"Effect of barley storage on respiration and Glucose - 6 – Phosphate dehydrogenase activity during malting"

P.Maharjan[1], **B.W.Woonton[2]**, and F.Sherkat[1]

1. RMIT University, GPO BOX 2476V, Melbourne, VIC, Australia
2. Food Science Australia, Werribee, VIC, Australia. E-mail: brad.woonton@csiro.au

Abstract

Malt is produced by the controlled, but limited germination of barley, with the extent of barley modification significantly affecting brewing yield and efficiency. Post harvest storage of barley alters barley germination characteristics during malting and subsequent endosperm modification levels. It has been suggested that the changes that occur during storage of barley are related to changes in the respiration rate of the barley. It has also been proposed that these changes are related to the absence of functional pentose phosphate pathway of which Glucose-6-Phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme. In fact, an increased activity of G6PDH has been correlated with increased germination vigour of cabbage seeds, rye seeds, tomato seeds and peanut seeds. In this study, we examined the effect of storage of Australian barley (cv. Gairdner) on germination parameters, α-amylase activity at the end of malting, respiration rate and G6PDH activity during malting. It was found that storage of barley for 7 months significantly increased germination vigour, reduce water sensitivity and increased a-amylase activity at the end of malting. However, storage for 7 months did not significantly influence the grain respiration rate or G6PDH activity during malting.

Keywords

Barley, dormancy, germination, respiration, glucose-6-phosphate dehydrogenase

Introduction

Malt is produced by the controlled, but limited germination of barley, with the extent of barley modification significantly affecting brewing yield and efficiency. One of the key qualities of malting barley (*Hordeum vulgare*) is its ability to germinate rapidly and synchronously[1]. Dormancy, a state in which viable seed are unable to germinate under optimal condition, can interfere with the rapid and uniform germination of barley, thereby reducing the resultant malt quality. Post harvest storage is a well practiced means of overcoming dormancy of malting barley. A better understanding of the physiological characteristics of dormancy decay may help predict the optimal maturity of barley for malting, which in turn would enhance malt quality.

Many hypotheses have been proposed to explain the mechanism of seed dormancy but because of its complexity, the fundamental basis of the induction, maintenance and termination of dormancy remains unknown[2]. It is thought that there is a relationship between the respiration rate of the germinating grains and endosperm modification during malting. Respiration not only supplies the necessary energy and carbon sources for synthesizing new proteins, but also participates in the degradation of inhibitors of germination[3]. Yosida *et al.* [4] found that a lowered respiration rate during steeping is correlated with slower malt modification. Thornton *et al.* [5] also ascertained that the respiration rate of embryos isolated from both dormant and non dormant grain was similar over the first 13 hours of imbibition, after which the oxygen uptake increased in the non-dormant sample and remained at the initial low level in the dormant sample. However, there is little information on the effect of dormancy decay on the respiration rate of barley during malting. The first aim of this study was to examine the aforementioned relationship.

Three respiratory pathways are assumed to be active in the imbibed seed, namely glycolysis, the pentose phosphate pathway (PPP) and the citric acid cycle[6]. The PPP is an alternative way to manipulate carbohydrate[7], and is thought to be the main oxidative pathway active during the initial stages of germination of various seeds such as lettuce, barley, oats, rice and kidney beans[8]. Glucose-6-Phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme of PPP[7,9], and increased G6PDH activity has been associated with dormancy decay and increased seed vigor of *Avena fatua* (wild oat)[10]; *Arachis hypogaea*

(peanut) seeds[11,12] tomato seeds[3] and *Brassica* (cabbage) seed[13]. Although the above literature would seem to indicate that the PPP is involved in dormancy decay, there has been little research conducted examining dormancy decay of barley and PPP activity. The second aim of this study was to examine the effect of barley storage on the rate limiting enzyme of the PPP; G6PDH.

Materials and Methods

Barley samples
Commercially grown Gairdner (2002 harvest) were obtained from Western Australia (WA). The barley sample was stored at room temperature (20-25°C) for 7 months and at 1, 3 and 7 months approximately 2 kg of each sample was sealed in a polyethylene bag and following a well established practice[14,15,16] stored at -18°C to halt further changes.

Germination testing
The 4mL and 8mL germination energy (GE) and germination index of the samples were determined according to European Brewing Convention Method 3.7[17].

Malting
Before micro-malting in an Automated Joe White Malting Systems Micro-malting unit (Perth, Australia), samples (≈100g) were removed from the freezer and left at room temperature for two hours in an air tight bottle. The samples were then micro-malted in two 50g lots, one for respiration studies and the other for α-amylase and G6PDH enzyme activity assays. The malting program constituted of 22h interrupted steeping program (12h submerged, 8h air rest (17°C, 70% airflow) and 2h submerged) at 17°C, and 95 h germination at 17°C, 70% airflow. To stagger the micro-malting of each sample, the first wet phase of the steeping process was carried out in a water bath at 17°C. Steeping air rest periods and the germination processes were undertaken in the Micro-malting unit. At 18h of germination, 6g of water was manually added to each sample. At 42h and 66h of germination, samples were made upto 46% moisture (w/w) by the manual addition of water.

Respiration measurement
At the end of steeping, 18h, 42h, 66h and 95h of germination the rate of grain respiration was measured using a Servomex 1400 Gas Analyser (Crowbrough, Sussex, England). Each sample was removed from the micro-malter, placed in the respiratory chamber and held at 17°C. The percentage carbon dioxide and oxygen in the circulating air was recorded at 2 minutes intervals over an 11 minute period. The carbon dioxide production and oxygen consumption rates were determined by the increase in carbon dioxide and decrease in oxygen concentration over this period. The Servomex Gas Analyser was calibrated using atmospheric air (O_2 = 20.9% CO_2 = 0.03%) and 9.2% CO_2 (0% O_2) at 101kPa.

G6PDH activity
The G6PDH activity was measured at 0h, at the end of steeping, 18h, 42h, 66h and 95h of germination using a method adopted from Shetty et al.[18]. Briefly, seeds (10) were homogenised in 10.0mL of cold enzyme extraction buffer (0.5% w/v polyvinyl pyrrolidone (PVP); 3 mM EDTA and 0.1 M potassium phosphate buffer at pH 7.5) using a cold mortar and pestle. A 1.0 mL aliquot of this homogenous sample was pipetted into a 1.5mL eppendorf tube and centrifuged at 10,000*g* for 10 minutes at 3° C. After centrifugation, 50μL of the enzyme extract supernatant was pipetted into a 1.5mL plastic cuvvete, to which a 1.00mL aliquot of enzyme reaction mixture (0.38 mM β-NADP; 6.3 mM magnesium chloride; 3.3 mM glucose-6-phosphate; 5 mM meleimide and 50 mM Tris HCl at pH 8.0) was added. The assay was carried out at 339nm and 25°C. The change in absorbance was recorded every minute over an 8 minute period. The enzyme quantity was measured in micromoles per minute per grain.

α-Amylase activity
The α-amylase activity of samples was determined at 95h of germination using AACC method 22-05[19]. Briefly, seeds (10) were homogenised in a cold enzyme extraction buffer (0.05M sodium malate; 0.05M sodium chloride; 2mM calcium chloride and 0.005% sodium azide) using an X620 homogeniser (Ingenieurburo CAT, M. Zipperer GmbH, Staufen, Germany). The samples were then centrifuged at 1,000*g* for 10 minutes at 3°C, and then the supernatant was suitably diluted (1:80). After dilution, 0.10 mL

of the diluted enzyme extract was pipetted into a 10mL V-bottom test tube (Technoplas Australia) and incubated at 40°C for 5 minutes. To each tube, 0.1mL of non-reducinig-end blocked p-nitrophenyl maltoheptasoside (BPNPG7) substrate solution (pre-incubated at 40°C for 15minutes; Megazyme Ltd., Ireland) was added directly to the bottom of the tube and incubated for exactly 10 minutes. At the end of incubation, 1.5mL of the stopping reagent (1% Trizma base (Sigma Aldrich) adjusted to pH of 11.0 (with 1M NaOH and 1M HCl) was added and the tube content was vortexed for 10s. The absorbance of the solution was measured at 410nm and the α-amylase activity calculated according to AACC method 22-05[19].

All the experiments were conducted in triplicate. The statistical analysis of variance (ANOVA) was undertaken using MINITAB software (Release 13.32).

Results and Discussion

Germination parameters

The 4mL GE is the percentage of grains that germinate over three days on filter paper soaked in 4mL of water and the general requirement for malting barley is that this value must be greater than 96%. The 4mL GE was greater than 96% from harvest (time zero) until the end of storage, with no significant difference between values during this time ($p<0.05$, Table 1). This is similar to the finding of Woonton *et al.*[16]. The high 4mL GE throughout storage may be due to the hot and dry grain maturation conditions in Australia. Germination can be reduced if barley encounters excessive levels of moisture during germination, a phenomenon called water sensitivity. The water sensitivity of barley is indicated by the 8mL GE, which is obtained by adding 4mL of additional water (total of 8mL) to the 4mL GE test. In this study, storage for 7 months significantly improved the 8mL GE ($p<0.05$, Table 1), indicating that post harvest storage reduced the grains water sensitivity. Woonton *et. al.* [16] and Briggs *et al.*[14] have indicated that both European and Australian grown barley overcome water sensitivity during post-harvest storage. The mechanisms involved in water sensitivity remain unknown, although the presence of microbes has been claimed to be one of the causes[20]. However, other factors also seem to be involved, as treatment of barely with anti-microbial agents does not consistently overcome water sensitivity[21].

Table 1. Changes in various germination parameters for WA Gairdner sample stored up to 7 months

Storage time	Germination parameters		
	4 mL GE	8 mL GE	GI
1 month	99 ± 0.6	70 ± 1	5.7 ± 0.1
3 months	100 ± 0	88 ± 1	6.6 ± 0.1
7 months	100 ± 0	95 ± 1	7.0 ± 0.2

NB: Mean ± SE of the mean (n=3)

The GI is the speed at which the grains in a GE test germinate and high GI values are essential for high quality and homogenous malt. In this study, storage of the sample for 7 months significantly increased the GI value ($p<0.05$, Table 1). Woonton *et. al.*[16] found similar results and also found that the GI was highly correlated with the ability of the barley to produce enzymes during malting and the final malt quality. The increases in the GI of these samples are likely to be associated with improvements in malt quality parameters.

α-Amylase activity

Post harvest storage of the sample significantly increased the α-amylase activity during malting ($p<0.05$, Figure 1). After 7 months of storage the α-amylase activity at the end of malting increased by 45%. This finding is similar to those of Woonton *et al.*[16] and indicates that storage of the sample was influencing not only germination, but also enzyme synthesis during malting.

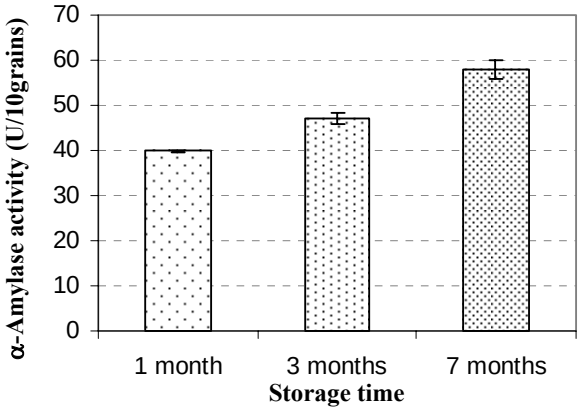

Figure 1. α-Amylase activity at the end of germination of WA Gairdner stored for up to 7 months after harvest. Error bars represent the SE of the mean (n =3)

The changes observed in 8mL GE, GI and α-amylase synthesis in this study may be due to a large number of factors. During germination, the growing barley embryo synthesizes and secretes gibberellins into the grain which triggers the aleurone to synthesise and secrete a range of hydrolytic enzymes. It may be that the changes observed in this study are due to changes in gibberellin production[22]. However, Schuurink *et al.*[23], and Briggs[24] have found that the dormancy state of the barley grain is correlated with the gibberellin responsiveness of isolated aleurone layer. Thus, the observed increase in the α-amylase activity during malting of stored samples may be due to an increased aleurone sensitivity to gibberellin.

Respiration rate

Respiration is the source of energy and carbon compounds required for germinations. Furthermore, respiration has been linked with the dormancy decay of European barley. For all samples the carbon dioxide production rate at the end of steeping (22h) was approximately 300 $mg.h^{-1}.kg^{-1}$ (dw) and increased to approximately 900 $mg.h^{-1}.kg^{-1}$(dw) by 88h of malting, after which it declined slightly (Figure 2). Similarly, for all samples, the oxygen consumption rate at the end of steeping was 300 $mg.h^{-1}.kg^{-1}$ (dw) and increased to approximately 1200 $mg.h^{-1}.kg^{-1}$ (dw) by 88h of malting. After this time the oxygen consumption decreased by a small extent (Figure 2). The observed respiratory pattern was similar to that found by After de Clerk (1940, as cited by Briggs, 1998).

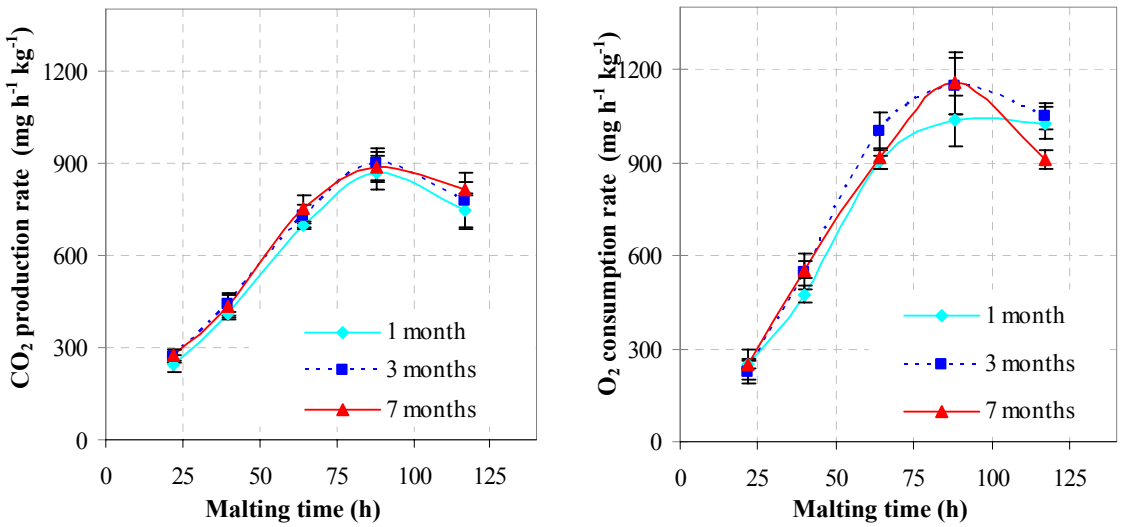

Figure 2. CO_2 production rate and O_2 consumption rate during malting of WA Gairdner stored for up to 7 months after harvest. Error bars represent the SE of the mean (n =3)

Post harvest storage of the sample for up to seven months did not significantly change the carbon dioxide production rate or the oxygen consumption rate during malting ($p>0.05$). This contradicts the finding of Thornton *et al.*[5] who found a difference in respiration rate between dormant and non-dormant European barley samples. Typically European barley has a more pronounced dormancy when compared to Australian barley, possibly due to the short days and cold climates in Europe[23]. The discrepancies between the results of this study and those of Thornton *et al.* [5] could be due to this fact.

G6PDH activity

The sample showed a small amount of G6PDH activity before malting (Figure 3) and a small increase in G6PDH activity during the first 40h of malting (i.e. during steeping and up until 20h of germination). After this time, the G6PDH activity of all samples increased in a linear fashion until the end of malting. This sudden increase coincides with the rapid growth of the acrospire during malting. The increase in the amount of living tissue within the grain could be a possible explanation for the linear increase in G6PDH activity after 40h of malting.

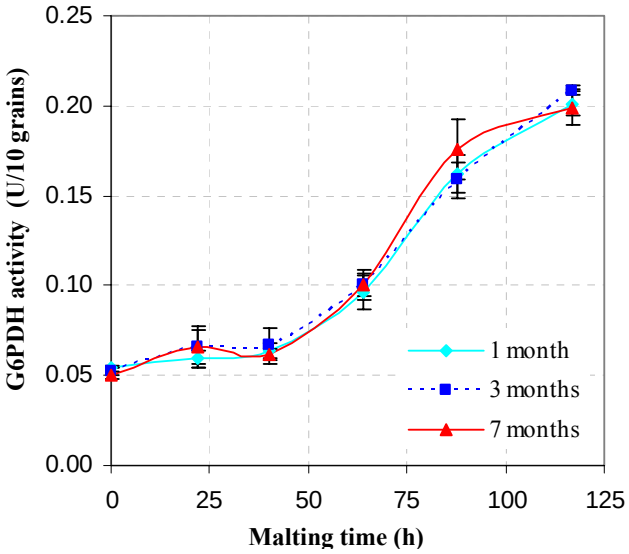

Figure 3. G6PDH activity during malting of WA Gairdner stored for up to 7 months after harvest. Error bars represent the SE of the mean (n =3)

Storage of all samples had no significant effect on the G6PDH activity during malting ($p>0.05$). This contradicts the findings of Betty & Finch-Savage[13], Gui *et al.*[3], and Swamy & Sandhyarani[11], who found that increased activity of G6PDH was correlated with increased germination vigour of cabbage, rye seeds, tomato seeds and peanut seeds. This discrepancy may be due to barley having different dormancy decay and germination vigour pathways. Alternatively, the favorable growing conditions in Australia may lead to barley with minimal dormancy and thus no or only very minor differences in G6PDH activity during malting.

Conclusions

In conclusion, storage of barley at room temperature causes dormancy decay and significant improvements in germination characteristic and increases in α-amylase production during malting. However, storage of the barley grain does not significantly influence the respiration rate or the G6PDH activity during malting.

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