Beta-D-Glucan in Developing and Germinating Barley Kernels

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ABSTRACT

The levels of β-D-glucans in barley kernels were followed during the growth of several varieties of barley and in kernels as they germinated over a four-day period. Of the two-row varieties, Birgitta and Piroline, Birgitta synthesized β-D-glucan much more rapidly than Piroline did. Similarly, the six-row variety Larker developed β-D-glucan somewhat more rapidly than did the variety Beacon. Of the western types of barley, the six-row Karl showed the most rapid decrease in β-D-glucan during germination, followed by the two-row varieties Klages and Piroline, then by the six-row variety Steptoe, and finally by Birgitta despite Birgitta's high β-glucanase activity. This indicated that high β-glucanase activity by itself may not be a meaningful indication of satisfactory β-D-glucan modification during malting. Of the midwestern six-row varieties, Dickson, Conquest, and Beacon showed a more rapid decrease in β-D-glucan during germination than did Larker and Morex.

Barley contains 4–8% β-D-glucan (Prentice et al 1980) located mainly in the cell walls of the endosperm, where it forms a complex matrix with protein (Forrest and Wainwright 1977). It is composed of β-1, 4-glucopyranosyl and β-1, 3-glucopyranosyl units in the proportion of about 7:3 (Bathgate and Dalgliesh 1975). During malting and the mashing stage of brewing, these gums must be hydrolyzed to low molecular weight products; otherwise filtration of the brewer's mash is difficult. Therefore, the amount of β-D-glucan in barley and the activity of β-glucanases in malt are important quality factors for malting barleys.

β-D-glucans appear to be important in the nutritional properties of barley. They restrict weight gain when present in the diet of the chick (Burnett 1966, Laerdal et al 1960) through an increased viscosity of the intestinal fluid, which contributes to the characteristic sticky feces of birds on a barley diet. Under this condition, nutrient uptake and water relationships in the digestive tract are impaired.

β-D-glucans, which are generally present at higher levels in barley than in other cereals, may have a role in lipid metabolism (Qureshi et al 1980) because barley diets markedly lowered cholesterol synthesis and stimulated fatty acid formation by enzymes in the liver.

In the present work we have followed the level of β-D-glucan in the developing barley kernel in the field and during the subsequent germination of the grain under malting conditions.

MATERIALS AND METHODS

Preparation of β-Glucanase (3.2.1.6 and 3.2.1.4)

β-Glucanase was prepared from "Cellulase Onazuka SS," a crude culture filtrate of *Trichoderma reesei* obtained from Yakult Biochemicals, Inc., Nishimoniya, Japan. The crude β-glucanase complex of enzymes in this preparation was purified as described by Prentice et al (1980).

β-Glucanase was prepared also from *T. reesei* QM 9414. The fungus was obtained from the U.S. Army (Natick Laboratories, Natick, MA 01760). It was cultured in 1-L portions of cellulose medium for seven days according to Mandels and Weber (1969). The composition of the medium was as follows: 2.0 g of KH_2PO_4, 1.4 g of $(NH_4)_2SO_4$, 0.3 g of urea, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2$, 5.0 mg of $FeSO_4 \cdot 7H_2O$, 1.56 mg of $MnSO_4 \cdot H_2O$, 1.4 mg of $ZnSO_4 \cdot 7H_2O$, 2.0 mg of $CoCL_2$, 31 g of Whatman powdered cellulose (Whatman Co., Clifton, NJ), 10 g of Difco peptone (Difco Laboratories, Detroit, MI), 0.5 g of Tween 20 (Fisher Scientific Co., Fair Lawn, NJ), and 1 L of water.

The suspension was adjusted to pH 5.0 before autoclaving. It was inoculated with 10 ml of *T. reesei* spore suspension from potato-dextrose agar slants and oscillated at 150 rpm for seven days at 20°C. The pH was 3.0 after the incubation period. The culture was filtered through glass wool, and the filtrate was dialyzed against distilled water. The nondialyzable portion was freeze-dried and taken up in 0.02 *M* acetate, pH 4.6, so that the solution contained 1,500 μg of N per milliliter. The solution was dialyzed against the same buffer. A portion (12–15 ml) of the solution was treated with diethylaminoethyl cellulose as described previously (Prentice et al 1980) to purify the β-glucanase.

Barley β-D-Glucan

This was purchased from Biocon, Inc., Lexington, KY 40507.

Barleys

The barleys were grown in rod-row plots at Fargo, ND, Aberdeen, ID, St. Paul, MN, and Madison, WI.

Germination Procedure

Cleaned barleys (175 g, db) were steeped to 45% moisture at 16°C and germinated at this temperature in the dark. The germination chamber was a modified drum that slowly rotated 36 perforated cans containing the samples. Moisture-conditioned air was circulated through the chamber during the germination period. Portions of the barleys were removed after one, two, three, and four days of germination and were lyophilized to 4–8% moisture. Rootlets were not removed.

Harvest of Developing Kernels

Each variety of barley was grown in three rod-row plots. Heads from a portion of each plot were harvested by hand at each development period. The heads were frozen and lyophilized to 4–8% moisture. Awns were removed from the dry, intact kernels.

β-D-Glucan Assay

The barleys were assayed with the *Trichoderma* β-glucanase as described by Prentice et al (1980). β-D-glucans were extracted at 80°C for a prolonged period under alkaline conditions so that essentially all the material became soluble. Polymers with molecular weights above 10^4 were measured under the conditions of the assay. All assays were done in quadruplicate, and the results were evaluated by variance analysis.

Barley β-Glucanase Activity

β-Glucanase was extracted from barleys by the method of Ballance et al (1976). The substrate solution was 1% barley β-D-glucan in 0.1 *M* acetate, pH 5.0. Reaction mixtures consisted of 20 ml of substrate solution containing an appropriate quantity (usually 100–300 μg) of enzyme nitrogen such that the logarithm of

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the viscosity in centipoises was linear with enzyme concentration. Viscosity was measured at 1-min intervals at 30° C for 20–30 min with a Brookfield Synchro-lectric Viscometer, model LVF (Brookfield Engineering Laboratories, Inc., Stoughton, MA) operated at 6 rpm. The logarithm of viscosity (in centipoises) was plotted against reaction time. The β-glucanase activity was calculated as the change in the logarithm of the viscosity per minute per gram of germinated barley.

RESULTS AND DISCUSSION

Larker, Beacon, Dickson, and Conquest had similarly decreased β-D-glucan levels with germination time (Table I). In fact, no significant difference was found between Dickson and Conquest throughout the four days. Beacon had a slightly higher β-D-glucan level at two days but did not differ significantly thereafter from Dickson and Conquest. The similarity of Beacon to Dickson and Conquest is not surprising because Beacon is a selection from a cross of Conquest and Dickson. Larker, which, like Conquest, has UM570 germplasm, maintained a high β-D-glucan level at three and four days, which caused the interaction between varieties and times to be significant.

Larker and Beacon, although from different crop years, had β-D-glucan levels (Table II) not greatly different from the corresponding values for these varieties grown in 1978 (Table I). Again, the level in Beacon decreased quickly with germination. The performance of the Morex sample was similar to that of Larker from either crop year. Thus, the β-D-glucan level in Morex does not appear to be a factor in its characteristically high extract, which is higher than that of Larker.

The western barleys in Table III are all two-row barleys except Karl and Steptoe, which are six-row. All are malting varieties except Steptoe, a rather poor feed barley. Only Birgitta had a significantly high level of β-D-glucan before germination. Marked differences in the changing β-D-glucan levels are evident. The quantity of β-D-glucans in Karl decreased quickly to a low level at four days. The performances of Klages and Piroline were similar, whereas Steptoe at four days had significantly more β-D-glucan than did any variety except Birgitta. Birgitta is characteristically high in β-D-glucan (Prentice et al 1980), and the present data show that the relatively high level is maintained during germination.

The data of Table IV for Piroline and Birgitta grown in 1979 clearly parallel the corresponding data for the 1975 crop, although the level for Birgitta at harvest in 1979 was somewhat less than that in 1975.

Commercial malts do not contain rootlets, whereas the rootlets were not removed from the freeze-dried, germinated barleys in this work. The fibrous rootlets represent about 3% of dried malt by weight and may contain about 7% cellulose (Hough et al 1971). Thus, glucose from the cellulose, if completely hydrolyzed by the fungal β-glucanase, would represent only about 0.2% of the germinated barley after four days germination and considerably less at shorter germination times when rootlet growth was not as advanced. Such a value should not affect the interpretation of the data in Tables I–IV.

β-D-glucan developed more rapidly during the growth of Birgitta than during that of Piroline (Table V). Although Larker and Beacon were similar in the rate of β-D-glucan synthesis during early development, Beacon had significantly less β-D-glucan than did

TABLE I
Beta-D-Glucan (%)[a] in Germinating Midwestern Barleys (ND-78)

Variety	Germination Time, days				
	0	1	2	3	4
Larker	4.8	4.3	3.6	2.6	1.7
Beacon	4.9	4.7	3.6	1.9	1.0
Dickson	5.4	4.3	3.0	1.7	0.8
Conquest	5.3	4.4	2.8	1.8	0.7

[a]LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.4 and 0.5, respectively, and between times, 0.3 and 0.4, respectively. The interaction varieties × times is significant at the 1% level.

TABLE II
Beta-D-Glucan (%)[a] in Germinating Midwestern Barleys Grown in Different Locations and/or Years

Variety	Germination Time, days				
	0	1	2	3	4
Morex (MN-79)	4.5	4.9	4.0	2.3	1.5
Beacon (ND-73)	4.8	4.3	2.8	1.1	0.4
Larker (ND-76)	5.1	5.6	4.0	2.6	1.7

[a]LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.5 and 0.7, respectively, and between times, 0.3 and 0.5, respectively. The interaction varieties × times is significant at the 1% level.

TABLE III
Beta-D-Glucan (%)[a] in Germinating Western Barleys (ID-75)

Variety	Germination Time, days				
	0	1	2	3	4
Birgitta	7.7	7.6	6.5	4.3	3.2
Klages	5.9	4.8	4.1	2.5	1.8
Karl	6.0	5.1	3.8	2.2	0.8
Steptoe	5.8	5.3	4.6	3.1	2.4
Piroline	6.0	5.3	4.9	3.3	1.7

[a]LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.3 and 0.5, respectively, and between times, 0.3 and 0.5, respectively. The interaction varieties × times is significant at the 1% level.

TABLE IV
Beta-D-Glucan (%)[a] in Germinating Western Barleys (ID-79)

Variety	Germination Time, days				
	0	1	2	3	4
Birgitta	6.2	5.7	5.2	4.5	3.4
Piroline	5.0	4.8	3.8	2.7	2.2

[a]LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.6 and 0.9, respectively, between times, 0.2 and 0.5, respectively. The interaction varieties × times is significant at the 5% level.

TABLE V
Beta-D-Glucan (%)[a] in Developing Western Barleys (ID-79)

Variety	Development Time, days after anthesis				
	12	19	26	29	36
Birgitta	0.9	3.2	5.3	7.1	7.2
Piroline	0.8	2.5	3.4	5.8	5.8

[a]LSD at $P = 0.05$ and $P = 0.01$ between varieties are 1.0 and 1.5, respectively, and between times, 0.5 and 0.7, respectively. The interaction varieties × times is significant at the 1% level.

TABLE VI
Beta-D-Glucan (%)[a] in Developing Midwestern Barleys

Location and Variety	Development Time, days after anthesis			
	13	18	24	34
Madison, WI				
Larker	0.4	1.2	3.5	5.5
Beacon	0.4	1.1	2.9	4.3
Fargo, ND				
Larker	1.9	3.8	5.8	6.0
Beacon	1.0	3.2	4.0	4.2

[a]For WI location, LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.3 and 0.4, respectively, and between times, 0.2 and 0.3, respectively. For ND location, LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.9 and 1.4, respectively, between times, 0.6 and 0.8, respectively. For all locations, LSD at $P = 0.05$ and $P = 0.01$ between varieties are 0.6 and 1.0, respectively, and between times, 0.6 and 1.0, respectively. The interaction varieties × times is significant at the 1% level in all cases.

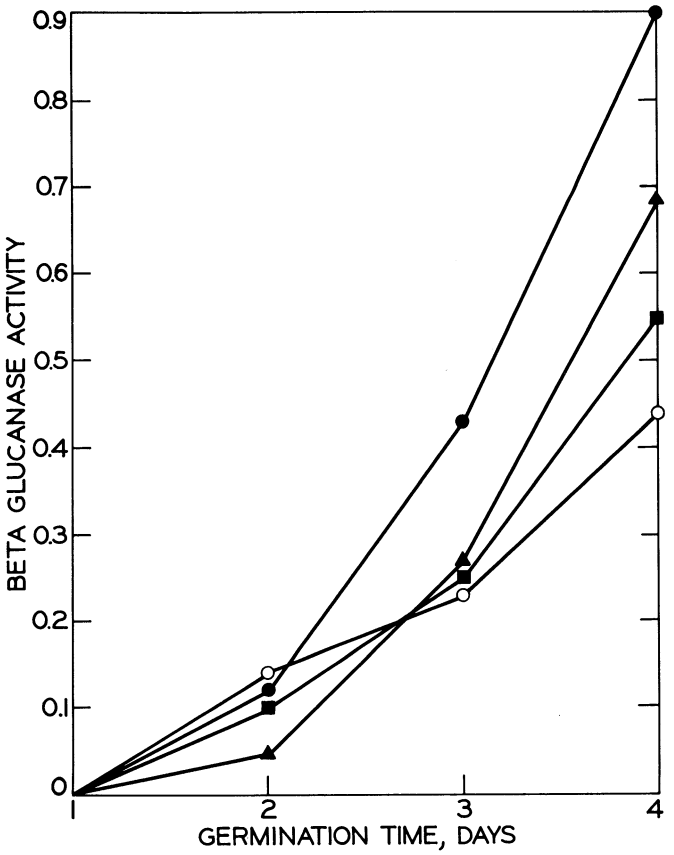

Fig. 1. Beta-glucanase activities during germination of four varieties. Activity = Δ log viscosity (cps)/ min × g barley. ●—● = Birgitta, ▲—▲ = Piroline, ■—■ = Larker, o—o = Conquest.

Larker at the 24-day and 36-day periods (Table VI). This agrees with our previous data (Prentice et al 1980), which show that the mature grain of Beacon has a consistently low level of β-D-glucan.

Figure 1 shows representative β-glucanase activities in the germinating barleys. The values for Beacon and Dickson (not shown) are between the curves for Piroline and Conquest. These data indicate that β-glucanase activity as measured conventionally may not be, in itself, a sufficient indicator of a satisfactorily low β-D-glucan level in malt. The β-D-glucan in barley is not homogeneous. Molecular weights of 2×10^5–2×10^7 have been reported (Bourne and Pierce 1970, Ducroo and Delecourt 1972), and the proportion of 1:4 bonds to 1:3 bonds is variable (Fleming and Kawakami 1977). Part of the β-D-glucan is water-soluble and part water-insoluble (Anderson 1978). Scott (1972) has suggested that the water-insoluble portion may be resistant to β-glucanase during malting and the initial stages of mashing but may become soluble during the high temperatures at the conversion stage of mashing when β-glucanase is inactivated. Thus, the satisfactory lowering of the β-D-glucan level during malting will depend upon such factors as the amount and solubility of β-D-glucan present, the spectrum of molecular weights, the proportion of 1:4 to 1:3 glucosidic bonds, and the amount and specificities of the β-glucanases. For example, a high proportion of water-insoluble β-D-glucans in Birgitta could account for its consistently high β-D-glucan level during malting despite the appreciable β-glucanase present.

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