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

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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, reduced 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 major attributes 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. An insight into physiological basis of dormancy decay might assist in predicting the optimal maturity of barley for malting, thereby leading to subsequent improvement in malt quality.

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. Yosida *et al.* [3] found that a reduced respiration rate during steeping is associated with slower malt modification. Thornton *et al.* [4] also found 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 limited information on the effect of dormancy decay on the respiration rate of barley during malting. The first aim of this study was to look at the association between the respiration rate of germinating grains and dormancy decay.

Three respiratory pathways are assumed to be active in the imbibed seed, namely glycolysis, the pentose phosphate pathway (PPP) and the citric acid cycle[5]. The PPP is an alternative way to manipulate carbohydrate[6], and is thought to be the main oxidative pathway active during the initial stages of germination of various seeds[8]. Glucose-6-Phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme of PPP[6,8], and increased G6PDH activity has been associated with dormancy decay and increased seed vigor of *Avena fatua* (wild oat)[9]; *Arachis hypogaea* (peanut) seeds[10,11] tomato seeds[12] and *Brassica* (cabbage) seed[13]. The above literature would indicate the involvement of the PPP in dormancy decay.

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) was obtained from Western Australia (WA). The barley sample was stored at room temperature (20-25°C) for 7 months and at 1 and 7 months approximately 2 kg of each sample was sealed in a polyethylene bag stored at -18°C to halt further changes[14].

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[15].

Malting
Before micro-malting in an Automated Joe White Malting Systems Micro-malting unit (Perth, Australia), samples were removed from the freezer and left at room temperature for two hours in an air tight bottle. The samples were then micro-malted. The malting program constituted of 22h interrupted steeping program (12h wet, 8h dry and 2h wet) at 17°C, and 95 h germination at 17°C. 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 carbon dioxide production and oxygen consumption rates were determined by the increase in carbon dioxide and decrease in oxygen concentration over the period of 11 minutes.

G6PDH activity
The G6PDH activity was measured at various stages of malting using a method adopted from Shetty et al.[16]. Briefly, seeds (10) were homogenised in 10.0mL of cold enzyme extraction buffer (0.5% w/v polyvinyl pyrrolidone; 3 mM EDTA and 0.1 M potassium phosphate buffered 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 MgCl2; 3.3 mM G-6-P; 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.

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 prerequisite for malting barley is greater than 96%. The 4mL GE for both one month and seven month storage were greater than 96% (Table 1), indicating no significant changes with post harvest storage. This is similar to the finding of Woonton *et al.*[17]. Water sensitivity is a phenomenon which reduces germination when barley receives excessive levels of moisture during germination. The water sensitivity of barley is indicated by the 8mL GE and storage of this barley for 7 months significantly improved the 8mL GE ($p<0.05$, Table 1). This implies that storage of grain can significantly reduce water sensitivity and is in accordance with that of Woonton *et. al.* [17] (Australian barley) and Briggs *et al.*[14] (European barley). The mechanisms involved in water sensitivity remain unknown. However, it is suggested that the major cause of the phenomenon is the presence of microorganisms on

the surface of the grain, which compete with the embryo for available oxygen[18]. Nevertheless, it seems that other causes are also involved, as treatment of barely with anti-microbial agents does not consistently overcome water sensitivity[19].

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
7 months	100 ± 0	95 ± 1	7.0 ± 0.2

NB: Mean ± SE of the mean (n=3)

The GI is the average speed at which the grains in a GE test germinate. High GI are crucial if barley is to be speedily and efficiently converted to homogenous malt. The significant increase in the GI of sample with storage ($p<0.05$, Table 1) indicates that the post harvest storage improved the germination vigor. This finding is similar to that of Woonton *et. al.*[17].

Respiration rate

Respiration has been associated with the dormancy decay of European barley. For both samples the carbon dioxide production rate and the oxygen consumption rate at the end of steeping (22h) was approximately 300 $mg.h^{-1}.kg^{-1}$ (dw) and both the parameters gradually increased till 88h of malting, after which it decreased slightly (Figure 1).

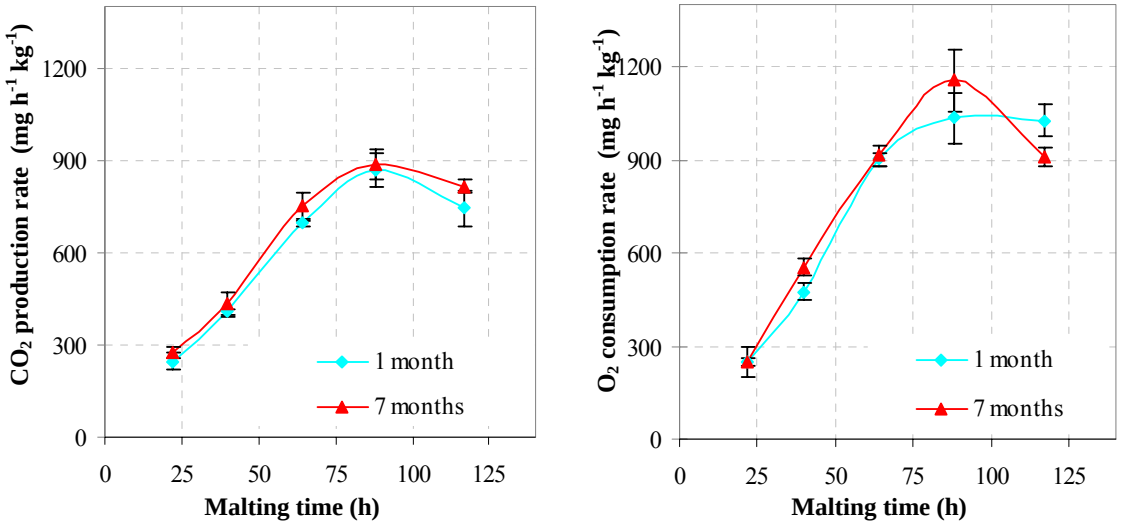

Figure 1. CO_2 production rate and O_2 consumption rate during malting of WA Gairdner stored for up to 7 months after harvest. Error bars represent the SE of the mean (n =3)

Post harvest storage of the sample did not cause any significant difference in either the carbon dioxide production rate or the oxygen consumption rate during malting ($p>0.05$). This contradicts the finding of Thornton *et al.*[4] who found a variation in respiration rate between dormant and non-dormant European barley samples. Characteristically, European barley has strong dormancy compared to Australian barley, possibly due to the short days and cold climates in Europe[20]. The inconsistency in our findings and that of Thornton *et al.*[4] is most probably due to this fact.

G6PDH activity

The samples showed a small amount of G6PDH activity before malting and a small increase in G6PDH activity during the first 40h of malting (Figure 2). After this time, the G6PDH activity of both samples increased in a linear fashion until the end of malting. This increase in the G6PDH activity coincides with the expansion of the acrospire during malting. The proliferation of living tissue is most likely responsible for the escalation of the G6PDH activity after 40h of malting.

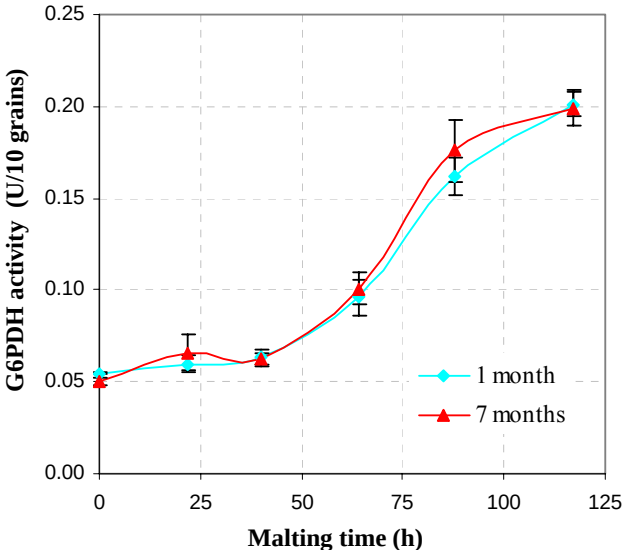

Figure 2. G6PDH activity during malting of WA Gairdner stored for up to 7 months after harvest. Error bars represent the SE of the mean (n =3)

Storage of barley 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.*[12], and Swamy & Sandhyarani[10],who found that increased activity of G6PDH was correlated with increased germination vigor of cabbage, rye seeds, tomato seeds and peanut seeds, respectively. This difference may be due to favorable growing conditions in Australia, leading to barley with minimal dormancy and thus no or very little changes in G6PDH activity. Furthermore, barley may not have the same biochemical mechanisms/regulators of germination vigor and dormancy decay.

Conclusions

In conclusion, storage of barley at room temperature causes dormancy decay and significant improvements in germination vigor and significant reductions in water sensitivity. However, storage of the barley grain does not significantly influence the respiration rate or the G6PDH activity during malting.

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