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The impact of terroir on barley and malt quality – a critical review

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Abstract

Why was the work done: With respect to terroir, 'To be or not to be,' (Hamlet, Shakespeare) is a key question for maltsters and brewers for malt and beer quality. Terroir is a sparsely studied aspect of malt quality, despite it being an important component of added market-value in wine since ancient times. The 'sense of place' imbued by terroir is an expression of the growth of a grape variety in a specific region with respect to local climate, soil, microbiome, elevation/aspect of the vineyard, viticultural and wine making methods. Similar corollaries to wine terroir can be drawn for hops and malting barley.
How was the work done: A comprehensive review of the literature was undertaken to identify reports of terroir in barley/malt quality. Where possible, the discussion was extended by consideration of appropriate unpublished data.
What are the main findings: The primary influence of terroir on malt is grain protein content (GPC). This appears, in part, to be controlled by the daylength during grain maturation. Increasing day length typical of grain maturation in Australia tends to be associated with a lower GPC, while decreasing daylength during grain maturation in Canada is associated with a higher protein content. GPC is positively correlated with diastatic power, *beta*-amylase activity and foam positive proteins such as protein Z4 and hordeins. Conversely, GPC is inversely correlated with extract and Kolbach Index (KI). Interestingly, lower protein barley tends to produce higher KI malts that result in wort with greater flavour complexity and desirability. The level of ionic micronutrients (cations, anions) including calcium and zinc are understudied aspects of malt quality. It is evident that there is significant variation in the ionic micronutrient content of malt produced from different international regions and between regions of the same country which would be an expression of terroir. Lastly, the microbiome of barley/malt shows influences of terroir such as the deleterious impacts of *Fusarium* head blight on malt quality including gushing and mycotoxins. Variation in terroir will also have more subtle impacts, both desirable and undesirable, on malt quality for the contribution of beneficial enzymes (e.g., cell wall degrading enzymes) or for the propensity of barley to impart malt components into wort (e.g., arabinoxylan) that have been implicated in premature yeast flocculation (PYF) and undesirable beer quality.
Why is the work important: The concept of terroir in malt quality has important implications for the efficiency of mashing, lautering, fermentation and beer quality.

Keywords:

terroir, malt quality, protein, extract, Kolbach index, diastatic power, flavour, foam proteins, microorganisms, micronutrients

Introduction

The concept of terroir is generally interpreted as a 'sense of place' and is considered to have originated over 2000 years ago from the winemaking and viticultural practices of ancient Greece and Rome (Dodd 2020; Robinson 2006). Terroir encompasses all the factors that affect the production of grapes at the vineyard or vineyard block, from the relationship between the local climate, soil, elevation/aspect of the vineyard to the viticultural and wine making methods. A further facet of terroir is the interaction between the grape varieties and terroir. For terroir to be a useful indicator of variations in grape/wine quality, the weather and the genome x environment (G x E) must be relatively consistent or have a dependable thread between production years.

A useful case study of wine terroir is Château Y'quem, which is in the 'Bordeaux Wine Official Classification of 1855' (Markham 1998) and was the only Sauternes 'la maison' given the 'Superior First Growth' rating. The prominence of Château Y'quem in producing Sauternes style wine (sweet) is largely derived from susceptibility to infection by 'noble rot' (*Botrytis cinerea*), determined in part by consistent (seasonal) vineyard leaf moisture conditions. Château Y'quem has regularly dominated the wine market as the most expensive white wine sold at auction. A bottle from 1811 was sold in July 2011 for £75,000 ($117,000) (Chow, 2011). However, such are the vagaries of grape growing and noble rot infection, that not every vintage reaches the required quality to attain the Chateau Y'quem label. The production years 1910, 1915, 1930, 1951, 1952, 1964, 1972, 1974, 1992 (Anon 2022) and 2012 (Anon 2012) were disastrous vintages for Château Y'quem where no wine was produced. In contrast, the success of the 2008 and 2009 vintages, provided both excellent quality and quantity, as evident by the number of barrels in the cellar (Figure 1). These vintages were described by Château Y'quem as being 1 in 100 years of production. Therefore, fluctuations between years occur, even though terroir results in relative stability for both yield and quality (flavour).

Provocatively, Matthews (2016) has described wine terroir as a myth concieved through marketing and lacking rigorous scientific validation and precise definition. However, evidence is mounting that the influences of terroir on wine quality has been substantiated for grape borne microbiota (Belda et al. 2017; Bokulich et al. 2014, 2016; Pretorius 2020), or soil/climate/aspect related drivers of wine quality (Bramley et al. 2020; Brillante et al. 2020; Choné et al. 2001; Fernández-Marín et al. 2013; Pinu 2018; Retallack and Burns, 2016; van Leeuwen et al. 2018, 2020). Terroir has also been claimed for other food products including berries (Zoratti et al. 2015), coffee (Avelino et al. 2005; Oberthür et al. 2011), olive oil (Kalua et al. 2007) and cheese (Turbes et al. 2016). Indeed, Rochefort cheese was one of the earliest recognised food products determined by terroir, as on 4th June 1411 Charles VI granted a monopoly for the ripening of this cheese to the people of Roquefort-sur-Soulzon (Donnelly et al. 2013; Robuchon et al. 1996).

From a brewing perspective it is reported that terroir influences the production of hop varieties (van Holle et al. 2021). This was established when hop producers sought to grow classic or noble hop varieties (Tettnanger, Saaz, Hallertau and Fuggles) away from their traditional areas of production in Europe. Further, differences in hop flavour and aroma can be observed between regions (Rodolfi et al. 2019; Staples et al. 2022; van Holle et al. 2017; van Simaeys et al. 2022a,b). These differences were characterised in terms of α- and β-acids, xanthohumol, essential oil yield and composition (for example mycene, linalool, geraniol).

Direct evidence for terroir in malting barley is more elusive and is limited to studies on malt flavour (Bettenhausen et al. 2018; Herb et al. 2017a; Li et al. 2022; Stewart et al. 2023). The evidence appears to be primarily linked to protein modification during malting (Kolbach Index or FAN) (Herb et al. 2017a; Stewart et al. 2023). In addition, the Waterford Distillery in Ireland claims terroir, due to the meticulous selection of local barley as its malt source (Griffiths 2017).

It is not surprising that barley researchers have been slow to consider the impact of terroir on malt quality. The *modus operandi* of national and international maltsters has been the consistency

Figure 1.

Terroir is a function of and modulated by seasonal weather conditions even for a wine terroir such as Château Y'quem (Premier Cru Supérieur), Appellation Sauternes, Bordeaux, France.

Wine cellars reflecting the juice yield in (A) vintage 2008 cellar maturation with an average juice yield of 8 hL/ ha and (B) vintage 2009 cellar maturation with an average juice yield of 23 hL/ ha. Both vintages were rated by Château Y'quem as 1 in 100 years for quality and production.

and homogeneity of malt to meet the needs of brewing companies. Unlike wine production, the skill of the maltster and brewer is measured by their ability to achieve similar malt quality between seasons such that the flavour and quality of beer remains consistent from year to year. Maltsters have developed malting protocols and quality specifications for barley that are suited to producing consistent malt and, in effect, minimise any effects of terroir. As such, one of the key barley/malt parameters is the grain protein content (GPC). In Australia, the typical acceptable GPC for the premium malt is 9-12% (Meghan Sheehy, personal communication). In comparison in Canada, the acceptable GPC for malting barley is 10-13%, although maltsters prefer 11-12.5% (Yueshu Li, personal communication). Although it is considered that the GPC of Australian malts are typically low while Canadian malts are high, both make high quality malt when compared with standard malt quality parameters (Evans et al. 2022).

The emergence some 30 years ago of craft malting and brewing enables malt diversity to be embraced. Rather than malting on the large scale with 50-400 tonne batches with barley from several grain growers, craft malting involves small batches (<10 tonne) of barley. Indeed, in this case, beer can be produced using barley and malt sourced from a single field. Such location/terroir identity are potentially useful for product differentiation and marketing. As such, craft maltsters and brewers need to be cognoscente that significant variation in barley quality can be evident within a field, such as for protein content (Sheehy et al. 2009).

The manifestation of consistent abiotic, biotic factors and stress on the growth of barley can vary from acute to minor in terms of impact and subsequent malt quality. Acute abiotic stress includes irregular and infrequent events such as drought and frost that can interfere with the formation of the barley grain proteins and carbohydrates (starch and cell wall polysaccharides). Within these abiotic stresses there are less drastic influences that may apply differently between barley growing seasons or varieties that subtly modify the accumulation and composition of the grain protein and carbohydrate. Biotic factors include infection with disease, but as seen at with wine at Château Y'quem, diseases such as noble rot can add value to the product. With barley, *Fusarium* head scab has an undesirable impact on grain quality contributing mycotoxins and potential gushing of beer (Geißinger et al. 2022; Sarlin et al. 2005; Schwarz and Han 2003).

The influence of terroir on barley/malt and subsequent beer quality, requires a more forensic

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approach to tease out the contribution of terroir and its effect on barley/malt quality to brewing efficiency and beer quality. In this review, the impact of terroir on barley/malt quality is considered in terms of the following:

- Malt/barley grain protein content (GPC) - quality
- Kolbach Index (KI), extract
- DP enzymes
- Starch
- Flavour
- Foam proteins (LTP1 and proteins Z4 and Z7)
- Ionic micronutrients
- Microorganisms

The insights from will be of interest to maltsters and brewers. Large maltsters/brewers will benefit from the identification of terroir based malt quality components that require greater attention for process amelioration or barley selection to improve the consistency of malt quality. Small scale 'craft' maltsters/brewers will benefit from insights as to barley/malt quality characteristics from differences in terroir to support marketing claims and product differentiation. Terroir influenced malt characters may impact on process efficiency (extract, lautering, DP), product quality (flavour, foam proteins) or conceivably interactions between efficiency and quality (GPC). Further, an understanding as to whether these differences are positive or negative to the product and how these may be mitigated or utilised is a novel opportunity.

Grain protein content and protein

Grain protein content (GPC) is one of the key quality specifications for the selection of both barley and grading of malt. Although GPC does not differ significantly between barley and malt (Yousif and Evans 2020), the proteins in barley and malt differ markedly due to proteolysis of existing proteins and protein synthesis (Brennan et al. 1997; Enari and Sopanon 1986; Folkes and Yemm 1958; Luo et al. 2019). Critically, it is known that barley/malt GPC is negatively correlated with malt extract yield (Bishop 1930; Briggs 1978; Cai et al. 2013; Smith and Lister 1983; Smith 1990), within genetically similar varieties, but positively correlated with diastatic power (DP) (Arends et al. 1995; Delcour and Vershaeve 1987; Erdal et al. 1993; Evans et al. 2022; Gibson et al. 1995). Free amino nitrogen (FAN) in wort ranges from 100 to 220 mg/L and is required to support yeast nutrition during fermentation (Hammond 2000). Higher levels of FAN are required from malt when used with unmalted adjuncts (rice or corn), which have a low FAN (Meilgaard 1976; O'Rourke 1999). A practically useful prediction of the relationship between GPC and FAN has been reported to assist in the procurement of barley for malting (Axcell, 2018). The 'Axcell' equation (below) can be used to estimate the levels of barley nitrogen required to produce malt with greater than the minimum FAN within the range of satisfactory Kolbach Index.

$$TN = FAN/(2.3\ x\ KI)$$

It follows that barley/malt with higher GPC at similar levels of protein modification (KI = 39-45%) will result in higher FAN levels which is of value when using non-malted starch adjuncts.

Stepping back from malting/brewing, the key determinant of a barley growers' profit is yield. The primary driver for yield in Canada is usually nitrogen, as in an average or better rainfall season, growers seek to translate the use of nitrogen by the plant into yield (Anbessa and Juskiw 2012). Conversely, in Australia, there is a finer balance between nitrogen and water availability (Sadras et al. 2016). This reflects the rainfall in Australia being more variable between seasons than in Canada, and the generally shallower depth of Australian soil and water retaining capacity. However, the adoption of low/no-till cropping systems over the past two decades has dramatically improved water conservation in Australian cropping zones by increasing soil organic matter (Dang et al. 2015 a,b). However, if the balance between nitrogen fertilisation and water availability is wrong, the growers may suffer losses in yield and/or high barley GPC as the process of 'haying off' may occur (van Herwaarden et al. 1998b). Haying off 'describes the premature ripening of cereal crops in conditions of high soil nitrogen and post anthesis drought' (Colwell 1963). This physiological effect occurs due to the reduction in re-translocation of water soluble carbohydrates into the grain resulting in reduced wheat yields of 34-50% (van Herwaarden et al. 1998a).

Luo et al (2019) reported trials with two Australian (Buloke, Commander) and Canadian (CDC Meredith, Bentley) malting varieties at sites in both Australia (Walebing and Cunderdin, Western Australia, WA) and Canada (Lancombe, Alberta) across two to three growing seasons. In each case, the trials were planted in soils depleted of nitrogen by the proceeding crops of canola and the subsequent trials were fertilised at varying rates of nitrogen (0-100 kg/ha). The primary aim of this study was to examine how the growing location and rate of nitrogen fertilisation impacted malt protein quality. They observed that a lower proportion of protein was modified (KI) during malting in the Canadian varieties that were associated with genetic factors that dominated protein variation, while the environment was reported to affect the protein composition of the grain (Luo et al. 2019).

What was not published in the Luo et al (2019) study was the wealth of data on background yield and malt quality that lends itself to further forensic analysis. Figure 2 provides an insight from one growing season (2014) into the influence of genome x environment (G x E) and growing site on barley protein composition. While there was relatively wide variation in protein composition for the four varieties grown at Lancombe (Canada) site, the variation between the varieties at the Walebing (Australia) site was relatively limited. Interestingly, the higher GPC for Buloke and Commander at Lancombe (Figure 3A, B), was matched with a lower proportion of water/saline soluble albumins and globulins, but higher proportions of water insoluble hordeins. Overall, the tendency with increasing nitrogen fertilisation was a decrease in the proportion of albumins and globulins, with a corresponding increase in the proportion of hordeins (Figure 2 A-D).

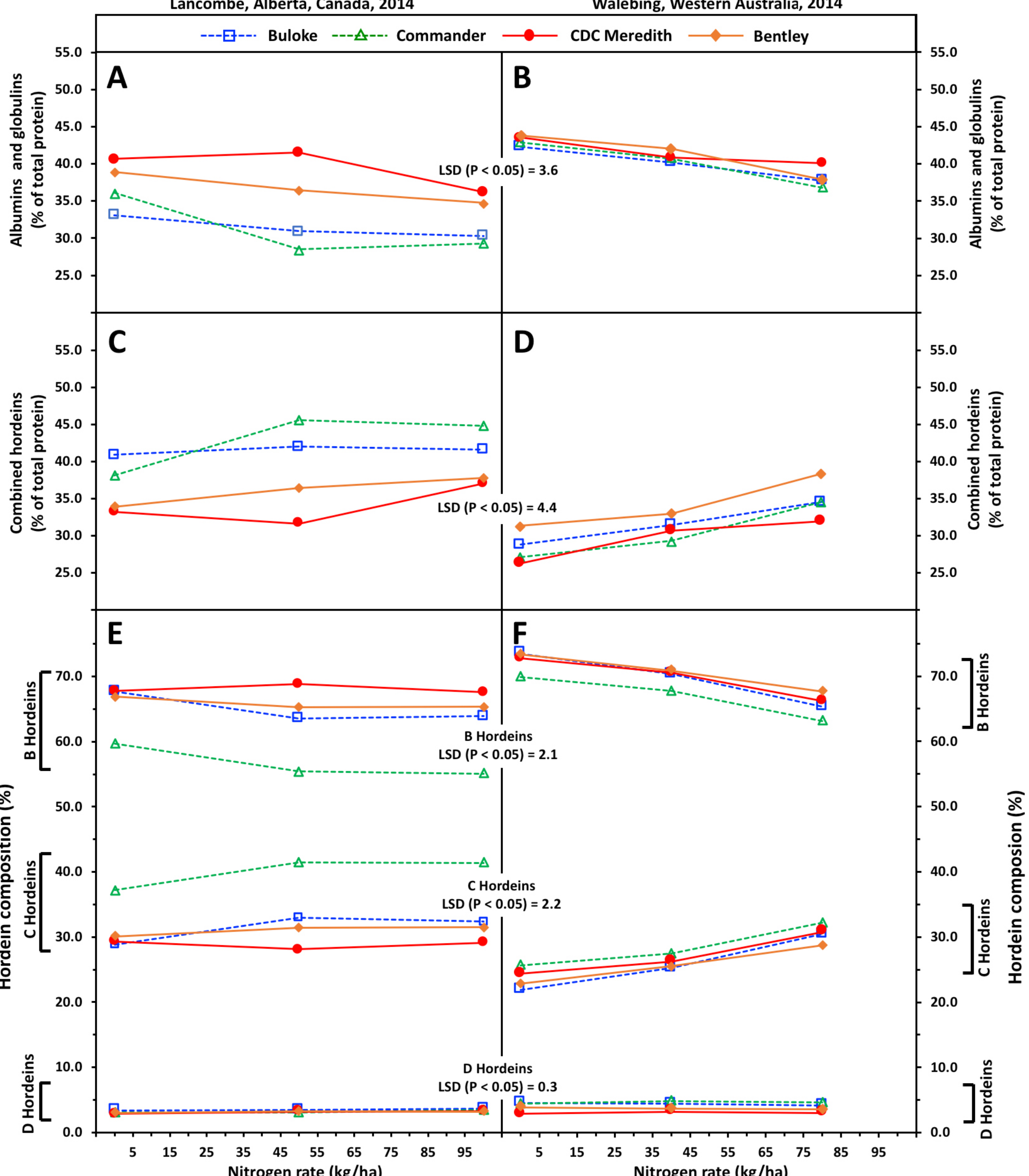
With respect to hordein (Figure 3E, F), Buloke and Commander tended to have a higher proportion of C and D hordeins but a lower proportion of B hordeins. At the Lancombe site (Figure 3E), Buloke and Bentley had similar compositions of B, C and D hordeins while Commander and CDC Meredith had markedly different compositions of B, C and D hordeins. Interesting as the results are, further investigation is required to understand the impact of terroir on protein composition.

It is well known that the hordein component of GPC is strongly associated with variety, such that it has been considered as an option for varietal identification (Marchylo et al. 1986; Smith and Lister 1983; Yamaguchi et al. 1998). However, the level of hordeins varies according to the region the barley is grown in, the amount of nitrogen fertilisation and, presumably, terroir (Luo et al. 2019; Molina-Cano et al. 2004; Smith 1990). Molina-Cano et al (2004) observed lower proportions of hordein in barley varieties grown in Spain compared to Canada. Further it has been observed that hordein and other barley proteins can impact on wort separation (Barrett et al. 1973; Rennie and Ball 1979; Smith 1990), milling energy/friability (Chandra et al. 1999; Ferrari et al. 2010; Leach et al. 2002), foam stability (Evans et al. 2003; Vaag et al. 1999, 2000), and haze (Evans et al. 2003). In addition, varieties with lower proportions of D hordein tend to have improved malt extract at equivalent levels of GPC (Howard et al. 1996). Some of these characteristics could impact on beer quality (foam, haze stability) while other characters (extract, wort separation, milling energy/friability) influence brewing efficiency and profitability. As such, small and large maltsters would be expected to value these characteristics differently. Again, more study is required to elucidate the putative influence of terroir on these malt quality parameters.

Any potential influence of terroir on the composition of grain protein, is likely to have an impact on wort amino acid composition with implications for yeast nutrition and beer flavour (Donhauser and Wagner 1990; Gibson et al. 2009; Yin et al. 2017). Wort amino acid composition would change, as each of the protease targeted hordeins have a different amino acid composition (Shewry 1993). In contrast, proteins such as protein Z and LTP1 (2-3% total protein), are present post wort boiling since they are resilient to proteolysis (Evans and Hejgaard 1999). It is well known that the absorption of amino acids from wort is selective (Jones and Pierce 1964, Briggs et al. 2004), while the amino acid composition in the wort impacts fermentability and yeast health (Edney and Langrell 2005; Gibson et al. 2009; Blanca-Gomez and Edney 2011). In addition, the most intense Maillard browning occurs with the amino acid lysine followed by tyrosine, tryptophan and glycine (Ajandouz and Puigserver 1999).

Figure 2.

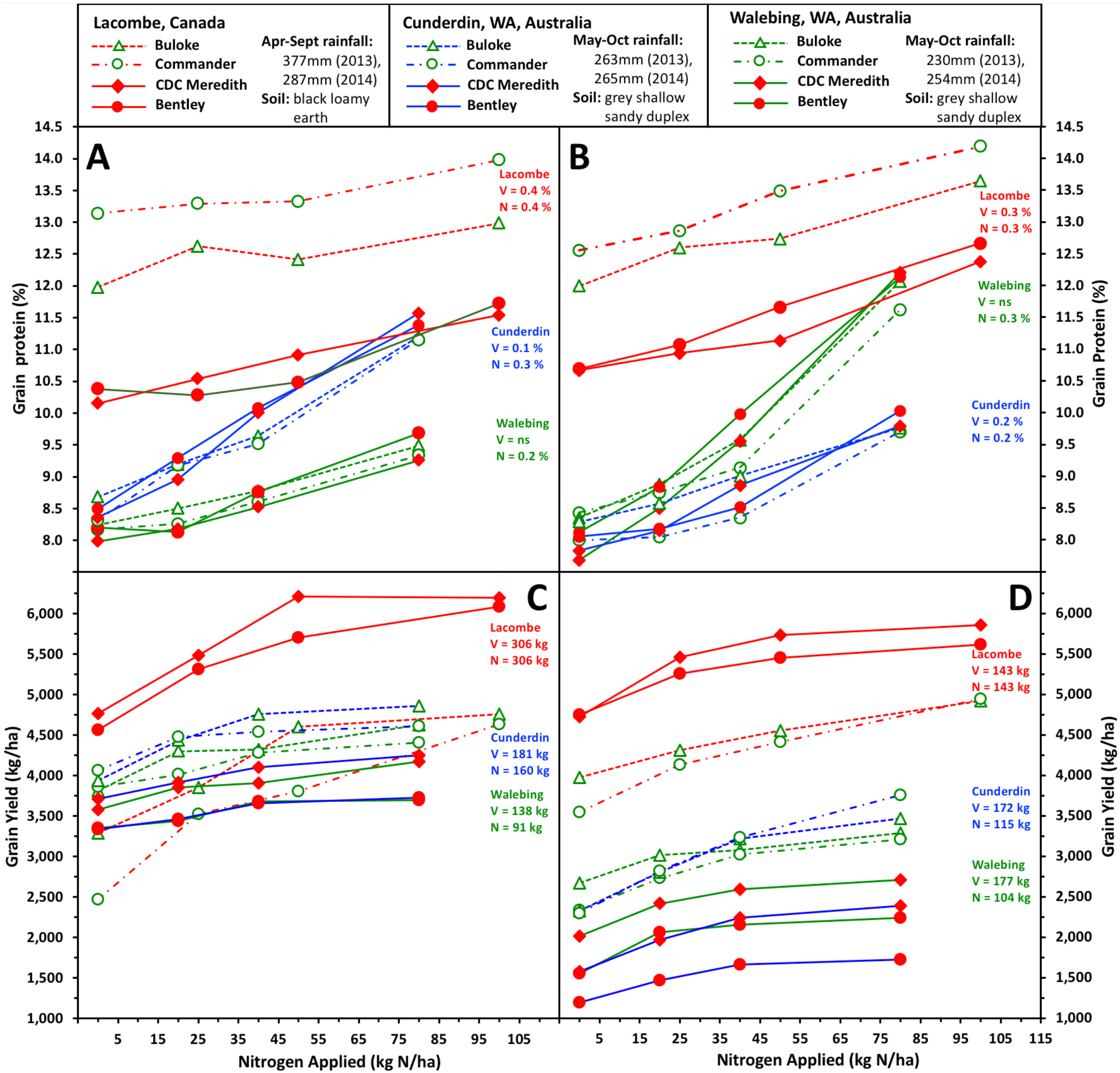
The impact in 2014 of growing location (Western Australia or Canada) and nitrogen fertilisation on barley yield characteristics for two mainstream Australian (Buloke, Commander) and Canadian (Bentley, CDC Meredith) barley varieties.



Proportion of albumins and globulins in (A) Lancombe, Canada and (B) Walebing, Western Australia.
Combined hordeins in (C) Lancombe, Canada and (D) Walebing, Western Australia.
Hordein composition in (E) Lancombe, Canada and (F) Walebing, Western Australia.
Unpublished data from Luo et al (2019).

Figure 3.

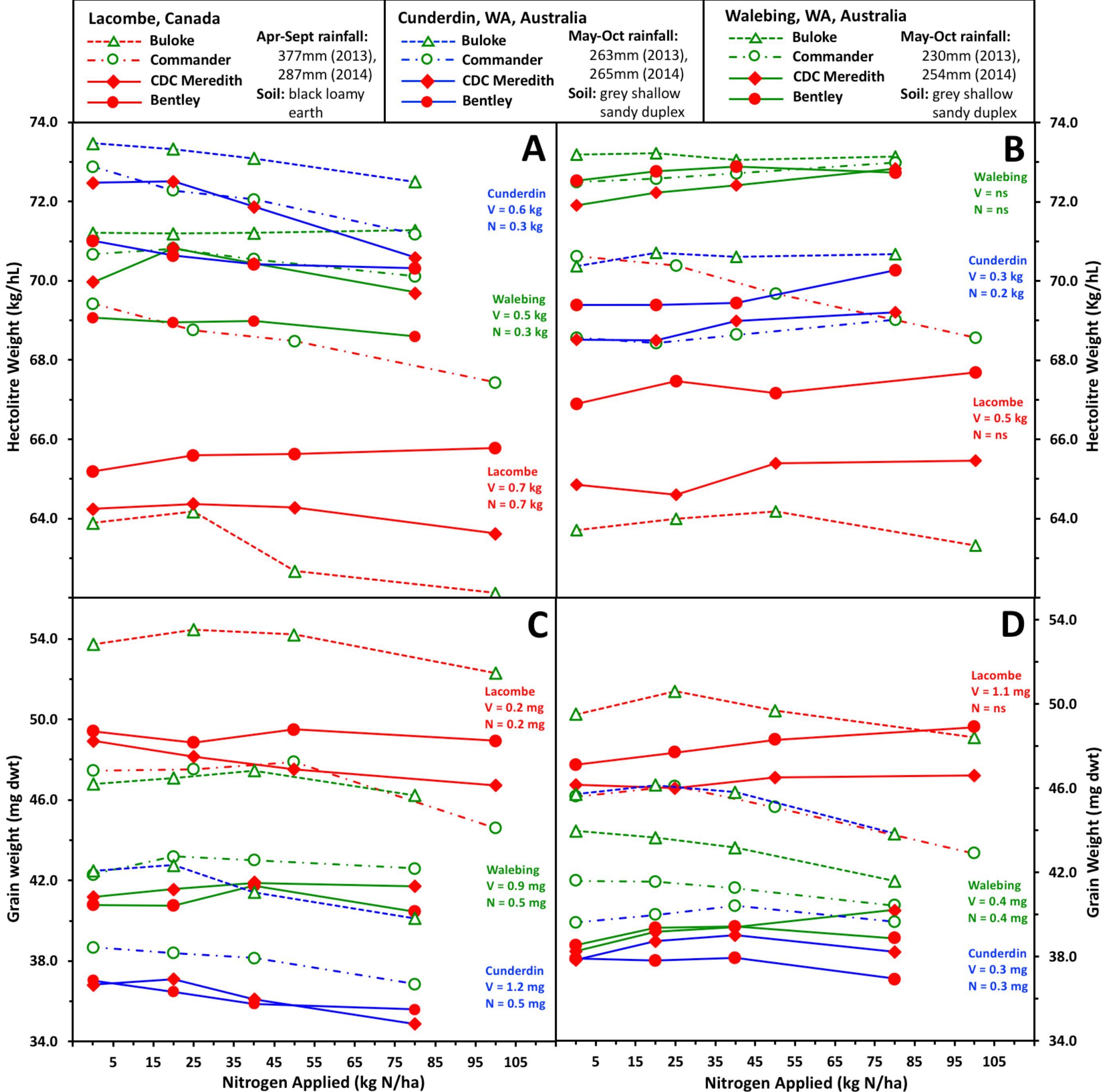
The impact of growing location (Western Australia or Canada) and nitrogen fertilisation on barley yield characteristics for two Australian (Buloke, Commander) and Canadian (Bentley, CDC Meredith) barley varieties.



Grain protein: 2013 (A) and 2014 (B)
Grain yield: 2013 (C) and 2014 (D).
LSD (P < 0.05), where V = variety and N = nitrogen application, ns = not significant.
Unpublished data from Luo et al (2019).

Figure 4.

The impact of growing location (Western Australia or Canada) and nitrogen fertilisation on barley yield characteristics for two mainstream Australian (Buloke, Commander) and Canadian (Bentley, CDC Meredith) barley varieties.



Grain hectolitre weight; 2013 (A) and 2014 (B)
Grain weight 2013 (C), and 2014 (D) growing seasons.
LSD (P < 0.05), where V = variety and N = nitrogen application, ns = not significant.
Unpublished data from Luo et al (2019).

Water stress conditions are dependent on the tolerance to drought of barley varieties, with the more tolerant varieties exhibiting elevated proline levels in the grain (Hayat et al. 2012). As proline is poorly metabolised by brewing yeast (Briggs et al. 2004), such a change in wort amino acid composition would be expected to have ramifications for fermentation efficiency and beer flavour. Again, the consequences of the influence of changes in terroir mediated protein composition is another aspect worthy of further investigation.

The impact of nitrogen fertilisation on grain yield and GPC, was shown to vary according to genetics and the growing location (Figure 3). Interestingly high rates of nitrogen fertilisation did not result in haying off at the Canadian or the Australian sites in either growing season. We suspect that Luo et al (2019) were hoping to induce haying off, at least at the Australian sites, to maximise experimental variation. That haying off did not occur was reinforced by both the grain size and hectolitre weight data, where there were no dramatic reductions at higher nitrogen fertilisation (Figure 4). A relatively gradual increase in GPC was observed with increasing nitrogen fertilisation at both growing sites with all varieties (Figure 3 A, B). Similarly grain yield was observed to increase up to 40-50 kg nitrogen/Ha, after which it plateaued.

The response of GPC to nitrogen fertilisation, growing location and variety showed clear differences (Figure 3 A, B). Firstly, compared to the Canadian varieties, the Australian varieties had significantly higher levels of GPC when grown in Canada in both growing seasons. The lower GPC for Canadian varieties grown in Canada signifies the success of the Canadian breeders to target lower GPC in that environment. The Canadian varieties also achieved substantially higher yields compared to the Australian varieties under the same Canadian growing conditions. However, the Canadian and Australian varieties grown in Australia tended to have a similar GPC. Not surprisingly, the grain yields in Australia favoured the Australian varieties, particularly in season 2014 (Figure 3 C, D). Combined, the GPC was higher for all barley varieties grown in Canada compared to Australia at comparable levels of nitrogen fertilisation.

Presumably, the higher level of GPC for the Australian varieties was accompanied by a lower proportion of albumin/globulin but a higher proportion of hordeins with C hordeins and a lower proportion of B hordein (Figure 2). These observations suggest that GPC and protein composition were terroir associated traits.

Comparatively, the seasonal trials in 2013 and 2014 in Australia provided quite different outcomes in terms of GPC and grain yield (Figure 3). Noticeably, the average yield for the four varieties across the Walebing and Cunderdin sites was 63% higher in 2013 than 2014 (Figure 3C, D). Interestingly, the GPC response to nitrogen fertilisation at Cunderdin was substantially steeper than Walebing in 2013. The reverse relationship for GPC between the sites being observed in 2014. These results were found despite the rainfall in the growing season being very similar at Cunderdin (263 vs 265mm) and Walebing (230 vs 254mm). Although perplexing, this may suggest factors beyond total rainfall influence GPC, such as the timing of rainfall and evapotranspiration (wind/temperature).

Typically, the grain grown in Canada had the lowest hectolitre weight but somewhat higher grain weight (Figure 4). Interestingly, the Australian variety Buloke, when grown in Canada, had the lowest grain hectolitre weight but still the highest grain weight, while its compatriot Commander was the opposite. Comparing grain grown in Canada and Australia showed that grain weight was higher in Canada for all varieties (Figure 4 C, D). These results are suggestive of genetic outcomes but also the subtle influence of terroir.

In Tasmania Australia, climatic conditions allow growers to plant barley after the seasonal late Autumn sowing (May/June – December/January harvest) or Spring sowing (September – February/ March harvest) including supplementary irrigation. Australian grain traders regularly comment that Spring sown Tasmanian barley has substantially higher grain GPC. Table 1 shows data from Tasmanian barley breeder (Meixue Zhou, personal communication) comparing three local barley varieties sowed in Autumn and Spring. The Spring sowed barley had on average 29.2% higher GPC than the Autumn sowed barley. As would be expected,

the Spring sowed barley had higher DP (23.1%) and wort viscosity (5.1%) but lower extract (4.7%). A similar advantage was observed over four growing years with respect to commercial barley received by the Tasmanian Agricultural Producers (Supplementary information Table 1S). It was noted that the difference between commercial Autumn and Spring sowed crops was not as obvious in Table 1S compared to the controlled trial of Table 1. This blurring between Autumn and Spring sowed barley was not surprising. Growers have on-farm storage to ameliorate delivery time to ensure the highest price and would use agronomic management to limit the GPC of Spring sowed barley.

A bimodal GPC distribution has observed between Australia (low GPC) and Canada (high GPC) by Emebiri et al (2005). Hwang and Lorenz (1986) also observed that the GPC of US inter-mountain and Western Canadian Prairie region was substantially lower than grain from the Central and Eastern Canadian Prairie regions. In contrast, Molina-Cano et al (2004) conducted a trial on low and high GPC barley in the 1998/99 growing seasons in Spain and Canada using the European varieties Alexis (two row) and Dobla (six row) together with the North American varieties Harrington (Canada, two row) and Stander (US, six row). The crop was planted at the Spanish site (Lleida, Latitude 41.25°N) in mid-November and harvested mid-June (an increasing photoperiod). In 1998, the rainfull in the growing season was 240mm with 94mm during grain fill, while in 1999 it was 297mm with 157mm during grain fill. The crop was planted at the Canadian site (Kernen Farm, Saskatchewan, Latitude 52.9°N) in mid-May and harvested mid-August (decreasing photoperiod) where in 1998 the rainfall was 220 mm during the growing season, with 137mm during grain fill, and in 1999, 282 mm with 141mm during grain fill for a crop planted. Although Molina-Cano et al (2004) did not distinguish between varieties, they found that Canadian grown barley had lower levels of GPC and the three hordein fractions than those grown in Spain.

The level of GPC appears to be influenced by photoperiod. Figure 5 shows that Spring sowed Tasmanian and Canadian barley ripened and matured in relatively long day lengths that are shortening to harvest producing higher GPC. In contrast, the Tasmanian Autumn sowed barley and most Australian barley, ripens and matures at shorter daylengths that are increasing to harvest with lower GPC. Qi et al (2005) found differing Spring sowing dates increased GPC significantly but by a small amount. Emebiri et al (2005) also referred to a link between GPC and photoperiod. In contrast, the Molina-Cano et al (2004) Canadian/ Spanish study observed that a higher GPC was found in the Spanish grown barley where the day length was increasing. The rainfall recorded for this trial was substantially lower compared to Luo et al (2019) which may suggest factors relating to haying-off, although no data was reported on yield, grain or hectolitre weight to understand these potential controlling factors. A comprehensive examination of the agronomic impact of photoperiod and the specific quantitative trait loci involved in the response of barley varieties were outlined by Boyd et al (2003).

Other variations in field light conditions can also impact the accumulation of GPC. Firstly, high GPC in grain was produced in glasshouse trials, where a cycle of light (16 hours) and dark (eight hours) was used (Macnicol et al. 1993). Discussions with Southern Western Australian barley growers indicated that the relatively high rainfall in the 2022 growing season resulted in abnormally low GPC, particularly in specific varieties. Interestingly, the literature shows that 'shading,' either by clouds, aerosols (pollution) or shade cloth can influence both yield and GPC (Gao et al. 2017; Shimoda and Sugikawa 2019; Yang et al. 2013). Indeed, Shimoda and Sugikawa (2019) observed that a cloudier growing site in Japan (Kitahonami) compared with a less cloudy site (Yumechikara) produced lower yields but a higher GPC. Whether terroir, photoperiod and light intensity determines GPC, requires further investigation with respect to impact it may have in different geographic regions (e.g., comparisons of grain protein content between Australia, North America, Argentina, Europe).

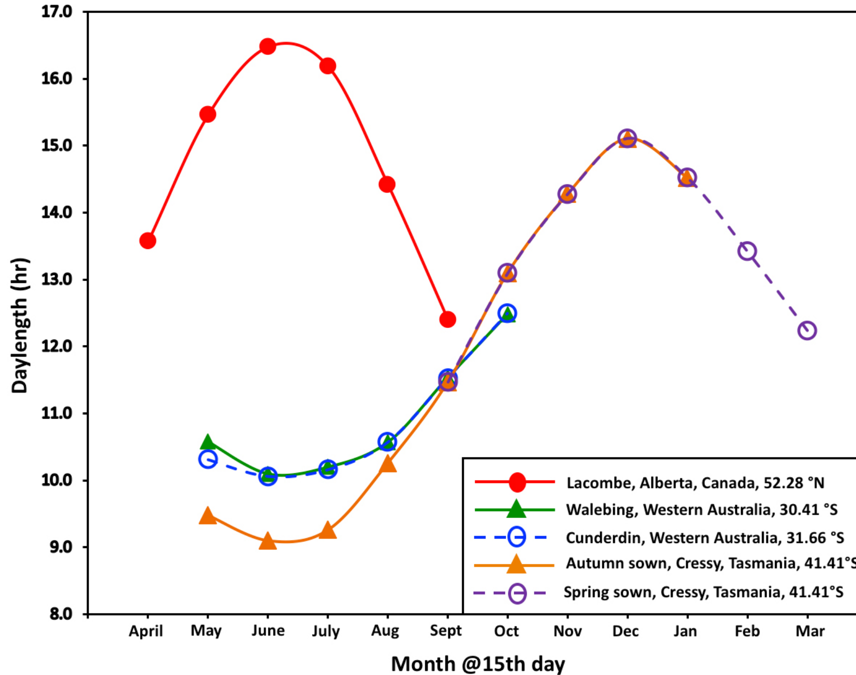
Grain protein content and extract

The inverse relationship between grain protein and extract yield (Bishop 1930; Briggs 1978; Cai et al. 2013) is well known by maltsters and brewers. Also, it has been noted that lower proportions of D hordein tend to improve extract levels through varietal variation or greater proteolytic modification

Figure 5.

Regional day lengths for the growing sites during barley growing and grain fill periods in Canada, Western Australia and Tasmania, Australia.

(https://www.timeanddate.com/)



during malting (Howard et al. 1996; Smith and Lister 1983; Smith and Simpson 1983). Examination of the data from Luo et al (2019) (Figure 6A) shows the expected and significant inverse correlation between GPC and extract (r = -0.711, P < 0.01). However, additional analysis of the data - variety, growing season and site - revealed further insights (Figure 6A). This approach increases the GPC and extract correlation to r = -0.984 to -0.882 with no loss of significance. Further, it was observed that at the Australian growing sites that there was a clear genetic difference between Meredith-Bentley (Walebing 2012; Cunderdin 2013, r = -0.963, P < 0.01) and Buloke-Commander (Walebing 2012; Cunderdin 2013, r = -0.882, P < 0.01). Interestingly, a quite different relationship, maintaining the Canadian/ Australian varietal divide, was observed for the Walebing site in the 2014 season (Figure 6A), for Meredith-Bentley (r = -0.963, P < 0.01) and Buloke-Commander (r = -0.984, P < 0.01). The Walebing 2014 season shows a substantial deviation in the GPC-nitrogen fertilisation plot compared to that of Cunderdin 2014 (Figure 3B) whereas the opposite relationship between Cunderdin and Walebing (2013) (Figure 3A) did not show divergence in the varietal GPC to extract relationship (Figure 6A). Finally, all four varieties when grown in Canada in the 2013/2014 seasons (Figure 6A) had similar GPC to extract relationships (r = -0.915, P < 0.01). Such observations show interaction between genotype and growing conditions but also suggest there was a subtle interaction between genotype, season and terroir (Canada vs Australia).

Grain protein content and Kolbach index

Axcell et al (1984) were one of the first to report that Kolbach index (KI), GPC and variety should be taken into account in malt specifications. In the Luo et al (2019) data, the overall correlation for samples was r = - 0.473 (P < 0.01, Figure 6B). However, it should be highlighted that these samples were malted using the same micro-malting protocol and equipment. This is quite different to commercial maltings where the maltster would actively modify the protocol to ameliorate the KI and other malt quality parameters to the standard 39-45% range or that specified by their customers. Tables 2 and 3 examine the relationship between malt protein and KI for commercial malts (and in Table 3 some micro malts) from Australia and elsewhere over a two-decade period. In Table 3, the correlations are reported between malt protein and KI (r = -0.381 to 0.189, P < 0.05). In Table 4, a weak correlation was found between malt protein and KI for each Australian malting year (r = -0.518 to -0.256, P < 0.05). Table 4 went one step further by separating each data set by variety which improved the degree of correlation (r = -0.713 to -0.389, P < 0.05). These observations support and extend those of Axcell et al (1984) regarding the Axcell equation and the relationship between KI, GPC, and variety.

Figure 6B shows GPC and KI data with respect to variety, growing season and site which showed an correlation of r = - 0.473 (P < 0.01). This selective analysis resulted in an increase in the correlation

Figure 6.

Relationship between grain protein and Congress (EBC) extract (A) and KI (B) for malt from barley grown in Western Australia (Cunderdin - 2013 and Walebing – 2012/2014 sites) and Canada (Lacombe - 2013/14) using Australian (Buloke, Commander) and Canadian (Bentley, Meredith) barley varieties in the 2012 to 2014 growing seasons, * = P < 0.05 ** = P < 0.01. Unpublished data from Luo et al (2019).

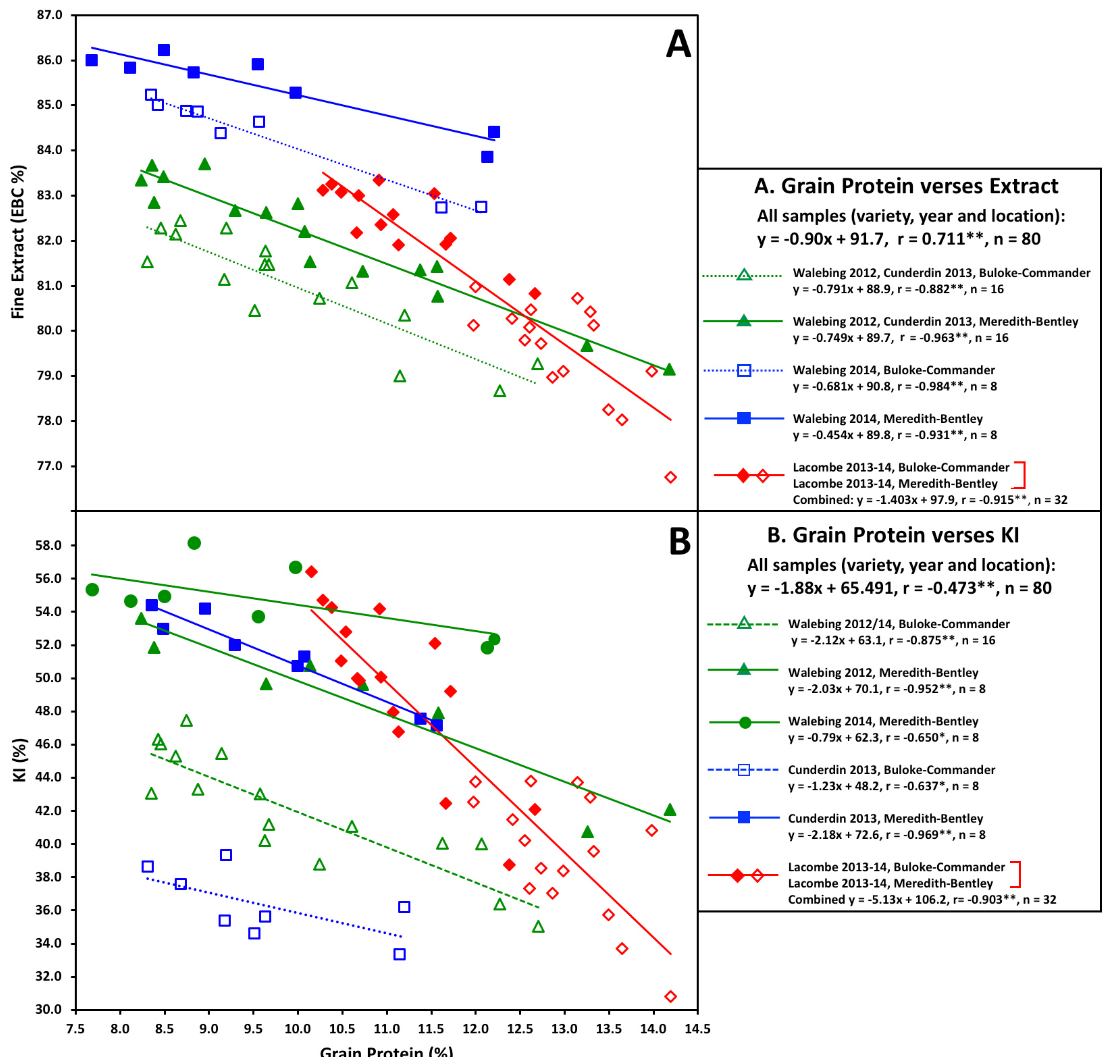


Table 1.

Barley characteristics for three varieties (duplicate plots) grown in Northern Tasmania (Cressy Research Station) during the 2003/2004 growing season.

Sowing time	Variety	Protein (%)	DP (°WK)	Fine extract Congress (%)	Wort viscosity (mPa.s)
Autumn	Franklin	12.4 ± 0.8	538 ± 7	79.1 ± 0.6	1.502 ± 0.017
	Gairdner	12.3 ± 0.4	384 ± 64	79.6 ± 1.6	1.536 ± 0.004
	Macquarie	11.7 ± 0.9	295 ± 8	80.0 ± 0.5	1.484 ± 0.010
Spring	Franklin	16.0 ± 0.8	576 ± 93	76.0 ± 1.1	1.644 ± 0.095
	Gairdner	16.2 ± 0.4	547 ± 8	74.0 ± 0.0	1.517 ± 0.012
	Macquarie	14.8 ± 0.7	375 ± 36	77.5 ± 0.2	1.595 ± 0.009

Data is the mean ± standard deviation.

The authors are indebted to Prof Meixue Zhou (Tasmanian Institute of Agriculture, University of Tasmania, Australia) for this data.

(r = -0.969 to -0.637, P < 0.05). Seasonal effects were observed comparing Cunderdin 2013 to Walebing in 2012/14 for Buloke/Commander, and the difference for the Australian grown Meredith/Bentley compared to Buloke/Commander. Interestingly the Canadian trials for GPC vs KI, like the GPC vs extract plot (Figure 6A), showed the same inverse correlation determined by GPC. The slope of the regression line was steeper than for the other relationships. Also in Figure 2 A-D, in the 2014 season at Lancombe, the proportion of hordein was higher and albumin/globulins lower for the barley grown at Lancombe (Canada) compared to those grown at Walebing (WA). Luo et al (2019) commented that Canadian grown barley was slower to modify protein. The greater proportion of hordein compared to albumin/globulin in the Lancombe grown barley may partially explain this observation. With respect to the Canada-Australian differences, a more predictive inverse relationship was found between GPC and KI when variety, and growing season (presumably terroir) were considered. Also, in addition to the protein to KI relationship, there may be a relationship between KI and the key producing proteinases which respond to gibberellic acid (Evans et al. 2009c; Jones 2005; Mikola 1987; Wallace et al. 1988).

Grain protein content, diastatic power and enzymes

There is a positive relationship between grain protein and diastatic power (DP) (Arends et al. 1995; Delcour and Verschaeve 1987; Erdal et al. 1993; Evans et al. 2022; Gibson et al. 1995). Table 2 shows that this relationship was observed (r = 0.324 to 0.622, P < 0.05) across a wide range of (largely) commercial malts over more than 15-year period. Of the DP component enzymes, *beta*-amylase is the primary determinant of DP, with lesser and intermittent contributions from α-amylase and limit dextrinase (Arends et al. 1995; Evans et al. 2005b, 2014b, 2022; Gibson et al. 1995). Unsurprisingly Table 2 shows that DP was primarily correlated with *beta*-amylase activity (r = 0.540 to 0.903, P < 0.01), such that *beta*-amylase activity was also correlated with GPC (r = 0.361 to 0.733, P < 0.01) in most data sets. Table 2 also shows that α-amylase (r = 0.288 to 0.749, P < 0.05) and limit dextrinase (r = 0.250 to 0.706, P < 0.05) were intermittently correlated with DP. Finally of the DP enzymes, α-amylase (r = 0.198 to 0.643, P < 0.05) and limit dextrinase (r = 0.369 to 0.805, P < 0.05) were better correlated to KI than *beta*-amylase (r = -0.280 to 0.389, P < 0.05) (Table 2). Figure 7A shows that there was a significant correlation between GPC and DP (r = 0.830 to 0.805, P < 0.01). Further analysis of the Luo et al (2019) data by variety, growing year and location does result in small improvements in correlation (r = 0.671 to 0.978, P < 0.01), but this was minor compared to GPC and KI or extract (Figure 6 A, B). Figures 7B and C show that levels of α-amylase and LD were poorly correlated with GPC. This was not unexpected as gibberellin stimulation of α-amylase and LD synthesis during 'germination' was substantially boosted (Evans et al. 2009b; Hardie 1975). However, *beta*-amylase activity (not measured by Luo et al 2019), and by correlation DP, were unresponsive to gibberellin.

There is a report of possible 'terroir' for limit dextrinase in a survey of Australian malthouses in 2005-06 (Evans et al. 2008a) where the level of the enzyme was unexpectedly high in malt house WA-A (Figure 8). Evans et al (2022) compared the levels of *beta*-amylase, α-amylase and limit dextrinase activity, primarily in Australia, over nearly 20 years and eight surveys. It was observed that variation in the mean levels of *beta*-amylase and α-amylase activity remained relatively constant, however the level of limit dextrinase was consistently high from the WA-A malthouse across the surveys. The level of limit dextrinase was also found to be consistently high for malt produced by Pilot Malting Australia (PMA) and for Australian and Chinese malted barley from the 2017/18 malt year (Figure 8).

The PMA malting used 100 kg pilot malter with high levels of rousing (5 minutes/hour) to avoid imbibing barley corns jamming the turning screw of the Unimalter (with steeping, germinating and kilning in the same vessel). Further, the WA-A malthouse had a two-step steeping operation where after about 16 hours the grain was dropped into second steeping vessel below (Yousif and Evans 2020). Both the PMA rousing and the WA-A double steep operation would be expected to increase the level of dissolved oxygen in the steep water and this would promote the increased synthesis of limit dextrinase, but not α-amylase (Evans et al. 2022; Yousif and Evans 2020). It was further suggested that the high levels of limit dextrinase in the 2017/18 malts was due to

Table 2.

Correlation coefficient matrix for malt protein, KI, DP and DP enzyme levels protein compared over 20 years of studies with commercial malts (unless indicated).

Year malted	Sample #	Parameters	DP (°WK)	KI (%)	*beta*-amylase (U/g)	α–amylase (U/g)	Limit dextrin. (U/kg)	Source and description
			Correlation coefficients (r)					
2002-03	n = 40	**Malt prot. (%)**	ns	ns	ns	ns	ns	Evans et al. (2005)
		DP (°WK)	-	ns	0.890**	0.749**	0.706**	Australian barley/malted
		KI (%)	-	-	ns	0.447*	ns	
2005	n = 275	**Malt prot. (%)**	0.346**	-0.381**	0.464**	0.464**	0.214*	Evans et al. (2008a)
		DP (°WK)	-	ns	0.540**	0.361**	0.298**	All Gairdner variety samples
		KI (%)	-	-	0.389**	0.198*	ns	
2005	n = 22	**Malt prot. (%)**	ns	na	ns	ns	ns	Evans et al. (2008a)
		DP (°WK)	-	na	ns	ns	ns	All Gairdner, WA-A malthouse
2006/07	n = 29	**Malt prot. (%)**	ns	ns	ns	ns	ns	Evans et al. (2011)
		DP (°WK)	-	0.254*	0.903**	0.569**	0.403**	Australian barley/malted
		KI (%)	-	-	0.313*	0.282*	0.369**	
2018	n = 76	**Malt prot. (%)**	0.386**	ns	0.361**	ns	ns	Evans et al. (2022)
		DP (°WK)	-	ns	0.531**	ns	ns	Australian barley/malted
		KI (%)	-	-	ns	0.271*	ns	
2008-10	n = 54	**Malt prot. (%)**	0.380*	ns	ns	ns	ns	Evans et al. (2014b)
		DP (°WK)	-	ns	0.851**	0.288*	0.302*	Australian barley/malted
		KI (%)	-	-	-0.280*	ns	ns	
2008-10	n = 42	**Malt prot. (%)**	ns	ns	0.733**	ns	0.365*	Evans et al. (2014b)
		DP (°WK)	-	0.413**	0.455**	ns	ns	Europe & N. American malt
		KI (%)	-	-	ns	ns	0.443**	
2005	n = 111	**Malt prot. (%)**	0.504**	0.189*	0.426**	ns	ns	Evans et al. (2008a)
		DP (°WK)	-	0.518**	0.619**	ns	ns	N. Zealand & N. Hemisphere
		KI (%)	-	-	0.388**	0.329**	0.540**	
2018	n = 19	**Malt prot. (%)**	ns	ns	-	0.575**	ns	Evans et al. (2022)
		DP (°WK)	-	ns	-	0.529*	0.733**	Aust. Barley, malted China
		KI (%)	-	-	-	ns	ns	
2008	n = 109	**Malt prot. (%)**	0.622*	ns	0.560**	0.251*	ns	Evans et al. (2010)
		DP (°WK)	-	0.228*	0.889**	0.395**	0.250*	Breeders lines micro-malted
		KI (%)	-	-	ns	0.465**	0.596**	Waite Program, S. Australia
2008	n = 88	**Malt prot. (%)**	0.324**	-0.229*	0.556**	ns	ns	Evans et al. (2010)
		DP (°WK)	-	0.410**	0.898**	0.535**	0.456**	Breeders lines micro-malted
		KI (%)	-	-	ns	0.633**	0.805**	WA Program, W. Australia

The data is the mean ± standard deviation.
The authors are indebted to Prof Meixue Zhou (Tasmanian Institute of Agriculture, University of Tasmania, Australia) for this data.

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Table 3.

Correlation coefficient (r) between Australian commercial and breeding program malts for protein, KI and foam proteins over three years.

		Australian malting year: 1998 (r, with GPC)					Australian malting year: 1999 (r, with GPC)			
Variety	n	KI (%)	Protein Z4 (μg/g)	Protein Z7 (μg/g)	LTP1 (μg/g)	n	KI (%)	Protein Z4 (μg/g)	Protein Z7 (μg/g)	LTP1 (μg/g)
All samples	71	-0.282*	ns	ns	ns	138	-0.518**	0.268**	ns	0.221*
Arapiles	17	ns	0.845**	0.829**	ns	48	-0.389**	0.419**	0.548**	ns
Franklin	26	-0.640**	0.588**	0.752**	ns	33	-0.468**	0.562**	0.693**	0.429*
Gairdner	na	na	na	na	na	11	-0.713**	0.674*	ns	0.699**
Schooner	28	-0.601**	0.466*	0.494**	ns	46	-0.620**	0.538**	0.534**	0.036
Mean ± δ		45.2 ± 5.7	1048 ± 332	154 ± 70	423 ± 93		41.2 ±5.9	1127 ± 453	148 ± 75	674 ± 258
Malt prot. (%) mean ± δ		10.5 ± 1.1					10.6 ± 1.0			

		Australian malting year: 2000 (r, with GPC)			
Variety	n	KI (%)	Protein Z4 (μg/g)	Protein Z7 (μg/g)	LTP1 (μg/g)
All samples	56	-0.256*	ns	0.273*	ns
Arapiles	10	ns	ns	0.790*	ns
Franklin	12	-0.618*	0.611*	ns	ns
Gairdner	14	na	ns	ns	ns
Schooner	20	-0.542*	ns	ns	ns
Mean ± δ		41.8 ± 4.5	1178 ± 412	147 ± 87	429 ±73
Malt prot. (%) mean ± δ		11.0 ± 0.9			

Unpublished data (E. Evans), Measurement of Protein Z4, Protein Z7 and LTP1 by ELISAs according to Evans and Hejgaard (1999). * = P < 0.05, ** P < 0.01 according to the Pearson Product-Moment correlation coefficient critical values, na = not available, ns = not significant (P < 0.05).

Table 4.

Correlation coefficient matrix for malt protein, KI, FAN and wort colour levels across 15 years of studies with commercial malts and micro-malted malts.

Year malted	Sample #	Parameters	Correlation Coefficient (r): FAN (mg/L)	Correlation Coefficient (r): Wort Colour (°EBC)	Source and description
2002-03	n = 40	**KI (%)**	0.569*	0.435*	Evans et al. (2011)
		FAN (mg/L)	-	ns	Australian barley/malted
2006/07	n = 29	**KI (%)**	0.440**	ns	Evans et al. (2005)
		FAN (mg/L)	-	ns	Australian barley/malted
2018	n = 76	**KI (%)**	0.573**	0.454**	Evans et al. (2022)
		FAN (mg/L)	-	ns	Australian barley/malted
2008	n = 109	**KI (%)**	0.880**	ns	Evans et al. (2010)
		FAN (mg/L)	-	ns	Breeders lines micro-malted Waite Program, Sth Australia
2008	n = 86	**KI (%)**	0.850**	ns	Evans et al. (2010)
		FAN (mg/L)	-	0.335*	Breeders lines micro-malted WA Program, West. Australia

* = P < 0.05, ** P < 0.01 according to the Pearson Product-Moment correlation coefficient critical values.

ns

maltsters using higher rates of steep aeration to avoid premature yeast flocculation (PYF, Axcell et al. 1986, 2000). This insight into limit dextrinase activity is a salutatory warning (and opportunity) for those searching for terroir effects, since differing malting protocols can result in unexpected impacts on malt quality. These impacts maybe desirable or undesirable depending on customers requirements (Evans et al. 2007).

There has been an increasing interest in the contribution of base malts to beer flavour, particularly from craft brewers (Craine et al. 2021; Kraus-Weyermann et al. 2020). Much of this interest has been generated by the heritage variety Maris Otter, which has been prominent in the grist bill of champion beers (12 out of 18) at the annual CAMRA Great British Beer Festival between 2002 and 2019 (Stewart et al. 2023). Of course, it has long been understood that specialty malts can influence both beer flavour and colour due in part to Maillard (browning) reactions (Evans 2021; Féchir et al. 2021).

Wort/beer flavour, Kolbach index and GPC

Over the past decade, a number of groups have sought to define the molecular basis for differences in malt flavour by SPME GC/LC-MS in wort (Stewart et al. 2023), whisk(e)y (Kyraleou et al. 2021) and beer (Bettenhausen et al. 2018; Dong et al. 2015; Herb et al. 2017b; Li et al. 2022; Stewart et al. 2023). Although most studies have concentrated on differences in flavour between varieties, four publications have considered the influence of the barley growing environment on beer flavour (Bettenhausen et al. 2018; Herb et al. 2017a,b; Li et al. 2022). In a study of a two-year growing season in Canada with four commercial varieties, Li et al (2022) observed that despite significant differences in malt quality parameters (GPC, KI, etc), there were limited significant differences found with a sensory panel, mass spectrometry and NMR assessment for aroma and non-volatile beer compounds (acetaldehyde). However, the limited difference may reflect the experimental design where the Canadian Prairie presents similar growing conditions coupled with the relative similarity of the malt varieties.

Herb et al (2017b) went further by concluding that malt modification was the key outcome in malt flavour from the genotype x environment interaction. Figure 9 shows that the non-parametric regression plots for KI ($r = 0.679$, $P < 0.05$) and GPC ($r = -0.618$, $P < 0.05$) were correlated to the overall complexity of wort flavour. In this study, Stewart et al (2023) observed that Maris Otter had the highest overall wort flavour complexity and desirability as determined by a tasting panel while the Canadian grown AC Metcalfe and Chinese grown Gan Pi (both malted in China) scored the lowest for flavour complexity and desirability. Morrissy et al (2021) also concluded that there were positive benefits of the Maris Otter genotype for beer flavour. Accordingly, studies searching for terroir and environmental influences on malt flavour should pay heed to variation in protein modification (KI, FAN), given the relationships outlined above and links between the growing photoperiod, GPC (Figure 3A, 5, Table 1) and GPC and KI (Figure 6B, Table 3). In addition, the compositional differences in protein (Figure 2), with respect to potentially influencing KI should be considered.

Caution is needed when considering the malt KI quality parameter in isolation. A higher KI will increase FAN (Axcell 2018), so changes in wort colour and flavour are suggested outcomes (Herb et al. 2017a). Undoubtedly, higher levels of small peptides/amino acids (particularly lysine and glycine) would be expected to drive Maillard reactions during the conducive conditions found towards the end of kilning (higher temperature, lower moisture) (Ames 1988). Certainly, Table 4 shows a correlation between KI and FAN ($r = 0.440$ to 0.880, $P < 0.05$) but any relationship between wort colour and KI/FAN was unconvincing. However, sorting the data for genotype, environment and terroir (Figure 6B) may improve the strength of relationships. Overall, it would not be suprisiong if like grapes (Bramley et al. 2020; Brillante et al. 2020; Choné et al. 2001; Fernández-Marín et al. 2013; Pinu et al. 2018; Retallack and Burns 2016; van Leeuwen et al. 2018, 2020) and hops (Forster et al. 2014; Green 1997; Rodolfi et al. 2019; Staples et al. 2022; van Holle et al. 2017; van Simaeys et al. 2022a,b) that malt flavour was not influenced by terroir. Particularly, as van Leeuwn et al (2020) concludes, the soil terroir effects the aromas from grapes in wine which are primarily associated with water availability and nitrogen supply.

Figure 7.

Relationship between grain protein and (A) DP, (B) α-amylase and (C) limit dextrinase for malt produced from barley grown in Western Australia (Cunderdin 2013 and Walebing 2012/2014 sites) or Canada (Lacombe site 2013/14) using mainstream Australian (Buloke, Commander) and Canadian (Bentley, Meredith) barley varieties in the 2012 to 2014 growing seasons, * = P < 0.05 ** = P < 0.01, ns = not significant. Unpublished data from Luo et al (2019).

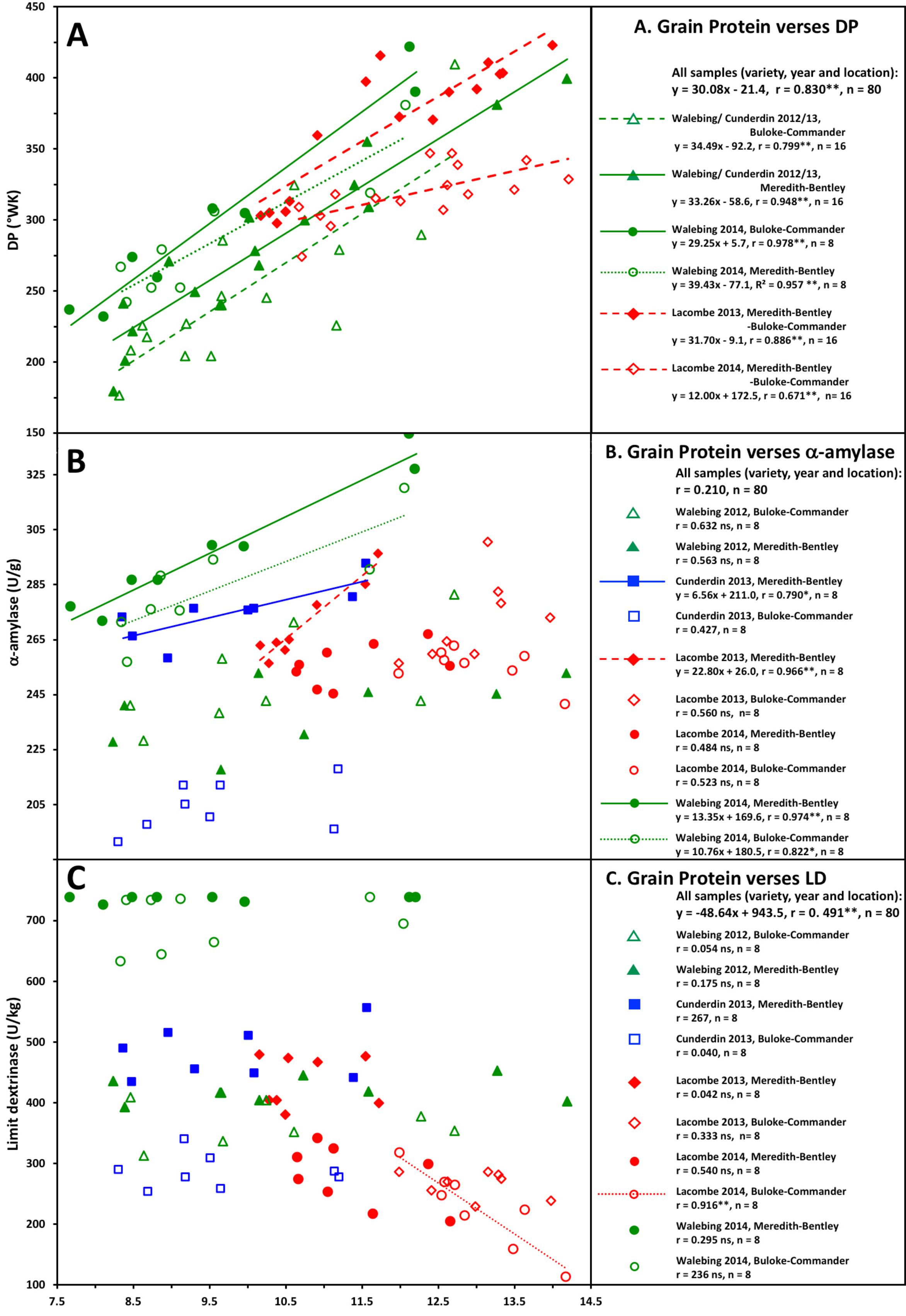
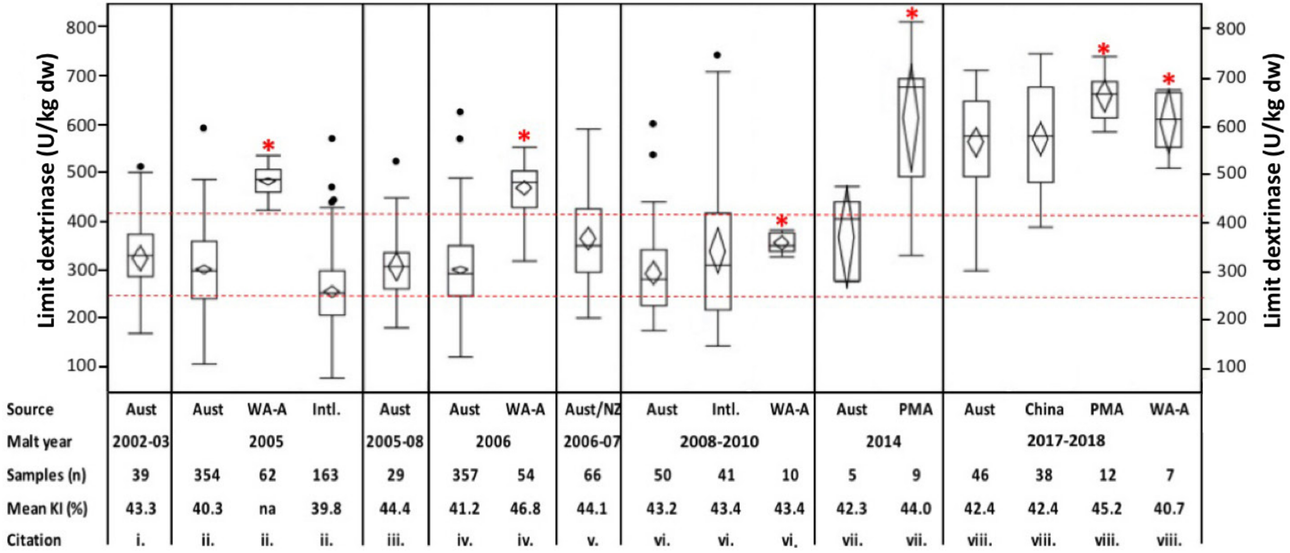


Figure 8.

Box-plot distributions over almost two decades of the levels of limit dextrinase in commercial malt samples. Horizontal dashed lines indicate range within which most data means were historically observed.
Data from Evans et al (2022).



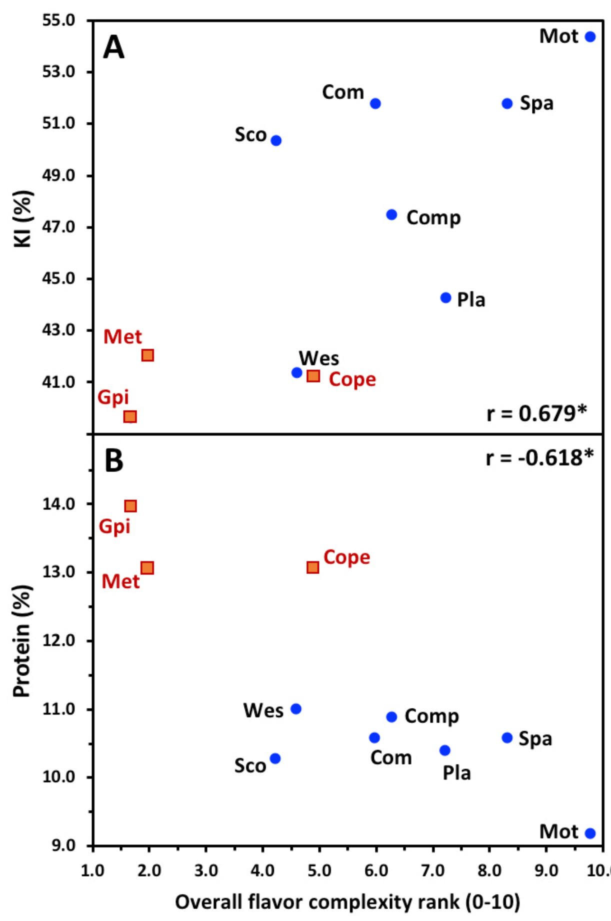
Malt Sources: Aust = Australia, WA-A = Western Australian malthouse with consistently high malt limit dextrinase (Evans et al, 2008a), Intl. = international malt samples from Europe and North America, PMA = Pilot Malting Australia (100 kg batch), China = Chinese malt from malting (primarily) Australian and Canadian barley. * denotes WA-A or PMA malt samples that have consistently high limit dextrinase levels.

Data from (i) Evans et al (2005), (ii) Evans et al (2008a), (iii) Evans et al (2011), (iv) Evans et al (2008a), (v) Evans and Finn 2008 unpublished (vi), Evans et al (2014b), (vii) Cooper et al (2016), (viii) Evans et al (2022).

Figure 9.

Scatterplots of ranking wort flavour complexity/intensity and (A) malt protein and (B) malt KI. Non-parametric correlations determined by Freidman's test (Spearman's correlation coefficient, r, * = P <0.05).

Malting barley varieties AC Metcalfe (Met) and CDC Copeland (Cop), were grown in Canada and malted in China, while Gan Pi was grown and malting in China. Compass (Comp), Commander (Com), Planet (Pla), Scope (Sco), Sparticus (Spa) and Westminster (Wes) were grown and malted in Australia, while Maris Otter (Mot) was grown and malted in the UK. Figure extracted from Stewart et al, (2023).



In recent years there has been an increasing interest in biotransformation, especially hop thiol and sulphur compounds that can enhance 'tropical fruit' flavours in beer (Chenot et al. 2023, Wallen et al. 2021). Candidates for potential enzyme include C-S lyase, β-lyase and β-glucosidase (glycoside cleaving). These enzymes originate from yeast and the substrates from hops. Currently there is excitement from the commercial yeast providers for a genetically modified 'thiolised' yeast that can enhance flavour biotransformation.

To date, malt has been neglected as a source of flavour contributing enzymes and substrates. However, some key flavour compounds have been identified in green malt, such as the glutathione S-conjugate of 3-sulfanylheptanol (Chenot et al. 2022) or, in unboiled wort, linalool and damascenone at lower levels than found in hops (Stewart et al. 2023). Significant variation in the level of β-glucosidase in malt has also been observed (Evans et al. 2022). Overall, the question of flavour substrates and the enzymes that release them may be another facet of terroir that would merit further investigation.

Foam stabilising proteins

Foam stabilising proteins from malt are quality constituents that most brewers like to increase to improve beer quality (Evans et al. 2009a). There is a caveat that there are some idiosyncrasies associated with beer style, and the gender or location of consumers (Bamforth 2000b; Roza et al. 2006; Smyth et al. 2002). The main foam positive proteins are protein Z4 (Z7), 'hordeins' and lipid transfer 1 (LTP1) (Evans et al. 2009a).

Foam stabilising proteins: lipid transfer protein (LTP1)

The determination of the level of lipid transfer protein (LTP1) in malt is not straight forward, as LTP1 does not have a simple association between barley genotype or protein content (Table 3, Evans et al. 2003). One insight is that barley grown in humid or wet environments has higher levels of LTP1. Table 5 shows that the level of LTP1 in barley grown in the dry Australian environment has substantially lower levels of LTP1 than barley grown in Mississippi (USA); a more humid environment. This observation was not surprising as there is a growing consensus that a putative biological function of LTP1 is as a plant defence protein (Douliez et al. 2000). It follows that in environments where barley is challenged by pathogens or insects, the level of LTP1 would be higher. While the selection of malt grown in humid environments with a high LTP1 would increase foam stability, this course of action may not be viable. Research has suggested that conditions that stimulate the accumulation of plant defence proteins will increase the level of LTP1, but also the level of lipoxygenase (de Almeida et al. 2005). Ironically, lipoxygenase produces foam damaging fatty acid hydroperoxides (Kobayashi et al. 1994; Kuroda et al. 2002; Schwarz and Pyler 1984; Yang et al. 1995). Accordingly, increasing LTP1 via growing barley in hot and humid environments may be self-defeating, particularly if too much of the lipid binding capacity of LTP1 is lost during boiling, thereby reducing foam stability (Evans et al. 2009a; Robinson and David 2008; van Nierop et al. 2004b).

Foam stabilising proteins - protein Z

Protein Z describes a small family of foam positive proteins termed serpins (Evans and Hejgaard 1999). The two primary isoforms of protein Z in barley are protein Z4 and protein Z7, where protein Z4 is generally (but not always) the dominant varietal isoform representing 80% of the total. In terms of amino acid sequence, protein Z4 and Z7, have been 70% similarity (Brandt et al. 1990; Rasmussen et al. 1996). Surprisingly, given this, Iimure et al (2012) reported that protein Z7 was associated with reduced foam stability. Whether this observation was functionally based or by association is yet to be determined.

The levels of protein Z4 can be expressed as high (>1000 µg/g malt), intermediate (200-1000 µg/g malt) and low (< 200 µg/g malt) (Table 6). Similarly, the levels of Protein Z7 can be subdivided into high (> 150 µg/g malt) and low (< 150 µg/g malt). These categories underline the necessity to consider malt samples by variety or, at least, similar varieties (Table 3). Interestingly, the level of protein Z4 appears to be coordinated (Evans unpublished data) with the thermostability the three allelic forms of barley *beta*-amylase - Sd2L (intermediate), Sd2H (high), or Sd1 (high, Eglinton et al. 1998). These insights suggest that intermediate levels of protein Z4 are associated with the Sd2L type of *beta*-amylase

Table 5.

Comparison of LTP1 in malt made from barley grown in Australia (1997) and grown in Mississippi (1996).

[1]Includes breeder's lines, Morex, Robust, Stander and Excel. Malt provided by Dr Berne Jones, USDA, Madison WI. Determined using ELISA (Evans and Hejgaard 1999) and reproduced from Evans and Bamforth (2009a) with permission.

	LTP1 (µg/g)	
Statistic	**Australia**	**Mississippi, USA[1]**
Mean	434	1082
σ	140	132
minimum	230	796
maximum	898	1315
n	169	39

Table 6.

Summary of protein Z4 and Z7 categories for malt from a selection of varieties surveyed in 1998, 1999 and 2000 (Protein Z4 and Z7).

Variety from [A] Australia, [E] Europe, [U] USA, [C] Canada, [J] Japan. Determined using ELISA (Evans and Hejgaard 1999) and reproduced from Evans and Bamforth (2009a) with permission.

High protein Z4 (>1000 µg/g malt)		High protein Z7 (>150 µg/g malt)	
Alexis[E]	Grimmett[A]	Arapiles[A]	Moravian III[U]
Arapiles[A]	Harrington[C]	Barque[A]	Morex[U]
Barque[A]	Lindwall[A]	Bowman[U]	Parwan[A]
Bonanza[U]	Picola[A]	Chebec[A]	Pirkka[E]
Bowman	Sloop[A]	Franklin[A]	Sloop[A]
Caminant[E]	Tallon[A]	Grimmett[A]	Stirling[A]
Chariot[E]	Unicorn[J]	Harrington[C]	Unicorn[J]
Gairdner[A]		Karl[U]	

Intermediate protein Z4 (200-1000 µg/g malt)	
Chebec[A]	Schooner[A]
Fitzgerald[A]	Stirling[A]
Parwan[A]	

Low protein Z4 (<200 µg/g malt)	
Karl[U]	Morex[U]
Moravian III[U]	Pirkka[E]

Low protein Z7 (<150 µg/g malt)	
Alexis	Gairdner[A]
Barque[A]	Lindwall[A]
Bonanza[U]	Picola[A]
Caminant[E]	Schooner[A]
Chariot[E]	Tallon[A]
Fitzgerald[A]	

while high levels of protein Z4 are associated with Sd2H and Sd1. Exceptions are descendants of Morex (Sd1, USA) and Pirkka (Sd1, Finland) that have low levels of protein Z4 (Evans unpublished data). However, malt from these low protein Z4 varieties does not necessarily produce beer with poor foam (Gibson et al. 1996).

It has been well established that an increase in GPC within a variety increases protein Z (Evans and Hejgaard 1999; Giese et al. 1984). Table 3 shows that there was generally an acceptable correlation between malt protein Z4 and Z7, and GPC when correlated within individual varieties (Table 6). Given the durability of protein Z4 and Z7 in both malting (proteolysis) and brewing (boiling) it was not surprising that Evans et al (1999) found that the level of protein Z was little influenced by modification during malting. Accordingly, it would be expected that terroir could influence the level of protein Z via the influence on GPC.

Hordeins (Shewry 1993) have so far proved to be elusive for foam improvement as only one 17 kDa hordein (epsilon-1-hordein) has been linked with the stability of beer foam (Evans et al. 2009a; Vaag et al. 1999; Vaag et al. 2000). Overall, hordeins are the dominant barley protein (>50% GPC) and the primary target of proteases during malting (Baxter 1976; Folkes and Yemm 1958). Hordein fragments have been found to partition and concentrate in beer foam using techniques including foam collection, foam concentration towers or sequential re-foaming (Evans et al. 2003; Hao et al. 2006; Sheehan and Skerritt 1997; Sorensen et al. 1993). However, being composed of high levels of proline (and glutamine) (Asano et al. 1982; Pollock et al. 1959), the downside of hordeins is their role in forming undesirable hazes in beer (Siebert et al. 1996).

In addition to the positive influence of foam positive proteins, malt with a higher KI (Kolbach Index) is associated with reduced foam stability (Back et al. 1997; Evans et al. 1999). This inverse relationship with KI is mediated through the proteolysis of hordeins (during malting) (Baxter 1976; Evans 2021) as LTP1 and protein Z4 are largely intact after malting and brewing (Evans et al. 1999). Indeed, Evans et al (2009a) suggested that for each percentage point increase in KI resulted in a decrease of one second in Rudin head retention value. However, the method used to measure foam stability is important, as each is biased towards specific contributors to foam stability; in the case of Rudin, foam positive proteins (Evans et al. 2008b). Bamforth (1999) sagely commented that 'perhaps the biggest need is for a decent method for assessing foam quality. Many methods have been suggested (a sure sign that none of them approach perfection) but none is universally accepted as a yardstick of foaming performance.' As such, the consumer impact of malt KI on foam stability needs to be compared with foam assessment by a 'natural' method akin to on-trade dispense (Evans et al. 2012; Kosin et al. 2012; Viejo et al. 2018).

Hordein content increases with higher GPC (Lancombe grown, Figure 2), like protein Z4, Z7 and *beta*-amylase (Kirkman et al. 1982; Luo et al. 2019; Qi et al. 2006). However, the impact of protein modification during malting has obscured the identity of any contribution from hordein to foam stability. Thus, although there would possibly be an influence of terroir on hordein content and composition, outcomes in terms of improvement in beer foam stability are not clear.

Grain filling temperatures – starch formation

Starch content (50-60% of barley) is an important component of malt quality (Bamforth and Fox 2020; Fox 2018; Kessler et al. 2008; Perez and Bertoft 2010; Zhu 2017) but is not routinely assessed. Starch is comprised of two polymers of glucose; amylose (less branched) and amylopectin (more branched) (Bamforth and Fox 2020).

These polymers are packed into starch granules to form growth rings of alternating crystalline and amorphous lamellae. In wheat starch, granules include small amounts of protein (0.2%) and lipid (1%) (Schofield et al. 1987; Skerritt et al. 1990) either on the surface of the granule or internally. It follows that higher levels of starch associated hordein would affect malt friability (Bamforth and Fox 2020). Starch granule composition includes small amounts of proteins including B/D hordeins (Boren et al. 2004; Slack et al. 1979), hordoindoline (puroindoline/grain softness protein wheat) (Dubreil et al. 1998; Douliez et al. 2000; Bloch et al. 2001; Evans et al. 2002), protein Z4, and soluble/granule bound starch synthases I and II. There is an added degree of complexity in that the small (20-25% by weight) and large starch granules have different gelatinisation properties during mashing (de Schepper et al. 2020; Langenaeken et al. 2019). Barley variety, genetics, and environmental conditions have been shown to alter the ratio of amylose: amylopectin, the degree of branching and branch length for amylose/amylopectin, lipid association, the ratio of small to large granules and the degree of starch granule - protein association (Bamforth and Fox 2020; Gous et al. 2014; Kessler et al. 2008; Oliveira et al. 1994; Tester et al. 1991; Zhu 2017).

Environmental and varietal starch variation manifests itself practically in changes in starch gelatinisation temperature ('*Tp*' temperature of peak gelatinisation determined by Densitometric Scanning Calorimetry), that can carry over during the mashing of undegraded small starch granules. Undegraded small starch granules combine with

undigested protein to reduce lautering efficiency (Barrett et al. 1973). In general, malt starch has a *Tp* between 59-64°C (Bamforth 2003; Evans et al. 2005). Lipid associated starch has been reported to decrease *Tp* (lower lipid) (Myllarinen et al. 1998), or increase *Tp* (higher lipid) (Ao and Jane 2007). It has also been shown that small starch granules (62.8°C) have a higher *Tp* than large starch granules (59.6°C) (de Schepper et al. 2022), while the ratio of amylose:amylopectin can influence *Tp* (Gous et al. 2014; Källman et al. 2015). Previous investigators have reported a higher *Tp* (75-80.0°C) for small granules (Palmer 1989). What appears to be critical in terms of starch hydrolysis during mashing is that the gelatinisation temperature for starch remains below 65°C, the optimum temperature starch gelatinisation and combined DP (diastatic power) enzyme action (Evans et al. 2005; Evans 2021).

When growing conditions result in starch gelatinisation temperatures greater than 65°C, wort is produced with an undesirably low attenuation (Bekkers et al. 2007; Stenholm et al. 1996a; Stenholm et al. 1996b; Stenholm et al. 1998). This is not surprising since *beta*-amylase and limit dextrinase are relatively thermostable up to about 65°C but their activity rapidly declines as the mash temperature increase over 70°C, leaving starch hydrolysis to α-amylase (Evans et al. 2005; Evans et al. 2017). The optimal balance between starch gelatinisation and thermostability of the DP enzymes is at a typical commercial mash thicknesses (1:2.5 to 1:4, grist:water ratios) (Evans 2021b). Where starch gelatinisation temperature increases above 65°C, the enzyme balance is pushed in favour of the more thermostable α-amylase, potentially altering the glucose:maltose ratio of the wort. Any change in this ratio will influence yeast ester metabolism and impact on beer flavour (Verstrepen et al. 2003). Of course, knowledgeable brewers, knowing the properties of their malt, would select mashing temperature protocols to modify their beers flavour but also the mouthfeel by adjusting the level of residual dextrins (Langstaff and Lewis 1993; Ragot et al. 1989; Rubsam et al. 2013).

Growth temperature (and location) appears to be the key determinant of starch quality and *Tp* (Fox 2019; Hawker and Jenner 1993; Myllarinen et al. 1998; Savin et al. 1997; Wallwork 1997). In an investigation with a controlled environment, the 'normal' temperatures were 21°C day/16°C night with the experimental treatment of 35°C day/25°C night (Hawker et al. 1993; Savin et al. 1997; Wallwork 1997). In addition, Gous et al (2014) observed that crop moisture (drought) and increased nitrogen supply were implicated in producing a shorter branch length for amylopectin and longer branch length of amylose related in increases in *Tp*. Hot conditions during the barley growing season reduce the level of soluble starch synthase enzyme. This key synthetic enzyme has also been implicated in either increasing or decreasing the starch *Tp* in rice (Miura et al. 2018; Waters et al. 2006). In the wheat and barley endosperm, heat treatment resulted in greater number of small starch granules (Gous et al. 2014; Hurkman et al. 2003; Wallwork et al. 1997). It was concluded that heat treatment had a small influence on protein content, while the impact on soluble starch synthase resulted in less starch and elevated levels of protein and DP. This is agreement with van Herwaarden et al. (1998a) with regard to 'haying off'.

The key question is what role terroir might have in barley quality with respect to starch? As noted, the growing environment can impact the amylose: amylopectin ratio, the degree of branching and branch length, lipid association, small to large granules and starch granule-protein association (Bamforth and Fox 2020). All these variations may impact the starch *Tp*. Higher temperature growth conditions, as found in drought situations, impact on brewing efficiency by reducing wort fermentability (Bekkers et al. 2007; Stenholm et al. 1996a; Stenholm et al. 1996b; Stenholm et al. 1998) and by changing the ration of glucose:maltose ratio in fermentation (Verstrepen et al. 2003). As drought is usually a transitory weather condition, it is not a consistent influence on barley quality, so variations in starch quality would not be covered by 'terroir'. However, knowledge of the impact of temperature on starch quality should be recognised with respect to the annual barley intake to produce consistent malt quality.

Micronutrients

Ionic micronutrients (cations and anions) and the pH of brewing water are important practical factors in brewing. It is recognised that, in certain regions, the ionic ground water composition is fundamental to the style of beer produced (Table 7). For instance,

the pilsner produced with the soft water found in Pilsen (Czech Republic) whereas, in contrast, the English Pale Ale is made with hard water (rich in calcium and magnesium sulphate) from Burton-on-Trent (UK). These days brewers treat their water using a variety of treatments and processes such as lime precipitation, filtration, reverse osmosis, ion exchange and activated carbon filtration (Eumann and Schildbach 2012). The required salts can then be added back to the brewing liquor according to the beer style to be brewed. An extended discussion of the contribution of brewing water to beer production and quality can be found in Palmer (2018) and Evans (2021b).

Not only does the availability of water and geography influence grain quality, but so does the anions and cations in the soil in terms of deficiencies or toxicities of key micronutrients (Alloway et al. 2008; Welch and Graham 2002). Examples include toxicity of manganese (Fernando and Lynch 2015) and boron (Brennan and Adcock 2004; Cartwright et al. 1986) or deficiencies in iron, zinc, molybedum, manganese, copper and/or cobalt (Alloway et al. 2008). Other micronutrients such as potassium impact on barley production, and interact with variety and presumably are reflect terroir (Azzawi et al. 2021). Of course, genetic manipulation of crops can alleviate such toxicity, for instance boron (Hayes et al. 2015). It has also been proposed that crops could be bred with enhanced micronutrient content so as to augment human nutrition (Welch and Graham 2002). Similarly, consideration should be given to the levels of vitamins E and B (Do et al. 2015; Farag et al. 2022; Gupta et al. 2010; Loskutov and Khlestkina 2021). Such differences in micronutrient and vitamin composition may have consequences for beer quality, yeast performance and human nutrition. With regard to pH, Bamforth (2001) reminds us that the net pH is the result of the inherent pH of the grist and the chemical changes and interactions that take place during mashing. Briggs et al (2004) provide a useful summary of the range of cations (aluminium to zinc) and anions (chloride to sulphate) in beer. There is little information on the impact of variations in malt on ionic micronutrients, pH and vitamins on the quality of the beer. However, malt provides between a quarter (mash) to 10% (beer) of the total weight with the balance primarily water. It stands to reason those malts from different genetic backgrounds, grown in different environments (soil, season) and, accordingly, terroir will influence beer in terms of cations and anions.

Given the importance of ionic micronutrients in the production and quality of beer, it is ironic there are few studies on barley (Farag et al. 2022; Loskutov and Khlestkina 2021; Turner et al. 2022; Wietstock et al. 2015) or wheat (Dikeman et al. 1982; Peterson et al. 1983). Justus (2017) investigated the influence of location on the anion and cation composition of wort (Table 8). Long et al (1999) reported small scale mashes made with malt from Canada, England and Germany with comprehensive analysis of ionic micronutrients for the same barley variety

Table 7.

Ionic composition of water from different brewing locations.

Component (mg/L)	Burton-on-Trent (UK)	London (UK)	Pilsen (Czech Rep)	Dublin (Ireland)	Munich (Germany)	Vienna (Austria)
Calcium	352	52	7	119	80	200
Magnesium	24	32	8	4	19	60
Sodium	54	86	3	12	?	8
Sulphate	820	32	6	54	6	125
Chloride	16	34	5	19	1	12
Bicarbonate	320	104	37	319	333	120
beer style	English Pale	British	Pilsner	Stout	Dunkel,	Vienna

From Bamforth (2006) and Palmer (2006) - reproduced with permission from Evans (2021).

(Schooner) over a range of growing sites, nutrient fertilisation and (two) growing years (Table 9). In these samples, the level of Ca^{2+} (average 345 mg/kg) is of note as being less than half of that observed for samples from North America (500-800 mg/kg) (Turner et al. 2022, Table 10). This begs the question - is the difference in Ca^{2+} evidence or a 'smoking gun' for differences related to terroir?

A key question is how much of these micronutrients survive the malting and brewing process and influence efficiency and beer quality? For instance, for yeast growth and maintenance, Hammond (2000) outlines the roles of vitamins (biotin, pantothenate, inositol), anions (SO_4^{2-}, PO_4^{3-}) and cations (Zn^+, Mn^{2+}, Mg^{2+}, Ca^{2+}, $Cu^{+/2+}$, K^+, $Fe^{2+/3+}$).

Ca^{2+} stimulates yeast growth but its main contribution is to yeast flocculation (Speers et al. 1992). In mashing, the role of Ca^{2+} includes removing oxalic acid as calcium oxalate (beer stone) and in the thermostability of the dominant α-amylase II enzyme (Bertoft et al. 1984). Zn^+ is arguably the most important wort cation as a key yeast enzyme co-factor in multiple catalytic centres with levels below 0.1 mg/L resulting in slow fermentation and above 0.6 mg/L depressing yeast growth, however an excess can be ameliorated by higher concentrations of Mn^{2+} (Hammond, 2000).

Others micronutrients that are important for beer flavour and staling are low levels of transition metals such as Fe, Cu and Mn (Mertens et al. 2022). Finally, from a flavour perspective, Mg^{2+} has been observed to accelerate Maillard reactions during brewing (Omari et al. 2020). Not only are ionic micronutrients important in isolation but there is the potential for synergism and antagonism between them. Correspondingly, with respect to plant growth under waterlogging or salinity stress, the interaction between Ca^{2+}, Zn^+, Mn^{2+} and $Fe^{2+/3+}$ substantially influence plant growth (Dittmann et al. 2022; Mckee and McKevlin 1993).

The level of ionic micronutrients and vitamins in malt and barley is only half of the brewing equation. Equally important is their release and retention in wort and beer to the point at which their levels impact beer production and quality. As a guide, Wietsock et al (2015) observed that under brewing conditions, of Ca^{2+} was lost in the spent grain (86.3%), in the whirlpool (6.7%) and during fermentation (0.6%). Similarly, 86.2% of Zn^+ was lost with the spent grain, 3.9% in the whirlpool, 9.5% during fermentation. Regrettably, there has not been a similar survey on the fate of malt vitamins during brewing. Overall, Wietsock et al (2015) concluded that, 'malt was found to be the biggest source of metal ion uptake considering the amount used for

Table 8.

The potential influence of the geographic source of malt on anion and cation composition of wort.

	Canada	England	Germany	Wheat	Raw Barley
Wort gravity (°P)	10.1	10.6	10.6	9.5	2.6
SO_4^{2-} (mg/L)	110.0	45.0	96.0	54.0	27.0
Cl^- (mg/L)	210.0	200.0	190.0	160.0	90.0
Ca^{2+} (mg/L)	24.7	24.0	25.0	19.2	18.8
Mg^{2+} (mg/L)	68.8	63.4	75.7	71.6	49.1
Na^+ (mg/L)	40.6	7.2	23.6	10.7	10.8

Mashing with malt (200g) fine ground and steeped with 1200 mL water at 63.8-65.6°C in an insulated, closable flask (thermos) for 60 minutes before mash separation. Reproduced with permission from Justus (2017).

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Table 9.

Ionic composition of Schooner barley grain grown in South Australian trials in 1997/98.

			Barley ionic components (average ± SD) measured by Inductively Coupled Plasma spectroscopy (ICP)												
Trial site	Trial Year	Fertilizer Treatment	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)	S (mg/kg)	Mo (mg/kg)	B (mg/kg)
Sandalwood	1997	P 0-24	55 ± 9	10.6 ± 0.7	1.6 ± 0.2	14.5 ± 0.9	300 ± 6	1000 ± 64	165 ± 20	4071 ± 174	1984 ± 287	1296 ± 76	< 1.1	< 1.8
Paruna	1997	P 0-24	45 ± 5	11.7 ± 0.4	3.1 ± 0.7	15.7 ± 1.9	281 ± 23	1034 ± 45	156 ± 23	3988 ± 261	1940 ± 283	1528 ± 120	< 1.1	< 2.0
Callington	1997	Zn 0-15	51 ± 1	20.4 ± 0.7	3.8 ± 0.1	21.3 ± 1.3	361 ± 16	1174 ± 16	189 ± 12	4071 ± 183	3413 ± 461	1545 ± 26	< 1.2	< 1.9
Marion Bay	1997	Mn 0-35, +/+Mn	40 ± 10	7.5 ± 2.5	2.9 ± 0.5	31.8 ± 7.5	399 ± 41	1170 ± 34	467 ± 109	4200 ± 143	2448 ± 89	1445 ± 84	4.4 ± *02*	< 2.2
Lameroo	1997	Zn 0-15, +/-Mn	67 ± 3	16.1 ± 0.7	6.9 ± 0.2	23.6 ± 4.1	335 ± 19	1591 ± 49	215 ± 52	6256 ± 109	5264 ± 108	2081 ± 25	5.2 ±*0.2*	3.8 ± *0.6*
Geranium	1998	N 0-50, S 0-50	47 ± 3	12.5 ± 1.4	1.7 ± 0.2	24.2 ± 3.5	302 ± 19	1388 ± 25	162 ± 17	4422 ± 48	3781 ± 90	1381 ± 54	< 1.1	< 4.0
Charlick	1998	N 0-50, S 0-50	33 ± 2	8.7 ± 0.3	3.6 ± 0.1	18.7 ± 0.8	435 ± 11	1190 ± 25	324 ± 8	4471 ± 164	2904 ± 108	1487 ± 72	< 1.1	< 1.9

Reproduced with permission from from Long et al (1999, 2001). Treatments with four replicates at each site with watering by rainfall. Metal cations were analysed by ICP (Rengel et al. 1999). Additional cations were detected towards the ICP's effective limit of detection, Co (<1.1 mg/kg), Ni (<1.7 mg/kg), Al (<7.0 mg/kg) and Cd (<0.48 mg/kg)

Table 10.

Malt quality and ionic composition compared with beer characteristics related to beer foam stability.

	Beer Quality Characteristics			Malt Protein Characteristics					Malt Ionic Composition[4]										
Variety[1]	HRV (sec)	Protein (μg/ml)[2]	Viscosity (mPa.s)	Protein (%)[3]	KI (%)	Prot Z4 (μg/g)[3]	Prot Z7 (μg/g)[3]	LTP1 (μg/g)[3]	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Al (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)	S (mg/kg)
Morex (NAm)	111.2	156	1.359	12.7	43.2	72	421	514	40	17.3	4.9	17.7	830	1330	15.3	44	2200	3700	1250
Harrington (NAm)	110.0	232	1.358	12.2	38.8	1569	249	554	49	12.8	4.7	22.2	520	1300	10.6	31	2500	3100	1450
Harrington (WA)	104.0	190	1.380	10.0	46.6	1184	223	442	47	16.2	2.4	17.4	380	1120	6.5	90	3200	2800	1330
Gairdner (SA)	102.3	196	1.373	10.5	41.3	1333	114	367	45	13.2	2.4	14.7	370	1180	6.7	155	3700	2900	1450
Fitzgerald (SA)	101.0	158	1.342	10.6	46.5	678	111	323	40	12.1	2.0	14.1	330	1110	6.6	152	3500	2700	1520
WI 3102 (SA/MM)	108.2	180	1.394	11.0	49.0	600	140	334	34	6.7	5.2	20.1	480	1290	8.8	123	2900	3600	1230
Franklin (SA)	107.0	196	1.362	10.7	44.3	1200	276	333	39	11.1	3.5	18.4	360	1210	6.5	92	3600	3500	1190
Chebec (SA)	109.1	228	1.382	10.9	42.6	566	96	408	42	14.3	4.9	21.8	390	1270	9.6	128	3900	3500	1370
Sloop (SA)	104.8	214	1.366	10.5	48.1	1270	101	481	43	13.6	4.6	20.0	480	1190	6.7	124	3700	3300	1380
Schooner (SA)	105.7	268	1.348	10.1	45.5	752	157	392	38	13.2	4.6	16.4	450	1200	9.2	130	3600	3200	1170
Arapiles (SA)	101.4	224	1.354	9.6	49.4	1035	96	380	36	12.7	3.6	16.3	310	1140	6.7	119	3400	3100	1130
Average	**105.9**	**204**	**1.365**	**10.8**	**45.0**	**933**	**180**	**412**	**41**	**13.0**	**3.9**	**18.1**	**445**	**1213**	**8.5**	**108**	**3291**	**3218**	**1315**
Minimum	101.0	156	1.342	9.6	38.8	72	96	323	34	6.7	2.0	14.1	310	1110	6.5	31	2200	2700	1130
Maximum	111.2	268	1.394	12.7	49.4	1569	421	554	49	17.3	5.2	22.2	830	1330	15.3	155	3900	3700	1520

Addition of exogenous cell wall degrading enzymes (Bio-Glucanase® and Bio-Cellulase®) to a small-scale mash. Unpublished data and data summarised in Evans and Sheehan, (2002), reproduced with permission.
[1] Variety and malt origin (malting year 1997), NAm = North America, SA = South Australia, SA/MM = South Australia micro-malted, WA = Western Australia. [2] Bradford assay, BSA as standard (Bradford, 1976). [3] Dry weight basis.
[4] Metal cations were analysed by ICP (Rengel et al. 1999).

brewing.' Brewers of course make additions of salts to condition their brewing liquor, typically Ca^{2+} at 40-70 mg/L (Evans 2021). The observations of Wietsock et al (2015) compared to the contribution of brewers salts with average potential malt contributions of Ca^{2+} and Zn^{+} are reported in Table 11. Overall, an average Australian malt would make a contribution similar to that of adding liquor salts with a level of Zn^{+} (0.35 mg/L) within the 0.1-0.6 mg/L range recommended by Hammond (2000).

Hughes and Simpson (1995) proposed that metal cations such as Mn^{2+}, Al^{3+} and Ni^{2+} effectively cross-link iso-α-acids to strengthen the foam bubble film. This is explored In Tables 10 and 11 wherre modified commercial malts and one micro-malt from Australia, together with eight from the USA, were used in a small-scale study on Rudin beer foam stability (Bishop et al. 1975). Inductively coupled plasma spectroscopy (Rengel et al. 1999) was used for elemental analysis of the malts. Table 10 shows that there was a wide range of metal contents in malt. It was assumed that the greater proportion of these metals would be in their ionic form. To

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remove the influence of beer viscosity on foam analysis the brews were treated with Bio-Glucanase® and Bio-Cellulase®

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Regression analysis of small-scale beers (Table 10) showed significant correlations between Rudin HRV and the malt content of Cu, Zn, Ca, Mg, P and Al, along with negative correlations with Na and K (Table 12). A strong positive correlation was also observed for malt protein content. The content of the ionic elements in beer (Table 10), shows that the level of elements was substantially lower than for malt due to losses during brewing (Wietstock et al. 2015) and the mashing of 142.5g of grist to produce ca. 700ml of wort. Surprisingly, the correlation was not observed with the ionic elements in beer (data not shown), which suggests that the relationship between foam stability and malt metal content may not have been a result of improvements in iso-α-acid cross-linking or, alternatively, perhaps an artefact of the sensitivity ICP analysis.

The concemtraion of metal elements has been shown to influence the accumulation of protein in cereals (Dikeman et al. 1982; Peterson et al. 1983; Rengel et al. 1999). The Rudin foam stability of the beers did correlate well correlated with the content of protein Z4, protein Z7 and LTP1 (Table 12) in the malt. It is suggested that barley metal nutrition may influence malt protein content, composition (hordeins) or some other unidentified factor. Certainly, the strong correlation between HRV and barley protein content (Table 12) would support this. However, a later repeat of this experiment using different malt samples did not show a strong relationship between ionic elements and Rudin foam stability (data not shown). Accordingly, these results make the case for further investigation to clarify (or not) any relationships..

In combination, the content of vitamins and ionic micronutrients in malt has been neglected in brewing research. This review highlights the contribution of malt to levels of vitamins and ionic micronutrients in wort and beer. It would be anticipated that future studies will identify substantive variation in the levels of these micronutrients that could reflect the impact of terroir.

Table 11.

Cation levels from mashing to cold wort (Evans, unpublished data).

					100% utilisation	
	Total Volume	**Brew outcomes**		**potential**	**Ca mg/L**	**Zn mg/L**
Mashing 3:1 (water:grist)	3 L mash	3.05 L (+malt moisture)			113.1	7.02
Typical Ca add to mash liquor					40-70 mg/L	
Typical wort Zn levels[1]						0.1-0.6 mg/L
After mash separation (1:8)	8 L wort+SG	6.4 L wort	8.8°P	3.8% ABV	43.1	2.68
					Cold wort	
After mash separation (1:6)	6 L wort+SG	4.8 L wort	11.7°P	5.1 % ABV	56.6	3.51
Real attenuation adjust (0.81)				*4.1 % ABV*		
Cation levels after process losses[2]					**Brewing losses considered**	
Ca in beer	*losses: 86.3% Spent Grain, 6.7% Whirlpool, 0.6% ferment*				3.6	
Zn in fermentable wort	*Losses: 86.2% Spent Grain, 3.9% Whirlpool*					0.35
Based Modified IoB mash	50g grist, (g:w 1:8 final)		320ml wort	8.8°P	3.8% ABV	

Malt has 80% fine grind extract, moderate fermentability, with ~345 mg/kg Ca and ~21.4 mg/kg Zn (kg/grist).

[1] Zn range wort low to excessive, but excess can be ameliorated by higher concentrations of Mn (Hammond, 2000).
[2] Cation losses based on Weitstock et al. (2015) and discussions with Daniel Carey, New Glarus. Brewing, WI. SG = spent grains, alc = alcohol.

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Table 12.

The influence of metals on beer foam stability with the addition of exogenous cell wall degrading enzymes (Bio-Glucanase® and Bio-Cellulase®) to a small-scale brewing procedure mash.

Characteristic	Correlation coefficient (r)[1]
Beer:	**HRV (sec)**
Protein (µg/ml)	0.03
Viscosity (mPa.s)	0.30
Malt:	
Protein (%)	0.81**
KI (%)	-0.46
Protein Z4 (µ g/g)	-0.33
Protein Z7 (µ g/g)	0.63*
LTP1 (µ g/g)	0.53
Fe (mg/kg)	0.07
Mn (mg/kg)	0.83**
Cu (mg/kg)	0.79**
Zn (mg/kg)	0.75**
Ca (mg/kg)	0.73*
Mg (mg/kg)	0.93**
Al (mg/kg)	0.80**
Na (mg/kg)	-0.70*
K (mg/kg)	-0.58*
P (mg/kg)	0.78**
S (mg/kg)	-0.15

From Evans and Sheehan (2002) published with permission. Beer and malt defined in Table 10.
[1] * = P < 0.05, ** = P < 0.01, Spearman rank correlation, n = 11. HRV = Rudin Head Retention Value.

Microorganisms

The microbiota of barley and malt are - in the words of Shakespeare's Hamlet - an 'undiscovered country'. Petters et al (1988) showed that the viable counts (colony forming units) on agar plates of bacteria and fungi on the barley grain increase exponentially with the addition of water and favourable temperatures for microbial growth during steeping and germination. As would be expected, kilning results in a substantial reduction in the viable count of microorganisms.

According to Flannigan (2003), barley provides an ecological niche (especially the husk) for a diverse range of microorganisms, with the microbiota of different barleys are similar to each other and to other cereals. Barley microbiota at harvest has been found to comprise the same limited number of microbial species. Studies of (culturable) fungi associated with South African malt showed that *Alternaria* were the most prevalent species and the same as those found elsewhere in the world; however, the counts of these fungal taxa, especially *Fusarium* and *Penicillium* species, were significantly lower than those reported in the Northern Hemisphere (Ackermann 1998; Rabie and LüBben 1993). Other microbial surveys of barley or malt have been reported for the USA (Follstad and Christensen 1962; Prentice and Sloey 1960), Sweden (Gyllang and Martinson 1976), the UK (Petters et al. 1988), Canada (Turkington et al. 2002) and Spain (Medina et al. 2006). The microbial community structure of barley malt can be influenced by other factors including growing location (Birgitte et al. 1996), climatic conditions (Backhouse and Burgess 2002; Doohan et al. 2003, Krstanović et al. 2005), malting techniques (Flannigan et al. 1982), and storage and handling environments (Hill and Lacey 1983; Laitila et al. 2003). However, as ever, the techniques for detection and enumeration of microorganisms may result in different conclusions (Jarvis and Williams 1987: Rabie et al. 1997), especially as some taxa may be unculturable, quiescent or require specific cultural conditions.

Kaur et al (2015) used terminal restriction fragment length polymorphism (TRFLP) fingerprinting and clone library analyses of ribosomal RNA genes which is not culture dependant. Figure 10 shows the canonical analysis of principal coordinates (CAP) to survey the microbiota of malts from locations in both the Southern and Northern hemispheres. This analysis shows distinctly different groupings between the individual malt samples. The Australian malt grouping have some association with the other Southern hemisphere growing areas of South Africa and Argentina being to the left side of the plot, although those samples are generally lower with respect to the vertical dimension compared to Australia. The circled samples from Finland (four samples), South Africa (one sample) and North America (one sample) were deliberately inoculated with *Fusarium* and group towards the left of the plot. The French, Danish and Belgian samples tend locate in the centre vertically but on the bottom compared to the Slovakian and Russian samples that group into the right and upper quadrant. Overall,

Figure 10.

Canonical analysis of principal coordinates (CAP) of fungal TRFLP peak data from malt samples combined based on a Bray Curtis similarity matrix grouped by geographical location of the samples.

Legends with circles around them represent DON and OTA inoculated Finnish malts, a standard gushing malt sample from South Africa, and a Fusarium head blight infected North American malt sample. Vector overlays indicate Pearson's correlations between the ordination axes and individual taxa (only taxa with a >0.55 correlation are shown). Figure from Kaur et al (2015).

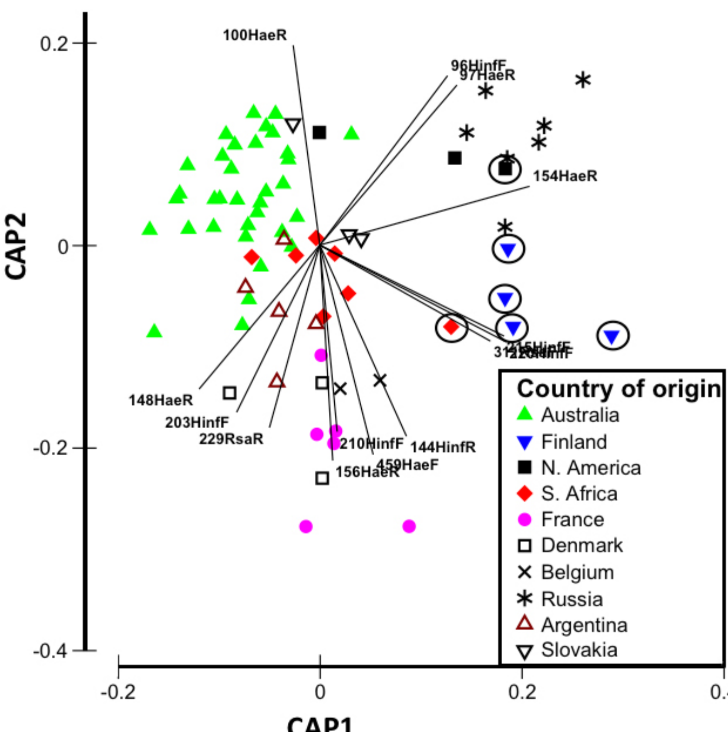

Figure 10 suggests that differences in location and presumably climatic conditions influence the diversity of malt microbiota which may be linked with terroir.

In grapes, terroir influences wine quality by the action of grape borne yeasts and bacteria contributing to fermentation (Belda et al. 2017; Bokulich et al. 2014, 2016; Pretorius 2020). In contrast, viable microorganisms contributed by malt or barley are reduced during kilning and mashing and killed during wort boiling. Therefore barley/malt microorganisms make their contribution during malting and mashing via the contribution of active enzymes, micronutrients or barley degradation products. The dry malt or barley in store has too low a moisture content (14% lower limit, Flannigan et al. 2003) to sustain microbial growth. Laitila (2008) contends that the contribution of barley/malt microbiota is 'more good than bad.' For example, the positive contribution of microbes to malting and brewing include plant growth regulators that enhance germination (gibberellin), vitamins, antioxidants and enzymes (proteases, amylases, β-glucanases, xylanases). Conversely, the potential negative impacts of microorganisms include inhibition of barley germination and products that impact wort quality including organic acids (pH), exopolysaccarides (wort separation, beer filtration), factors causing premature yeast flocculation (PYF), gushing factors that cause beer over-foaming, allergens and toxic metabolites (mycotoxins).

Flannigan (2003) underlined the importance of achieving a grain moisture content of 14% to limit the production of mycotoxins such as ochratoxin A that accumulates through the growth of moulds such as *Aspergillus* and *Penicillium*. In several northern hemisphere growing regions harvest conditions are humid such that barley moisture contents of 14% are common. Should barley moisture content exceed 14%, then grain dryers are required. However, this process dries the grain to control mould growth but can also impact on grain germinability and dormancy (Bishop 1944; Jayas and Ghosh 2006). In contrast, the lead up to harvest in Australia is typically hot and dry such that grain moisture contents are in the range 9-11.5%, whereas in Tasmania, the moderate conditions result in higher grain moisture levels (Evans et al. 2014b; Woonton et al. 2005). Given this, the Australian malting industry has yet to detect ochratoxin A in routine quality assurance testing (Kaur et al. 2009).

The barley fungi that are best known are species of *Fusarium* (Schwarz et al. 2003), often being manifest as '*Fusarium* head blight/head scab.' This is undesirable as these filamentous fungi produce trichothecene mycotoxins (such as deoxynivalenol or DON) and gushing factors such as hydrophobins.

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In terms of mycotoxins, routine screening of malt is required for ochratoxin A and DON to assure malt safety (Kaur et al. 2009). Conversely, *Fusarium* can potentially produce a wide range of enzymes that can be beneficial in malting and mashing including amylases, β-glucanases, xylanases and a broad range of proteases (Geißinger et al. 2022; Schwarz et al. 2002).

Gushing is a serious beer quality problem defined as the spontaneous over-foaming of carbonated beverages on opening the can or bottle. Gushing can be due to the presence of calcium oxalate in the beer but more concerningly is due to barley/malt fungal hydrophobins in conjunction with non-specific LTPs and protein Z4 (Christian et al. 2011; Deckers et al. 2010; Sarlin et al. 2007; Shokribousjein et al. 2011). In Europe, gushing can be major problem, but is dependent on the weather in the growing season. One major European testing facility using the Carlsberg 'mineral water' test (Garbe et al. 2009; Rath 2009), suggests that gushing effects about 15% of malt samples with considerably more in bad years (wet grain maturation and harvest).

The presence of *Fusarium* in barley, appears to have a terroir aspect. *Fusarium* infection resulting in gushing and mycotoxin issues are detected regularly in barley production areas such as Europe and North America (the Red River Basin including Eastern North Dakota, North-western Minnesota, and North-eastern South Dakota and southern Manitoba in Canada). Conversely, the primary Australian barley growing regions rarely report *Fusarium* head blight (FHB) outbreaks, issues with mycotoxins or gushing. Backhouse and Burgess (2002) observed that the primary causes of FHB - *F. graminearum*, *F. pseudograminearum* and *F. culmorum* - were endemic in the Australian grain belt. However, given the prevailing environmental terroir around anthesis, the temperature and humidity were too low to result in the manifestation of FHB (Backhouse and Burgess 2002; Geißinger et al. 2022).

Premature yeast flocculation (PYF) is an intermittent malt quality problem associated with susceptible yeast strains (Evans and Kaur 2009; Lake and Speers 2008; van Nierop et al. 2006). The consensus is that the PYF problem is caused by fungi that produce a xylanase to liberate arabinoxylan of a particular size (Herrera et al. 1991a, b; Koizumi et al. 2008, 2009; Shang et al. 2014, 2020; van Nierop et al. 2004a; Xie et al. 2022). An alternative hypothesis for PYF is that causal fungi produce antimicrobial peptide factors that negatively affect yeast metabolism (Evans and Kaur 2009; Lake and Speers 2008; Okada and Yoshizumi 1970; Okada et al. 1970; van Nierop et al. 2006). Malting conditions are also implicated in making a PYF+ve malt. Here, the focus has been on steeping, with either too high a pressure on the germinating barley (Yoshida et al. 1979) and/or the use of flat bottom style steeps (Axcell et al. 1986). The current view is that some flat bottom steeps require careful attention to ensure optimal aeration of water to avoid production of PYF+ve malts. Anecdotally, there is a view that a PYF malt can be produced from any barley by applying the appropriate steeping conditions although this was is tempered by some barleys being easier to make PYF+ve malts than others. Presumably, in this case, barley had higher levels of the 'PYF inoculum' due to prevailing field conditions at harvest encompassing temperature, humidity and rain conditions (Lake and Speers, 2008).

The quest for causal microorganisms triggering PYF has been ongoing since the identification of the problem over 40 years ago (Okada and Yoshizumi 1970; Okada et al. 1970). Investigations have associated an array of bacteria, fungi and yeast with PYF including *Lactobacillus fermentum* (Zarattini et al. 1993), *Aspergillus sp*. and *Fusarium culmorum* (van Nierop et al. 2004a), *F. culmorum* and *F. graminearum* (Blechová et al. 2005), *A. fumigatus*, *Fusarium sp*. and *Rhizopus sp.* (Wang et al. 2007), *A. candidus*, *Cladosporium cladoporiodes* and *Penicilllium sp.* (Sasaki et al. 2008), *Cochliobolus sativus* and *F. graminearum* (Macintosh et al. 2014), *Pichia pastoris* (Shang et al. 2022), and *Aureobasidium pullulans*, *A. flavus*, and *Alternaria tenuissima* (Xie et al. 2022). These studies used culture dependant techniques which limit the identification of non-culterable microorganisms. Culture independent genetic methods overcome such concerns.

For example, culture independent terminal restriction fragment length polymorphism (TRFLP) was used to fingerprint the barley malt microbiome using the 16S rRNA and D1/D2 domain of 28S rRNA genes for respectively bacterial and fungal

communities. This method has limitations as the fingerprint sequences are short (100's) and it is time consuming and expensive. However, this approach identified the microbial communities that were associated with the presence of PYF in 41 geographically diverse PYF+ve and PYF negative (PYF-ve) commercial barley malts (Kaur et al. 2012). This study suggested that the cause of PYF was fungal and yeast based but not bacterial. Kaur et al (2012) identified, 'fifteen HaeIII TRFs of the 360–460 bp TRFLP region were tentatively identified as *Aureobasidium pullulans*, *Candida intermedia*, *Candida natalensis*, *Candida Kabatiella microsticta*, *Kazachstania exigua*, *Rhodotorula glutinis*, *Sporobolomyces roseus*, and *Wickerhamomyces anomalus*.' Kaur et al. (2012) concluded that to find a 'needle in a haystack,' there is a need for 'greater in-depth sequencing data generation to better cover the fungal diversity associated with PYF+ve and PYF-ve malts in future studies, especially considering the relatively large number of samples examined in this study and the relatively low proportion of PYF associated fungal taxa apparent in the TRFLP profile data.'

More recently, next generation pyrosequencing has been used to probe the microbiota of PYF+ve and PYF-ve malt. This approach uses single DNA strands attached to beads in individual wells where nucleotides are introduced one at a time, along with DNA polymerase. If the correct nucleotide is introduced and is added to the strand, a chain of chemical reactions is initiated, resulting in the emission of light. The conserved fungal sequences targeted were the large rRNA gene (28S subunit) and the internal transcribed spacer region (5.8S region) to produced discrimination down to the fungal genera level. The samples that were studied were collected from Asia, North America, Asia Pacific and Europe where 15 samples were identified as as PYF +ve and 17 samples PYF-ve.

On average, some 20,000 sequences per malt sample were obtained which enabled a powerful bioinformatic comparison including fungal BLAST searches of genera putatively identified from the PYF +ve and PYF -ve samples. In this work, two wild yeast genera, for which qPCR primers were constructed, enabled PYF designation with a PERMANOVA P value for qPCR gene copy number data ($P < 0.0014$) to be achieved with the 32 defined PYF +ve/-ve malts (Evans et al. 2014a).

It follows that the occurrence of PYF+ve malt is putatively related to a combination of steeping conditions during malting (Axcell et al. 1986; Yoshida et al. 1979) and the level of PYF inducing inoculum on the barley. As such, the PYF inoculum levels will vary between seasons and annual rainfall (Lake and Speers 2008, van Nierop et al. 2006), as those conditions produce malts which are likely to have a terroir factor.

Conclusions

Terroir or a 'sense of place' is a concept rooted in subtle organoleptic variations that can be traced back to wine in Greco-Roman times. Terroir encompasses all the factors that go into producing a food crop, from the relationship between the climate and soil together with the elevation/aspect of the production area. A further aspect of terroir are the interactions between specific varieties and terroir factors. Despite reasonable scepticism, the case for the effect of terroir has been demonstrated in crops as diverse as wine grapes, coffee, berries, olive oil and hops.

GPC (grain protein content) is the lynch pin of the expression of terroir in malting barley. Declining day length during maturation is related to higher levels of GPC, such as Canadian growing practices are compared with those in Australia. Other factors such as seasonal light intensity (clouds) may also play a contributory role. In this, Canadian barley breeders have been successful in genetically ameliorating high grain protein content. GPC has long been known to be inversely correlated with extract although positively correlated with DP. Of the DP enzymes, only *beta*-amylase was consistently and highly correlated with expression of terroir in malting barley. The levels of α-amylase and limit dextrinase were correlated with KI (Kolbach Index), as reflects their gibberellin induced expression. The foam positive proteins, protein Z4, Z7 and hordeins, also increase with GPC. Further, where the barley variety is grown, and its variety may influence the relative proportion of hordein to albumin/globulins. Yet, the foam positive status of protein Z7 has yet to be conclusively demonstrated. The other main foam protein, LTP1, does not seem to be linked to barley genetics or protein but does appear to

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respond to humid or wet conditions during grain maturation.

Grain starch content and quality are influenced by seasonal growth conditions that may be a component of terroir. In cases of high temperature and water stress, the ratio of large to small starch granules may decrease, along with the length of the amylose and amylopectin branches, and the ratio of amylose:amylopectin. Such changes appear linked to a reduction in the activity of starch synthases during grain fill. An additional factor is the quality and level (higher GPC) of grain proteins, presumably those which can bind to starch granules. Such starch levels and quality changes influence the potential production of fermentable sugars and the *Tp* of starch gelatinisation temperature. It has been observed that a *Tp* > 65°C will reduce wort fermentability due to reduced mash persistence together with the activity of *beta*-amylase and limit dextrinase.

Other malt quality related components can be linked with terroir. The Kolbach index (KI) or protein modification has a largely inverse correlation with GPC, which is impacted by terroir. An improved understanding of the influence that terroir has on the GPC - KI relationship in malt quality should be a priority for future research. It is noteworthy that lower protein barley tends to produce higher KI malts that generate worts with the highest flavour complexity and desirability.

In terms of terroir, the topic that has been most neglected in malting and brewing are cation and anion micronutrients. It is well known that some regions and soils have deficiencies or toxicities that impact on plant growth and the quality of the grain. With the limited number of studies reported on wort and malt/barley, it is clear there is the potential for substantive variation in these components in beer.

It is abundantly clear, that the population, ecology and diversity of microorganisms present on barley and malt differs significantly, as it does in grapes. For example, there are regions where *Fusarium* head blight is endemic (North America, Europe) in a proportion of the crop resulting in the undesirable gushing and presence of mycotoxins. In contrast, other regions (Australia) have climatic conditions and growing practices that do not support infection by *Fusarium*. Further, it is reasonable that the microbiota to produce premature yeast flocculation may be determined by the conditions of barley growing and harvest (humid and wet) in combination with undesirable malting practices.

The characteristics of malt quality considered in terms terroir can be broadly divided into those influencing the efficiency of the malting and brewing process (extract, DP enzymes, wort separation characteristics etc) and those affecting consumer perception (beer flavour, foaming proteins, gushing/mycotoxins, etc).

An understanding of the influence of terroir on barley and malt quality could be applied differently by malting and brewing operations. In general, large and international malting (e.g., Boortmalt, Malteurop etc) and brewing companies (for example AB Inbev, Miller-Coors, Heineken, Asahi, Tsingtao, etc) could use a knowledge of terroir to ameliorate natural variation in malt quality so as to assure the consistency of malt and beer. Such companies would be interested in opportunities that improve the efficiency of the brewing process (extract, mash separation efficiency). Conversely, other brewers (e.g, Boston Brewing, Sierra Nevada etc) might focus on characteristics of malt quality that influence the flavour and aroma of beer that may enable the differentiation from other brands. In addition, some malting/brewing companies might maximise the connection with terroir by linking a release of beer to the fields in which the barley was grown.

In conclusion, this review identifies malt quality attributes such as protein, extract, Kolbach index, foam proteins, DP enzymes, starch, ionic micronutrients and differences in microbiota that are, or are most likely, influenced by the genetic, environmental, agronomic and production variables that can collectively be attributed as 'terroir'. Maltsters and brewers have the opportunity to harness this understanding to improve the consistency of their products, quality characteristics and impact on the efficiency of their processes.

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Author contributions

Evan Evans: conceptualisation, methodology, writing (original draft)
Blakeley Paynter: unpublished data, writing (review and editing).
Marta Izydorczyk: unpublished data, writing (review and editing).
Chengdao Li: unpublished data, writing (review and editing).

Conflict of interest

The authors declare there are no conflicts of interest.

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