

# Barley Malt Limit Dextrinase: Varietal, Environmental, and Malting Effects<sup>1,2</sup>

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## ABSTRACT

A new assay procedure was used to study the limit dextrinase activity of barley malt. Variety, location, and crop year were shown to significantly affect the production of limit dextrinase. Enzyme activity remained at a very low level during steeping and up to the third day of germination but increased substantially after the fifth day. Gibberellic acid had a stimulatory effect on limit dextrinase activity. No loss in activity occurred during kilning at 49°C for 24 hr, but activity declined as kilning temperatures increased. In ASBC mashes, 100% of the activity remained after 30 min at 45°C but began to decline as mash temperatures rose to 70°C. Ground malts containing varying levels of limit dextrinase activity were added to fermenting wort, and alcohol production and the extent of fermentability of the wort were closely related to limit dextrinase activity.

**Key words:** Dextrins, Limit dextrinase, Wort fermentability

Limit dextrinase (E.C.3.2.1.10) is one of many enzymes involved in the degradation of starch and starch-derived oligosaccharides. This enzyme specifically attacks the  $\alpha$ -1,6-glucosidic linkage, which occurs in  $\alpha$ -glucans such as amylopectin, pullulan, and amylopectin  $\beta$ -limit dextrin, as well as in starch-derived  $\alpha$ -limit dextrins.

Limit dextrinase has been studied in barley, malt, and other plant materials. Some of the earliest reports on the limit dextrinase activity of barley and malt are those of Kneen (11), Kneen and Spoerl (12), and Lowry et al (14). Other studies were reported by Hopkins and Wiener (10), MacWilliam and Harris (15), and Greig (6). More recently, Manners and co-workers (5,9,16-20,26) made extensive studies of the enzyme from malt and various other plant sources.

Much of the older literature on limit dextrinase contains data obtained using reducing power assays and amylopectin or amylopectin  $\beta$ -limit dextrin as the substrate. These assays have been criticized by Manners and Yellowlees (20) because either the substrate itself or the products derived from the limit dextrinase-mediated reaction were suitable substrates for  $\alpha$ - and  $\beta$ -amylase, which are present in crude malt extracts in variable amounts. The attack by the amylases on these substrates releases additional reducing groups, which may lead to erroneous results.

An improvement in the assay was realized when pullulan was introduced as the substrate by Manners and Yellowlees (20). The exclusive product of the reaction between pullulan and limit dextrinase is maltotriose. Although maltotriose is a relatively poor substrate for the amylases, it is degraded at a measurable rate by malt extracts, by the amylases, and perhaps by endogenous  $\alpha$ -glucosidase. The additional reducing groups produced may again lead to erroneous results (13). A viscometric technique that is not affected by the presence of amyloglucosidase has been described (8), but viscometric techniques may not be suitable for the assay of large numbers of samples.

We described elsewhere (13) a new procedure for the estimation of limit dextrinase activity that does not suffer from these defects. In this procedure, a crude malt extract is dialyzed to reduce blank values and is then incubated with pullulan. During this digestion, limit dextrinase attacks pullulan to produce maltotriose, which is further attacked by other enzymes present in the crude extracts (Fig. 1). These enzymes also attack starch-derived, nondialyzable

oligosaccharides, producing new reducing groups.

After this initial digestion, the reaction mixture is heated to destroy all enzyme activity, and an aliquot is treated with  $\alpha$ -glucosidase to convert maltotriose, maltose, and other starch-derived substrates to glucose, which we have estimated enzymatically. A blank containing no pullulan is run concurrently to estimate starch-derived glucose. Thus, this procedure provides an estimate of limit dextrinase activity, which is unaffected by the presence of  $\alpha$ - and  $\beta$ -amylase and endogenous  $\alpha$ -glucosidase.

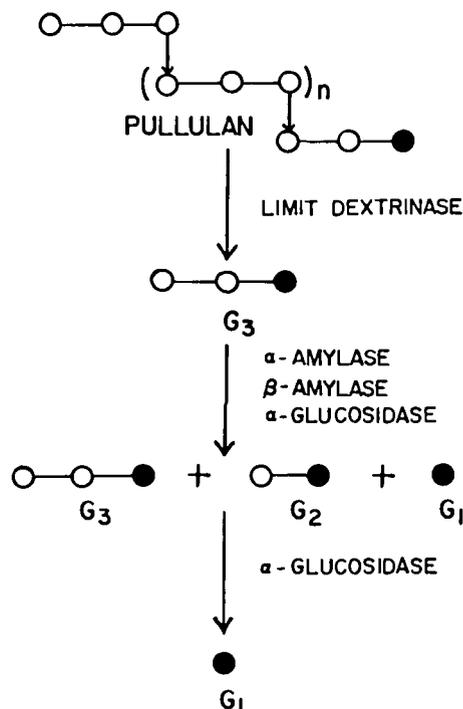
Using this assay procedure, we proceeded to study limit dextrinase with respect to its development during germination, the effect on its activity of kiln and mashing heat, its effects on  $\alpha$ -limit dextrins and wort fermentability, and the variability in its activity due to varietal and environmental effects.

## MATERIALS AND METHODS

Barley samples of the varieties Larker, Morex, Glenn, Klages, Summit, and Azure, and the advanced blue aleurone line, ND 1156, were grown at Fargo, Langdon, Minot and Williston, ND, in 1979, 1980, and 1981. In addition, malts produced from Morex grown in 1981 were obtained from commercial maltsters and brewers.

Barley samples were cleaned by hand and were steeped at 16°C to a moisture content of 45%. After germination for variable periods at 16°C and 100% relative humidity, the samples were either freeze-dried or kilned, freed of rootlets, and ground in a Udy mill through a 0.5-mm screen. When gibberellic acid was used, it was added to the steep water at the levels specified.

Extracts of malt were prepared by treating 5.0 g of malt flour with 25 ml of 0.02M phosphate-citrate buffer (pH 6.8) for 2.5 hr at 20°C. Insoluble material was removed by centrifugation (10,000



**Fig. 1.** Assay procedure for limit dextrinase activity. G = glucose, G<sub>2</sub> = maltose, G<sub>3</sub> = maltotriose.

<sup>1</sup>Presented at the 49th Annual Meeting, Nashville, TN, April 1983.

<sup>2</sup>Published with the approval of the Director of the Agricultural Experiment Station, North Dakota State University, Fargo, as Journal Series No. 1264.

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× g, 2° C, 15 min), and 12.5 ml of the clear supernatant was dialyzed overnight at 4° C against distilled water. The dialyzate was made up to 25 ml with distilled water and re-centrifuged before assay for limit dextrinase activity. One unit of limit dextrinase activity will produce 1 mg of maltotriose in 60 min at 37° C and pH 5.0 when 1-g equivalent of malt is incubated with 0.5% pullulan.

When the activity of malt flour, as opposed to malt flour extracts, was estimated, malt flour (2.0 g) was dispersed in 10 ml of phosphate-citrate buffer at 20° C for 2.5 hr. The suspension was then dialyzed overnight at 4° C and made up to 25 ml. To this was added 25 ml of 0.5% pullulan, and the mixture was incubated at 37° C for 1 hr. Following incubation, the mixture was placed in a boiling water bath for 10 min to inactivate the enzymes present and was then filtered. The filtrate was treated with  $\alpha$ -glucosidase, as previously described (13).

To determine the influence of varietal and environmental factors on limit dextrinase activity, 78 barley samples grown at four locations over three crop years were steeped in acidified steep water ( $H_2SO_4$ , pH 5.0) (21), germinated for 10 days, and freeze-dried as described above. Extracts were assayed for limit dextrinase activity, and soluble protein was determined by a UV-spectrophotometric procedure (22). The resulting data were subjected to statistical analysis, using the 10V procedure of the Biomedical Computer Programs distributed by the Health Science Computing Facility (UCLA, Los Angeles, CA).

TABLE I  
Effect of Various Chemicals on Enzyme Extraction

Chemicals	Activity (units per gram of malt)	Change in Activity (%) <sup>a</sup>
Sodium chloride (0.5%)	16.5	...
Cysteine hydrochloride (0.1%)	23.5	+42
Triton X-100 (0.25%)	22.5	+36
Phosphate citrate buffer (0.02M, pH 6.8)	22.1	+34
Papain (0.1%)	16.7	+ 1

<sup>a</sup> Compared to sodium chloride extract.

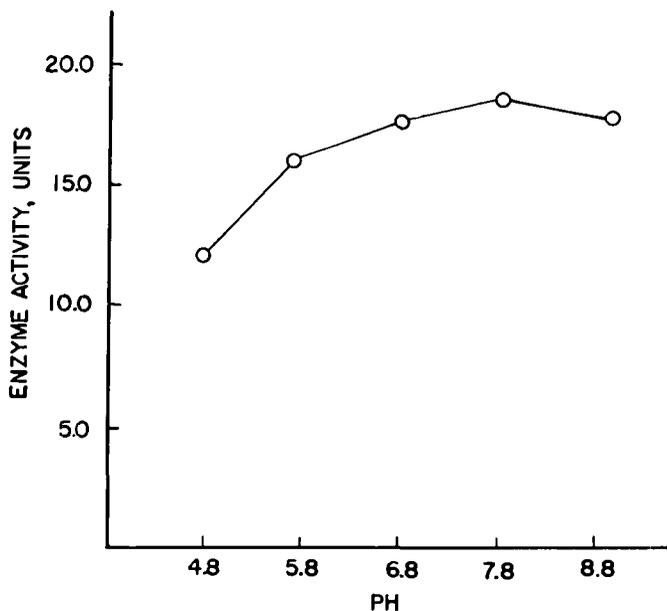


Fig. 2. Effect of pH on the extractability of limit dextrinase activity.

The effect of germination time on the development of limit dextrinase activity was determined by steeping a sample of Morex to 45% moisture at 16° C and germinating individual samples for up to 11 days at 16° C. A concurrent study was performed to determine the effect of gibberellic acid on limit dextrinase activity.

The effect of kilning temperature was determined by subjecting seven-day green malt to kilning at different temperatures for varying periods of time.

To study the effect of limit dextrinase activity on  $\alpha$ -limit dextrin and during fermentation, four malts exhibiting varying limit dextrinase activities were prepared by methods described above. Five-gram samples of the finely ground malt flour were extracted, and the extracts were dialyzed. A reaction mixture consisting of 2 ml of extract and 2 ml of  $\alpha$ -limit dextrin (3.0%) in 0.067M phosphate-citrate buffer was incubated at 37° C for up to 6 hr. Aliquots were removed at intervals and were analyzed by high performance liquid chromatography (HPLC) (23).

In addition, 5 g of flour from these malts was added to individual 200-ml aliquots of unboiled wort prepared from a commercial sample of Morex that had been mashed according to the standard ASBC procedure (MALT-4) (1). The worts were subjected to rapid fermentation (WORT-5) (1), and alcohol production (BEER-4) (1) and fermentability (BEER-6C) (1) were determined.

$\alpha$ -Limit dextrin was prepared from barley starch by the method of Whelan (24). This material was not further attacked by commercial preparations of  $\alpha$ - and  $\beta$ -amylase as determined by HPLC (13).

## RESULTS AND DISCUSSION

### Extraction of Limit Dextrinase Activity

Bathgate et al (2) reported that limit dextrinase activity was difficult to extract from malt using a cold water extraction method, but consistent results were obtained by extraction in 3% potassium chloride containing mercaptoethanol, followed by freeze-pressure extraction. We have examined some of the factors involved in the extraction of the enzyme, including pH, temperature, extraction time, and extracting medium composition.

Table I shows the results obtained when various extraction media were used. Sodium chloride (0.5%) extracted the least amount of activity, and the addition of papain (0.1%) did not increase this level. Cysteine hydrochloride extracted the greatest amount of activity, followed closely by Triton X-100 and phosphate-citrate buffer at pH 6.8.

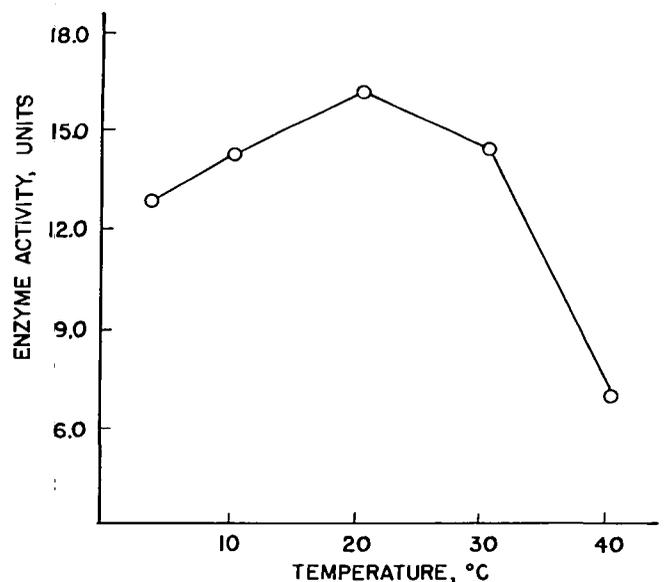


Fig. 3. Effect of extraction temperature on limit dextrinase activity.

Figures 2, 3, and 4 show the effects of changes in pH, temperature, and extraction time, respectively. Only small changes in extractability were noted as the pH of the phosphate-citrate buffer was adjusted between 6.8 and 8.8. Extraction was optimum at 20° C, and maximum activity appeared to be effectively extracted after 2.5 hr. The standard procedure chosen for extraction used phosphate-citrate buffer at pH 6.8 at 20° C for 2.5 hr.

Not all of the activity is extracted, even under these conditions, as demonstrated by the data in Table II. In this study, the malt flour was not removed from the extracting medium until after the incubation with pullulan was complete. For malts with low limit dextrinase activities, no differences were observed between the activities of the extract and the whole flour. However, for samples with higher levels of activity, the flour was considerably more active than the extract. One possible explanation for this behavior may be that the enzyme occurs in malted barley partially bound to some insoluble material, similar to the way in which  $\beta$ -amylase occurs in unmalted barley. We have not further investigated this phenomenon.

#### Effect of Germination Time

Kneen and Spoerl (12), Greig (6), Manners and Yellowlees (20), and Pratt et al (21) demonstrated the early appearance of limit dextrinase activity during germination. Measurable levels of activity were developed during the first day of germination in each study. Maximum activity was observed after four (20), seven (6), or 11 (21) days of germination; Kneen and Spoerl (12) found no maximum after six days of germination.

Hardie (7) demonstrated that gibberellic acid was necessary for the synthesis of limit dextrinase in germ-free barley tissue slices. Manners and Yellowlees (20) showed that exogenous gibberellic

acid had little effect on limit dextrinase activity during the first two days of germination but increased the level of activity thereafter, resulting in a maximum level after four days which was 178% of the untreated control.

Figure 5 shows the effects of germination time and gibberellic acid level on the development of limit dextrinase activity observed in our study. No activity could be detected for the first three days of germination, but the enzyme developed rapidly after that, continuing to rise in activity even after 11 days of germination in the absence of exogenous gibberellic acid. Significantly higher levels of activity were developed in the presence of 2 mg/kg gibberellic acid,

TABLE II  
Effect of Using Malt Flour on Enzyme Activity

Malt Sample	Extract (units per gram of malt)	Flour (units per gram of malt)	Increase in Activity (%)
Commercial malt <sup>a</sup>	0.9	0.0	...
Larker	4.4	4.4	...
Morex	11.9	22.0	85
Azure	11.9	15.4	29

<sup>a</sup>Prepared from Morex barley.

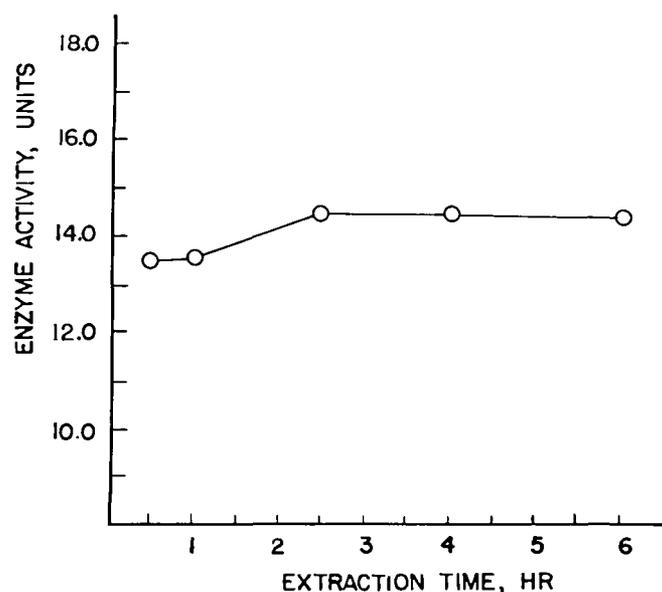


Fig. 4. Effect of extraction time on limit dextrinase activity.

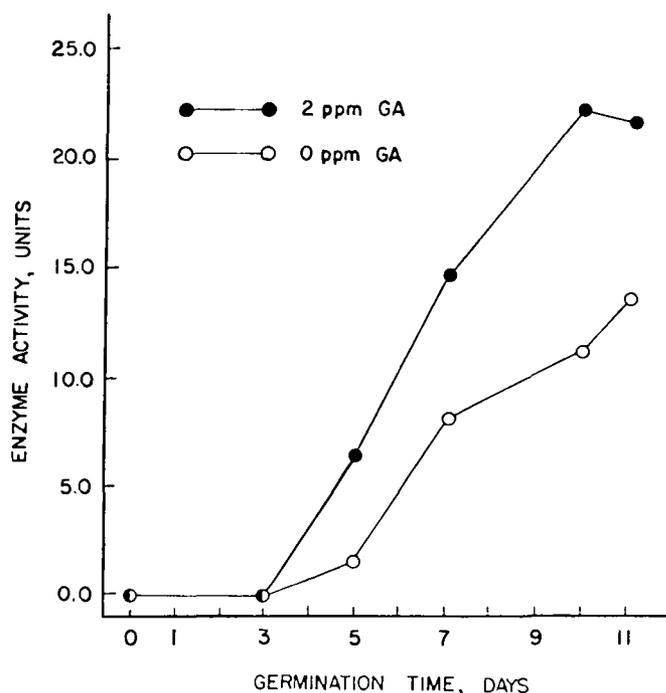


Fig. 5. Development of limit dextrinase activity during germination with and without added gibberellic acid.

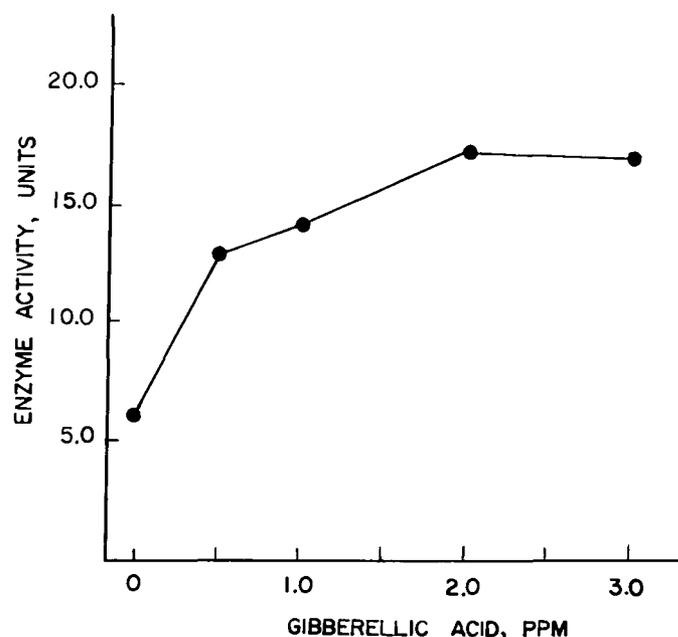


Fig. 6. Effect of gibberellic acid level on the development of limit dextrinase activity during germination.

almost twice as much after seven and 10 days, but the presence of the hormone did not result in the earlier appearance of the enzyme. Figure 6 shows the effect of increasing levels of gibberellic acid on the level of limit dextrinase activity developed. Enzyme levels increased as the level of gibberellic acid in the steep water increased to 2 mg/kg, and no further increase was detected at a level of 3 mg/kg.

#### Effect of Kilning Temperature

Kneen and Spoerl (12) reported losses of 32% in activity after kilning green malt for 3 hr at 70°C, and losses of 59% after 3 hr at 75°C, even though both samples had previously been kilned for 24 hr at 50°C with no loss in activity. Manners and Yellowlees (20), on the other hand, found very little loss in activity upon kilning for an unspecified period at 88°C.

To determine the effect of kiln heat on limit dextrinase activity, a sample of Morex was divided into six parts, each of which was steeped in the presence of gibberellic acid (2 mg/kg) and germinated for seven days. The samples were kilned according to the schedule shown in Table III.

The results of this study are presented in Fig. 7. Limit dextrinase activity was quite stable at 49°C, showing almost no loss in activity over 24 hr. When the temperature was raised to 65°C, only a slight decline in activity was noted, but a dramatic decline was observed at a kilning temperature of 82°C.

Manners and Yellowlees (20) attributed the differences between their observations and those of Kneen and Spoerl (12) to the unspecific assay procedure used by the latter investigators to estimate limit dextrinase activity.

They suggested that the apparent decline in limit dextrinase activity noted by Kneen and Spoerl was due to the thermal inactivation of  $\alpha$ - and  $\beta$ -amylase during kilning. However, our

TABLE III  
Kilning Schedule

Treatment	Nature of Kilning	
	Time (hr)	Temperature (°C)
A	8	49
B	16	49
C	24	49
D	C + 3	65
E	D + 3	82

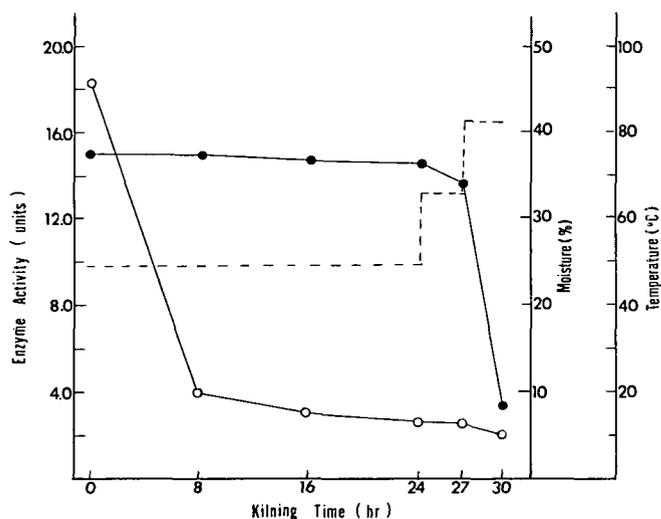


Fig. 7. Changes in limit dextrinase activity during kilning (• = limit dextrinase activity; o = malt moisture content; --- = kiln temperature).

data, using the modified assay procedure which is unaffected by  $\alpha$ - and  $\beta$ -amylase, agree with those of Kneen and Spoerl, although the similarity in results may be merely fortuitous. The reasons for the discrepancy between our data and those of Manners and Yellowlees are not obvious, but their assay procedure for limit dextrinase suffers from the effects of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities, both of which are present in their extracts.

#### Temperature Stability of Limit Dextrinase

To evaluate further the temperature stability of limit dextrinase, the extracts of a single malt were held at constant temperatures of 45, 55, or 65°C for periods of up to 1 hr. Samples were removed at 15-min intervals and cooled to 37°C for assay. The results of this study are presented in Fig. 8.

Limit dextrinase is quite stable at temperatures of 45 and 55°C, although there is a slight loss in activity after 1 hr at 55°C. The enzyme is quite unstable at 65°C, losing more than half of its activity after 15 min.

Manners and Rowe (19) found a lower and rather narrow optimum temperature range for purified preparations of malt limit dextrinase at about 40°C, with significantly lower activity on either side of the optimum. The somewhat higher temperature stability found in our study may be the result of the protective action of other components of our unpurified preparations.

To test the temperature stability of limit dextrinase under mashing conditions, a dry malt with high activity was ground to ASBC fine-grind specifications and was mashed according to the standard ASBC schedule. Mashers were terminated at various points during the process and were immediately cooled, filtered, and assayed for limit dextrinase and  $\alpha$ -amylase activities and diastatic power. The results of this study are presented in Fig. 9.

All three activities were stable during the 30-min rest at 45°C, with limit dextrinase and  $\alpha$ -amylase activities increasing slightly, probably because of further extraction from the grist. Diastatic power levels declined rapidly as the temperature began the rise to 70°C, and then more slowly during the 70°C rest.  $\alpha$ -Amylase activity showed substantially greater temperature stability, declining only after the mash had reached 70°C.

Limit dextrinase activity was very similar to diastatic power in its temperature response, beginning to decline as the temperature rose toward 70°C, and becoming undetectable after 30 min at 70°C.

The results of these temperature-response studies are consistent, showing limit dextrinase to be similar to  $\beta$ -amylase in this respect.

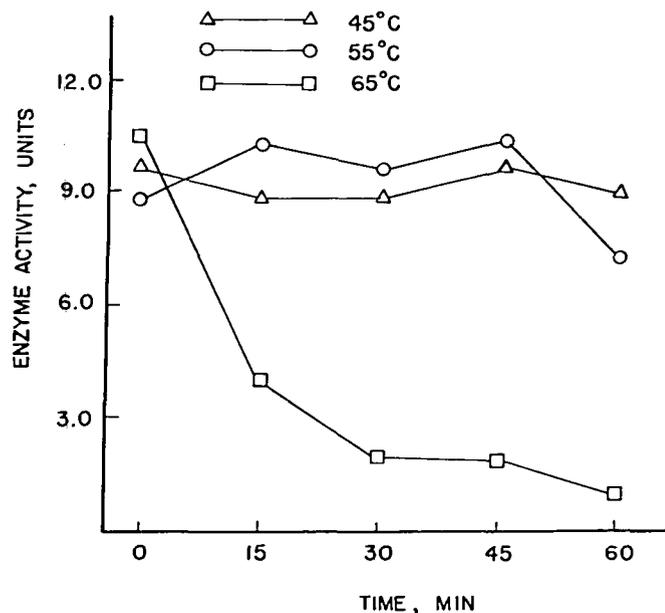


Fig. 8. Temperature stability of limit dextrinase.

In each case, the enzyme is stable at temperatures up to about 50–55°C, above which temperature it undergoes rapid, temperature-induced inactivation. These data also suggest that limit dextrinase will, like  $\beta$ -amylase, be active during mashing at low temperatures and will be able to effect changes in starch, which has been solubilized by  $\alpha$ -amylase during malting and the early stages of mashing, only during this initial period. As the temperature of the mash rises toward 70°C, limit dextrinase, like  $\beta$ -amylase, will be inactivated before starch gelatinization temperatures are reached and thus will only be able to realize a small portion of its potential effectiveness.

#### Effect of Limit Dextrinase on $\alpha$ -Limit Dextrin

Enevoldsen (3) and Enevoldsen and Schmidt (4) have demonstrated the effect of the enzyme pullulanase on the distribution of oligosaccharides produced during mashing. Worts produced upon addition of pullulanase had higher levels of maltose and maltotriose and reduced levels of oligosaccharides of DP 8-14 due to the cleavage of 1,6 linkages in the oligosaccharides and the subsequent degradation of the resulting products by  $\alpha$ - and  $\beta$ -amylase.

$\alpha$ -Limit dextrins represent the simplest substrates available for limit dextrinase. To determine the effect of variable levels of limit dextrinase activity on limit dextrins, extracts were prepared from malts that showed low, intermediate, and high levels of limit dextrinase activity. The substrate,  $\alpha$ -limit dextrin, had been shown by HPLC to be resistant to the action of  $\alpha$ - and  $\beta$ -amylases (13). Dialyzed malt extracts and substrate were incubated at 37°C, and aliquots were removed at intervals and were assayed for glucose, maltose, and maltotriose by HPLC. The results of this study are presented in Table IV.

The extract representing low-limit dextrinase activity was prepared from a commercial malt. During a 4-hr incubation period, only small amounts of fermentable sugar were produced, mainly maltose and a trace of maltotriose. The intermediate and high limit dextrinase extracts, however, produced considerable amounts of maltose and readily detectable amounts of both glucose and maltotriose. The decline in maltotriose levels and appearance of glucose is the result of the action of  $\alpha$ - and  $\beta$ -amylase and  $\alpha$ -glucosidase, because limit dextrinase will not directly produce glucose.

#### Limit Dextrinase Activity of Commercial Malts

In view of the demonstrated effect of kilning temperature and length of germination on limit dextrinase activity, one might expect commercial brewers' malts to have only low levels of limit

dextrinase activity.

Malts were obtained from seven commercial maltsters and brewers who were asked to supply samples of Morex brewers' malt from the 1981 crop from commercial production runs. The samples were assayed for limit dextrinase activity as described above. These malts did indeed exhibit very low levels of limit dextrinase activity, due, in part, to the controlled growth and modification schedules typical for such malting and, in part, to the elevated kilning and curing temperatures employed in commercial production. The malts varied from a low of zero units of limit dextrinase activity (two malts) to a high of 0.8 units per gram of malt, with four of the seven malts being in the range of 0.2 to 0.5 units per gram of malt.

#### Effect of Limit Dextrinase on Fermentability and Alcohol Production

Several studies have demonstrated the effectiveness of debranching enzymes in increasing the fermentability of worts and subsequent alcohol production. Greig (6) reported no relationship between the activity of limit dextrinase and the yield of alcohol. Kneen and Spoerl, however, demonstrated a relationship between limit dextrinase activity and alcohol production in eight malts (12).

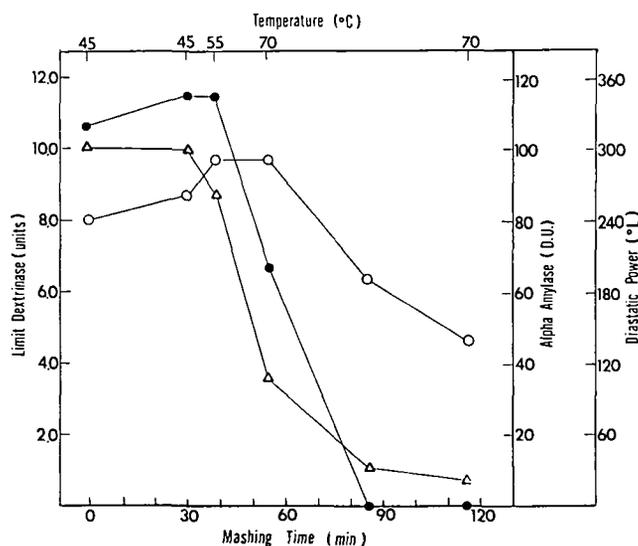


Fig. 9. Fate of limit dextrinase (●),  $\alpha$ -amylase (○), and diastatic (Δ) activities during mashing.

TABLE IV  
Fermentable Sugars Produced by the Digestion  
of  $\alpha$ -Limit Dextrin with Malt Extract

Malt Samples	Limit Dextrinase (units per gram of malt)	Sugar <sup>a</sup>	Fermentable Sugar Production		
			Digestion Time		
			1 hr	2 hr	4 hr
High-enzyme malt I	18.3	G	None	Trace	1.0
		G <sub>2</sub>	5.7	7.2	9.5
		G <sub>3</sub>	3.5	3.2	1.0
		Total	9.2	10.4	11.5
High-enzyme malt II	11.1	G	None	Trace	1.3
		G <sub>2</sub>	5.2	7.1	8.7
		G <sub>3</sub>	3.3	3.1	1.5
		Total	8.5	10.2	11.5
Commercial malt	0.5	G	None	None	None
		G <sub>2</sub>	Trace	1.5	2.5
		G <sub>3</sub>	Trace	Trace	Trace
		Total	Trace	1.5	2.5

<sup>a</sup>G = glucose, G<sub>2</sub> = maltose, G<sub>3</sub> = maltotriose, in milligrams per milliliter.

Hopkins and Wiener (10) showed that unboiled wort produced greater alcohol yields upon fermentation than boiled wort, even when the boiled wort was supplemented with purified  $\alpha$ - and  $\beta$ -amylase. They concluded that an enzyme other than  $\alpha$ - or  $\beta$ -amylase present in the unboiled worts was responsible for the increase in alcohol production. Enevoldsen (3) showed that the addition of the debranching enzyme pullulanase effectively reduced residual extract and increased alcohol levels in fermenting wort and that the extent of these changes depended on the levels of pullulanase. Willox et al (25) also showed improved attenuation when enzyme preparations containing pullulanase were added to worts after lautering but before boiling or fermenting the worts.

The effect of malts high in limit dextrinase activity on the fermentability of worts and on alcohol production was evaluated. One commercial and four experimental malts were finely ground, and 5 g of each flour was added to individual Morex worts that had been prepared according to the usual ASBC mashing schedule. The worts were then fermented according to the ASBC rapid fermentation procedure. In addition to limit dextrinase activity, the malt flours were assayed for  $\alpha$ -amylase activity and for diastatic power. The results are presented in Table V.

There appears to be no relationship in these samples between limit dextrinase activity and either  $\alpha$ -amylase activity or diastatic power. The experimental Morex malt, although highest in  $\alpha$ -amylase and diastatic activities, is the lowest of the experimental malts in limit dextrinase activity.

Also, no significant relationship is apparent between alcohol production or degree of fermentability and either  $\alpha$ -amylase activity or diastatic power. The data do demonstrate, however, a strong relationship between both alcohol production and degree of fermentability and the activity of limit dextrinase. Correlation coefficients of 0.89 and 0.90 were calculated between the activity of this enzyme and the degree of fermentability and alcohol production, respectively.

TABLE V  
Analyses of Malts and Fermentability in the Presence  
of High-Enzyme Malt

Samples	Limit Dextrinase	$\alpha$ -Amylase (20° DU) <sup>a</sup>	Diastatic Power (° ASBC)	Fermentability	
	(units per gram of malt)			Alcohol (%, vol)	Degree (%)
Glenn	18.0	95.6	261	4.09	75.2
Klages	11.7	96.0	234	4.11	75.5
Azure	11.1	87.0	251	4.01	75.1
Morex	8.6	115.9	322	3.91	73.4
Commercial malt	5.0	51.5	143	3.16	68.3

<sup>a</sup>Dextrinizing units.

### Factors Affecting Limit Dextrinase Levels

One objective of this study was to determine the effect of variety and agronomic factors on the development of limit dextrinase activity. Kneen and Spoerl (12) examined the influence of variety and growth location on the limit dextrinase activity of eight barley varieties grown at seven locations in the upper Midwest. While the data were not treated statistically, they concluded that, while there appeared to be a varietal influence on limit dextrinase activity, environment seemed to play only a minor role.

The results of an analysis of variance of data obtained from seven varieties grown at four locations over three crop years are presented in Table VI. Each of the single factors evaluated—variety, location, and year—had a significant effect on limit dextrinase activity, whether expressed as units per gram of malt or as units per milligram of soluble protein. No significant effects were noted when the activity of the enzyme was expressed as units per gram of total protein. Neither of the varietal interactions was significant, but the interaction between location and year was.

These results suggest that differences exist in the ability of these varieties of barley to produce limit dextrinase. Considering only currently acceptable malting barley varieties, Morex produces high levels of activity, while Glenn and the two-rowed variety Klages produce intermediate levels (Table VII).

Table VII also shows that, of the four locations studied, Fargo produced samples high in limit dextrinase activity, and Minot produced low levels of activity. In addition to this location effect, samples grown over all locations showed high levels of activity in 1979 and lower levels in the two succeeding years. The analysis of variance showed a significant year–location interaction. This interaction is responsible for the fact that, although Fargo produced the highest levels of activity in 1979 and 1980 (Table VIII), it was only third highest in 1981. Thus, although the effect of growth location is significant, it is unpredictable because annual climatic conditions may override this effect.

### CONCLUSIONS

Limit dextrinase activity is difficult to extract fully from ground malt, malt flour showing significantly higher levels under the extraction conditions thus far examined. The enzyme is not present in detectable amounts in barley or in green malt until after three days of germination. The level of activity then rises and continues to increase through 11 days of germination. The activity of the enzyme is stimulated by gibberellic acid.

The enzyme is stable in green malt kilned at 49° C for 24 hr and maintains its activity after 3 hr at 65° C. The activity declines dramatically at kilning temperatures of 82° C. In solution, the enzyme is relatively stable for 1 hr at 55° C but is rapidly inactivated at 65° C. Its activity during mashing is similar, remaining stable during the 45° C rest and declining as the temperature of the mash begins to rise to 70° C. No activity was detected after 30 min at

TABLE VI  
Analysis of Variance: The Effect of Variety, Location, and Year on Limit Dextrinase Activity

Source	Degrees of Freedom	Activity (units per gram of malt)	Mean Square	
			Activity (units per milligram of soluble protein)	Activity (units per milligram of total protein)
Mean		3110.3465	4.1570	13.2425
V (variety)	6	51.4457**	0.0731** <sup>b</sup>	10.1491
L (location)	3	253.4527** <sup>b</sup>	0.5368** <sup>b</sup>	16.8878
Y (year)	2	122.9073** <sup>b</sup>	0.2150** <sup>b</sup>	16.5688
VL (variety × location)	18	13.4218	0.0246	18.1233
VY (variety × year)	12	15.8826	0.0233	18.7098
LY (location × year)	6	159.8754** <sup>b</sup>	0.2803** <sup>b</sup>	17.1552
Error	30	15.0743	0.0190	19.9563.

\*\* Significant at  $P = 0.05$ .

\*\* Significant at  $P = 0.01$ .

**TABLE VII**  
Average Limit Dextrinase Activity  
of Experimental Malts

	N	Limit Dextrinase (units per gram of malt)
Variety		
Morex	11	10.4
ND 1156	12	8.7
Klages	11	7.3
Glenn	10	5.7
Azure	12	5.5
Summit	11	3.9
Larker	11	3.9
Location		
Fargo	19	11.5
Williston	17	6.0
Langdon	21	4.9
Minot	21	4.1
Year		
1979	23	9.7
1980	27	5.7
1981	28	4.6

**TABLE VIII**  
Location Means of Limit Dextrinase  
Activity over Years

Location	Year	Limit Dextrinase (units per gram of malt)
Fargo	1979	20.8
	1980	11.8
	1981	3.2
Williston	1979	9.8
	1980	2.2
	1981	8.1
Langdon	1979	3.8
	1980	8.8
	1981	2.9
Minot	1979	6.2
	1980	1.8
	1981	4.3

70° C.

Malt limit dextrinase is capable of producing fermentable sugars from  $\alpha$ -limit dextrans. Good correlations were established between fermentability and alcohol production and the limit dextrinase activity of malt flour when the flour was added to fermenting wort.

Commercial brewers' malts have low levels of limit dextrinase activity due to restricted physical growth during germination and

to high kiln temperatures.

The level of activity is a varietal characteristic that is influenced by both climatic and environmental conditions.

#### ACKNOWLEDGMENTS

The authors would like to thank the following malting and brewing companies for graciously supplying malt samples: Anheuser-Busch, Inc., Fleischmann Malting Co., Kurth Malting Corp., Ladish Malting Co., Minnesota Malting Co., Rahr Malting Co., and Schreier Malting Co. Appreciation is also expressed to Leigh Murray, Department of Mathematical Sciences, North Dakota State University, for her aid in the statistical analysis of the data.

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[Received May 13, 1983]