric acid spectrophotometric method (12).

RESULTS

Malting Analyses

The analyses of the malts are shown in Table I.

For the 5-day malting time the difference between the fine grind extract and coarse grind extract is small for Larker. This small difference shows that extensive hydrolysis of the high-molecular-weight constituents of the kernel (good modification) has occurred during malting. These differences are large for Trebi and Feebar malts, and show that these barleys modified poorly during malting. Trebi and Feebar compare unfavorably with Larker for enzymatic activity as shown by the values for soluble nitrogen, α-amylase, and diastatic power (Table I). Under normal conditions these barleys would not be used for commercial malting.

The three barleys did not differ much in acrospire development, as shown by the growth indices, except at the 3-day stage when Larker may have had slightly less acrospire. Rootlet development for Feebar exceeded that of the other two varieties, which had about the same amount of rootlet growth (Table I).

TABLE III. INVERTASE IN EMBRYO TISSUE

Barley Variety	Soluble		Insoluble	
	units/kernel	units/mg. tissue	units/kernel	units/mg. tissue
		Scutella		
Larker	0.75	0.63	0.56	0.47
Trebi	0.37	0.33	0.61	0.54
Feebar	0.41	0.40	0.54	0.53
		Roots		
Larker	1.4	2.1	2.1	3.1
Trebi	1.0	1.4	2.5	3.3
Feebar	1.1	1.3	3.6	4.4
		Shoots		
Larker	2.3	2.0	0.64	0.56
Trebi	1.5	1.3	0.37	0.33
Feebar	1.2	1.3	0.31	0.34
		Axes		
Larker	2.5	2.5	1.9	1.9
Trebi	2.2	1.7	2.7	2.0
Feebar	1.9	1.7	2.9	2.6

Invertase Activity during Germination

Because the invertases in germinating barley are entirely in the embryo (10), comparison of barleys with different kernel weights can be made best on a per kernel basis. The amylase and invertase activities of the three barleys, calculated on a kernel basis, are shown in Table II. In ungerminated barley the soluble invertase was at a low level for all varieties, and it did not change significantly after 1 day of germination. After 3 days of germination, the activities of the soluble invertase increased. At this stage there was no difference between Larker and Trebi, but Feebar had a higher activity. After 5 days of germination, a different pattern became evident. Both Trebi and Feebar had dropped to a low level, whereas Larker maintained its activity. The α-amylase and diastatic power of Trebi and Feebar were consistently lower than those of Larker.

The activities of the insoluble invertase presented a somewhat different picture. Appreciable levels were present in all ungerminated barleys, but these activities dropped after 1 day of germination. However, after 3 days of germination, activities increased, particularly in Feebar, and after 5 days the poorer malting varieties Trebi and Feebar were more active than Larker.

When the individual embryo tissues were examined after 5 days of germination (Table III), there were low levels of soluble invertase in all tissues of Trebi and Feebar. The levels of the insoluble enzymes in all tissues of Trebi and Feebar, except shoots, were at least as great as those of Larker.

Electrofocusing

The separation of the soluble acidic and basic invertases from Larker is shown in Fig. 1. Essentially the same pattern was obtained for the soluble enzymes from Trebi. The isoelectric pH for the acidic invertase is approximately 4, and

presumably this enzyme comes from the shoot as demonstrated previously (10).The enzymes responsible for the peak at pH 10 probably consist of the basic invertase from rootlets and embryo, isoelectric at pH 10, and the basic invertase from shoots, isoelectric at pH 9.5 (10).

Measurement of the total activity of the acidic and basic enzymes and calculation of activity on a per kernel basis revealed that Larker had 0.7 units of acidic invertase and 3.1 units of basic invertase, whereas Trebi had only 0.4 units of acidic and 2.3 units of the basic enzymes. The sum of the two activities for each variety (3.8 units per kernel for Larker and 2.7 units per kernel for Trebi) agrees reasonably well with the measurement for the total soluble invertase at the 5-day stage before electrofocusing, i.e., 4.1 units for Larker and 2.8 units for Trebi (Table II).

Sucrose Levels in Embryo Tissues

The levels of sucrose in the scutella after 3 and 5 days of germination are shown

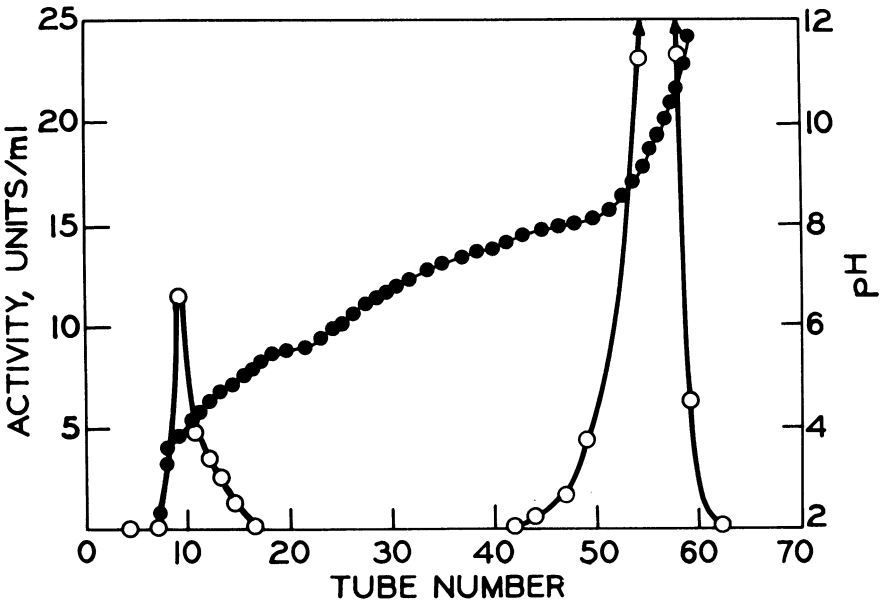

Fig. 1. Electrofocus of Larker soluble invertase. •, pH; o, activity.

TABLE IV. , SUCROSE IN SCUTELLA

Barley Variety	Germination Time			
	3 days		5 days	
	γ/kernel	γ/mg. tissue	γ/kernel	γ/mg. tissue
Larker	64	62	122	108
Trebi	60	50	135	113
Feebar	75	72	100	98

in Table IV. After 3 days the levels for Larker and Trebi were comparable and the level for Feebar was somewhat higher; after 5 days of germination the levels for all varieties were similar.

DISCUSSION

In higher plants invertases are found with widely differing properties, and their role in metabolism is not completely understood. In corn radicle (13), bean root (14), and pea root (15) high activity is associated with the elongation of cells where the presumed functions of invertase are to 1) increase the osmotic pressure of the cell during water uptake and 2) to supply substrate and energy for the synthesis of new tissue. The insoluble invertases commonly found in higher plants are presumed to be associated with cell-wall and membrane materials and to play a role in their synthesis (15), whereas the soluble forms appear in the cytoplasm and vacuole.

In pea and carrot roots (15,16) an invertase with pH optimum 4.5 is in both the soluble and insoluble fractions, whereas a soluble enzyme with pH optimum 7.4 is in the cytoplasm. The former invertase is presumed to be active in cells with a high demand for sucrose hydrolysis such as rapidly elongating cells. This enzyme may be similar to the soluble and insoluble enzymes of germinating barley that have a pH optimum 4.1. Germinating barley, however, has no invertase with pH optimum 7.4.

Part of the insoluble enzyme in the barleys examined may be related to root development because after 5 days of germination all barleys had about the same growth of roots and about the same level of insoluble invertase (Tables I and II). These data support previous work (10) that showed that the ratio of the insoluble to the soluble enzymes is greatest in the roots. If invertase is involved with the development of amylases and proteases, it must be one or both of the soluble invertases that reach a final low value in the poorer malting varieties. Inasmuch as the sucrose concentrations in the scutella of the three varieties are not greatly different, the low amylase and protease of Trebi and Feebar could not likely be caused by the sucrose-inhibited gibberellin secretion described by Radley (1,2). Similarly, the low amylase and protease of Trebi and Feebar were not likely caused by the accumulation of low-molecular-weight carbohydrates near the aleurone cells, as discussed by Jones and Armstrong (5) for Himalaya barley.

Literature Cited

1. RADLEY, M. Site of production of gibberellin-like substances in germinating barley embryos. Planta 75: 164 (1967).
2. RADLEY, M. The effect of the endosperm on the formation of gibberellin by barley embryos. Planta 86: 218 (1969).
3. EDELMAN, J., SHEBKO, S. I., and KEYS, A. J. The role of the scutellum of cereal seedlings in the synthesis and transport of sucrose. J. Exp. Bot. 10: 178 (1959).
4. PALMER, G. H. Sucrose and raffinose utilization during the early stages of barley germination. J. Inst. Brew. 75: 505 (1969).
5. JONES, R. L., and ARMSTRONG, J. E. Evidence for osmotic regulation of hydrolytic enzyme production in barley seeds. Plant Physiol. 48: 137 (1971).
6. NOLTE, H., and KIRCHDORFER, A. M. Zur Kenntnis der Saccharase in Braugersten. Brauwissenschaft 7: 161 (1954).
7. HOFFMANN, E., and GÜNZEL, G. Uber die Verteilung einiger Karbohydrasen in Keimen der Gerste. Brauwissenschaft 8: 2 (1955).
8. AMERICAN SOCIETY OF BREWING CHEMISTS. Methods of analysis. The Society: Madison, Wis. (1958).

9. SHANDS, H. L., DICKSON, A. D., DICKSON, J. G., and BURKHART, B. A. The influence of temperature, moisture, and growth time on the malting quality of four barley varieties. Cereal Chem. 18: 370 (1941).
10. PRENTICE, N. Invertase of germinated barley. J. Agr. Food Chem. 20: 764 (1972).
11. SMITH, I. Chromatographic techniques. Interscience: New York (1958).
12. DUBOIS, M., GILLES, K., HAMILTON, J. K., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350 (1956).
13. HELLEBUST, J. A., and FORWARD, D. F. The invertase of the corn radicle and its activity in successive stages of growth. Can. J. Bot. 40: 113 (1962).
14. ROBINSON, E., and BROWN, R. The development of the enzyme compliment in growing root cells. J. Exp. Bot. 3: 356 (1952).
15. LYNE, R. L., and ap REES, T. Invertase and sugar content during differentiation of roots of *Pisum sativum*. Phytochemistry 10: 2593 (1971).
16. RICARDO, C. P. P., and ap REES, T. Invertase activity during the development of carrot roots. Phytochemistry 9: 239 (1970).

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