3

Chemical Composition in Barley Grains and Malt Quality

G. P. Fox
Department of Primary Industries & Fisheries
Queensland Grains Research Laboratory
PO Box 2282
Toowoomba Qld 4350 Australia
glen.fox@dpi.qld.gov.au

3.1 Introduction

Barley is used for a wide range of traditional and novel end-uses (Edney 1996; Sparrow *et al.*, 1988). In most countries, the major portion of barley is fed to animals, particularly cattle and pigs. Human food uses of barley are more limited, although recent trends in the use of barley varieties, high in dietary fibre, have been identified. A significant high-value use is to produce malt as a raw material for the brewing industries, including beer and whiskey.

Improved barley production requires barley breeding programs to provide varieties with the combination of reliable and efficient production characteristics and grain quality attributes suited to these uses. Selection of varieties, with the correct range of traits, is a difficult process. It is necessary to produce high quality malt for beer and other fermented beverages. However, a number of factors influence the final quality of the barley grain as well as the malt. Testing of end product quality for each breeding line is not only expensive but requires the availability of larger quantities of barley than is available from single plants or lines at an early stage in barley breeding. Biochemical or molecular tests that predict likely feed or malting and brewing quality are therefore needed to allow rapid development of barley cultivars.

Research continues to unravel the biochemistry behind areas of grain development (gene to protein to product) which relates to end product quality. Research into well-established components such as starch and protein, improves our knowledge of the relation between barley, malt and beer. However,

the genetic, chemistry and synergistic relationship between some components that are important in malt and beer quality are still unknown.

Many of the traditional testing and evaluation methods aim to ensure a consistency of quality without a link between the attribute measured and the end-use quality being known. This results in attributes of uncertain value being assessed to reduce the risk of adopting or using barley that causes difficulties in processing or end product quality. This process may discriminate against barley with superior processing traits and will only be overcome by improved understanding of the basis of barley quality. This is highlighted when comparing our understanding of barley carbohydrates or proteins and the enzymes that degrade them being tested in conditions that do not duplicate the conditions of the in-situ reaction. A number of these key enzymes have inhibitors and some aspects of the role and function of these inhibitors also are still unclear.

A number of quality attributes for malt barley are critical for the identification and release of malt varieties. These are hot water extract, viscosity, Kolbach index, wort β-glucan, fermentability, and diastatic power. Additional parameters include α-amylase, β-amylase, free amino nitrogen, friability, and β-glucanase. However, the value of parameters currently used to measure malt quality has been questioned (MacGregor, 1996; Palmer, 1983). The limitations of some of these traits in predicting brewery performance have led to suggestions that specific brewery tests were also needed to adequately describe malt quality (Axcel, 1998). More recently, Evans *et al.* (2007) demonstrated that the sum of individual starch degrading enzymes (as well as the thermostability of these enzymes) was a better indicator of diastatic power and that these enzymes also correlated better to fermentability.

The barley grain is comprised of three main components, namely the germ or embryo, the outer layers (husk, pericarp, testa and aleurone layers), and the endosperm. Fig. 3.1 shows the overall structure and individual components of the grain, with the endosperm being the dominant component. However, the main living organ in the grain is the embryo, with the aleurone layers (also a living tissue) surrounding the endosperm.

This review covers recent research in barley structure and chemistry as well as highlighting new research from a number of excellent reviews published over the last two decades (Duffus and Cochrane, 1992; Duffus and Cochrane, 1993; Swanston and Ellis, 2002). Also, for a number of barley components such as starch or protein, more detailed reviews are provided in later chapters of this book.

3.2 Physical Structure

Some physical structures affecting germination are associated with malt quality. Here grain size, dormancy and hardness will be in detail discussed.

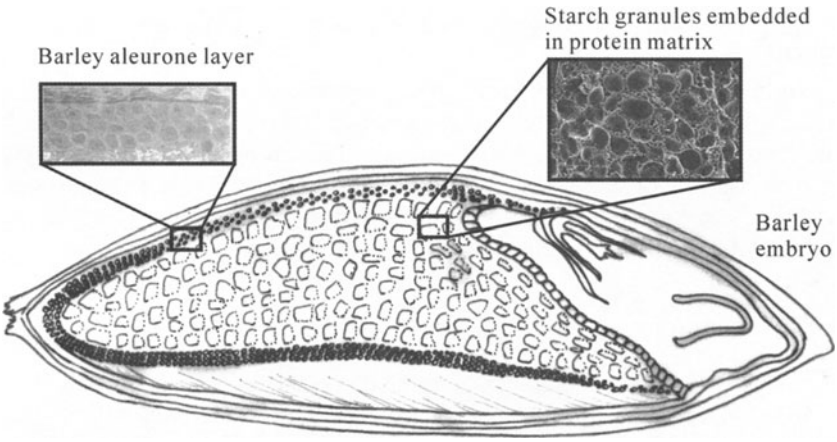

Fig. 3.1. Structure of barley

3.2.1 Grain Size

Grain size is an important descriptive trait based on the physiology of the grain. The final grain size is determined by environmental effects, which affect the biochemical components within the grain itself (Coventry *et al.*, 2003b). For thousands of years, when grain was used specifically for human consumption, the grain was selected based on size. Improvement in grain size and weight has been shown to have occurred through human selection rather than through evolutionary events (Ferrio *et al.*, 2006). Within the last century, barley breeders have continued to target large grain cultivars in association with improved yield and grain quality attributes. In Australia, the measurement of grain size is generally based on 4 fractions: <2.2 mm (screenings), >2.2 mm, >2.5 mm, and >2.8 mm. Fox *et al.* (2006a) demonstrated the genetic and environmental effects for improving grain size. Selection for increased plump grain (>2.8mm) provided a useful strategy in increasing overall grain size. Increased grain size also provided an increase in the important malt quality trait of diastatic power (Agu *et al.*, 2007). However, there were no corresponding protein determinations to ascertain if the increase in DP was related to protein content. Emebiri *et al.* (2007) reported a strong correlation between increased grain size and yield when using genotypes with low protein content alleles. Passarella *et al.* (2005) demonstrated the impacts of temperature during grain fill on the level of screenings with high temperature reducing the overall size. Fox *et al.* (2006a) also showed that from breeding trials, where sites suffered from terminal moisture and/or heat stress, grain size was significantly reduced. The negative impacts of heat and drought stress on grain size and weight have also been shown in Australian barleys under controlled experiments (MacNicol *et al.*, 1993; Savin and Nichols, 1996; Savin *et al.*, 1999; Savin and Nichols, 1999). The negative effects on heat during grain filling on

grain quality and starch synthesis was reported by Wallwork *et al.* (1998a 1998b).

Industry standards on large grain are based on the amount of grain > 2.5mm. Smaller grain generally has lower starch and higher protein levels, thus reducing the extract/feed potential. Large grains conversely have increased levels of starch and therefore more potential extract. However, excessively large grain could impact on malt quality particularly on the rate of water hydration and modification during malting.

3.2.2 Dormancy

Dormancy is not a biochemical component such as starch or protein that can be isolated and measured from grain. However, an understanding of the level of dormancy in barley is critical in terms of malt quality. Malting barley is one of the few grains where the seed is required to germinate for product development, ie. production of malt. The failure of barley to germinate at an acceptable level, i.e. <95%, has the potential to cause problems during the malting process.

The physiological and biochemical components of dormancy are very complex. Han *et al.* (1999) reported on four possible gene regions related to dormancy, with Seed Dormancy (SD) 1 and 2 on chromosome 5H, SD 3 on 7H and SD4 on 4H. Li *et al.* (2003) confirmed genetic associations of dormancy on chromosome 5H. The role of hormones and enzymes in dormancy has been documented in recent reviews by Briggs and Woods (1993) and Benech-Arnold (2002), with the latter author describing the most significant and relevant aspects of dormancy and resistance to pre-harvest sprouting. Several biochemical mechanisms have been associated with dormancy, including an antagonistic effect between abscisic acid (ABA) and gibberellic acid (GA). For example, Weidner *et al.* (1993) demonstrated a relationship between phenolic acids and barley dormancy with an increase in total and free phenolic acid content coinciding with the pattern of dormancy after flowering and post ripening. In addition, it was demonstrated that ferulic and sinapic acid retarded germination of ripening barley embryos, and Wang *et al.* (1995) showed that an increased level of hydrogen peroxide reduced the inhibitory effect of ABA. The biochemistry of dormancy is complex and covered in a wide range of comprehensive literature.

Dormancy refers to a live grain not germinating, even under ideal conditions. However, for malting barley it is preferable for the grain to go into its dormant state prior to harvest as this dormant state can help in preventing the grain from sprouting in the head prior to harvest. Rain at harvest can cause the grain to sprout, resulting in a condition known as pre-harvest sprouting (PHS). This condition results in malt barley being downgraded to feed. Two methods currently can be used to assess the level of any pre-harvest sprouting, namely the Falling Number test and the Rapid Visco Analyzer (RVA) test. Both of these tests relate to the starch in a ground barley sample being

gelatinized and the level of resistance from the gelatinized starch being an indication of how "sound" the grain is.

Recent studies have linked genetic regions for resistance to sprouting and dormancy (Ullrich *et al.*, 2005; Hori *et al.*, 2007) with a high level of correlation between tests that indicate pre-harvest sprouting resistance and grain dormancy (measured by germination tests). Controlling dormancy would contribute to the control of pre-harvest sprouting. Lin *et al.* (2008) also demonstrated varietal differences in dormancy which could be correlated to differences in PHS resistance. The differences were also linked to the level of α-amylase as well as measured by the Stirring Number method (similar method to RVA).

3.2.3 Grain Hardness

Grain hardness is a trait normally considered when looking into the structure and composition of barley. It is not routinely measured when evaluating barley grain quality. However, hardness in wheat is related to the texture of the grain endosperm where starch granules are either readily separated from the protein matrix (soft) or the granules resist separation (hard). In general, malting barley cultivars can be classified as soft, whereas non-malting or feed cultivars are classified as hard (Alison *et al.*, 1976). Hardness has also been associated with the level of modification of malt, which would imply that grain components within the endosperm directly affect modification. Specific proteins that interact with starch granules such as hordoindolines, including friabilin, have also been implicated in grain hardness (Darlington *et al.*, 2000).

Milling energy (ME) has been used as a measure of barley grain hardness. Finished malt may be analysed either for ME or for "friability", thereby providing an indication of malt endosperm modification. Recent studies have demonstrated the relationships between barley hardness (milling energy) and grain and malt quality parameters (Swanston *et al.*, 1995). Grain protein and β-glucan have been positively correlated with hardness (Henry and Cowe, 1990), with malt extract and endosperm modification correlated negatively to hardness (Swanston and Taylor, 1988; Swanston *et al.*, 1995). B and C hordein have been shown to be associated with milling energy, where an increase in C hordein along with a decrease in β-glucan corresponded to a decrease in ME. The reverse was reported for B hordein. However, that study was conducted with a limited dataset (Molina-Cano *et al.*, 1995). The effects of environmental impacts on grain components such as glucan and protein have been associated with changes in ME (Henry, 1985; Swanston and Cowe, 1989; Henry and Cowe, 1990).

Another aspect of grain hardness associated with endosperm structure is the steely or mealy appearance (glassy or chalky). A number of studies have highlighted this effect and how it related to protein content, composition and other parameters and its impact on malting quality. The effect is mostly by influenced growing environment; however, some varieties have been shown to

be more prone to expressing this effect (Chandra *et al.*, 1999; Broadbent and Palmer, 2001; Koliasou and Palmer, 2003; Halopainen *et al.*, 2005).

Recent studies have investigated the role of grain hardness for malt and/or feed quality. These studies have used a different technology from the ME one. The measurement of particle size after milling, as well as the measurement of the resistance to crushing of single kernels (the Single Kernel Characterisation System (SKCS)), offered alternative hardness determinants (Osborne *et al.*, 2003; Fox *et al.*, 2007b; Psota *et al.*, 2007; Osborne *et al.*, 2007). While there were genetic and environmental effects on hardness, there were also correlations to malt and feed quality traits. Results from a recent study using SKCS also showed a strong relationship between endosperm cell wall composition, namely β-glucan, and barley hardness.

In wheat, there is a strong relationship between hardness and a single gene locus (two genes). However, in barley at the gene level, three genes encode the hardness proteins (hordoindolines). These three genes are *hina*, *hinb1* and *hinb2*. A number of researchers have explored the allelic variation between genotypes with reports that, unlike wheat, polymorphisms in the gene sequence did not relate to functional changes in malt or feed quality (Beecher *et al.*, 2000; Darlington *et al.*, 2001; Beecher *et al.*, 2002; Caldwell *et al.*, 2004; Fox *et al.* 2007a). Fox *et al.* (2007a) detailed the homology between a number of domesticated genotypes and wild accessions and land races, based on literature and database (Genbank) entries. There was a considerable diversity in the wild accessions and land races, which offers opportunities to exploit possible hordoindoline variation and functionality.

The indoline proteins have a tryptophan rich motif, which has been shown to bind lipids (see review Douliez *et al.*, 2000). While at this stage there is no comprehensive data in barley, it is thought that these proteins are involved in binding the endosperm storage proteins, lipid and starch granules. Further evidence of this lipid binding capacity has been shown through the wheat indolines binding to yeast membrane lipids (Evrard *et al.*, 2007). Douliez *et al.* (2000) also highlighted the similarity of indolines with Lipid Transfer Proteins.

3.3 The Internal Structure

The internal structure of barley grains associated with malt quality is involved in embryo, aleurone and husk.

3.3.1 The Embryo

The barley embryo is the most important living tissue within a barley grain. A comprehensive review of the formation and structure of the embryo was provided by Duffus and Cochrane (1992) and Duffus and Cochrane (1993). The embryo is the fertile part of the barley seed, which also contains starch, protein and lipids. These are used during the development of the embryo after

fertilisation and as an initial food source when the harvested seeds commence germination. The dormancy level and viability of the embryo is critical for the next generation of seeds. A number of factors can have a negative impact on dormancy and viability including:

- environmental conditions after anthesis and during grain fill, i.e. heat (heat wave) or cold (frost) stress;
- plant moisture (avoiding drought conditions);
- harvest conditions (i.e, excessive abrasions during threshing in the harvester or pre-harvest rain);
- storage conditions (excessive heat in storage or high grain moisture can reduce germination potential).

Further, the development of hulless barley provides a number of positive characteristics for malting, food and feed end-uses. However, in hulless barleys, as the husk is removed during the harvesting process, physical damage can occur to the germ.

The germination process is initiated by the embryo imbibing water, from which a cascade of biological systems is initiated. The sole purpose of the embryo is to generate a plant which will produce new seeds. The biological systems initiate the breakdown of the endosperm reserves to produce sugars, amino acids and fats for the growing embryo to consume. These systems trigger the development of complex enzyme pathways (including the development of hormones) that will produce proteins (enzymes) for the breakdown of the endosperm cell walls, storage proteins and starch granules and subsequent transport of those products to the embryo.

Most of these systems are controlled by growth hormones including gibberellic acid, abscisic acid and salicylic acid. Variation in the levels of these hormones will impact on the expression of the enzymes. This variation can be influenced by the development of genetic variants that alter the level of the hormones produced. For example, α-amylase is one of the most important enzymes in the breakdown of starch during germination. Studies have shown that through a change in the gibberellic and/or abscisic acids' pathways the α-amylase can be up or down regulated. In terms of malt quality, most of the important components produced by the embryo are related to the breakdown of endosperm reserves. However, two enzymes that can have a negative impact on beer quality are lipoxygenase 1 and 2 (LOX 1 and LOX2). LOX has been related to beer staling with the production of trans-2-nonenal (cardboard flavour). A number of reviews have been published on LOX with Morrison (1993) providing a comprehensive summary. Both LOX isoforms have been identified during the grain fill period (Schmitt and van Mechelen 1997). While early work showed that LOX1 was present in resting barley embryos, both LOX1 and LOX2 were present in germinating embryos and the specificities of these enzymes on lipid substrates were different (Holtman *et al.*, 1997). Wu *et al.* (1997) showed there was genetic variability for LOX1 and

LOX2 although the ratios were similar. While Hirota *et al.* (2006) reported it was possible to produce germplasm free of LOX (Hirota *et al.*, 2006).

Yan *et al.* (1999) clearly showed the level of effect that the embryo, endosperm and the interaction between the two tissues had on malt quality. As a consequence they noted it was important to have an understanding of the impact of the embryo and endosperm for future developments in barley quality improvement.

3.3.2 The Aleurone

The thickness of the barley aleurone layer varies around the kernel depending upon the tissue its covers. The aleurone layer is two to three cells thick and encases the endosperm. The thickness of the aleurone is more variable in the crease and is approximately one cell thick at the germ. The aleurone cells are living tissue and contain protein, starch and lipids. The cell walls of the aleurone contain β-glucan, arabinoxylan and phenolic acids. However, the ratio of β-glucan to arabinoxylan is 25:75, which is the reverse for the endosperm cell walls. The sub-aleurone which varies in the number of cells is located between the aleurone and endosperm. The sub-aleurone also contains protein embedded in starch granules (Macewicz *et al.*, 2006). Macewicz *et al.* (2006) proposed that some of these proteins were isoforms of a storage protein (hordein B3) (see section on storage proteins below) as well as a serine proteinase inhibitor (serpin) protein.

A comprehensive description of the aleurone is presented in Duffus and Cochrane (1992, 1993). The aleurone plays a critical role in the expression of endosperm degrading enzymes during germination, including:

- β-glucanases (no inhibitors reported at this stage);
- Proteinases (Jones 2005a), and peptidases;
- α-amylase;
- Limit Dextrinase (Sissions *et al.*, 1992);
- α-glucosidase;
- Inhibitors (proteinases (Jones 2005b), trypsin/amylase, limit dextrinase (Macgregor *et al.*, 2002b; Stahl *et al.*, 2007).

A number of important transcript factors that regulate the synthesis of α-amylase and other enzymes include gibberellin oxidase (GAox) and GAMYB which are expressed in aleurone cells. Up-regulation of GAMYB results in increased expression of α-amylase and (1→3,1→4)-β-Glucan endohydrolase (Murray *et al.*, 2006), while inhibition of GA-oxidase proteins can stop the expression of amylase and other enzymes. Similar responses can also be observed when transcription factors in the abscisic acid pathway are affected.

The growing environment affects aleurone cell integrity. Excessive heat stress can induce the development of some proteins while having little effect on others. Heat Shock Proteins (HSP) are expressed in high temperatures.

These proteins can in turn impact on the expression of other proteins (Harju *et al.*, 2003).

In addition, important trace elements including zinc and iron, are stored in aleurone cells (Brinch-Pedersen *et al.*, 2007).

3.3.3 The Barley Husk

Barley is one of only four commercial species of grass that retains a husk after harvest. Rice, oats and millet are the other "covered" grains. The hull amounts to approximately 13% of grain weight but can range between 7%~25% depending upon cultivar, growing environment and grain size (Evers *et al.*, 1999). Variation occurs between row types, with six-row having a larger proportion than two-row barley (Evers *et al.*, 1999). Additionally, winter barleys have higher husk content than spring barleys and the husk content increases in regions closer to the equator (Evers *et al.*, 1999). Thin hulled barley cultivars exist but these can suffer from "skinning" during harvest. Hulless barley cultivars are also commercially available. For these barleys, the hull is removed during the harvesting process.

The hull plays an important role before and after harvest. During the later stages of grain ripening, the hull has been considered to have a role in grain dormancy and therefore pre-harvest sprouting resistance (Benech-Arnold *et al.*, 1999). During harvest, the husk acts to protect the germ during the abrasive threshing process in the harvester (Olkku *et al.*, 2005). Post harvest, the husk plays a role in processing for the malting, brewing and feed industries. In terms of the malting industry, the husk aids during the malting process by protecting the germ from physical abrasion during handling and preventing the growing acrospire from being damaged during germination and kilning (Meredith *et al.*, 1962). In the brewing process, the husk aids during filtration of the brewers extract from the lautering process. Huskless barley can "gum" up filters and reduce filtration rate, thereby adding to the cost of production and potentially impacting on the quality of beer (Edney *et al.*, 1998; Evans *et al.*, 1999b). However, Edney and Langrell (2004) recently demonstrated that good quality malt could be produced from hulless barley if the "appropriate malting conditions were used".

The intensive livestock industry also benefits from using a hulled barley grain. The hull aids in holding crushed or pressed grain together. A thinner hull is desirable as thicker hulls will result in higher levels of deleterious compounds, in particular lignin, which have been shown to have a negative impact on feed performance in ruminants (Kaiser, 1999).

3.4 The Barley Endosperm

Endosperm is a major part of the barley grain, and its chemical composition is directly related to malt quality.

3.4.1 Barley Carbohydrates

Barley carbohydrate composition has been one of the most studied aspects in terms of barley quality and its relation to feed, malt, and beer quality. Henry (1988) reviewed the current knowledge of barley carbohydrate composition in terms of malt quality, concluding that despite many years of research, our knowledge of barley carbohydrate chemistry was incomplete. Further work was required to fully understand barley carbohydrates before significant genetic gain could be reached. More recent reviews have covered the topic in similar detail (Duffus and Cochrane, 1992; Duffus and Cochrane, 1993). In this section we will cover the basics of barley carbohydrates as well as review the most recent research.

3.4.1.1 Starch

Starch is the most abundant component of the endosperm , comprising around 60% of total grain weight. Starch consists of two polymers, amylose and amylopectin. Amylose is a linear polymer made up of glucose molecules linked via α-(1-4) glucosidic bonds. AmylopectinindexAmylopectin. is the larger polymer with α-(1-4) glucosidic and α-(1-6) glucosidic linkages, which form a branched structure (Hough, 1985). The ratio of amylopectin to amylose is around 3:1 (Palmer, 1983). Both amylose and amylopectin polymers are present in the barley endosperm starch granules. The large granules, designated A type, are round in shape and contain 70%~80% amylopectin. The small, spherical, B type granules, contain 40%~80% amylose (May and Buttrose, 1959; Evers *et al.*, 1999).

The formation of starch is a complex pathway with amylose and amylopectin being formed simultaneously (Fig. 3.2 Rahman *et al.*, 2000). The genes responsible for particular proteins are expressed shortly after anthesis. For example, starch branching enzymes I and II (SBEI and II) have been shown to express 12 days post anthesis (Mutisya *et al.*, 2003).

Dosage of starch synthesis (SS) IIb has been shown to affect amylose synthesis in wheat (Konik-Rose *et al.*, 2007), while mutation of the SSIIa gene in barley resulted in a novel high amylose phenotype. The SSIIa gene is located near the barley sex6 mutation (Morrell *et al.*, 2003).

Recently, the gelatinisation properties of starch were reviewed by Evers *et al.* (1999). For barley, gelatinisation temperature plays an important role in the quality of malt and hot water extract. The temperature at which gelatinisation occurs varies between 55°C and 65°C. MacGregor *et al.* (2002) presented data on the effects of gelatinisation temperature on normal, high, low, and zero amylose starches. Each starch type was found to vary, with the high amylose and waxy starches having gelatinisation temperatures higher than that for the normal starch, whereas high amylose starch showed a high level of resistance to enzymic attack in a slow ramp mash. Ellis *et al.* (1979) had

previously demonstrated that high amylose barley had an increased gelatinisation temperature. In "waxy" barley, the amount of amylopectin increases to >90% (Evers *et al.*, 1999). However, waxy barleys generally have lower hot water extract values, and higher β-glucan content and slower modification rates (Ullrich *et al.*, 1986; Swanston, 1996). These factors complicate any interpretation of the impact of changes on starch properties. The main requirement seems to be for a high level of starch, which is not lost during malting and was readily convertible to fermentable sugars in the brewery (low gelatinisation temperature).

Limited starch breakdown occurs during malting, although Allosio-Ouarnier *et al.* (2000) reported increased levels of maltose, maltotriose, and maltotetraose during germination. Starch was degraded more in the mashing process by the hydrolytic enzymes α-amylase, β-amylase, β-glucosidase, and limit dextrinase. High temperature infusion mashes readily solubilise the starch but limit the activity of thermolabile enzymes, in particular α-glucosidase and β-amylase (Osman *et al.*, 1996a). Oliverira *et al.* (1994) related the effect of starch properties, namely granule volume and size, to hot water extract.

3.4.1.2 Non-starch polysaccharides

The major constituent of barley endosperm cell walls is β-D-(1→3), (1→4)-glucan (75%), with a minor component identified as arabinoxylans (20%) (Fincher, 1975; Fincher and Stone, 1986; Henry, 1987). The arabinoxylan fraction is usually referred to as pentosan. The solubility of β-glucan in beer varies according to the number and arrangement of (1→3) and (1→4) linkages (Izawa *et al.*, 1993) as well as the size of the molecules. The range in barley for β-glucan is 2%~10% of total grain weight (Henry, 1987). Cultivar and environmental influences on the content of β-glucan have been reported (Henry, 1987; Stuart *et al.*, 1988; Zhang *et al.*, 2001; Li *et al.*, 2008).

Recent studies have presented models of the structure of endosperm cell walls. Autio *et al.* (1996) suggested that the cell walls of the endosperm have a layered structure. Bamforth and Kanauchi (2001) presented a model whereby the outer cell wall was made up of xylan, arabinose, and ferulic and acetic acids, with the inner layer composed of β-glucan. These results indicate that enzymic hydrolysis of the arabinoxylan layer would be critical to ensure that β-glucanase could hydrolyse the β-glucan layer. Vietor *et al.* (1991) and Han and Schwartz (1996) reported that arabinoxylan survives into malt and beer, albeit in smaller oligosaccharide forms. Similarly, it was shown that xylanases could release all pentosan as well as a limited amount of β-glucan from cell walls (Kanauchi and Bamforth, 2002). During germination or malting, the "modification" of the endosperm relates to the breakdown of the cell wall and endosperm components. Although endosperm modification is measured through the solubilisation of endosperm protein reserves, access to those reserves is only possible after cell wall breakdown. Henry (1987) measured the breakdown of β-glucan during malting. The results indicated that by day 4 of

germination, barley β-glucan had decreased from 5% to around 1%. Similarly, Allosio-Ouarnier *et al.* (2000) reported an increased level of sugars derived from β-glucan and arabinoxylan components during malting. Although these studies analysed the complete malting process, Walker *et al.* (2001) suggested that by day 2 of germination, the β-glucan level could be used to indicate if a cultivar had desirable extract potential. Stewart *et al.* (1998, 2000) demonstrated that both β-glucan and pentosan impact on wort viscosity and beer filtration rates. Thus, the enzymic breakdown of β-glucan and pentosan during malting is critical for efficient brewing. Mashing temperature has an influence on the solubility of β-glucan. Palmer and Agu (1999) demonstrated the difference in solubility of β-glucan when malt was mashed at 45°C or 65°C, with an increased level of solubilisation at the latter temperature.

The level of β-glucan has been shown to have a relationship with other malt quality traits. Importantly, high β-glucan levels may not result in higher or lower extract but relate to other malt quality traits such as Kolbach Index (ratio of soluble to total protein), viscosity or the speed of filtration (Evans *et al.*, 1999). Views differ on the relationship between β-glucan and foam stability (Lusk *et al.*, 2001).

3.4.2 Grain Protein

Barley protein accounts for 8%~13% (dry basis) of the total composition of malt barley, with a more desirable range of 10%~11% for brewers using a liquid adjunct or for those using a low protein solid adjunct such as rice in which the protein content could be 12%~13%. Of total protein content, around 50% is the prolamin (hordein) protein, with the other three protein fractions, albumins, globulins and glutelins making up the remaining protein. In this review, the hordein protein component will be the main focus. However, structural proteins will be mentioned in the context of their interactions with specific components of barley.

The synthesis of protein commences quite early after anthesis, although there are different reports on the actual timing of these protein components. The hordein component and the individual fractions that make up this component have received the most attention (Shewry, 1992). In addition, a number of the enzymes responsible for the synthesis of the barley proteins have been identified and, most recently, the individual genes and allelic variation have been identified.

Protein accumulates during grain fill at the same time as other grain components. However, there are a number of factors that influence the final protein content and composition, including genotype, growing environment and fertilizer. Nitrogen fertilizer at particular growth stages has been shown to affect final protein content and composition (Sardana and Zhang, 2005; Zhao *et al.*, 2007). In addition, sulphur fertilizer also affects the total protein content and composition (Zhao *et al.*, 2006). Shewry *et al.* (2001) noted that there was little data describing the impact of sulphur fertilizer on malt or beer quality.

However, Zhao *et al.* (2006) reported effects on a range of malt parameters including germination, modification and beta-glucan levels, on malt produced from sites where there was yield response to sulphur fertilizer.

There are reported genetic variations in the starch protein relationship with some genotypes maintaining high protein content regardless of the final starch content whereas others can express low protein levels, which also seem independent of the starch content. This is potentially an important factor for barley breeders to consider. Barley protein has a complex interaction with quality. High protein is undesirable because of the strong correlation with low carbohydrate (starch) levels and thus low extract values (Bishop, 1930). However, if the protein content of malt is too low, brewing performance may be impaired through poor yeast amino acid nutrition. Protein, peptides and amino acid levels in packaged beer are also considered important, positively enhancing foam stability but negatively influencing shelf life by contributing to chill hazes. Many proteins have been identified with specific functions in terms of grain and malt quality, whereas a number have yet to have their function clearly defined.

3.4.2.1 Low protein barleys

Protein content has been shown to be controlled by environment as well as genotype. In addition, low protein content has been shown to be an inherited trait. Karl, a six-rowed variety was released in 1979, and was clearly shown to have low protein content across a range of environments. See *et al.* (2002) demonstrated a negative correlation between low protein and yield, as well as a number of genome regions for yield, maturity and protein. These relationships were also reported by Emebiri *et al.* (2003) using two-rowed breeding lines, one with Karl as a parental relative. Emebiri and Moody (2004) demonstrated the low protein content effect when eight varieties were grown on dryland and irrigated sites, using a range of N fertilizer applications. Again there was a strong relationship between yield and low protein content. Emebiri *et al.* (2004) further confirmed the close linkage between low protein content and agronomic traits, yield, maturity and plant height. Further, Moralejo *et al.* (2004) also identified genomic regions between low protein content, high yield and maturity (earliness). The authors concluded that the low protein content could be related to nitrate reductase genes and not hordein genes. Emebiri *et al.* (2007) reported a strong low protein Genotype x Environment x Management for improved yield, and concluded that through selection of low protein genotypes and higher nitrogen fertilizer application, significant yield gains could be achieved.

Low protein will have a strong effect on malt quality (see below). However, based on the relationship between yield and low protein content, it would be desirable to select for low protein content, while increasing grain yield (and grain size). Selection for low protein content would be a consideration when selecting for malt quality in that low protein content relates to a high extract.

A number of studies have explored the relationship between low protein content breeding and malting quality. Goblirsch *et al.* (1996) identified it was possible to select for low protein and kernel discolouration but the cost was a decrease in diastatic power (DP) using Karl as a parent. Similarly, Canci *et al.* (2003) also reported a close linkage between low protein content and kernel discolouration resistance. Emebiri *et al.* (2004) reported a negative correlation between protein and extract and a positive correlation between protein and diastatic power, using a low protein breeding population. Further, a number of genomic regions common for these traits were identified.

3.4.2.2 Storage proteins

Storage proteins exist in all cereals. This protein component forms a matrix around starch granules in the endosperm and provides a source of nitrogen for the growing embryo if germination occurs. These proteins are generally rich in the amino acids proline and glutamine (hence the term prolamine). In barley, the major storage protein is called hordein, and this comprises 40%~50% of total grain protein. This component is soluble in aqueous alcohol and comprises 4 fractions designated D, C, B, and A. The diversity in the hordein family has made the analysis of these fractions very useful in varietal identification. This diversity can be explained through differences within the B and C hordeins that occur between cultivars, as well as grain protein levels and environments (Benetrix *et al.*, 1994; Molina-Cano *et al.*, 2000b). Several hordein proteins have been purified and sequenced. However, the roles of the specific subunits remain undefined for B-, C- and D-hordeins.

Of these hordeins sub-groups, the A-hordein has been investigated for its possible role as a trypsin/amylase inhibitor and a possible protein involved in beer haze formation. First reports on the existence of five components of chloroform/methanol (CM) extracted proteins was in the early 1980s (Saledo *et al.*, 1980; 1982; Aragoncillo *et al.*, 1981; Paz-Ares *et al.*, 1983; Lazaro *et al.*, 1985). These five proteins have been identified as CM (a~e). Saledo *et al.* (1984) first reported on the genetics of these CM proteins and its close homology to A-hordein. Subsequent studies have confirmed the role of some of these individual proteins and their inhibitory role on trypsin as well as insect α-amylase. Moralejo *et al.* (1993) reported genetic variability in at least one specific protein, namely CMe. Additional roles have been identified for these proteins, including inhibiting serine proteinases in malting (see review by Jones 2005b).

3.4.2.3 Hordeins, malting and brewing quality

For over 100 years researchers have attempted to improve our understanding of the relationship between protein and hot water extract. Smith (1990), Shewry (1993) and Tatham and Shewry (1995) provided excellent reviews of previously published results on this subject, although initially Bishop (1930)

concluded that there was a negative relationship between protein and hot water extract. Details from these reviews showed that each hordein group had some relationship to extract or final beer quality. In particular, aggregation of the sulfur-rich B and D groups forms gels which cause filtration problems in brewing. Individual B and C (sulfur-poor) groups have variable effects on extract (Skerritt and Janes, 1992; Janes and Skerritt, 1993). Initial studies by Marchylo *et al.* (1986) demonstrated the difference in hordein breakdown during the malting of two cultivars of differing quality. The malting cultivar was reported to give a higher level of modification than the non-malting. This was reflected in the hordein analysis where the malting cultivar exhibited a higher level of hordein breakdown. Recently, several studies have described a negative correlation between D hordein and hot water extract (Howard *et al.*, 1996; Molina-Cano *et al.*, 2000b). In contrast, Brennan *et al.* (1998) found that with D hordein isogenic lines there was no effect on extract, although the presence or absence of a D hordein allele had an impact on gel protein formation. Further, Brennan *et al.* (1998) related grain hardness, in the form of milling energy, to specific B and C hordein alleles. However, two cases noted a positive relationship between individual hordein fractions and malt quality. Janes and Skerritt (1993) reported a B hordein fraction with a positive effect on hot water extract, whereas Molina-Cano *et al.* (1995) suggested that a C hordein had a positive effect on water uptake during malting. Many of these observations may be explained by indirect effects of the levels of one group of proteins on total protein or the levels of other specific protein components.

Several studies have examined the effect of hordein breakdown products on beer quality. Using monoclonal antibodies, Kauffman *et al.* (1994) identified the presence of D- and B-hordein components in lager foam, whereas C-hordein components could not be detected. ELISAs (The Enzyme-Linked ImmunoSorbent Assay) have also been used to identify polypeptides derived from B, C, and D hordein in beer and beer foam (Sheehan and Skerritt, 1997). Further, a member of the A-hordein sub-group, a CMe protein has been identified as being involved in beer haze formation (Robinson *et al.*, 2005), where malts produced from varieties without the CMe protein failed to produce a haze under forced aging.

Although large proline-rich polypeptides influence chill haze formation (Asano *et al.*, 1982), smaller hydrophobic polypeptides have positive effects on foam formation. Hence, several important factors should be considered when investigating beer and beer foam quality, including the initial barley hordein profile, malt hordein profile, level of modification, mashing conditions, and the possible interaction of polyphenols and additional proteins such as protein Z and lipid transfer proteins (LTPs). Silva *et al.* (2007) also monitored hordeins (and other proteins) during malting, wort and beer production. There was a significant reduction in hordeins but other proteins (LTP and Protein Z) survived the malting process.

3.5 Malt Quality

Malt quality is derived from the impact of the malting process on components and enzymes within the barley grain. However, a number of these cannot be predicted from the analysis of the barley grain.

3.5.1 Diastatic Power

Diastatic Power (DP) is the term used to describe the collective activity of starch degrading enzymes in malt. Four enzymes, α-amylase, β-amylase, limit dextrinase and α-glucosidase, have been identified during malting and mashing (Osman *et al.*, 1996). β-amylase is the only enzyme in resting barley. The remaining enzymes are synthesised during germination. There are genetic and environmental effects on α-amylase, β-amylase and limit dextrinase (Arends *et al.*, 1995). Industry methods used to measure diastatic power vary considerably in a number of aspects including substrate, pH and assay temperature. These variations may impact on one or more enzymes. Most methods only provide data solely on the enzyme potential under those conditions which are far removed from industrial mashing conditions (Henry, 1984).

3.5.2 α-amylase

α-amylase is an endohydrolase that randomly cleaves α-(1-4) glucosidic bonds in starch. The level of this enzyme is not detectable in barley grain, however it increases once germination commences (Bathgate and Palmer, 1973). In most cereals, the α-amylase I form appears shortly after anthesis. The level declines during grain maturation. When germination commences, a second form, α-amylase II, appears. In the first hours of germination, α-amylase II is released from the scutellum. After the first day of germination the aleurone becomes the main source of α-amylase (Munck *et al.*, 1981). The level of α-amylase II formation is highly dependant upon GA. Studies have shown that this form was synthesized in an embryo-less kernel with GA as a stimulant (Freeman, 1984). In the presence of GA, both enzymes continue to be secreted by the aleurone (MacGregor, 1987). Multiple forms of amylase I and II exist and these forms can vary between varieties (MacGregor, 1987).

A third form of α-amylase (III) has been reported. It is a complex between amylase II and an amylase inhibitor protein (MacGregor 1987). However, recent evidence on the location of expression of the inhibitor gene suggests that the binding of this inhibitor to α-amylase is probably not significant *in vivo* (Furtado *et al.*, 2002).

The pH optima for α-amylase is also below mashing pH. α-amylase II is highly dependant upon the level of calcium ions present. During mashing the enzyme was shown to be highly active, albeit a number of variables influenced the total activity. The optimal temperature for α-amylase II is around

65°C(Briggs *et al.*, 1981; Hoseney, 1986) which would allow the enzyme to perform efficiently under most mashing conditions. However, consideration has been given to improving its thermostability in barley by introduction of an alien genetic form of the enzyme from bacteria (Vickers *et al.*, 1996). The preliminary results suggested that mashing could be carried out at temperatures as high as 75°C. In a 65°C infusion mash, the activity of the bacterial enzyme was found to be 1.5 times that of the barley enzyme (Vickers *et al.*, 1996).

3.5.3 β-amylase

β-amylase is an exoenzyme that cleaves the disaccharide, maltose, from the non-reducing end of amylose and amylopectin. The enzyme activity alone catalyses the hydrolysis of approximately 70% of the amylose and 50% of the amylopectin fractions of barley starch (Hoseney, 1986). Two forms (free and bound) are present in barley. The bound form is linked to the hordein matrix and results suggest that the bound form was released by proteinase activity during germination (Sopanen and Lauriere, 1989; Grimes and Briggs, 1996; Buttimer and Briggs, 2000a; 2000b). A cellular pH of 5.3 is optimal for the enzyme. However, conditions during mashing of > 6.0 are not optimal and impact significantly on its action in commercial mashes. The thermostability of β-amylase decreases rapidly at temperatures above 55°C (Hoseney, 1986). For decoction style mashes (a low initial mash-in temperature followed by rapid heating) β-amylase remains active until the mash temperature exceeds 55°C. In comparison, for a mashing style where the infusion temperature is greater than 65°C, the activity of β-amylase is reduced to a few minutes. Fix (1989) has shown that in high temperature mashes, with a low grist liquor ratio, the maltose level in the final wort is higher. This suggests substrate protection of the enzyme within a thick mash.

An increase in the thermostability of β-amylase would provide brewers with opportunities to increase efficiencies in wort production. Two forms (SD1 and SD2) have been observed in commercial barley varieties. A third form (SD3) with increased thermostability has recently been identified in wild barley types (Eglington *et al.*, 1998). This enzyme has been shown to remain active at temperatures greater than 60°C under simulated commercial mashing conditions.

3.5.4 Limit Dextrinase

Limit dextrinase catalyses the hydrolysis of the α-(1-6) glucosidic linkages of amylopectin. The enzyme produces an increased number of smaller linear oligosaccharide chains that subsequently are rapidly hydrolysed by α-amylase and β-amylase. Limit dextrinase has been extracted from ungerminated barley (Manners and Yellowlees, 1973; Yamada, 1981; Lenior *et al.*, 1984; MacLeary, 1992). However, its level increases during germination, with maximum activity

being obtained after eight days. The observed increase in total limit dextrinase activity is due to a bound form being released by the action of proteinase (Longstaff and Bryce, 1993). Purified limit dextrinase has been found to have an optimal pH of 5.5 and temperature at 50°C (Sissions *et al.*, 1992). Stenholm and Home (1999) have shown that malt limit dextrinase, under programmed mashing conditions, has a similar pH optimum but higher temperature optimum of between 60°C to 63°C. These results have been confirmed by Osman *et al.* (1996). Malt limit dextrinase has been observed to have a positive influence on wort fermentability. This is presumably due to an increase in the production of linear oligosaccharides, allowing α-amylase and β-amylase to carry out their roles (Stenholm and Home, 1999). It is also proposed that limit dextrinase inhibitors play a role in malt quality (MacGregor *et al.*, 2002) as well as starch synthesis (Stahl *et al.*, 2004).

3.5.5 α-glucosidase

α-glucosidase (EC 3.2.1.20) is the fourth enzymic activity involved in hydrolysis of starch during mashing. The enzyme catalyses the release of single glucose from maltose and higher sugars. Like α-amylase, α-glucosidase is synthesised during germination and dependant upon GA. The role of α-glucosidase in malting and mashing has not been clearly defined. Agu and Palmer (1997) reported an increase in the level of α-glucosidase and also in glucose levels during malting. Osman *et al.* (1996) demonstrated that α-glucosidase hydrolyses oligosaccharides preferentially over starch polymers. This suggests that α-glucosidase would rely on the availability of oligosaccharide substrates released by the prior action of the other three starch degrading enzymes. The pH optima for α-glucosidase appears to be dependant upon substrate, namely 4.5–4.6 for maltose substrate but 5.0 for starch as a substrate (Osman *et al.*, 1996a; Agu and Palmer, 1997). Under mashing pH conditions, α-glucosidase activity is reduced, suggesting that activity would be limited during mashing and dependant upon the activity of α- and β-amylases (Osman *et al.*, 1996a). The amount of α-glucosidase activity present in a hot water extract was found to be considerably lower than the activity of other starch degrading enzymes (Osman *et al.*, 1996b). Overall, the efficiency of α-glucosidase during mashing is dependant upon temperature, pH and the availability and form of substrate (Osman *et al.*, 1996a; 1996b; Agu and Palmer, 1997).

3.5.6 β-glucanase

β-(1→3), (1→4)-glucan-4-glucanhydrolases (EC 3.2.1.73) or β-glucanase has received considerable attention by researchers over the past 50 years. The function of β-glucanase is to hydrolyse β-glucan during germination. Two isoenzymes, EI and EII, have been identified and their functional properties reported (Woodward and Fincher 1982). β-Glucanase is produced during grain germination in the aleurone and scutella, with EII and EI produced in the

aleurone and EI produce from the scutella (Stuart *et al.*, 1986). The level of this enzyme increases upon the addition of GA.

This first stage of endosperm modification is the breakdown of cell walls. This is one of the critical steps in producing good quality malt. The hydrolysis of β-Glucan during germination has a significant impact on the final malt quality. The mechanism and enzymes involved in cell wall modification remain controversial (Bamforth and Barclay, 1993). However, it is clear that an increase in malt β-glucanase levels results in reduced levels of β-glucan in wort. Slow and/or incomplete breakdown of barley β-glucan has been shown to have a negative impact on hot water extract (Henry, 1986; Stuart *et al.*, 1988) as well as causing viscosity and filtration problems in the brewhouse (Stewart *et al.*, 1998, Stewart *et al.*, 2000).

β-glucanase, as with most enzymes in cereals, was not designed to be active at high temperatures. Woodward and Fincher (1982b) reported the optimal temperatures for EI and EII to be approximately 37°C and 45°C, respectively. β-glucanase activity is considerably reduced during the kilning process, and in the initial stages of high temperature mashing (Woodward and Fincher, 1982b; Loi *et al.*, 1988). All activity is lost after approximately 15 minutes under these conditions. Hence, under industry commercial brewing conditions either high levels of β-glucanase are required through addition of enzymes or an increase in thermostability of the native enzymes.

β-glucan hydrolysis still remains an important target in barley breeding programs. High levels of β-glucanase would be required for high temperature mash breweries as well as breweries where unmalted barley is used as an adjunct. The development of varieties processing high levels of β-glucanase or increased thermostabilities will remain an important breeding target.

3.5.7 Proteinase

The influence of storage protein within the grain and malt on beer quality has been well documented (Jones and co workers), with Jones providing a comprehensive review recently (Jones, 2005a). The mechanisms and control of the breakdown of storage proteins has only been partially unravelled. Early studies were limited to general proteinase activity. Recently, assays have been developed that can more precisely describe the specificity of individual proteinases under germination or mashing pH and temperature optima (Schmitt and Budde, 2007) which could finally help breeders in developing varieties with optimal storage protein degradation.

Four classes of proteinases namely, cysteine, serine, aspartic and metallo, have been identified in barley. The most abundant are the cysteine enzymes (Zhang and Jones, 1995a, 1995b). Studies on individual proteinase classes have been limited and the roles of the various classes have not yet been clearly defined. Jones and co-workers have carried out a number of studies attempting to characterise the role of these enzymes during malting and mashing (Poulle and Jones, 1988; Worbel and Jones, 1992; Worbel and Jones, 1993; Zhang and

Jones, 1995a; 1995b; Fontanini and Jones, 2001; Jones and Marinac, 2002). Most of the work previously reported used non barley protein substrates. In contrast, Osman *et al.* (2002) characterised the major endoproteinase activities in malted barley using isolated native substrates, specifically hordein and glutelin. These activities were all found to be of the cysteine class. The temperature optimum for endoproteinases with glutelin and hordein substrates was 50°C and 40°C respectively. Osman *et al.* (2002) further investigated the proteinase activity in a range of genotypes. Obvious differences were observed between genotypes with the activity again found to be dependant upon the type of substrate. However, no statistical data was provided to indicate any significance between genotypes.

In the most recent review, Jones (2005a) showed that of the four proteinase classes, cysteine- and metallo-proteinases were the only two involved in storage protein breakdown during germination of mashing. However, Schmitt and Marinac (2007) reported that serine proteinase may be involved in the degradation of the hydrolytic enzyme β-amylase using gel studies on green malt.

3.5.8 Hot Water Extract

The hot water extract (HWE) of wort, commonly called malt extract (ME), is the most important trait whether selecting potential new malting varieties or trading malt. The quality of the extract is influenced by four factors. The first is the quality of barley which is affected by environmental factors such as growing conditions, temperature, fertiliser, available nitrogen or moisture. These factors do not impact on extract directly. Their effect is on content and compositions of traits that influence extract, particularly protein and starch.

The second is a number of physical and biochemical components that influence the final level of extract such as two or six rowed type, husk thickness, grain size, protein, starch, non-starch polysaccharides and enzyme production. Varieties with the optimum combination of these traits will consistently produce higher extract even with minor negative effects from either environmental or physiological factors.

The third factor is the malting process. Nischwitz *et al.* (1999) outlined the effects of malting on final beer quality. Most aspects of grain modification affect final beer quality, including important aspects such as clarity and foam stability. During malting, enzymes which have an impact on the degradation of substrates, are either synthesised or cleaved from their bound forms. The range of enzymes produced includes those that degrade cell wall components, proteins and starch. The process of malt production varies between countries with four day germination schedules in Australia and five to six day germination schedules in most overseas countries. The objective for most maltsters is to maintain high extract levels and yet somehow achieve relatively low protein modification levels ($< 50\%$).

Mashing is the fourth factor that influences extract. Within the mashing process, there are a number of physical factors that affect the resultant extract.

These are pH, mash time, mash temperature, grist/particle size and grist to liquor ratio. Cook (1964) and Briggs *et al.* (1981) presented reviews detailing previous and current knowledge and technology available on mashing. While all of the above aspects of mashing would influence the quality of the final extract, most result from the genetic attributes of the starting barley. For example, high DP varieties produce high levels of malt DP under optimal conditions. This in turn impacts on starch hydrolysis and the final fermentable sugar profile. Low DP barley varieties only produce low to moderate levels of starch degrading enzymes which affect the fermentable sugars profile.

A number of studies have presented results detailing the relationship between grain and malt physical attributes and extract. Some of these attributes include protein levels and the type of protein fractions (Smith, 1990; Howard *et al.*, 1996; Brennan *et al.*, 2000; Molina Cano *et al.*, 2001), hardness and milling energy (Ellis *et al.*, 1979; Alison, 1986; Swanston and Taylor, 1988), starch properties (Glennie-Holmes, 1995a,b,c,d), non-starch polysaccharides (Henry, 1985; Molina Cano *et al.*, 1995) and husk thickness (Roumeliotis *et al.*, 2000). The specifics of the relationships between extract and these attributes have been discussed in the sections above.

3.6 Feed Barley Quality Traits

The major portion of the Australian barley grain crop (around 70%) is used as feed grain. This is made up of a combination of poor quality malting cultivars and specific feed cultivars. A high portion of barley is used as the feed by the animal industries in developed countries. Overall, feed cultivars are those that either do not the pass industry malt quality standards or were bred for specific improved agronomic performance. These are simply designated as feed cultivars, not necessarily possessing desirable feed quality attributes.

Current understanding of true feed quality is somewhat limited. However, recent studies indicate that a number of grain traits in barley are relevant to feed quality. A number of reviews have listed specific traits that could be related to feed quality, including a high starch level, low protein, acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Overnell-Roy *et al.*, 1998a,b,c;). A special edition of the Australia Journal of Agricultural Research (Volume 50, 1999) published a comprehensive set of reviews on the current knowledge of feed quality. Areas covered were grain composition and attributes that contribute to feed quality. These included both processing (Rowe *et al.*, 1999; Kaiser, 1999) and methodology (Petterson *et al.*, 1999; Wrigley, 1999). The general consensus was that there were critical differences between grain types and feed quality which have an impact on animal performance (both ruminants and monogastrics). Hussien *et al.* (2004) identified a number of genetic regions associated with hardness, fibre and an *in-sacco* dry matter digestibility method.

Starch, protein and fibre (ADF, acid detergent fibre; NDF, neutral detergent fibre) are the main grain attributes in barley that have a direct impact on feed potential. All of these traits are influenced by both cultivar and environment. Thus it can be assumed that it would be possible to genetically select for feed quality. However, from a breeding perspective it is still unclear as to the definitive range of values for grain traits that would have a direct and positive influence on animal performance. To date, only one "fee" quality cultivar (Valier) has been released with claims of a direct improvement in animal performance (Bowman *et al.*, 1999). Particle size, ADF and starch as well as rate and extent of fermentation were considered to be the four most significant factors influencing performance (Bowman *et al.*, 2000).

Within the malting industry, processing of the grain has a direct impact on malt and beer quality. Similarly, processing of grain in the feed industry would also appear to impact on feed quality. Barley hardness is proposed associated with improved feed grain quality. Bowman *et al.* (1996) have reported a negative correlation between particle size index (hardness), acid detergent fibre and dry matter digestibility (DMD), but a positive correlation to daily live weight gain. Rowe *et al.* (1999) has also found that particle size has a direct impact on animal performance. Dehghan-Banadaky *et al.* (2007) detailed the effects of processing on particle size and cattle feed quality. Further, treatment of the grain via heating or cooking, as well as the use of supplements, has been found to have a positive effect on animal performance.

Limited data has been published on the relationship between feed and malt quality. Crosbie and Portman (1977) have reported on the relationships between EBC style malt extract with fibre. Significant ($p < 0.05$) negative correlations were obtained between extract and both ADF and NDF. Further analysis is required on well designed sample sets to define the relationships between barley traits for malt and feed quality. These, in turn, would be useful for breeding programs to select for feed quality cultivars. It has been proposed to use the EBC extract as an indicator of feed quality (Molino-Cano *et al.*, 1997) in a combined feed/malt quality testing program within a barley breeding program (Fox *et al.*, 2008).

Overall, the feed industry has yet to define quality in any terms that can be used practically for improved breeding selection. Until then, feed cultivars will remain second class quality grain.

3.7 Conclusion

Barley remains one of the world's economically important crops for its uses in food, feed and beverage production. While the main components are quite well understood, the minor components such as enzymes and inhibitors, their functions and roles and the synergistic interactions within the grain and when used in making value-added products remain to be completely unravelled.

The opportunity to exploit wild and exotic barley germplasm, as well as possibly genetically enhanced barleys, will have two effects. This will allow researchers to continue to improve barley cultivars and gain future knowledge of the chemistry and biochemical processes of barley.

Another aspect in gaining further understanding of barley will be the use of the most modern technologies, such as Nuclear Magnetic Resonance, Infrared Technologies, improved gene studies, i.e. Single Nucleotide Polymorphsims and Diversity Array Technologies.

References

Agu R C, Palmer G H (1997) α-Glucosidase Activity of Sorghum and Barley Malts. J Inst Brew, 103: 25-29

Agu R C (2003) Some Relationships between Malted Barleys of Different Nitrogen Levels and the Wort Properties. J Inst Brew, 109: 106-109

Agu R C, Brosnan J M, Bringhurst T A, Palmer G H, Jack F R (2007) Influence of Corn Size Distribution on Diastatic Power of Malted Barley and its Impact on Other Malt Quality Parameters. J Agr Food Chem, 55: 3702-3707

Alison M J, Cowe I, McHale R (1976) A Rapid Test for the Prediction of Malting Quality of Barley. J Inst Brew, 82: 166-167

Alison M (1986) Relationships between Milling Energy and Hot Water Extract Values of Malts from Some Modern Barleys and Their Parental Cultivars. J Inst Brew, 92: 604-607

Allosio-Ouarnier N, Quemener B, Bertrand D, Boivin P (2000) Application of High Performance Anion Exchange Chromatography to the Study of Carbohydrate Changes in Barely during Malting. J Inst Brew, 106: 45-52

Arends A M, Fox G P, Henry R J, Marschke R J, Symons M H (1995) Genetic and Environmental Variation in the Diastatic Power of Australian Barley. J Cereal Sci, 21: 63-70

Asano K, Shinagawa K, Hashimoto N (1982) Characterization of Haze-formation Proteins of Beer and Their Roles in Chill Haze Formation. J Am Soc Brew Chem, 40: 147-154

Australian Lot Feeders Association (2001) Lotfeeding, Annual Report, September 14-17

Autio K, Mannonen L, Pietila K, Koskinen M, Siika-Aho M, Linko M, Morgan A (1996) Incubation of Barely Kernel Sections with Purified Cell Wall Degrading Enzymes. J Inst Brew, 102: 427-432

Axcell B (1998) Malt Analysis: Prediction or Predicament, Tech Q, 35: 28-30

Bamforth C W, Kanauchi M (2001) A Simple Model for the Cell Wall of the Starchy Endosperm in Barley. J Inst Brew, 107: 235-240

Bason M L, Wrigley C W, McLeary B V (1993) Modelling the Effects of Sprouting Damage on Malting Barley, Based on Viscometric or Amylase Analysis. In: Walker-Simmons M K, Reid J L (eds.) Preharvest Sprouting in Cereals. American Society of Cereal Chemists, St Paul, 417-425

Bathgate G N, Palmer G H (1973) the *in vivo* and *in vitro* Degradation of Barley and Malt Starch Granules. J Inst Brew, 79: 402-406

Beecher B, Smidansky E D, See D, Blake T K, Giroux M J (2001) Mapping and Sequence-Analysis of Barley Hordoindolines. Theor Appl Genet, 102: 833-840

Beecher B, Bowman J, Martin J M, Bettge A D, Morris C F, Blake T K, Giroux M J (2002) Hordoindolines Are Associated With a Major Endosperm-texture QTL in Barley (*Hordeum Vulgare*). Genome, 45: 584-591

Benech-Arnold R L, Giallorenzi M C, Frank J, Rodriguez V (1999) Termination of Hull-imposed Dormancy in Developing Barley Grain Is Correlated in Change With Embryonic ABA Levels and Changes. Seed Sci Res, 9: 39-47

Benech-Arnold R (2002) Bases of Pre-harvest Sprouting Resistance in Barley: Physiology, Molecular Biology and Environmental Control of Dormancy in the Barley Grain. In: Slafer G A, Molina-Cano J L, Savin R, Araus J L, Romagos I (eds.) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality. Food Products Press, New York, 481-502

Benetrix F, Sarrafi A, Autran J C (1994) Effects of Cultivar and Nitrogen Nutrition on Protein Aggregates in Barley. Cereal Chem, 71: 75-82

Bhullar S S, Jenner C F (1996) Effects of Temperature on the Conversion of Sucrose to Starch in the Developing Wheat Endosperm. Aust J Plant Physiol, 13: 605-615

Bowman J G P, Blake T K, Surber L M M, Habernicht T K, Daniels T K, Daniels J T (1996) Genetic Factors Controlling Digestibility of Barley for Ruminants. Proc West Sec Am Soc Anim, 47: 257-260

Bowman J G P, Blake T K, Surber L M M, Habernicht D K, Bockelman H (2001) Feed Quality Variation in the Barley Core Collection of the USDA National Small Grains Collection. Crop Sci, 41: 863-870

Brennan C S, Harris N, Smith D, Shewry P R (1996) Structural Differences in the Mature Endosperms of Good and Poor Malting Barley Cultivars. J Cereal Sci, 24: 171-177

Brennan C S, Smith D B, Harris N, Shewery P R (1998) the Production and Characterisation of Hor3 Null Lines of Barley Provides New Information on the Relationship of D Hordein to Malting Performance. J Cereal Sci, 28: 291-299

Briggs D E, Hough J S, Stevens R, Young T W (1981) Malting and Barley Brewing Science, Volume 1, 2nd Edition, Chapman and Hall, London

Briggs D E, Woods J L (1993) Dormancy in Malting Barley: Studies on Drying, Storage, Biochemistry and Physiology. HGCA Project Report 84, 146

Briggs D E, Woods J L, Favier J F (1994) Drying and Storage Treatments for Overcoming Dormancy in Malting Barley. J Inst Brew, 100: 271-278

Briggs K G (1974) Combining Ability for Kernel Plumpness in a Diallel Cross of Five Canadian Barley Cultivars. Can J Plant Sci, 54: 29-34

Brinch-Pedersen H, Borg S, Tauris B, Holm P B (2007) Molecular Genetic Approaches to Increasing Mineral Availability and Vitamin Contents of Cereals. J Cereal Sci, 46: 308-326

Broadbent R E, Palmer G H (2001) Relationship between α-amylase Activity, Steeliness, Mealiness, Nitrogen Fractions on the Barley Grain. J Inst Brew, 107: 349-354

Buttimer E T, Briggs D E (2000a) Characterisation of Solubilized Forms of Bound Amylase Released By Various Agents. J Inst Brew, 106: 71-82

Buttimer E T, Briggs D E (2000b) Mechanisms of the Release of Bound Amylase. J Inst Brew, 106: 83-94

Caldwell K S, Langridge P, Powell W (2004) Comparative Sequence Analysis of the Region Harbouring the Hardness Locus in Barley and its Collinear Region in Rice1. Plant Physiol, 136: 3177-3190

Cakir M, Poulsen D, Galwey N, Ablett G, Chalmers K, Platz G, Park R, Lance R, Panozzo J, Read B J, Moody D, Barr A, Johnston P, Boyd W J R, Grime C R, Appels R, Li C, Jones M G K, Langridge P (2003) Mapping and QTL Analysis in the Barley Population Tallon X Kaputar. Aust J Agric Res, 54: 1155-1162

Canadian Wheat Board (2004) Barley - Malting Barley. Http://www.cwb.ca/en/growing/barley/12-malting_barley.jsp

Chandra G S, Proudlove M O, Baxter E D (1999) the Structure of Barley Endosperm - An Important Determinant of Malt Modification. J Sci Food Agric, 79: 37-46

Choct M, Selby E A D, Cadogan D J, Campbell R G (2004) Effects of Particle Size, Processing and Dry or Liquid Feeding on Performance of Piglets. Aust J Agric Res, 55: 237-245

Chowdhury S L, Wadlaw I F (1978) the Effect of Temperature on Kernel Development in Cereals. Aust J Agric Res, 29: 205-23

Cowe I A, Cuthbertson D C, Swanston J (1989) the Effect of Moisture and Nitrogen Levels on Milling Energy of Barley. J Inst Brew, 95: 423-425

Crosbie G B, Portman P A (1977) A Comparison of Screening Tests for Feed and Malting Quality for Barley. J Aust Inst Agri Sci, 43: 160-161

Dehghan-Banadaky M, Corbett R, Oba M (2007) Effects of Barley Grain Processing on Productivity of Cattle. Anim Feed Sci Tech, 137: 1-24

Dahl S W, Rasmussen S K, Hejgaard J (1996) Heterlogous Expression of Three Plant Serpins With Distinct Inhibitory Specificities. J Biol Chem, 271: 25083-25088

Darlington H F, Tesci L, Harris N, Griggs D L, Cantell I C, Shewery P R (2000) Starch Granule Associated Proteins in Barley and Wheat. J Cereal Sci, 32: 20-21

Darlington H, Rouster J, Hoffman L, Halford N G, Shewry P R, Simpson D J (2001) Identification and Molecular Characterisations of Hordoindolines from Barley Grain. Plant Mol Biol, 47: 785-794

Doan D N P, Fincher G B (1992) Differences in the thermostabilities of Barley (cv Clipper)(1→3, 1→4)β-glucanases Are only Partly Determined By N-glycosylation. FEBS Lett, 309: 265-271

Douliez J P, Michon T, Elmorjani K, Marion D (2000) Structure, Biological and Technological Functions of Lipid Transfer Proteins and Indolines, the Major Lipid Binding Proteins from Cereal Kernels. J Cereal Sci, 32: 1-20

Duffus C M, Cochrane M P (1992) Grain Structure and Composition. In: Shewry P R (eds.) Barley: Genetics, Biochemistry, Molecular Biology and Biotechnolog. CAB International Oxon, 291-318

Duffus C M, Cochrane M P (1993) Formation of the Barley Grain - Morphology, Physiology and Biochemistry. In: MacGregor A W, Bhatty R S (eds.) Barley-Chemistry and Technology. AACC, Minnesota, 31-72

Edney M (1996) Barley. In: Henry R J, Kettlewell P S (eds.) Cereal Grain Quality. Chapman & Hall, the University Press, Cambridge, 113-146

Edney M J, Mather D E (2002) Quantitative Trait Loci Affecting Germination Traits and Malt Friability in a Two-rowed By Six-rowed Barley Cross. J Cereal Sci, 39: 283-290

Edney M J, Langrell D E (2004) Evaluating the Malting Quality of Hulless CDC Dawn, Acid-dehusked Harrington and Harrington Barley. J Am Soc Brew Chem, 62: 18-22

Eglinton J K, Langridge P, Evans D E (1998) Thermostability Variation in Alleles of Barley Beta-amylase. J Cereal Sci, 28: 301-309

Ellis R P, Swanston J S, Bruce F M (1979) A Comparison of Some Rapid Screening Tests for Malting Quality. J Inst Brew, 85: 282-285

Emebiri L C, Moody D B, Panozzo J F, Chalmers K J, Kretschmer J M, Ablett G A (2003) Identification of QTLs Associated With Variations in Grain Protein Concentration in Two-row Barley. Aust J Agric Res, 54: 1211-1221

Emebiri L C, Moody D B (2004a) Potential of Low-protein Genotypes for Nitrogen Management in Malting Barley Production. J Agric Sci, 142: 319-325

Emebiri L C, Moody D B, Horsely R, Panozzo J, Read B J (2004b) the Genetic Control of Grain Protein Content Variation in a Double Haploid Population Derived from a Cross between Australian and North American Two-rowed Barley Lines. J Cereal Sci, 41: 107-114

Emebiri L C, Moody D B, Panozzo J F, Read B J (2004c) Mapping of QTL for Malting Quality Attributes in Barley Based on a Cross of Parents With Low Grain Protein Concentration. Field Crop Res, 87: 195-205

Emebiri L C, Moody D B, Black C, Van Ginkel M, Hernandez E (2007) Improvement in Malting Quality Barley Grain Yield By Manipulation of Genes Influencing Grain Protein Content. Euphytica, 156: 185-194

Evans E, Stenholm K, Vilpola A, Home S, Hughes G (1998) Pilot Scale Evaluation of Mash Filter Performance of Malts With Variable Quality. Tech Q, 35: 189-195

Evans D E, Hejgaard J (1999) the Impact of Malt Derived Proteins on Beer Foam Quality Part I: the Effect of Germination and Kilning on the Level of Protein Z4, Protein Z7 and LTP1. J Inst Brew, 105: 159-169

Evans D E, Sheehan M C, Stewart D C (1999a) the Impact of Malt Derived Proteins on Beer Foam Quality Part I: the Influence of Malt foam-positive Proteins and Non-starch Polysaccharides on Beer Foam Quality. J Inst Brew, 105: 171-177

Evans D E, Vilpola A, Stewart D C, Stenholm K, Poyri S, Washington J M, Barr A R (1999b) Pilot Scale Investigation of the Importance of the Barley Husk for Mash Filtration. Tech Q, 36: 375-382

Evers A D, Blakeney A B, O'Brien L (1999) Cereal Structure and Composition. Aust J Agric Res, 50: 629-650

Evrard A, Lagarde V, Joudrier P, Gautier M F (2007) Puroindoline-a and Puroindoline-b Interact With the Saccharomyces Cerevisiae Plasma Membrane Through Different Amino Acids Present in Their Tryptophan-rich Domain. J Cereal Sci, 48: 379-386

Ferrio J P, Alonso N, Voltas J, Araus J (2006) Grain Weight and Changes Over Time in Ancient Cereal Crops: Potential Roles of Climate and Genetic Improvement. J Cereal Sci, 44: 323-332

Fincher G B, Stone B A (1986) Cell Walls and Their Components in Cereal Grain Technology. In: Pommaranz Y (ed.) Advances in Cereal Science and Technology. American Society of Cereal Chemists, St Paul, 8: 207-295

Fincher G B (1975) Morphology and Chemical Composition of Barley Endosperm Cell Walls. J Inst Brew, 81: 116-122

Fontanini D, Jones B L (2001) Study of Metallopeptidase Isoenzymes from Malted Barley (*Hordeum Vulgare* Cv Morex). J Agric Food Chem, 49: 4903-4911

Fox G P, Kelly A M, Poulsen D M E, Inkerman P A, Henry R J (2006a) Genetic and Environmental Effects on Selecting Improved Barley Grain Size in Dry Environments. J Cereal Sci, 43: 198-208

Fox G P, Kelly A M, Cakir M, Bloustein G, Poulsen D M E, Inkerman P A, Henry R J (2006b) Impact of the Husk on Barley Grain Quality. J Inst Brew, 112: 101-107

Fox G P, Nguyen D L, Bowman J G P, Poulsen D M E, Inkerman P A, Henry R J (2007a) Relationship between Hardness Genes and Quality in barley (Hordeum Vulgare). J Inst Brew, 113: 87-95

Fox G P, Osborne B G, Bowman J G P, Kelly A M, Cakir M, Poulsen D M E, Inkerman P A, Henry R J (2007b) Measurement of Genetic and Environmental Variation in Barley (*Hordeum Vulgare*) Grain Hardness. J Cereal Sci, 46: 82-92

Fox G P, Bowman J, Kelly A, Inkerman P A, Poulsen D M E, Henry R J (2008a) Assessing for Genetic and Environmental Effects on Ruminant Feed Quality in Barley (*Hordeum Vulgare*). Euphytica, 163: 249-257

Fox G P, Kelly A, Bowman J G, Poulsen D M E, Inkerman P A, Henry R J (2008b) Relationship between Malt and Feed Quality in Barley (*Hordeum Vulgare*). Euphytica, Submitted

Furtado A, Henry R J, Scott K J (2003) the Promoter of the asi Gene in Transgenic Barley. Plant Mol Biol, 52: 787-799

Gamlath J, Aldred G P, Panozzo J F (2008) Barley (1→3; 1→4)-β-glucan and Arabinoxylan Content Are Related to Kernel Hardness and Water Uptake. J Cereal Sci, 47: 365-371

Gibson C E, Evans D E, Proudlove M O (1996) Protein Z4 and Beer Foam. Ferment, 9: 81-84

Glennie-Holmes M (1995) Studies on Barley and Malt With the Rapid Viscoanalyser I: the Effect of Variations in Physical and Chemical Parameters. J Inst Brew, 101: 11-18

Glennie-Holmes M (1995) Studies on Barley and Malt With the Rapid Viscoanalyser II: the Effects of Modification on Viscograms. J Inst Brew, 101: 19-28

Glennie-Holmes M (1995) Studies on Barley and Malt With the Rapid Viscoanalyser III: the Prediction of Malting Potential from Viscograms. J Inst Brew, 101: 29-32

Glennie-Holmes M (1995) Studies on Barley and Malt With the Rapid Viscoanalyser IV: Amyloclasis, Amylolysis and Cytolysis of Malt and Malting Quality. J Inst Brew, 101: 33-38

Goblirsch C A, Horsley R D, Schwarz P B (1996) A Strategy to Breed Low-protein Barley With Acceptable Kernel Color and Diastatic Power. Crop Sci, 36: 41-44

Grimes K H, Briggs D E (1995) Release and Activation of Barley Amylase. J Inst Brew, 101: 337-343

Grimes K H, Briggs D E (1996) the Release of Bound amylase By Macromolecules. J Inst Brew, 102: 261-270

Guerin J R, Lance R C M, Wallace W (1992) Release and activation of Barley Beta-amylase By Malt Endopeptidases. J Cereal Sci, 15: 5-14

Holopainen U, Wilhelmson A, Salmenkallio-Marttila M, Peltonen-Sainio P, Rajala A, Reinikainen P, Kotaviita E, Simolin H, Home S (2005) Endosperm Structure Affects the Malting Quality of Barley (Hordeum Vulgare L.). J Agric Food Chem, 53: 7279-7287

Han F, Ullrich S E, Clancey J A, Romagosa I (1999) Inheritance and Fine Mapping of a Major Barley Seed Dormancy QTL. Plant Sci, 143: 113-118

Han F, Ullrich SE, Romagosa I, Clancy JA, Froesth JA & Wesenberg DM (2003) Quantitative Genetic Analysis of Acid Detergent Fibre Content in Barley Grain. J Cereal Sci, 38: 167-172.

Han J Y, Schwartz P B (1996) Arabinoxylan Composition in barley, Malt and Beer. J Am Soc Brew Chem, 54: 216-220

Harju M A, De Souzza S, Brodl M R (2003) the Heat-shock Response of Developing Barley Aleurone Layers. Seed Sci Res, 13: 229-238

Hartl L, Kipp A, Baumer M, Schweizer G, Friedt W (2000) QTL-Mapping of Malting Quality Parameters in Spring Barley. In: Logue S (eds.) Proceedings of the 8th International Barley Genetics Symposium, Volume 3, 246-248

Hejgaard J, Kaesgaard P (1983) Purification and Properties of the Major Antigenic Beer Protein of Barley Origin. J Inst Brew, 89: 402-410

Henry R J (1984) A Rapid Method for the Determination of Diastatic Power. J Inst Brew, 90: 37-39

Henry R J (1986) Genetic and Environmental Variation in the pentosan and β-glucan Contents of Barley and Their Relation to malting Quality. J Cereal Sci, 4: 269-277

Henry R J (1987) Pentosans and (1-3) (1-4)-β-glucan Concentrations in Endopserm and Whole Grain of Wheat, Barley, Oats and rye. J Cereal Sci, 6: 253-258

Henry R J, Cowe I (1990) Factors Influencing the Hardness (Milling Energy) and Malting Quality of Barley. J Inst Brew, 96: 135-136

Hirota H, Kuroda H, Takoi K, Kaneko, T, Kaneda H, Yoshida I, Takashio M, Ito K, Takeda K (2006) Brewing Performance of Malted Lipoxygenase-1 Null Barley and Effect on the Flavour Stability of Beer. Cereal Chem, 83: 250-254

Hockett E A, White L M (1984) Simultaneous Breeding for feed and Malting Quality. Proceedings of the 4th International Barley Genetics Symposium, 234-241

Holtman W L, Vredenbregt-Heistek J C, Schmitt N F, Feussner I (1997) Lipoxygenase-2 Oxygenates Storage Lipids in Embryos of Germinating Barley. Euro J Biochem, 248: 452-458

Hori K, Sato K, Takeda K (2007) Detection of Seed Dormancy QTL in Multiple Mapping Populations Derived from Crosses Involving Novel Barley Germplasm. Theor Appl Genet, 115: 869-876

Hoseney R C (1986) Minor Constituents of Cereals. In: Principles of Cereal Science and Technology. American Society of Cereal Chemists, St Paul Minnesota, 89-110

Howard K A, Gayler K R, Eagles H A, Halloran G M (1996) the Relationship between D-Hordein and Malting Quality in Barley. J Cereal Sci, 24: 47-53

Hough J S (1985) the Biotechnology of Malting and Brewing. In: Cambridge Studies in Biotechnology 1. Cambridge University Press, Cambridge

Hussein A H (2004) Genetic and Mapping of Quantitative trait Loci of Feed Quality-related Traits in Barley (*Hordeum Vulgare* L.). Ph D Thesis, Montana State University

Izawa M, Kano Y, Koshino S (1993) Relationship between Structure and Solubitity of (1-3) (1-4)-β-glucan from Barley. J Am Soc Brew Chem, 51: 123-127

Izydorczyk M S, Macgregor A W, Billiaderis C G (2001) Effects of Malting on Phase Transition Behaviour of Starch in Barley Cultivars With Varying Amylose Content. J Inst Brew, 107: 119-128

Janes P W, Skerritt J H (1993) High Performance Liquid Chromatography of Barley Proteins: Relative Quantities of Hordeins Fractions Correlate With Malt Extract. J Inst Brew, 99: 77-84

Jones B L (1991) Partial Purification and Characterisation of Two Barley Fractions That Inhibit Malt Endoproteinases. J Am Soc Brew Chem, 49: 158-161

Jones B L (1999) Malt Endoproteinases and How They Affect Soluble Protein Levels. in Proceedings of the 9th Australian Barley Technical Symposium, Melbourne

Jones B L (2001) Interactions of Malt and Barley (*Hordeum Vulgare* L.) Endoproteinases With Their Endogenous Inhibitors. J Agric Food Chem, 49: 5975-5981

Jones B L (2005a) Endoproteases of Barley and Malt. J Cereal Sci, 42: 139-156

Jones B L (2005b) the Endogenous Endoprotease Inhibitors of Barley and Malt and Their Roles in Malting and Brewing. J Cereal Sci, 43: 271-280

Jones B L, Marinac L A (1995a) A Comparison of Barley and Malt Polypeptides That Inhibit Green Malt Endoproteinases. J Am Soc Brew Chem, 53: 160-166

Jones B L, Marinac L A (1995b) Barley LTP1 (PAPI) and LTP2: Inhibitors of Green Malt Cysteine Endoproteinases. J Am Soc Brew Chem, 53: 194-195

Jones B L, Marinac L A (2000) Purification and Partial Characterisation of a Second Cysteine Proteinase Inhibitor from Ungerminated Barley (*Hordeum Vulgare* L.). J Agric Food Chem, 48: 257-264

Jones B L, Marinac L A (2002) the Effect of Mashing on Malt Endoproteolytic Activities. J Agric Food Chem, 50: 858-864

Juge N, Svensson B (2006) Proteinaceous Inhibitors of Carbohydrate Active Enzymes in Cereals: Implication in Agriculture, Cereal Processing and Nutrition. J Sci Food Agric, 86: 1573-1586.

Kaiser A G (1999) Increasing the Utilisation of Grain When Fed Whole to Ruminants. Aust J Agric Res, 50: 737-756

Kanauchi M, Bamforth C W (2002) Enzymic Digestion of Walls Purified from the Starchy Endosperm of Barley. J Inst Brew, 108: 73-77

Kenn D A, Dagg A H S, Stuart I M (1993) Effect of Environment and Cultivar on the Fermentability of Malt Produced from Four Australia Barley Cultivars. J Am Soc Brew Chem, 51: 119-222

Koehler S M, Ho T H D (1990) A Major Gibberellic Acid-induced Barley Aleurone Cystein Proteinase Which Digest Hordein. Plant Physiol, 94: 251-258

Koliatsou M, Palmer G H (2003) A New Method to Assess Mealiness and Steeliness of Barley Cultivars and Relationship of Mealiness with Malting Parameters. J Am Soc Brew Chem, 61: 114-118

Kühbeck F, Dickel T, Krottenthaler M, Back W, Mitzscherling M, Delgado A, Becker T (2005) Effects of Mashing Parameters on Mash β-glucan, FAN and Soluble Extract Levels. J Inst Brew, 111: 316-327

Lee W J, Pyler R E (1984). Barley Malt Limit Dextrinase: Varietal, Environmental and Malting Effects. J Am Soc Brew Chem, 42, 11-17

Lenior P, MacGregor A W, Moll M, Duassant J (1984) Identification of Debranching Enzymes from Barley and Malt By Isoelectric Focusing. C.R. Academy of Science Series, 298: 243-248

Li J, Baga M, Rossnagel B G, Legge W G, Chibbar R N (2008) Identification of Quantitative Trait Loci for β-glucan Concentration in Barley Grain. J Cereal Sci, 48: 647-655

Lin R, Horsley R D, Schwartz P B (2008) Associations between Caryopsis Dormancy, α-amylase Activity, and Pre-harvest Sprouting in Barley. J Cereal Sci, in Press 48: 446-456

Longstaff M A, Bryce J H (1993) Development of Limit Dextrinase in Germinated Barley (*Hordeum Vulgare* L.). Evidence of Proteolytic Activation. Plant Physiol, 101: 881-889

Lusk L T, Duncombe G R, Kay S B, Navarro A, Ryder D (2001) Barley β-glucan and Beer Foam Stability. J Am Soc Brew Chem, 59: 183-186

MacNicol P K, Jacobsen J V, Keys M M, Stuart I M (1993) Effects of Heat and Water Stress on Malting Quality and Grain Parameters of Schooner Barley Grown in Cabinets. J Cereal Sci, 18: 61-68

MacGregor A W (1987) α-amylase, Limit Dextrinase and β-glucosidase Enzymes in Barley and Malt. CRC Critical Reviews in Biotechnology 5: 117-128

MacGregor A W (1996) Malting and Brewing Science: Challenges and Opportunities. J Inst Brew, 102: 97-102

MacGregor A W, Balance D L (1980) Hydrolysis of Large and Small Starch Granules from Normal and Waxy Barley Cultivars By β-amylases from Barley Malt. Cereal Chem, 57: 397-402

MacGregor A W, Bazin S L,Izydorczyk M S (2002) Gelatinisation Characteristics and Enzyme Susceptibility of Different Types of Barley Starch in the Temperature Range 48°C-72°C. J Inst Brew, 108: 43-47

MacGregor A W, Bazin S L, Schroeder S W (2002) Effect of Starch Hydrolysis Products on the Determination of Limit Dextrinase Inhibitors in Barley and Malt. J Cereal Sci, 35: 17-28

Macewicz J, Orzechowski S, Dobrzynska U, Haebel S (2006) Is Quantity of Protein in Barley Forms Determined By Proteins Localized in the Subaleurone Layer? Acta Physiol Plant, 2: 409-416

Maghirang E B, Dowell F E (2003) Hardness Measurement of Bulk Wheat By Single-kernel Visible and Near-infrared Reflectance Spectroscopy. Cereal Chem, 80: 316-322

Maguire T, Collins G, Sedgley M (1994) A Modified CTAB DNA Extraction Procedure for Plants Belonging to the Family Proteaceae. Plant Mol Biol Rep, 12: 106-109

Manners D J, Yellowlees D (1973) Studies on Debraching Enzymes. I. the Limit Dextrinase Activity of Extracts of Certain Higher Plants and Commercial Malts. J Inst Brew, 79: 377-385

Marchylo B A, Kruger J E, Hatcher D (1986) High-performance Liquid Chromatographic and Electrophoretic Analysis of Hordein during Malting for Two Barley Cultivars of Contrasting Malting Quality. Cereal Chem, 63: 219-231

May L H, Buttrose M S (1959) Physiology of Cereal Grain. II. Starch Granule Formation in the Developing Barley Kernel. Aust J Biol Sci, 12: 146-159

McLeary B V (1992) Measurement of the Content of Limit Dextrinase in Cereal Flours. Carbohyd Res, 227: 257-268

McLeod L C, Duffus C M (1988) Reduced Starch Content and Sucrose Synthase Activity in Developing Endosperm of Barley Plants Grown at Elevated Temperatures. Aust J Plant Physiol, 15: 367-75

Meredith W O S, Anderson J A, Hudson L E (1962) Evaluation of Malting Barley. In: Cook A H (eds.) Barley and Malt. Biology, Biochemistry and Technology. Academic Press, NewYork, 207-270

Molina-Cano J L, Ramo T, Ellis R P, Swanston J S, Bain H, Uribe-Echieverria T, Perez-Vendrell A M (1995) Effect of Grain Composition on Water Uptake By Malting Barley: A Genetic and Environmental Study. J Inst Brew, 101: 79-83

Molina-Cano J L, Francesch M, Perez-Vendrell A M, Ramo T, Voltas J, Brufau J (1997) Genetic and Environmental Variation in Malting ad Feed Quality of Barley. J Cereal Sci, 25: 37-47.

Molina-Cano J L, Rubio A, Igartua E, Gracia P, Montoya J L (2000a) Mechanisms of Malt Extract Development in Barleys from Different European Regions I. Effect of Environment and Grain Protein Content on Malt Extract Yield. J Inst Brew, 106: 111-115

Molina-Cano J L, Polo J P, Sopena A, Voltas J, Perez-Vendrell A M, Romagosa I (2000b) Mechanisms of Malt Extract Development in Barleys from Different European Regions II. Effect of Barley Hordein Fractions on Malt Extract Yield. J Inst Brew, 106: 117-123

Molina-Cano J L, Polo J P, Romera E, Araust J L, Zarco J, Swanston J S (2001) Relationships between Barley Hordeins and Malting Quality in a Mutant of Cv. Triumph I. Cultivar By Environment Interaction of Hordein Content. J Cereal Sci, 34: 285-294

Moralejo M A, Garcia-Casado G, Sanchez-Monge R, Lopez-Otin C, Romagosa I, Molina-Cano J L, Salcedo G (1993) Genetic Variants of the trypsin Inhibitor from Barley Endosperm Show Different Inhibitory Activities. Plant Sci, 89: 23-29

Moralejo M, Swanston J S, Munoz P, Prada D, Elia M, Russel J R, Ramsey L, Cistue L, Codesal P, Casas A M, Romagosa I, Powell W, Molina-Cano J L (2004) Use of New EST Markers to Elucidate the Genetic Differences in Grain Protein Content between European and North American Two-rowed Malting Barleys. Theor Appl Genet, 110: 116-125

Morell M K, Kosar-Hashemi B, Cmiel M, Samuel M S, Chandler P, Rahman S, Buleon A, Batey I L, Li Z Y (2003) Barley Sex6 Mutants Lack Starch Synthase IIa Activity and Contain a Starch With Novel Properties. Plant J, 34: 172-184

Morgan A G, Riggs T J (1981) Effects of Drought on yields and on Grain and Malt Characters in Spring Barley. J Sci Food Agric, 22: 339-346

Morris C (2002) Puroindolines: the Molecular Genetic Basis of Wheat Grain Hardness. Plant Mol Bio, 48: 633-647

Morris C F, Masa A N (2003) Puroindoline Cultivars of the U.S. National Institute of Standards & Technology Reference Material 8441, Wheat Hardness. Cereal Chem, 80: 674-678

Murray F, Mathews P, Jacobsen J, Grubler F (2006) Increased Expression of HvGAMYB in Transgenic Barley Increases Hydrolytic Enzyme Production By Aleurone Cells in Response to Gibberellin. J Cereal Sci, 44: 317-322

Mutisya J, Sathish P, Sun C X, Andersson L, Ahlandsberg S, Baguma Y, Palmqvist S, Odhiambo B, Aman P, Jansson C (2003) Starch Branching Enzymes in Sorghum (*Sorghum Bicolor*) and Barley (*Hordeum Vulgare*): Comparative Analyses of Enzyme Structure and Gene Expression. J Plant Physiol, 60: 921-930

National Association Commodity Marketing Authorities, 2001. National Barley Standards

Oliveira A B, Rasmusson D C, Fulcher R G (1994) Genetic Aspects of Starch Granule Traits in Barley. Crop Sci, 34: 1176-1180

Olkku J, Kotaviita E, Salmenkallio-Marttila M, Sweins H, Home S (2005) Connection between Structure and Quality of Barley Husk. J Am Soc Brew Chem, 63: 17-22

Osborne B G, Anderssen R S (2003) Single-kernel Characterization Principles and Applications. Cereal Chem, 80: 613-622

Osborne B G, Fox G P, Kelly A M, Henry R J (2007) Measurement of Barley Grain Rheology for the Prediction of Malting Quality. J Inst Brew, 113: 135-141

Osman A M, De Jersey J, Inkerman P A (1996a) A Novel Approach to a Differential Assay of Barley Malt α-glucosidases, Maltase and Malt Oligosaccharide α-glucosidase. In: Wrigley C W (eds.) Proc. of the 46th Australian Cereal Chemistry Conference. Royal Australian Chemical Institute, Melbourne, 172-175

Osman A M, De Jersey J, Inkerman P A (1996b) the Specific Measurement of Starch Degrading Enzymes in a Common Extract from Barley Malt. In: Wrigley C W (eds.) Proc of the 46th Australian Cereal Chemistry Conference. Royal Australian Chemical Institute, Melbournea, 208-212

Osman A M, Coverdale S M, Cole N, Hamilton S E, De Jersey J, Inkerman P A (2002) Characterisation and Assessment of the Role of Barley Malt Proteases during Malting and Mashing. J Inst Brew, 108: 62-67

Palmer G H (1983) Malting and Mashing. In: An Introduction to Brewing and Science Technology. the Institute of Brewing, London, 10-27

Palmer G H, Agu R C (1999) Effect of Mashing Temperature and Endo-β-glucanase on β-glucan Content of Malt Worts. J Inst Brew, 105: 233-235

Passarella V S, Savin R, Slafer G A (2005) Breeding Effects on Sensitivity of Barley Grain Weight and Quality to Events of High Temperature during Grain Filling. Euphytica, 141: 41-48

Perez-Vendrell A M, Brufau J, Molina-Cano J L, Francesch M, Guasch J (1996). Effects of Cultivar and Environment on β-(1,3)-(1,4)-D-glucan Content and Acid Extract Viscosity of Spanish Barleys. J Cereal Sci, 23: 285-292

Poulle M, Jones B L (1988) A Proteinase from Germinating Barley. I. Purification and Some Physical Properties of a 30kD Cysteine Endoproteinase from Green Malt. Plant Physiol, 88: 1454-1460

Psota V, Vejraka K, Famera O, Hrcka M (2007) Relationship between Grain Hardness and Malting Quality of Barley (*Hordeum Vulgare* L.). J Inst Brew, 113: 80-86

Robinson L, Juttner J, Milligan A, Lahnstein J, Eglinton J K, Evans D E (2007) the Identification of a Barley Haze Active Protein that Influences Beer Haze Stability: Cloning and Characterisation of the Barley SE Protein as a Barley Trypsin Inhibitor of the Chloroform/methanol Type. J Cereal Sci, 45: 348-352

Roumeliotis S, Collins H M, Logue S J, Willsmore K L, Jefferies S P, Barr A R (1999) Implication of Thin Husk. In: Proceedings of the 9th Australian Barley Technical Symposium, Melbourne, http://www.cdesign.com.au/proceedings%5Fabts1999/papers/S_Roumeliotis.pdf

Salcedo G, Fra-Mon P, Molina-Cano J L, Aragoncillo C, Garcia-Olmedo F (1984) Genetics of CM-proteins (A-hordeins) in Barley. Theor Appl Genet, 68: 53-59

Sardana V, Zhang G P (2005) Genotypic Variation in Some Quality Traits of Malt Barley (*Hordeum Vulgare* L.) Caused By Time of Nitrogen Application and Kernel Position Within the Spike. Cereal Res Commun, 33: 817-823

Savin R, Nicolas M E (1996) Effects of Short Periods of Drought and High Temperature on Grain Growth and Starch Accumulation of Two Malting Barley Cultivars. Aust J Plant Physiol, 23: 201-210

Savin R, Stone P J, Nicolas M E, Wardlaw I F (1997) Grain Growth and Malting Quality of Barley 1. Effects of Heat Stress and Moderately High Temperature. Aust J Agric Res, 48: 615-624

Savin R, Nicolas M E (1999) Effects of Timing of Heat Stress and Drought on Growth and Quality of Barley Grains. Aust J Agric Res, 50: 357-364

Schmitt H F, Van Mechelen J R (1997) Expression of Lipoxygenase Isoenzymes in Developing Barley Grains. Plant Sci, 128: 141-150

Schmitt M R, Budde A D (2007) Improved Methods for High-throughput Extraction and Assay of Green Barley Malt Proteinases Activity Facilitating Examination of Proteinases Activity Across large-scale Barley Populations. Cereal Chem, 84: 313-319

Schmitt M R, Marinac L (2008) Beta-amylase Degradation By Serine Endoproteinases from Green Barley Malt. J Cereal Sci, 47: 480-488

See D, Kanazin V, Kephart K, Blake T (2002) Mapping Genes Controlling Variation in Barley Grain Protein Concentration. Crop Sci, 42: 680-685

Sheehan M C, Skerritt J H (1997) Identification and Characterisation of Beer Polypeptides Derived from Barley Hordeins. J Inst Brew, 107: 297-306

Shewry P R (1993) Barley Seed Proteins. In: MacGregor A W, Bhatty R S (eds.) in "Barley: Chemistry and Technology". Am Soc Cereal Chem, ST. Paul MN, USA, 131-197

Shewry P R, Tatham A S, Halford N G (2001) Nutritional Control of Storage Protein Systhesis in Developing Grain of Wheat and Barley. Plant Growth Regul, 34: 105-111

Silva F, Nogueria L C, Goncalves C, Ferreira A A, Ferreira I, Teixeira N (2007) Electrophoretic and HPLC Methods for comparative Study of the Protein Fractions of Malts, Worts and Beer Produced from Scarlett and Prestige Barley (*Hordeum Vulgare* L.) Varieties. Food Chem, 106: 820-829

Sission M J, Lance R C M, Sparrow D H B (1992) Studies on Limit Dextrinase in Barley. I. Purification of Malt Limit Dextrinase and Production of Specific Monospecific Antibodies. J Cereal Sci, 16: 107-116

Skerritt JH & Henry RJ (1988) Hydrolysis of Barley Endosperm Storage Proteins during Malting. II. Quantification By Enzyme- and Radio-immunoassay. J Cereal Sci, 7: 265-281.

Skerritt J H, Janes P W (1992) Disulphide-bonded "gel Protein" Aggregates in Barley: Quality-related Differences in Composition and Reductive Dissociation. J Cereal Sci, 16: 219-235

Smith D B (1990) Barley Seed Protein and its Effects on Malting and Brewing Quality. Plant, Var Seeds, 3: 63-80

Sopanen T, Lauriere C (1989) Release and Activity of Bound Beta-amylase in a Germinating Barley Grain. Plant Physiol, 89: 244-249

Sparrow D H B, Lance R C M, Henry R J (1988) Alternative End Uses of Barley. Royal Australian Chemical Institute, Melbourne

Stahl V, Coates S, Bryce J H, Morris P C (2004) Antisense Downregulation of the Barley Limit Dextrinase Inhibitor Modulates Starch Granule Size Distribution, Starch Composition and Amylopectin Structure. Plant J, 39: 599-611

Stahl Y, Alexandra R D, Coates S, Bryce JH, Jenkinson H R, Morris P C (2007) the Barley Limit Dextrinase Inhibitor: Gene Expression, Protein Location and Interaction With 14-3-3 Protein. Plant Sci, 172: 452-461

Stenholm K, Home S (1999) A New Approach to Limit Dextrinase and its Role in Mashing. J Inst Brew, 105: 205-210

Stevnebø A, Sahlström S, Svihus B (2006) Starch Structure and Degree of Starch Hydrolysis of Small and Large Starch Granules from Barley Cultivars With Varying Amylose Content. Anim Feed Sci Tech, 130: 23-38

Stewart D C, Hawthorne D, Evans D E (1998) Cold Sterile Filtration: A Small Scale Filtration Test and Investigation of Membrane Plugging. J Inst Brew, 104: 321-236

Stewart D C, Freeman G, Evans D E (2000) Development and Assessment of a Small-scale Wort Filtration Test for the Prediction of Beer Filtration Efficiency. J Inst Brew, 106: 361-366

Stuart I M, Loi L, Fincher G B (1988) Varietal and Environmental Variations in (1→3, 1→4)-β-glucan and (1→3, 1→4)-β-glucanase Potential in Barley: Relationships to malting Quality. J Cereal Sci, 7: 61-71

Swanston J S (1995) Effects of Barley Grain Size, Texture and Modification during Malting Associated With Three Genes on Chromosome 1. J Cereal Sci, 22: 157-161

Swanston J S (1996) Associations of the Waxy (wx) Gene With Malting Quality Characteristics in Random Inbred Lines of Barley. J Inst Brew, 102: 355-358

Swanston J S, Taylor K (1988a) the Milling Energy of Malted Barley and its Relationship With Hot Water Extract and Amylase Activity. J Inst Brew, 94: 143-146

Swanston J S, Taylor K (1988b) A Comparison of Some Rapid Tests to Assess Extent of Modification during a Laboratory Scale Malting Procedure. J Inst Brew, 94: 311-314

Swanston J S, Cowe I A (1989) A Rapid Technique to Predict Malting Quality Prior to Harvest. Ann Appl Biol, 115: 529-532

Swanston J S, Ellis R P, Rubio A, Perez-Vendrell A, Molina-Cano J L (1995) Differences in Malting Performance between Barleys Grown in Spain and Scotland. J Inst Brew, 101: 261-265

Swanston J S, Molina-Cano J L (2001) Beta-amylase Activity and Thermostability in Two Mutants Derived from the Malting Barley Cv. Triumph. J Cereal Sci, 33: 155-161

Swanston J S, Ellis R P (2002) Genetics and Breeding of Malt Quality Attributes. In: Slafer GA, Molina-Cano J L, Savin R, Araus J L, Romagosa I (eds.) In, Barley Science, Recent Advances From Molecular Biology to Agronomy of Yield and Quality. Food Products Press, New York, 85-114

Tatham A A, Shewery P R (1995) Mini Review: the S-poor Prolamins of Wheat, Barley and Rye. J Cereal Sci, 22: 1-6

Turnbull K M, Gaborit T, Marion D, Rahman S (2000) Variation in Puroindoline Polypeptides in Australian Wheat Cultivars in Relation to Grain Hardness. Aust J Plant Physiol, 27: 153-158

Ullrich S E, Clancy J A, Lee H, Han F, Matsui K, Del Blanco I A (2005) Genetic Analysis of Preharvest Sprouting in Barley. Proceedings of the 18th North American Barley Researchers Workshop, and 4th Canadian Barley Symposium, Red Deer: Canada.

Vickers J E, Hamilton S E, De Jersey J, Henry R J, Marschke R J, Inkerman P A (1996) Assessment of Bacillus Licheniformis β-amylase as a Candidate Enzyme for Genetic Engineering of Malting Barley. J Inst Brew, 102: 75-78

Vietor R J, Angelino S A G F, Voragen A G J (1991) Arabinoxylans in Barley, Malt and Wort. Proceedings of the 23rd EBC Congress, Lisbon, 139-146

Walker C, Dickie K, Biawa J P, Ueda T, Muller R E (2001) Prediction of Extract Potential in New Barley Cultivars By Measuring Cell-wall Breakdown. J Inst Brew, 107: 167-174

Wallwork M A B, Logue S J, MacLeod L C, Jenner CF (1998a) Effects of a Period of High Temperature during Grain Filling on the Grain Growth Characteristics and Malting Quality of Three Australian Malting Barleys. Aust J Agric Res, 49: 1287-1296

Wallwork M A B, Logue S J, MacLeod L C, Jenner C F (1998b). Effect of High Temperature during Grain Filling on Starch Synthesis in the Developing Barley Grain. Aust J Plant Physiol, 25: 173-181

Wang M, Heimovaara-Dijstra S, Van Duijn B (1995) Modulation of Germination of Embryos Isolated from Dormant and Non-dormant Barley Grains By Manipulation of Endogenous Abscisic Acid. Planta, 195: 586-592

Weidner S, Paprocka J, Kamieniecki B, Zadernowski R (1993) In: Walker-Simmons M K, Reid J L (eds.) the Role of Phenolic Acids in Dormancy of Barley Caryopses. Proceedings of the 6th International Conference on Pre-Harvest Sprouting, American Society of Cereal Chemists, USA, 200-211

Woodward J R, Fincher G B (1982) Substrate Specificities and Kinetic Properties of Two (1-3) (1-4) -D-Glucan Endohydrolyase from Germinating Barley. Carbohyd Res, 106: 111-122

Worbel R, Jones B L (1992a) Appearance of Endoproteolytic Enzymes during the Germination of Barley. Plant Physiol, 100: 1508-1516

Worbel R, Jones B L (1992b) Electrophoretic Study of Substrate and PH Dependence of Endoproteolytic Enzymes in Green Malt. J Inst Brew, 98: 471-478

Worbel R, Jones B L (1993) Identification and Partial Characterisation of *high Mr Neutral Proteinases* from 4-day Germinated Barley Seeds. J Cereal Sci, 18: 225-237

Wu Y, Schwartz P B, Doehlert D C, Dahleen L S, Horsley R D (1997) Rapid Separation and Genotypic Variability of Barley (*Hordeum Vulgare* L.) Lipoxygenase Isoenzymes. J Cereal Sci, 25: 49-56

Yamada, J (1981) Purification of Oat Debranching Enzyme and Occurrence of Inactive Debranching Enzyme in Cereals. Agric Biol Chem, 45: 1013-1015

Yan Y, Zhu J, Xu S, Xu Y (1999) Genetic Effects of Embryo and Endosperm for Four Malting Quality Traits of Barley. Euphytica, 106: 27-34

Zhang G, Chen J, Wang J, Ding S (2001) Cultivar and Environmental Effects on (1→3, 1→4)-β-D-glucan and Protein Content in Malting Barley. J Cereal Sci, 34: 295-301

Zhang N, Jones B L (1995a) Characterisation of Germinated Barley Endoproteolytic Enzymes By Two-dimensional Gel Electrophoresis. J Cereal Sci, 21: 145-153

Zhang N, Jones B L (1995b) Development of Proteolytic Activities during Barley Malting and Their Localisation in the Green Malt Kernel. J Cereal Sci, 22: 147-155

Zhang N, Jones B L (1999) Polymorphism of Aspartic Proteinases in Resting and Germinating Barley Seeds. Cereal Chem, 76: 134-138

Zhang Y, Darlington H, Jones H D, Halford N G, Napier J A, Davey M R, Lazzeri P A, Shewery P R (2003) Expression of the Gamma-zein Protein of Maize in Seeds of Transgenic Barley: Effects on Grain Composition and Properties. Theor Appl Genet, 106: 1139-1146

Zhao F J, Fortune S, Barbosa V L, McGrath S P, Stobart R, Bilsborrow P E, Booth E J, Brown A, Robson P (2006) Effects of Sulphur on Yield and Malting Quality of Barley. J Cereal Sci, 43: 369-377