

# Barley and Malt Proteins and Proteinases: III. A Simple Method for Estimating the Combined Actions of Malt Proteinases and the Extent of Protein Degradation during Malting

Abdalla M. Osman

## ABSTRACT

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The activities of barley and malt proteinases have been measured using haemoglobin and the highly degradable barley protein fraction (HDBPF) in malting and feed barley varieties. In conjunction, the barley and malt total protein and its components: hordein, glutelin, soluble proteins and free amino nitrogen (FAN) as well as Kolbach index were investigated. The comparative analysis of results revealed that the general grain modification index of Kolbach (KI), which was higher in malting varieties, was much more strongly associated with the levels of hordein degraded during malting than any other parameter investigated. The KI levels were also correlated with the increase in the levels of FAN, but not with the increase in the levels of soluble protein or changes in the glutelin component. The changes in total proteinase activity were low and cannot account for the increase in KI or the degraded hordein. The levels of total proteinase activity in both feed and malting barley varieties were similar.

The results suggest that estimation of the levels of degraded hordein, during malting, is a sensitive indicator of the total proteolytic action of proteinases as well as the degradability of the reserve proteins. Therefore, we recommend measuring the amounts of hordein degraded during malting for the assessment of the impacts of grain protein and proteinases on malting barley quality of different varieties, in addition to KI and FAN.

**Key words:** Barley, degradability, HDBPF, hordein, malt, protein, proteinases.

## INTRODUCTION

Barley is the preferred choice grain for brewing because of its desirable natural components and the technologically advantageous features that complement the technology used in the malting and brewing industry<sup>10,12</sup>. However, not all barley grain varieties are equally suitable for malting and brewing. Maltsters and brewers use a variety of methods to evaluate the quality of their barley and malt. These include the methods of estimating the levels of the hydrolytic enzymes such as the starch degrading and

cell wall degrading enzymes. The desire to also include the measurement of the levels of the protein degrading enzymes has been frustrated by the lack of suitable natural barley protein substrates and the effect of inhibitors<sup>5,10,17</sup>.

Usually the proteinases are assayed using synthetic or animal protein substrates, casting doubt on whether the results would be the same as using the natural barley protein substrates<sup>4,18</sup>. Attempts to rectify this unsatisfactory situation were not always successful<sup>3,11,18</sup>. In this series, we reported a new method using a highly degradable barley protein fraction (HDBPF) as the natural substrate of choice. The results with this and other protein substrates indicate that the levels of proteinase activities in all malting barley varieties investigated are very similar and do not reflect the grade of barley quality or its variations with total grain protein content<sup>13,18</sup>.

In contrast, the amount of protein (hordein) degraded during malting seems to be much more variable and associated with FAN and KI<sup>15–17,19,21</sup>. This is not unusual as both the enzyme and the substrate can affect the catalytic process (see the first paper in this series). In the case of the malting barley it seems that the variation in the proteolytic process was more affected by the protein substrate than the proteinases. It is possible that varietal quality difference is caused by the variations in the amounts of readily degradable hordeins, inhibitors or the effect of different proteinase products<sup>5,7,8,15</sup>.

In addition, it has been shown that the use of barley and malt hordein in calibration of NIR machines gave more accurate prediction values for barley and malt protein estimation than the use of other protein standards<sup>4</sup>.

The objective of this study was to find an alternative method, which will measure the total activity of all proteinases and the protein modification during malting, simultaneously. Estimation of the quantity of hordein degraded during malting was found to meet this requirement, appropriately. We report here, the procedure to extract and estimate the quantity of hordein degraded during malting.

## MATERIALS AND METHODS

### Barley and malt

Four malting barley (Franklin, Grimmett, Schooner and Tallon) and three feed barley (Mackay, Tantangara and Valier) samples were obtained from the Northern Barley

Leslie Research Centre, Farming Systems Institute, Queensland Department of Primary Industries, Agency for Food and Fibre Sciences, P O Box 2282, Toowoomba, QLD 4350, Australia  
Corresponding author. E-mail: Abdalla.Osman@dpi.qld.gov.au

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Improvement Program in Australia. The samples were micro malted using automatic Phoenix micromalter according to the Australian standard procedure<sup>2</sup>. Barley and malt samples were finely ground using a Buhler Maig mill (0.2 mm)<sup>14</sup>.

### Chemicals

All chemicals used in this study were of the highest analytical grade, purchased from Sigma-Aldrich, Australia.

### Estimation of the quantity of hordein degraded during malting (Australian malting)

**Extraction of hordein from barley and malt.** Hordein was extracted from both barley and malt flours (at 60°C) in 70% ethanol containing 0.5% dithiothreitol (DTT) after the removal of water and salt soluble proteins. Two grams of barley or malt flour were weighed into three centrifuge tubes for each variety. Six mL 0.1 M NaCl was added to each tube, mixed with vortexing at 5 min intervals for 30 min at room temperature and centrifuged at 4000g for 20 min. After centrifugation the supernatants were collected for globulin measurements and the pellets were washed with 8 mL distilled water and centrifuged, as before. Again the supernatants were collected for albumin measurements and the pellets were resuspended in 5 mL 70% hot (60°C) ethanol containing 0.5% DTT and kept in a water-bath at 60°C, with vortexing every 5 min for 1 h. Then the extracts were centrifuged as before. This time the supernatants were carefully collected into pre-weighed and marked centrifuge tubes and the pellets were reused for the extraction of glutelins<sup>15</sup>.

**Sodium chloride precipitation.** To each tube with alcohol extract of hordein, 5 mL ice-cold 1 M NaCl was added, thoroughly mixed and kept at 4°C for 1h. The precipitated hordein was separated with centrifugation. The supernatants were discarded and the precipitates were washed with distilled water. The washed tubes were allowed to drain by standing them on filter paper. Then the hordein containing tubes were dried in the oven, overnight, at 110°C. By weighing and subtracting the empty tubes weights, barley and malt hordeins were measured.

**Calculation of the quantity of the degraded hordein fraction.** Next morning all the tubes were weighed and the weights recorded. The mean values for barley and malt of each variety were calculated. The quantity of degraded hordein was calculated by subtracting the mean malt hordein values from those of the corresponding mean barley values, as follows:

$$\begin{aligned} \text{Degraded hordein} &= \text{Barley-hordein} - \text{Malt-hordein} \\ &= \text{-- mg/g} \end{aligned}$$

Moisture data can also be incorporated easily in the above equation, by calculating each mean weight per dry weight unit.

### Enzyme assay

Proteinases were assayed with HDBPF and haemoglobin as substrates, according to the previously described procedure<sup>13</sup>.

### Estimation of soluble proteins and glutelin

The levels of barley and malt soluble protein and glutelins were measured using the colour method of Folin and Ciocalteu's reagent<sup>9,14</sup>.

### Measurement of FAN and KI

Both FAN and KI were measured using the recommended EBC methods<sup>1</sup>.

## RESULTS AND DISCUSSION

The results of the comparative study of the total proteinase activity, measured with haemoglobin (EP/Hb) and highly degradable barley protein fraction (EP/HDBPF), the total barley grain protein (G. protein) and its composites in the two groups of feed and malting barley varieties are summarised in Fig. 1. In this investigation we compared the mean values of total grain protein content, which were very similar in both groups, while the rates of modifications (KI) were highly different. Similarly, the mean levels of the degraded hordein were significantly different between these two groups. The latter is in full agreement with the widely accepted fact that the stored hordein fraction is the major component of protein degraded during malting<sup>15,18,20</sup>. However, it is not clear why in similar contents of total barley grain protein, the levels of degraded hordein are so different. It is possible within the equal total protein; the amounts of different composites are different. Nevertheless, it appears that the KI values and the levels of hordein degraded (LHD) are closely associated.

These results suggest that the total protein content does not seem to be the cause of the discrepancy in the KI and LHD values between the two barley variety groups. Moreover, the total malt proteinase activity measured with either haemoglobin (Hb) or barley protein (HDBPF) does not appear to explain the difference either. Although the total proteinase activity was slightly less in the feed group it cannot account for the magnitude of variation in KI or LHD levels. In addition, the levels of increase in the soluble protein and FAN components were different in the two groups of barley. While the soluble protein levels were slightly higher in the feed group, the increase in FAN levels was disproportionately lower. It is surprising the sum of soluble proteins and FAN in the two groups, although arithmetically equal or very similar; do not have the same effect on KI. This reflects the fact that feed varieties, usually, have higher total protein contents and that is why although the feed and malt groups have similar combined soluble protein and FAN contents, their KI values are different. This may also suggest that the levels of exoproteinases might be involved in causing the difference. Although, it is generally believed that endoproteinases are the rate limiting enzymes and not exoproteinases<sup>13,19</sup>.

It is clear from these results that the interaction between the barley grain proteins and the proteinases is much more complex than any other grain component and their degrading enzymes. Proteins and the respective proteinases have the largest variation in form, structure, functional specificity, distribution and complexity. Therefore, it is not surprising that their estimation is also complex.

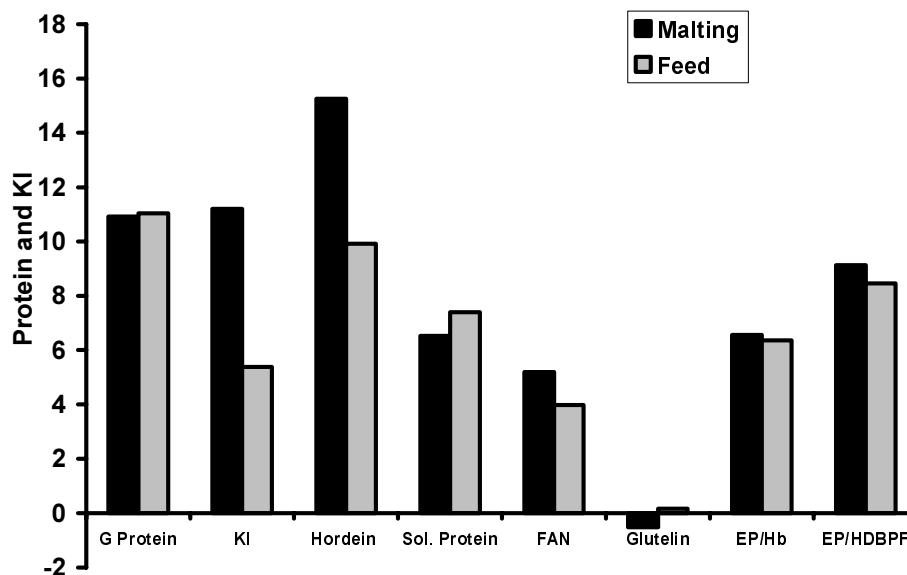


Fig. 1. The mean values of grain protein (%), KI the percentage levels above 30%, hordein degraded during malting (mg/g) and the increases in the levels of soluble proteins (mg/g), FAN (mg/10 g), glutelin (mg/g) and proteinases measured with haemoglobin (EP/Hb) and with highly degradable barley protein fraction (HDBPF) in units (U/g).

It was contemplated that the use of natural barley protein substrates for the assay of proteinases will offer the chance to discriminate between the positive and negative impacts of grain protein and proteinases on malting quality<sup>1,9,12</sup>. However, the finding that barley grain develops very similar levels of proteinase activities, makes it untenable to differentiate between varieties<sup>2,12,17,19</sup>. It appears that much more valuable information can be obtained about the proteins and proteinases by estimating the levels of hordein degraded during malting or germination. The concept is similar to the use of diastase estimation for the diastatic power (DP) for starch-degrading enzymes<sup>13</sup>. It will be possible and easier to germinate a few grams of barley and measure the amount of hordein degraded. This will enable breeders to rapidly and simply test for the effects of grain proteins and proteinases on malting quality.

The use of the degraded hordein measurement will be even more advantageous, if it is combined with NIR technology, the merit of which has already been shown<sup>4</sup>. Furthermore, the use of HDBPF in calibrating the NIR machines, as the standard protein for barley and malt proteins, will enhance the accuracy and closely associate the results from the two methods.

## CONCLUSION

The estimation of the levels of hordein degraded during malting truly reflects the changes in proteins during malting and can measure the difference in barley varieties related to proteins and their degrading enzymes. It will enable one to determine the impacts of proteins and proteinases on malting quality. No other protein component or proteinases offered a similar opportunity for their role assessment. This method is simpler, faster and more specific than KI. Moreover, there is a great opportunity to use this protein (HDBPF) as the standard protein for calibra-

tion of NIR machines, for the estimations of barley and malt proteins. This method is recommended, as a fast and simple technique for the evaluation of the impact of proteins and proteinases on malt and beer quality.

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