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125th Anniversary Review: Barley research in relation to Scotch whisky production: a journey to new frontiers

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Human experience with barley has been well established for several millennia and barley research has been fundamental to our understanding of raw materials for malting, brewing and distilling. Distillers have long been indebted to malting, brewing and distilling researchers for information on barley (and malt) relevant to their operations. Originally distilling barley research was focussed on the parameters defining barley quality and plant performance, but it has developed to further our understanding of the properties and genetics of barley and malt. Through the years, several strategic milestones can be identified showing a progression of related research themes, culminating in our current state of knowledge of barley. These include the development of the fermentability method, together with the biochemistry and enzymology underlying starch and cell wall hydrolysis, which resulted in a greater understanding of processing properties and subsequent improvements in performance. Ethyl carbamate is a barley-derived carcinogen present in a range of potable spirits, which has been a concern for distillers; the identification of the genetic marker for the barley precursor epiheterodendrin laid the foundations for the application of modern (non-GMO) genetics to developing improved barley varieties, which will benefit the whole supply chain. Together these approaches underline the mutual interdependence of applied research and genetic approaches in achieving substantial advances in our knowledge.

Keywords: Scotch whisky; barley; quality; processing; epiheterodendrin (EPH); research; genetics

Introduction

It is a great pleasure to celebrate the 125th Anniversary of the Institute of Brewing and Distilling by providing a modest review of the major milestones in our understanding of the properties of barley and malt that are best suited for Scotch Whisky distilling.

Barley is the fundamental raw material for both beer and whisky production and has been used for this purpose for many centuries, starting with the original use of (semi-)wild landraces such as bere barley, and continuing with modern advanced high-quality multi-use barley varieties, such as Concerto, and its successors, which have been specifically developed, selected and customized for both distilling and brewing. The historical development of brewing and distilling technology and practice has been concisely summarized by Anderson *(1)*, Hume and Moss *(2)* and Bathgate *(3)*. Fundamental aspects of the production of Scotch whisky and the cereal raw materials used are described by Dolan *(4)* and Bringhurst *et al. (5)*. In parallel with this there has also been a long and illustrious heritage of fundamental malting barley research in the UK, as well as globally, primarily disseminated by the Institute of Brewing and Distilling (IBD), and which has been well documented over the last century (and before), particularly for malting and brewing, with major contributions from a very wide range of researchers, either as individuals or as members of both small and large research groups. Scotch whisky distillers have long been substantial beneficiaries of this vast body of research, which has been essential in providing information to help understand the properties of barley and malt, so that they can be used effectively in the distillery. However, it must be acknowledged that distillers and brewers have different quality requirements, with distillers laying more emphasis on maximizing alcohol production, while brewers are more focussed on high levels of extracted sugars and dextrins, to provide both alcohol and flavour (and body) in the final product. The fundamental difference between distilling and brewing production practices is that Scotch whisky distillers do not boil their worts, since they need to retain the maximum levels of endogenous enzyme activity into the fermentation process, to ensure that as much of the starch as possible is hydrolysed to fermentable sugars, which can be converted efficiently to alcohol by yeast.

Many aspects of the science underlying brewing and the production of Scotch whisky have been studied for a very long time, at least since the start of the twentieth century, and a full review of this massive body of work would fill many volumes; indeed the archives

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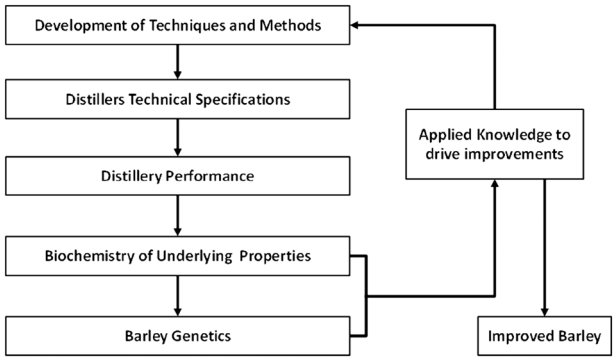

Figure 1. Understanding distilling barley properties.

of the *Journal of the Institute of Brewing* are filled with useful, and still relevant information about barley and malt. In this paper I will try to focus on a relatively small number of major milestones in barley research specifically relating to the production of Scotch whisky and attempt to show how these have helped to improve our understanding of the special characteristics of distilling barley, and how they have driven improvements in the quality of cereal raw materials, which have not only resulted in substantial increases in both alcohol yield and production efficiency, but also helped the industry to respond effectively to issues affecting product safety, such as minimizing the chances of contamination of spirits with materials such as nitrosodimethylamine and ethyl carbamate.

The progress of understanding the properties of distilling barley can probably be best described in a series of distinct stages (Fig. 1): (a) the development of techniques and methods to select barley and malt to meet distillers' technical specifications; (b) understanding the biochemistry and genetics of the processes underlying these properties; and (c) using that understanding to drive the development of new and improved techniques and materials. For distilling (and brewing), aside from the purely technological advances in processing equipment, these developments have encouraged stakeholders to consider the possibilities for more advanced barley varieties that are better suited to their specific needs, and which can provide substantially higher alcohol yield and give improved process efficiency in the distillery (and the brewhouse).

Origins and dispersal of barley

Nettleton's book, written in 1913 *(6)*, still provides an essential, and remarkably, still relevant, highly detailed account of the basic production processes for both malt and grain whisky, which emphasizes the importance of breeding good barley varieties

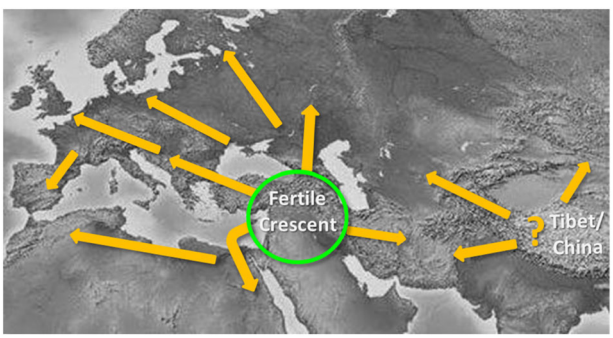

Figure 2. Origins and gradual spread of barley cultivation (10,000 BCE onwards) (original map available from www.freeworldmaps.net).

to provide a reasonable level of alcohol production and good processing characteristics, all of which are still a very high priority for modern Scotch whisky distillers.

Barley (*Hordeum vulgare*) was one of the first cereals domesticated by humans, probably in the 'fertile crescent' in the Middle East (Turkey/Lebanon/Syria/Iraq/Iran) at least 10,000 years ago *(7)*, before gradually spreading to Egypt and other areas, although there is an increasing body of evidence to suggest an independent origin further east *(8)*, perhaps as far away as Tibet *(9)* (Fig. 2).

Current opinions surmise that the origins of baking and 'brewing' were intrinsically linked to the domestication and spread of cereals even at this pre-historical period *(10)*. Nelson *(11)* points to the development of pottery at around 6000 BCE, as being a major watershed in the early development of beer ('malting' and 'brewing') and appears to have been well established in Mesopotamia and Egypt by the start of the fourth millennium BCE. Pottery shards, possibly containing 'beer' residues have been identified that have been dated to 3500–2900 BCE (Godin Tepe, Iran) *(12)*. Similarly, storage vessels from Crete dating from 2000 BCE have been found to have contained a barley-derived product that might have been beer *(11)*. Traces of cereals and other organic residues from Neolithic pottery fragments found in various locations in Scotland, and in other regions (e.g. Jutland) dating from 3000–1500 BCE suggest an independent origin of 'brewing' in northern Europe *(11)*. These early traces indicate that cereals have been used to make beverages similar to beer (at least in principle) for a very long time indeed, showing that this activity has been fundamental to humans, from the very beginnings of civilization to the present day.

Shewry *et al. (13)* and Palmer *(14)* provide studies on some Egyptian material obtained from 3000 to 1000 BCE, which indicates some similarities to modern varieties particularly in the visible protein structures and amino acid composition *(13)*, although the effects of time have made it somewhat unclear whether some of the detailed structures are truly comparable. However, Palmer *(14)* suggests that the visible remains of the grain structures and endosperm of the material from ancient Egypt shows some evidence of some form of 'malting'.

Modern genetic studies *(8)* suggest that most of the modern barley now grown in northern Europe derives from landraces originating from these aboriginal forms, which have spread from these areas. In the transition from wild to domesticated barley the amount of starch in the endosperm was effectively doubled *(15)*. The wide diversity of these wild progenitors of modern barley is considered to provide a strong potential for improved tolerance to biotic and abiotic stresses *(9)*. It has been shown that there was a close genetic relationship between East Asian and northern Mediterranean two-rowed cultivars, which suggested that germplasm exchange between these regions, for example through barley trading, helped to ingress a high degree of cold tolerance and spring habit, which allowed them to spread into large areas of Europe and Scandinavia *(9)*. The broad genetic diversity that has been associated with barley is an important factor in the development of the modern barley varieties that are now used commercially for distilling and brewing (as well as for other purposes), and it is interesting to note how the diversity arising from some of these wild exotic and sometimes ancient cultivars is now providing germplasm that is being used to address fundamental challenges arising from climate change and drought tolerance *(16)* and could potentially provide new varieties with a wider range of potential applications, which could also be utilized for brewing and distilling as well as for feed/food use.

One of the key areas of research into the breeding of new barley varieties is in the application of modern molecular

biology techniques *(17)*, which have been developed as a result of advances in our understanding of the genetics of barley. This has resulted in the development of genetic markers that can be used to select for important phenotype traits, such as for hot water extract (HWE), fermentability and epiheterodendrin (EPH), the barley precursor of ethyl carbamate *(18)*. The importance of this approach is that it facilitates the selection of parent varieties with complementary genetic properties that can be used to ingress the desired phenotype characteristics in the progeny using classical breeding techniques to develop new, improved barley cultivars more rapidly and precisely than was previously possible *(19)*.

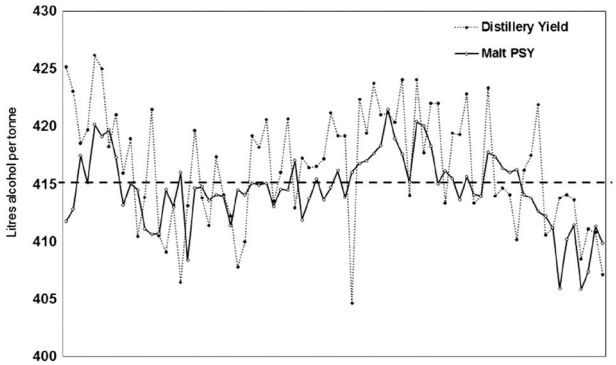

Figure 3. Chart showing the relationship between distillery alcohol yield and malt predicted spirit yield (PSY). (Production data from a Scotch whisky distillery).

Barley malt performance specifications

Almost 40 years ago Dolan *(20)* was able to highlight the impact of brewing science on providing fundamental information to support the specific needs of distillers. He selects three major areas where the application of brewing research can give important support to distillers in pursuit of their production requirements. These are all still highly relevant. The first of these is to ensure that the raw materials are of the most suitable (i.e. best) quality. The second is to ensure that the process plant is kept as clean as possible to minimize bacterial contamination in an essentially non-sterile system. Finally, paramount to all, there is a need to ensure that the process is operated as efficiently as possible.

In order to achieve these, it is necessary to look objectively at each factor influencing the process, by analysing samples, whether they are barley, malt or other process samples. The development of the set of laboratory methods, which was compiled as the Institute of Brewing Recommended Methods of Analysis *(21)* [now re-issued by the European Brewery Convention (EBC) as part of the standard Analytica-EBC Methods *(22)*], was a critical factor in achieving this.

Dolan *(20)* selects a relatively small number of key parameters that can be used to effectively characterize malted barley, which have been fundamental on malt specifications. These are HWE, total nitrogen (protein), soluble nitrogen ratio, wort fermentability and thousand corn weight. While also acknowledging that (starch degrading) enzyme levels {α- and β-amylase [dextrinizing units (DU); diastatic power (DP)]} are important, Dolan points out that these are less critical in malt since, if barley is malted properly, there will be sufficient availability of these and the other important malt enzymes (such as β-glucanases) to provide satisfactory distillery performance.

During this period it was highlighted that there was a need for a reliable method to predict the potential alcohol yield of barley malt directly, rather than from an equation *(20)*. Griffin *(23)* had proposed a fermentability method, based on the fermentation of the unboiled Institute of Brewing hot water extract wort. Gray and Dewar *(24)* reported the evaluation of a fermentability method based on residue gravity, which at that time was undergoing collaborative trials by distillers and maltsters. The fermentability method that was finally developed, gave values for both fermentable extract and predicted spirit (alcohol) yield (PSY) *(25)* and was adopted as a standard Institute of Brewing Recommended method (Method 2.16). Over the years there have been minor changes to the fermentability method, principally with the change in the HWE procedure from 515 mL mash, to 450 g mash *(26)*. However, the method has been re-assessed and validated periodically *(27–29)* and is still considered to be fit for purpose. One of the fundamental properties of the fermentability method is that the PSY of the malt gives a value that can be easily interpreted and realistically compared with trends in the actual alcohol production of the distillery *(28–30)* (Fig. 3).

Figure 3 shows some 'typical' commercial production data from a Scotch whisky distillery, which highlights the relationship between the malt potential (PSY) and distillery yield over a significant period (about 18 months). During this period, on average, the differences between the distillery yield and the malt predicted spirit yield were around 1% (ca. 4 L of alcohol per tonne). While the mapping of these two important parameters is not perfect [correlation (r) = 0.475; significant at p = 0.001], the values for the distillery yield generally keep track with the malt predicted spirit yield, but can also be higher or lower owing to 'random' or batch variations in both barley malt quality and distillery performance [cf. Dolan *(29)*]. The chart also highlights the high degree of variability in barley supplies over this relatively long period, which also reflects changes between consecutive barley harvest seasons, and how these can impact on distillery production.

The fermentability method has been in constant use for trading and assessment of distilling malt for more than 30 years and is still highly relevant. Dolan *(30)* summarizes the key analytical parameters for pot still malt for malt distilleries, which often appear on distillery malt specifications. These are moisture, friability, homogeneity, soluble extract (or HWE), fermentable extract and PSY, and these provide a realistic benchmark for measuring and comparing the potential performance of barley malt in the distillery. This information is also a fundamental cornerstone in defining the desirable attributes for barley that have been agreed by distillers and communicated to barley breeders to guide the development of new improved varieties for both brewers and distillers *(30)*. The list of desirable attributes has been refined over the years by the Scotch whisky industry, but the essential attributes for a Scotch whisky distillers' barley wish-list are high alcohol yield, good enzyme levels and non-glycosidic nitrile (non-GN) producing varieties.

There is now also a caveat to ensure that any new varieties will enhance the sustainability of the industry. Table 1 summarizes the attributes for both malt distilling (pot still) and grain distilling barley that are currently agreed by the Scotch whisky distilling industry.

Table 1. Attributes of Scotch whisky malt and grain distilling barley varieties agreed by the Scotch Whisky industry

Malt distilling barley	Grain distilling barley
Process efficiency Maximum alcohol yield potential [Predicted spirit +yield (PSY); fermentability; hot water extract (HWE)] *Ease of processing in the distillery* (a) Cell wall modification [friability (homogeneity); low wort viscosity; β-glucans] (b) Protein modification (TSN; SNR; free amino nitrogen) *Product integrity/regulatory issues* Minimize ethyl carbamate precursors [glycosidic nitrile (GN); screen for epiheterodendrin (EPH) non-producers using genetic marker] *Sustainability* Identify varieties likely to be resilient to the predicted effects of climate change and to contribute to lowering the carbon footprint of distilling (improvements compared with established distilling control varieties)	*Process efficiency* Potential to convert cereal starch into fermentable sugars [diastatic power (DP); dextrinizing units (DU)] *Protein modification* Potential for protein degradation [total soluble nitrogen (TSN); soluble nitrogen ratio (SNR)]

The fermentability method that is currently used is time consuming and labour intensive, so there is now increasing interest in more rapid methods, such as near infrared (NIR) analysis, for basic intake parameters, but it will be some time before some NIR analysis models for predicted spirit yield are considered fully reliable and will be universally accepted for the commercial trading of barley and malt.

Distilling quality of barley and malt

Jamar *et al. (31)* show a diagram summarizing the desirable characteristics of malting barley, which is a useful guide to understanding the complexity of the parameters that are considered when defining malting quality. This has been adapted to highlight the factors defining distilling quality, and Fig. 4 shows the main parameters that are considered by Scotch whisky distillers to be the most important determinants of barley (and malt) distilling quality. These fall into several categories: (a) general requirements, which summarize those on the Scotch Whisky Association barley wish list; (b) product protection and regulatory requirements, which include conformance with the definition of Scotch whisky, the preference for low/no glycosidic nitrile and other due diligence requirements; and (c) structural and biochemical factors that will affect alcohol production and process efficiency. Some of these aspects will be discussed later in this paper.

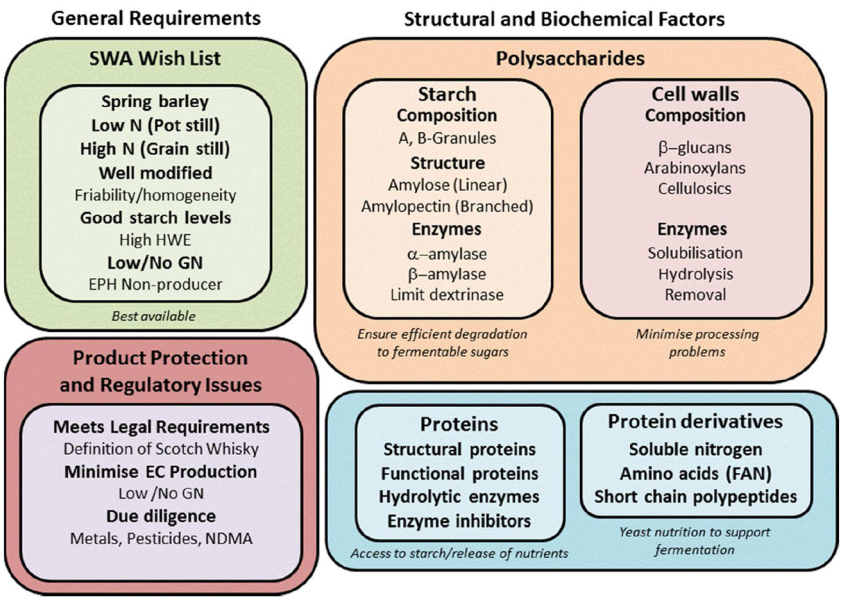

Figure 4. Characteristics defining distilling quality of barley [adapted from Jamar *et al. (31)*].

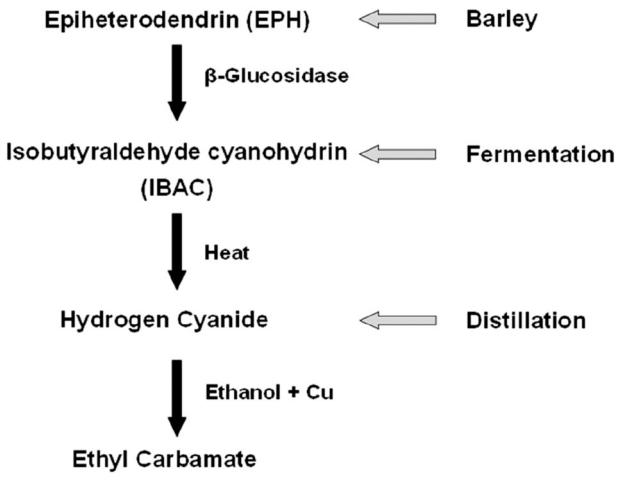

Figure 5. Ethyl carbamate formation in distilled spirits from epiheterodendrin (EPH) in barley [after Cook *et al. (41)*].

Precursors of ethyl carbamate from barley

In 1985, the Liquor Control Board of Ontario discovered that certain alcoholic beverages, including whisky, contained relatively high levels of ethyl carbamate, a known animal carcinogen, and a guideline maximum value of 150 ppb was established for distilled spirits, including Scotch whisky *(32)*. At the same time, in the USA a 'voluntary' limit of 125 ppb was applied to whisk(e)y, and a maximum level of 400 ppm was set in Germany for all alcoholic beverages *(33)*). Since this time, maximum limits have been set internationally (e.g. Czech Republic 150 ppb) *(34)*. These limits are under continuous review by international regulating authorities and could potentially be reduced at any time in the future.

The identification of the ethyl carbamate issue resulted in intensive research efforts by Scotch whisky distillers to find the main sources of ethyl carbamate in distilled spirits such as Scotch whisky, and to find ways of minimizing levels in their processes and products. Work by Riffkin *et al. (35–37)*, Aylott *et al. (38)*, Mackenzie *et al. (39)*, and McGill and Morley *(40)* confirmed that ethyl carbamate could be formed from precursors at various points in the process, principally during fermentation and distillation, with the resulting trace levels of hydrogen cyanide (measurable cyanide) being converted to ethyl carbamate during and post-distillation (during maturation) *(38)*, highlighting the importance of careful distillation practice and the presence of copper in the stills to ensure that ethyl carbamate levels in new-make spirits were kept under control.

However, important findings by Cook *et al. (41)* confirmed that the primary source of ethyl carbamate in Scotch whisky new-make spirit was a cyanogenic glycoside precursor from malted barley. This was identified as a glycosidic nitrile, known as Epiheterodentdrin (EPH), which could be determined as measurable cyanide in barley malt *(41)*. The glycosidic nitrile precursor of ethyl carbamate from barley develops in the living tissues of barley (leaf, roots and shoots) as it grows, and high levels of ethyl carbamate precursors in malt were associated with extended germination periods under malting conditions promoting excessive acrospire and rootlet growth *(41)*. The reaction pathway is summarized in Fig. 5 *(42)*.

Various tests, based on the measurable cyanide assay have been developed over the years *(41,43,44)*. Brown and Morrall *(45)* established a relatively simple, rapid glycosidic nitrile (GN) assay that remains the official Analytica-EBC method, which is used to determine the ethyl carbamate potential of production batches of barley malt, and which is an important parameter used for the commercial trading of barley malt between maltsters and Scotch whisky distillers.

Measurements of GN in barley malt showed that certain malt varieties were associated with high levels of this compound and could potentially generate high levels of ethyl carbamate, while other barley varieties produced lower levels of GN. Certain varieties produced very low levels of GN, close to the detection limits of the assay, and were identified as GN (or EPH) non-producers *(41)*.

It is now known that some barley varieties do not produce epiheterodendrin and that GN production is under genetic control *(44)*. Fundamental research into the biochemistry underlying the production of cyanogenic glucosides in plant tissues is ongoing, since this is an important aspect of plant defence

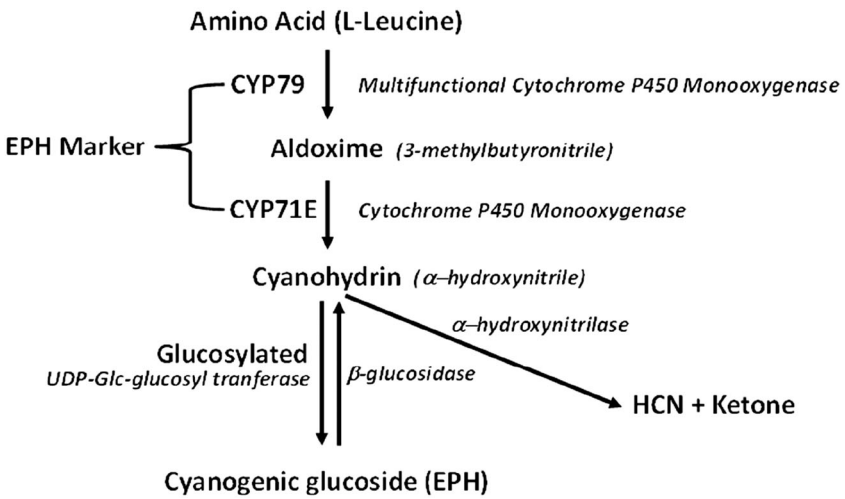

Figure 6. Synthetic pathway for cyanogenic glucosides in the barley plant highlighting the genetic marker for the cytochrome P450 monooxygenases (CYP79 and CYP71E) controlling the two critical steps in EPH formation [adapted from Nielsen *et al. (46)* and Møller *(49)*].

mechanisms against pathogens and herbivores *(46–49)*. There are several types of cyanoglucosides (hydroxynitriles) present in barley and other plants, but only α-hydroxynitriles are cyanogenic. Epiheterodendrin is a cyanogenic α-hydroxynitrile glucoside, which is inherently unstable and can release cyanide under biotic and abiotic stress conditions *(46)* as well as in the presence of endogenous β-glucosidase, which is compartmentalized separately in plant tissues and which is essential *in vivo* for the release of hydrogen cyanide *(48,49)*.

In barley, the cyanogenic glucoside is present in the living tissues such as leaf and shoots, while the β-glucosidase that is responsible for the cyanide release is located in the endosperm *(46)*. Epiheterodendrin is now considered to be the only cyanide-releasing α-hydroxynitrile glucosidase present in barley *(46,49)*. In barley, epiheterodendrin derives from leucine via a multistep reaction mediated by multifunctional P450 cytochromes (CYP79 and CYP71E) to form a cyanohydrin, which is then glucosylated to form epiheterodendrin *(46,50)* (Fig. 6). In certain barley varieties, one or more of these cytochromes are down-regulated, so no epiheterodendrin will be produced *(50)*. This makes it possible to identify and select barley varieties that do not produce epiheterodendrin.

With growing interest in the links between genetics and barley quality traits *(17,51)* there was an opportunity to use a molecular breeding approach to identify quantitative trait loci (QTL) associated with epiheterodendrin (GN) production *(18)*, which it was considered could identify the barley genes associated with this trait. This would allow the ultimate control of the ethyl carbamate problem by eliminating its precursors at the source, and selecting barley cultivars that would produce either very low levels of epiheterodendrin or, preferably, would not produce it at all *(41)*. Further work reported by Swanston *et al. (18)* was able to identify a potential QTL marker for epiheterodendrin production, which was well suited for selecting non-producing varieties in barley breeding programmes. This was developed into a more precise and reliable genetic marker for epiheterodendrin production by Hedley *et al. (50)*. The EPH marker is now a well-established tool that is routinely used in the selection of new malting barley varieties directed at the Scotch whisky distilling market. With the advent of this marker it is now the agreed policy of Scotch whisky distillers to protect their products by specifying that all *new* distilling barley varieties entering the official IBD Brewing Malting Barley trials are confirmed as EPH non-producers using the genetic marker.

Table 2 gives a list of the non-GN barley varieties that have been identified or approved for Scotch whisky production in the UK by the IBD Malting Barley Committee, since the genetic marker for epiheterodendrin was introduced *(52,53)*.

It is worth highlighting that all modern non-GN distilling barley varieties are tested under the same rigorous trial conditions as the other malting barley varieties that have successfully achieved approval by the IBD Malting Barley Committee, and can thus be considered as 'elite' UK barley varieties. There is no evidence that non-production of epiheterodendrin is associated with any other negative effects on malt quality or distillery performance.

Starch hydrolysis (enzymes, sugars and dextrins)

Coupled with the development of our understanding of the quality requirements of barley and malt, over the last 25–30 years there has been an expansion in our understanding of the biochemistry underlying the quality of barley malt. Bathgate and Bringhurst *(54)* provide a short review of our recent understanding of starch structure and functions, as well as the starch-degrading enzymes that are important to distillers, primarily α-, β-amylase and limit dextrinase. This reference provides an important link between the biochemistry of barley and malt and the quality parameters influencing the behaviour of the barley malt in the distillery.

Researchers are now able to understand more about the structure and degradation of starch and the impact of other non-starch polysaccharides as a result of improved instrumentation and analysis. Pérez and Bertoft *(55)* provide a comprehensive review of the molecular structures of the various starch components and the architecture of starch granules, which underlie current thinking in this area.

Early work by Bathgate *et al. (56)* describes some of the fundamental biochemistry underlying the fermentability method,

Table 2. Spring barley varieties identified as non-producers of glycosidic nitrile (GN) and entered into the Institute of Brewing and Distilling (IBD) Malting Barley Committee (NL/RL) approval systems, or used by Scotch Whisky distillers (1990–2014)

Current RL (2013–2014) (approved for distilling)	Belgravia, Concerto, Glassel, Moonshine, Odyssey, Overture
No longer at RL	**Appaloosa**, **Chronicle**, **Derkado**, **Forensic**, **Minstrel**, **Oxbridge**, **Shuffle**, **Tartan**, **Troon**
Grain distilling (no longer current; *Scandinavian varieties)	**Belgravia**, (**Decanter**, **Delibes**, **Forensic**, **Grit**, Maresi, Mirja, Toucan; *SW Catriona, *SW Markof)
Others (no longer available or not progressed to commercial use)	(**Aboyne**, **Alveston**, **Renaissance**, **Jagger**, **Barrel**, **Benchmark**, **Berlioz**, **Bogart**, **Cairn**, **Century**, **Checkmate**, **Chime**, Knightsbridge, **Mirage**, **Momentum**, Spike, Spire, Turnberry, **Universal**)
Pre-1990 [Cook *et al. (41)*]	Alis, Athos, **Corniche**, Delita, Fergie, **Grit**, Joline, Kaskade, Patty, **Pipkin**, Signal
Others (Bmac 213 predictive marker[a])	Aramir, Emir, Heron, Lada, Maris Mink

[a]Earlier predictive marker [now superseded by modern single nucleotide polymorphism (SNP) marker].
Bold: EPH non-production status confirmed by genetic SNP marker *(51,52)*.

highlighting the contribution of starch-degrading enzymes, as well as emphasizing the importance of wort sugars and dextrins in controlling the wort fermentability, and shows how the degree of malt modification has a strong impact on fermentability. In the development of well-modified malts there is a balance between high fermentability and hot water extract and the fermentability is depressed as the proportion of assimilable sugars drops, with a corresponding increase in amino acids and peptides. In slightly under-modified malts, there is a high level of enzyme activity, but the maximum fermentable extract may not be achieved without very fine milling, since not all of the starch will be accessible. In contrast, over-modified malts will give higher malting losses, lower HWE, fermentability and fermentable extract as more carbohydrate is lost and more soluble nitrogen compounds (amino acids and peptides) are produced. Bathgate argues that the reducing sugar content of the wort is the most useful, rapid guide to the fermentable extract of well modified malt, and that this is directly dependant on the enzymic content of the malt, which is primarily defined by the DP.

Towards the start of the 1970s, work by Enevoldsen and coworkers *(57–59)* was beginning to elucidate the relative amounts of sugars and dextrins in beers and (brewing) worts, primarily using the (then) developing technique of gel chromatography. Enevoldsen and Schmidt *(59)* were able to resolve individual dextrins and identified the opportunity for enzymic hydrolysis of branched dextrins deriving from starch amylopectin using an external, commercial debranching enzyme such as pullulanase. This work was able to quantify the relative levels of dextrins in brewing worts and suggest reasons why α-amylase was unable to fully hydrolyse α-limit dextrins.

Since the 1970–1980s, there have been major contributions to our understanding of barley physiology and biochemistry, endosperm cell walls, starch structure and the actions of the principal starch-degrading enzymes in the hydrolysis of oligosaccharides such as amylose and amylopectin in the context of brewing and distilling [e.g. Palmer *(60)*; Manners and Yellowlees *(61)*; Fincher *(62)*, to name a few], which continues today as their successors continue to add to our knowledge in this area. Briggs and MacDonald *(63)* and Palmer *et al. (64)* provided important fundamental information about the action of the principal starch-degrading enzymes in barley grains during malting that highlight the importance of both the aleurone layer and the embryo in influencing the patterns of endosperm modification. This contributes fundamentally to our current understanding of the processes occurring during malting. MacGregor and Fincher *(65)* and Fincher and Stone *(66)* provide useful summaries of the structure and functions of barley starch, carbohydrates and non-starch polymers and endosperm cell wall materials that are still relevant 20 years later.

We now know that the major enzymes influencing the efficient hydrolysis of starch are α- and β-amylase, which act together to break down the starch into fermentable sugars, but which cannot hydrolyse α-(1–6) links in amylopectin, which requires more specific de-branching enzymes such as limit dextrinase to fully hydrolyse the starch (Fig. 7). Muller *(67)* emphasizes that the main starch-degrading enzymes show enhanced thermostability in thicker, more concentrated mashes.

Other enzymes, such as α-glucosidase, have been suggested as also having a role in starch hydrolysis *(68)*, although it is now generally accepted that the impact of α-glucosidase during mashing is limited *(69)*. Duke *et al. (15)* consider that this enzyme has a synergistic effect with α-amylase on native (ungelatinized) starch granules, but suggest that, because of its low thermostability, the main effect of this enzyme on starch degradation would only be apparent early on in mashing, perhaps before the starch is gelatinized.

Until fairly recently, DP was primarily defined as the sum of α and β-amylase, but Fox *et al. (53)*, and Evans *et al. (70)* note that the concept of DP enzymes can be extended to embrace a wider range of starch-degrading enzymes, including α-glucosidase and limit dextrinase.

During the last decade of the twentieth century there was considerable interest in the effects of limit dextrinase, which was known to develop in barley during malting *(61,71,72)*. This interest was stimulated by the development of a simple assay for this enzyme by McCleary *(73)* and the subsequent

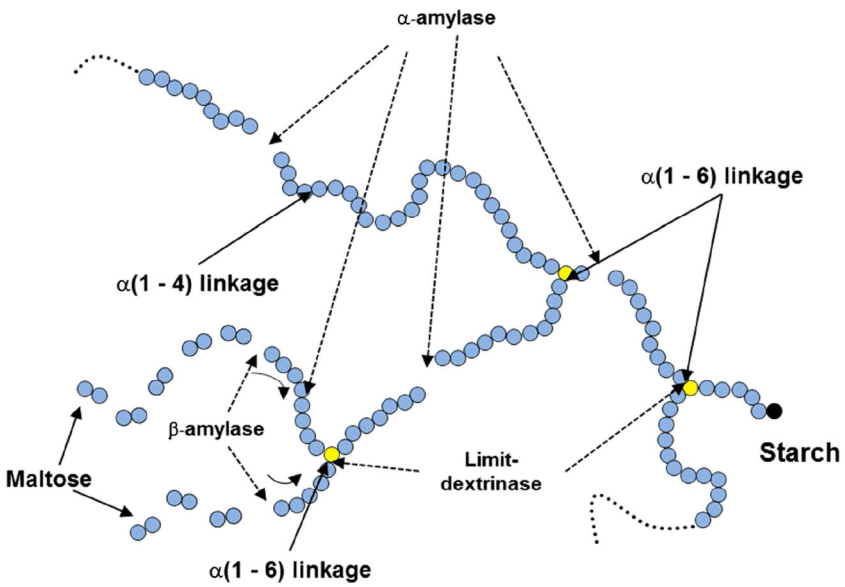

Figure 7. Action of α-, β-amylase and limit dextrinase in degrading starch [after Bringhurst *et al. (5)*].

commercial availability of a test kit supplied by Megazyme®. Prior to this work, it was assumed that, under normal malting and brewing conditions, limit dextrinase had little impact on the composition of the wort, since in barley malt it was primarily present in an inactive form *(71–74)*. It was known that the enzyme was linked to an inhibitor, which prevented the enzyme from acting fully under normal brewing conditions. Longstaff and Bryce *(74)* showed that limit dextrinase was released from its inhibitor *in vivo* by a cysteine proteinase. Unravelling the relationship between the enzyme and its inhibitor has been a key factor in improving our understanding of the importance of limit dextrinase during malting, mashing and subsequent processes. Stenholm and Home *(75)* considered the role of limit dextrinase during mashing, and confirmed that, while the purified enzyme (temperature optimum 50 °C) was rapidly denatured under the temperature and pH conditions encountered during mashing, the enzyme that was extracted from wort had a higher temperature optimum (60–62.5 °C), and a pH optimum of 5.5 and was able to survive and show debranching activity during mashing by increasing the wort fermentability. While this suggested that the enzyme was relatively heat stable, work by Walker *et al. (76)* confirmed that most of the limit dextrinase extracted during mashing (at 65 °C) was protected in its bound inactive form, where it was gradually released from its inhibitor as the mash progressed. Since, in contrast to brewing, distillers' wort is not boiled after mashing, this means that limit dextrinase was able to survive mashing and the active form of the enzyme was released and was free to operate under the temperature and pH conditions associated with the early stages of fermentation *(76)*. Further work on industrial distilling worts *(76,77)* confirmed that, in conjunction with α- and β-amylase, limit dextrinase was able to hydrolyse branched dextrins during fermentation, resulting in a dynamic turnover of linear and branched dextrins and other oligosaccharides into fermentable sugars. Subsequent work reported by McCafferty *et al. (78)* and Bryce *et al. (79)* demonstrated that the release of the active enzyme from its inhibitor was mediated by the pH of the wort/wash, during the early stages of fermentation when it dropped from about pH 5.5 to pH 4.2–4.4 and that this was critical in ensuring that the free, active enzyme was available to convert branched dextrins to linear dextrins that would ultimately be hydrolysed to fermentable sugars, by α- and β-amylase during fermentation. McCafferty *et al. (78)* also confirmed that, as the free enzyme is released from its inhibitor, it becomes more vulnerable to the effects of low pH and temperature, so that the availability and action of the enzyme occur within a relatively narrow window, during the early stages of fermentation (normally within 24 h). As result of this body of work, it is now clear that α- and β-amylase are the principal enzymes controlling starch degradation, and that limit dextrinase acts in consort with these other enzymes to provide higher levels of fermentability by hydrolysing barley starch more efficiently.

Shewry and Morell *(80)* provide a comprehensive general summary of the main endosperm structures in barley (and wheat), together with their development and composition, which is aimed at identifying opportunities for improving end-user properties. Bamforth *(69,81)* provides a fairly recent review of starch and its hydrolysis by malt enzymes from the perspective of brewing, which also brings together information supporting our current understanding of the degradation of starch into fermentable sugars. Further work by more recent researchers such as Evans (and co-workers) *(70)* and Vriesekoop *et al. (82)* has added additional detail regarding the roles of limit dextrinase and the other starch-degrading enzymes (DU, i.e. α-amylase, and DP, i.e. the sum of α- and β-amylase) in hydrolysing starch and dextrins in the brewing process. Although a simulated distillery process was reported by Vriesekoop *et al. (82)*, the fermentation conditions encountered in the Scotch whisky process were not fully replicated in this work, hence the results for these studies did not entirely agree in relation to maltotriose utilization, with those of Bringhurst *et al. (77)*, who looked at actual industrial fermentations in Scotch whisky distilleries. However, the Vreisekoop *et al. (82)* results largely reflect the general trends observed in previous studies, and provide important additional detailed information confirming the complexity of the turnover of dextrins in a Scotch whisky type fermentation.

Evans *et al. (70,83–85)* have been able to propose some predictive models based on the relative levels of these enzymes, which it is suggested can be used to quantify their effects on fermentability (apparent attenuation limit), in the context of developing barley breeding programmes. Evans *et al. (70)* note that, since levels of heat stable α-amylase are generally high in barley malt, and those of the active form of limit dextrinase (which is heat labile) are very low under normal malt processing conditions, it is the available amount of relatively heat labile β-amylase that is limiting, and this is critical in ensuring that starch degradation proceeds efficiently and hence has the biggest impact on wort fermentability *(67,70)*. The view that, in properly modified malt, β-amylase is the most important and possibly limiting starch-degrading enzyme is supported by more recent work by Duke *et al. (15)*, which highlights its importance during the early stages of mashing.

Improving the heat stability of this enzyme, which is relatively heat labile at temperatures above 55 °C, has been identified as a key target for genetic studies into the breeding of new barley varieties that are more adapted to brewing and distilling process conditions. Eglinton *et al. (86)* highlight that there are several forms of β-amylase for which QTL have been identified in barley genetics studies, and which are associated with greater thermostability at temperatures above 60 °C. This work assesses the natural variation of β-amylase in a number of barley varieties to identify the potential of selecting for barley with enhanced β-amylase thermostability, which can be used to breed new generations of barley varieties that would be more suited to industrial distillery and brewery processing.

Cell wall hydrolysis

Understanding how cell wall components such as β-glucans and arabinoxylans impact on malting efficiency and distillery processing is of fundamental importance to maltsters, distillers and brewers. Aside from the processes involved in the utilization of barley starch, the structure, functions and hydrolysis of barley endosperm cell walls are probably some of the most complex facets of barley and malt relating to performance in the malting, brewing and distilling processes. This area of research is probably one of the most well-documented aspects of barley research, but almost certainly the least understood, primarily owing to the complexity of the mechanisms and pathways controlling the release and degradation of these materials and how they impact on the efficiency of the malting, brewing and distilling processes. The efficient degradation of these materials is essential to the production of good-quality barley malt and to avoid problems with the processes involved with brewing and distilling *(87,88)*.

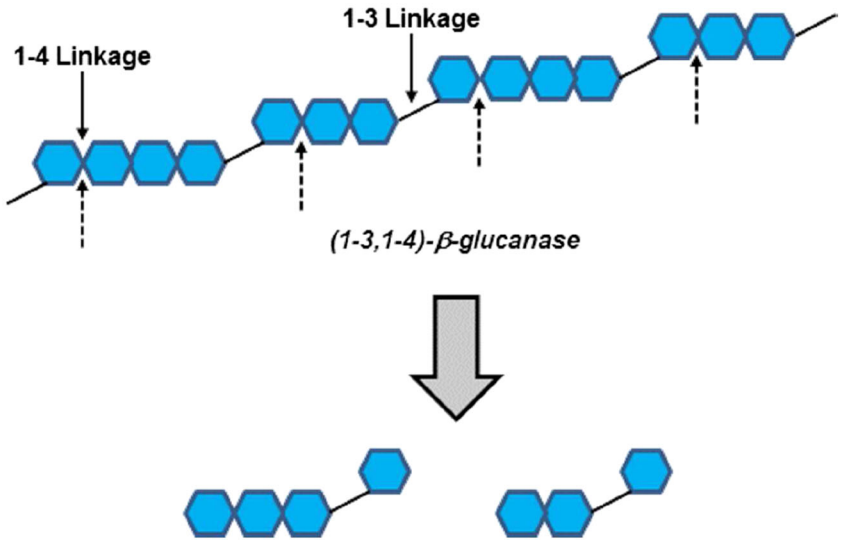

Figure 8. Hydrolysis of (1–3,1–4)-β-glucan by endo (1–3,1–4)–β-glucanase [adapted from Jamar *et al. (31)*].

Barley endosperm walls are mainly composed of (1–3,1–4)–β-D-glucans (ca. 75%) and arabinoxylan (ca. 20%) *(89)*, together with about 2% cellulose and 2% glucomannan *(31)*. The cell walls in the aleurone layer contain much more arabinoxylan (71%), and less β-glucan (26%) with 3% cellulose and glucomannan *(31)*. In barley, β-glucans [(1–3,1–4)–β-glucans], are unbranched linear chains of glucose (β-D-glucopyranose residues) with the ratio of β-(1–4) links to β-(1–3) links in the range 3.2:1–6.6:1 *(31)*. Barley also contains a small amount of β-(1–3) glucan. Within the β-glucan chain, groups of either two or three (1–4)–β-glucosyl residues are linked by single (1–3)–β-glucosyl residues (Fig. 8). Adjacent (1–3)–β-glucosyl residues are not observed in barley. Some of the water-soluble (1–3,1–4)–β-glucan from barley starchy endosperm cell walls (ca. 10%) consists of contiguous 'cellulosic' blocks of between 4 and 14 (1–4)-β-glucosyl residues *(90)*.

Barley arabinoxylan (pentosan, pentose gum, hemicellulose) consists of a backbone of xylan (D-xylanopyranosyl) units connected by β-(1–4) bonds, to which L-arabinofuranose (arabinose) units are attached by α-(1–2) or α-(1–3) links, which are designated C(O)2, or C(O)3, depending whether the arabinofuranose units are attached to the second or third carbon (after the oxygen) in the xylose units *(91)* (Fig. 9).

The combined action of a range of enzymes, including endo- and exo-xylanases, β-xylosidases and α-arabinofuranosidases, is necessary to achieve complete degradation of endosperm cell wall arabinoxylan. Significant changes in the arabinoxylan composition occur during malting and the water-soluble components are degraded initially and then oligosaccharides released through the enzymic degradation of the endosperm cell walls during malting are further degraded as germination progresses *(31)*. The embryo itself can synthesize arabinoxylan *(31)*. Most of

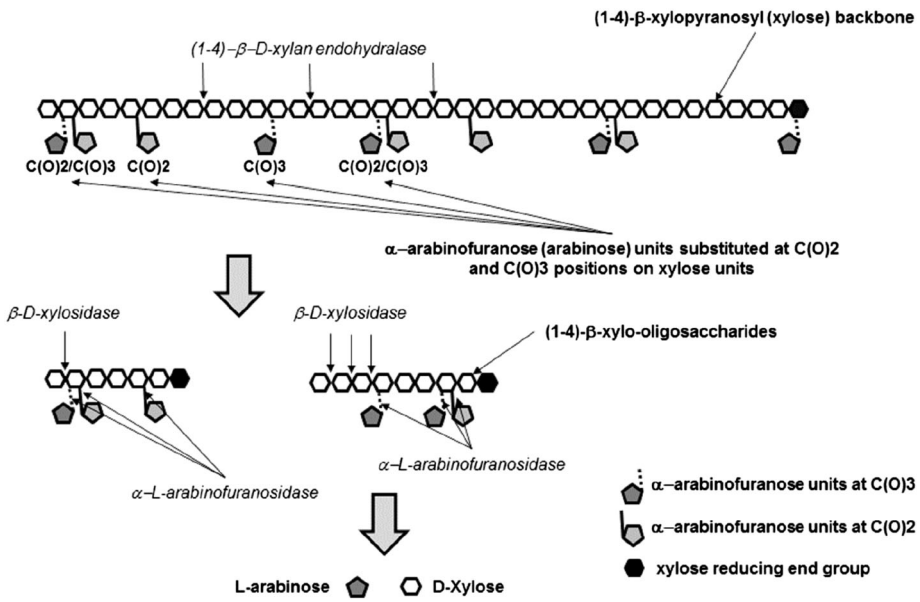

Figure 9. Typical structural features of arabinoxylan and the principal enzymes mediating its hydrolysis [adapted from Jamar *et al. (31)*].

the enzymes necessary to degrade arabinoxylan are synthesized during germination, although there is a view that arabinoxylan is not completely degraded during malting [Allosio-Ouarnier *et al*. *(92)* quoted by Jamar *et al*. *(31)*].

Arabinoxylans are hydrolysed by three main types of enzyme and these are: (a) (1–4)-β-xylan endohydralases, which catalyse the hydrolysis of the (1–4)-β-links in the arabinoxylan polymer (xylan backbone), to give smaller xylo-oligosaccharides and reduce the viscosity of water soluble arabinoxylan; (b) β-xylosidases, which are responsible for hydrolysing the β-(1–4) xylosidic linkage within xylo-oligo saccharides; and (c) arabinofuranosidases, which are responsible for the release of the α-(1–2) and α-(1–3) linked arabinofuranose units. Other enzymes involved in the hydrolysis of arabinoxylan are acetyl esterase and ferulic acid esterases, which can alter the accessibility and solubility of arabinoxylan *(31)*. Jamar *et al*. *(31)* also mention that exo-xylanases are also involved in arabinoxylan degradation.

The first of these enzymes, (1–4)–β-xylan endohydralase, attacks the (1–4)–β-links in the arabinoxylan polymer chain at random, to produce xylo-oligosaccharides, which can be further hydrolysed by β-xylosidases, to release individual D-xylose units. While (1–4)–β-xylan endohydralase is an endo-acting enzyme, it can also release xylose units from polymeric xylans well as from small xylo-oligosaccharides released by the endoxylanases.

There are two isoenzymes of (1–4)–β-xylan endohydralase expressed during germination. The genes controlling the most important one (X-I) are expressed during the later stages of grain filling and develop in the aleurone layer surrounding germinating barley. In contrast the genes for the second isoenzyme (X-II) are expressed in the early stages of grain filling and this isoenzyme is mainly associated with the developing shoot and root of the seedling embryo, rather than with the aleurone layer *(31)*.

Stuart *et al*. *(93)* emphasize the importance of the stresses resulting from climatic and environmental conditions, such as ambient rainfall and temperature as well as soil structure, particularly after flowering, and suggests that high levels of water (drought) stress will have important effects on grain composition, starch structure, starch granule distribution and the release of (1–3,1–4)-β-glucans and other endosperm cell wall materials, such as arabinoxylans, which would impact on the efficiency of mashing. This work also suggests that there is an important varietal component in these interactions.

The extent of modification of the barley endosperm during malting is the key factor underlying the performance of barley malt in the brewhouse *(94,95)*. Modification is the degradation of the barley endosperm cell walls and the protein matrix supporting the starch granules by a large complex of enzymes that include proteinases, β-glucanases, xylanases and arabinofuranases to allow the starch-degrading enzymes (amylases) to access the starch granules. Barley β-glucanase comprises at least two isoenzymes of (1–3,1–4)β-D-glucan 4-glucanohydralase *(96)*. The main compounds present in barley that need to be hydrolysed are starch, protein and cell wall polysaccharides. As well as the standard starch and protein-degrading enzymes, the malting process provides all of the necessary enzymes that are necessary to achieve malt modification *(31,97)*.

Fincher (62) and Fincher and Stone (66) highlight the importance of the endosperm cell wall composition, identifying β-glucans and arabinoxylans as the major components of barley cell walls that impact on processability, while Izydorczyk *et al*. *(98,99)* provide more detail about the structures and functions of these components. Bamforth and Kanauchi *(89)* and Kanauchi and Bamforth *(88)* provide models for composition of barley starchy endosperm cell walls, underlining the importance of β-glucans and arabinoxylans (pentosans) and the range of enzymes that can degrade them, with particular emphasis on the use of externally added (exogenous) enzymes such as xylanases and xyloacetylesterase to elucidate the structures and functions of the cell wall components, together with the challenges with degrading them, to provide better access to the starchy components. Hrmova *et al*. *(90)* highlight the main enzymes involved in the hydrolysis of cell walls and defines their properties. Hrmova and Fincher *(100)* provide a model summarizing the interrelationships between the different enzyme systems and their substrates, as well as highlighting the complex of enzymes involved in the pathways in the complete degradation of cell wall components to glucose. This emphasizes the importance of interactions with arabinoxylan in restricting the access of hydrolytic enzymes to cell wall (1–3,1–4)–β-D-glucans. Bamforth and Kanauchi *(89)* further highlight the interaction between β-glucan and arabinoxylan and stress the importance of the architecture and fine structure of barley endosperm walls, and postulate that components that are normally associated with arabinoxylan, such as phenolic acids, are accessible on the outer surfaces of cell walls; they propose a model that suggests that a layer of pentosan is located in the outer regions of the cell wall and in which the access to arabinoxylan is hindered by the presence of attached ferulic acid and acetic acid moieties. Kanauchi and Bamforth *(101)* indicate that xylanases can promote the release of β-glucan from purified barley cell walls, while β-glucanases will not release pentosan. This confirms that some of the glucan is masked by cell wall pentosan. Hence enzymes that remove ferulic acid and acetic acids, which are normally associated with arabinoxylan, may not release pentosans but can release glucan, showing that these components partially restrict the extractability of β-glucan *(88,101)*. Jamar *et al*. *(31)* provide a more recent review of the cell wall polysaccharide components in barley and malt and emphasize the wide range of enzymes involved in glucan hydrolysis.

Cell wall hydrolysis, which provides the first step in removing the physical barriers between enzymes and their substrates, is considered to be one of the most important of the transformations resulting in the modification barley during malting. However, some aspects of the solubilization of cell wall polysaccharides are still not fully understood *(31)*. Recent models of barley cell wall structures *(89,100)* indicate that β-glucans are enwrapped in arabinoxylan *(31)*, and that the pentosan limits the solubility of the glucans *(89)*. Bamforth and Kanauchi *(89)* also suggest that the covering layer of pentosan is incomplete and that this allows glucanase enzymes to access the glucan substrate. This suggests that the release of β-glucan is not necessarily dependent on complete digestion of the pentosan layer. A range of enzymes, among them xylanases and enzymes capable of removing arabinosyl and ester side-chains from pentosan, will release β-glucan from the walls surrounding starchy endosperm cells of barley *(88)*.

Modern studies *(31,88,97,100)* highlight the complexity of the links between barley cell wall arabinoxylans and β-glucans and other polymeric materials (such as proteins), and how these interactions impact on how the cell wall are degraded. Kanauchi and Bamforth *(97)* emphasize the fundamental importance of malting in the development of the enzymes that will digest

the endosperm cell walls and ensure the optimal solubilization and removal of β-glucans, in order to minimize potential problems with viscosity and wort separation.

Figure 10 [adapted from Hrmova and Fincher *(100)* and Jamar *et al. (31)*] summarizes our current understanding of the principal enzymes and pathways involved in the solubilization and hydrolysis of β-glucans. In this model, cell wall β-glucans are partly embedded in arabinoxylan, and are released from the barley cell walls and subsequently hydrolysed in a two-stage process consisting of an initial solubilization, or 'increased accessibility' of the β-glucans by a range of enzymes showing different thermostabilities. This is followed by their further degradation into smaller oligosaccharides and monomers by a complex of endo- and exo-β-glucan hydrolysing enzymes *(89,97)*.

Initial solubilization of β-glucan is mediated by a range of solubilase enzymes *(100)*. These enzymes (labelled Endo X) have not yet been fully characterized, and currently there is no strong evidence that a specific enzyme is responsible for this solubilization *(31)*. However, these enzymes are considered to be a complex of (1–3)–β-glucanases, phospholipases, (1–4)-endoglucanases, ferulyl esterases, xyloacetyl esterases and arabinofuranosidases *(31)*. The (1–3,1–4)–β-D-glucans released from the cell walls are then hydrolysed to oligosaccharides by (1–3,1–4)–β-glucanases and the resulting oligosaccharides are degraded to glucose, by β-glucan exohydralase and β-glucosidase.

Normally the hydrolysis of β-glucans primarily takes place during malting *(88)* while arabinoxylans are released during brewing (mashing) *(31)*. While β-glucosidase and endo(1–4)–β-glucanase are present in raw barley, like endo-(1–3,1–4)–β-glucanase, endo (1–3)–β-glucanase and exo-(1–3)–β-glucanase increase in activity during malting, with the latter developing very late during germination *(97)*. Endo(1–3,1–4)–β-glucanase, the most important of these enzymes, is known to be heat labile and is not active during mashing, so it is essential that these are utilized effectively during malting to avoid the viscosity problems associated with β-glucans *(87)*.

Unless the viscogenic components of the cell walls are effectively degraded during malting, the principal enzymes developed during germination will be unable to fully access their substrates and this will result in poor or uneven modification. If these cell wall polysaccharides are released before they are fully hydrolysed, this will result in increased process viscosity and will have a serious impact on brewhouse (and distillery) performance *(31)*. It should be emphasized that, since the principal enzymes are heat labile, changes to the mashing or brewing processes cannot improve the synthesis of the enzymes *(31)*.

Given the restrictions on the use of additives, specified by the legal definitions of Scotch whisky *(102,103)* where process aids are not permitted, once the malt is finished, there is little the distiller can do to improve its processing characteristics and there are no 'magic bullets' that will improve its processing performance after the barley has been malted and hence there is no simple adjustment to the mashing procedure that will dramatically improve the heat stability of β-glucanase during mashing *(96)*.

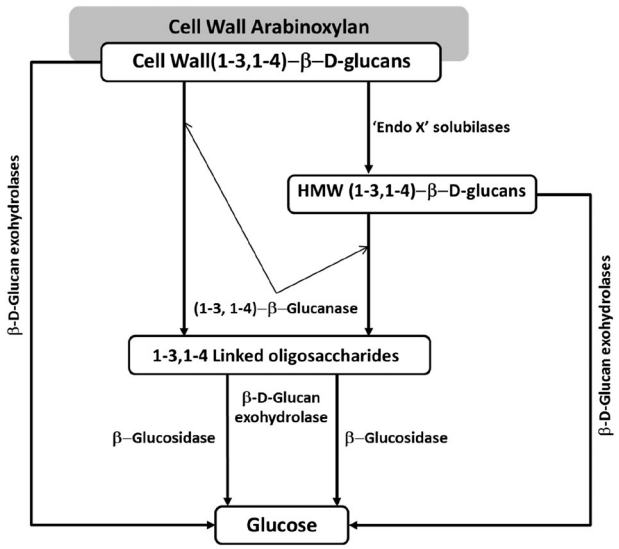

Figure 10. Solubilization and hydrolysis of endosperm cell walls (1–3,1–4)-β-D-glucans [adapted from Hrmova and Fincher *(100)* and Jamar *et al. (31)*].

Assessing barley processing performance

There have been several ways of assessing the processing performance of barley malt, one of these is the V_{max} test developed by the Campden Brewing Research Institute *(104)*. These procedures tend to require a fairly specialized apparatus, and although useful, can be time consuming and labour intensive, and hence are not well suited to the rapid throughput of large numbers of samples. As a result, simpler more rapid instruments such as the Rapid Visco-analyser® (RVA) and the friabilimeter have found useful applications in helping to elucidate and predict the processing properties of cereals such as barley and malt.

Stuart *et al. (93)* and Cozzolino *et al. (105)* highlight the usefulness of the RVA in characterizing the starch properties of barley, as well as providing information on the gelatinization temperatures and the pasting time, which could also be related to the malt extract. Nevertheless, the correlations for barley flour were not strong enough to support the development of a robust RVA test for HWE, although it was suggested that it could be used effectively in breeding programmes for the rapid identification of barley cultivars with potentially higher extract. However, the RVA instrument has been a useful tool for comparing the properties of starch in cereals that are important to distillers and comparisons with alcohol yield data *(106)* confirm that there is a relationship with the RVA peak viscosity that is influenced by the amount and composition of the starch that is available.

The RVA instrument can also be applied to malted barley, which yields a profile that is very different from that of the unmalted cereal. It normally shows a single major peak characteristic for starch gelatinization and gives a measurement of the gelatinization temperature. It can vary for individual barley varieties grown under different seasonal/harvest conditions. The peak height and area, obtained at different stages of the germination process, provide a qualitative measure of the level of starch present in the grains, and the impact of the DP enzymes. Recent work has shown that RVA peak viscosity will decline as the barley endosperm is modified during germination and that the RVA profile can provide a rapid, visual indication of the progress of modification during germination *(107)* (Fig. 11). Together with friability data, this can provide a more detailed picture of malting progress and highlight the potential impact on malt quality and processability *(108–110)*.

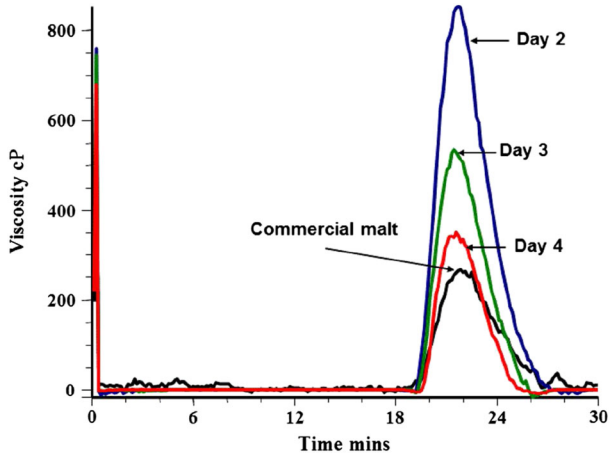

Figure 11. Rapid visco-analyser® (RVA) profile of barley malt after 2, 3 and 4 days of germination [SWRI data, adapted from Bryce *et al. (110)*].

It is well established that the main components of barley (and malt) affecting processability in the distillery (and the brewhouse) are variations in the levels of β-glucans and the degree of protein breakdown during malting *(111)*. It has been suggested that 'standard' malt analysis parameters are not sufficiently sensitive to pick up significant variations within individual batches of malt, since the individual grains making up a batch are non-uniformly distributed *(111)* and it is possible to show that this non-homogeneity is present at all levels, from the original field from which the barley was grown through to the malting and eventual processing of the malt in the brewhouse *(112)*.

In recent years, much applied barley research has focussed on the impact of barley quality and variety on the malting and processing properties of barley, highlighting the potential variability of commercial barley and malt supplies. Overall, much of this work emphasizes the complexity of the processing aspects of barley quality ranging from the effect of nitrogen on malting quality performance *(113)* and the impact of the growing environment and location, even within the same field *(112,114)*, to the impact of kernel size *(109)*, varietal differences and malting conditions on malting performance *(110)* and subsequent distillery processing *(115)*. This underlines how much there is still to understand before we can fully predict practical outcomes from standard measures of barley quality.

Studies have also been carried out on novel types of barley, such as hull-less or naked barley, which has confirmed that these would present significant process problems for conventional mashing processes, although the use of mash filters would probably help *(108,116)* if difficulties with growing, harvesting and malting could be overcome. Edney *et al. (116,117)* confirmed that it is possible to develop hull-less barley with acceptable enzyme levels, but that these may be deficient in other ways, such as in reduced levels of certain free amino nitrogen components. Edney *et al. (116)* and Agu *et al. (108)* both indicate that conventional malting procedures may need to be adjusted to get the best out of hull-less barley. However, Agu *et al. (109)*, who worked with pre-production research varieties supplied by a plant breeder, showed that it would be possible to produce malt from a relatively high-nitrogen hull-less barley with sufficient enzyme activity for use in a grain distillery, where no wort filtration stage would be necessary.

The study of barley starch and its subsequent breakdown into fermentable sugars, together with the components influencing processability in the distillery (and brewery), remains an important goal of applied research and will continue to be high on the agenda for researchers with interest in malting, brewing and distilling. However, in recent years it has become clear that the biggest impact of barley research will continue to be from an increased understanding of the genetic factors underlying barley properties, taking into account inputs from end users such as maltsters, distillers and brewers, as well as agronomists, and this will continue to be used by plant breeders to deliver real improvements in barley quality and will ultimately increase the commercial availability of a wider range of barley varieties that are better suited to the end users' processes.

Importantly, it is essential that these genetic studies are well supported by continuing research into the physiological factors and the underlying biochemistry influencing the properties and processing characteristics of barley. These are important in relating important quality traits to the genetic factors controlling them and validating them so that reliable and more specific genetic markers can be developed to identify and select for elite barley varieties more suited to the specific end-user quality requirements of maltsters, distillers and brewers.

Towards improved modern distilling barley varieties

Over the last 15 years or so, there has been increasing integration between traditional applied malting, distilling, brewing and malting research and genetic studies stimulated by advances in molecular biology techniques, which have resulted in the development of new technologies to simplify methods of studying the genes of barley and other cereals that can be used to understand and potentially predict their properties. During this period, large collaborative research projects have been developed to address the complexity of relating end user to quality parameters to the genetic factors controlling them. Typically these 'big research' projects are large, finite, government/industry funded supply chain collaborative projects involving agronomists, plant breeders, genetic researchers and end-users such as distillers, maltsters and brewers, where the scale of investment enables research partners to extend their research horizons in a focussed manner to achieve tangible outcomes that will benefit all of the research partners.

Recent advances in the knowledge of the genetics of barley have stimulated a significant interest in applying molecular biology approaches to barley quality (Swanston *et al.* 1999) *(18,19,44)*. This started in the late 1990s with the identification of genetic markers, known as QTL, on barley chromosomes that could be associated with important barley quality traits. This resulted in the generation of very complex genetic maps *(17)*, which could be used to relate particular sets of genes to real-world, macroproperties of barley. In theory, it was considered that, if such markers could be identified, then it would be possible to breed new barley varieties that were more suited for particular end-user qualities, more rapidly than before. The key was that, if the correct QTL marker could be identified, it would be possible to cross suitable parent varieties carrying the target QTL, using classical breeding techniques to express the desired properties in the offspring, and hence provide a new generation containing the new traits that could be passed on to subsequent

generations. This technique, while circumventing the genetic modification (GMO) approach, provided opportunities for barley breeders to accelerate their conventional breeding programmes in a more precise and targeted way than was previously possible.

An AgroScience LINK project, funded under the Ministry of Agriculture, Fisheries and Food Agro-Food Quality LINK scheme, was undertaken to study this more closely *(116)* and initial work *(18,19)* indicated that it would be possible to map QTL markers for malt fermentability and the production of the ethyl carbamate precursor [GN (or EPH)], which were quality traits that were considered particularly important for Scotch whisky distillers.

Swanston *et al. (18,19)*, Meyer *et al. (118)* and Thomas *(17)* reported that, as a result of the study, genetic markers had been successfully identified for important traits such as GN (EPH) and for HWE, fermentability and other important grain quality traits. However, the challenge was to validate the phenotype traits using analysis data deriving from a relatively large subset of barley and malt samples that were generated during the study. The results of the study confirmed that the genetic marker for GN could be used effectively in barley breeding programmes and a more precise, refined EPH marker *(50)* is now used routinely and effectively to select for new non GN distilling barley varieties.

The markers for HWE and fermentability were less well defined and were to some extent mutually antagonistic, where varieties with high HWE tended to give lower fermentabilities and this suggested that there was some way to go before these could be reliably applied to barley breeding. However, the project indicated that the largest and most significant effects on fermentability were associated with a QTL located in close proximity to one for β-amylase (*sdw*l), confirming that there might be an opportunity to identify and select for this in future barley varieties *(18,19)*.

Molecular plant breeding based on naturally occurring allelic diversity (association genetics) is now a powerful tool for improving self-pollinated crops such as barley. This is a result of linkage disequilibrium, which is the non-random association of alleles at various loci on the barley genome. Knowing the degree of linkage disequilibrium is an important factor in detecting relationships between genotypes and phenotypes in a given sample population, and this can be used for gene discovery in plants such as barley *(119)*.

Barley was found to be particularly well suited to molecular biology/genetics studies and there has been a great deal of interest in these approaches from barley researchers, since there are also large amounts of QTL linkage data and many reference mapping populations, which could be used to form the basis of an association genetics approach. Hence, rather than using a relatively small number of genotypes as parental material, as in QTL studies, it was possible to use an association genetics approach to analyse very large germplasm arrays using increasingly affordable, versatile and widely available genotyping platforms such as the Illumina GoldenGate Assay® to facilitate the rapid evaluation of large numbers of single nucleotide polymorphisms (SNPs), which could be used to map and explore the barley genome in much more detail, and would provide a more precise way of mapping genetics data to a wider range of agronomic and end user (phenotype) traits. The presence of a suitable degree of linkage disequilibrium allows for the detection of SNPs that are functional determinants of phenotype properties, which can be manifested *in vivo (119)*.

Rostoks *et al. (120)* identified an opportunity to use association genetics to study modern elite northwest European barley, which has been naturally subjected to strong selection for favourable alleles, and which can be effectively exploited by using whole-genome scans to map phenotype traits, particularly those distinguishing European winter and spring barley genotypes. This work formed the fundamental basis for important studies into the Association Genetics of UK Elite Barley (AGOUEB) *(121)*. Bringhurst *et al. (122)* described this approach to look at the way UK winter and spring barley varieties can be distinguished as well as identifying significant associations with a range of physiological, distinction, uniformity and stability grain characteristics. A key aspect of this project was that a very large quantity of end-user phenotype data was necessary to validate the SNP markers, and this set a limit on the detail that could be resolved to give the required associations.

The AGOUEB project was highly successful in allowing plant breeders to understand the genetics underlying the properties of elite UK barley lines by providing a more accurate view of the genomic regions of barley controlling end-user traits, so that they can now exploit new genetic combinations by identifying the causal genes that would deliver significant improvements in the malting quality of barley. However, the most important outcome of the project was the development of very large genetic databases that can be accessed by plant breeders to accelerate the breeding of new malting barley varieties, which are now beginning to make their way into commercial production. The resulting establishment of a strong supply chain-based research network capable of generating such expanded databases also provides a platform from which to develop future projects, both in the field of malting barley and in the wider aspects of barley production.

Importantly, the study has highlighted the relatively narrow genetic base for many of the modern malting quality barley varieties, which was particularly apparent in spring barley. This is of some concern to both end-users and plant breeders, since it shows that, if the current situation were to continue, there would be a limit to the scope of improvements to barley that would be available. This situation has highlighted the need to increase the genetic diversity in the genotype stocks available to UK plant breeders. This can be done by accessing a wider range of material from international gene banks so that more exotic barley varieties can be included in modern breeding programmes *(122)*.

Much of the association genetics work developed in the AGOUEB project has been facilitated by the mapping of the rice genome sequence by the International Rice Gene Sequencing Project *(123)*, which allowed barley researchers to apply the synteny between the functional genes of rice and barley to identify common chromosome sequences in rice to locate the equivalent genes in barley. This ultimately resulted in the sequencing of the barley genome by the International Barley Genome Sequencing Consortium *(124)*, which now provides a platform for both genome-assisted research and improvement of contemporary crops.

The work started by the AGOUEB Project is continuing in a wide range of other similar projects that are taking place around the world, and which are now using similar approaches to AGOUEB. The benefits of selecting suitable barley varieties are clear.

Data generated in the AGOUEB project, from a range of historical and more recent barley varieties (1980 and 2005) that were

grown under similar site conditions, shows that there have been significant improvements in a range of barley and malt quality parameters, particularly with the HWE showing strong upward trends for both spring and winter barley varieties *(122)* (Fig. 12).

These trends are reflected in commercial malt analysis data collated for the period since 1991 (Fig. 13), which shows corresponding improvements in the PSY of micro-malts prepared by a commercial distilling company over the period 1991–2013. This shows a sustained increase in barley malt PSY and HWE, primarily as a result of the development of improved barley varieties. As can be seen, there is a strong relationship between the PSY and HWE [correlation (r) = 0.84], which can be exploited more easily by barley breeders in future research, since it is easier to identify genetic markers for HWE, than fermentability and PSY. The drop in PSY and HWE for 2012 and 2013 is the result of the particularly challenging growing and harvest conditions in those years, but overall the picture shows an increasing trend, for both HWE and PSY, which is expected to continue as new varieties enter the market for distilling barley.

While in recent years the sustained increase in alcohol yield seems to be tending to a limit, this has to some extent been influenced by the greater variations that have been observed recently in the Scottish growing environment. Researchers are

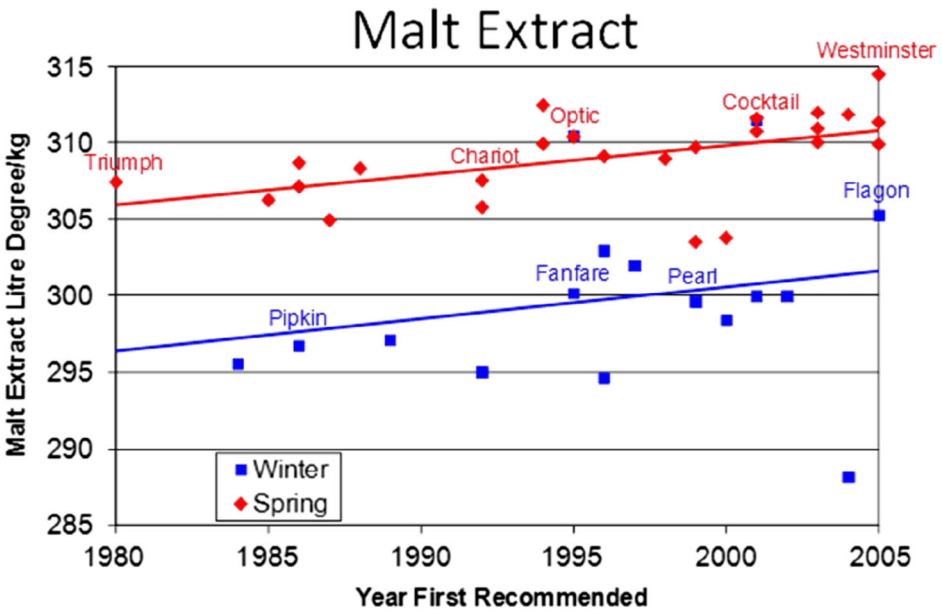

Figure 12. Malt hot water extract (HWE) of Institute of Brewing and Distilling (IBD) approved spring and winter barley varieties plotted against their first year of recommendation in the UK. AGOUEB data derived from common trials grown in 2006–2008 and provided by Maltsters Association of Great Britain (MAGB) member companies. Source: SCRI [James Hutton Institute, Bringhurst *et al. (122)*].

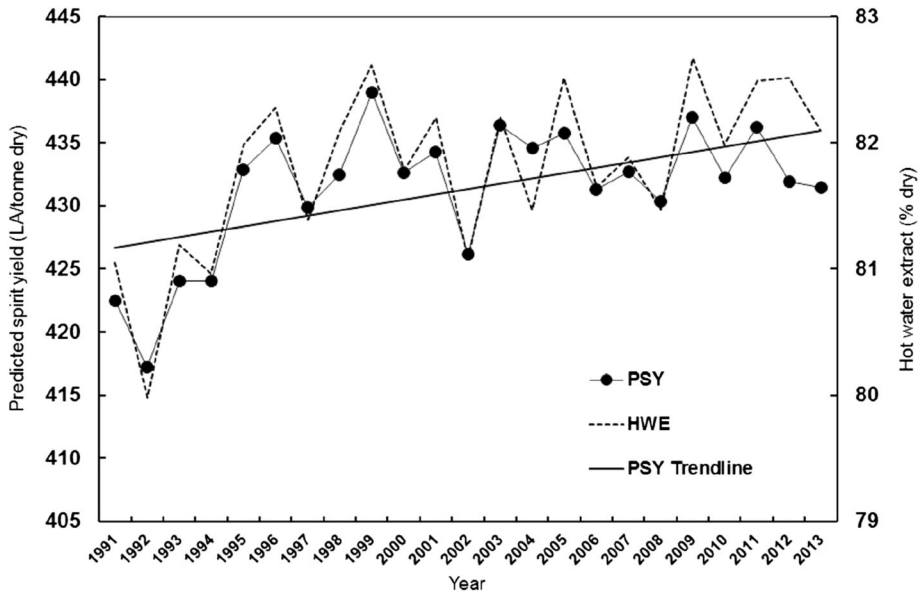

Figure 13. Increasing trend in malt PSY and HWE (1991–2013) (commercial distillery data).

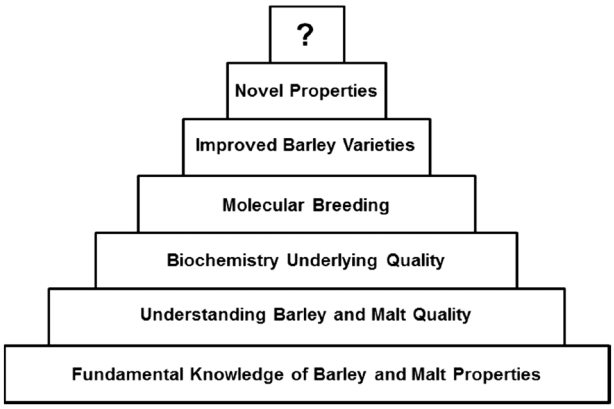

Figure 14. Building on fundamental barley research.

confident that new distilling barley varieties will continue to provide a sustainable increase in the distilling quality of barley in the longer term, particularly with the identification of novel germplasms, which can be used to increase the genetic diversity and robustness of modern malting barley.

Conclusions

Barley and malt research has been progressing for at least the 125 year lifespan of the Institute of Brewing and Distilling, and it is certain that this will continue for many years ahead. I am aware that this review is not comprehensive, since to do this subject proper justice would require a very large volume by itself. There are many researchers, whom I have not specifically mentioned, particularly outside the UK, who are currently actively working in the field of barley research, extending our range of knowledge about barley in terms of malting, brewing and distilling applications, as well as, importantly, in the wider context of human food and the impact of global environmental challenges such as disease pressure and resistance to biotic stresses, as well as increased tolerance to abiotic stresses such as drought and flooding.

Over the last 25 years, research on barley has progressed rapidly by building on earlier work, and by applying 'new' technologies to more traditional approaches, researchers have been able to make substantial progress in our understanding of the biochemistry, physiology and genetics of barley and malt. This report is very selective, and aims only to highlight some of the important stages in our understanding of barley and to show how research into distilling barley and malt has moved forward by building on our 'simple' understanding of end-user quality in terms of analytical parameters, extending through a more complex understanding of the biochemistry and genetics of barley that can help us obtain real and sustainable improvements in barley quality that will ultimately benefit everyone. In the context of the global picture of barley research, the genetics approach is probably the one that will provide the greatest amount of understanding for the future use of barley.

As Fig. 14 shows, our progress in barley research can be likened to a pyramid, with each layer of research building on earlier principles. Fundamental knowledge of the properties of barley and malt provides the major cornerstone for our understanding of malting and end-user quality, for which we need to unravel the biochemistry underlying the parameters defining the essential quality traits required by maltsters, brewers and distillers, as well as other stakeholders in the barley supply chain. Now that we have a sound knowledge of the science underlying barley and malt quality, we can identify genetic or molecular markers for specific phenotype traits that are of major economic importance, and use (non-GMO) molecular breeding techniques to develop new improved barley varieties. Ultimately, as we learn more about the structure and functions of starch and its analogues (e.g. phytoglycogen), it will be possible to develop commercially sustainable barley varieties with novel properties *(125)*, in which the starch might be easier to gelatinize, or which conceivably might not require to be malted at all. Work is already well progressed in molecular breeding studies in this area and will ultimately result in novel material entering commercial breeding programmes *(126)*.

The results of all this research have brought significant benefits for distillers as well as maltsters and brewers (and growers), in terms of a much better understanding of the properties of barley, and has resulted in important modern malting barley varieties, such as Concerto and Odyssey and their successors, that can now deliver significant improvements in both production performance and easier processing characteristics.

We are at the cutting edge of barley (and cereals) research. However, we are still not even close to approaching the threshold of our potential knowledge of barley, and continuing barley and malt research is essential since there is still a great deal we need to understand, even for something as small and seemingly insignificant as a single barley grain. We now have much better and more precise tools that will provide better opportunities for an ever increasing expansion of our detailed understanding of barley and its properties.

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