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EFFECTS OF VARIETY, AGRONOMY, MALTING AND MASHING CONDITIONS ON DIASTATIC POWER OF BARLEY

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by

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Summary

A method to measure diastatic power (DP), α- and β-amylase rapidly from the same barley malt sample has been developed. A separate method to assay the levels of 'free' and 'total' limit dextrinase (LD) in malt has also been implemented. These methods were modified to measure the 'total' DP in barley, by incorporating 100mM cysteine in the extraction buffer. This was used to predict the DP produced by the final malt.

A wide range of barley varieties, grown at several sites in England, Wales and Northern Ireland were malted on the 350g scale. The resultant malts were analysed for DP, α- and β-amylase and 'free' and 'total' LD. The results show that DP is highly correlated with β-amylase. DP and β-amylase are more dependent on the variety of barley, whereas α-amylase and LD are more dependent on where the barley is grown.

The steep regime, aeration, germination time and temperature, use of gibberellic acid and kilning schedules were varied to optimise DP production. Of these parameters, kilning regime had the greatest effect on DP, with standard ale kilning reducing both β-amylase and LD dramatically. The other parameters can be used to increase the DP potential of barley, with aeration, low germination temperature for an extended period, addition of gibberellic acid and high cast moistures being optimal.

The effect of DP on various mashing regimes was investigated. Malt with a DP greater than 100°IOB could convert up to 80% adjunct readily. Mashing regimes could be used to influence the carbohydrate spectrum of the wort. Mashing with a proportion of high 'free' LD malt, such as 9-day germinated

barley, generated an increased proportion of fermentable sugars at the expense of branched dextrins. This wort was brewed to produce a low carbohydrate beer with no detrimental flavour effects from 'green' notes.

1.0 Introduction

There is a world-wide demand for high diastatic barleys, malts and malt extracts by maltsters, brewers, distillers and the food industry. For maltsters high diastatic power (DP) barleys and their malts are important for the domestic market and export. Within brewing and distilling high DP products are vital for the maximum production of fermentable sugars, in particular when high levels of un-malted cereal adjuncts are used during mashing. These high DP products obviate the need to add 'processing enzymes' and are useful for the production of low carbohydrate 'Diat' beers.

Diastatic power is the combined activity of several amylolytic enzymes, the most important being α- and β-amylase and limit dextrinase (LD). These enzymes act together to hydrolyse gelatinised starch into a spectrum of fermentable sugars during mashing.

α-Amylase is produced *de novo* during germination by the aleurone layer . The enzyme is capable of hydrolysing intact starch granules with the formation of soluble products *in vivo* . However the enzyme is much more active on gelatinised starch present during mashing. It hydrolyses this soluble starch in a random fashion to produce a mixture of linear and branched dextrins. Thus it is capable of rapidly lowering the viscosity of starch solutions (MacGregor, 1990). α -Amylase is relatively heat stable and it usually retains a residue of activity even after prolonged mashing periods, before being denatured during the boil.

β-Amylase is synthesised during grain filling. It initially occurs as a freely soluble form, but as maturation proceeds a portion is rendered insoluble and becomes attached to the surface of large starch granules (Lauriere *et al*, 1985). Therefore mature kernels contain a mixture of free and bound forms of β-Amylase, with the actual proportion of each present varying with barley cultivar (Bendelow, 1964). As with most barley storage proteins β-amylase is not synthesised in barley kernels during germination (Hardie, 1975), but the insoluble form is released by a combination of proteases (Guerin *et al*, 1992). This process can be achieved *in vitro* by the use of a suitable reducing agent or protease, such as papain (Pollock and Pool, 1958), although recent work has suggested that a combination of the two is required for complete release (Grime and Briggs, 1995). β-amylase is relatively heat labile and significant amounts of activity may be lost during kilning (Narziss *et al.* 1973) and especially mashing (Muller, 1991).

LD is a debranching enzyme that can catalyse the hydrolysis of the α (1-6) glucosidic linkages in branched α dextrins derived from the amylopectin component of starch. LD is produced mainly *de novo* during germination (Hardie, 1975). It is present in a 'free' (fully active), 'latent' (soluble-bound) and a 'bound' form that remains in the pellet after extraction (Kristensen *et al*, 1993). During germination the level of the free form increases, possible due to release by a cysteine protease (Longstaff and Bryce, 1993), but prolonged periods of germination are needed before significant levels are released (Lee and Pyler, 1984). LD is also heat labile (Sissons *et al*, 1995). Thus levels of activity in standard malt are generally insufficient to allow the complete

conversion of branched dextrins in mashing (Enevoldsen and Schmidt, 1973). However recent work (Stenholm *et al*, 1996) has indicated that that LD is more heat stable than previously thought and the use of malt with high free LD activity can lead to the production of low dextrin beers.

The potential DP in malt is limited by the genetic potential of the barley (Arends *et al*, 1995). However, environmental conditions can modify realisation of this genetic potential. It is thought that long daylight hours and cool temperatures during grain filling promote high enzyme levels (Kenn *et al.* 1993). This can be seen in barley grown in high latitude Scandinavian countries producing extremely high DP barleys. Rainfall also affects DP production, with an optimal value being present within a band between too much rainfall which leads to nitrogen stripping of the soil, and too little which leads to water stress. However it must be noted that rainfall is usually associated with cooler climates and hence these two can have a synergistic effect (Henry and Kettlewell. 1996). The application of nitrogen fertiliser has been reported to increase DP within a single variety (Eagles *et al.* 1995), although no correlation with nitrogen application has been observed between different varieties (Goblirsch *et al*, 1996). This is an important observation as it indicates that barley could be bred to have a high DP whilst retaining a low overall protein content. This would be useful in a brewing context.

Steeping, which is the first stage of malting, involves alternate immersions and air resting of grain to allow uptake of water by the embryo and adequate distribution of this water into the endosperm. The steeped grain is then allowed to germinate, typically for a four day period at 16°C. The temperature and duration of both these stages are thought to influence the production of

DP enzymes. Thus higher steep temperatures have been reported to increase DP (Reeves *et al.* 1980), possibly by raising the casting moisture (Ballesteros and Piendl, 1977). On the other hand long cool germination periods have been associated with higher DPs (Guglielminetti *et al.* 1995, Ballesteros and Piendl, 1977). Sissons (1993) has suggested that up to 9 days germination is required for optimal LD release. The use of processing aids such as gibberellic acid to stimulate germination is recognised as increase the production of several hydrolytic enzymes but especially α-amylase (Paleg 1960).

The final stage in malting is kilning, where the grain is dried using hot air, at temperatures starting around 40°C and rising as high as 100°C for some ale malts. It is well recognised that kilning can destroy the more heat liable enzymes such as ß-amylase and LD (Bathgate 1973), although more recent work suggests that enzyme preservation can be enhanced by using high initial air flows and low temperatures (Karababa, 1993).

High DP malts are used in 'Diat' beer production and in a grain distilling context. For both of these end uses an efficient mash regime must be used to maximise the fermentable extract produced. Maximal yield of fermentable extract is generally considered to be obtained at 65°C, at a pH of 5.3 - 5.4, at a mash thickness of about 2.5:1 (Briggs, 1981). These mash conditions were arrived at by trial and error, but following detailed studies of the stability of individual enzymes (Muller, 1991) we can now relate them to the optimal conditions required by the enzymes involved.

Production of low carbohydrate beers frequently relies on the use of exogenous debranching enzymes such as amyloglucosdiase (Enevoldsen, 1986) or diluting the branched dextrin in the addition of high proportions of sugar adjuncts. Highly attenuating yeasts including genetically modified yeast (Hammond, 1995) have also been used. High DP malts have also been used (Gjertsen and Hartley, 1995) but these have the disadvantage that the endogenous barley debranching enzyme LD, has generally been found to be difficult to enhance, and is rapidly inactivated during mashing. The use of high DP malt in grain distilling has a slightly different role in that it is used for the conversion of high levels of cereal adjunct into fermentable sugars during the mash. Hence high levels of all the relevant enzymes are necessary (Bathgate *et al.* 1978). Any ability to decrease the amount of malt needed to perform the conversion will lead to substantial cost savings.

The aim of this project was to maximise DP in malt for both brewing and distilling purposes. The genetic and environmental factors influencing the individual components in barley which contribute to DP in malt have been investigated. Particular attention has been paid to ß-amylase, which has been studied to a much lesser extent than α-amylase. The extent to which this potential may be optimised by suitable malting conditions has been established. Special attention has been given to LD, in terms of quantifying its influence on DP and identifying possible routes whereby its effective activity can be increased. Finally, alternative mashing procedures for the production of high fermentability worts have been developed.

2.0 Objectives

To assess the effect of barley varieties, environmental parameters and malting and mashing conditions on the potential diastatic power of barley for use in the malting, brewing and distilling industries.

Specifically:

1. To establish methods for measuring β-amylase and limit dextrinase in the presence of α-amylase.
2. To measure levels of these enzymes in barleys during malting and in final commercial and control malts.
3. To determine the activity of β-amylase and limit dextrinase in malts produced from different barley varieties grown under a wide range of growing conditions. To relate diastatic enzymes activities to varietal and environmental parameters
4. To establish the effects of malting protocols (including steeping regime, moisture, temperature, aeration, germination time, gibberellic acid and kilning) on the levels of β-amylase and limit dextrinase in relation to α-amylase levels and overall malt quality.
5. To correlate enzyme levels in final malt with mash performance in order to produce high fermentability worts.
6. To consider techniques for the production of high diastatic malt extracts.

3.0 Materials and Methods

3.1 Materials

Red Pullulan and Betamyl were obtained from Megazyme Pty Ltd (N.S.W., Australia); β-limit dextrin from Rank Hovis Ltd (Southampton, UK); L-cysteine, papain (from papaya latex), 2-mercaptoethanol, *p*-hydroxybenzoic acid hydrazide, 3,5-dinitrosalicylic acid, dithiothreitol (DTT), Lintner starch and iodoacetamide from Sigma (Poole, UK). Gibberellic acid was supplied as a 3.33%w/v solution in isopropanol (Biocon, England). All other chemicals were of the highest grade available and all water used was deionised.

Barley was supplied by Pauls Malt Ltd (Kentford, UK), H.Rudebeck & Co. Ltd.(West Sussex, UK), Bairds Malt (Witnam, Essex), Crisp Malt (Norfolk), the NIAB (Cambridge, UK), Kirin Malt (Western Australia).
Malt extracts were supplied by British Diamalt (Newark, UK) and EDME.

3.2 Methods

3.2.1 Enzyme Assays

Samples (barley, malt, green malt) were ground for 10s in a Moulinex coffee grinder and the grist sieved through a 0.5mm screen.

LD activity was determined essentially according to McCleary (1992) as adapted by Sissons (1996). Ground samples were mixed with 0.2M acetate buffer, pH 5.0, containing 100mM L-cysteine for total LD assays and 0.4%

(w/v) iodoacetamide for free LD assays, and extracted at 30°C for 16h with occasional vortexing. The slurry was centrifuged at 2000g for 10 min and the resultant supernatant assayed immediately. Ethanol, 78% (v/v), was used to terminate the reaction and the non-hydrolysed sugar/dye conjugate was removed by centrifuging at 2000g for 10min. Supernatants were measured spectrophotometrically at 510nm.

α-amylase was determined by a method modified from Briggs (1961). Grist (2g) was extracted in 40ml of 6mM ammonium hydroxide at room temperature for 30min. This was centrifuged at 2000g for 10min and samples of the supernatant were diluted 1:100 in acetate buffer (100mM NaCl, 10mM $CaCl_2$ and 50mM sodium acetate, pH 5.7). Diluted extract (0.5ml) was added to 0.6ml acetate buffer and pre-incubated at 35°C for 10min. β-limit dextrin (4%w/v in acetate buffer) was added to start the reaction. The reaction was terminated after exactly 10min by the addition of 7.5ml of working KI/I_2 solution [3g KI and 10ml stock KI/I_2 solution (10g KI and 2.54g I_2 in 100ml deionised water) in 1L deionised water] and the fall in absorbence was measured at 607nm. This was compared to a substrate blank where working KI/I_2 solution was added prior to the enzyme extract.

β-amylase was measured using one of four procedures as described below.

1. Megazyme Betamyl assay.

β-amylase activity was determined using a *p*-nitrophenyl maltopentoside substrate according to McCleary and Codd (1989).

2. Dinitrosalicylic Acid Assay (Bendelow, 1963)

Grist (0.5g) was extracted in 5ml of extraction buffer (60mM sodium acetate, 30mM sodium chloride, 3mM DTT and 30mM EDTA, pH 4.8) at 4°C for 1h. This was centrifuged at 2000g for 10min and samples taken from the supernatant were used for the assay. Incubations consisted of 0.1ml extract and 1.9ml extraction buffer, equilibrated at 20°C for 30min, followed by the addition of 1ml of 2%(w/v) Lintner starch to start the reaction. After 3min, 3ml of chromogen reagent (5gDNS, 100ml 2M NaOH, 150g potassium sodium tartrate made up to 500ml) was added. Samples were boiled for 5min, then cooled to 20°C before being diluted with 20ml water, vortexed and OD measured at 540nm. Blanks were prepared by boiling an aliquot of extract for 5min before performing the incubation. The number of reducing sugar equivalents present were calculated from a maltose standard curve.

3. PAHBAH Assay (adapted from Bajomo and Young. 1990)

Grist (0.5g) was extracted in for free β-amylase (i) 5ml of 0.5M tri-sodium citrate, 0.3M ammonium oxalate (pH 4.8) containing 2.5% (w/v) potassium chloride, 5mg bovine serum albumin (BSA) and 5mg polyvinylpolypyrrolidone (PVPP), or for total β-amylase in (ii) 5ml of 0.5M tri-sodium citrate, 25mM 2-mercaptoethanol (pH 8.2) containing 5mg PVPP and 5mg BSA, at room temperature for 2h with occasional shaking. The extract was centrifuged at 2000g for 10min and samples of the supernatant were used for the assay. Samples of extract (0.5ml) were incubated with 10ml of 2% (w/v) Lintner starch solution for 30min at 20°C and the reactions stopped by transferring

0.5ml of the mixture into 4.5ml of 0.1M sodium hydroxide. The tubes were vortexed and 0.1ml of the reaction mixture was transferred to a boiling tube containing 5ml of fresh PAHBAH reagent (5g/l of alkaline diluent, comprised of 0.05M tri-sodium citrate, 0.01M calcium chloride hexahydrate and 0.5M sodium hydroxide). They were then boiled for 5min, cooled to 20°C and 4.9ml of water added before reading at 415nm. The number of reducing sugar equivalents present were calculated from a maltose standard curve.

4. PAHBAH Assay (adapted from Delcour and Verschaeve, 1987)
Grist (2g) was extracted in 40ml 6mM ammonium hydroxide for 30min at room temperature, then centrifuged at 2000g for 10min. An equal volume of the supernatant was added to 0.2M ammonium oxalate and incubated at 40°C for 2h. 400µl of this solution was added to 20ml 2%(w/v) Lintner Starch pre-equilibrated at 20°C . The mixture was incubated for 10min and the reaction termination by the addition of 0.5ml 0.5M sodium hydroxide. 0.2ml of the reaction mixture was then added to 5ml PAHBAH reagent in a boiling tube and boiled for 4min. The tube was cooled to 20°C and 10ml of deionised water added. The tubes were vortexed and the absorbance was read at 415nm. The activity of the sample was calculated in °IOB from a calibration curve (Figure 1).

Two procedures were used to measure **DP**; the Institute of Brewing (IOB) recommended ferricyanide assay and an adaptation of the American Society of Brewing Chemists (ASBC) recommended rapid method (Malt-6).

The latter method was based on the use of the PAHBAH reagent. 2g of grist are extracted in 6mM ammonium hydroxide for 30min at room temp, with occasional gentle swirling. After centrifugation at 2000g for 10min, 0.2ml of the supernatant was added to 20ml 2%(w/v) starch solution pre-equilibrated at 20°C. The mixture was incubated for 10min at 20°C, and the reaction stopped by the addition of 0.5ml 0.5M sodium hydroxide. 0.2ml of the reaction mixture was then added to 5ml PAHBAH reagent in a boiling tube and boiled for 4min. The tube was cooled to 20°C and 10ml of deionised water added. The tubes were vortexed and the absorbance was read at 415nm. The activity of the sample was calculated in °IOB from a calibration curve (Figure 1).

In order to simplify established methods, the following procedure was adapted : this allowed α- and β-amylase to be measured from the same extract within a single day. The grist (2g) was extracted in 40ml of 6mM ammonium hydroxide for 30min at room temperature and divided into three aliquots for analysis. The first sample was used to perform the ASBC method for DP. The second was mixed with an equal volume of 0.2M ammonium oxalate and incubated at 40°C for 90min. This inactivates any α-amylase present by chelating Ca^{2+} ions. The PAHBAH assay for DP can then be performed to give a measure of β-amylase. The third aliquot was used for the α-amylase assay.

3.2.2 Malting

Micro-malts were prepared by steeping 2kg barley at various temperatures and steep regimes as given in the text. Where indicated 0.2ppm gibberellic acid was sprayed on at cast and grains were germinated for periods of up to 9 days at various temperatures, with mixing twice daily. The green malt was either dried in a forced-draught oven at temperatures up to 60°C over 39h, until a moisture content of between 4-6% was reached, or frozen and kept at -20°C until needed. After drying the roots and shoots were removed.

Lager malt was prepared in the BRFI pilot maltings (50kg scale) by steeping barley under various steep regimes and germinating at different temperatures for up to 6 days. Green malt was kilned for 39h with a finishing temperature of 60°C.

Moisture was measured by the IOB Recommended Method. Pre-weighed ground samples were dried at 105-106°C for 3h, cooled in a desiccator and re-weighed.

3.2.3 Brewing

Premium lagers were produced in the BRFI pilot (1hl) brewery in a mash conversion vessel at 47°C, ramped to 64°C at 1°C per min, for a 1h stand and then ramped to 78°C. After lautering wort was boiled for 90min, cooled then fermented at 12°C for 6 days. The beer was filtered, pasteurised, bottled and stored at 4°C until organoleptically assessed.

3.2.4 Mashing

A number of small scale (50g) mashes were performed using a BRF mashing bath as described by Buckee and Long (1981), except that the mash temperature and thickness were varied. Adjunct, when used, consisted of wheat flour mixed in the desired proportion to the grist. Mashes were carried out to an IOB HWE format, except that mercuric chloride was added immediately after cooling the mash to 20°C to give a final concentration of 100μg/ml. Concentrations of fermentable sugars were measured by Dionex HPLC.

4.0 Results and Discussion

4.1 Repeatability of β-amylase Assays

Assays of this enzyme using the Megazyme Betamyl test kit gave good repeatability on a single day but, when the same malt sample was analysed over 4 consecutive days the repeatability was poor. This lack of day-to-day repeatability was considered too imprecise, a finding echoed by the EBC analysis sub-committee (Erdal, 1993). Alternative assays for β-amylase, using DNS or PAHBAH have therefore been evaluated. Whilst they measure a less definable product, (*i.e.* reducing power of hydrolysate) they were very repeatable and can be measured easily and quickly using a spectrophotometer. They are also relatively inexpensive when compared to the Megazyme test.

Tables 1,2 and 3 show the standard error of each method both on a single day and over a four day period for ten extracts of the same commercial lager malt. All the assays gave a standard error <11% when performed on a single day. However when the standard error was calculated using data over the four day period, the Megazyme assay gave a standard error of 18%.
From this data the PAHBAH assay gave slightly higher standard daily errors than the DNS assay but simplifying the procedure corrected this. With an in-house check malt a standard error of <4% was obtainable over 20 separate extractions. Although the DNS method gave the lowest error in this trial, several authors have noted that substances present in barley extracts can interfere with the assay (Delcour and Verschaeve, 1987, Etokakpan and

Palmer, 1990). Hence the PAHBAH β-amylase assay was be used for the remainder of this work.

4.2 Limit Dextrinase Assay

The Megazyme Limit-dextrizyme method (McCleary, 1992) has been used in-house for measuring the amount of LD activity in malt. However some development work was performed to optimise both the extraction of "total" LD and to give an indication of the "free" LD which would be active in the mash. In work to optimise the full extraction of "total" LD, not reported here, it was found that 100mM cysteine was needed to obtain maximal levels rather than the 29mM reported in the Megazyme method. Also it was found that in the long extraction of 16h, some additional LD activity was released due to the inherent reducing activity of the malt extract, leading to a over estimation of the actual levels of "free" LD present. This was overcome by using iodoacetamide , a sulphydryl inhibitor, in the extraction medium.

4.3 Use of a Rapid Method for Measurement of DP

The present IOB Recommended Method for DP is labour intensive and is a lengthy procedure to perform. An alternative rapid procedure is offered by the ASBC Malt-6 method, based upon the PAHBAH reagent. It was found to be both easy to use, rapid and as reliable as the IOB method. Figure 1 demonstrates the relationship between A_{415}, a measure of the amount of reducing sugar equivalents produced from starch hydrolysis, with DP in °IOB

units. The plot gave a correlation coefficient of 0.996, enabling the method to accurately reflect DP in °IOB units.

Repetition of this method by one person on a check malt has produced standard errors of less than 4% over 20 separate extractions.

4.4 Measurement of DP, α- and β-Amylase from a Single Extract of Malt in One Day

To determine the relative contributions of α- and β-amylase in the measurement of DP it would be useful to perform all three assays using the same malt extract, to save time and to produce more comparable results. It has been shown that over 80% of starch degrading enzymes in malt are released in the first 10min of their extraction in 6mM ammonium hydroxide (34). The extract can then be divided into three samples. One is used for a PAHBAH DP assay, the second, (after the addition of an equal volume of a 0.2M ammonium oxalate solution at 40°C for 90min to inhibit α-amylase activity), can be used to measure β-amylase and the third is subsequently used to measure α-amylase by using a β-limit dextrin substrate in conjunction with KI/I_2 assay. Example results in Table 1, show that the assay is able to measure DP over a wide range of values, and that the individual contributions of α- and β-amylase can be clearly distinguished.

4.5 Measurement of Amylolytic Enzyme Levels During Malting

4.5.1 'Free' and 'Total' DP in NIAB Trial Barleys

The only major amylolytic enzyme present in barley is β-amylase, since both α-amylase and LD are synthesised *de novo* during germination. Thus β-amylase is the main component of DP in unmalted barley present in both a free and a bound form. The 'free' form is easily extracted by 6mM ammonium hydroxide, whilst the bound form requires the addition of 100mM cysteine in the extraction buffer. Tables 5 and 6 give the 'free' and 'total' DP activity present in the spring barleys from two growing sites. For these varieties about 40% of the total β-amylase is present as a soluble 'free' form. The actual proportion of 'free' to 'total' DP appears to be cultivar dependant, with a maximum of 62% and a minimum of 33%.

Table 7 displays similar data for winter barleys. Here we can see that the average proportion of 'free' to 'total' β-amylase is much greater, around 64%, with a maximum of 71% and a minimum of 58%. In addition the levels of both "free" and 'total' DP are elevated in these winter barleys. In both spring and winter varieties the TN range was very similar.

In all these cases the 'total' DP values are quite substantial, approaching and in some cases exceeding the levels of DP in standard commercial malts (approximately 50-60°IOB for ale malts and 75-85°IOB for lager malts). This measurement of "total" β-amylase in barley gives a good approximation of the DP developed in the final malt, under standardised malting conditions. This could be utilised by barley breeders or maltsters to select for high DP

varieties without the necessity for micro-malting. This correlation will be reported fully in section 3.6.1.

4.5.2 Amylolytic Enzymes in Micro-malted NIAB Barleys

Tables 8,9,10 and 11 show amylolytic enzymes in malts prepared from these barleys, using a standardised 4-day micro-malting regime, with air drying in a forced draught oven (see Methods section 2.2.2). The malts are the 1994 and 1995 harvests from Dorset, Hampshire, Clwyd and Lincolnshire.

The malts from Dorset barleys give a typical example of the results obtained. DP ranged from 76 to 123°IOB. These are higher levels than would be found in commercial malts due to the gentle low temperature drying methods employed. The β-amylase levels range from 39 to 75, and correlate well with DP (section 3.6.2), thus on average DP is composed of 60% β-amylase activity. Comparing the β-amylase activity in these malts with the total β-amylase activity present in the corresponding barley (Table 5) there is again a very good correlation (section 3.6.1). The levels of α-amylase are measured in units of mg β-limit dextrin hydrolysed/ min/ g, and range from 1559 to 2214, with an average of 1846. Although it is apparent that α-amylase only comprises about of 40% of DP, it does make a contribution especially when at elevated levels. When we compare Chariot and Delibes, two barleys with similar β-amylase activities, 75 and 71°IOB respectively, we can see that the increased level of α-amylase in Delibes, (2214 compared with 1823 units in Chariot) results in an overall increase in DP, 123 compared to 112°IOB.

Hence we can see that DP is composed of differing amounts of α- and β-amylase activity, with β-amylase making the greatest contribution. The data also indicates that α- and β-amylase activities are not linked, that is, it is possible to have a high β-amylase and a modest α-amylase, for example Optic, or a high α-amylase and a modest β-amylase, for example Cooper. The importance of the α : β amylase ratio will be further discussed in section 3.10 on mashing.

The LD results demonstrate that whilst total LD is abundant, the levels of free LD which would be active in mashing are only some 5-10% of the total. The LD activities are fairly uniform although there is some correlation between LD and α-amylase activity (section 3.6.2) with Delibes, Alexis and Derkado. Although the debranching role of LD activity is of obvious importance to mashing it is clear that it contributes very little directly to the measurement of DP, that is the production of reducing sugars from soluble starch.

Results for spring varieties given in tables 9,10 and 11 display similar trends. For example Pitcher gave the highest DP at each of the sites in 1995.

Table 12 gives data for the micro-malts produced from the 1995 winter barleys from Northern Ireland. The levels of enzymes are within the same ranges as those found for the spring varieties. Table 13 gives a summary of the comparison between spring and winter barleys for each enzyme. Although the average DP (102 and 99°IOB respectively) and LD are very similar there is a difference in the ratio of α to β-amylase. The winter varieties possess more of both 'free' and 'total' DP than do spring varieties, and the percentage of the DP which is 'free' is also greater. This is echoed in the malt results

where β-amylase comprises 75% of DP on average compared to 62% for the spring varieties. Thus winter barleys could be used to adjust the ratio of ß to α-amylase in the mash. It must be stressed, however that these findings are made using samples malted on a small scale, where hydration problems sometimes encountered with winter varieties are easily overcome. In addition, the winter data is obtained from one years harvest at a single site. Thus there is a possibility of environmental effects.

This data will now be analysed to obtain the correlations between the various enzymes and the amount of variation in DP associated with variety and growing environment.

4.6 Correlation Between the Major Amylolytic Enzymes and DP

4.6.1 Correlation Between 'Free' and 'Total' DP in Barley and 'Final' DP in Malt

Table 15 gives the correlations between 'free' and 'total' DP in barley with the amylolytic enzymes subsequently developed in the malt. The most interesting observations were the very strong correlation between 'total' DP in barley (mainly due the presence of β-amylase) and the final level of DP in the malt. This is depicted in figure 2. This correlation is perhaps to be expected as we have shown DP to be mainly β-amylase and so the level of β-amylase present in the barley would have a major impact on the resultant level of DP in malt. Nevertheless it is a significant result as it could have commercial implications in the prediction of DP in barley without the need to micro-malt. Certainly barley breeders could rapidly perform a PAHBAH 'total' DP assay on as little

as 2g of barley to determine the DP potential of the final malt. Maltsters could also predict which of a selection of barleys have the tendency to produce high DP malts.

α-amylase is not correlated with either 'free' or 'total' DP in barley. This is perhaps not surprising since it is primarily synthesised *de novo* during germination.

4.6.2 Correlations Between Amylolytic Enzymes in Malt

Table 16 gives the correlation between the various amylolytic enzymes in malt. Here we see a very significant correlation between DP and β-amylase in malt (R^2=0.87 - all barleys). This again demonstrates the very close relationship between β-amylase and DP, and that DP is in fact largely a measure of β-amylase. DP is less well correlated with α-amylase (R^2=0.54 - all barleys), although as with previous data in this report, it can be seen that α-amylase does make a contribution to DP. 'Free' LD does not correlate with DP (R^2=0.18 - all barleys) however 'total' LD does show a weak correlation of 0.54 for all barleys examined. It follows that there is little correlation between β-amylase and either LD or α-amylase. There is some relationship between α-amylase and 'total' LD (R^2=0.54 - all barleys), probably accountable by the fact both enzymes are synthesised *de novo* during germination.

These results again emphasise the importance of β-amylase levels in barley both for breeding for high DP barley and for selecting barley for high DP malt production.

4.6.3 Correlations Between Amylolytic Enzymes and TN

Table 17 shows the correlation of TN with DP in both barley and malt. It appears that in all cases TN is only weakly correlated with DP. This is especially obvious for final DP in malt (R^2 = 0.12 - all barleys). However although TN does not correlate with DP between varieties, within a variety increasing the TN increases DP in an almost linear manner. This result is significant in terms of breeding for DP in that it should be possible to breed a line of high DP barley with low TN as they do not seem to be directly linked genetically.

4.7 Environmental Versus Varietal Effects on Variation in DP

For breeding and selection purposes it would be desirable to know whether it is environment, (overall growing conditions), or variety, (inheritable genetic factors), which plays the most important role in determining potential DP in barley. If the growth environment is most important in determining DP then the site could be focused upon. Conversely if genetics play the major role then barley variety should be the focus. The proportion of total variation which is attributable to these two parameters can be measured by subjecting the data generated during this work to ANOVA statistical analysis. Due to the changing barley varieties that appear each year, only 10 varieties (Alexis, Brewster, Chariot, Cooper, Cork, Delibes, Derkado, Heron, Optic, Riviera) grown at 4 different sites (Dorset, Hampshire, Clwyd, Lincolnshire) over 2 harvests (1994 and 1995) were included in the analysis. These 10 varieties were compared for the amount of variation in enzyme levels due to environment and the variation due to variety. From the results presented in

Table 18, an interesting trend can be seen. For both DP and β-amylase the major source of variation is the variety of the barley grown. However for α-amylase and 'free' and 'total' LD the major source of variation appears to be the environment the barley is grown in.

Therefore these results indicate that a high DP variety barley could be grown under a wide range of growing conditions and still produce high DP malt. However the levels of α-amylase and LD would be influenced to greater extent by the growing conditions. As will be shown later in this work (section 3.9) β-amylase is released very easily in malting since it is already present in the grain. Thus malting conditions will not affect final β-amylase or DP to any great extent. However α-amylase and LD are synthesised during malting and hence can be more readily affected by the malting process. Therefore the variation attributed to environmental factors could feasibly include some environmental influences on the "maltability" of the grain, rather than upon the α-amylase or LD potential *per se*.

4.7.1 Statistical Analysis of DP Data on Malting Barley Varieties - A Report by Biomathematics and Statistics Scotland, University of Edinburgh

As part of the investigation into the genetic and environmental variation in DP a statistic report was commissioned to the Biomathematics and Statistics Scotland, at the University of Edinburgh. They examined data on TN, DP and Dextrinising Units (DU - industry measure of α-amylase) from 42 spring and 34 winter varieties grown at 13 sites in Scotland over a four year period and

malted and analysed at a number of laboratories. Overall, the report supports the findings reported here from the analytical work at BRFI, that is DP, and hence β-amylase is more dependant on variety than environment, whilst α-amylase appears to be more variable across different environments. Other conclusions of this statistical report, which is included in its entirety as Appendix 1, are that there are no correlations between DP v TN, DU v TN, and DP v DU. Again this echoes the findings from the present BRFI study and reinforces the observation that DP and α-amylase are not reliant on TN. It should therefore be possible to breed low nitrogen barleys with high DP potential. One significant correlation the report did observe was between 'earliness' in ripening and DU. This correlated at a 99% confidence level, suggesting that early ripening barleys will tend to produce high levels of α-amylase in malting. This data was also analysed for interrelationships, correlations and components of variation. Table 19 shows the components of variation for TN, DP and DU, when split into the variation attributable to variety, year, site, and combinations of these factors. This highlights the relative importance of environmental effects (site. year interactions). This is similar for DU, however for DP there is a much larger variation due to variety. However the report did also highlight some problems in examining data of this nature. It was obvious from the treated data that as the barleys were analysed in a variety of analytical laboratories an unquantified but significant amount of variation will be due to inter-laboratory error. This makes it difficult to define the true amount of variation between samples. In addition, because commercial varieties are constantly changing, few varieties were included in all the trials. This obviously affects the statistical interpretation. It would seem

that the ideal way to produce and analyse data for work of this kind would be to collect a wide range of samples and then micro-malt and analyse them using identical conditions within one laboratory. This is indeed what we have managed to do for this study by using the NIAB barleys grown at a wide range of sites.

4.8 Comparison of Amylolytic Activity in a Range of Malts and Barleys from non-UK Sources

Table 20 gives the levels of the amylolytic enzymes from a range of malts and barleys from several important barley growing areas across the world. The Australian and Canadian varieties show a very similar range of enzyme levels to British barleys and malts. The Canadian barleys have high levels of 'total' DP, resulting in a high level of DP activity in the malts. These are commercial samples and hence the effect of kilning on the heat labile β-amylase can be seen with certain samples e.g. Australian Stirling. The Finnish varieties, Pokko and Kilta show a very high level of β-amylase within the barley, as evidenced by 'total' DP. This results in extremely high levels of β-amylase in the malt. High levels of α-amylase are also developed during malting which contributes to extremely high DP levels in the malt. It is interesting that in these malts the β-amylase levels exceed the 'total' level measured in barley. This differs from the majority of the malts examined in this work, where the β-amylase usually remains at a very similar level, or decreases slightly in the malt. This is most likely due to the extremely high level of β-amylase activity within the grain, resulting in incomplete extraction from the barley. An

alternative explanation is that there may be secondary production of β-amylase during germination. The elevated levels of amylolytic enzymes in these Finnish barleys is probably due to a combination of a high genetic potential to produce DP, coupled with ideal growing conditions, long daylight hours, cool temperatures and plentiful rainfall, to fulfil this potential.

4.9 Effect of Malting Conditions on DP

4.9.1 Effect of Aeration and Steep Regime on DP

Table 21 shows the effects on two barleys of four different steep regimes, designed to produce an increasing level of cast moisture, with and without aeration. The beneficial effect of aeration during the steeps is pronounced over all the steep regimes for Alexis, largely by the effect on α-amylase, with only a small increase in β-amylase. Aeration was markedly less effective for Cooper. Steep regime itself has a pronounced effect on DP and α- and β-amylase for both barleys. Steep regimes resulting in increased cast moistures produced increased levels of α- and β-amylase and hence DP. However the effect appears to be more complicated then a straightforward linear relationship, with the length of wet steep being significant. This is probably due to the induction of aerobic stress during long immersions. It is also noteworthy that Alexis with a short two steep regime (8.16.8) is nearly capable of attaining the levels of DP produced by the longer three steep regimes. Again this is an indication that the quality of the hydration (i.e. the

distribution of water within the grain) (Chandra *et al*, 1996) is more important than the final moisture content.

The variety Cooper was included in this work as it had been reported as a good malting quality barley that suffered from a low DP potential. These results show that it has an acceptable level of α-amylase, that can be increased under the right conditions, however it has a low β-amylase content, with a limited potential for improvement via optimum malting conditions. This displays the importance of the genetic potential of barley to produce DP. If this genetic potential is poor then the scope for improvement by optimising growing and malting conditions is limited.

4.9.2 The Effect of Reduced Aeration on DP

Table 22 shows the conditions used to generate the results depicted in Figure 3. Chariot barley was steeped under four conditions which resulted in a decreasing level of aeration, with the last regime being in an enclosed glass jar which would result in nearly anaerobic conditions by the end of germination. From Figure 3 we can see effect of these steep and germination conditions at the end of day one of germination and in the final dried malt. The level of DP decreases steadily both at day one and in the final malt. The levels of β-amylase show a very much less marked decrease over these steep and germination conditions. The effect on α-amylase is dramatic with virtually no activity being produced in the oxygen stressed regimes. It is clear from these results that it is this effect on α-amylase that leads to the decrease in overall DP. The marked differences that the degree of aeration has on α-

and β-amylase is indicative of the relative times of their production, and again displays that malting conditions are likely to have a greater impact on α-amylase than on β-amylase.

4.9.3 The Development of Amylolytic Enzymes During Germination

A typical profile of the development of DP, α- and β-amylase with time is shown in figure 4 (Optic barley germinated over 6 days at 14°C). It can be seen that there is a steady development of DP, levelling off as germination time extends beyond day 4. This DP curve is closely mirrored by β-amylase development, again emphasising the close relationship between these two measurements. The divergence of the DP and β-amylase curves around day 2 of germination is due to the rapid production of α-amylase at this time. This sigmoidal shaped curve of α-amylase development is typical of the majority of the timecourse investigations performed during this work. Overall this graph nicely highlights the production of the main amylolytic enzymes, demonstrating the close relationship between DP and β-amylase and clearly showing that for maximal DP production a germination length of about 6 days is needed, although the bulk of DP is produced by around day 4. Extending the germination period to a total of 9 days to attempt maximal LD release did not result in any further gains in DP.

4.9.4 The Effect of Germination Temperature on DP

Figures 5 to 9 show the effect of increasing germination temperature on the levels of DP, α-and β-amylase produced in Chariot, malted on the 2kg scale.

These graphs nicely depict the effect of temperature during germination. At all temperatures with the exception of 12°C there is the typical steady development of DP, which is mirrored by the production of β-amylase. At 12°C there is a major temperature effect on α-amylase. The curve loses its typical sigmoidal shape and enzyme activity continues to increase in a linear fashion up to day 6 of germination, producing an elevated level of α-amylase in the final malt. This increase in α-amylase at this temperature has a clear effect on DP, which also continues to increase up to day 6, instead of plateauing between days 5-6. Thus low germination temperatures (12°C) appear to decrease the rate of α-amylase production but to allow production to be sustained for a longer period resulting in enhanced final levels of both α-amylase and DP. These results were echoed in Alexis barley, DP production again maximal at the lowest temperatures, and α- and β-amylase showing concomitant decreases with increasing temperature. A summary of the data is shown in Table 23.

4.9.5 The Effect of Gibberellic Acid on DP

Figure 10 shows that extending the time of malting, beyond the normal 4-5 days, results in a marked increase in LD activity. This could be further enhanced by the addition of relatively high concentrations of GA_3, (1mg/kg), giving almost a doubling in the level of LD by day 8 compared with malt germinated for the usual 4-5 days without GA_3. By comparison, α-amylase activity was also substantially increased by 1mg/kg GA_3 but this occurred

earlier in germination, peak stimulation being at about day 5, with the effect being noticeable from day 2.

The later development of LD activity compared to α-amylase is consistent with other reports (Lee and Pyler, 1984; Manners, 1985) and could be due to a slow synthesis of the enzyme (Lee and Pyler, 1984; MacGregor, 1987). Recent findings, however, indicate that a large amount of LD is bound to an inhibitor (Marcri *et al*, 1983) and it is possible that this increase in LD is due to conversion of bound to free enzyme. It is possible that GA_3 enhances the release of bound enzyme via stimulation of a cysteine protease which has been reported to be responsible for the release of LD from a bound form (Longstaff and Bryce, 1993).

In a separate experiment the effects of 0.1mg/kg GA_3 on pilot (50kg) scale malting is depicted in table 24. Here we can see that for both barleys the addition of GA_3 increases DP, α- and β-amylase beyond the level that would occur if the barley was malted for 6 days. The striking feature of these results was the effect on both 'free' and 'total' LD. The 'free' values increased ten-fold for Optic and four-fold for Alexis, relative to the level in barley malted for 6 days without GA_3. This could be effective in releasing LD which would be active during mashing.

4.9.6 The Effect of Kilning on DP

The pilot scale facilities at BRF International allow up to 50kg of malt to be kilned under conditions which closely approximate to commercial processing.

The effects of kilning on enzyme activity are complex and are related to the temperature and grain moisture throughout the grain bed.

Figure 11 shows that in the early phase of kilning, when moisture is being removed from the outside of the grain, thus cooling the grain because of the latent heat of evaporation, no loss in LD activity occurred. Once the bed temperature exceeded about 55°C and the grain had started to lose a significant amount of bound water, LD activity also began to decrease. Sampling at different positions in the kiln shows that LD activity is lost more quickly at the bottom of the bed, where temperature and moisture loss are the highest. Once the grain temperature had evened out throughout the bed, at 55°C, activity loss was equal across the bed. The relationship between temperature and moisture at the top, middle and bottom of the bed can be seen in the lower two graphs. The temperature differential between the top and bottom during the early stages is due to evaporative cooling of the upper layers of the grain. Limit dextrinase activity of the green malt was reduced by 24% during kilning, which is consistent with the results of Kristensen *et al* (1993) but is less than that previously reported by Lee and Pyler (1984). Figure 12 depicts the loss in activity of LD in malt taken from the middle of the kiln, following an ale kilning schedule. We can see that the increased temperatures used in ale malt production, with a final curing temperature of 105°C caused a much greater loss of activity of 85%. The effect of lager and ale kilning on β-amylase can be seen in Figures 13 and 14 respectively. As with LD, lager kilning caused minimal loss of activity, whereas ale kilning resulted in losses up to 77%. This work indicates that to preserve the heat

labile enzymes β-amylase and LD, a gentle kilning regime should be employed, especially at the start of kilning when moisture levels are high.

4.9.7 Summary of Ideal Malting Conditions for the Production of High DP Malt

Table 25 displays a summary of the optimal conditions for maximising DP, as indicated by the present investigation, compared with the routine conditions used in the BRFI pilot plant to produce lager malt to standard commercial specifications. The table highlights the need for high cast moistures, with aeration during the steeps, extended germination at low temperatures followed by very gentle kilning, with relatively low temperatures and high air-flows. Two varieties of barley, Chariot and Alexis, which both exhibit good genetic potential for β-amylase production, were malted in the pilot plant using optimal conditions as described above. The amylolytic activity in these malts is shown in Table 26, together with a similar data set for a standard Chariot lager malt. The data shows a near doubling of DP, α- and β-amylase and 'total' LD in the high DP malts compared to the control malt. The 'free' LD improved 3-fold in the Chariot high DP malt whilst the high DP Alexis only showed a marginal increase. These results demonstrate the opportunities to improve the DP of a malt by altering malting conditions.

The extent to which DP can be further maximised by variation in mashing conditions is described in the next section 3.10. The high DP pilot malts have also been used in pilot brewery studies in order to demonstrate the relationship between malt DP and fermentation performance and to identify

any possible negative effects, for example on beer flavour which might result from the kilning conditions employed (Section 3.10.9).

4.10 Mashing and Pilot Scale Brewing

4.10.1 Effects of Mash Temperature and Thickness on the Production of Fermentable Sugars

Laboratory scale (50g) mashes were performed at single temperatures (isothermal mashing) between 61 and 69°C, for two different commercial malts to predict optimal conditions for fermentable sugar production. Table 27 gives a summary of the isothermal mashes, showing the Hot Water Extract (HWE), the total amount and profile of fermentable sugars produced. The ratio of total fermentable sugar to HWE is given for reference. The results indicate that HWE increases with mash temperature, although this is more marked for the lager malt (Chariot), than the more modified ale malt (Puffin). The distribution of the fermentable sugars is fairly uniform, with the major difference being at a temperature below 63°C which probably reflects a reduction in the proportion of gelatinised starch granules.

Because there was no clear optimum temperature, mashing conditions were investigated further by examining the effects of mash thickness at 65°C. Table 28 shows that increasing mash thickness by reducing the liquor to grist ratio from 4:1 to 2:1 has no effect on HWE, but has an appreciable effect on fermentable sugar produced. For both malts the total fermentable sugar increased with a thicker mash, resulting in a higher fermentable:HWE ratio.

This is due to the increased enzyme stability which occurs with increasing mash thickness.

4.10.2 Micro-Mashes of Pilot High DP Malts

The two malts produced at BRFI on the pilot scale using optimal malting conditions for high DP (see section 3.9.7), were mashed on the laboratory scale using IOB Recommended Methods, with a 0.7mm mill setting. The wort was analysed for fermentable sugars (see Table 29). The Chariot malt in particular shows a low HWE, probably due to increased malting loss incurred by the long germination time used. The distribution and amount of fermentable sugars was similar to the commercially malted Puffin and Chariot malts, with a similar fermentable sugar:HWE ratio. Thus higher levels of diastatic enzymes in the malt do not necessarily result in more fermentable sugars in the wort in laboratory mashes. This conclusion was supported by further experiments in which pilot malted high DP Malt (Alexis 701P) was added to a standard mash to make up 10 or 20% of the grist. Results are given in Table 30. The addition of small amounts of high DP malt to an all malt grist did not significantly alter the HWE or the amount or distribution of fermentable sugars produced.

One explanation for the lack of effect of high DP malts in laboratory scale mashes is that under these conditions the β-amylase and LD are inactivated by temperature before sufficient starch is gelatinised to provide a substrate. An experiment was therefore performed in which high DP malt was added to a laboratory scale mash 10 minutes after the start of mashing (see below).

4.10.3 Delayed Addition of High DP Malt to a Standard Malt Micro-Mash

The main enzymes that are likely to increase fermentable sugars are β-amylase and LD, which are both heat labile. They also both have the common requirement that starch granules must first be gelatinised and be subjected to initial attack by α-amylase in order to produce suitable substrates for them. Therefore much β-amylase and LD may be inactivated by heat in the early stages of mashing before they can be fully active due to the lack of available substrates. Hence delaying their addition by 10min will allow the starch granules to gelatinise and α-amylase to partially hydrolyse the starch thereby ensuring that the enzymes are fully active throughout their limited lifespan in a mash. The results from Table 31 suggest small increases in the amount of fermentable sugars produced. Although these results are not conclusive they indicate a possible trend which may be accentuated on the large scale (see section 3.10.9)

4.10.4 HWE and Fermentable Sugar Production in Temperature Programmed Mashes

In commercial brewing practice, temperature programmed mashing, (that is starting the mash at a lower temperature to allow more enzyme activity), is often used to enhance the performance of under-modified lager malts. Table 32 shows the effect of a temperature programmed mash on the HWE and total fermentable sugar produced in standard Puffin and Chariot malt, pilot malted high DP Alexis and Chariot malt, and a mixed grist composed of 20% high DP Alexis and a Puffin standard malts. All the malts have a superior

HWE and release more fermentable sugars when compared to their equivalent isothermal mashes. This is due to enhanced accessibility of starch due to improved cell wall/ protein matrix breakdown at 45°C. Starch would not be gelatinised at this temperature so there would be no extra DP activity during this stand. Hence temperature programmed mashing would seem to be a more efficient system for some malt than traditional isothermal regimes. The addition of 20% high DP malt did not enhance the production of either HWE or total fermentable sugars.

4.10.5 Effect of added DP in all Malt Mashes

Table 33 shows the relationships of HWE and total fermentable sugars in laboratory mashes of a range of malts with increasing DP. Figure 15A, B and C demonstrate the relationship between DP and HWE, DP and total fermentable sugars and between HWE and total fermentable sugar, respectively. From these figures it can be seen that there is no relationship between DP and HWE and between DP and total fermentable sugar. This is in accordance with several findings in the literature which state that there is sufficient α- and β-amylase in standard malts to fully convert up to 67% non-malted adjunct in a standard mash. There is a higher, although still not significant correlation, between HWE and total fermentable sugar.

4.10.6 Effect of Additional DP in 80% Adjunct Mashes

Table 34 shows the relationship of HWE and total fermentable sugars in laboratory mashes containing 80% unmalted adjunct for a range of malts with

increasing DP. Figure 16A, B and C demonstrate the relationship between DP and HWE, DP and total fermentable sugars and between HWE and total fermentable sugars respectively. From Figure 16A it can be seen that under high adjunct conditions, DP levels of less than 95°IOB are limiting. After this point the results are similar to an all malt mash in that addition increase in DP do not cause a further increase in HWE. This is echoed in Figure 16B where the malt with the lowest DP of 66°IOB produced significantly less fermentable sugars than malts possessing higher DPs. Figure 16C again shows that at the lowest DP, both the HWE and the total fermentable sugars were restricted.

4.10.7 Significance of Releasing LD on the Wort Carbohydrate Profile

It is believed that LD activity in mashing is minimal due to the small amount that is in the free form and the fact that LD activity is rapidly lost during mashing. Hence a mashing protocol which maximises the amount of free LD in a mash might alter the carbohydrate profile and hence increase fermentability by hydrolysing more of the branched dextrins present. In previous work at BRFI it was shown that releasing more LD in a thin mash (liquor:grist ratio, 7:1) did significantly alter wort carbohydrate composition (Sissons 1995).

Table 35 indicates that thicker mashes can increase both free LD and that which is extractable by papain/ME. Release of bound LD by papain/2-mercaptoethanol was better in thicker mashes, with a 3:1 ratio being optimal. Subsequent work (data not shown) indicated that papain was equally effective at releasing LD and since the addition of this to the mash would be

more acceptable to brewers, this was used in all subsequent studies unless indicated.

Thick mashes introduce difficulties with analytical measurements. For example, the determination of extract is often less precise than with thin mashes. Analysis of the wort carbohydrates showed greater standard deviations in the values (Table 36) compared to that reported with thin mashes (Sissons *et al* 1995). The percentage of glucose and maltotriose was higher in the thicker mashes, whereas the percent maltose and dextrin was lower compared to thin mashes.

It has been reported that the longer barley is germinated for, the easier it is to extract LD fully (Macgregor *et al* 1994). Thus Longstaff & Bryce (1993) found that barley germinated for 9 days did not require reducing agents, probably because the bound enzyme is converted to the free, active form during germination (Longstaff and Bryce 1993). It must be emphasised that this 9-day germinated barley was very different from commercial malt. The barley was germinated under high moisture conditions for long periods and then only lightly oven dried. Such a procedure will optimise the levels of LD in the final material. These workers found that a long extraction at 45°C with the addition of dithiothreitol (DTT) (25mM) was required to obtain maximum LD from malt (table 37). In contrast, DTT was not required to extract LD from 9-day germinated barley, and maximum activity (which was 3-4 times greater than in malt) was obtained with a 1-2h extraction. Thus, 9-day germinated barley would be a good source of high levels of LD, which is extracted quickly and without the need for reducing agents.

The use of extracts of this 9 day germinated malt as an alternative to using papain to enhance the LD activity of a malt mash was explored in a series of decoction mashes described in Table 38.

Significant degradation of the endosperm starch would be expected in barley germinated for 9 days, due to the presence of maximum activities of all the starch degrading enzymes. A 45°C extract of such barley (mash A) contained high levels of glucose and fructose and a low proportion of dextrin. Presumably, the very high LD activity in 9-day germinated barley would catalyse the hydrolysis of the α-1,6 bonds in the dextrins which could then be degraded by amylases, leaving small quantities of dextrin. In contrast, a wort prepared from a conventional malt mash (Mash B) contained more dextrin and maltose but lower levels of glucose and fructose. When a 45°C mash prepared from 9-day germinated barley was mixed with an all malt mash and then the mixture mashed for 1h at 65°C, the resulting carbohydrate profile of the wort was dramatically altered (Mash D). The dextrin content was low as in the 9-day mash while levels of maltose were higher than in the malt mash (Mash B, Table 38). The wort carbohydrate profile from Mash E (where papain was used to extract LD in a preliminary incubation at 45°C) produced a similar profile to Mash D, but had a higher dextrin content and a lower percentage of maltose. This can be explained by the 3-4 fold lower level of LD produced by adding papain to malt (Mash C), compared to as is extracted from 9-day germinated barley.

These mashing procedures are overly complex and would not be practical to a brewer. An alternative strategy might be to prepare mixtures of grist from

malt and 9-day germinated barley and then mash at 65°C for 1h. Ideally, only a small proportional of 9-day grist should be mixed with malt, so as not to alter the final beer quality. Such a procedure may produce a wort with a very high fermentable sugar content but may alter the properties of the beer adversely. In conclusion, much of the limit dextrinase in malt is bound, which limits its effectiveness during mashing. Release from the bound form requires reducing conditions or the addition of papain. Extending the length of germination of barley leads to much higher levels of free LD. Mashing an extract of 9-day germinated barley with an all malt mash produced a wort with a very low dextrin and high maltose content.

4.10.8 The Effect of Modified Mashing Procedures on Fermentation in Pilot Brewing Studies

To assess the practicality of modified mashing routines, pilot brews were performed. Hammer milled high DP malt (701P) was added to make up 20% of the grist in a standard BRFI premium lager brew. In brew A, the high DP portion was mixed with the grist in the grist hopper and the brew proceeded normally. In brew B the high DP portion of the grist was held back until 10min after the mash had been standing at 64°C (to allow gelatinisation of the starch), then added to the mash converter and mixed thoroughly. A third brew was undertaken where 30% of the grist was composed of 9-day germinated barley which was high in 'free' LD (Table 39). This was brewed normally. Full analysis and tasting data were recorded and compared to a BRF standard premium lager brew. Figure 21 shows the attenuation profiles of the four brews. It can be seen that all the brews fermented in a broadly similar

manner, but the brews made with the delayed addition of 20% high DP malt and the brew containing high LD 9-day germinated barley attenuated further, demonstrating the increased fermentability of the wort. The levels of residual dextrins were also lower in each case compared with the standard beer (Table 40).

Each beer was tasted by BRFI's trained beer profile panel. There was an absence of undesirable 'green' notes in the beers and the mashing regimes did not adversely affect beer quality. In particular the beer produced by the delayed addition of high DP malt had a 'clean', dry taste normally associated with low carbohydrate beers.

These brews demonstrate a practical application for high DP malt in making low carbohydrate beers, and maximising fermentable sugar without producing 'green' taints in the finished beer.

4.11 Production of High Diastatic Malt Extract

Malt extracts are natural products used to provide a valuable source of fermentable sugars, natural colouring and malt flavouring. High DP malt extracts are used to provide a valuable source of amylolytic enzymes, in production of various goods such as bread making, home brew kits and breakfast cereals.

High DP malt extracts are sold as a liquid form containing 80% solids and may be sold at a premium. Fermentability is expressed as °Linter and commercial high DP malt extracts are normally within the range from 20 to 400°L.

High DP extracts are produced from high DP malt (e.g. Plaisant) with a DP of at least 180°L. Typically the malt is mashed at 57°C in a 4:1 liquor to grist ratio, in relatively small batches. The mash is allowed to stand for about 10min before being lautered without sparge into a single pass falling film evaporator. 700kg of high DP extract is recovered in an 80% solids mixture. The extraction of the high DP malt is then completed, using a high temperature mash with sparging at 78°C and less gentle concentration, to give an extract which is low in DP. This can then be blended with the initial high DP extract to give the DP specifications required by individual customers.

Some samples of commercial high DP extracts have been examined for amylolytic enzyme activity using the methods described earlier in this report. Very little LD activity could be found, although high levels of β-amylase were present. Although it is possible that LD is being inactivated at the evaporation stage, it is more probable that the extraction stage is not extracting all the potential LD, which will be present in the malt mainly in the bound form. This would indicate that there could be scope for increasing the DP of these commercial malt extracts by introducing modification to the mashing regimes, as suggested in section 3.10.7 and 3.10.8 of this report.

5.0 Conclusions

DP is mainly composed of α-and β-amylase with limit dextrinase (LD) providing a de-branching role. β-amylase both in barley and in malt is highly correlated to DP and composes 60-75% of the total DP in malt. Thus the β-amylase content of barley can be used to predict the DP it will yield when malted. This could be particularly useful to plant breeders and also to maltsters when selecting suitable batches of barley for preparing high DP malts.

A method has been developed which allows the level of DP, α- and β-amylase to be measured rapidly and reliably from a single extract in a single day, providing information on the ratio of α : β-amylase. This method gives results which correlate well with those obtained with existing IOB methods, but is simpler and faster to use. Additional assays for LD have been developed to provide information on the 'total' level of this enzyme present in the malt and also on the level of 'free' LD that would be effective in mashing.

The level of β-amylase present in malt is more dependant upon the variety of the barley grown then the growing conditions. Conversely for α-amylase and LD the growing conditions had a greater impact than did the barley variety grown. Hence for high DP potential a high β-amylase variety should be selected, then grown under conditions where α-amylase and LD production are maximal.

Malting conditions are crucial to the maximal development of DP with high cast moistures, aeration and extended low temperature germination being optimal. α-Amylase, and to a lesser extent LD, are influenced by malting

conditions to a greater extent than ß-amylase. Kilning is especially important as β-amylase and LD are heat labile, with low temperatures and high air flows being necessary to preserve enzyme function.

The level of 'free' LD, that is the level that would be active in mashing, in the majority of malts is very low, usually under 10% of the 'total' value. The level of 'free' LD can be increased by using an extended germination time of up to 9 days. Also 'free' LD can be extracted by the use of proteases, such as papain, or reducing agents present in modified mashing regimes.

DP is unlikely to be limiting under normal mashing conditions in the brewery, with malts of 100°IOB being able to support mashes of up to 80% unmalted adjunct. However, DP would be limiting in such mashes for malts with less than around 66°IOB.

Mashing conditions can be adjusted in order to alter the final wort composition. For example, a proportion of high DP malt with naturally high levels of 'free' LD can be added in order to utilise more of the dextrins in the wort and thus to increase fermentability. This is particularly effective if the proportion of high DP/LD malt is held back until 10 minutes after the start of the 64°C stand. The absence of 'green' notes present in beer produced in this way indicates a good potential for the use of high DP and LD malt for the production of specific products, such as low carbohydrate or 'dry' beers.

6.0 References

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Table 1 Repeatability of Megazyme Betamyl Assays

	Day 1	Day2	Day 3	Day 4	Standard Error Between Days
β-Amylase Activity (U/g)*	441	685	546	546	555
Standard Deviation	33.3	24.2	58.3	27.1	100.0
Standard Error	7.5%	3.5%	10.6%	5.0%	18.0%

*Average activity of 10 extracts from the same commercial lager malt assayed in duplicate

Table 2 Repeatability of DNS β-Amylase Assays

	Day 1	Day2	Day 3	Day 4	Standard Error Between Days
β-Amylase Activity (U/g)*	228	211	260	217	229
Standard Deviation	8.6	8.1	6.0	8.0	21.8
Standard Error	3.8%	3.8%	2.3%	3.7%	9.5%

*Average activity of 10 extracts from the same commercial lager malt assayed in duplicate

Table 3 Repeatability of PAHBAH β-Amylase Assays

	Day 1	Day2	Day 3	Day 4	Standard Error Between Days
β-Amylase Activity (U/g)*	185	157	172	175	172
Standard Deviation	14.4	16.2	10.3	22.6	11.6
Standard Error	7.8%	10.3%	6.0%	12.9%	6.7%

*Average activity of 10 extracts from the same commercial lager malt assayed in duplicate

Table 4 Results of High and Low DP Malts Analysed Using Combined Amylolytic Method

Malt	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)
Swedish High DP Malt	222	187	2177
Finnish High DP Malt	167	127	2203
100% Vacuum Dried Malt	37	34	319

Table 5 Amylolytic Enzyme Activities in Spring Barleys (1994 Harvest - Dorset)

Barley	Total Nitrogen	"Free" Diastatic Power (°IOB)	"Total" Diastatic Power (°IOB)	Diastatic Power in Final Malt (°IOB)
Alexis	1.53	16	55	113
Brewster	1.39	14	40	76
Chariot	1.74	23	81	112
Cooper	1.62	15	36	74
Cork	1.40	19	67	117
Delibes	1.69	50	78	123
Derkado	1.70	23	45	88
Heron	1.56	26	48	98
Optic	1.58	26	76	117
Riviera	1.48	22	39	86
Average	1.57	23	57	100

Table 6 Amylolytic Enzyme Activities in Spring Barleys (1994 Harvest - Hampshire)

Barley	Total Nitrogen	"Free" Diastatic Power (°IOB)	"Total" Diastatic Power (°IOB)	Diastatic Power in Final Malt (°IOB)
Alexis	1.68	28	81	125
Brewster	1.60	23	70	99
Chariot	1.79	35	80	130
Cooper	1.57	17	49	86
Cork	1.61	24	78	137
Delibes	1.75	48	78	125
Derkado	1.76	30	54	83
Heron	1.78	20	51	82
Optic	1.66	28	78	94
Riviera	1.72	30	53	80
Average	1.69	28	67	104

Table 7 Amylolytic Enzyme Activities in Winter Barleys (1995 Harvest - Northern Ireland)

Barley	Total Nitrogen	"Free" Diastatic Power (°IOB)	"Total" Diastatic Power (°IOB)	Diastatic Power in Final Malt °IOB)
Angora	1.77	64	96	130
Fighter	1.71	40	62	83
Gleam	1.62	50	78	99
Halcyon	1.66	52	79	100
Hanna	1.68	39	61	61
Intro	1.76	64	102	121
Melanie	1.77	67	100	114
Prelude	1.70	42	61	87
Puffin	1.68	51	79	103
Regina	1.66	56	79	112
Sprite	1.78	39	68	78
Sunrise	1.59	41	71	94
Average	1.7	50	78	99

Table 8 Amylolytic Enzyme Activities in Spring Malts (1994 Harvest - Dorset)

Malt (4-day Germination)	Diastatic Power (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed /min/g)	Free Limit Dextrinase (mU/g)	Total Limit Dextrinase (mU/g)
Alexis	113	68	1997	-	1204
Brewster	76	44	1559	87	824
Chariot	112	75	1823	69	983
Cooper	74	39	1856	83	879
Cork	117	75	1938	68	967
Delibes	123	71	2214	73	1667
Derkado	88	47	1987	94	1239
Heron	98	54	1699	75	1084
Optic	117	74	1804	68	1105
Riviera	86	50	1584	-	1140
Average	100	60	1846	77	924

Table 9 Amylolytic Enzyme Activities in Spring Malts (1994 Harvest - Hampshire)

Malt (4-day Germination)	Diastatic Power (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed /min/g)	Free Limit Dextrinase (mU/g)	Total Limit Dextrinase (mU/g)
Alexis	125	75	1938	35	964
Brewster	99	68	1791	22	828
Chariot	130	95	1755	27	1095
Cooper	86	52	1926	28	1120
Cork	137	85	2154	26	1242
Delibes	125	86	2026	14	1409
Derkado	83	44	1771	35	1177
Heron	82	51	1776	16	956
Optic	94	64	1599	10	771
Riviera	80	51	1487	19	910
Average	104	67	1822	23	1047

Table 10 Amylolytic Enzyme Activities in Spring Malts (1995 Harvest - Clwyd)

Malt (4-day Germination)	Diastatic Power (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed /min/g)	Free Limit Dextrinase (mU/g)	Total Limit Dextrinase (mU/g)
Alexis	91	70	1429	26	763
Brahms	89	63	1657	38	930
Chad	84	61	1481	28	791
Chariot	107	79	1369	23	624
Chiefton	100	64	1797	16	671
Cooper	64	41	1736	16	579
Cork	107	81	1702	17	686
Dandy	72	56	1221	15	545
Delibes	88	69	1577	17	560
Derkado	76	53	1678	34	671
Felice	87	65	1397	19	572
Hart	70	55	1123	22	489
Heron	62	31	1368	21	493
Optic	86	67	1295	12	412
Pitcher	122	96	1532	21	551
Riviera	78	55	1285	32	505
Tankard	83	65	1343	37	714
Trinity	93	75	1299	8	343
Average	87	64	1461	22	606

Table 11 Amylolytic Enzyme Activities in Spring Malts (1995 Harvest - Lincolnshire)

Malt (4-day Germination)	Diastatic Power (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed /min/g)	Free Limit Dextrinase (mU/g)	Total Limit Dextrinase (mU/g)
Alexis	120	84	1717	16	681
Brahms	112	77	1686	37	822
Brewster	82	62	1170	14	477
Chariot	86	65	1296	25	613
Chiefton	86	59	1536	5	548
Cooper	71	41	1497	18	719
Cork	115	76	1711	13	716
Delibes	95	63	1575	23	570
Derkado	73	46	1492	16	566
Hart	66	48	995	15	527
Heron	67	48	1412		686
Optic	86	66	1418	17	711
Pitcher	132	97	1271	25	1019
Riviera	75	49	1625	8	670
Tankard	79	56	1463	16	638
Trinity	89	72	1381	9	661
ADRL1	67	50	1333	9	673
Average	83	59	1365	16	628

Table 12 Amylolytic Enzyme Activities in Winter Malts (1995 Harvest - Northern Ireland)

Malt (4-day Germination)	Diastatic Power (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed /min/g)	Free Limit Dextrinase (mU/g)	Total Limit Dextrinase (mU/g)
Angora	130	108	1688	48	1236
Fighter	83	59	1673	25	885
Gleam	99	78	1494	57	1098
Halcyon	100	77	1495	43	1123
Hanna	61	48	1410	26	424
Intro	121	94	1805	21	863
Melanie	114	85	1555	37	1600
Prelude	87	59	1744	85	1208
Puffin	103	73	1687	64	1469
Regina	112	82	1677	92	1548
Sprite	78	58	1269	18	618
Sunrise	94	70	1473	14	1044
Average	99	74	1581	44	1097

Table 13 Summary of Amylolytic Activity in Spring and Winter Varieties

	Barley		Malt					
	'Free' DP (°IOB)	'Total' DP (°IOB)	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-LD hydrolysed/ min/g)	'Free' LD (mU/g)	'Total' LD (mU/g)	Ratio of α to β-amylase
Av. Springs	26	62	102	63	1834	44	1078	29:1
Av. Winters	50	78	99	74	1581	44	1097	21:1

Table 14 Summary of Amylolytic Data Generated

Harvest	Site	No. Barleys Analysed	No. Malts Analysed
1994 Spring NIAB	Dorset	10	10
	Hampshire	10	10
1995 Spring NIAB	Clwyd	-	18
	Lincolnshire	-	17
1995 Winter NIAB	Northern Ireland	12	12
Totals		32	67

Table 15 Correlation Between Enzyme Activities in Barleys and Malts

Harvest	Site	'Free' DP in Barley v Final DP in malt	'Total' DP in barley v Final DP in malt	'Free' DP in barley v β-amylase in malt	'Total' DP in barley v α-amylase in malt
1994 Spring	Dorset Hampshire	0.56 0.44	0.90 0.86	0.40 0.52	0.51 0.34
1995 Winter	Northern Ireland	0.91	0.89	0.90	0.37
All Springs	-	0.49	0.84	0.48	0.30
All Barleys	-	0.33	0.71	0.63	0.02

Table 16 Correlation Between Amylolytic Enzymes

Harvest	Site	DP v β-Amylase	DP v α-amylase	DP v 'free' LD	DP v 'Total' LD	α-amylase v β-amylase	β-amylase v 'total' LD	α-amylase v 'total' LD
1994 Spring	Dorset Hampshire	0.89 0.95	0.59 0.66	-0.19 0.18	0.54 0.46	0.25 0.49	0.21 0.36	0.77 0.70
1995 Spring	Clwyd Lincolnshire	0.94 0.95	0.28 0.40	-.11 0.46	0.17 0.62	0.07 0.20	0.07 0.6	0.52 0.23
1995 Winter	Northern Ireland	0.97	0.58	0.25	0.69	0.48	0.54	0.50
All Springs	-	0.86	0.56	0.15	0.5	0.15	0.10	0.81
All Barleys	-	0.87	0.54	0.18	0.54	0.17	0.27	0.54

Table 17 Correlation of TN with Amylolytic Enzymes in Barley and Malt

Harvest	Site	TN vs 'Free' DP in Barley	TN v 'total' DP in Barley	TN v DP in Malt
1994 Spring	Dorset Hampshire	0.49 0.55	0.38 -0.03	0.21 -0.02
1995 Winter	Northern Ireland	0.41	0.48	0.25
All Barleys	-	0.54	0.46	0.12

Table 18 Analysis of Variance of Effect of Environment and Variety on Amylolytic Enzymes

Parameter	P-value Variety	P-value Environment	Major Variation
DP	5.3×10^{-5}	7.4×10^{-4}	Variety
β-amylase	3.3×10^{-5}	1.3×10^{-1}	Variety
α-amylase	5.1×10^{-3}	6.2×10^{-5}	Environment
'free' LD	6.0×10^{-1}	1.9×10^{-5}	Environment
'total' LD	1.6×10^{-2}	3.1×10^{-9}	Environment

N.B. Data amended by Biomathematics and Statistics Scotland to include site as a single variant

Table 19 Components of Variation - Malting Barley 1991-1994

	Spring Varieties			Winter Varieties		
Components of Variation	TN	DP	DU	TN	DP	DU
Variety	4	23	9	0	35	15
Year	16	0	0	28	0	7
Site	0	19	21	19	1	0
Site.Year	67	30	25	38	39	51
Variety.Year	4	8	8	8	6	0
Variety.Site.Year	9	20	37	7	19	27
Total	100	100	100	100	100	100

N.B. Data Amended by Biomathematics and Statistics Scotland to include site as a single variant.

Table 20 Amylolytic Activity in International Malts

Variety	Country	Barley 'total' DP (°IOB)	Malt DP (°IOB)	Malt β-amylase (°IOB)	Malt α-amylase (mg β-limit dextrin hydrolysed /min/g)
Harrington	Australia	67	105	68	1918
Stirling		92	99	64	1468
Franklin		78	100	74	1559
Harrington	Canada	102	111	93	1680
Manley		124	132	110	1758
B-1602		85	114	84	1661
Pokko	Finland	143	241	160	2336
Kilta		166	252	172	2380

Table 21 Effect of Aeration and Steep Regime on DP

		Aerated				Non-Aerated			
Barley	Steep Regime	Cast Moisture	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)	Cast Moisture (%)	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)
Alexis	8.16.8	39.5	131	82	2012	41.3	110	76	1827
	8.16.16	44.4	114	60	1849	43.9	103	59	1679
	8.16.8.10.2	44.8	144	103	1962	46.1	110	94	1761
	8.16.8.10.6	45.8	138	105	2104	46.4	125	102	1821
Cooper	8.16.8	39.1	75	44	1708	40.6	73	40	1587
	8.16.16	-	67	37	1579	43.1	78	41	1661
	8.16.8.10.2	43.7	89	62	1859	44.3	85	54	1669
	8.16.8.10.6	43.6	90	55	1779	42.6	89	56	1783

Table 22 Effect of Reduced Aeration on DP

Steep Regime	Aeration	Aeration Level
7.17.7.17.1	Air-Sparged	****
7.17.7.17.1	Open	***
7.17.7.17.1	Sealed	**
8.16.24	Sealed	*

Standard germination for 8 days, lightly kilned.

Table 23 Summary of the Effect of Germination Temperature in Alexis Barley

Germination Temperature	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)
12	150	82	1776
14	144	73	1954
16	133	68	1593
18	117	64	1723
20	106	62	1267

Table 24 Effect of 0.1mg/kg GA_3 on DP in Optic and Alexis 50kg Pilot Scale Malt

	Added Gibberellic Acid						No Added Gibberellic Acid				
Barley	Day of Germination	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)	'free' LD (mU/g)	'total' LD (mU/g)	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)	'free' LD (mU/g)	'total' LD (mU/g)
Optic	Barley	24	23	0	-	-	24	23	0	-	-
	1	61	52	210	6	56	61	54	270	11	62
	2	103	81	586	50	310	99	68	399	28	498
	3	122	89	1550	313	485	109	76	1519	48	472
	4	127	93	1796	350	682	127	84	1698	45	374
	5						137	86	1938	66	511
	6						136	89	1861	81	492
	Malt	141	94	2015	587	1237	128	80	1892	45	659
Alexis	Barley	28	24	5	-	-	28	24	6	-	-
	1	80	58	305	4	31	75	61	277	13	97
	2	126	90	1508	28	251	102	80	406	30	377
	3	153	99	2043	126	441	113	84	1612	71	544
	4	162	99	2241	270	592	127	94	2029	168	689
	5						140	103	2220	155	682
	6						133	94	1982	161	697
	Malt	163	100	2295	392	1157	152	100	2151	109	1017

Table 25 Summary of Optimal Malting Conditions for the Production of High DP Malt

Process	Standard	Optimal	Actual
Steeping	14.12.14 Aerated	High Cast Moisture Aerated	8.16.8.10.6 Aerated
Cast Moisture	43-44%	48%+	45%
Germination	15 -18°C 4 days	Low Temperature Extended Germination Time	12°C 6 days
Kilning	Final Curing Temperature 85°C	Low Temperatures High Air Flows	8h at 45°C 16h at 55°C

Table 26 Pilot Production of High DP Malt

Malt	DP (°IOB)	β-amylase (°IOB)	α-amylase (mg β-limit dextrin hydrolysed /min/g)	'total' LD (mU/g)	'free' LD (mU/g)
Standard Chariot	77	62	1125	475	44
High DP Chariot	141	112	1895	1051	158
High DP Alexis	145	112	1936	942	65

Table 27 Isothermal Micro-Mashing of Commercial Chariot and Puffin Malts

Temperature	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
61°C								
Puffin	292.8	4.8	0.35	2.2	36.9	10.2	54.5	0.19
Chariot	269.2	4.5	0.37	2.3	35.4	7.8	50.4	0.19
63°C								
Puffin	297.0	5.5	0.48	3.7	46.9	13.4	70.0	0.24
Chariot	279.0	5.6	0.51	3.8	47.2	10.8	67.9	0.24
65°C								
Puffin	297.0	4.3	0.30	2.1	44.2	12.5	63.4	0.21
Chariot	286.4	3.5	0.12	2.0	43.2	9.6	58.4	0.2
67°C								
Puffin	299.2	4.8	0.39	4.4	46.3	13.1	69.0	0.23
Chariot	287.1	4.2	0.33	4.1	48.0	11.7	68.3	0.24
69°C								
Puffin	299.2	5.1	0.35	4.9	47.4	14.0	71.8	0.24
Chariot	290.8	4.4	0.26	4.5	49.8	12.7	71.7	0.25

Table 28 Effect of Mash Thickness at 65°C

Thickness	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
Puffin 2:1	297.7	5.2	0.42	3.9	44.8	13.5	67.8	0.23
Puffin 4:1	298.8	3.1	0.24	3.3	39.3	11.0	56.8	0.19
Chariot 2:1	290.9	4.1	0.34	3.6	48.0	12.0	68.0	0.22
Chariot 4:1	290.5	2.8	0.22	3.2	41.4	9.7	57.2	0.20

Table 29 Micro-Mashes of Pilot Puffin and Chariot High DP Malt

Temperature	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
Chariot 700P	261	5.3	0.52	2.8	39.9	9.6	58.2	0.22
Alexis 701P	291	5.0	0.55	3.0	43.5	10.2	62.3	0.21

Table 30 Addition of High DP Malt to Standard Mashes

Addition	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
Control Puffin	297.7	5.3	0.41	3.7	39.0	12.7	61.1	0.21
Puffin +10% Alexis	300.1	5.1	0.34	3.6	41.3	12.6	62.5	0.21
Puffin +20% Alexis	297.5	4.2	0.29	3.1	38.2	10.8	56.5	0.19
Control Chariot	286.5	5.6	0.48	3.6	45.7	11.3	66.6	0.23
Chariot +10% Alexis	288.0	5.6	0.42	3.6	45.6	11.2	66.3	0.23
Chariot +20% Alexis	288.9	5.4	0.42	3.5	44.7	11.1	65.0	0.22

Table 31 Effect of Addition of High DP Malt 10min After Start of Mashing

Addition	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
Control Puffin	298.3	5.3	0.36	4.0	43.7	13.6	66.91	0.22
Puffin +10% Alexis	297.3	5.8	0.46	4.0	44.2	14.1	68.4	0.23
Puffin +20% Alexis	298.7	6.1	0.44	4.3	45.6	14.6	70.9	0.24
Control Chariot	286.0	4.3	0.32	2.9	41.5	10.1	59.1	0.21
Chariot +10% Alexis	287.8	4.2	0.24	2.9	40.0	9.9	57.2	0.20
Chariot +20% Alexis	290.9	4.7	0.33	3.4	43.5	10.9	62.8	0.22

Table 32 Total Fermentable Sugars and HWE in a Temperature Programmed Mash

Malt	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Fermentable. Sugar/HWE
Puffin	314.7	7.9	1.1	3.0	46.8	13.4	72.2	0.23
Chariot	307.4	6.8	1.1	2.3	45.7	11.2	67.1	0.22
Pilot Alexis high DP malt	310.9	8.3	1.3	1.8	47.1	10.7	69.2	0.23
Pilot Chariot high DP malt	304.8	9.2	1.5	1.4	46.0	11.3	69.4	0.22
Puffin + 20% high DP Alexis malt	312.8	7.3	1.0	3.0	46.2	12.6	70.1	0.22

Table 33 Correlation of DP with Fermentable Sugar Production and HWE

Sample	DP	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Total Fermentable Sugars/HWE
Hart	66	280.8	3.4	0.18	2.8	39.8	8.5	54.7	0.19
Riviera	75	280.3	3.5	0.40	2.5	41.1	9.5	57.0	0.20
Tankard	79	307.1	4.0	0.44	2.9	42.5	10.6	60.4	0.20
Trinity	89	307.8	4.2	0.39	3.3	45.6	9.9	63.4	0.21
Delibes	95	300.5	4.3	0.45	3.3	43.1	10.6	61.8	0.21
Cork	107	307.3	3.8	0.23	3.6	44.4	9.5	61.5	0.20
Alexis	120	305.2	3.9	0.39	3.6	44.4	9.6	61.9	0.20
Pitcher	132	302.2	5.7	0.56	4.8	50.9	12.4	74.4	0.25
Chariot	141	308.5	6.1	0.64	3.3	45.1	11.6	66.7	0.22
Finnish Malt	167	291.8	6.0	0.76	3.4	39.7	10.6	60.5	0.21
Swedish Malt	222	290.4	6.3	0.62	3.9	38.7	9.0	58.5	0.20

Table 34 Correlation of DP with HWE and Total Fermentable Sugars in an 80% Adjunct Mash

Malt	DP (°lOB)	HWE (l°/kg as is)	Fermentable Sugars (g/l) Glucose	Fructose	Sucrose	Maltose	Maltotriose	Total Fermentable Sugars (g/l)	Tot. Ferm. Sugar/HWE
Hart	66	257.1	1.0	0.0	0.8	52.7	5.8	60.7	0.24
Riviera	75	287.0	1.6	0.0	1.1	57.4	7.4	67.5	0.24
Tankard	79	280.6	1.8	0.1	1.0	55.8	7.7	66.4	0.23
Delibes	95	293.4	1.7	0.0	0.9	55.5	7.6	65.7	0.23
Cork	107	294.9	1.7	0.0	1.3	58.5	7.7	69.2	0.23
Alexis	120	294.0	1.6	0.0	1.1	56.3	7.3	66.2	0.23
Pitcher	132	294.2	1.7	0.0	1.1	57.7	7.5	68.0	0.24
Chariot	141	296.0	2.5	0.1	0.8	57.4	9.1	69.9	0.23
Finnish Malt	167	292.9	3.0	0.1	1.0	54.4	9.6	68.1	0.23
Swedish Malt	222	293.1	2.6	0.0	1.0	55.1	8.4	67.1	0.23

Table 35 Effect of Mash Thickness and Papain/ME on release of Bound LD

	Limit Dextrinase Activity (mU/g)	
Liquor:Grist Ratio	- Papain/ME	+ Papain/ME
2:1	97	142
3:1	96	151
4:1	93	136
5:1	95	138
6:1	79	123
7:1	84	118

Table 36 Effect of Papain on Wort Composition in Thick Mashes

	Percent of total Sugars				
Sample	Dextrin	G3	G2	G1	Fructose
No papain	14.18 ±0.64	14.45 ±0.24	55.93 ±02.36	13.89 ±2.42	1.55 ±0.26
Papain Added	9.32 ±0.45	15.68 ±1.23	58.47 ±1.68	14.09 ±0.33	2.44 ±0.28

Table 37 Limit Dextrinase Activities in Malt and 9-day Germinated Barley

Sample	Extraction Length (h)	Limit Dextrinase Activity -DTT (mU/g)	+DTT
Malt	1	N.D	N.D
	2	N.D	N.D
	24	121	228
9-day germinated barley	1	606	749
	2	693	550
	24	620	434

N.D - Not detectable

Table 38 Wort Carbohydrate Profiles Obtained from Decoction Mashing Experiments

	Percent of total sugars					
Mash	Dextrin	G3	G2	Glucose	Fructose	LD
A	4.57	12.84	48.82	28.58	5.17	824
B	19.58	13.25	56.10	10.05	1.02	16
C	25.03	10.13	50.70	11.55	2.60	227
D	5.11	13.26	67.74	12.06	1.63	34
E	9.18	12.85	65.44	11.59	0.93	17

Key : A : 9-day germinated barley mash - 2h 45°C
B : Malt Mash - 1h 65°C
C : Malt Mash - 2h 45°C +10mg/ml papain
D : Mash A + Mash B - 1h 65°C
E : Mash C + Mash B - 1h 65°C

All mashes 3:1 Liquor to Grist Ratio
Samples were taken at the end of mashing and divided into two. One was boiled for 10min, cooled and centrifuged and the supernatant stored at -20°C until later analysis of carbohydrates by HPLC. The other portion of sample was cooled immediately on ice, centrifuged and the supernatant analysed for LD activity.

Table 39 Amylolytic Activity in 9-day Germination Barley

Barley	DP (°IOB)	β-Amylase (°IOB)	α-Amylase (mg β-limit dextrin hydrolysed /min/g)	'free' LD (mU/g)	'total' LD (mU/g)
Alexis	149	85	2108	65	1204
Chariot	156	96	2187	158	1298

Table 40 Dextrin Content of Beers Produced by a Modified Mashing Regime

Mashing Regime	Dextrin Content (g/l)
Control	18.1
Control + 20% high DP	15.7
Control + 20 % high DP (Delayed)	15.5
Control + 30% high LD	14.3

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Figure 12 : Effect of Ale Kilning on LD Activity
The effect of ale kilning on LD activity in Chariot malt on the BRFI pilot scale (50kg) kiln. Samples were taken from the top, middle and bottom of the kiln at 0, 5, 11, 20 and 24 hours into the kiln.

Figure 13 : Effect of Lager Kilning on β-amylase Activity
The effect of lager kilning on β-amylase activity in Chariot malt on the BRFI pilot scale (50kg) kiln. Samples were taken from the top, middle and bottom of the kiln at 0, 5, 11, 20 and 24 hours into the kiln.

Figure 14 : Effect of Ale Kilning on β-amylase Activity
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Figure 15A : Correlation of DP and HWE in an All Malt Mash
The correlation of DP with HWE in an all malt mash.

Figure 15B : Correlation of DP and Total Fermentable Sugars in an All Malt Mash
The correlation of DP with total fermentable sugars in an all malt mash.

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The correlation of HWE and total fermentable sugars in an all malt mash.

Figure 16A : Correlation of DP and HWE in a 80% Adjunct Mash
The correlation of DP and HWE in an 80% Adjunct (wheat flour) mash.

Figure 16B : Correlation of DP and Total Fermentable Sugars in a 80% Adjunct Mash
The correlation of DP and total fermentable sugars in an 80% adjunct (wheat flour) mash.

Figure 16C : Correlation of HWE and Total Fermentable Sugars in a 80% Adjunct Mash
The correlation of HWE and total fermentable sugars in an 80% adjunct (wheat flour mash)

Figure 17.: Attenuation Profiles of Pilot Brews using Modified Mashing Procedures

Four pilot brews were undertaken on the BRFI 1hl scale. Brew A consisted of 80% BRFI premium lager grist and 20% of hammer milled high DP Alexis malt (Table 26) mashed normally. Brew B consisted of the same grist composition, however the 20% high DP malt was held back until the mash had been allowed to stand for 10min at 64°C. Brew C consisted of 70% BRFI premium lager grist and 30% high 'free' LD malt (Table 39) mashed normally. Brew D was a standard BRFI premium lager brew, included for reference.

Appendix A

Statistical Analysis of Diastatic Power Data on Malting Barley Varieties

A report by the Biomathematics and Statistics Scotland.

University of Edinburgh

STATISTICAL ANALYSIS

OF DIASTATIC POWER DATA ON

MALTING BARLEY VARIETIES

REPORT

on behalf of

BREWING RESEARCH FOUNDATION INTERNATIONAL

by

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INTRODUCTION

This report summarises the results of an investigation into the contribution of genetics and the environment to diastatic power and dextrinising units in malting barley.

The report has been commissioned by Brewing Research Foundation International (BRFI) as part of a wider investigation which is being undertaken to assess the effects of a range of factors on the potential diastatic power of barley for use in the malting, brewing and distilling industries.

The study is based on data from barley variety trials in Scotland conducted by the Scottish Agricultural College (SAC) over the period 1991-94.

BACKGROUND

Malt produced for the distilling industry is often used as a source of enzymes to break down starch from unmalted cereal adjuncts which are added at the mashing stage. For this purpose varieties are required which have the ability to produce very high levels of activity of the starch-attacking enzymes. Such varieties are said to have high diastatic power (DP).

While DP is associated with levels of alpha-amylase activity another important source of starch-attacking enzymes relates to beta-amylase activity which is measured in terms of dextrinising units (DU). Varieties with both high DP and DU are desirable in malt production.

There is an interest in establishing the extent to which genetics and the environment contribute to DP and DU in those barley varieties which are currently in commercial use or which have recently been tested in the UK National/Recommended List cereal variety trials system. If genetics contributes a substantial proportion of total variation then farmers could be advised to select appropriate varieties and barley breeders might be encouraged to give higher priority to selecting for high DP and DU.

Two other variables which are also studied are total grain nitrogen and the ratio of DP to DU.

THE DATA

The data come from National/Recommended List trials of spring and winter barley varieties conducted in Scotland in the years 1991-94 by SAC. A total of 44 spring and 34 winter barley varieties were studied and details of the data are given in Table 1. Samples of grain from the trials were submitted for micro-malting tests in a co-ordinated programme conducted under the aegis of the Institute of Brewing's Scottish Micro-malting Committee.

SUMMARY OF RESULTS

Variety means

Variety means over trials are presented in Tables 2a and 2b for each of the characteristics DP, DU, DP/DU and total N.

The results are presented in descending order of variety mean DP. In general, those spring barley varieties which are currently recommended have levels of DP slightly lower than the average of varieties which were submitted to micromalting tests in the period 1991-94. On the other hand, the recommended winter barley varieties have generally high levels of DP.

Variance analysis

For each of the measures the total variation has been separated into parts attributable to the following factors:

- varieties;
- years, i.e. between seasons;
- sites, i.e. between locations within years;
- varieties x years, i.e. due to changes in variety performance from year-to-year;
- varieties x sites within years, i.e. the differences in variety performance from site-to-site within a season.

The variances are in Table 3 where, for both spring and winter barley, the components can be broadly ranked in order of importance as:

1. Overall influence of site factors which affect all varieties sown at a site, i.e. the site.year component;

2. The extent to which variety performance changes from site-to-site within a year, i.e. the variety.site.year component;

3. average differences between varieties, i.e. the genotypic component.

The other two components, season and differences in variety performance between years, are negligible in these data, though season plays a bigger role in winter barley than in spring barley. To see more clearly the patterns we may consider how the variation underlying a single assessment on a sample of grain from a variety at a site is made up. In Table 4 the total variation of a single observation has been broken into its parts in percentage terms. It is apparent that there are broadly similar breakdowns of the components of variance for each of the measures.

It was not possible, from the present data, to examine between-laboratory or within-laboratory variation in assessments of the same sample. However, using other data from tests of bulked samples submitted to several laboratories, it has been possible to get estimates of analytical error, and these are presented at the bottom of Table 4. These figures suggest that a substantial part of the site-to-site variation identified earlier may be attributable in part to differences in analytical technique between laboratories.

Relationships between varietal characteristics

The relationship between variety means for DP, DU, DP/DU and N are explored in Figures 1-3. The evidence suggests that there are no clear associations between the characteristics although there are some indications that varieties with high DU levels also have high DP levels. (Figure 4)

Table 5 examines the correlation between DP, DU and selected agronomic characteristics. In the case of winter barley there is a statistically significant ($p < 0.01$) relationship between earliness in ripening and DU levels.

CONCLUSIONS

1. There are substantial and consistent differences both in DP and DU levels amongst barley varieties undergoing official trials in the period 1991-94.

2. Currently recommended winter barley varieties have higher than average DP levels while spring barley varieties have slightly lower DP values when compared with the bulk of varieties undergoing tests in 1991-94.

3. Apart from variety differences the other major factor affecting DP and DU is associated with site-to-site differences.

4. There is evidence of considerable inter-laboratory variation in the assessment of DP and DU, particularly in the case of spring barley. While this does not affect varietal assessments it may indicate that some of the site-to-site differences identified in 3. above may be due to differences between laboratories in analytical procedures.

5. Varieties producing grain with high levels of N tend also to give slightly higher levels of DP and DU.

APPENDIX A

TABLES

1. Extent of data
2. Variety means
3. Components of variance
4. % components of variation
5. Correlations between characteristics

Table 1a: Extent of Spring Barley data over trials 1991-94

Variety	Number of trials 1994	1993	1992	1991
TRINITY	3	3	0	0
PITCHER	4	3	0	0
PLATOON	0	0	0	3
MENTOR	4	3	0	0
SALUTA	0	0	4	4
EMINENT	0	0	0	5
KATHERINA	0	0	0	4
CORK	0	3	1	0
TAIN	0	0	0	5
NEVADA	0	0	0	5
OPTIC	0	3	0	0
CHARIOT	5	0	0	0
DELIBES	0	0	4	5
BRAHMS	4	0	4	0
FIRE	0	0	0	5
PORTIA	0	0	0	5
BREWSTER	0	0	4	5
LORDMANSHIP	0	0	0	5
POLYGENA	4	3	0	0
OTIS	0	0	4	5
WREN	3	3	0	0
BIANKA	0	0	0	3
VINTAGE	0	0	0	3
PRISMA	0	0	0	8
GOLDIE	0	0	0	3
REGGAE	0	3	4	0
WOODCOCK	0	0	0	3
HERON	0	0	4	4
CHIEFTON	4	3	0	0
GLEN	0	3	0	0
AMBER	0	3	4	0
CAMARGUE	5	5	8	0
JUNO	0	0	4	5
VODKA	0	0	0	3
COOPER	0	0	4	4
CARRON	0	0	0	5
RIVIERA	0	3	0	0
TROIKA	0	0	0	5
CHEVIOT	0	3	4	0
AUDIO	0	0	0	5
TANKARD	4	3	0	0
SHAMU	0	0	4	5

Table 1b: Extent of Winter Barley data over trials 1991-94

Variety	Number of trials			
	1994	1993	1992	1991
GENEVA	0	0	1	1
ADAGIO	0	0	1	0
REGINA	2	3	0	0
FAKIR	0	0	0	2
MUSCAT	2	3	0	0
MELANIE	0	3	2	0
GLEAM	2	3	0	0
ANGORA	2	3	2	0
CORSA	2	3	0	0
INTRO	0	0	0	2
THERESA	2	3	0	0
HALCYON	1	5	5	3
CHESTNUT	0	0	0	2
SUNRISE	0	3	2	0
EPIC	0	0	2	0
FINCH	0	0	2	0
SWIFT	0	0	0	2
SILK	0	0	2	1
LINNET	0	0	3	1
TOKYO	2	3	0	0
PRELUDE	2	3	0	0
TEMPO	0	3	1	0
VELVET	0	0	1	1
TARKA	0	0	0	1
KELIBA	0	0	1	1
BABYLONE	0	0	1	1
ARTIC	2	3	0	0
LACE	0	0	1	1
TOSCA	0	3	1	0
WILLOW	0	0	0	2
ALLURE	0	0	1	1
FANFARE	0	3	2	0
HANNA	0	3	1	0
GAELIC	0	0	3	1

Table 2a: Spring Barley variety means over trials 1991-94

Variety	Mean (No of trials) Total N	DP	DU	DP/DU
TRINITY	1.63 (6)	116 (7)	39.7 (5)	3.21 (5)
PITCHER	1.71 (7)	115 (7)	43.2 (5)	2.77 (5)
PLATOON	1.60 (3)	115 (3)	44.7 (2)	2.49 (2)
MENTOR	1.68 (7)	112 (7)	46.3 (5)	2.49 (5)
SALUTA	1.69 (8)	111 (4)	51.3 (3)	2.20 (3)
EMINENT	1.63 (5)	111 (3)	52.5 (3)	2.17 (3)
KATHERINA	1.73 (4)	110 (2)	43.2 (2)	2.59 (2)
CORK *	1.48 (4)	108 (3)	46.5 (2)	2.46 (2)
TAIN	1.57 (5)	106 (3)	45.8 (3)	2.32 (3)
NEVADA	1.63 (5)	105 (3)	40.8 (3)	2.76 (3)
OPTIC * †	1.53 (3)	105 (3)	44.9 (2)	2.20 (2)
CHARIOT * †	1.67 (5)	105 (6)	44.0 (5)	2.48 (5)
DELIBES *	1.55 (9)	104 (5)	48.1 (4)	2.13 (4)
BRAHMS	1.66 (8)	103 (6)	45.3 (5)	2.27 (5)
FIRE	1.59 (5)	103 (3)	46.8 (3)	2.19 (3)
PORTIA	1.66 (5)	102 (3)	44.9 (3)	2.30 (3)
BREWSTER *	1.61 (9)	102 (5)	39.8 (4)	2.66 (4)
LORDMANSHIP	1.65 (5)	102 (3)	43.6 (3)	2.38 (3)
POLYGENA	1.64 (7)	101 (7)	42.0 (5)	2.43 (5)
OTIS	1.63 (9)	101 (5)	40.7 (4)	2.61 (4)
WREN	1.63 (6)	101 (7)	40.6 (5)	2.68 (5)
BIANKA	1.63 (3)	101 (3)	45.1 (2)	2.15 (2)
VINTAGE	1.61 (3)	101 (3)	39.8 (2)	2.70 (2)
PRISMA * †	1.61 (8)	100 (5)	52.3 (4)	1.85 (4)
GOLDIE	1.66 (3)	99 (3)	46.4 (2)	2.22 (2)
REGGAE	1.50 (7)	99 (5)	47.0 (4)	2.02 (4)
WOODCOCK	1.64 (3)	98 (3)	42.9 (2)	2.31 (2)
HERON	1.63 (8)	98 (4)	47.0 (3)	2.07 (3)
CHIEFTON	1.68 (7)	97 (7)	50.5 (5)	2.05 (5)
GLEN	1.62 (3)	96 (3)	46.7 (2)	1.93 (2)
AMBER	1.58 (7)	94 (5)	41.0 (4)	2.26 (4)
CAMARGUE *	1.71 (18)	94 (15)	45.5 (10)	2.06 (10)
JUNO	1.61 (9)	93 (5)	45.5 (4)	2.03 (4)
VODKA	1.61 (3)	92 (3)	44.0 (2)	2.01 (2)
COOPER * †	1.60 (8)	90 (4)	42.8 (3)	2.15 (3)
CARRON	1.66 (5)	90 (3)	39.0 (3)	2.39 (3)
RIVIERA * †	1.60 (3)	89 (3)	44.1 (2)	1.95 (2)
TROIKA	1.55 (5)	88 (3)	43.1 (3)	2.07 (3)
CHEVIOT	1.62 (7)	88 (5)	49.9 (4)	1.70 (4)
AUDIO	1.63 (5)	88 (3)	43.5 (3)	2.04 (3)
TANKARD * †	1.63 (7)	85 (7)	42.6 (5)	2.03 (5)
SHAMU	1.59 (9)	81 (5)	40.1 (4)	2.13 (4)
SED - average	0.084	12.1	7.3	0.42
- max	0.117	15.7	10.0	0.56
- min	0.053	7.2	4.3	0.26

* These varieties are included in the UK cereals recommended list for 1996

† Recommended varieties which have a malting grade of 8 or 9

Table 2b : Winter Barley variety means over trials 1991-94

Variety	Total N	DP	DU	DP/DU
	Mean (No of trials)			
GENEVA	1.71 (2)	128 (2)	42.9 (2)	2.80 (2)
ADAGIO	1.72 (1)	117 (1)	43.9 (1)	2.45 (1)
REGINA * †	1.70 (5)	114 (4)	40.5 (4)	2.72 (4)
FAKIR	1.70 (2)	112 (2)	41.7 (1)	2.49 (1)
MUSCAT *	1.70 (5)	111 (3)	46.4 (3)	2.30 (3)
MELANIE * †	1.69 (5)	110 (4)	45.2 (3)	2.40 (3)
GLEAM * †	1.71 (5)	108 (4)	38.7 (4)	2.74 (4)
ANGORA * †	1.70 (7)	108 (6)	42.0 (5)	2.49 (5)
CORSA	1.69 (5)	107 (3)	44.4 (3)	2.30 (3)
INTRO *	1.70 (2)	106 (2)	39.0 (1)	2.51 (1)
THERESA	1.69 (5)	105 (3)	40.7 (3)	2.48 (3)
HALCYON * †	1.72 (14)	104 (12)	40.5 (10)	2.65 (10)
CHESTNUT	1.70 (2)	103 (2)	39.3 (1)	2.60 (1)
SUNRISE * †	1.69 (5)	102 (4)	39.1 (3)	2.54 (3)
EPIC *	1.70 (2)	98 (2)	41.4 (2)	2.46 (2)
FINCH	1.69 (2)	98 (2)	35.7 (2)	2.79 (2)
SWIFT	1.70 (2)	98 (2)	37.0 (1)	2.50 (1)
SILK	1.71 (3)	97 (3)	40.4 (3)	2.39 (3)
LINNET *	1.71 (4)	96 (4)	42.8 (4)	2.30 (4)
TOKYO	1.69 (5)	95 (3)	42.1 (3)	2.23 (3)
PRELUDE * †	1.71 (5)	94 (4)	43.2 (4)	2.18 (4)
TEMPO	1.69 (4)	94 (4)	44.4 (3)	2.20 (3)
VELVET	1.69 (2)	93 (2)	42.7 (2)	2.26 (2)
TARKA	1.71 (1)	92 (1)	38.8 (1)	2.46 (1)
KELIBA	1.70 (2)	90 (2)	39.2 (2)	2.37 (2)
BABYLONE	1.69 (2)	90 (2)	47.6 (2)	2.05 (2)
ARTIC	1.70 (5)	89 (3)	36.1 (3)	2.52 (3)
LACE	1.71 (2)	89 (2)	42.7 (2)	2.25 (2)
TOSCA	1.71 (4)	85 (3)	40.5 (2)	2.20 (2)
WILLOW	1.71 (2)	83 (2)	37.0 (1)	2.47 (1)
ALLURE	1.70 (2)	81 (2)	41.9 (2)	2.11 (2)
FANFARE * †	1.70 (5)	77 (4)	39.7 (3)	2.23 (3)
HANNA *	1.72 (4)	76 (4)	42.7 (3)	1.97 (3)
GAELIC *	1.68 (4)	72 (4)	37.5 (4)	2.15 (4)
SED - average	0.0083	12.4	5.8	0.48
- max	0.119	17.8	8.7	0.70
- min	0.048	6.7	3.1	0.26

* These varieties are included in the UK cereals recommended list for 1996

† Recommended varieties which have a malting grate of 8 or 9

Table 3: Components of variance of malting assessment data 1991-94

(a) Spring Barley

Components of variation	N	DP	DU	DP/DU
Variety	0.0037	119.2	22.9	0.162
Year	0.0074	0.0	0.0	0.000
Site.year	0.0421	326.6	72.6	0.112
Variety.year	0.0014	15.6	1.4	0.000
Variety.site.year	0.0061	175.3	57.5	0.231

(b) Winter Barley

Variety	0.0000	210.8	17.8	0.082
Year	0.0017	84.0	9.3	0.087
Site.year	0.0085	173.4	70.3	0.000
Variety.year	0.0021	0.0	0.0	0.033
Variety.site.year	0.0050	141.1	36.1	0.194

Table 4: Percentage of the total variance attributable to each component - malting assessment data 1991-94

(a) Spring barley

Components of variation	N	DP	DU	DP/DU
Variety	6	19	15	32
Year	12	0	0	0
Site.year	69	51	47	22
Variety.year	2	2	1	0
Variety.site.year	10	27	37	46
Total	100	100	100	100
Between labs	2	22	60	36
Within labs	1	8	13	13

(b) Winter barley

Variety	0	35	13	21
Year	10	14	7	22
Site.year	49	28	53	0
Variety.year	12	0	0	8
Variety.site.year	29	23	27	49
Total	100	100	100	100
Between labs	56	12	7	22
Within labs	12	12	9	29

Table 5: Correlation between variety characteristics and variety mean DP and DU levels

	Coefficient of correlation			
	DP		DU	
	Spring barley	Winter barley	Spring barley	Winter barley
Earliness	-0.30	0.41	0.38	0.57**
Malt grate	-0.06	0.36	-0.14	0.20
Specific wt	-0.19	-0.13	-0.41	-0.34
1000 grain wt	0.14	-0.17	0.25	-0.33

Note: ** significantly different from zero at 1% level

APPENDIX B

FIGURES

1. DP v N
2. DU v N
3. DP/DU v N
4. DP v DU

Figure 1a: DP v N spring barley variety means

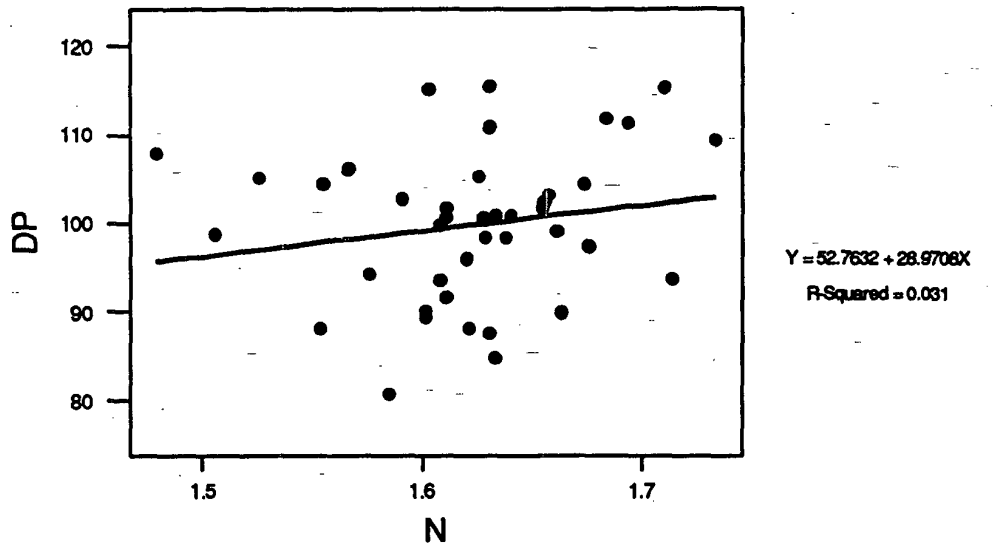


Figure 1b: DP v N winter barley variety means

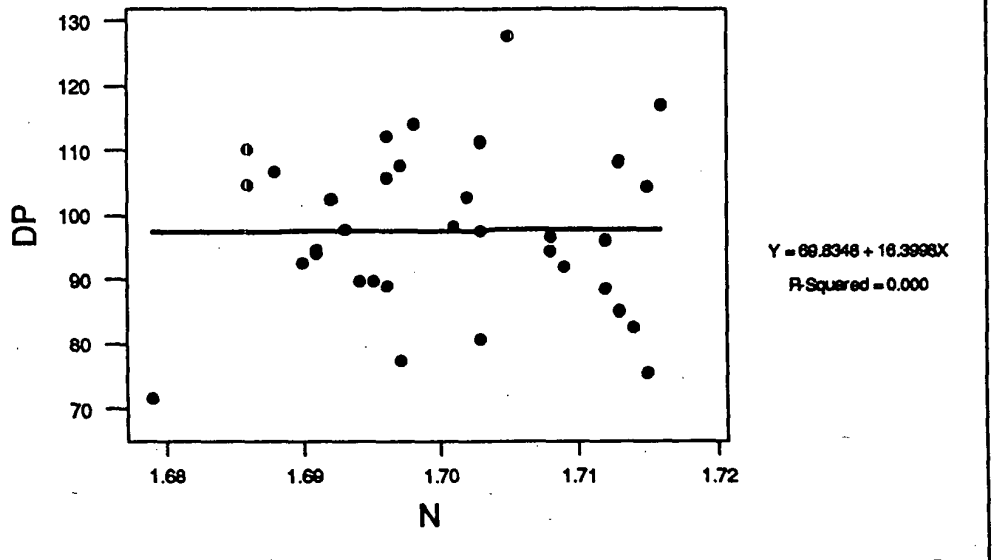


Figure 2a: DU v N spring barley variety means

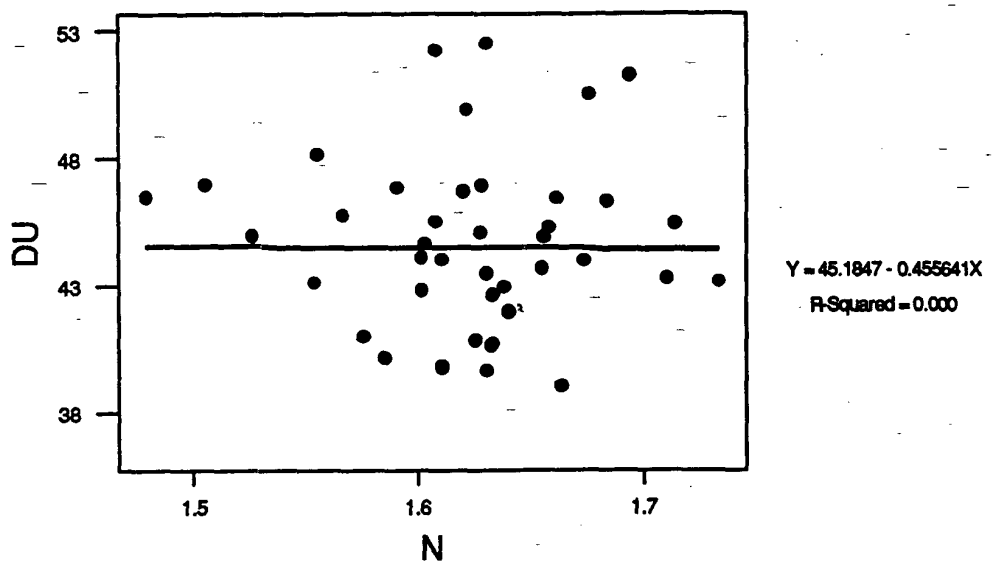
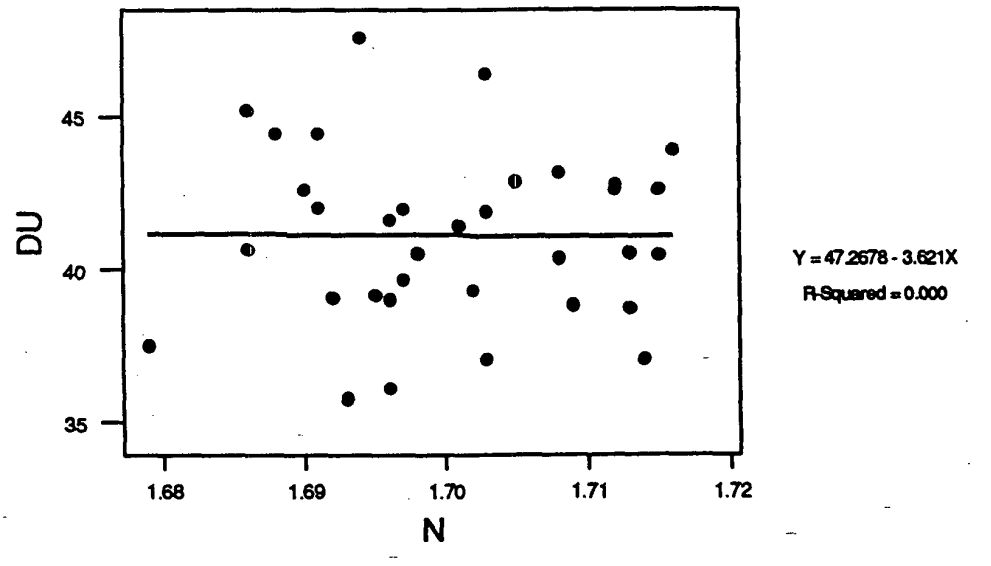


Figure 2b: DU v N winter barley variety means



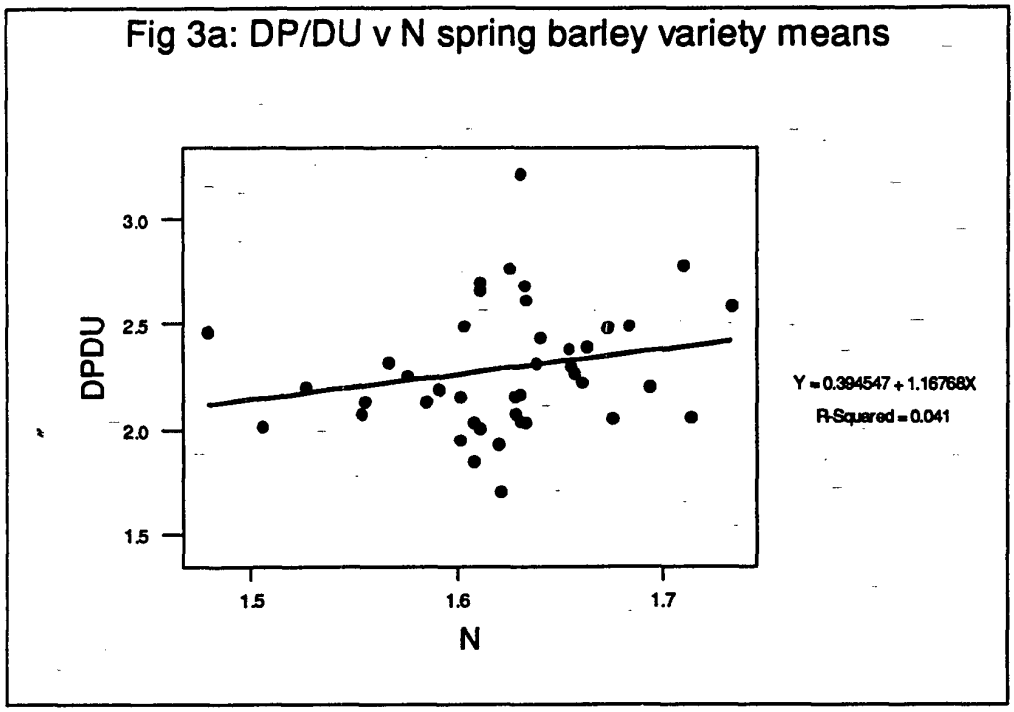


Fig 3a: DP/DU v N spring barley variety means
DPDU
3.0
2.5
2.0
1.5
1.5
1.6
1.7
N
Y = 0.394547 + 1.16768X
R-Squared = 0.041

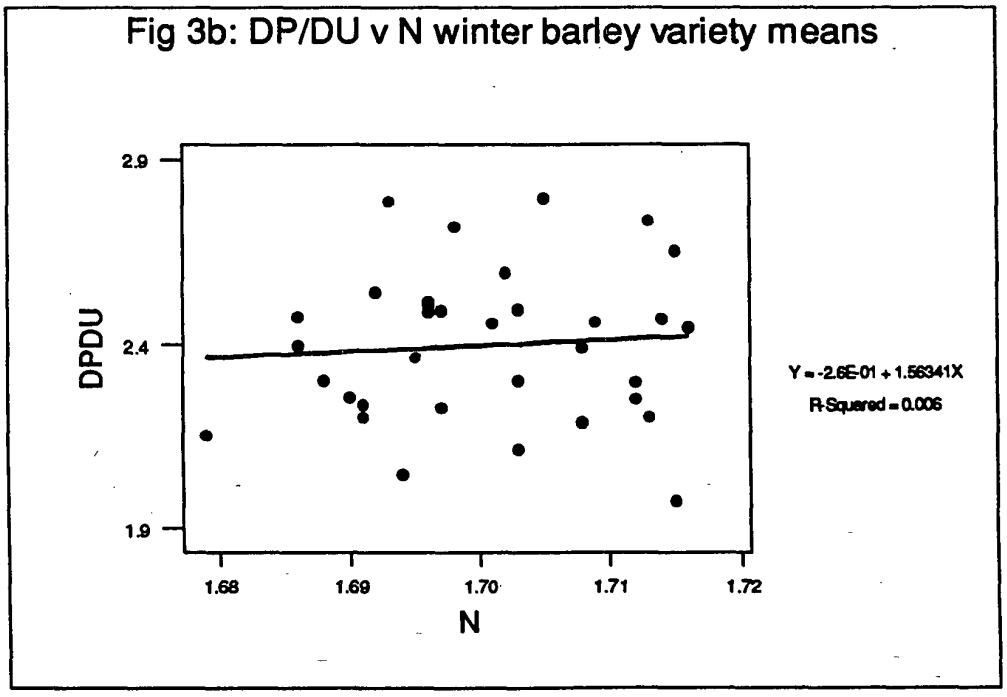


Fig 3b: DP/DU v N winter barley variety means
DPDU
2.9
2.4
1.9
1.68
1.69
1.70
1.71
1.72
N
Y = -2.6E-01 + 1.56341X
R-Squared = 0.006

Fig 4a: DP v DU spring barley variety means

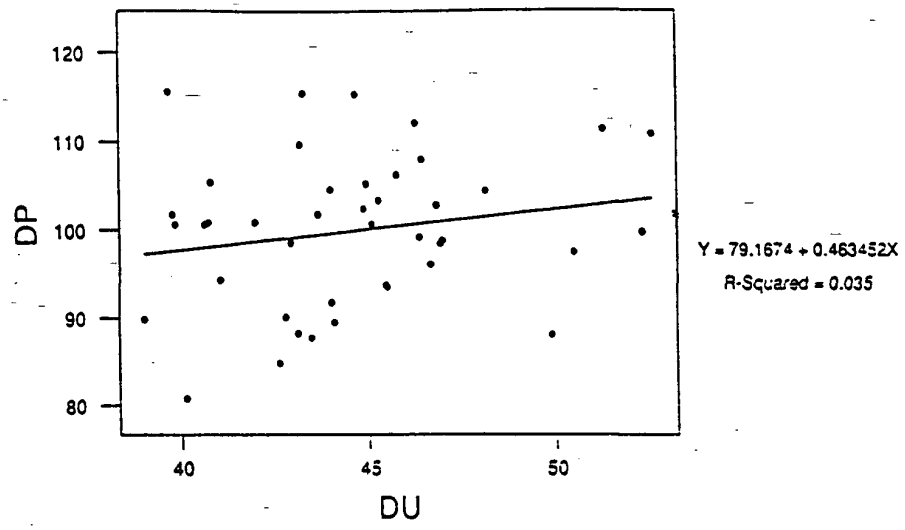
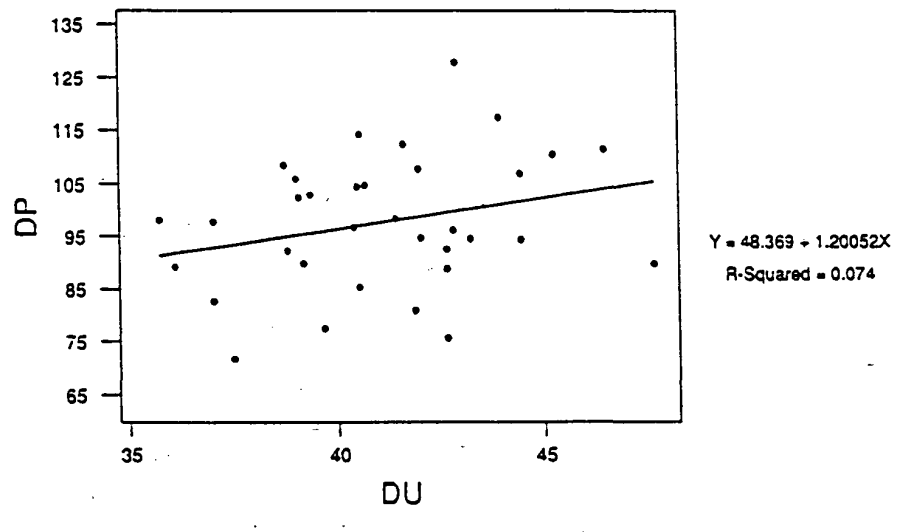


Fig 4b: DP v DU winter barley variety means



APPENDIX C

STATISTICAL METHODS

The main method applied in analysing the data is that of Residual Maximum Likelihood (REML). REML is a technique for estimating means in data tables which have gaps, as is the case with the present data where any one variety is grown in only a small proportion of trials over the four years. REML also provides estimates of the main sources of variation in data from multi-way tables.

The REML model used in analysing the data has all factors including varieties as random terms.

Figure 1 PAHBAH Calibration Curve

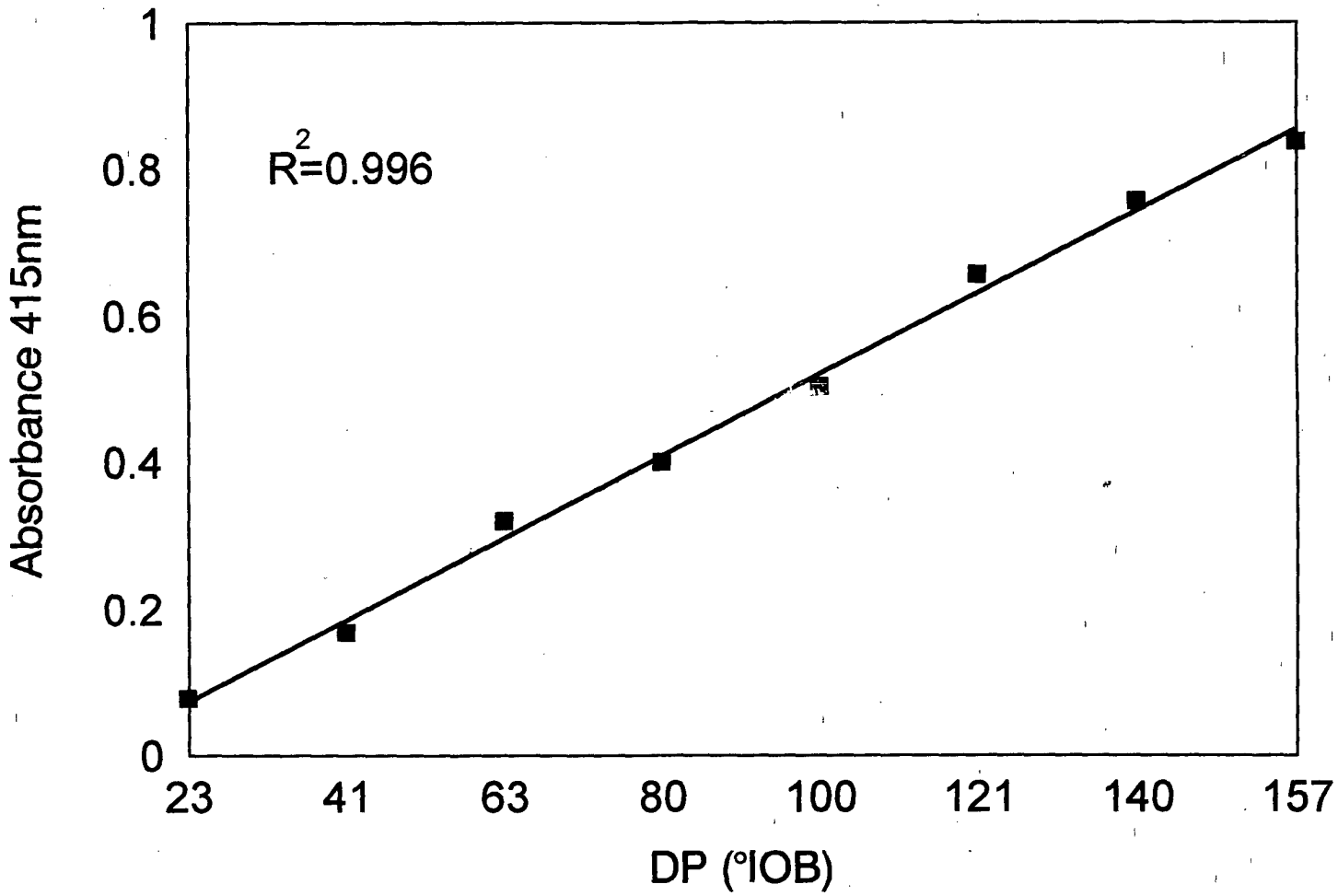


Figure 2 Correlation of 'Total' DP in Barley with Final DP in Malt

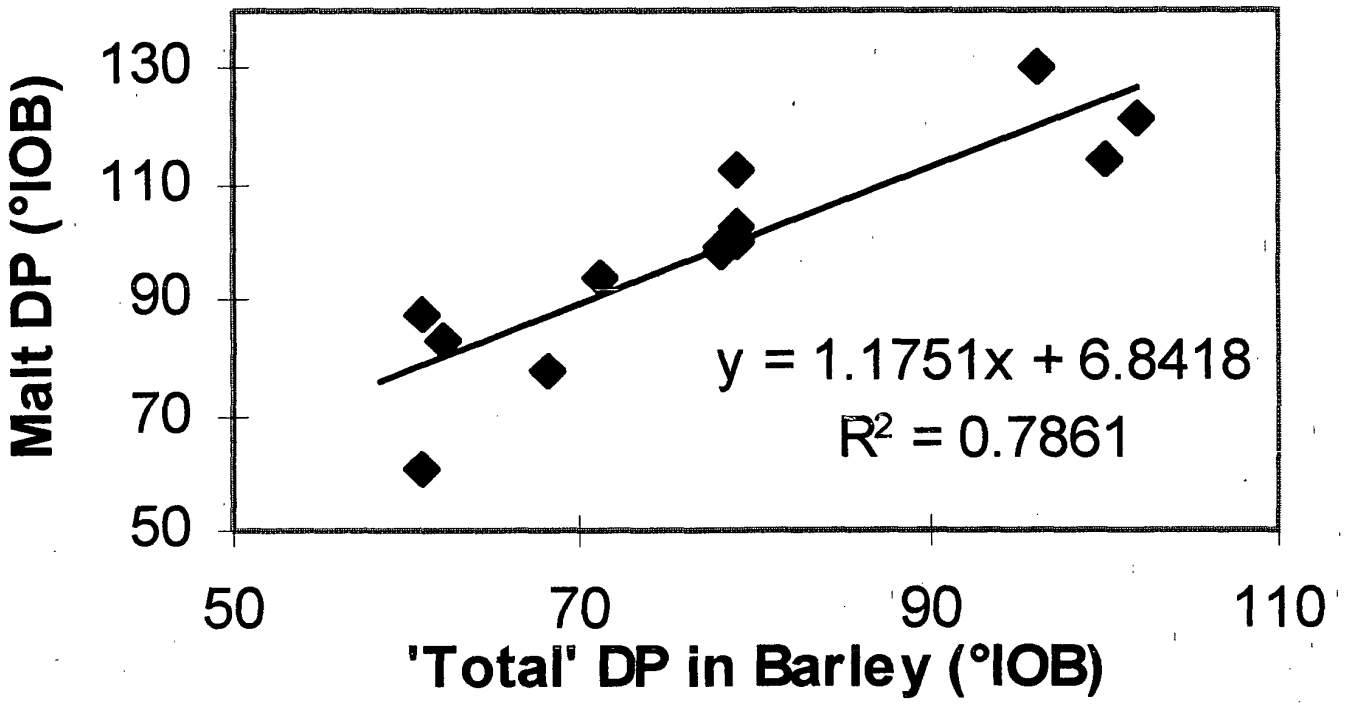


Figure 3 : Effect of Reduced Aeration During Germination on Chariot Barley

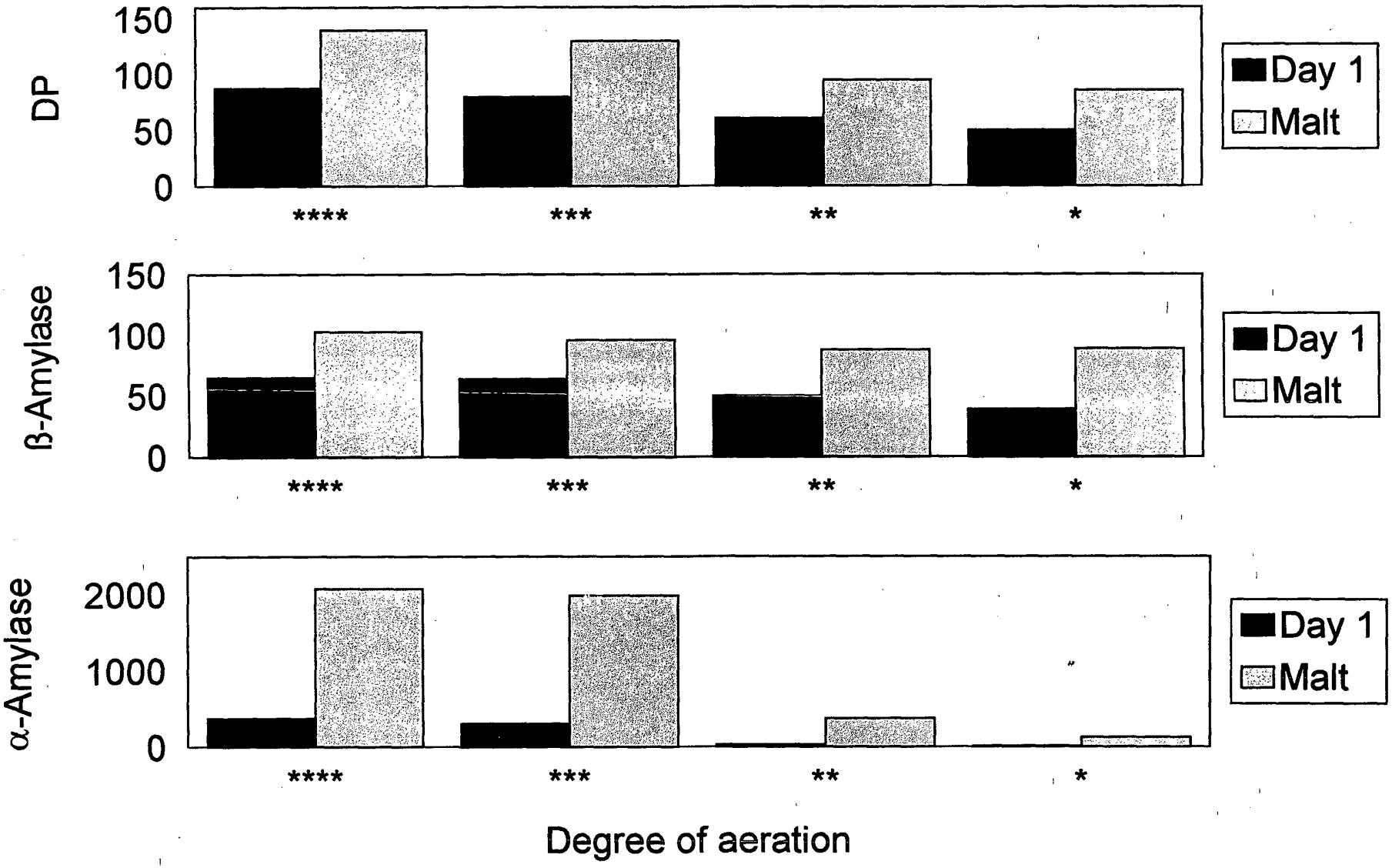


Figure 4 Development of DP Enzymes

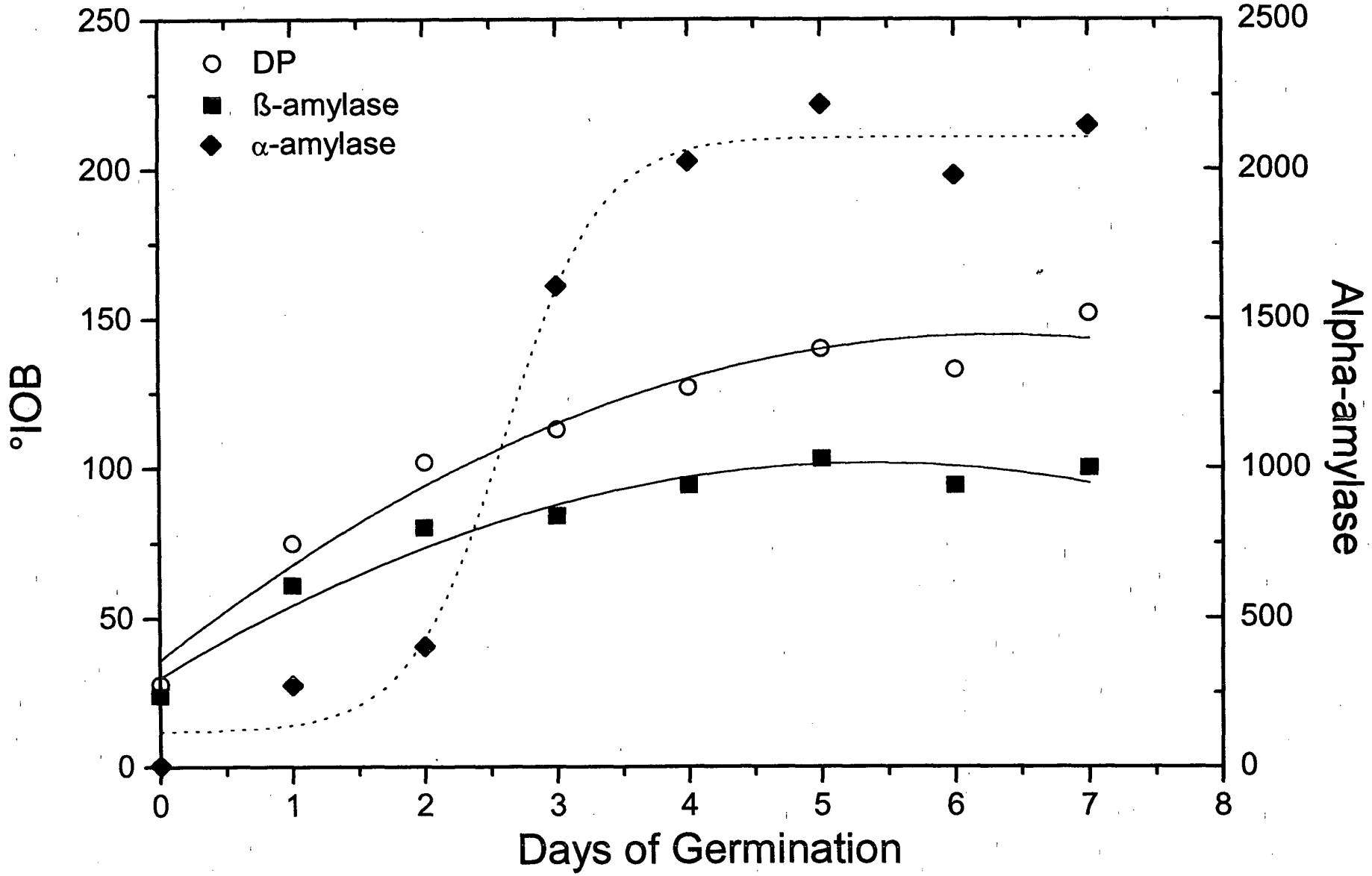


Figure 5 Development of DP - Chariot 12°C

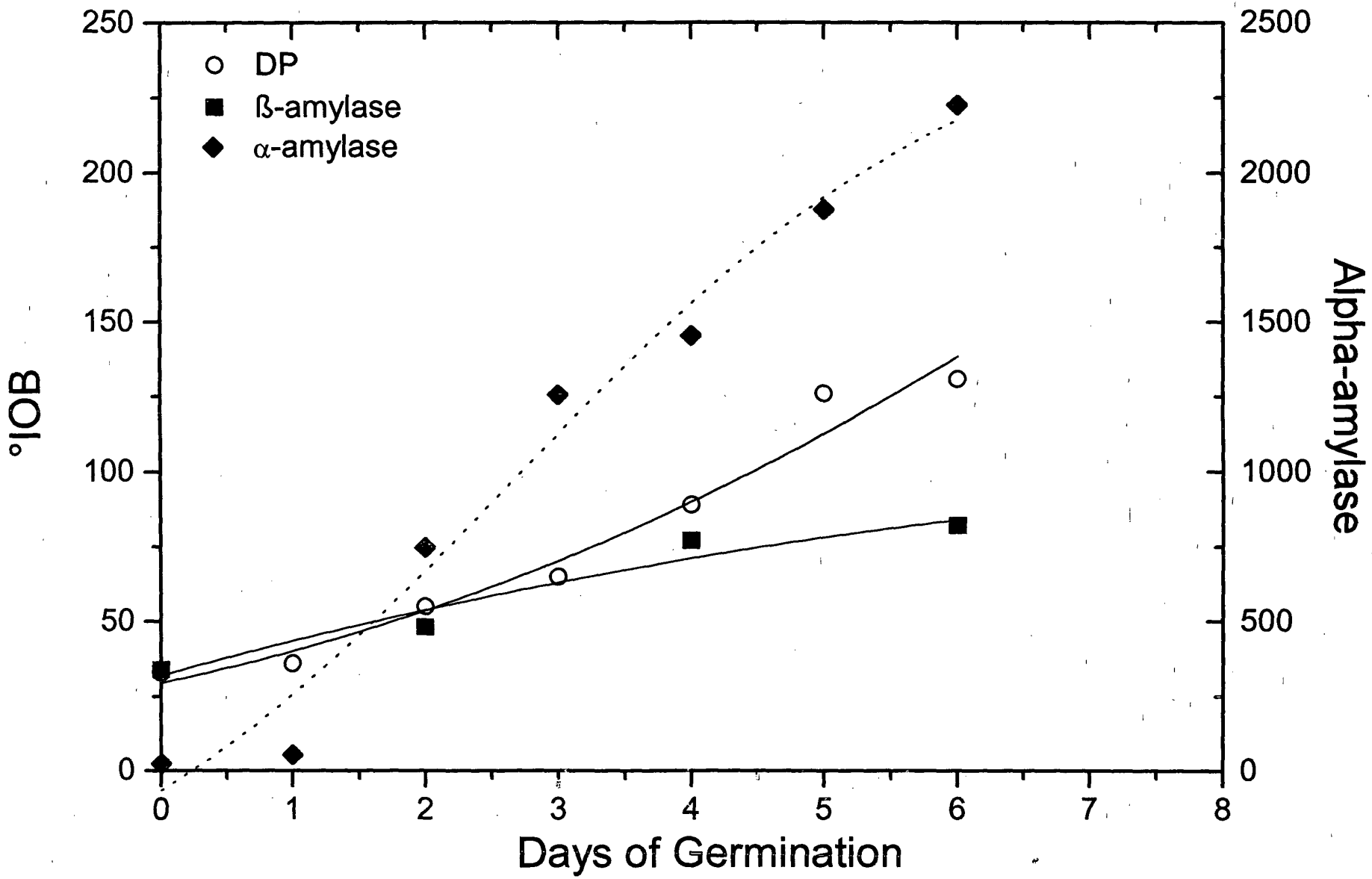


Figure 6 Development of DP - Chariot 14°C

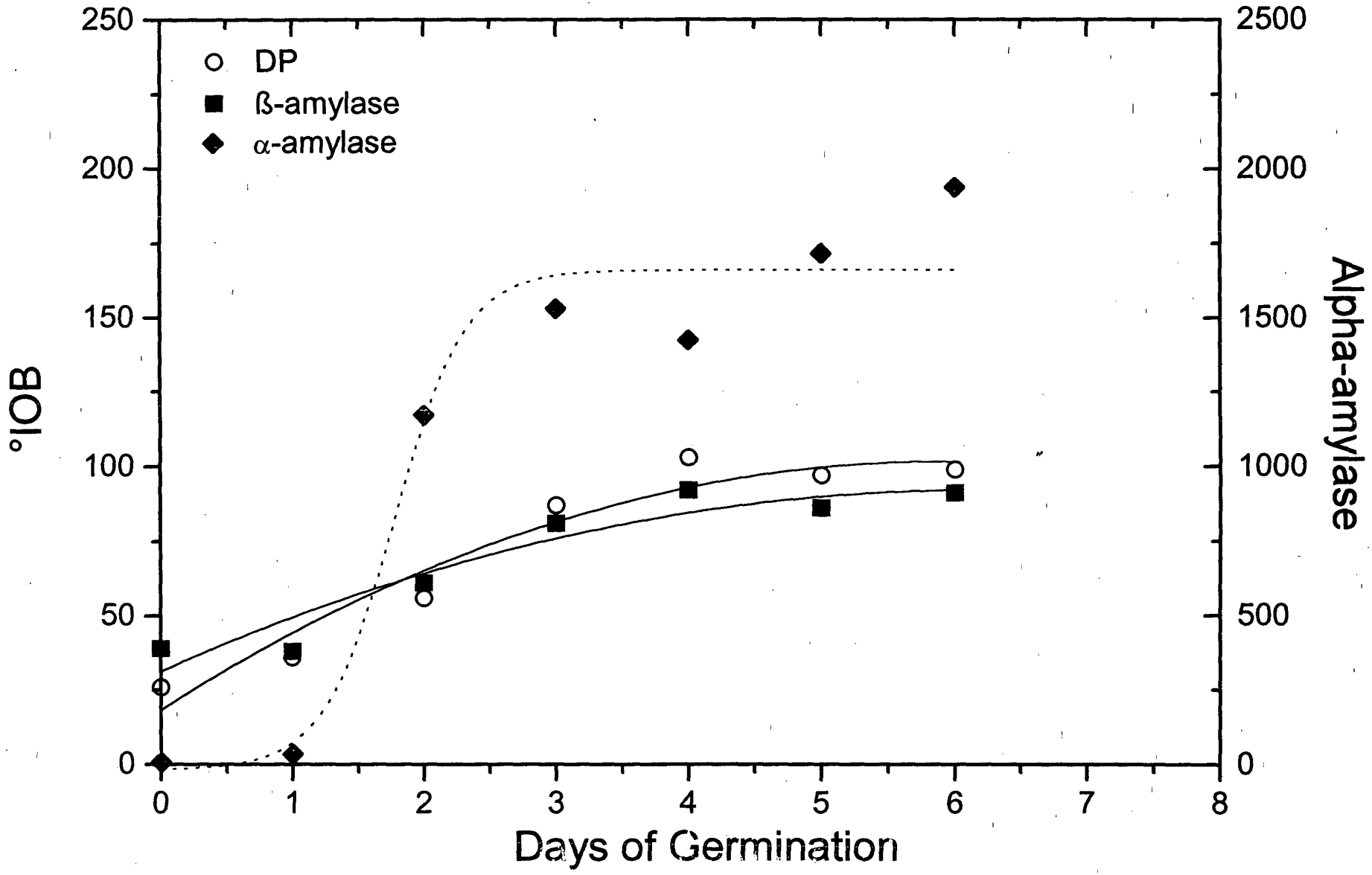


Figure 7 Development of DP - Chariot 16°C

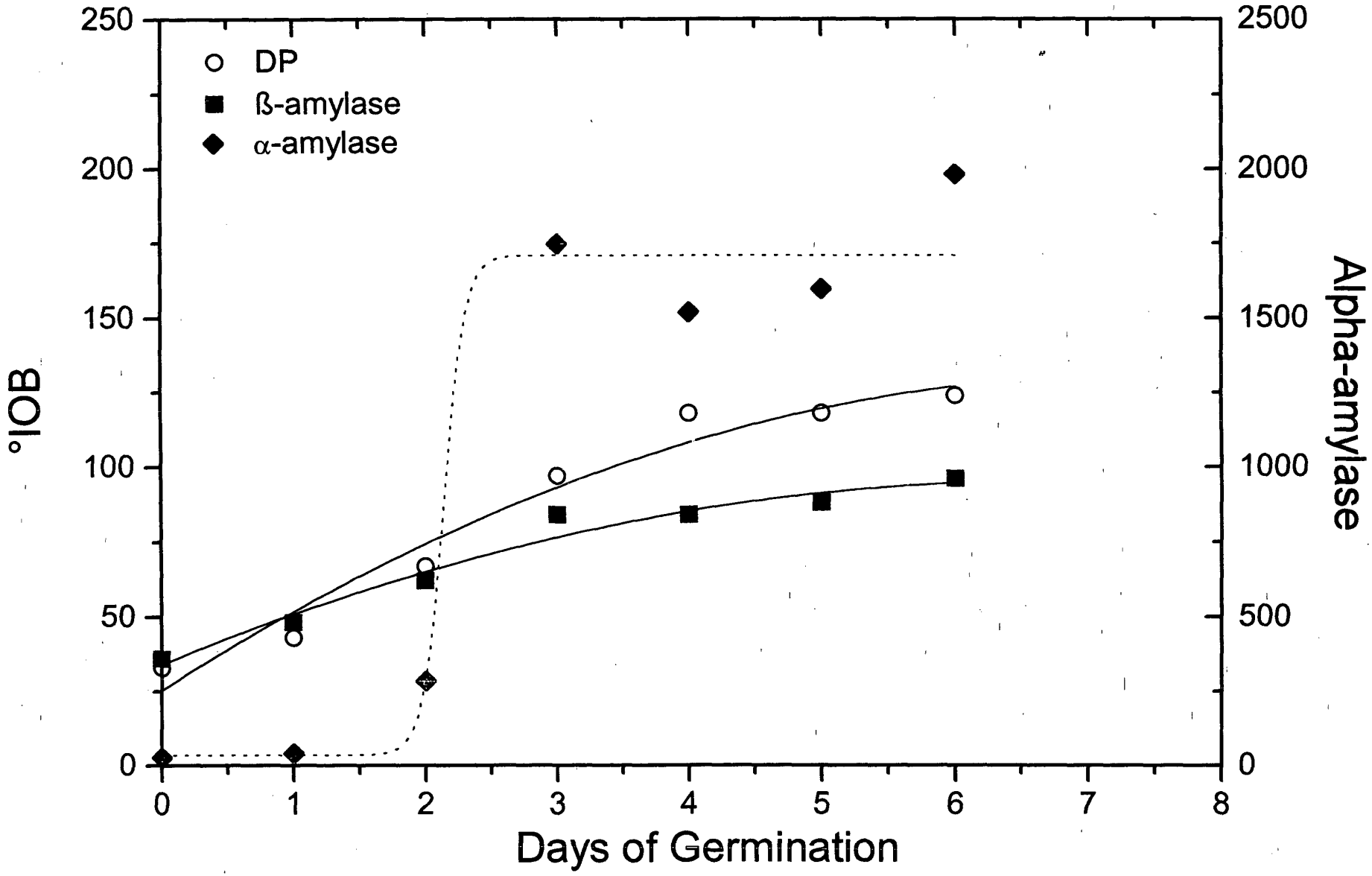


Figure 8 Development of DP - Chariot 18°C

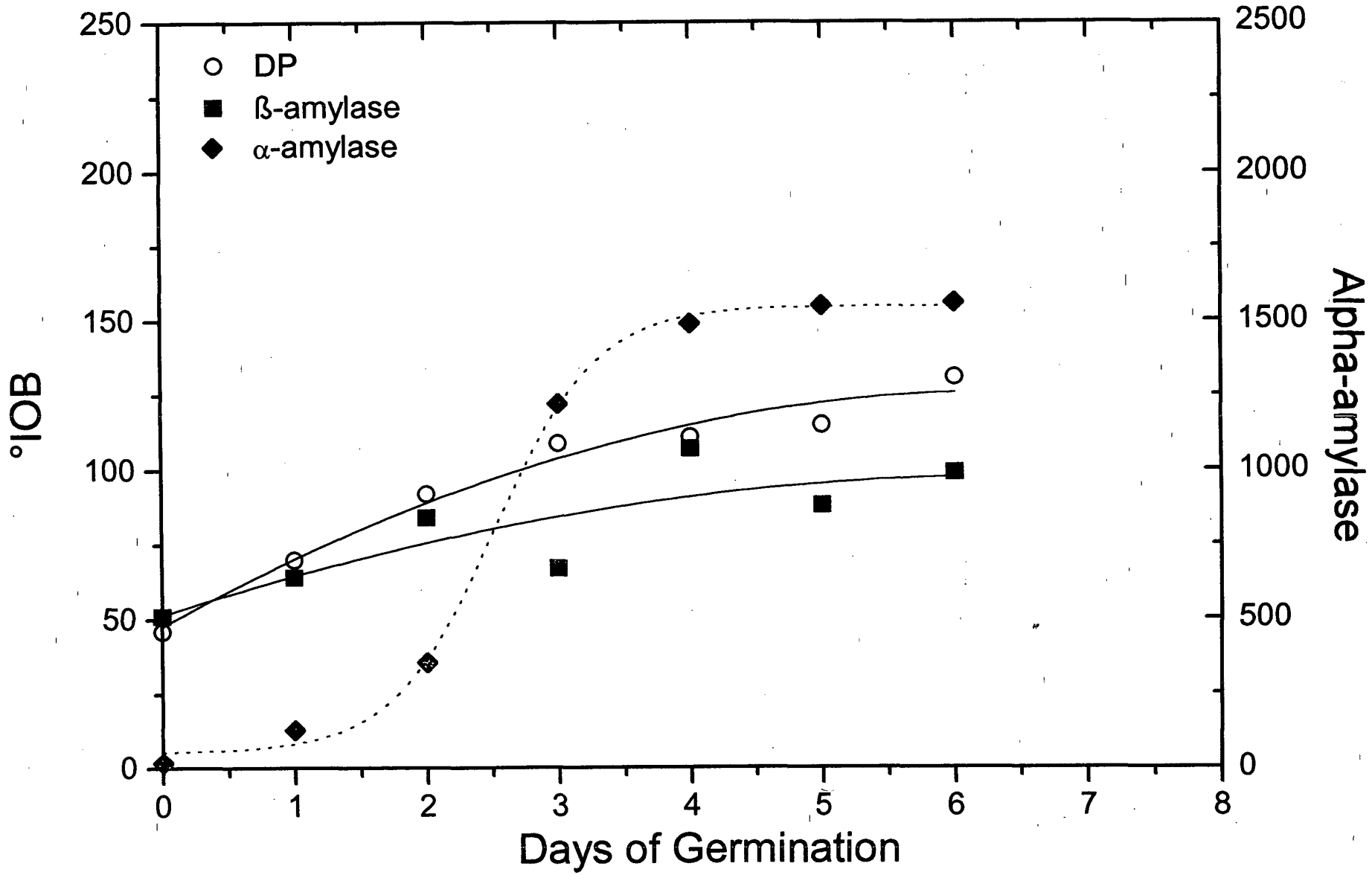


Figure 9 Development of DP - Chariot 20°C

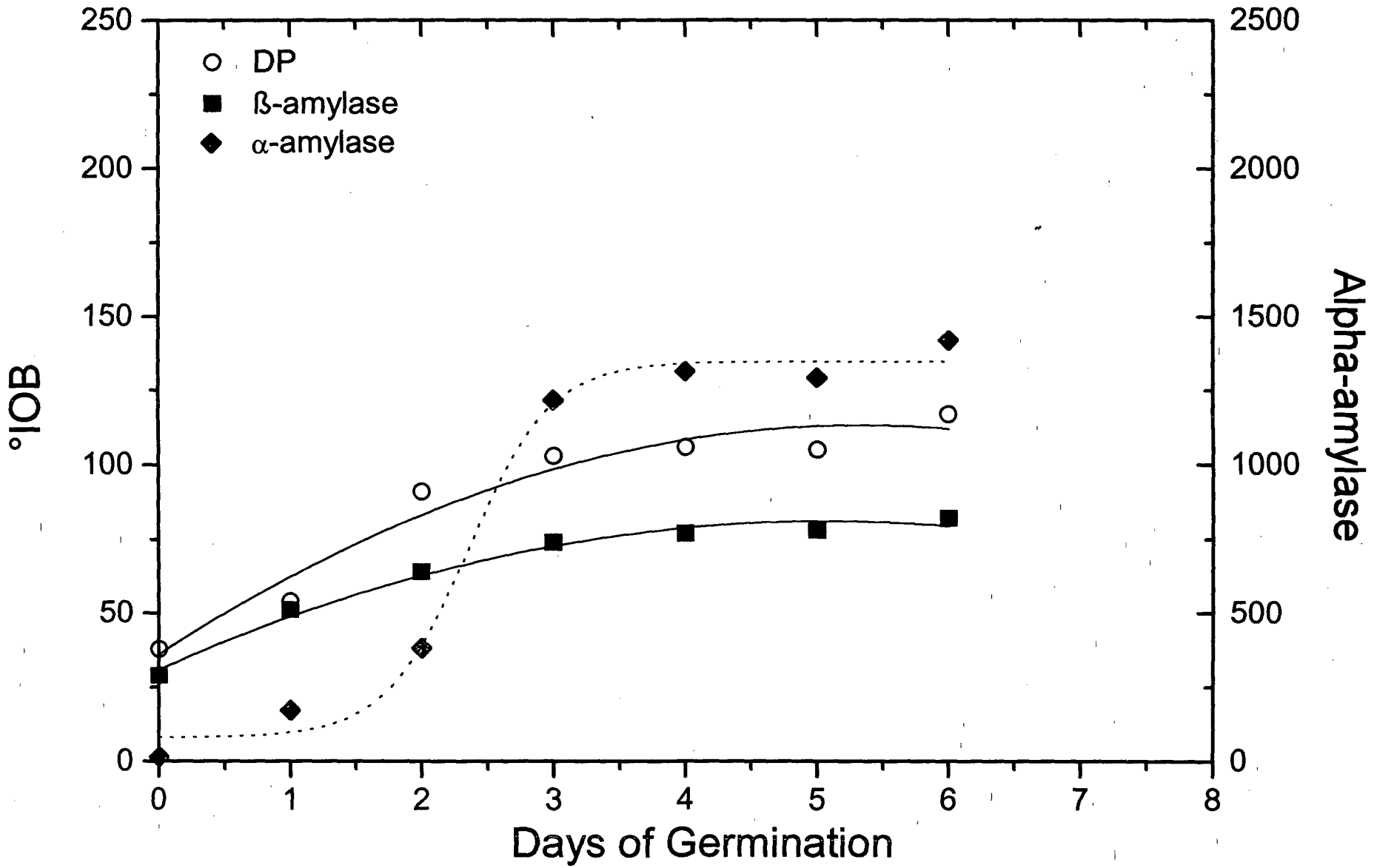


Figure 10 Effect of 1mg/kg Gibberellic Acid on DP

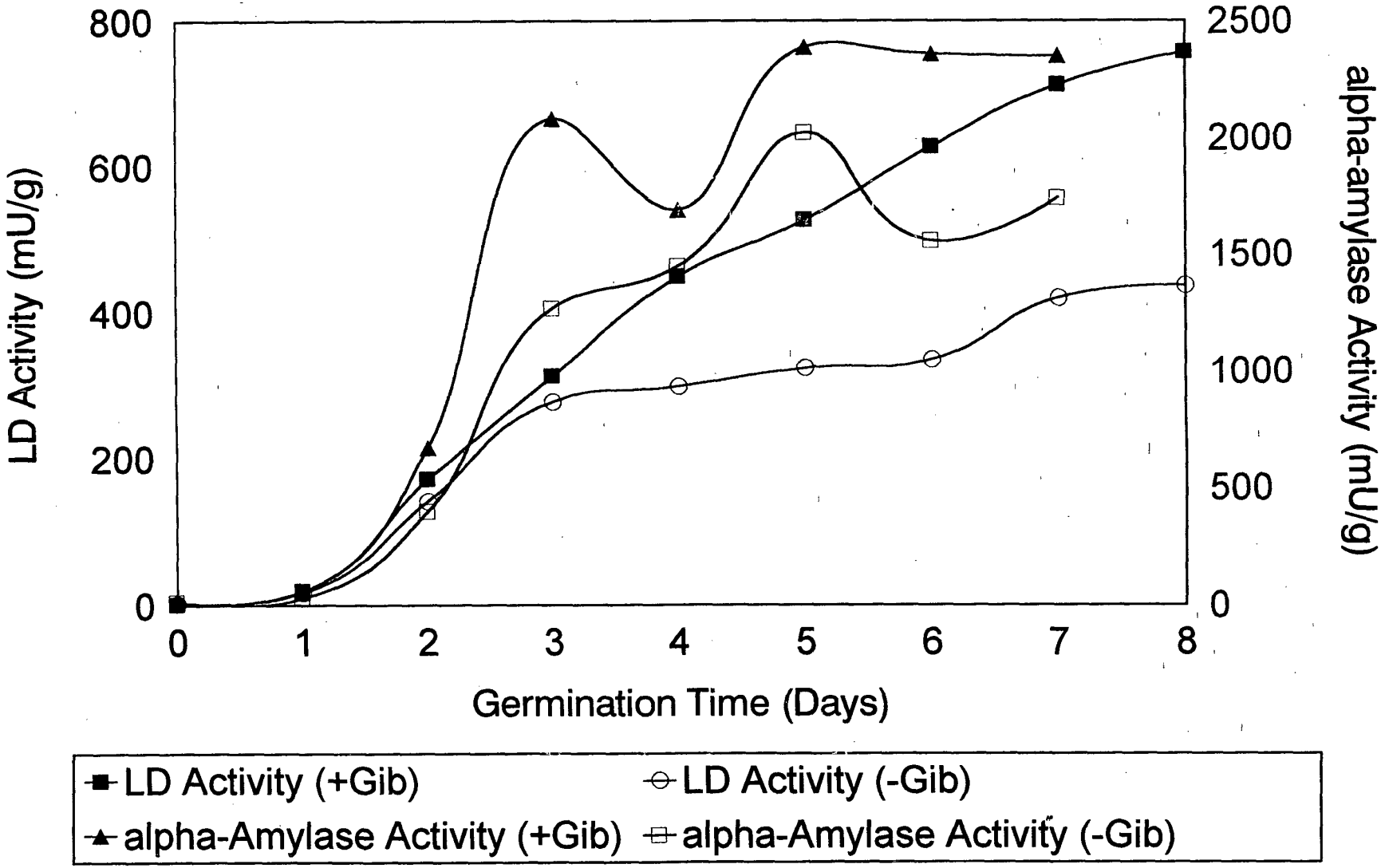


Figure 11 Effect of Lager Kilning on LD

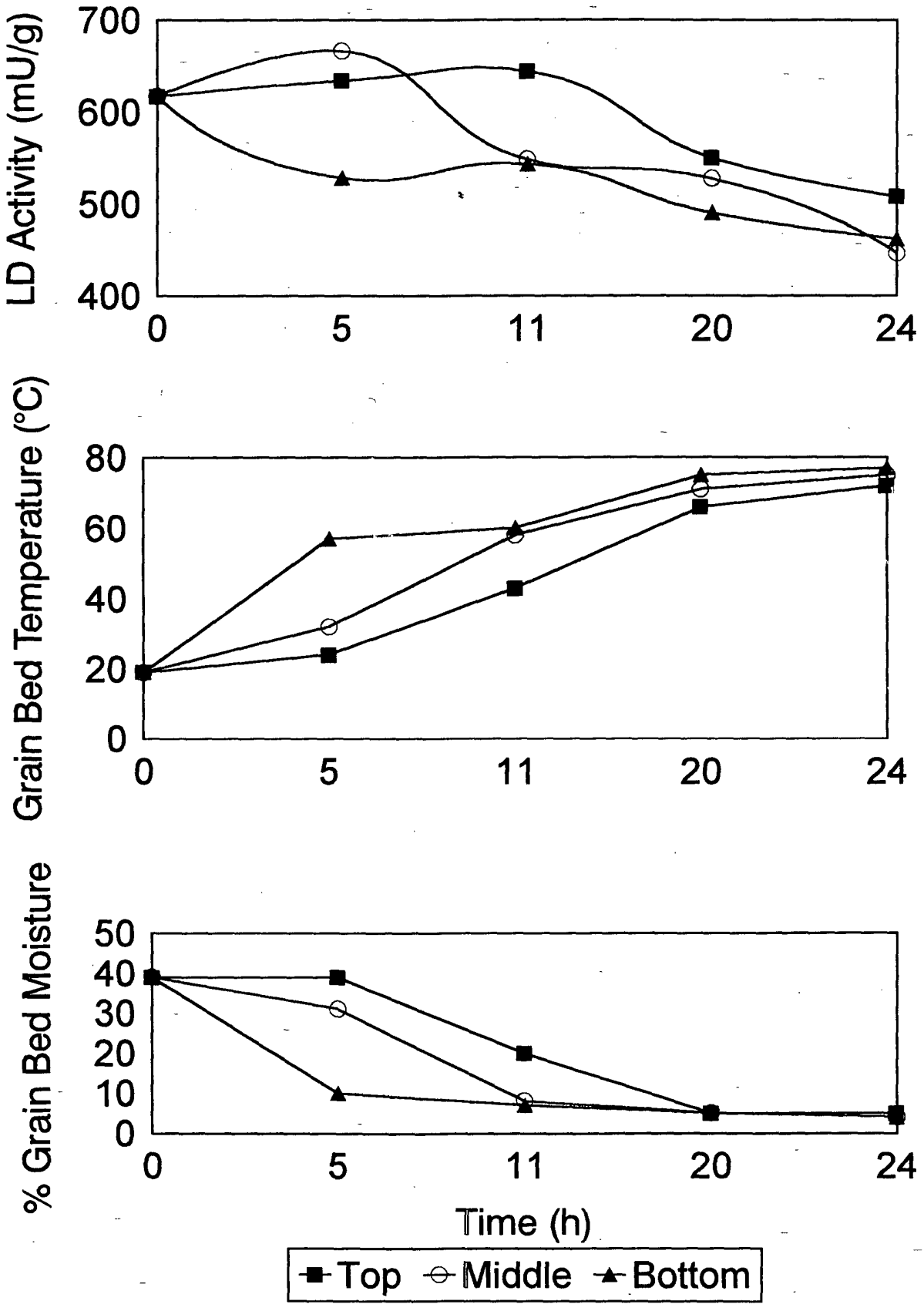


Figure 12 Effect of Ale Kilning on LD Activity

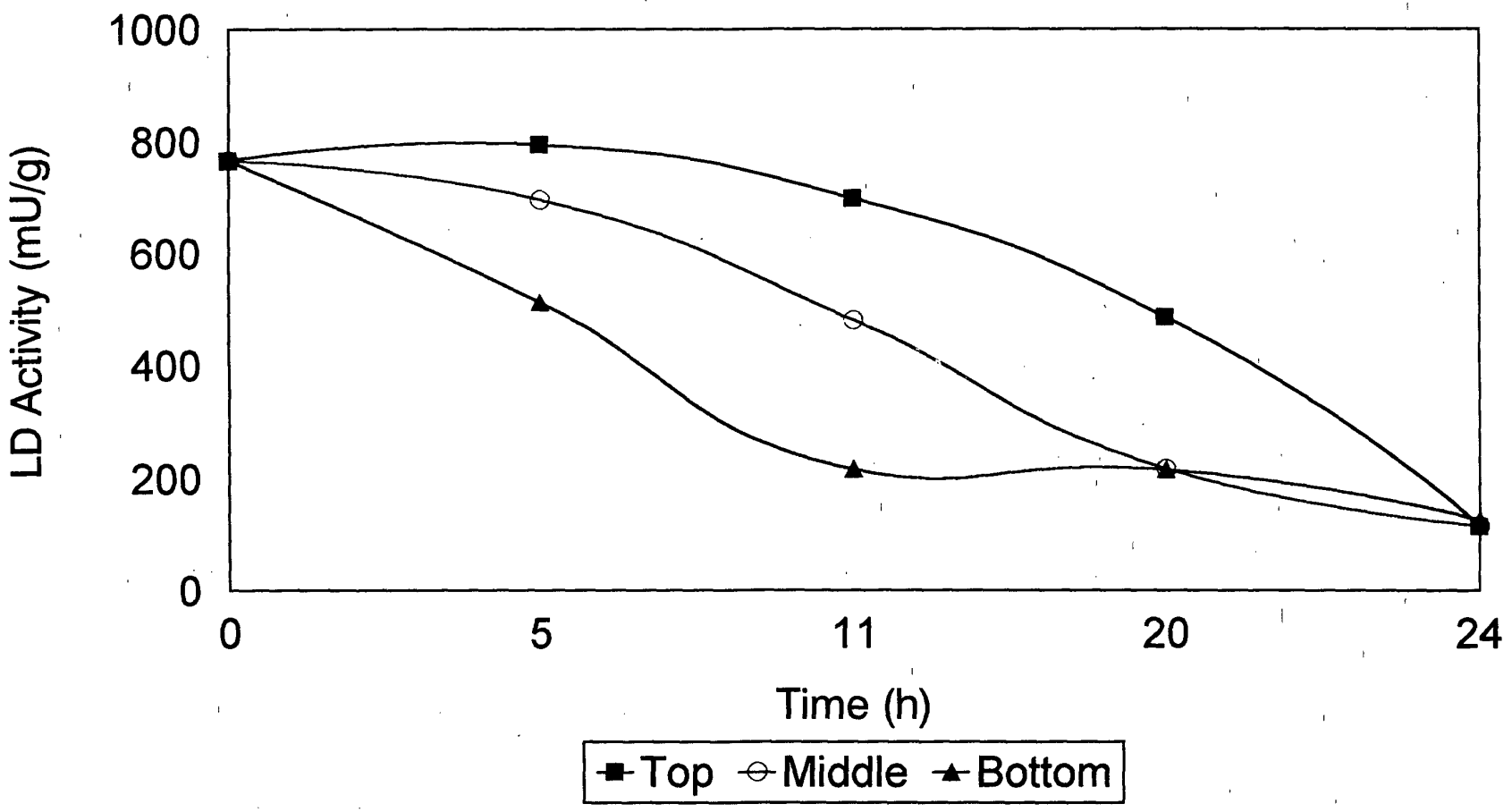


Figure 13 Effect of Lager Kilning on ß-Amylase Activity

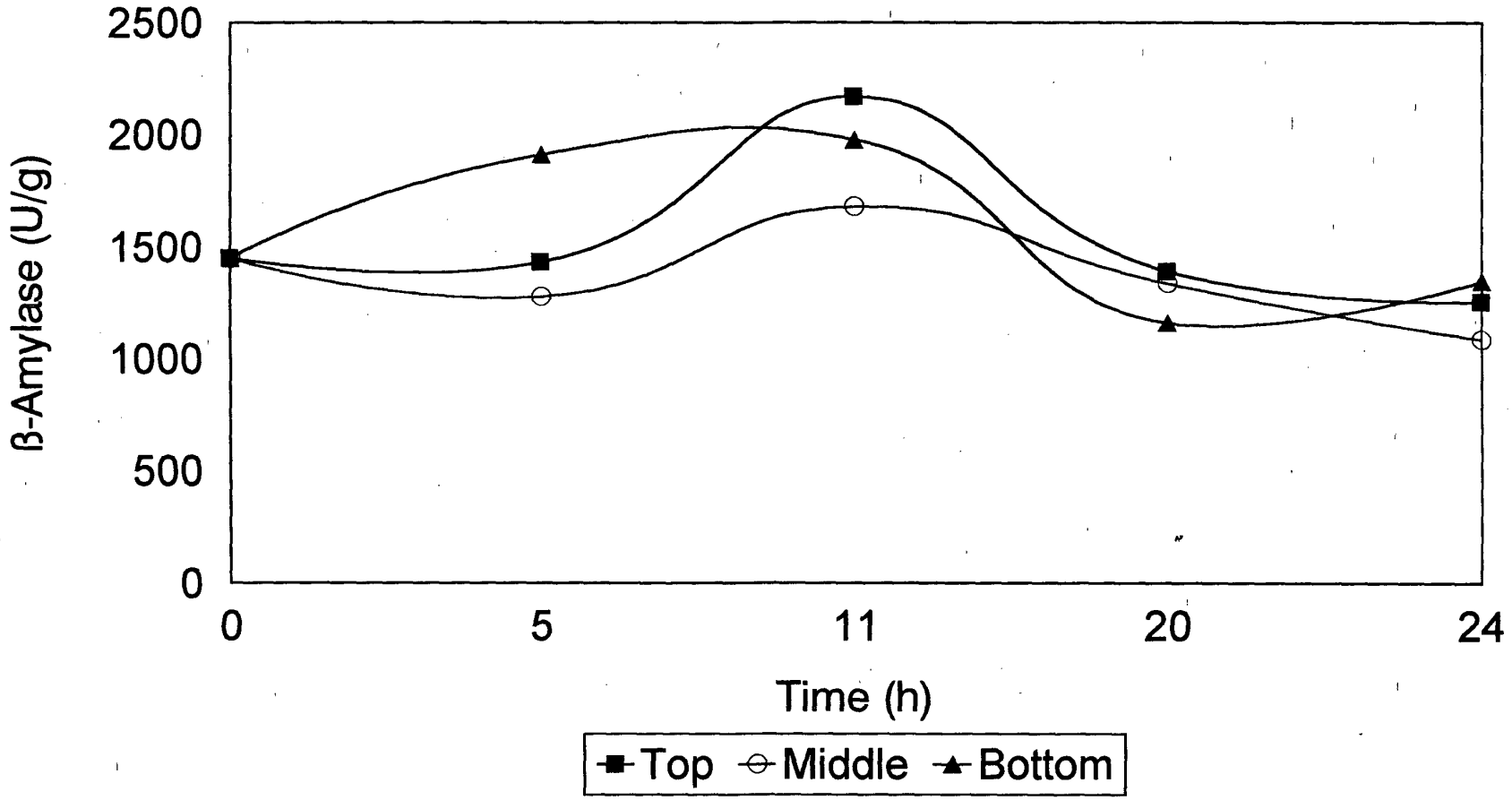


Figure 14 Effect of Ale Kilning on ß-Amylase Activity

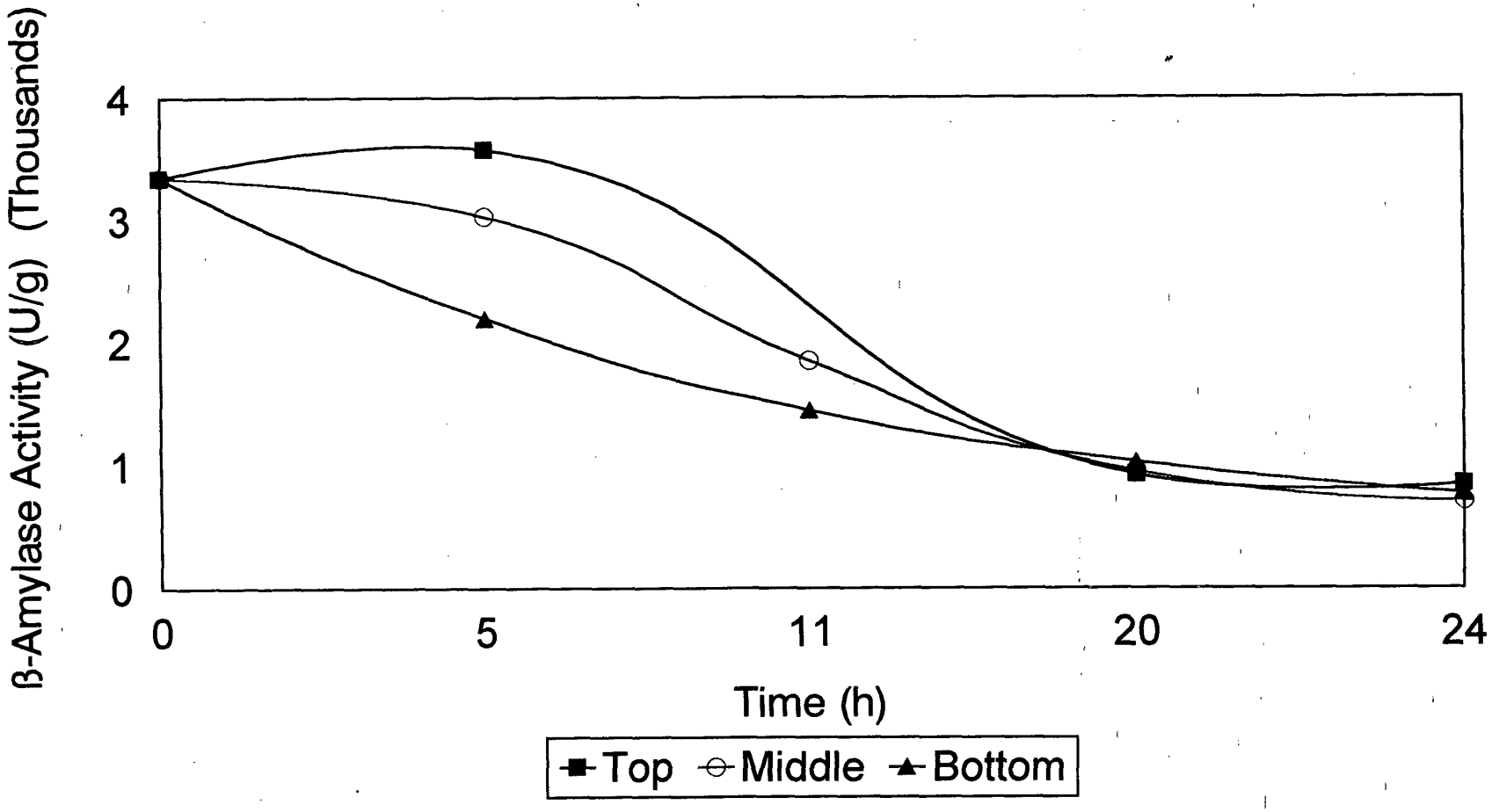
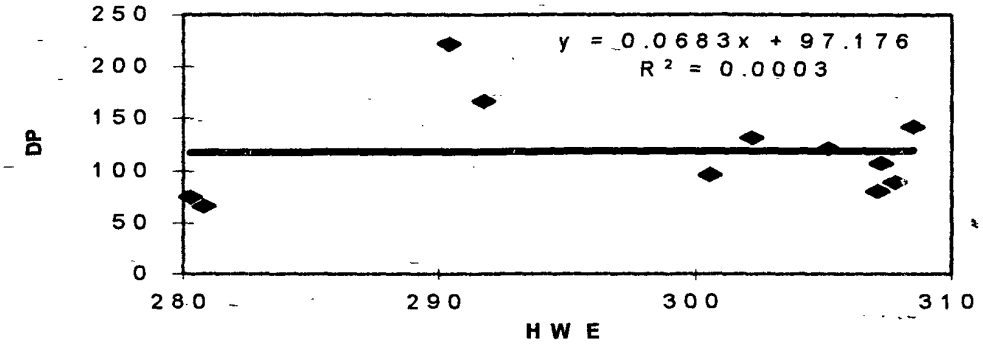
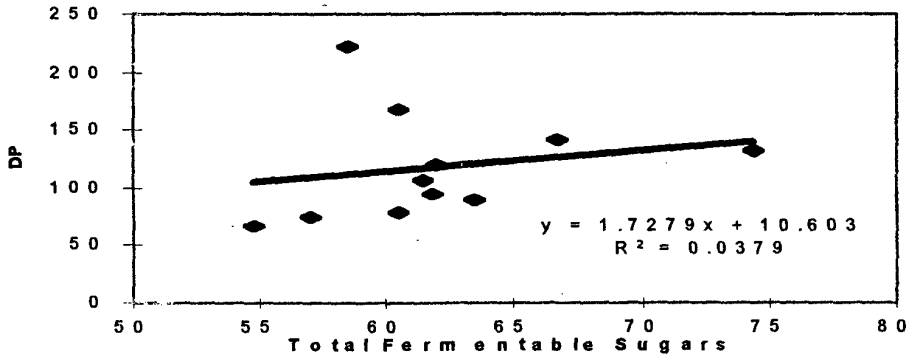


Figure 15
A : Correlation of DP vs HWE in an All Malt Mash



B : Correlation of DP vs Total Fermentable Sugars in an All Malt Mash



C : Correlation of HWE vs Total Fermentable Sugars in an All Malt Mash

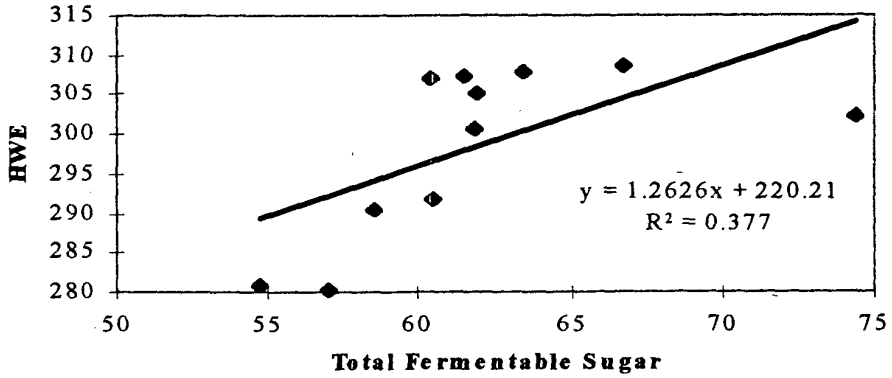
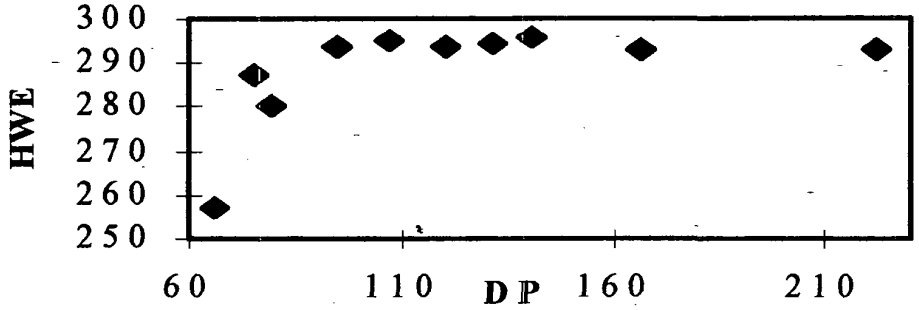
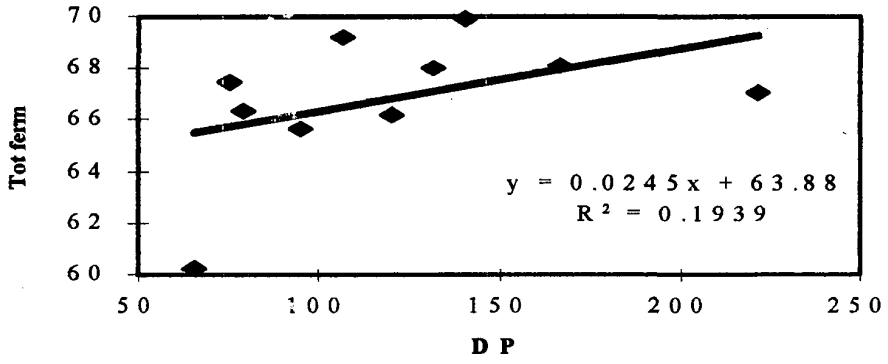


Figure 16

A :Correlation of DP vs HWE in a 80% Adjunct Mash



B: Correlation of DP and Total Fermentable Sugars in an 80% Adjunct Mash



C: Correlation of HWE and Total Fermentable Sugars in an 80% Adjunct Mash

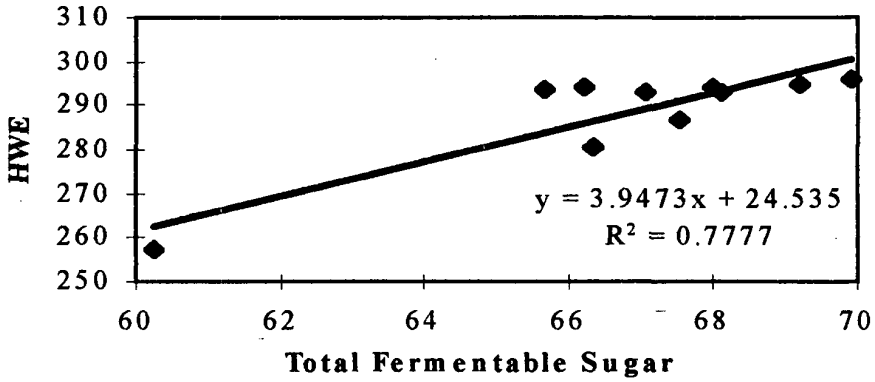


Figure 17 Attenuation Profiles of Pilot Brews Using Modified Mashing Procedures