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What causes low barley protein modification and low wort free amino nitrogen - proteins or proteinases?

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Abstract

Preliminary data analysis from this study of barley grain proteins, endoproteinases and the extent of protein modification (Kolbach index, KI) during malting confirmed the inverse relationship between the protein content and KI. Similarly the results showed an inverse relationship between the levels of endoproteinase activity and KI, but the magnitude was smaller. The increase in grain protein content and the levels of endoproteinase activity was influenced more by late sowing than the application of higher levels of nitrogen fertiliser. Further investigation of the contribution of the different protein constituents to the increase in grain protein content revealed that the hordein fraction is the most dominant contributor. Hence, it is concluded that the increase in the hordein component accounts for the inverse relationship between KI and protein content.

Levels of free amino nitrogen (FAN) in malt extracts were found to be closely associated with the amounts of protein degraded during malting. In current, readily modifying, commercial malting varieties, about 50 percent of the hordein fraction was degraded while in slower modifying new lines the level of degradation was lower than 50 percent. In the worst case, the level of degradation was as low as 25 percent

Levels of endoproteinase activity were very similar in all varieties investigated. In addition, these levels rose slightly with increases in protein contents. Therefore, it is unlikely that the levels of endoproteinase activity are the cause of slow modification or low wort FAN levels. It is more likely that the content or availability of the fraction of barley protein degraded during malting is the limiting factor. This fraction is genetically controlled and environmental influenced.

Introduction

Commercial malting is the preparatory step to modify barley grain for brewing. It entails the imbibition and the activation of the embryo, the scutellum and aleurone layer. The latter two produce and secrete the hydrolytic enzymes necessary for the mobilisation of the nutrients from the endosperm to initiate and sustain the embryo development. The most important features of grain modification for brewing purposes, apart from the production of hydrolytic enzymes, is cell wall breakdown and protein degradation. Indeed, protein degradation is the origin and precursor of all events during malting. It provides amino acids for the *de novo* synthesis of proteins, including hydrolytic enzymes, and other essential nitrogenous compounds as well as adequately providing for the yeasts' nutritional needs. However, the benefits of protein degradation transcend the nutritional aspects to facilitate the whole process of grain modification. Proteolysis enhances cell wall breakdown by the action of solubilase, liberates starch granules from protein bodies and matrix (Enari and Sopanen, 1986; Palmer, 1989). It also activates enzymes, releases important proteins such as β-amylase, and foam-forming and foam-stabilising proteins (Palmer, 1989; Guerin *et al* 1992 and Evans and Hejgaard, 1999).

Protein degradation during malting is achieved by a multitude of different proteinases, including, endo- and exoproteinases acting in a concerted and specialised manner (Zhang and Jones, 1995). Endoproteinases have been characterised as the key as well as the rate limiting enzymes in the mobilisation of the grain storage proteins. Hordeins constitute about 40 percent of the total grain protein and the bulk of the storage proteins. Only part of the storage proteins, usually equivalent to about 20 percent of the total, is degraded during malting. It is the interplay between the proteins and the proteinases, which determines the rate and the extent of degradation. However, it is not yet clear which of the two has the greater influence on the final outcome. Therefore, the objective of this study is to investigate whether either proteins or proteinases have the greater effect on the rate of protein modification and the level of wort FAN.

Materials and Methods

Barley samples of the cultivars, B%-1302, CMO/KORU-85, CMO/KORU-123 (collectively designated, new lines {NL}), Grimmett, Lindwall, Schooner and Tallon (collectively designated, commercially malted varieties [CMV]), were obtained from Jondaryan site of 1999 season planting (The Northern Barley Improvement Program). These varieties had been treated with three levels of nitrogen fertiliser (90, 120 and 150 kg/Ha) and sowed at two different dates (May and July). Malts were prepared by micro malting according to MBIBTC standard procedure (1995) and total grain protein was measured by the Dumas combustion method.

Extracts of fine-ground samples of barley and malt were used in the measurements of FAN, hordeins, soluble proteins and the enzymes. FAN was determined by the ninhydrin method (EBC, 1997). Hordeins were extracted by warm 70 % alcohol, precipitated by salt, oven dried and weighed. Soluble proteins were filtered through 10 DG Bio-Rad columns and estimated by the Lowry method. Endoproteinases were measured using barley proteins as the substrate.

Results and Discussion

The results of barley grain protein content and levels of malt endoproteinase activity indicate that the sowing date has a more profound effect, especially on protein content, compared to nitrogen fertiliser (Figure 1). Late (July) sowing significantly increased grain protein content compared to earlier (May) sowing. The effect of nitrogen fertiliser was most evident only in the increase at the medium level (120 kg/Ha) of July sowing. In contrast, the effects of both nitrogen fertiliser and the date of sowing were very mild, not exceeding 5 percent, on the levels of endoproteinase activity. The latter rose with

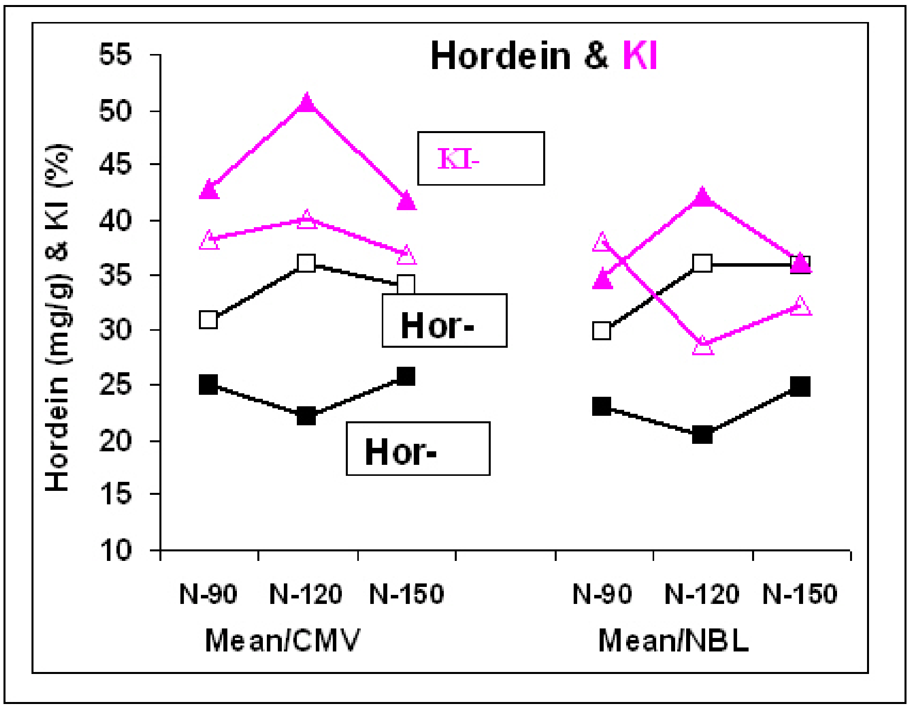

Figure 1. Mean grain protein (GP) content (May ■ & July sowing □) and mean endoproteinase (EP) levels of activity (May ▲ & July sowing ∆), for the commercially malted varieties (CMV) and new breeding lines (NL). MS - May sowing and JS - July sowing.

the increase in total protein content, accompanying the July sowing, but only in very limited ranges. In a similar fashion the hordein fraction (Figure 2) closely mimicked the patterns observed with the total proteins. In contrast, the protein modification rates (KI, Figure 2) decreased reversing the pattern followed by the protein, hordein fraction and endoproteinases.

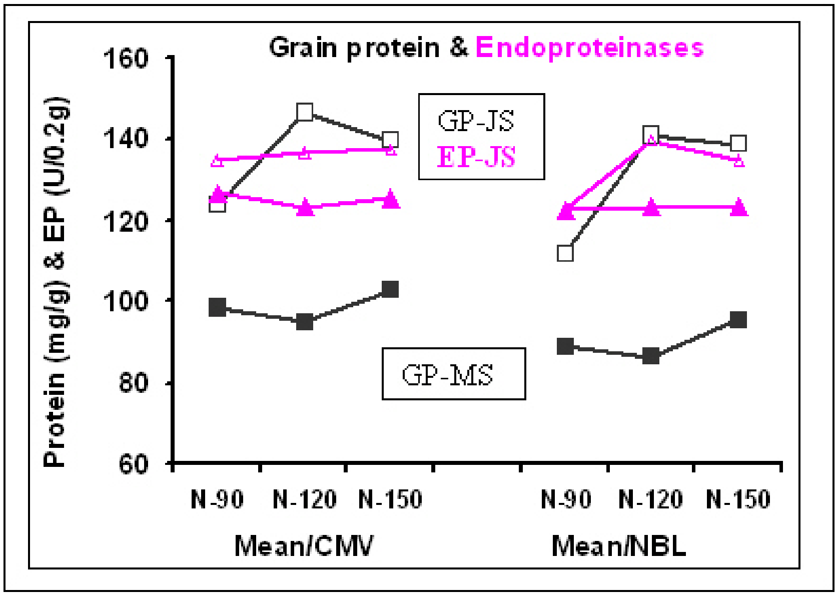

Figure 2. The hordein fraction (May ▲ & July sowing Δ), and modification rate (May ■ & July sowing □), for the commercially malted varieties (CMV) and new lines (NBL).

Because the increase in grain protein was due mainly to the increase in the hordein fraction, it is reasonable to assume that the inverse relationship between protein content and its modification rate could be precipitated by hordeins. On the other hand, it appears that the levels of endoproteinase activity, which are similar in all varieties, are unlikely to be the cause of variation in modification-rate.

However, the amounts of protein degraded and the levels of FAN produced during malting were lower in the slow modifying new lines (Figure 3). Despite the fact that grain protein and hordein contents as well as the levels of endoproteinase activities in both commercially malted varieties and the new breeding lines, were similar (Figure 4). These results suggest that the new lines might either contain higher levels of proteinase inhibitors or the endoproteinases degrade preferentially certain types of hordeins and these are limiting. Although the increase in malt soluble proteins during malting was higher in the new lines, suggesting that exoproteinases might be limiting, the total malt soluble proteins was lower in this group (Figure 4). In addition, both the levels diastatic power (DP) and α-amylase activity were lower in the new lines. Therefore, we believe that the amount of degraded protein within the hordein fraction holds the key to both low KI and FAN. This is not unusual as hordeins are composed of different components with the probability of different appeal to the endoproteinases. Thus the total amounts of hordeins may be the same in all varieties but with different mixtures of components.

In normal modifying varieties about 50 percent of the hordein fraction was degraded during malting. Whereas in slow modifying varieties it was lower, declining to about

25 percent, in the worst case. Consequently, both the rate of modification (KI) and levels of FAN closely followed the trend in protein degradation during malting. This supports our conclusion that the levels of endoproteinases are unlikely to be the cause of slow modification rate or the low levels of FAN. It seems more likely that malt endoproteinases prefer to degrade certain barley proteins faster than others. Hence the content, availability and/or easy access to these proteins influences the rate of modification.

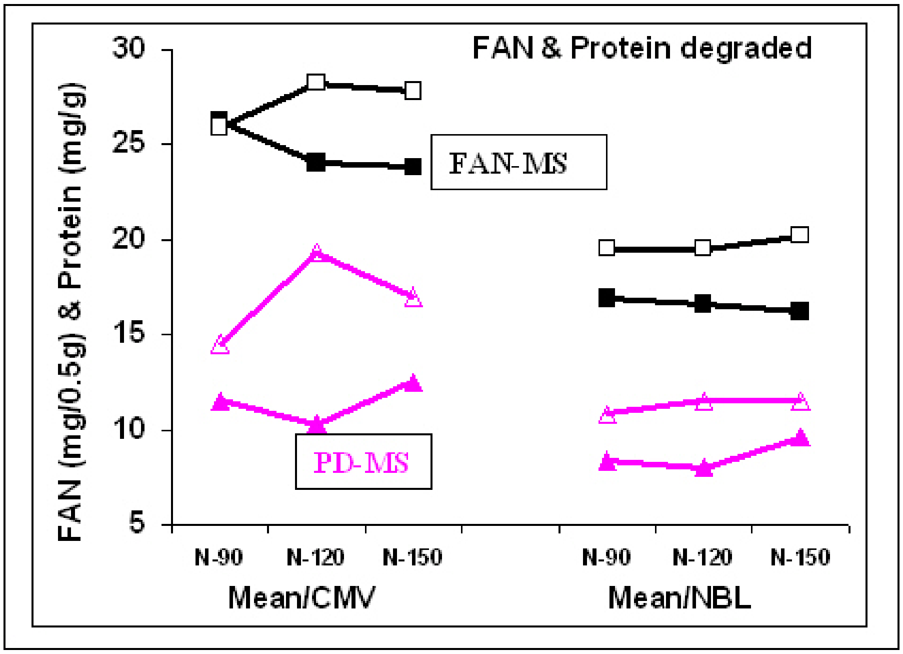

Figure 3. The means of protein degraded during malting (May ▲ & July sowing Δ) and the levels, of FAN (May ■ & July sowing □)

These findings confirmed the generally accepted fact that barley grain protein content influences malt modification rate with the negative ramification on extract yield and FAN levels, as was observed recently by Agu and Palmer, (2001). Moreover, the results reveal that this influence is more closely associated with the hordein fraction and in particular the amount of protein degraded during malting, rather than the total barley protein or total hordein. The latter appears to be genetically controlled and amenable to environmental influence. This explains why the high quality malting varieties, despite having a higher protein content, exhibit a higher modification rate and FAN than the new lines (Figure 4). This is strongly supported by the matching patterns of well-correlated levels of protein degraded during malting with the rate of modification and the levels of FAN, diastatic power (DP) and α- amylase activity (AAA) (Figure 4).

The importance of barley protein and its content within defined levels (9 - 11 %) for malting and brewing is well recognised. However, it is a challenge to produce barley with protein content within the defined-desirable levels. Efforts are made to combine the application of nitrogen fertiliser and sowing date to manage the production of high quality malting barley. In this study the effects of both nitrogen fertiliser and date of sowing were investigated. Our findings indicate that late sowing rather than extra nitrogen fertiliser increased barley grain protein content to higher levels. However, higher than the desirable levels have the detrimental potential of lowering the modification rate and the extract yield. In addition the increase in protein did not achieve better rates of modification or FAN levels in slow modifying varieties. Nevertheless, it opens new opportunities of, synchronised levels of nitrogen fertiliser and date of sowing, to manage and produce high quality malting barley, within the limits set by the malting and brewing industries.

Conclusion

The levels of endoproteinases are similar in all varieties tested and adequate enough to achieve the desirable levels of protein modification and wort FAN under similar conditions. In contrast, the levels of protein degraded during malting, from similar levels of grain protein, were lower in slow malting varieties. Malt endoproteinases appear to have higher affinity to degrade certain barley proteins faster than others. Therefore, the quantity of these proteins or their availability to proteinases in different varieties determines the rate of modification and wort FAN level, in each variety.

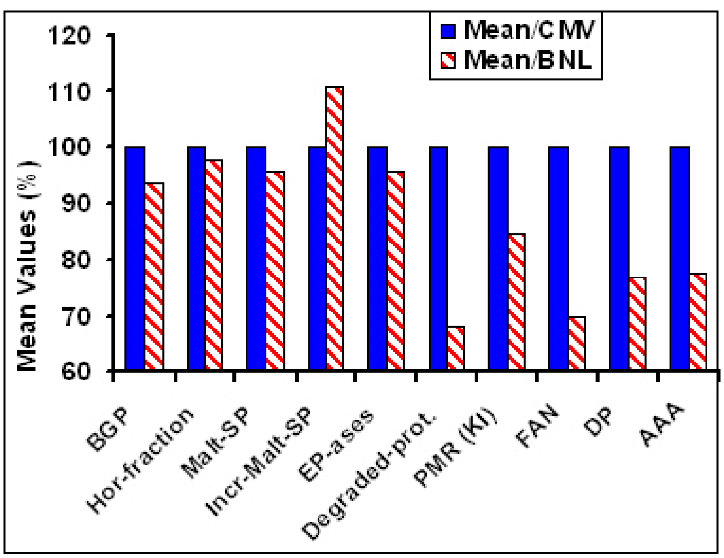

Figure 4. The mean values of grain protein content (BGP), levels of endoproteinase activity (EP-ases), Barley hordein fraction (Hor-Fr.), amount of protein degraded during malting (Deg.Prot.), Protein modification rate (PMR [KI]), levels of free amino nitrogen (FAN), malt soluble proteins (MSP), increase in malt soluble proteins over barley-levels (Incr. MSP), diastatic power (DP) and α-amylase activity (AAA).

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