The effect of storage conditions on post-harvest maturation and maltability of barley

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Abstract

Storage conditions have a major influence on the rate at which dormancy breakdown occurs. The rate of breakdown depends mainly on grain moisture content, storage temperature, storage time and the characteristics of individual batches of barley. Data presented in this paper are a significant addition to the existing knowledge on post harvest maturation of Australian malting barley.
Germination of *Gairdner* and *Sloop* increased at low moisture content (mc) at storage temperatures above 20ºC, as water sensitivity progressively broke down. In contrast, *Grimmett* germination declined under similar conditions. *Stirling* was very robust and maintained its germination even under high temperature storage, while *Tallon* had to be stored below 25ºC to prevent loss in barley quality. Samples with high water sensitivity and dormancy were less vulnerable to germination loss. Dormancy and water sensitivity could be overcome by matching storage conditions and barley characteristics. *Gairdner*, *Sloop* and *Stirling*, all samples with some level of dormancy and water sensitivity, showed improved maltability after storage, while *Tallon* and *Grimmett*, samples with high pre-storage germination, decreased in maltability under most storage conditions. Longer term storage under mild conditions generally resulted in better malting outcomes. Storage affected Kohlbach index, wort β-glucan, diastatic power and apparent attenuation limit of malt. Changes in germination and maltability need to be considered in combination to develop optimum storage strategies for breaking dormancy and water sensitivity. In dormant grain, enzyme activity of α-amylase and β-glucanase changed with storage, while β-amylase measurements showed no clear trends. Enzyme activity decreased at higher mc and temperatures concurrent with germination loss. Changes in enzyme activity were highly sample-dependent.
An understanding of the vulnerability of particular cultivars to damage in storage will assist in increasing and preserving value. Barley with pronounced levels of dormancy and/or water sensitivity can be safely stored at 25° to 30°C for short periods to increase germination. Care must be taken to monitor germination during storage, and moisture content should be kept at or below 12%. Dormant barley stored at low temperatures may not reach malting quality, even over a 12 month period.

Key Words

Storage, germination, malting, amylase, glucanase, dormancy, water sensitivity

Introduction

Storage conditions largely determine the rate at which quality parameters of Australian barley varieties change after harvest. Initial kernel condition, temperature, moisture content and storage time, are major factors influencing changes in malting quality [1]. Depending on storage conditions, Australian malting barley can take several months to reach optimum malting quality while dormancy and water sensitivity are broken down.
Research has identified several options for managing barley dormancy to provide opportunities to malt and export barley earlier, such as use of agricultural chemicals or application of dry heat [2]. Alternatively, by understanding and carefully manipulating the storage process, post harvest dormancy breakdown can be accelerated without compromising barley quality.
Changes during storage of dormant and water sensitive barleys can be understood as after ripening, also referred to as post harvest maturation. Storage conditions have a major influence on the rate at which dormancy breakdown occurs [3]. The rate of breakdown depends mainly on grain moisture content and storage temperature [4, 5, 6]. Research in this area has been predominantly carried out on European malting barley varieties, and has led to the construction of models now available to European maltsters to predict dormancy decay during storage [7, 8, 9]. Much less information is available to maltsters and grain handlers working with Australian varieties. Information available is conflicting and the methods used in experiments

are open to question [10, 11]. New data is presented in this paper that contributes considerably to the knowledge on post harvest maturation of Australian malting barley.

Methods

Sources and characteristics of barley varieties

Barley samples from the 2001/2002 harvest used in the storage trials were *Stirling* from Western Australia, and *Sloop* and *Gairdner* from South Australia. *Grimmett* and *Tallon* samples were obtained from the 2002/2003 Queensland harvest. These samples exhibited some initial degree of dormancy or water sensitivity (Table 1).

Storage experiments

Grain was stored at 10, 12 and 14% mc and 15, 20, 25 and 30°C for a period of up to 12 months. All samples had been graded as malting barley, obtained immediately after harvest, and were conditioned to the required moisture content (mc) before storage under controlled laboratory conditions.

Germination tests

Germination testing was based on the germination energy (GE) and water sensitivity (WS) tests recommended by the European Brewery Convention [12] and improved by Doran and Briggs [13], and methods described in the International Rules for Seed Testing [14].

The germination index (GI) of the samples was calculated according to EBC methods [12].

To define dormancy status, germination with water only was compared to germination in the presence of 0.05% gibberellic acid (GA_3) solution. Dormant (but not dead grain) will germinate in the presence of GA_3. Dormancy was also evaluated by stratifying kernels (4°C for 4 days) that had failed to germinate after conventional tests. Seed moisture content (mc) was determined using the International Standards Organisation (ISO) standard oven dried method No. 712 [15].

Micromalting and enzyme assays

Samples were micromalted with a Joe White Micromalting System. Steep temperature was 17°C, germination was carried out at 15°C for 4 d. Kilning was carried out for 21 h (program: min. 55°C, max. 82°C). Malt quality was assessed following protocols of the EBC (1998). All micromalting and malt quality analyses were carried out by Barrett Burston Malting Central Laboratory.

Activity of α-amylase was determined according to the Royal Australian Chemical Institute (RACI) standard method no. 05 06 using the Ceralpha assay kit available from Megazyme [16]. β-amylase activity was determined using RACI method no. 05 07 and the Megazyme Betamyl assay kit, and β-glucanase activity according to RACI method 05 03, using the Malt β-D-glucanase assay kit [16].

Results

Changes in barley germination

Germination tests showed losses of germination energy (GE) at mc above 10% after 3 to 6 months of storage at higher temperatures. For all samples, storage at 30°C and 14% mc resulted in germination being reduced to below 10%, or completely lost after 3 months. *Gairdner* germination improved with storage, except when grain with a mc of 12% was stored at 30°C, or grain with a mc of 14% was stored at 25 to 30°C (Figure 1). For *Gairdner* storage at low mc led to continued improvements in GE and breakdown in water sensitivity, but only storage temperatures above 20°C were sufficient to reach GE greater than 95% (Figure 1 A). At 12% mc, 25°C was the ideal storage temperature for the grain to reach high germination within 3 months of storage. At 20°C GE improved sufficiently after 9 months of storage, but water sensitivity (WS) remained too high (Figure 1 B). At 14% mc storage temperatures had to be 15° to 20°C to preserve or slightly improve germination; temperatures above 20°C led to the deterioration of barley quality (Figure 1 C).

Grimmett showed very different storage characteristics to *Gairdner*. *Grimmett* was already quite mature before storage and displayed low levels of water sensitivity. Storage at 10% mc had little effect on germination, but a slight decrease in germination could be observed after 12 months storage at 30°C. At 12% mc, *Grimmett* deteriorated with increased storage time unless stored at 15-20°C. Storage at 14% mc led to loss of grain quality, although storage at 15°C allowed safe storage for 3 months.

The effects of storage on the germination of *Sloop* were similar to that of *Gairdner*. At low mc, storage improved germination by increasing GE and WS, and at 12% mc; storage at temperatures below 30°C increased germination. Storage at 14% mc decreased germination unless grain was stored cool, but *Sloop* appeared slightly less vulnerable to high mc and temperature storage than *Gairdner*.

Stirling appeared the most robust of the samples tested and maintained its germination except when stored at 30°C for 3 months at 12-14% mc. Storage at 25°C at 14% mc did not affect germination for 9 months, but an additional 3 months of storage caused a loss in germination. *Tallon* in contrast was the most vulnerable sample and needed to be stored below 25°C to prevent deterioration. At 14% mc, *Tallon* could not be stored without loss of germination.

Changes in maltability

Samples that exhibited some degree of dormancy and water sensitivity before storage (*Gairdner, Sloop* and *Stirling*) showed increased maltability after storage at low mc and at or below 25°C. Micromalting results for barley stored at 12% mc and 25°C are summarised in Table 2.

Tallon and *Grimmett* showed decreased germination during storage, which was reflected in a decrease in malting quality. Short term storage at 12 and 14% mc at 30°C, and longer term storage at 14% and 25°C was detrimental to maltability. Storage at lower temperatures of 20°C and 15°C tended to maintain or improve maltability over the long term. *Tallon* and *Grimmett* showed some decrease in maltability when stored at 14% mc and 20°C.

There were no clear trends that could attribute changes in hot water extract, wort viscosity or wort colour to storage conditions. There were however changes in Kohlbach index, wort β-glucan, diastatic power and apparent attenuation limit, which could be related to characteristics of the samples prior to storage, to subsequent changes in grain quality during storage, and to the interaction between in-take quality and storage. Although there was a strong relationship between GE and maltability, the interaction between these factors was complex, and therefore not readily defined. Rapid changes in maltability could largely be attributed to the presence of unmodified or under-modified kernels. Where grain samples were stored at 25° to 30°C at a high mc, no malting data could be obtained due to substantial reductions in GE.

Changes in the Kohlbach Index (KI)

The Kohlbach index (KI) of *Grimmett* decreased with storage (Figure 2). The KI of *Tallon* slightly decreased with storage under conditions that maintained germination, and more rapidly when stored at high temperatures and mc. In *Gairdner*, increases in KI were observed at storage temperatures of 20-25°C and mc of 10-12%, but storage at 30°C decreased KI. Storage at 10% mc at 30°C led to a steady increase in KI that reached a plateau after 12 months. Unlike *Grimmett* and *Tallon*, *Sloop* showed an increase in KI under most storage conditions (Figure 3). Storage at 25°C led to a drop in KI when the grain was stored for more than 9 months, but at 30°C and 10% mc KI increased at a rate similar to *Gairdner*. *Stirling* showed a similar response. KI increased where the germination remained high. *Sloop* exhibited the greatest change with *Gairdner* and *Stirling* showing slight increases. *Tallon* and *Grimmett*, which showed a loss in germination, exhibited a decrease in KI and produced hazy wort.

Changes in Wort β-glucan (WG)

In *Grimmett,* wort β-glucan (WG) remained steady or increased slightly when stored at mc below 14%. Storage at 14% mc and temperatures above 15°C led to an increase in WG content of worts made from the malt (Figure 4). This reflected damage to the germination ability of the grain. Measurements of *Tallon* malt led to similar findings. *Gairdner* displayed some slight decreases in WG at lower storage temperatures and mc, but this trend was reversed with increasing storage time. The increases in measurements at high mc were less pronounced than in *Tallon* or *Grimmett*. *Sloop* samples had much lower WG than other barley samples. There was an overall trend for wort WG to remain stable or decrease slightly with storage, and increases at high mc were much less pronounced than in other samples. In *Stirling* samples there were clear changes in wort WG even under storage conditions that maintained or increased germination. While results were inconsistent, with pronounced drops after 3 months of storage at 15 to 25°C, there was a general trend for wort WG to increase for 6 months and then drop again as storage continued. Overall, the level of WG in the wort decreased with storage.

Changes in Diastatic Power (DP)

In *Grimmett*, diastatic power (DP) initially increased with storage at 10% mc, but dropped as storage progressed. At 25°C, the change in DP varied with an overall decreasing trend in measurements. At 30°C DP decreased, but this trend needs to be seen in the context of germination loss. Samples of *Tallon* showed an increase in DP when stored at 15°C. Higher storage temperatures generally led to decreases in DP, but in low mc samples recovery was observed following longer-term storage. *Gairdner* had similar characteristics, but

maintained higher DP values after storage at 14% mc. The DP of *Sloop* increased with storage at 10% mc for 12 months. Increases were also apparent at 12% for storage of up to 6 months, but this was followed by reduced DP values (Figure 5). *Stirling* stored at 10 to 12% mc at temperatures of 15 to 25°C, DP increased over 6 months of storage and then decreased with further storage. Overall, there was an increase in DP where germination increased with storage. DP decreased concurrently with germination in *Tallon* and *Grimmett*.

Changes in the Apparent Attenuation Limit (AAL)

The apparent attenuation limit (AAL) of *Grimmett* remained relatively constant in samples stored at 15°C. At higher storage temperatures, there was a trend for AAL to decrease slightly over 6 months of the storage. This was followed by a slight increase over the next 6 months in samples with mc of less than 14% (Figure 6). Results for *Tallon* were similar, but the decrease in AAL at 14% mc was more pronounced. *Gairdner* displayed minimum changes in AAL with storage time, with a slight increase at higher storage temperatures. Similar findings were made for *Sloop*. *Stirling* showed a slight increase in AAL with storage time under most of the conditions tested.

Changes in enzyme activity

Malts of the five varieties analysed displayed considerable differences in enzyme activity. *Grimmett* had higher levels of α-amylase than *Sloop*, *Stirling* or *Tallon*, and higher levels of β-amylase and β-glucanase than other samples. *Tallon* had low levels of β-amylase activity, and *Stirling* had low concentrations of β-glucanase.

Storage conditions of the grain were shown to influence enzyme activity of the malts. At 12% mc, it became apparent that enzyme activity of *Sloop* and other varieties depended on storage temperature and time (Table 3, Figure7). Germination of *Sloop* was found to increase with storage time and temperature, and the resulting α-amylase and ß-glucanase enzyme activity followed this trend (Table 3). Similar increases in enzyme activity were seen in other varieties stored at 10%. Samples stored at 14% mc showed an initial increase in enzyme activity with storage, but at 25°C and 30°C activity decreased.

At 15°C, the α-amylase activity of *Sloop* decreased with storage time (Table 3), while the activity of *Stirling* increased after 3 month of storage and then dropped below initial concentrations. *Tallon* increased after 9 months of storage, but subsequently increased to pre-storage levels. The activity of *Grimmett* remained relatively constant. The β-amylase content of *Stirling* and *Tallon* increased with time; however, there were no clear trends for *Grimmett*. β-glucanase activity of *Grimmett* and *Tallon* remained high, while activity in *Stirling* remained low, and that of *Sloop* increased slightly.

At 25°C, the α-amylase content of samples were similar to storage at 15°C. In *Sloop,* the activity of α-amylase increased (Table 3). For other samples, α-amylase activity remained relatively constant. The β-amylase activity of *Tallon* was more variable at 25°C compared to 15°C; otherwise activities of β-amylase and β-glucanase were similar at both temperatures. β-amylase activity in *Grimmett* was reduced over 12 months, while activity in *Stirling* remained relatively constant. The β-glucanase activity of *Grimmett* was found to decline at 25°C, while the activity of *Tallon* remained close to levels measured before storage.

After 12 months of storage at 30°C, α-amylase activity of all samples except for *Tallon* was higher than before storage. For β-amylase, *Tallon* and *Sloop* displayed an increase in activity over a 12 month period, while *Stirling* had a spike in activity after 3 months and increased its activity over 12 month. *Grimmett* displayed irregular spikes and troughs, but showed decreased activity after 12 months. Finally, there was a tendency for an increase in β-glucanase activity during storage at low mc and high temperature. However, activity of *Tallon* was higher after 12 months then before storage, and peak activity of *Gairdner* occurred after 9 months of storage (Figure 6).

Table 1. Samples used in storage trials

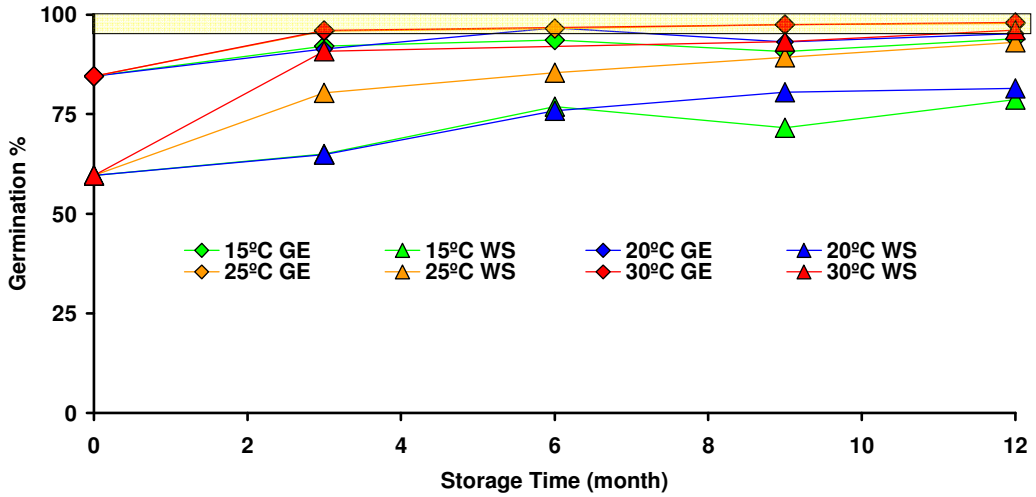
Sample	Origin	Harvest	MC (%)	GE	WS
Gairdner	SA	2001/02	11.7	76.1	49.9
Grimmett	Qld	2002/03	11.1	98.0	93.5
Sloop	SA	2001/02	11.8	64.7	37.8
Stirling	WA	2001/02	12.1	85.0	71.5
Tallon	Qld	2002/03	11.1	96.2	91.9

Table 2. Micromalting data of three barley varieties stored for 12 months at 12% mc and 25°C

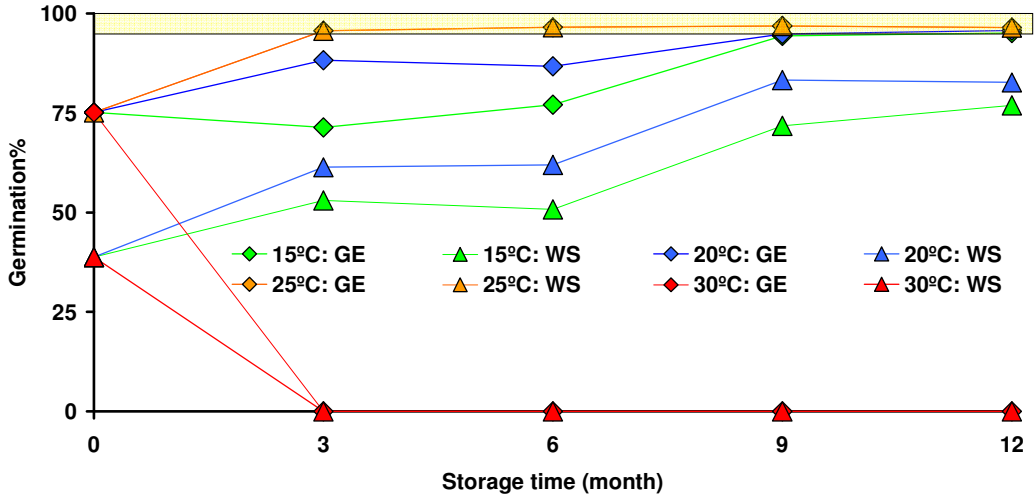
Variety	Storage (months)	GE (%)	KI	AAL (%)	ß-Glucan (mg/L)	DP (WK)
Gairdner	0	75.2	37	80.1	333	261
	3	95.6	37	80.4	253	278
	6	96.5	41	80.3	195	289
	9	96.9	39	79.9	290	282
	12	96.5	39	80.5	319	282
Sloop	0	75.3	44	78.5	170	254
	3	97.9	44	81.2	114	261
	6	98.5	48	81.2	92	282
	9	99.5	49	81.8	112	278
	12	99.9	55	82.2	136	243
Stirling	0	89.8	30	74.9	628	226
	3	99.5	30	77.3	351	243
	6	99.8	31	77.0	520	261
	9	99.5	32	77.5	497	208
	12	99.8	33	78.4	589	243
Tallon	0	97.4	40	78.9	401	184
	3	94.5	40	78.6	400	149
	6	87.6	37	76.8	369	173
	9	80.4	38	77.4	390	156
	12	68.9	34	76.4	445	156
Grimmett	0	98.0	42	80.7	211	254
	3	96.6	41	80.7	286	226
	6	93.4	38	79.2	297	229
	9	89.1	38	78.1	375	215
	12	78.8	35	77.7	404	198

Table 3. Enzyme activity of malt from barley variety *Sloop* stored for 12 months at 10% mc.

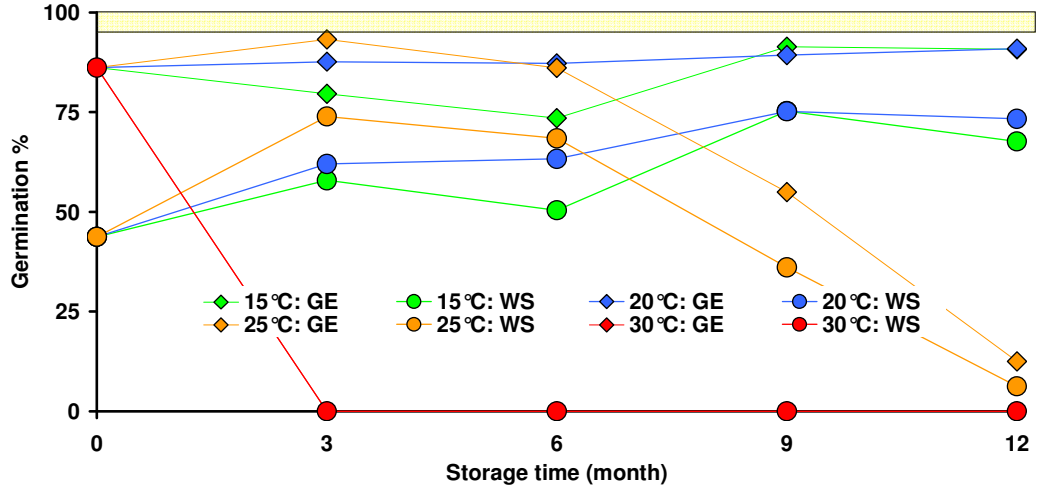
Temperature (˚C)	Time (months)	GE	GI	α-amylase (U/g malt)	β-amylase (U/g malt)	β-glucanase (U/kg malt)
	0	88.3	6.5	155	666	279
15	3	93.0	7.6	147	817	236
	6	94.7	7.2	126	631	297
	9	94.0	6.8	146	752	327
	12	97.4	7.1	121	442	424
20	3	93.2	7.3	155	767	250
	6	97.2	7.6	165	646	336
	9	94.9	7.6	161	756	330
	12	97.2	7.7	184	385	468
25	3	97.5	8.3	170	922	280
	6	99.6	8.5	194	668	363
	9	98.9	8.8	192	700	357
	12	99.4	8.7	223	687	523
30	3	98.9	8.7	167	637	274
	6	99.6	9.2	189	644	312
	9	99.3	9.5	219	681	431



A: 10% mc



B: 12% mc



C: 14% mc

Figure 1. Germination Energy (GE) and Water Sensitivity (WS) of *Gairdner* stored at 10, 12 and 14% mc. The narrow yellow band in the graph indicates 95-100% germination.

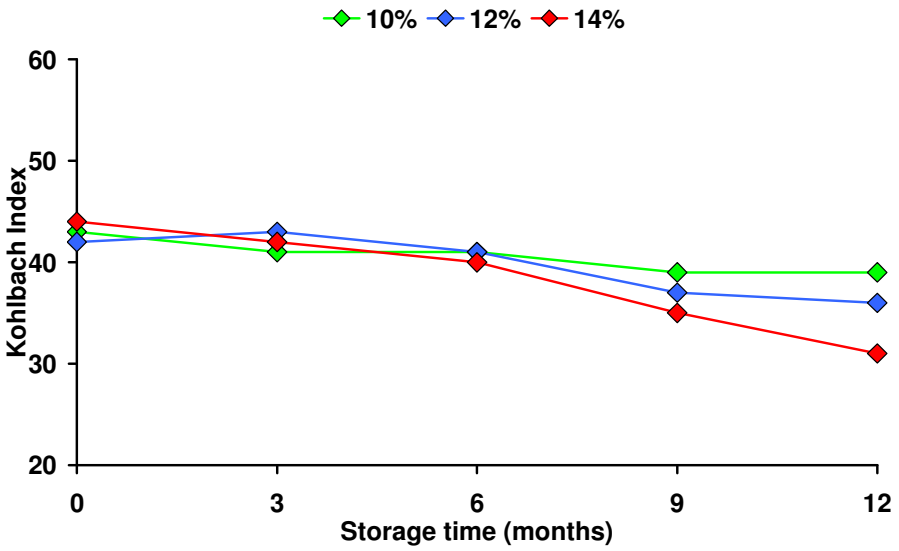


Figure 2. Kohlbach Index of malt derived from *Grimmett* stored at 20°C

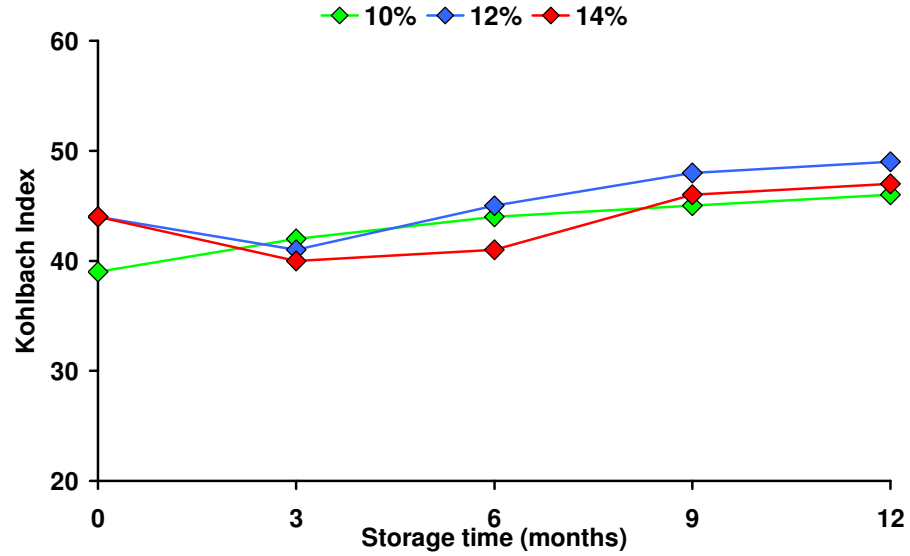


Figure 3. Kohlbach index of malt derived from *Sloop* stored at 20°C

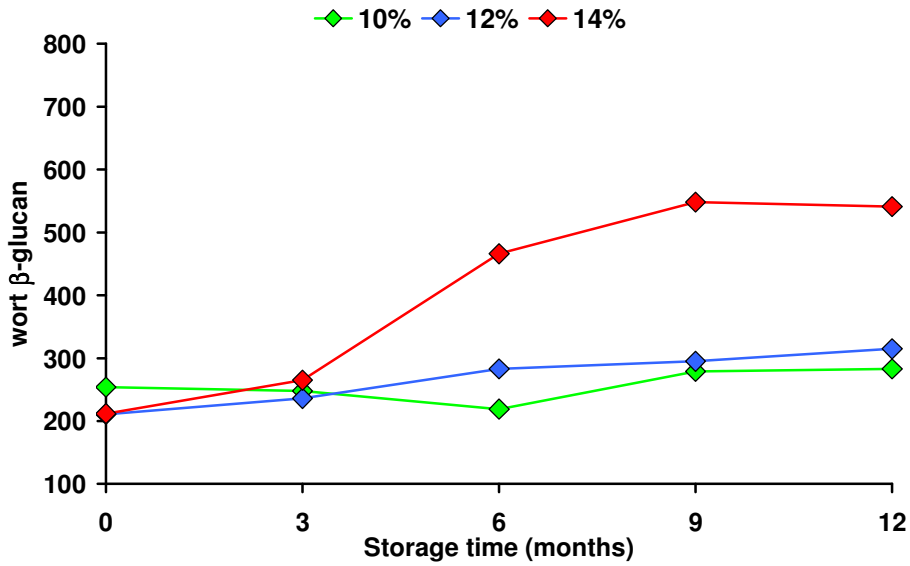


Figure 4. Wort β-glucan of malt derived from *Grimmett* stored at 20°C

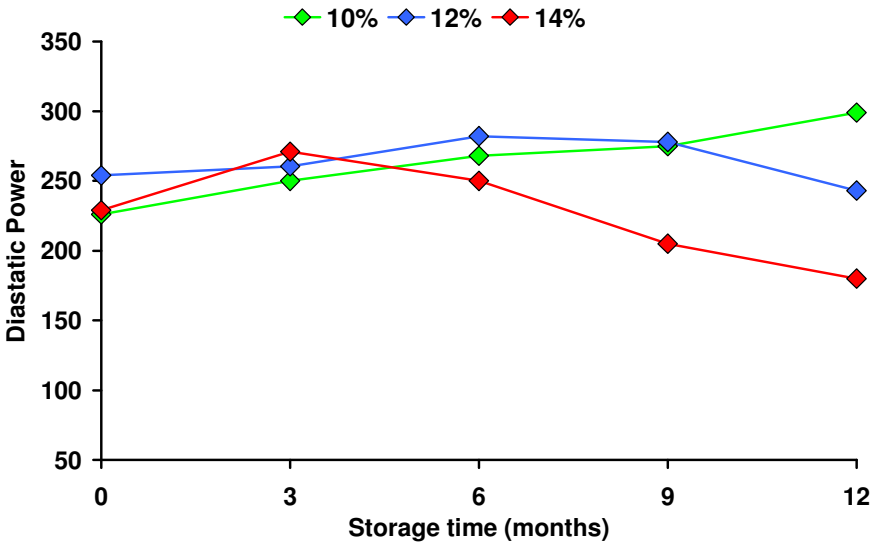


Figure 5 Diastatic power of malt derived from *Sloop* stored at 25°C.

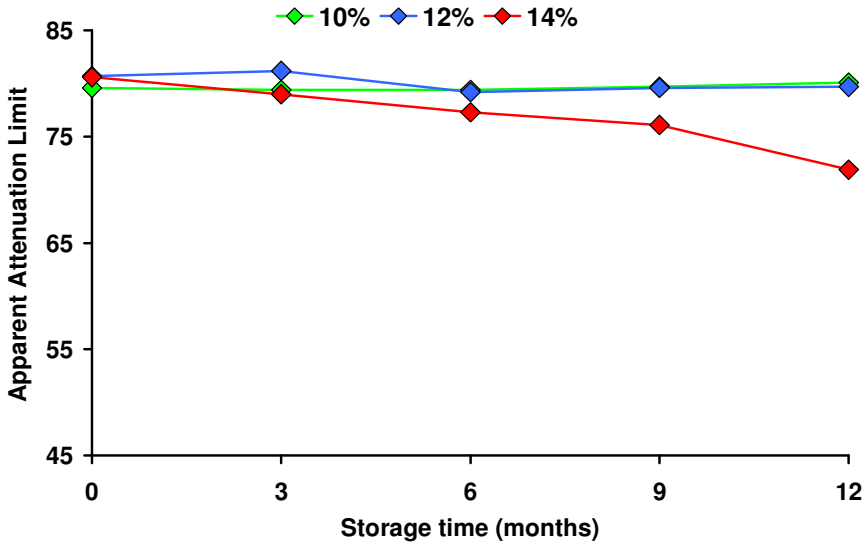


Figure 6 Apparent attenuation limit of malt derived from *Grimmett* stored at 20°C.

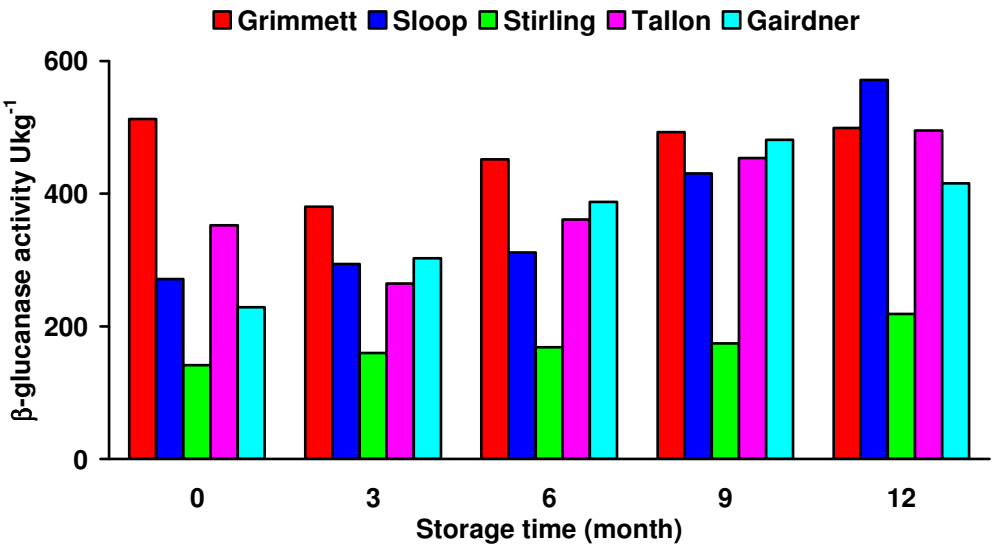


Figure 7. β-glucanase activity of malt derived from barley samples stored at 30ºC and 10% mc

Discussion and Conclusions

There have been substantial efforts in understanding barley germination ability and subsequent malt quality [7, 8, 9]. We have shown that, given the correct storage parameters are maintained, germination of Australian barley varieties can be improved during storage under Australian conditions. In barley with no dormancy and minimal water sensitivity, changes in quality were more linear than changes in barleys that expressed dormancy or water sensitivity. In immature samples, trends in bio-deterioration processes were mitigated by activities related to the grain reaching its full maturity, and consequently its maximum commercial value.

The breakdown of water sensitivity and increase in GE observed were consistent with findings by Briggs [7] and Woonton et al [8]. The storage potential however was dependent on the type of barley stored. *Gairdner* and *Sloop* germination increased at low mc at storage temperatures above 20ºC, as water sensitivity progressively broke down. In contrast, *Grimmett* germination declined under similar conditions. *Stirling* was very robust and maintained its germination even under high temperature storage at 14% mc, while *Tallon* had to be stored below 25ºC to prevent a loss in barley quality. The maturity of grain at harvest was shown to be an important factor, as samples with high water sensitivity and dormancy were less vulnerable to germination loss. If dormancy and water sensitivity levels were known, it would be possible to improve the germination of freshly harvested barley by matching storage conditions and barley characteristics.

The maltability of samples with water sensitivity and dormancy can also be improved by storage under favourable conditions. *Gairdner*, *Sloop* and *Stirling*, all samples with some level of dormancy and water sensitivity, showed improved maltability after storage, while *Tallon* and *Grimmett*, samples with high pre-storage germination, decreased in maltability under most storage conditions. Importantly, storage conditions that improved the GE of samples did not necessarily improve maltability, and longer term storage under mild conditions generally resulted in better malting outcomes. Although the relationship between hot water extract, wort viscosity, wort colour and storage was unclear, storage did affect the KI, WG, DP and AAL of malt. The maltability of samples was dependent on the characteristics of the sample before storage which reflected the response obtained for germination. Changes in germination and maltability therefore need to be considered in combination to develop optimum storage strategies for breaking dormancy and water sensitivity. At a minimum, germination testing must be carried out at intake. Ideally, monitoring of GE and WS should continue during storage. In practise, cost effective and rapid methods for measuring barley quality are required to allow growers and handlers to make safe and effective decisions on the handling of malting barley. Methods worth investigating include the Rapid Viscosity Analyser, Near Infrared Spectroscopy and electronic noses [19, 20]. Manipulations in storage conditions can be realised on-farm or in the bulk handling system by delaying aeration cooling for a short period, or by raising the temperature of grain bulk by aeration with warm ambient air, followed by cooling after dormancy is broken.

The enzyme activity of malted samples varied considerably before storage and then changed depending on storage conditions. In dormant grain, α-amylase and β-glucanase changed with storage, β-amylase measurements showed no clear trends. This reflected the fact that β-amylase is deposited in the endosperm during grain development, while α-amylase is very actively synthesized during germination [21]. Enzyme activity decreased at higher mc and temperatures concurrent with germination loss. As with germination and malt quality, changes in enzyme activity were highly dependent on the sample. At lower storage temperatures, α-amylase activity decreased in some samples, but initially increased in other samples. β-amylase increased in *Stirling* and *Tallon* under some storage regimes, but in *Grimmett* no clear trends could be observed. When stored at 25°C, the β-glucanase activity of *Grimmett* was reduced over 12 months, while the activity of Stirling was more constant at 25°C than at lower storage temperatures.

In view of these results, to appropriately store malting barley, the GE and WS of each particular batch of grain needs to be known before storage. An understanding of the vulnerability of particular cultivars to damage in storage will assist in increasing and preserving value. Barley with pronounced levels of dormancy and/or water sensitivity can be safely stored at temperatures between 25° and 30°C for short periods to increase germination. Care must be taken to monitor germination during storage and moisture content should be kept at or below 12%. Dormant barley stored at low temperatures may not reach optimum malting quality, even over a 12 months period.

The storage options reported here need to be evaluated in on-farm storage situations. To safely and effectively store barley, the options and restraints resulting from varietal and environmental characteristics of batches of grain need to be better understood. In this context, ongoing research into the post harvest handling of barley varieties, with particular reference to Genetic x Environmental type interactions, and its effect on germination and maltability is essential.

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