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# Genotypic and environmental variation in barley limit dextrinase activity and its relation to malt quality*

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**Abstract:** Variation in the limit dextrinase activity of barley malt, and the relationships between limit dextrinase activity and malt quality parameters were investigated using eight cultivars grown at seven diverse locations in China for two successive years. Limit dextrinase activity varied with genotype and location, with the levels ranging from 0.245 U/g to 0.980 U/g. The results showed that the variation in limit dextrinase activity was more attributable to the environment (location and year) than to the genotype. The response of limit dextrinase activity to the environment differed markedly among cultivars, and was reflected by large difference in coefficient of variation of cultivars across diverse locations. Regression analysis showed that limit dextrinase activity was negatively correlated with malt viscosity ($r$=−0.52, $P$<0.01), positively correlated with Kolbach index ($r$=0.38, $P$<0.01) and malt extract ($r$=0.30, $P$<0.05), but had no significant correlation with malt protein content and diastatic power.

**Key words:** Barley (*Hordeum vulgare* L.), Limit dextrinase activity, Genotype, Environment, Malt quality
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## INTRODUCTION

There exist four important starch hydrolytic enzymes in barley malt, i.e. $\alpha$-amylase, $\beta$-amylase, limit dextrinase and $\alpha$-glucosidase, with complete degradation of starch to fermentable carbohydrates during mashing requiring the action of these enzymes (Evans *et al*., 2003; MacGregor *et al*., 1999; 2002; Fincher, 1989). $\alpha$-amylase rapidly hydrolyzes starch in a random fashion to a mixture of linear and branched dextrins. Linear dextrins, in turn, are hydrolyzed further by $\beta$-amylase to maltose, with $\alpha$-glucosidase being also an exo-acting enzyme that primarily cleaves $\alpha$-1,4-linkages to produce glucose. However, branched dextrins are incompletely hydrolyzed because neither $\alpha$- nor $\beta$-amylase can hydrolyze the $\alpha$-1,6 bonds originating from amylopectin component of starch (MacGregor and Dushnicky, 1989). Thus limit dextrinase is required to cleave these bonds and so render the branched dextrins susceptible to further hydrolysis by $\beta$-amylase (Serre and Lauriere, 1989; Enevoldsen and Schmidt, 1973).

Limit dextrinase (LD), also known as pullulanase or $\alpha$-dextrin 6-glucanohydrolase (EC 3.2.1.41), specially catalyses hydrolysis of $\alpha$-1,6 glucosidic bonds, which are found in pullulan, amylopectin and amylopectin limit dextrins (Manners and Yellowlees, 1973; Lee and Whelan, 1971). In barley, LD is synthesized in the aleurone layer and released into the endosperm (Lee and Pyler, 1984). During grain development, LD activity increases shortly after anthesis, and reaches a maximum at middle filling, then decreases rapidly (Sissons *et al*., 1993). At maturity, very small amounts of limit dextrinase exist in barley grains, as a result of their binding to the inhibitors expressed later during seed maturation (McCleary, 1992; MacGregor and Dushnicky, 1989), therefore, its activity is very low. However, LD activity in-

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creased sharply during malting due to release of LD from a bound form to a free form (Ross *et al*., 2003; Longstaff and Bryce, 1993; Sissons *et al*., 1992a; 1993; 1994). Furthermore, there is abundant limit dextrinase mRNA present in the aleurone layer after germination (Burton *et al*., 1999; Kristensen *et al*., 1999), which indicates that not only is the bound form of LD released but substantial amounts of new LD is expressed during malting.

Compared to $\alpha$- and $\beta$-amylase, the LD activity is more closely correlated with wort fermentability than $\alpha$- or $\beta$-amylase activity (Stenholm and Home, 1999). Therefore, elevated limit dextrinase activity in malt may enhance hydrolysis of un-fermentable branched dextrins into fermentable sugars, leading to increased total fermentability of wort. However, increased limit dextrinase activity during mashing may need to be controlled carefully for some beer styles so as not to remove all branched dextrins because they contribute to mouthfeel and body in the final beer (Ragot *et al*., 1989).

There had been many studies on the extraction, purification and characterization of LD as well as its changes during grain development, germination and mashing (Ross *et al*., 2003; MacGregor *et al*., 1994; 2002; McCleary, 1992; Sissons *et al*., 1992b; 1993; 1994; Lee and Pyler, 1982), but little research had been done on its genetic and environmental variation (Arends *et al*., 1995). This paper reports a preliminary investigation of the effects of genotype and environment on limit dextrinase activity and its relation to barley malt quality.

## MATERIALS AND METHODS

### Plant materials

In the 2001~2002 barley-growing seasons, eight winter barley cultivars, currently being widely planted in southern China, were grown at seven locations with different ecological conditions. Each cultivar consisted of ten 2 m-length lines. At maturity, 8 lines in the medium of each cultivar were harvested and the grains were used as the sample for assay.

### Micro-malting and quality analysis

Barley grains were screened through a 2.2 mm sieve, with the grains remaining being used for micro-malting. Samples (200 g) were micro-malted in a Phoenix System Micro-malting Apparatus (Adelaide, Australia) with the regime: steeping (6 h, 16 °C), air-rest (14 h, 16 °C), steeping (8 h, 16 °C), air-rest (14 h, 16 °C), steeping (4 h, 16 °C); germination for 96 h at 15 °C; kilning for 24 h at 65 °C; derooting. Malt was milled using a Tecator Cyclone mill (Tecator AB, Hoganas, Sweden) fitted with a 0.5 mm screen. The malt quality parameters, extract, Kolbach index, viscosity and diastatic power (DP), were determined according to analytica EBC official methods (European Brewery Convention, 1975). Total protein was determined by the Kjeldahl method (AACC, 2000). Protein factions were extracted and their contents were determined according to Shewry *et al*.(1983).

### Limit dextrinase activity analysis

LD activity was assayed by the method of McCleary (1992) using limit-DextriZyme tablets (Megazyme Ltd., Ireland) as substrate, using 0.1 mmol/L sodium malate buffer (pH 5.5) containing 25 mmol/L dithiothreitol (DTT) as extraction/activation buffer. One unit of activity is defined as the amount of enzyme required to release one micromole of glucose reducing-sugar equivalents per minute from pullulan under the defined assay conditions. Malt limit dextrinase activity was determined by reference to the standard curve to convert absorbance to milli-units per assay and then calculated as follows:

$$\text{Units/kg malt}=\text{milli-units per assay (i.e. per 0.5 ml)} \times(1/1000)\times 32,$$

where 1/1000 is conversion from milli-units to units; 32 is conversion from activity/0.5 ml of extract to that in 1 g of malt. Flour was extracted with 16 ml of buffer per gram of flour, and the assay was performed on 0.5 ml solution.

### Statistical analysis

Analysis of variance was performed with SPSS V.7.5 (SPSS, Michigan Avenue, Chicago, IL, USA). Differences among means were evaluated using the Duncan's multiple range test. Correlation coefficients were calculated between limit dextrinase activity and grain protein, malt quality parameters (Kolbach index, diastatic power, malt viscosity and extract).

RESULTS

**ANOVA of limit dextrinase activity**

The results of ANOVA for limit dextrinase activity of eight barley cultivars planted in seven locations for two successive years are shown in Table 1, showing that the variation for cultivar, location and year and all interactions between them were highly significant ($P<0.01$). When relative contribution of each variant to total variation of limit dextrinase was compared in terms of SS (sum of squares) proportion, SS of location, interaction between location and year were 34.58% and 25.13% of total SS, respectively, being larger than that of cultivar (17.58%), implying that variation in limit dextrinase activity was caused predominantly by environment (location). Moreover, the SS of year was only 6.27% of total SS, indicating that the variation between the year of 2001 and 2002 was relatively small. Similarly, the SS of interactions between cultivar and location, cultivar and year, and between cultivar, location and year, had small contribution to total SS (9.38%, 6.18%, and 5.93%, respectively).

**Table 1 ANOVA of limit dextrinase activity of eight barley cultivars in seven locations**

| Source variation | *SS* | *Df*[a] | *MS* | *F* value |
|---|---|---|---|---|
| Block | 893.3 | 1 | 893.3 | 3.21 |
| Cultivar (C) | 1079919.0 | 7 | 154274.2 | 554.75** |
| Location (L) | 2123933.0 | 6 | 353988.8 | 1272.90** |
| Year (Y) | 385003.7 | 1 | 385003.7 | 1384.44** |
| C×L | 576080.5 | 42 | 13716.2 | 49.32** |
| C×Y | 38065.9 | 7 | 5438.0 | 19.55** |
| L×Y | 1543447.0 | 6 | 257241.2 | 925.02** |
| C×L×Y | 364412.9 | 42 | 8676.5 | 31.20** |
| Error | 30868.7 | 111 | 278.1 | |
| Total | 6142624.0 | 223 | | |

[a] Degrees of freedom; ** Significant ($P<0.01$)

**Variation of limit dextrinase activity among locations**

The variation of limit dextrinase activity among locations between years is shown in Table 2, showing that there was significant difference in limit dextrinase activity between the seven locations, except for the difference between Nanchong and Yancheng. Moreover, the difference between two years was also significant, 2001 being higher than 2002, except for Hangzhou and Jingzhou, which had higher limit dextrinase activity in 2002 than in 2001. On average for all cultivars, limit dextrinase activity among seven locations ranged from 0.470 U/g in Tai'an to 0.637 U/g in Hangzhou in 2001 and from 0.323 U/g in Tai'an to 0.756 U/g in Jingzhou in 2002, with greater variation in 2002 than in 2001. Although Tai'an constantly ranked the lowest in both years, the other locations did not follow the same pattern. For example, Zhengzhou ranked the highest in 2001, but the fifth in 2002. Moreover, absolute difference (maximum vs minimum) and CV (coefficient of variation) of limit dextrinase activity also showed large difference between locations, indicating that the variation among cultivars was also dependent on the location. For example, in 2001, Putian and Tai'an had the lowest and highest CVs, respectively, while in 2002, Putian still ranked the highest in CV, but the lowest CV occurred in Nanchong.

**Variation of limit dextrinase activity among different cultivars**

Table 3 of the variation in limit dextrinase activity among different cultivars between years shows that there was also highly significant difference in limit dextrinase activity among cultivars, except for the differences between Suyinmai 2 and Yanyin 1, and Gangpi 1 and Zheyuan 18. For all locations and years on average, Dan'er ranked the highest in limit dextrinase activity (0.645 U/g), and ZAU 3 the lowest (0.433 U/g). Furthermore, the limit dextrinase activity in 2001 was much higher than that in 2002 for each cultivar, ranging from 0.467 U/g for ZAU 3 to 0.696 U/kg for Xiumai 3 in 2001 compared to 0.400 U/g for ZAU 3 to 0.594 U/g for Dan'er in 2002. The order of cultivars in terms of enzymatic activity varied with years. However, ZAU 3 ranked constantly the lowest in two years, while Dan'er ranked the second and first in 2001 and 2002, respectively. As expected from ANOVA, there was substantial difference in limit dextrinase activity for a given cultivar when grown in different locations, which was characterized by a large CV value. Hence, the CV for the cultivar grown at seven locations ranging from 24.02% for Suyimai 2 to 33.33% for ZAU 3, being larger than that of different cultivars grown at the same location (Table 2). Furthermore, the CV in 2002 was much higher than that in 2001 for each cultivar. For example, CV for

**Table 2 The variation of limit dextrinase activity (U/g) of eight barley cultivars grown in seven different locations**

| | | Hangzhou | Jingzhou | Putian | Zhengzhou | Nanchong | Yancheng | Tai'an |
|---|---|---|---|---|---|---|---|---|
| 2001 | Mean | 0.637 | 0.550 | 0.655* | 0.682* | 0.587* | 0.535* | 0.470* |
| | Min. | 0.525 | 0.490 | 0.572 | 0.560 | 0.472 | 0.245 | 0.262 |
| | Max. | 0.850 | 0.670 | 0.788 | 0.887 | 0.699 | 0.669 | 0.624 |
| | *CV* (%) | 17.85 | 8.19 | 7.49 | 17.90 | 10.75 | 24.81 | 27.16 |
| 2002 | Mean | 0.749* | 0.756* | 0.528 | 0.381 | 0.375 | 0.423 | 0.323 |
| | Min. | 0.609 | 0.626 | 0.258 | 0.292 | 0.293 | 0.305 | 0.277 |
| | Max. | 0.980 | 0.910 | 0.702 | 0.498 | 0.425 | 0.554 | 0.480 |
| | *CV* (%) | 17.41 | 13.41 | 25.80 | 16.82 | 12.37 | 23.96 | 19.46 |
| Average of 2001~2002 | Mean | 0.693 a[1] | 0.653 b | 0.592 c | 0.532 d | 0.481 e | 0.479 e | 0.397 f |
| | *CV* (%) | 19.23 | 20.30 | 20.72 | 33.98 | 25.09 | 27.02 | 31.29 |

[1] Values in the same line followed by different letters are significant ($P<0.05$); * Represents significant difference ($P<0.05$) between years for the same location

**Table 3 The variation of limit dextrinase activity (U/g) of the different cultivars over eight locations**

| | | Dan'er | Xiumai 3 | Suyinmai 2 | Yanyin 1 | Zhepi 4 | Gangpi 1 | Zheyuan 18 | ZAU 3 |
|---|---|---|---|---|---|---|---|---|---|
| 2001 | Mean | 0.695* | 0.696* | 0.607* | 0.609* | 0.571* | 0.523* | 0.536* | 0.467* |
| | Min. | 0.513 | 0.864 | 0.532 | 0.558 | 0.449 | 0.442 | 0.262 | 0.245 |
| | Max. | 0.887 | 0.500 | 0.681 | 0.672 | 0.661 | 0.572 | 0.654 | 0.602 |
| | *CV* (%) | 17.25 | 18.39 | 8.89 | 6.52 | 11.68 | 10.28 | 23.96 | 30.65 |
| 2002 | Mean | 0.594 | 0.570 | 0.551 | 0.548 | 0.464 | 0.472 | 0.441 | 0.400 |
| | Min. | 0.322 | 0.304 | 0.311 | 0.288 | 0.277 | 0.324 | 0.296 | 0.258 |
| | Max. | 0.980 | 0.910 | 0.853 | 0.906 | 0.778 | 0.691 | 0.692 | 0.626 |
| | *CV* (%) | 35.39 | 37.87 | 34.38 | 42.76 | 36.97 | 31.62 | 35.61 | 37.40 |
| Average of 2001~2002 | Mean | 0.645 a[1] | 0.632 b | 0.579 c | 0.578 c | 0.518 d | 0.497 e | 0.489 e | 0.433 f |
| | *CV* (%) | 27.37 | 29.08 | 24.02 | 29.00 | 26.81 | 22.73 | 30.47 | 33.33 |

[1] Values in the same line followed by different letters are significant ($P<0.05$); * Represents significant difference ($P<0.05$) between years for the same location

Suyinmai 2 and Yanyin 1 was 8.89% and 6.52% respectively in 2001, but the corresponding values were 34.38% and 42.76% respectively in 2002. The relatively low difference among cultivars and large difference among locations and between years in limit dextrinase activity indicated that the variation of the enzyme was more attributable to the environment than to the genotype.

**Relationship between limit dextrinase activity and malt quality parameters**

Correlation analysis was performed to investigate the relationship between limit dextrinase activity and protein content for eight barley cultivars grown at seven locations in 2001 and 2002. No significant correlation between limit dextrinase activity and total protein content, protein component was found.

The relationship between limit dextrinase activity and four malt qualities is shown in Fig.1. Higher limit dextrinase activity was associated with higher malt extract ($P<0.05$) and Kolbach index ($P<0.01$), lower viscosity ($P<0.01$), but had no significant correlation with diastatic power (DP). In addition, it may be seen from scatter points that distinct difference existed among genotypes in the relationship between limit dextrinase and each malt quality parameter, indicating the possibility of developing the genotypes with favorable association between the enzyme and malt quality.

## DISCUSSION

Few investigations had been done on the difference in limit dextrinase activity between barley varieties grown under similar conditions. Ross *et al*.(2003) studied four barley varieties differing in malting quality and found that there was significant difference in the level of total and free limit dextrinase activity among varieties during the course of

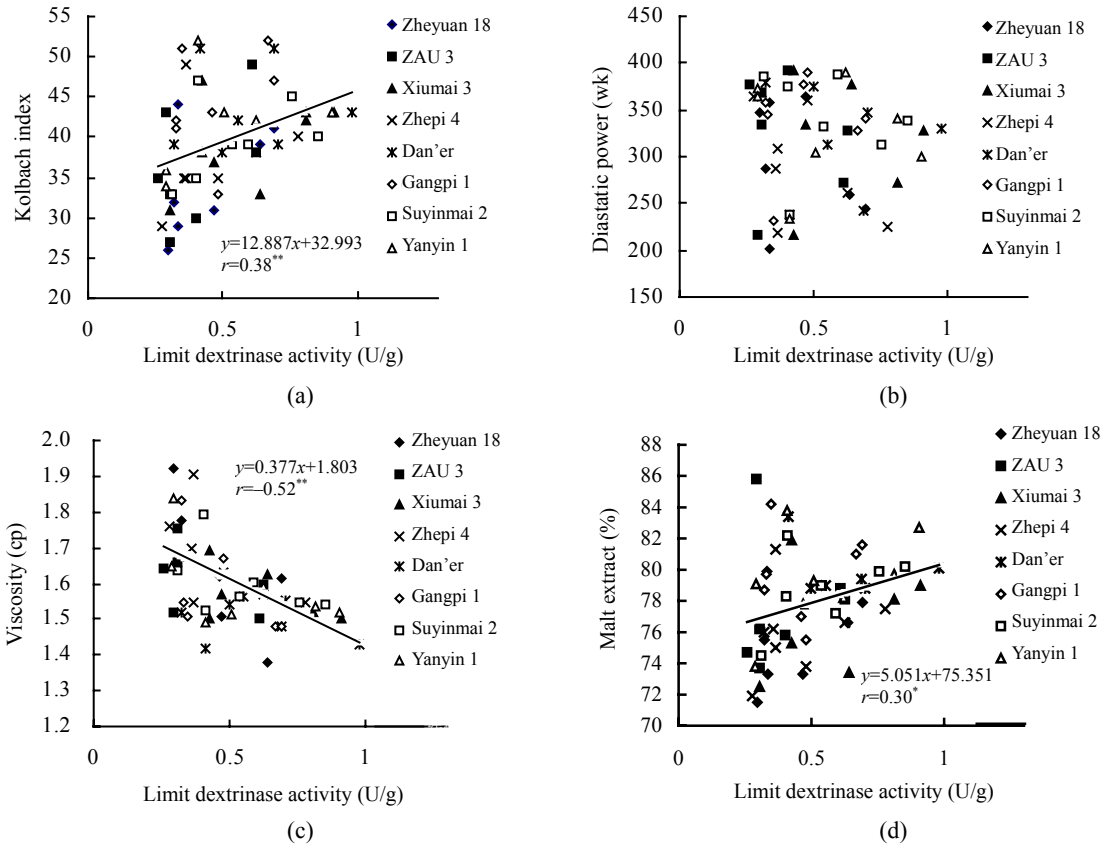


**Fig.1 The relationship between limit dextrinase activity and (a) Kolbach index, (b) diastatic power, (c) viscosity and (d) malt extract**
$^{*}P<0.05$; $^{**}P<0.01$

malting. In some previous studies, minor environmental effect of the enzymatic activity was reported (Kristensen *et al*., 1993; 1998; Longstaff and Bryce, 1991; 1993; Lee and Pyler, 1984). However, Arends *et al*.(1995) found significant variation among cultivars and locations in limit dextrinase activity in an experiment, where 11 Australian barley cultivars were planted at six diverse locations in Australia. In the current study, we planted eight barley cultivars, commonly used in southern China presently, in seven locations with large difference in ecological conditions (data not shown) for two successive years, and found that the total mean limit dextrinase activity across all cultivars, locations and years was 0.546 U/g and that the absolute difference in the enzyme activity was about 4-fold, i.e., from 0.245 U/g (ZAU 3 in Yancheng, 2001) to 0.980 U/g (Dan'er in Hangzhou, 2002). The higher total mean and greater variation in limit dextrinase activity in this experiment may be attributable to the greater difference among genotypes and locations. Moreover, the results of both ANOVA and variation analysis (CV) showed that limit dextrinase activity is more dependent on the environment than on the genotype.

High quality malt provides brewers with high levels of extract and efficiently produces the wort that is easily fermented by brewing yeasts (MacGregor *et al*., 1999). Diastatic power, the total activity of starch-degrading enzymes in barley malt, is considered to be an important quality characteristic for malting and brewing. Accordingly, improvement in the diastatic power in barley is becoming one of the most important traits in barley breeding. Arends *et al*.(1995) found that both $\alpha$-amylase and $\beta$-amylase were correlated positively with diastatic power, the latter making the most important contribution, but limit dextrinase was only weakly correlated with diastatic power. In our study, we found that total limit dextrinase activity had no significant correlation with diastatic power ($r$=0.12), being consistent with the

results of Manners and Yellowlees (1973). However, Evans *et al*.(2005) found that with commercial malts, *β*-amylase, *α*-amylase and limit dextrinase were highly correlated with DP. It may be suggested that the possible role of limit dextrinase is to supplement the action of *α*-amylase and *β*-amylase in degrading starch to fermentable sugars during mashing. However, MacGregor *et al*.(1999) using response surface methodology to determine the levels of *α*-amylase, *β*-amylase and limit dextrinase enzymes during mashing, found that levels of active limit dextrinase in malt mashes were below the optimum for efficient starch hydrolysis, while the other two enzymes were sufficient. Moreover, addition of limit dextrinase could increase substantially the levels of fermentable carbohydrates in mashes, particularly in mashes having high levels of *β*-amylase, indicating that the efficiency of any one starch-degrading enzyme in a mash is influenced by the degree of interaction with other starch degrading enzymes. Thus it is possible that malt with higher diastatic activity will not necessarily produce wort with higher levels of fermentable sugars in mash if the DP enzymes are not suitably balanced. Evans *et al*.(2005) found that increasing level of limit dextrinase could potentially result in a 2~4 folds percentage point increase in fermentability, and argued that wort fermentability is best predicted by *α*-amylase, total *β*-amylase and total limit dextrinase activity levels, Kolbach index, and *β*-amylase thermostability by multi-linear regression analysis.

Malt extract is a measure of the percentage of dry matter solubilized from malt grist during hot water extraction or mash, and is an important malting quality parameter. In the current study, there was a strongly positive correlation between limit dextrinase activity and malt extract. A similar conclusion was made by Collins *et al*.(2003). Starch accounts for a large portion of the dry matter in malt, so extract is considered to be a good indicator of the degree of modification of malt (i.e., of the degree of the protein matrix and cell walls in the endosperm that are hydrolysed during malting). Moreover, limit dextrinase activity showed significantly positive correlation with Kolbach index, and highly significant negative correlation with malt viscosity, which indicates that higher limit dextrinase assists in increasing the concentration of oligosaccharide in wort, and in turn, results in enhancing their hydrolysis into yeast fermentable sugars, thus enhancing wort fermentability.

In the current study, no significant correlation was found between limit dextrinase activity and total protein content, which was consistent with the results of Arends *et al*.(1995). Therefore, it is possible to develop barley cultivars with high limit dextrinase activity and moderate protein content. This is important as high protein content is negatively correlated with malt extract (Howard *et al*., 1996).

Previous studies showed that approximately 60% of limit dextrinase was inactivated due to being bound with inhibitors in malt (MacGregor, 1996; 2004). During mashing, the bound limit dextrinase continues to be converted into its free form that degrades limit dextrins into fermentable sugars and substrates for *α*- and *β*-amylase. It was found that the transformation of the bound form to free form varied between barley varieties (Ross *et al*., 2003), hence limit dextrinase activity in malt will be enhanced by selecting cultivars that can transform a greater proportion of bound limit dextrinase into the free form. A potential approach might be to lower the level of the inhibitor in malt, through breeding or optimizing malting or mashing conditions (Walker *et al*., 2001). Accordingly, it is necessary to investigate the genetic and environmental variation in free limit dextrinase activity during malting in order to select varieties with high proportions of the free form.

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## References

AACC, 2000. Approved Methods of American Association of Cereal Chemists, 10th Ed. St Paul, MN.

Arends, A.M., Fox, G.P., Henry, R.J., Marschke, R.J., Symons, M.H., 1995. Genetic and environmental variation in the diastatic power of Australian barley. *J. Cereal Sci*., **21**(1):63-70. [doi:10.1016/S0733-5210(95)80009-3]

Burton, R.A., Zhang, X.Q., Hrmova, M., Fincher, G.B., 1999. A single limit dextrinase gene is expressed both in the developing endosperm and in germinated grains of barley. *Plant Physiol*., **119**(3):859-871. [doi:10.1104/pp.119.3.859]

Collins, H.M., Panozzo, J.F., Logue, S.J., Jefferies, S.P., Barr,

A.R., 2003. Mapping and validation of chromosome regions associated with high malt extract in barley (*Hordeum vulgare* L.). *Aust. J. Agric. Res*., **54**(12):1223-1240. [doi:10.1071/AR02201]

Enevoldsen, B.S., Schmidt, F., 1973. Dextrins in brewing. II. Distribution of oligo- and megaoligosaccharides during mashing in wort and in beer. *Proc. Congr. Eur. Brew. Conv*., **14**:135-148.

Evans, D.E., Wegen, B., Ma, Y., Eglinton, J., 2003. The impact of the thermostability of $\alpha$-amylase, $\beta$-amylase, and limit dextrinase on potential wort fermentability. *J. Am. Soc. Brew. Chem*., **61**:210-218.

Evans, D.E., Collins, H., Eglinton, J., Wilhelmson, A., 2005. Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. *J. Am. Soc. Brew. Chem*., **63**:185-198.

Fincher, G.B., 1989. Molecular and cellular biology associated with endosperm mobilisation in germinating cereal grains. *Anal. Rev. Plant Physiol. Plant Mol. Bio*., **40**(1):305-346. [doi:10.1146/annurev.pp.40.060189.001513]

Howard, K.A., Gayler, K.R., Eaglest, H.A., 1996. The relationship between D hordein and malting quality in barley. *J. Cereal Sci.*, **24**(1):47-53. [doi:10.1006/jcrs.1996.0036]

Kristensen, M., Svensson, B., Larsen, J., 1993. Purification and characterization of barley malt limit dextrinase during malting. *Proc. Congr. Eur. Brew. Conv*., **24**:37-43.

Kristensen, M., Planchot, V., Abe, J.I., Svensson, B., 1998. Large-scale purification and characterization of barley limit dextrinase, a member of the alpha-amylase structural family. *Cereal Chem*., **75**:473-479.

Kristensen, M., Lok, F., Planchot, V., Svendsen, I., Leah, R., Svensson, B., 1999. Isolation and characterization of the gene encoding the starch debranching enzyme limit dextrinase from germinating barley. *Biochim. Biophys. Acta.*, **1431**:538-546.

Lee, Y.C., Whelan, W.J., 1971. The Enzyme. Academic Press, New York, p.191-221.

Lee, W.J., Pyler, R.E., 1982. Improved assay procedure for limit dextrinase in malt extracts. *Brewer's Digest*., **57**:24-27.

Lee, W.J., Pyler, R.E., 1984. Barley malt limit dextrinase: varietal, environmental and malting effect. *J. Am. Soc. Brew. Chem*., **42**:11-17.

Longstaff, M.A., Bryce, J.H., 1991. Levels of limit dextrinase activity in malting barley. *Pro. Cong. Eur. Brew. Conv*., **23**:593-600.

Longstaff, M.A., Bryce, J.H., 1993. Development of limit dextrinase in germinated barley (*Hordeum vulgare* L.). *Plant Physiol*., **101**:881-889.

MacGregor, A.W., 1996. Malting and brewing science: challenges and opportunities. *J. Inst. Brew*., **102**:97-102.

MacGregor, E.A., 2004. The proteinaceous inhibitor of limit dextrinase in barley and malt. *Biochim. Biophys. Acta*., **1696**:165-170.

MacGregor, A.W., Dushnicky, L., 1989. Starch degradation in endosperms of developing barley kernels. *J. Inst. Brew*., **95**:321-325.

MacGregor, A.W., Macri, L.J., Schroeder, S.W., Bazin, S.L., 1994. Limit dextrinase from malted barley: extraction, purification, and characterization. *Cereal Chem*., **71**:610-617.

MacGregor, A.W., Bazin, S.L., Macri, L.J., Babb, J.C., 1999. Modelling the contribution of $\alpha$-amylase, $\beta$-amylase and limit dextrinase to starch degradation during mashing. *J. Cereal Sci*., **29**(2):161-169. [doi:10.1006/jcrs.1998.0233]

MacGregor, A.W., Bazin, S.L., Schroeder, S.W., 2002. Effect of starch hydrolysis products on the determination of limit dextrinase and limit dextrinase inhibitors in barley and malt. *J. Cereal Sci*., **35**(1):17-28. [doi:10.1006/jcrs.2001.0408]

Manners, D.J., Yellowlees, D.S., 1973. Studies on debranching enzymes. Part I. The limit dextrinase activity of extracts of certain higher plants and commercial malts. *J. Inst. Brew*., **79**:377-385.

McCleary, B.V., 1992. Measurement of the content of limit dextrinase in cereal flours. *Carbohydr. Res*., **227**(1):257-268. [doi:10.1016/0008-6215(92)85076-C]

Ragot, F., Guinard, J.X., Shoemaker, C.F., Lewis, M.J., 1989. The contribution of dextrins to beer sensory properties. Part I. Mouthfeel. *J. Inst. Brew*., **95**:427-430.

Ross, H.A., Sungurtas, J., Ducreux, L., Swanston, J.S., Davies, H.V., McDougall, G.J., 2003. Limit dextrinase in barley cultivars of differing malting quality: activity, inhibitors and limit dextrin profiles. *J. Cereal Sci*., **38**(3):325-334. [doi:10.1016/S0733-5210(03)00048-1]

Serre, L., Lauriere, C., 1989. Limit dextrinase in cereal seeds. *Sci. Alim*., **9**:645-663.

Shewry, P.R., Franklin, J., Parmar, S., Smith, S.J., Miflin, B.J., 1983. The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *J. Cereal Sci.*, **1**:21-31.

Sissons, M.J., Lance, R.C.M., Sparrow, D.H.B., 1992a. Studies on limit dextrinase in barley. I. Purification on malt limit dextrinase and production of monospecific antibodies. *J. Cereal Sci*., **16**:107-116.

Sissons, M.J., Lance, R.C.M., Sparrow, D.H.B., 1992b. Studies on limit dextrinase in barley II. Application of an ELISA and immunoblotting to studies of genetic variability and malting effects. *J. Cereal Sci*., **16**:117-128.

Sissons, M.J., Lance, R.C.M., Sparrow, D.H.B., 1993. Studies on limit dextrinase in barley. 3. Limit dextrinase in developing kernels. *J. Cereal Sci*., **17**(1):19-24. [doi:10.1006/jcrs.1993.1003]

Sissons, M.J., Lance, R.C.M., Wallace, W., 1994. Bound and free forms of barley limit dextrinase. *Cereal Chem*., **71**:520-521.

Stenholm, K., Home, S., 1999. A new approach to limit dextrinase and its role in mashing. *J. Inst. Brew*., **105**:205-210.

Walker, J.W., Bringhurst, T.A., Broadhead, A.L., Brosnan, J.M., Pearson, S.Y., 2001. The survival of limit dextrinase during fermentation in the production of Scotch whisky. *J. Inst. Brew*., **107**:99-106.