



## Storage induced colour changes of barley grain

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# Abstract

Maltsters consider discolouring of barley a sign of microbial infection or weathering. Consequently, kernel colour is a criterion in the purchase of malting barley. Kernel colour also plays a role in determining the quality of barley for overseas markets, with the customer's colour preferences dependent on the end use of the grain. The effect of storage conditions on the colour of barley is therefore of considerable interest. Malting barley was stored at 25, 35 and 45°C for 104 weeks to investigate the relationship between barley colour and storage conditions. Samples were taken at regular intervals, and the colour of whole kernels was measured with a colorimeter. Aqueous extracts of the same samples were analysed with a scanning spectrophotometer. Finally, the browning products of the extracts were analysed by HPLC. Colour changes of barley in storage were pronounced and temperature dependent. Barley darkened and yellowed in storage. Differences between samples stored at different temperatures became quickly apparent. These changes were reflected in the absorption spectra of their aqueous extracts. Hydroxy methyl furaldehyde (HMF) was identified in those extracts and found to accumulate in short-term, high temperature storage and in long-term storage at moderate temperatures. In conclusion, storage of barley leads to measurable changes in the colour of barley, which may reduce the commercial quality of the commodity.

# Introduction

Kernel colour is a criterion in the purchase of malting barley, barley used to produce foods for human consumption other than malt, and feed barley. Maltsters perceive kernel discolouration as a sign of microbial infection and weathering (Goblirsch *et al.* 1996). The growing popularity of barley as a cereal food (eg as a rice extender) and the promotion of barley based functional foods has created interest in aspects of barley quality, including kernel colour (Doi and Tohnooka 1995, Bhatta and Rossnagel 1998). In feed barley, colour is considered an aesthetic factor and indicator

of microbial infection and has an impact on price (Edney *et al.* 1998). Some research has been carried out on the effect of storage on the colour of rice (Gras *et al.* 1989, Gras *et al.* 1990, Soponronnarit *et al.* 1998). Limited information on storage induced colour changes in legumes (Hughes and Sansted 1975, Iaderoza *et al.* 1989) and wheat flour (Srivasta and Rao 1994) is available. However, very little information is available on the effect of storage on kernel colour. The aim of this paper was to investigate the effect of storage temperature on barley kernel colour.

# Materials and Methods

## Storage of barley samples

The malting quality barley used in the trial was of the variety 'Arapiles'. The grain was in good condition with no weather damage, moulding or insect infestation. Germination rate was >99%. Samples of seed moisture content (mc) 12.1% were stored in sealed glass jars at 4°, 25°, 35° and 45°C. Sub-samples were taken on a fortnightly basis for 10 weeks and again at 28 and 104 weeks.

## Barley extracts

Approximately 2 g of barley grain was extracted into 25 mL of water for 48 hours. After extraction, samples were clarified with syringe filters and immediately measured by spectrophotometry or analysed by HPLC. Extraction of whole grain was the simplest and quickest method to use and the aim of the experiments was to monitor changes rather than develop an analytical method that quantitatively recovered Maillard Reaction Products (MRP) from grain. Some of the extraction procedures for MRP and other brown pigments have been reviewed by Poretta (1992).

## Kernel colour measurement

A Minolta CR-310 Colorimeter with build-in light-source was used for all measurements of kernel colour. Measurements of kernel colour have been expressed in the CIELAB colour space (Hunter and Harold 1987). Barley colour was measured at the beginning of the experiment (n = 20) and then in duplicate after 2, 4, 6, 8, 10, 28 and 104 weeks of storage (n = 70 per temperature).

## Spectrophotometry of grain extracts

All measurements were carried out in 1 cm cells using a Shimadzu UV-2401 spectrophotometer. Wavelengths from 200 to 800 nm were scanned at 550 nm min<sup>-1</sup>. Sampling intervals were 0.5 nm and slit width was 1 nm.

## HPLC analysis of HMF

Separation was performed on a 250 × 4.6 mm Alltima C18, 5 μ reversed phase column. The mobile phase was water and acetonitrile 95 + 5. (v/v) delivered by a Beckman System Gold Programmable Solvent Module 126 at a flow rate of 1 mL min<sup>-1</sup>. Absorption was measured at 280 nm with a Beckman System Gold Programmable Detector Module 166 UV detector. Peaks were identified by comparison to standard injections of 5-Hydroxymethyl-2-furaldehyde (HMF) (Source: Fluka Chemika) diluted in mobile phase. Concentrations of HMF were calculated from peak areas by comparing the response of the extracts to that of a standard curve.

# Results and discussion

## Kernel colour

Grain lightness decreased with increasing temperature and time (Figure 1A). At the beginning of the storage period, the L\* value of the stored barley averaged 62.4 (sd 0.50, n = 20). The grain that was stored at 25°C did not deviate from this value. However, the grain stored at 35°C and 45°C did decrease in lightness. There was a particularly sharp drop in lightness between weeks 10 and 28 weeks. This trend was more pronounced at 45°C than at 35°C.

The redness (a\* value) of grain increased in storage at all three storage temperatures (Figure 1B). Before storage, the a\* value of the barley samples averaged 3.0 (sd 0.05, n = 20). During storage, the value increased with the largest increase at the highest temperature (Figure 1B). At 25°C, a\* had increased to 3.5 (sd 0.06, n = 10) after 28 weeks of storage. Storage at 35°C led to a slight increase during ten weeks of storage and a pronounced increase after 28 weeks (4.3, sd 0.04, n = 10). At 45°C, a\* reached 3.9 (sd 0.06, n = 10) after two weeks of storage. Values increased with further storage (with the exception of a drop at eight weeks) and reached 5.4 (sd = 0.07, n = 10) after 28 weeks.

As with redness, the yellowness (b\* values) of barley increased in storage (Figure 1C). Up to ten weeks of storage, barley stored at 45°C was clearly more yellow than grain stored at lower temperatures, but the value decreased again in the longer term. Before storage, the b\* value of the barley averaged 21.0 (sd 0.16, n = 20). At the lowest temperature, the b\* value increased slightly until it reached 22.8 (sd 0.27, n = 10) after two years storage. At 35°C, the change was more pronounced. A slight decrease was seen after eight weeks storage. After the value had increased to 24.0 (sd 0.18, n = 10) after 28 weeks of storage it remained relatively constant. At 45°C, b\* values peaked after 10 weeks of storage (24.9, sd 0.18, n = 10) and then decreased with further storage.

Colour changes in barley were comparable to literature data reported for the effect of storage on the colour in rice (Gras *et al.* 1989, Soponronnarit *et al.* 1998), beans (Hughes and Sansted 1975, Iadederoza *et al.* 1989) and wheat flour (Srivasta and Rao 1994). Darkening of barley in storage was a good indicator of exposure to unfavourably high storage temperatures. The red-green axis is not usually used in

assessing the yellowing of commodities such as rice, as its usefulness is considered limited (Gras *et al.* 1990). However,  $a^*$  value appears to be a useful measurement for monitoring colour change of barley.

## Spectrophotometry

The wavelength of maximum absorption ( $\lambda_{\max}$ ) of barley water extracts prior to storage was 291 nm. The extracts taken from barley stored at 45°C showed an increase in wavelength becoming pronounced after 8 weeks and developing further with storage. Colour change of barley kernel extracts was found to be close to exponential. This made it likely that the rate of change was primarily dependent on the reaction of chemically alike compounds undergoing similar reactions. After two years storage at 45°C, the  $\lambda_{\max}$  of the extract had increased from 291 to 295 nm. Characteristics of extraction spectra suggested that these changes in absorbances could be due to browning reactions of the Maillard type (Richards 1956, Stenberg and Geddes 1960, Scott 1964).

## HPLC analysis of HMF

Hydroxy methyl furfuraldehyde (HMF) is formed at an early stage in non-enzymatic browning reactions and has been used to measure damage due to excessive heating of cereals (Poretta 1992, Albalà-Hurtado *et al.* 1997). It seemed likely that it could fulfil a similar function for assessing the effect of high temperature storage on barley grain. It was found that HMF accumulated rapidly during storage of barley at 45°C. After 8 weeks of storage at 45°C, 0.8  $\mu\text{g g}^{-1}$  (sem 0.11, n = 3) HMF had accumulated in the extracts. This increased to 5.6  $\mu\text{g g}^{-1}$  (sem 0.15, n = 3) by 10 weeks of storage and after two years reached 13.6  $\mu\text{g g}^{-1}$  (sem 0.21, n = 6). At the lower temperature, no HMF was detected in the short term, but after two years of storage 3.0  $\mu\text{g g}^{-1}$  were found in extracts from grain stored at 25°C and 35°C.

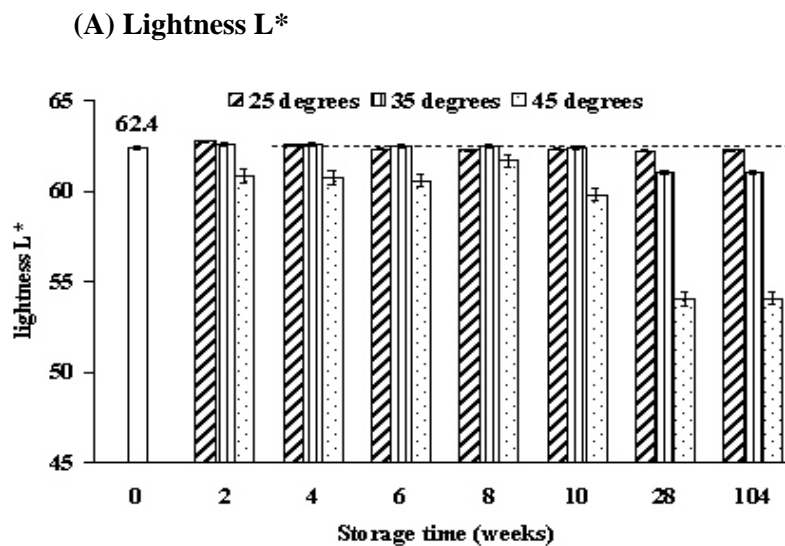
Accumulation of HMF due to heating has been reported for a number of products, including cereals (García-Villanova *et al.* 1993, Albalà-Hurtado *et al.* 1997, Kruskop 1998). Here the occurrence of HMF during the storage of barley under conditions commonly found in Australia was demonstrated. The accumulation of MRP in high temperature storage indicated storage conditions deleterious to seed quality. This agreed with the findings of Gras *et al.* (1989) who suggested a link between Maillard reactions and yellowing of rice in storage.

A relationship between accumulation of Maillard Reaction Products (MRP) and loss of viability has been previously reported for some commodities (Sun and Leopold 1995, Rao and Kalpana 1994). The potential nutritional implications of Maillard reactions in cereal storage are serious (Kim *et al.* 1984, Reddy and Pushpamma 1986). As shown by the rapid accumulation of HMF at 45°C, loss of biologically available amino acids from barley is likely in short term storage at high temperatures. In the longer term, even at 25, there was evidence of ongoing Maillard reactions; therefore, the nutritional value of barley was most likely reduced in storage. The possible effects of MRP on excretion, absorption and storage of minerals such as zinc (O'Brian and Morrissey 1989 and others) and their potential toxicity to animals (Lee *et al.* 1981 and others) are also of concern.

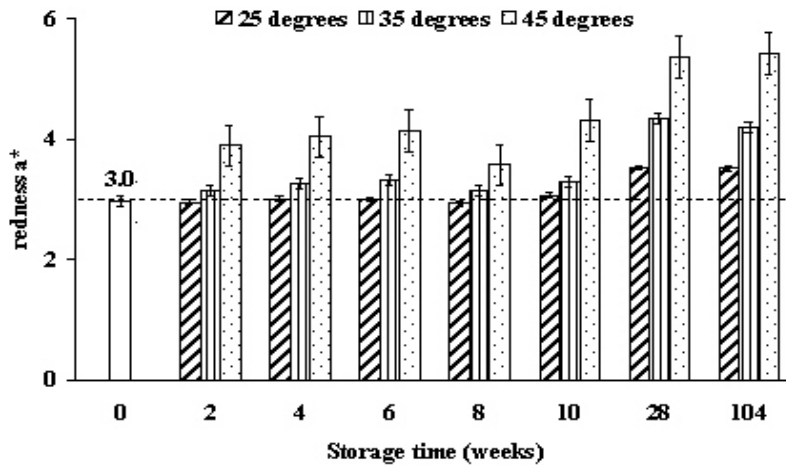
# Conclusion

Storage of barley caused measurable changes in the colour of barley grain, reducing the commercial quality of the commodity. Barley kernels darkened and yellowed in storage. These changes were reflected in the absorption spectra of the aqueous extract of whole barley grain. Hydroxy methyl furaldehyde was found to accumulate in short-term, high temperature storage and in long-term storage of barley at moderate temperatures. This most likely reduced the nutritional quality of barley grain.

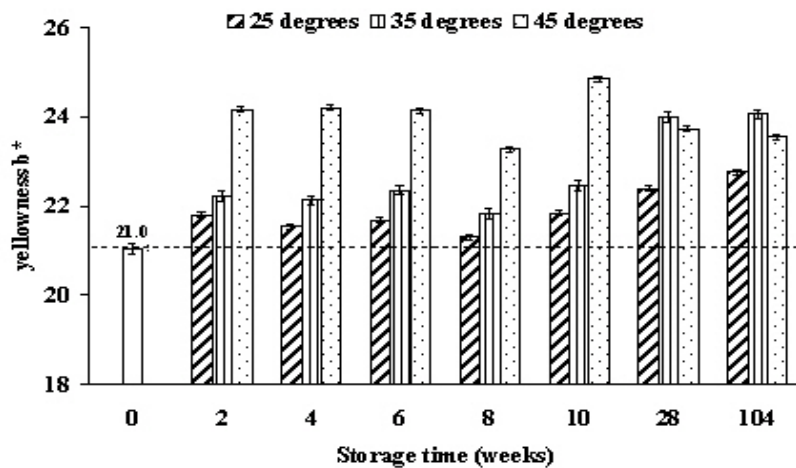
**Figure 1: Changes in the colour of whole barley stored under laboratory conditions for 104 weeks at three different temperatures.** Error bars show standard error of the mean (n = 70).



(B) Redness a\*



(C) Yellowness b\*



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