Green malt for a green future – Feasibility and challenges of brewing using freshly germinated (unkilned) malt - a review

Celina A. Dugulin[a], Gert De Rouck[b] and David J. Cook[a#]

[a] *International Centre for Brewing Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, UK*

[b] *KU Leuven, Bioengineering Technology, Laboratory of Enzyme, Fermentation and Brewing Technology,Technology Campus Ghent, Gebroeders De Smetstraat, 9000 Gent, Belgium*

[#]Corresponding author:
E-mail: david.cook@nottingham.ac.uk

ORCiD: https://orcid.org/0000-0002-4967-3287

Twitter: @DaveCookUoNbrew

Abstract

In malting, the kilning step is by far the dominant user of energy and has thus become the main target in reducing the carbon footprint of malting operations. Brewing beer using 'green' (germinated, but not dried) malt, thus saving the substantial energy input associated with kilning and conserving the water contained in the green malt, could improve the energy and water efficiency of malting operations. Furthermore, the high enzyme complement in green malt could enable more efficient brewing using unmalted cereals (e.g. raw barley) to create highly attenuated beers, without the use of exogenous enzymes. The objective of this review is to unravel the major advantages, but also quality and technical challenges, which could serve as a base for future studies on successfully brewing with green malt. Furthermore, the aim is to evaluate the impacts of such a process on beer flavour and flavour (in)stability. This is important to establish because one can make arguments that such a process will either improve flavour stability (reduced heat load should reduce the pool of staling aldehydes) or worsen it (since lipoxygenase activity and dimethyl sulphide potential can be regulated by heat treatment during kilning).

Keywords: green malt, energy efficient malting, flavour stability, sustainability

Introduction

Sustainability has been defined as *"development that meets the needs of the present without compromising the ability of future generations to meet their needs"* [1]. Annually, European maltsters produce around 9.7 million tonnes of malt - and this trend is increasing [2]. Malting barley production accounts for about 241 kg CO_2eq/t; malting itself adds 217 kg CO_2eq/t – doubling the total malt carbon footprint [3]. In the UK alone, emissions of more than 300,000 tonnes CO_2 per year are produced through the manufacturing of more than 1.6 million t of malt [2, 4] – this corresponds to annual CO_2 emissions of about 14,851 British households [5]. Thus, the malting industry is constantly exploring ways to improve its energy efficiency. To date, around 6-15% of the cost per ton of malt can be attributed to energy usage [4]. Moreover, a number of governments impose national-level energy or carbon taxes, calculated based on the carbon content. Hence, diminishing energy usage is not just an environmental driver but also a financial driver. While some research has been carried out on energy efficient malting [4, 6-10], to our knowledge only a few studies [11-16] focused on wort and beer production using green (germinated, undried) malt. The objective of this review is to evaluate the feasibility of brewing using freshly germinated (green) malt, with omission of the kilning step. Similarly, the main focus is on the influences on microbiological stability, extractability, as well as final beer flavour and flavour stability due to different biochemical compositions of germinated malt compared to kilned malt.

Considering the production of pale malted barley, the malting process comprises three main steps: steeping, germination and kilning. Malt quality and functionality is not solely defined by these classical stages. By changing the grade and type of barley, steeping and germinating under different conditions and by kilning or roasting at differing moisture contents and to different temperatures, a range of malt types are prepared [7, 17-19]. Malting is a process that modifies barley until it is suitable to produce beer (both due to physical modification of the grain and the development of key enzyme activities).

After harvest, barley can go through a phase of dormancy, usually a period of 6-8 weeks (depending on variety and the weather during harvest) where the seedling cannot germinate [20, 21]. Thus, after cleaning and grading, the barley is usually not malted directly but is stored until the actual malting process can start. If the moisture content in barley exceeds 12% the barley has to be dried to slow the rate of respiration and avoid microbial growth (e.g. fungi). Subsequently, the grain can be stored, e.g. in silos, whereby ventilation is necessary to maintain its viability. This effectively removes CO_2, water and heat, while simultaneously supplying the grain with oxygen.

Following cleaning and grading of the stored barley, the malting process is initiated with the steeping unit operation. During steeping, barley is immersed in water, to increase the moisture content, aiming to trigger germination, but also to clean the grain from dust, impurities and germination inhibitors [22, 23]. The steeping process consists of one or more wet and dry cycles. During the wet phase, the grain is submerged in water. The moisture uptake can change depending on barley variety, crop year, kernel size, nitrogen content, dormancy, water sensitivity, or applied steeping parameters (i.e. time, temperature, aeration) [24-26]. After a few hours of steeping, the water is drained off and the dry phase (air rest) commences (Figure 1). During this phase, moisture is distributed more evenly throughout the grain, helping to hydrate the compact starchy endosperm. Grain germinates more vigorously as a result of alternating wet and dry stands, than would be the case for continuous immersion. During steeping, the grain must be aerated, whilst the respiration product (CO_2) is removed; failure to do that could result in a so-called 'dead steep'. Following the required recipe of wet and dry stands steeping typically seeks to increase the moisture content of barley to between 40-46% w/w, at which point the barley is transferred ('cast') into a germination vessel. Here, the barley is allowed to germinate whilst cool, attemperated, 100% humidified air is circulated through the bed to remove the heat of germination, whilst not unduly drying the grain. Air-flow is regulated typically to maintain temperatures across the bed of germinating malt in the range 12-20°C. During germination, the required physical and biochemical changes occur. The initiation of germination triggers a hormonal and enzymatic cascade which effects the breakdown of endosperm components: cell wall materials, protein matrix and some limited breakdown of starch [17]. Longer and warmer germination conditions lead to increased rootlet growth, so it is important for maltsters to carefully control process temperature and time to deliver the required endosperm breakdown ('modification') and enzyme production, whilst minimising malting loss caused by excessive growth of the embryo. Germination is allowed to proceed until the desired degree of modification has been achieved (usually after 3.5 - 5 days of germination) when seed germination is arrested by a hot air drying process known as kilning. Furthermore, the thermal processing of malt on the kiln leads to the formation of desirable colour and flavour characteristics [17, 27, 28]. The main goal of kilning is to lower the moisture content of green malt down to around 4%, thus stopping germination and yielding a stable product that can be transported. In the production of base malts for brewing, it is important that key enzymes (e.g. amylases) should be protected by applying a 'gentle' kilning program (slow lowering of water content through stepwise increase in the air-on temperature). During conventional kilning, the initial moisture of > 40% is first reduced to approx. 12% by forcing dry air through a bed of grain with a stepwise increase in air-on temperature, starting at around 50°C and ramping gently to 70°C. In this stage, free water is being

removed. In the next phase, known as curing, the bound water from within grains is removed, lowering the moisture content to 4-5% by circulating air at temperatures above 80°C. After kilning, the malt is cooled, cleaned (rootlet removal) and stored for 3-4 weeks, to avoid lautering and fermentation difficulties, associated with the use of freshly kilned malt [29-32]. Subsequently, the brewer mashes milled malt in water to start the process of wort production in the brewery. Thus, viewed holistically, the malting and brewing processes are defined by the sequential addition and removal of water, which does not make a huge amount of sense when considering them as a joined-up process.

The most dominant contributors to the carbon footprint of the malting process are gas (or other process fuels) and electricity [4, 10, 33]. A UK Carbon Trust report [4] demonstrated that fuel use accounted for about 68% and electricity about 32% of the malting sector's CO_2 emissions. Electricity usage is spread over all process steps, whereas gas and coal are primarily used in kilning [33]. During the drying process, removal of free moisture from green malt is relatively easy until the grain reaches a moisture content of approximately 12%. To remove the remaining water in bound form a lot of energy is required. A review of the UK malting sector [4] reported average specific energy usage to be ca. 1,200 kWh/t malt and up to 80% of this energy was used in drying of malt (kilning). Hence, kilning is the most dominant user of heat and electricity, making it the main contributor to the carbon footprint associated with malting. Numerous previous studies have focused on ways in which to reduce the energy needed for kilning [4, 6, 10, 33-35]. The Carbon Trust's report evaluated different technologies, including kiln energy recovery, heat pumps or biomass burners as replacements for the heat energy used for kilning with regard to carbon emission reduction and payback periods for the industry. Although various technologies significantly reduce emission rates, not all of them are cost effective. Another option is the application of alternative heating methods, such as: electromagnetic heating [6], microwave drying [9], drying with supercritical CO_2 [36], or freeze-drying [35, 37]. In spite of this research and technology innovations that have been implemented to reduce specific energy usage across malting, there is still a demand to explore cost-effective methodologies to reduce the environmental footprint associated with malting and brewing.

Omission of the kilning process

Omitting the kilning process, thus producing beer with green malt, is one potential route to reduce the energy inputs required for malting. Additionally, de-carbonising and the reduction of primary energy usage through e.g. biomass CHP or hydrogen power could sit alongside the adoption of green malt in future strategy to meet environmental targets. Green malt differs from kilned malt in a number of respects. Green malt is not coloured green, it is a term used to refer to undried germinating malt (Figure

1C). Apart from being a dominant consumer of heat and electricity, the kilning process has many beneficial impacts on malt quality, which must be considered if it is to be omitted. These include reduction of lipoxygenase activity [19, 38-46], regulation of S-methyl methionine (SMM) levels [47-52], facilitating rootlet removal, diminishing unwanted "raw grain" characteristics [14] and, most importantly, developing the characteristic colour and flavours which malt imparts to beer. Furthermore, green malt, having a moisture content between 38-46%, is unstable and cannot be stored for prolonged periods. On the other hand, green malt is rich in β-glucanase [53-55] and diastatic enzyme activity [54, 56-58], hence it can very efficiently convert the starch of unmalted grains into fermentable sugars [11, 12]. Additionally, by removing the kilning process, the thermal heat load on malt is substantially reduced. As a consequence, green malt, is free of DMSO [50, 59], and contains lower concentrations of thermally generated compounds such as Maillard compounds or Strecker aldehydes, which are key agents in beer flavour change through shelf-life [60-66]. On this basis, potential benefits regarding beer staling can be expected in beers brewed from green malt. Table 1, compiled from multiple sources, provides a comparison between typical analytical specifications of a well germinated green malt and the corresponding kilned pale lager malt, however, analytical specifications of malts will vary depending on barley variety and malting protocol (temperature, time).

Well germinated green malt usually has a moisture content of 41-48%, depending on the malting procedure. The high moisture content of green malt is a perfect environment for microorganisms, making the grain unstable. The microbial growth on malt accelerates when stored at warm temperatures, for example the doubling time of the filamentous fungus *Geotrichum candidum* was shown to be as fast as 1.7 h at 25°C and just 1.1 h at 30°C [67]. Furthermore, moisture contents > 8% make the abrasion of rootlets difficult [68]. Malt rootlets are considered to impair the flavour of beer, therefore maltsters try to avoid excessive rootlet growth during germination and remove the rootlets, by abrading them after kilning. Thus, they form a malting loss of around 4%, usually sold as animal feed or organic fertilizer [17, 31]. Rootlets are low in phytic acid and polyphenols, but contain a high amount of fatty acids, tocopherols (Vit. E), B-vitamins and proteins (10-35%) [17, 69], calcium (19.9 g/kg), as well as DMS precursor [48] and lipoxygenase [41]. Rootlets of kilned malt are highly hygroscopic, due to their fibre content of up to 15% [17, 69]. Apart from the high water absorption they also highly absorb oil and have emulsification capacities [31]. However, malt rootlets (as analysed in kilned malts) show a high antioxidant potential [70-72]. The natural antioxidant phenolic compounds could potentially reduce the formation of free radicals, thus favouring wort and beer flavour stability. In the absence of a facile technique for rootlet removal from green malt, they are most

easily included in the brewing grist. Whilst this will lower malting losses, the foregoing quality issues associated with rootlet usage need to be addressed, or better evaluated, across a range of beer styles.

Processing green malt

Microbiological Stability

The brewing process presents numerous hurdles to the survival of microorganisms, of which mashing, wort boiling and the addition of hops, are considered the most effective. In addition, the composition of beer, mainly the presence of alcohol and CO_2, a low level of O_2 and the acidic pH, provides a very hostile environment for the growth of spoilage and pathogenic microorganisms [73, 74]. Wort, however, is a nutrient rich medium and represents an excellent environment for the growth and proliferation of microorganisms. The microbiology of malting and brewing is a complex topic, and little is known about the microbial stability of wort and beer made of green malt. Most of the microflora on barley in the field consists of bacteria (predominantly), wild yeast and filamentous fungi originating from the air and soil [75, 76]. Microbial colonisation of the grain is generally restricted to the outer layers, namely husk and between the husk and pericarp, although penetration into the endosperm does occur [77]. Green malts are covered in a complex microflora, with viable counts of various organisms that are 85-700 fold higher than measured on the original barley [78-81], their growth stimulated by dissolved nutrients, moisture, warmth, and aeration [80-82]. Before drying, an average of tens of thousands of fungi, hundreds of thousands of yeasts and millions of bacteria can be measured in just one gram of malting barley [17, 81]. Douglas and Flannigan [79] detected especially the yeast-like mould, *Geotrichum candidum*, in green malt. Usually, after kilning, the majority of microorganisms are destroyed [79-81, 83]. Mostly lactobacilli [80] and aerobic heterotrophic bacteria [81] are still being detected afterwards and counts further decrease steadily during mashing, with only thermotolerant microbes, such as homofermentative lactic acid bacteria [82], persisting. Table 2 compares representative numbers of microbes on barley, green malt and kilned malt according to Petters et al. [81].

Whilst the hurdles presented by the brewing process would be expected to overcome this higher initial presence of microbes and still present a sterile wort for colonisation with yeast, it is unclear whether the increased microbial loading of green malt would have any negative impacts on wort or beer quality. However, we do know that green malt as-is, is not microbiologically stable, hence it needs to be either processed directly, by mashing-in immediately or by reducing its moisture content to a microbiologically safe level (kilning, freeze-drying, electromagnetic heating) or alternative technologies [6, 9, 84]. In general, it is recommended to avoid making malts from barley that is heavily infected with fungi – this applies

especially to green malt brewing. It is recommended to store green malt cold and dry to reduce microbial activity and stabilise enzymatic activity until the grain is further processed – the sooner the better. Longer storage periods of green malt and the associated microbes present could greatly affect malt quality and thus impact beer quality [85-87]. Of particular concern are mycotoxins present on poor malts, which might survive into the final beer [86]. Alternatively, it was suggested [15] to mix proportions of 10-20% green malt with kilned malt or unmalted cereals, as the moisture content of the mixture would allow longer storage periods than green malt alone. Furthermore, Peterreins and Van Waesberghe [84] proposed two methods that could be used to stabilise green malt; either by exposing the green malt to a brief heat shock by applying water vapour or stabilising it with lactic acid bacteria at 45°C. Lactic acid bacteria and associated antimicrobial metabolites could potentially inhibit the growth of bacteria or fungi [88, 89]. These preserving effects may be due not only to the end products of their fermentative activity, such as lactic acid, but also to the formation of small, heat stable inhibitory peptides referred to as bacteriocins [90].

Milling

One of the first challenges when handling green malt is its sensitivity to the milling procedure, due to the high moisture content of green malt, hence it cannot be milled as for kilned malt. Previous studies [11-13, 16] already proved that extracts of green malt with equal quality to kilned malt can be achieved, provided a suitable mill is used. However, there is no general consensus on what is the most suitable milling technique. S.R. Duff [11] stated that higher extract yields are achievable by finely grinding the green malt. Furthermore, better yields were obtained by using a hammer mill rather than a roller mill. Unfortunately, no further information was given on the exact model or design of the hammer mill used in the study. In a standard hammer mill steel beaters rotate at speeds between 60-100 m/s, to form small particles which fall through holes in a sieve [31]. Therefore, this approach seems not well suited for green malt at a moisture content above 40%, as it would likely block the sieves. In general crushing rollers could be the most suitable option for handling green malt. Pre-soaking of barley or green malt before milling increased yield of extracts and facilitated the milling [12, 13], indicating that a wet milling system is a suitable technique for processing green malt. About 20 years ago, Meura (Belgium) developed in collaboration with Castle Malting (Belgium) the 'hydromill'; a disc mill which finely mills malt underwater, designed to process malts with a high moisture content [15, 91]. Recent pilot scale trials [92] indicated that green malt, used as 100% grist material, can be successfully milled using the Meura 'hydromill'. However, the spongy and cohesive structure of the resulting green malt mash caused significant losses of extract due to insufficient sparging when using the membrane assisted thin bed filter (Meura 2001). Green malt used as 100% grist material still poses a technical challenge for present day brewhouse designs. Probably by fine tuning of the liquor

to grist ratio or some optimisations in the mill design will enable to take full advantage of the highly fermentable worts of green malt. Most practically, only proportions of green malt could replace kilned malt to overcome the technical challenges faced.

Alternatively, standard kitchen meat grinders pose a more affordable option for homebrewers who would like to experiment with this 'novel' grist material, provided of course that the meat grinder is solely used for processing green malt (to avoid risks of cross contamination). Lars Marius Garshol [93] recently published a book on ancient brewing traditions and techniques; amongst them, brewing with homemade green (undried) rye malt, milled with an ordinary kitchen meat grinder and used as 100% grist material for mashing.

Mashing

As already highlighted, the malting and brewing process can be considered as a sequential adding and removing of water. If omitting the kilning process, thus brewing with green malt, the brewer could take advantage of the increased moisture content in the grain, hence less brewing water will be needed for mashing. However, brewing water adjustments are necessary to compensate for the increased water content in green malt. This would imply a temperature increase of the brewing liquor used for mashing, as well as lactic acid (pH regulation) and brewing salt addition (water hardness).

Filterability

As discussed previously, the thickness and structure of the (100%) green malt mash has caused filtration and sparging difficulties in recent pilot scale brewing trials [92]. However, Castle malting's research team has shown [15] that by replacing 20% of pilsner malt with green malt to a cereal recipe containing 30% unmalted barley, can significantly improve filterability. The resultant mash was filtered twice as efficient. Therefore, green malt has the potential to compensate for the relatively high β-glucan levels of unmalted cereals such as barley [17], or poor malts (>250 mg/L β-glucan in the mash [15]), and thus, improve filterability.

Green malt's (bio)chemistry – the good and the bad

Diastatic enzyme activity

During malting, starch degrading enzymes, are formed. These include (i) α-amylase, which hydrolyses α-(1-4) linkages in the long glucose chains of starch to yield smaller fragments (ii) β-amylase for the splitting of maltose from the non-reducing chain end and (iii) limit dextrinase to hydrolyse α-(1-6) linkages which form the branch points that are most prevalent in amylopectin molecules. The diastatic enzymes play a key role in brewing during the mashing process, where the starch is gelatinised to enable access of the

starch hydrolysing enzymes. The enzymatic breakdown of starch into simpler sugars provides the major energy source for the fermentation process. α-amylase is more thermostable than the other diastatic enzymes [54, 94, 95]. According to previous research [54, 96], kilning (80°C curing temperature) caused a significant loss of diastatic power (15%), limit dextrinase activity (25%), while α-amylase decreased only about 4% upon kilning. The thermosensitive β-amylase, on the other hand, suffered losses up to even 46% of initial activity during malt kilning [97]. Modern day breeding programs have ensured that elite barley cultivars are rarely deficient in diastatic enzyme potential when malted and used as the main grist component in brewing. However, the extra diastatic potential of green malt could favour the degradation of starch of unmalted adjuncts into fermentable sugars, novel malted cereals lacking diastatic enzyme activity (e.g. malted lentils [98]) or potentially be suitable for shortening the total mashing time, and thus further decreasing the total heat load.

β-glucanase activity

In addition to the diastatic enzymes, kilning causes significant losses of total β-glucanase activity [53-55, 96]. Previous research [54, 96] highlighted that β-glucanase was reduced by 43-44% when curing at 80°C, an inactivation that already started at the onset of kilning at a relatively low kilning temperature. Increasing the curing temperatures, as expected, further caused enzymatic activity losses [54]. Most of the necessary cytolytic degradation of barley (1,3)(1,4)-β-D-glucans via β-glucanases occurs during malting. Thus finished malts should contain low levels of β-glucan for a satisfactory brewing performance and to avoid the process problems associated with the elevated content of β-glucan (poor lautering performance [99, 100] and colloidal (in) stability of the finished beer [101, 102]). However, the increased β-glucanase content of green malt can be advantageous when unmalted adjuncts form part of the grist material used for brewing. Despite the enzymes heat-sensitivity and the recommendation to mash-in at >62°C to avoid LOX related off-flavours (see upcoming section), when using green malt as part of the grist material, previous research [53, 100] has demonstrated that significant quantities of β-glucanase can survive infusion mashing at 65°C. β-glucanases were discussed to be protected from heat by high concentrations of protein, the association with particles of malt or sugars [103], or reduced glutathione [53]. Additionally, thick mashes can offer protection to more fragile enzymes [104]. Thus, when β-glucan is continuously released from its binding to protein through the activity of the more heat stable β-glucan solubilase (inactivation temp. 73°C, [105]) during mashing, the malt β-glucanase can break down the β-glucan structure.

Anthocyanogenase

Claims have been made that beer produced from green malt and steeped barley has special advantages in connection with haze stability [12, 68, 106]. MacWilliam et al. [12] reported higher concentrations of anthocyanogens in wort prepared from kilned malt than from green malt. Anthocyanogens are polyphenolic compounds which play a role in the formation of chill haze in beer [107]. Green malt appears to contain the enzyme anthocyanogenase which will hydrolyse or degrade the anthocyanogens into simpler, less haze-inducing compounds [108]. Thus, the use of green malt for brewing might have beneficial effects on colloidal stability and beer shelf life.

Lipoxygenase

Significance to malting and brewing

The theory of lipid oxidation has been thoroughly discussed in previous research papers [39, 41, 46, 109-116] and reviews [64, 65, 117] and is an ongoing topic of research regarding the flavour stability of beer. In principle, a distinction is made in the peroxidation of fats between light-assisted photo-oxidation, enzymatic oxidation and finally radical autoxidation, although the products of all three reactions are partially similar or even of the same nature. One of the key quality concerns when handling green malt is enzymatic lipid oxidation (**Error! Reference source not found.**). Enzymatic lipid oxidation pathways are initiated by lipoxygenases (LOX). The oxidative activity of LOX is not limited to the free fatty acids, thus if LOX is present it can also oxidize the esterified fatty acids of the triacylglycerols and form lipid hydroperoxides, ultimately resulting in hydroperoxy fatty acids [111, 118]. Subsequently, the hydroperoxy fatty acids can undergo further degradations to mono-/di-trihydroxy fatty acids through several pathways [64]. The hydroxy fatty acids remain present in the beer [119] or can, in the presence of oxygen, be further degraded non-enzymatically to secondary metabolites known as ageing carbonyls; e.g. hexanal**,** *trans*-2-nonenal, which contribute to the staling of beer [112, 118]. In particular, one aldehyde is intensively investigated: *trans*-2 nonenal [43, 64, 112, 115, 120, 121]. *Trans*-2-nonenal is a major component for cardboard stale flavours in beer [43, 112, 122] and has a very low flavour threshold in the low ppb range (0.035 µg/L) [112, 123]. Furthermore, the amount of hexanal, which is correlated with green/grassy scent and a bitter, winey flavour [124], decreases with increasing malt colour [125]. In pilsner malt worts it was found in concentrations up to 50% higher than in wort made of dark malt samples [125].

Barley lipoxygenases

In barley, around 3-4% of the dry matter is lipid, of which almost 60% is linoleic acid (C 18:2), making it the major substrate for lipoxygenases [126]. However, the amount of free fatty acids in malt and barley is

quite low. Linoleic and linolenic acid constitute around 6% of the total fatty acid content in barley [126], around 70% are found as triglycerides and 20% as polar lipids (phospholipids and glycolipids) [127].

The lipoxygenase activity in germinating malt is contributed by two LOX isoenzymes: LOX-1 and LOX-2 [39, 41, 45, 46, 118, 128]. LOX-1 mainly oxidizes linoleic acid to 9-hydroperoxyoctadeca-10-12-dienoic acid (9-HPOD), whereas LOX-2 mainly forms 13-hydroperoxyoctadeca-9-11-dienoic acid (13 HPOD) from linoleic acid [41, 45, 128-130]. LOX- 1 is already present in sound barley and increases in activity during germination, whereas LOX-2 is solely formed during germination [39, 41, 128, 131]. During germination, both isoenzymes develop in the newly synthesised rootlets (only LOX-2) and acrospire (both isoenzymes) tissue [41]. LOX is relatively unstable to thermal processing and the activity remaining after kilning is due to the somewhat more heat-stable LOX-1 which is then transferred into the wort [39, 40].

Despite numerous efforts, little is known specifically about LOX from barley and malt, compared to LOX from other plants. As early as 1953, activities in different cereals including barley were measured [131]. The paper by Franke and Frehse showed that the activity from soybean far exceeds all other activities. Barley contains only 1.2% LOX activity compared with the activity in soybean. This membrane-bound enzyme had some similarities to LOX-1, such as its optimum pH, size, and preference to produce 13-hydroperoxy linoleic acid [132]. BLAST (Basic Local Alignment Search tool) alignment of amino acid sequences of $LOX_{Soybean}$ and $LOX_{Barley,}$ showed a degree of sequence similarity of 52.6% [133]. Although there is (to the best of our knowledge) no paper which explicitly proves the presence of bound state LOX in malt, it is reasonable to assume that plant cells contain both soluble and membrane bound lipoxygenases [132, 134, 135].

Lipoxygenase activities in green malt

Huge attention has been paid as to the role of LOX in beer flavour stability. However, these studies focused mainly on the LOX activity present in kilned malt, which is mainly contributed by the more heat stable isoenzyme, LOX-1 [39, 40]. Kilning reduces - depending on the drying protocol and intensity - the lipoxygenase enzyme activity by 96% of the initial activity found in green malt [136]. Hence, omitting the kilning step will result in significantly higher lipoxygenase activities [41, 43, 110, 136] and the usage of green malt in conventional brewing processes requires alternative techniques to reduce total LOX activity. An increase of LOX could result in elevated “rancidity” in the final beer, caused both by LOX-1 but also LOX-2. Furthermore, lipoxygenase worsens the foam stability of beer, possibly due to the production of trihydroxy octadecenoic acid (THOD), which is detrimental to foam stability [137, 138]. If LOX activity can be minimized at source, through adequate malting and/or mashing conditions, significant off-flavours in beer made

from green malt could be avoided. Therefore, it is important to understand the origin, development and activity of LOX.

Considerations when brewing with green malt

As already discussed, LOX is primarily destroyed during kilning. However, LOX is also proven to be pH sensitive and requires oxygen as a substrate. Researchers do not agree on the exact pH optima of both isoenzymes, but the general consensus is that the pH-optimum is on the alkaline side of typical wort pH. The pH-optima for LOX- 1 were reported to be around 6.3-6.5 [41, 45] and even 7.5 [128]; 6.5 [45] and pH 7.0-7.5 [128] for LOX-2. LOX-1 shows only 50% activity remaining at a pH of 5 whereas LOX-2 shows an activity rate close to zero, suggesting that LOX-2 is more pH sensitive than LOX-1 [39, 42, 43]. Another important criterion to avoid LOX-related side effects is to perform the brewing process under oxygen-free conditions. Oxygen is a substrate of LOX, hence oxygen and oxygen pick up should be avoided by all means when brewing with green malt, especially during the mashing step. Based on the kinetic information available for LOX-catalyzed reactions, it has been proposed [139], that the two substrates, unsaturated fatty acids as well as oxygen, are the limiting factor for lipoxygenase activity.

An important factor when brewing with green malt, is to consider the high lipoxygenase activity from the beginning of the process, meaning that milling and mashing need to occur in lipoxygenase hostile environments: e.g. mashing in at > 62 °C, pH: 5.2, under oxygen-free conditions [43, 66, 121, 136, 139]. Recent laboratory scale trials [56] indicated that re-steeping of green malt in combination with a LOX hostile mashing environment (62°C, pH 5.2 and oxygen-limited) could help to control LOX activity and the *trans*-2-nonenal potential of green malt [56].

Even though there are a number of process controlling methods, when brewing with green malt, Null-LOX [140, 141] or Low-LOX [137, 138, 142, 143] barley cultivars offer a further possible solution. However, low LOX cultivars need to be differentiated, because the term is principally used to refer to low LOX-1 cultivars, since LOX-1 activity is the main problem in kilned malt. However, regarding green malt brewing, the activity of LOX-2 should not be neglected. Beers made with a (kilned) lipoxygenase-1-less (LOX-less) malting barley variety had reduced levels of beer-deteriorating substances, such as *trans*-2 nonenal and THOD compared to beers made with the control malt [137, 138, 142, 143]. The sensory evaluation results indicated that LOX-less barley variety CDC PolarStar improved flavour stability without affecting other beer characteristics. Carlsberg's research in partnership with Heineken has shown that brewing beer using null-LOX barley minimises negative beer-staling components, provides stable, quality foam with no aged off-flavours, and keeps its fresh flavour for longer. Although lipoxygenases can have adverse impacts on beer flavor stability, the products of the LOX pathway play an important role in the plant itself. The physiological

function of LOX is associated with growth and development, mainly with lipid mobilisation (mainly via LOX-2) during seed germination [144], wound-induced or pathogen infection signaling for the local defense reaction [145] and participation in plant senescence [146]. However, since Null-LOX barley varieties are already in commercial production without any reported adverse effects during plant growth it might be suggested that these pathways are not insurmountable. Recent research confirmed [147] that a total loss of LOX-1 and LOX-2 function did not cause any obvious disadvantages for null-LOX cultivars over the traditional malt barley cultivar, in terms of grain yield, yield components, grain size, grain protein content and water use efficiency.

DMS and S-methyl methionine

Dimethyl Sulphide (DMS) is a highly volatile sulphur compound, with a boiling point of only 38°C. It has a characteristic flavour and odour usually described by brewers as cooked corn or cabbage-like. Although its odour plays an important role in some cooked vegetables, or contributes to the typical aroma of many lager style beers [148], in most other styles, or at an excessive level, DMS gives beer an undesirable flavour [47, 149, 150]. The flavour threshold is approximately 30 µg/L; however, the overall liking and acceptance of customers depends strongly on personal preferences. DMS originates from two possible precursors, S-Methyl Methionine (SMM) [49, 51] and DMSO [52, 59]. During germination, SMM, the thermal precursor of DMS, is produced from L-methionine and S-adenosyl-L-methionine catalyzed by L-methionine S-methyltransferase (MMT) [51, 151]. During barley germination, both the specific activity and the amount of MMT protein increase. SMM decomposes upon heating to yield free volatile DMS, and as a result, levels in malt are strongly regulated by the kilning stage which first breaks down SMM and then strips DMS into the exhaust gases [47, 48, 51, 152]. Besides SMM, as the thermal precursor of DMS, yeast can enzymatically reduce DMSO to DMS [59]. However, through this pathway DMS cannot be readily removed and a high proportion remains in the finished beer. DMSO can be formed by oxidation of DMS during kilning and concentrations increase at higher kilning temperature [153, 154].

Green malt is rich in S-methyl methionine [49, 50, 56], not in DMSO [50, 59], therefore the main focus when brewing with green malt is on the SMM pathway. Interestingly, according to a study by White and Wainwright [49], beers brewed from green malt had low levels of DMS, despite the significantly higher DMS potential, indicated by the high SMM levels in malt and wort (Section: Characteristics of wort and beers made of green malt). Hence, DMS levels in the pitching wort can be controlled, provided that there is a sufficient removal of DMS via evaporation during wort boiling and elimination through fermentation gases. Even though those study outcomes seem very promising, regarding DMS in beer made of green malt, the control of SMM-levels from the grist and throughout the process remains a significant issue to

control the potential for DMS formation. Precursor levels vary with the barley variety and depend on malting parameters used for steeping and germination as well as the kilning regime. As summarised by Bamforth [47] an enhanced germination, via higher temperatures or by the aid of gibberellic acid result in increased SMM levels. On the other hand, inhibitors of germination, e.g. potassium bromate (outlawed in foodstuffs in most countries), reduce embryo development and rootlet growth, hence lower SMM levels in green malt [155]. Interestingly the half-life of SMM at 100°C is 38 min at a pH of 5.2, whereas a half-life of 32.5 min is reported at a pH of 5.5, indicating that the chemical decomposition of SMM is not solely temperature but also pH-sensitive [152]. Furthermore, the use of a wort stripper could help to remove excessive DMS, but also purge other undesired volatiles [156]. Additionally, attention should be paid to the origin and localization of SMM in grist materials. SMM is mainly located in the seedling which is why wort production after fine milling gives higher SMM levels than after coarse milling since the seedling remains more intact after coarse milling of the malt. However, at the end of wort boiling, no differences in levels of DMS precursor and free DMS were found between fine milled-thin bed mash filter operations and coarse milled-lauter tun operations [157]. Additionally Heineken and Carlsberg described barley plants with combined traits of Null-LOX-1, Null-LOX-2 and NULL-MMT (L-methionine S-methyltransferase) within one plant [158]. Publications on brewing with green malt derived from malting the double-null-LOX-null-MMT cultivar would be highly interesting regarding flavour and flavour stability.

Flavour and aroma compounds

Apart from being an abundant source of starch and enzymes, malt delivers a wide range of flavour and aroma components, such as aldehydes, ketones, alcohols, organic acids and furans, to the wort and final beer [14, 18, 159-162]. The formation of flavour active compounds is largely promoted through thermally driven processes, such as Strecker degradation, Maillard reaction or caramelisation. Thus, if omitting the kilning process the brewer would introduce a grist material with a dissimilar mixture of volatile and non-volatile constituents compared to kilned malt. It is generally accepted that fermentation is the process step that most significantly determines finished beer flavour. However, green malt will most certainly induce subtle flavour changes in beers, principally through its different chemical composition (compared to pale kilned malt), acting as a feedstock for yeast metabolism. As apparent in Table 3, hot water extracts of green malt contained a series of lipid-derived aldehydes and alkenols as well as sulphur compounds, while concentrations in worts prepared from lightly kilned malt, were much lower [14]. Furthermore, traces of 4-vinylphenol and 4-vinylguaiacol were identified in a vacuum distillate of green malt. The final concentrations in beer were, however, far below those produced from phenolic off-flavour (POF+) producing yeasts. Moreover, malt is a major source of aldehydes, as well as aldehyde precursors and

intermediate products (e.g. amino acids, peptides, Schiff bases, bound state aldehydes, *etc*.) [61, 159, 161, 163], which were identified as contributors for stale flavour formation during beer ageing. The most important staling aldehydes comprise i) Maillard reaction aldehydes (e.g. furfural), ii) Strecker degradation aldehydes (e.g. 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde) and iii) fatty acid oxidation aldehydes (e.g. hexanal and *trans*-2-nonenal) [62, 64, 124, 164, 165]. The free aldehyde content in malt, as well as the heat load (TB-Index) were positively correlated with the rate of beer ageing [63]. These aldehydes were shown to increase in concentration during sprouting [159], dependent on germination time and temperature, according to Herrmann et al. [166]. Higher green malt moisture significantly increased the formation of malt volatiles, whereas high germination temperatures, on the other hand, lowered them [166]. Kilning or roasting greatly promotes the formation of Maillard compounds and Strecker aldehydes [19, 60, 61, 64-66, 115, 136, 159, 167, 168]. A wide range of Maillard compounds were determined in kilned, with only furfural, 1-acetylfuran and furfuryl alcohol identified in green malt [14]. LOX enzymes are thermally inactivated, thus a reduction in enzymatic oxidation of unsaturated fatty acids is expected particularly at the onset of the wort production process. However, during malting LOX activity was shown previously [169] to increase mainly in the first 2 – 6 hours of kilning, thus the risk to oxidise lipids remains at moderate kilning temperatures. Dong et al. [159], for example, showed that the *trans*-2-nonenal concentrations greatly increased when producing crystal malts compared to the corresponding green malt. Thus, it might be suggested that less *trans*-2-nonenal is introduced into the brewing process when using green malt. Hexanal and 2-hexenal, which are correlated with green/grassy scent and a bitter, winey flavour [124], on the other hand, were shown to be present in increased levels in green malt and decreased with increasing malt colour [125, 159].

Overall, from the perspective of green malt brewing, less aldehydes are introduced into the brewing process (except hexanal), thus, an improved endogenous ageing potential in green malt beers, may be hypothesised and will be further discussed in the following section (Beer flavour (in)stability factors). Further research is needed to define which of the flavour characteristics of green malt (in comparison to pale kilned malt) survives up- and downstream processing and has a direct (positive or negative) effect on the flavour and flavour stability of the finished beers.

Characteristics of wort and beer made from green malt

Early research on brewing with green malt [11, 13, 14, 16] reported that worts and beers from 100% green malt were perfectly normal in their analytical and physical characteristics. Unfortunately, in these papers no detailed brewing protocol or assessment of the resulting beer flavour or its' stability were published. Since

most of the literature available on brewing with green malt dates back as early as the 1960's, there was great interest for the scientific community for new research [92], particularly as analytical techniques have greatly improved since then.

Wort characteristics

Table 4 displays a summary of some characteristics of worts derived from green malt in comparison to kilned malt wort. According to Macwilliam et al. [12] the worts from green malt were more fermentable than those from kilned malt. This was related to the higher activities of α- and β- amylase in addition to the increased levels of limit dextrinase associated with green malt. Analysis of the wort carbohydrates further confirmed very high values for maltose and maltotriose at the expense of dextrins. Furthermore, proteolysis proceeded further when using green malt mashing than with conventional malts, which explained the high values for both soluble and amino nitrogen (Table 4). The anthocyanogen content was significantly lower in wort prepared from green malt than the control wort, possibly due to the still functioning anthocyanogenase in green malt [12, 68, 106]. The relatively high colour of the green malt wort (8-10 EBC, [12]) compared to the reference (3 EBC) was associated with this increased concentration of amino acids, which caused increased formation of melanoidins during wort production. Recently published brewing trials [92], on the other hand, reported that the colour of worts prepared from green malt was lower than the reference wort. However, EBC colour was not reduced as much as might have been predicted for green malt brews and the yellow colour could have potentially originated from polyphenols or riboflavin present in malt [68].

Interestingly, previous studies [49, 92] concluded that worts from green malt resulted in (expected) elevated DMS precursor levels, but surprisingly low levels of DMS in the pitching worts. White and Wainwright [48], on the other hand, reported DMS levels up to 500 µg/L in green malt pitching wort (no precursor levels reported).

Furthermore, it was found that the use of undried malts can have benefits in terms of hop economy. According to previous research [106, 170], the amount of humulone and isohumulone absorbed on the break of 'unkilned' malt wort was considerably less than on that of kilned malt wort.

Beer characteristics

Table 5 displays a summary of some characteristics of beers derived from green malt in comparison to kilned malt beers. Previous work has confirmed that the EBC colour was not reduced as much as might have been predicted for green malt brews [16, 92]. The origin of the unique pigments, hue and chroma yet

has to be determined. Furthermore, due to the high dimethyl sulphide (DMS) potential, overall DMS levels were expected to be higher compared to the control. Previous research [48, 49, 92] indicated that wort of green malt contains high concentrations of the DMS-precursor S-methyl methionine; however, DMS levels in final beers made of green malt were not higher than in beers prepared from pale kilned malt [48, 49, 92]. It is remarkable to note that the elevated DMS levels (up to 500 µg/L) in worts as reported by White and Wainwright [49] were gradually eliminated with the fermentation gases and resulted in DMS levels in beers closely matched to the control. Thus, it appears that DMS levels in finished beers can be controlled even when using green malt, given a sufficient removal of DMS via evaporation during wort boiling and through elimination of fermentation gases.

Even though these results seem very promising for the successful brewing of green malt there were still some substantial flavour differences that yet must be defined. Early studies [13, 16] who described 100% green malt beer, rather vaguely described their beer brewed from green malt 'green-malt-like'. The intensity of 'green flavour' increasing with increasing malt germination time. The flavour was further described as 'unpredictable', meaning that it was sometimes clean and other times 'green' [13]. The precise nature and sensory stimuli causing this reported 'green' sensation in green malt beers were not defined, but it is likely that these 'green' flavour is related to lipid-derived aldehydes abundantly present in green malt, as discussed previously [14]. Moir et al. [14] detected a grassy, beany taste in beers made of green malt, and proposed that this could have been a result of elevated levels of lipid-derived aldehydes. Although yeast is able to remove these aldehydes by reducing them to their saturated alcohol counterparts, the green grassy, pea-like character remained to some extent in green malt beer, with elevated amounts of 1-hexanol (reduction of hexanal and 2-hexanal) being detected [14]. Another possible route to explain the green flavours are the high number of alkenols found in green malt wort, formed by the action of a thermo-labile reductase. Yeast is not able to reduce the alkenols, which then remain in the beer [14].

Beer flavour (in)stability factors

Without the high temperatures from kilning, the heat load of the malt and future mash, wort and beer will be significantly lower. Additionally, the extra enzymatic potential of green malt, could potentially be suitable for reducing overall mashing process time, and thus implies additional reduction in total heat load. Heat load, expressed as TB-index, was correlated with the formation of beer staling compounds and reduced beer freshness [19, 60, 63, 64]. Thus, as discussed previously (Section: Flavour and aroma compounds) from the perspective of green malt brewing, less aldehydes are introduced into the brewing process (except hexanal). It is generally accepted that these aldehydes are greatly evaporated throughout wort

production (except of furfural)[161] and yeast metabolism can reduce aldehydes in the wort to their corresponding alcohols [171, 172]. However, aldehydes can bind to compounds such as bisulphites [173, 174] amino acids (formation of imines) [112, 173] or cysteine [175-178] during the wort production process, forming non-volatile 'bound-state aldehydes'. The current theory is that during beer storage, under specific conditions (temperature, pH value, redox potential, binding strength, thermodynamic stability), adducts may dissociate and release aldehydes in the free form [66, 112, 171, 173, 174, 177, 179-181]. Naturally, the formation of bound-state aldehydes is very complex, but in principle, fewer aldehydes might be available for adduct formation during the wort production process when using green malt, and thus, an improved endogenous ageing potential in green malt beers, may be hypothesised.

Furthermore, several authors [182-184] suggested that an increased formation of Maillard reaction products was associated with an acceleration of oxidative processes, and thus lower oxidative stability. Kunz et al. showed [185], that when using unmalted barley (lower total heat load) a lower content of specific Maillard reaction products led to a lower radical generation and thus better oxidative stability in the worts and beers measured. Green malt contains fewer Maillard reaction products due to the omission of the heating step, thus better oxidative stability might be expected in green malt wort and beer according to this hypothesis. Moreover, the pool of natural antioxidants which is enhanced in green malt could [186] potentially reduce the formation of free radicals. Hence, from this perspective, green malt wort could have a better oxidative stability than kilned malt wort, provided lipoxygenase activity can be controlled.

Transition metal ions, such as iron, copper and manganese, were identified to play a key role in the oxidative degradation of wort and beer, as they drive formation of reactive oxygen species (ROS) in the absence of antioxidants [187, 188]. Increased heat load on malt through kilning or roasting was shown to impact the content of transition metals with prooxidative effects in the wort [189-192]. Thus, it would be very interesting to further investigate the ionic composition and thus influence on oxidative stability when using green malt as the grist bill.

Furthermore, lower heat loads during brewing have been associated with improved free amino acid (FAN) assimilation during fermentation [193], resulting in lower residual FAN levels in finished beers, which was associated with an improved beer flavour stability.

Overall, green malt appears to be a very promising grist material to produce wort and beer with enhanced flavour stability metrics – provided lipoxygenase activity is controlled.

Industrial application of green malt in the brewing process

Malts with high enzyme contents have always been of interest to the brewer for the conversion of adjuncts in the mash. Thus, green malt could be used to complement both conventional malts and in processes utilising very high proportions of unmalted starch or raw grains [11-13]. Experiments were described, in which green malt was successfully used for brewing acceptable potable stout (20-80% green malt [11]), as proportion to 50% wheat flour [12, 13], 50% raw barley [12, 13] and 75% presteeped barley [12, 13]. Furthermore, Boston Beer Company and Weihenstephan brewery in Freising (Germany), brewed a champagne like beer with around 10% ABV, found on the market under the name Infinium. They developed and patented [194] a brewing process by taking advantage of the high enzymatic activity in green malt to brew a pale coloured beer, with a high alcohol content, strictly under the German Purity law (with no use of exogenous enzymes).

Recent pilot scale brewing trials [92] confirmed that beer without any significant taints of obvious defects can be prepared from 100% green malt. Naturally, the sensory profile and consumer acceptance still needs to be investigated, however, there is great potential for the industry to create a new market for green malt beers, as opposed direct flavour match to existing kilned malt beers. As consumer awareness and thus the demand for energy and water efficient products increases, there is the opportunity to market an environmentally friendly beer.

One widely acknowledged barrier to developing brewing processes using green malt is the instability of the raw material, biochemically and microbially, and thus limitations on its storage. To overcome these factors, malt production sites would need to be in close proximity to, and co-ordinated with, brewery production. Clearly these conditions do not apply to many current brewery sites, which would represent a limitation on the uptake of these approaches. One possible solution to overcome the instability issue would be the production of malt extracts. The traditional approach with malt extract production is to concentrate sweet wort, normally of kilned malt, under reduced pressure. The wort is further concentrated via flash- evaporation. By using green malt rather than kilned malt, this process would be a more economical alternative, due to the omission of kilning. A similar suggestion has been made by Peterreins and Van Waesberghe [84], who suggested the production of wort/malt extract or wort granulates through stabilised green malt. Shortages in mash tun capacity could be compensated for when using malt extract, or it could be used as a source of extract in some small breweries or temporary breweries.

Green malt has also been used in other alcoholic beverage production such as in whisky and vodka distilleries to avoid the cost of kilning and to take advantage of the full range of enzymes that are present.

595 This can assist with the digestion of other starchy materials which form the majority of the grist but are
596 relatively deficient in diastatic enzymes, as is the case in grain whisky production. Coppersea Distilling
597 (New York) for example used rye green malt to produce their first whiskies, while not having a kiln in situ.

598 ## Summary

599 This review placed emphasis on developing enabling technologies for green malt brewing which would
600 lower the carbon footprint of beer production. Successful technical solutions must meet several criteria;
601 they must be widely applicable, profitable to the maltsters and brewers, and, most importantly, must
602 produce beers which taste good and meet consumer expectations for the category. General principles,
603 which could form a basis for future studies on successfully brewing with green malt, are highlighted here:

604 - Green malt should be stored cold and dry to reduce microbial activity and stabilise enzymatic
605 activity until the grain is further processed – the sooner the better. It is either necessary to prepare
606 malt/wort extract or to process rapidly by having a brewery and maltings co-located (transport of
607 the high moisture commodity is not feasible). Longer storage periods of green malt and the
608 associated microbes present could greatly affect malt quality and thus impact beer quality [85-87].
609 - Pre-soaking of the grain increases the extract yield and facilitated milling, suggesting a wet milling
610 system is a suitable technique for milling the green malt [12, 13]. Meura (Belgium) developed in
611 collaboration with Castle Malting (Belgium) the 'hydromill'; a disc mill which finely mills malt
612 under water, designed to process malts with a high moisture content [15, 91]. On small scale,
613 (home)brewers could use an ordinary kitchen meat grinder [93].
614 - The malt and the milling installation should be sparged with CO_2 or N_2 to suppress enzymatic
615 oxidation via LOX enzymes throughout the wet milling process.
616 - Replacing 20% of pilsner malt with green malt to a cereal recipe containing 30% unmalted barley,
617 was shown to significantly improve filterability [15].
618 - The increased moisture content in the grain, means that less brewing water will be needed for
619 mashing. However, the brewing liquor used for mashing needs to be appropriately adjusted (e.g.
620 adjustment of temperature, calcium and lactic acid addition) to account for the extra water in the
621 grain.
622 - According to previous research [12], only 5% of green malt is needed to solubilise and convert the
623 carbohydrate of gelatinised barley. Thus, when brewing with 100% green malt (at optimal
624 technical conditions), it might be necessary to reduce the times spent at the ~62°C (mashing) rests

to strike the proper balance between fermentable and non-fermentable sugars. This could help to build a little more body to the beer.

- The EBC colour of beers produced from green malt was not reduced as much as might have been predicted for green malt brews [12, 16, 92]. Most practically, green malt would be used as a proportion of the grist and would, for example, be accompanied by small complements of roasted malts from which the colours and flavours of different beer styles would most economically be derived.
- Lipoxygenase activity should be controlled in order to avoid an increase in the staling potential of the final beer [39, 43], as well as impaired foam stability [137, 138]. Thus, mashing should be performed under LOX hostile mash conditions: > 62 °C, pH: 5.2, oxygen-free [43, 66, 121, 136, 139].
- LOX-less [137, 138, 142, 143] or Null-LOX [140, 141] barley varieties might offer advantages for brewing with green malt. Particularly in breweries where LOX-hostile conditions cannot be applied or additional costs for CO_2 injection want to be avoided.
- Previous research indicated that wort of green malt contains high concentrations of DMS-precursor, however, against expectations DMS levels in beer made of green malt were not higher than beers made of pale lager malt [48, 49, 92]. It appears that DMS levels in pitching wort can be controlled even when using green malt, given a sufficient removal of DMS via evaporation during wort boiling and through elimination in fermentation gases.
- The use of a wort stripper, could help to remove excessive DMS [156], and also purge other undesired volatiles, such as lipid-derived aldehydes or alkenols (grassy, beany taste) resulting from green malt [14]. In addition, an oversized chimney with condensate trap supports the removal and similarly prevents re-entrance of unwanted volatiles (e.g. DMS) during wort boiling [157].
- Additionally, Heineken and Carlsberg described barley plants with combined traits of Null-LOX-1, Null-LOX-2 and NULL-MMT (L-methionine S-methyltransferase) within one plant [158]. This barley variety avoids both LOX related off-flavour development but also suppresses DMS formation.
- The heat load of the malt and future mash, wort and beer made of green malt is significantly lower than for kilned malt beers and thus, an improved endogenous ageing potential in green malt beers, may be hypothesised:
    - Reduction of free staling aldehydes in the malt (particularly Maillard compounds and Strecker aldehydes) [19, 60, 61, 64-66, 115, 136, 159, 167, 168].
    - Lower chance of aldehyde adduct formation during the wort production process and thus lower release of free aldehydes during beer storage (?)

- The reduced content of specific Maillard reaction products in green malt could lower radical generation and thus improve oxidative stability in the worts and beers [182-185]
- Improved free amino acid (FAN) assimilation during fermentation, hence lower residual FAN levels in the finished beers [193].
- Furthermore, kilning or roasting was shown to impact the content of transition metal ions with prooxidative effects in the wort [189-192]. Thus, it would be very interesting to further investigate the transition metal ion composition in wort and thus influence on oxidative stability when using green malt as the grist bill.

Future work

Brewing with green malt represents a disruptive technology and multiple factors need to be considered to successfully brew with this 'novel' grist material. Most importantly, future research should focus on maximising the efficiency of brewing operations with green malt and the production of beers which are palatable for the consumer. This implies firstly the development of optimal processing of green malt, without any yield losses during filtration and sparging of the green malt 'cake'. Potentially, by technical optimisation of the milling system, or adjustment of the liquor to grist ratio, thus achieving a mash with optimal composition for the mash filter, these challenges can be overcome, and yield can be maximised. Furthermore, green malt beers should be subjected to detailed sensory analysis to collect flavour descriptors from a professional sensory panel, as well as consumer response. Brewing a pale lager-style beer with 100% green malt is most challenging technically. Most practically green malt could be used as a proportion of the grist and would, for example, be accompanied by small complements of kilned or roasted malts from which the colours and flavours of different beer styles would most economically be derived.

Conclusions

Despite all of the (theoretical) biochemical issues associated with green malt brewing, several researchers [11, 13, 14, 16, 92] have reported that beers brewed from 100% green malt were perfectly normal in their analytical and physical characteristics. The operating environment for the sector will increasingly necessitate that all options to improve the overall carbon footprint of malting and brewing are evaluated; either by using alternative low carbon fuels, novel drying technologies or by partially replacing kilned malt with green malt. Additionally, de-carbonising and reduction of primary energy usage can sit alongside adoption of green malt in future strategy to meet environmental targets. Nowadays, consumers are increasingly motivated to be more environmentally conscious and are exercising their power and voice

687 through the products they buy. This may develop a market for green malt beers based around perceptions
688 of their being environmentally friendly. As has been the case with the growing low or non-alcoholic beer
689 market, the perceived benefits associated with a category may mean that a group of consumers are willing
690 to accept, or even promote the virtues of a green malt beer, even where there is not a direct colour or
691 flavour match with existing products. Moreover, brewing with green malt has the potential to advance
692 current knowledge of the impacts of raw materials on beer staling, particularly regarding the impacts of
693 malt kilning on downstream flavour (in)stability.

Author Contributions

Celina Dugulin: PhD student. Research and writing – original draft of this review.

Gert De Rouck: Funding acquisition, conceptualisation and writing – review and editing.

David Cook: Funding acquisition, supervision of PhD, conceptualisation and writing – review and editing.

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie gran agreement No. 722166.

The authors declare there are no conflicts of interest.

Word Count

15406

References

1. United Nations World Comission on Environment and Development. 1987. Our Common Future. Oxford University Press:383.

2. Euromalt. 2017. Malt Production Capacity from 2012 to 2017. http://www.euromalt.be/list_infos/euromalt%20statistics/1011306087/list1353668317.html (last accessed August, 2020)

3. Muntons. 2019. Sustainability at Muntons. https://www.psmuntons.com/wp-content/uploads/2019/04/190424-Sustainability-Statement-2019.pdf (last accessed September, 2019)

4. CarbonTrust. 2011. Industrial Energy Efficiency Accelerator - Guide to the maltings sector. Malting Sector Guide CTG053.

5. Buchs M, Sylke S. 2013. UK Households' Carbon Footprint: A Comparison of the Association between Household Characteristics and Emissions from Home Energy, Transport and Other Goods and Services. Journal 2020.

6. Ferrari-John RS, Katrib J, Zerva E, Davies N, Cook DJ, Dodds C, Kingman S. 2017. Electromagnetic Heating for Industrial Kilning of Malt: a Feasibility Study. Food and Bioprocess Technology 10:687-698. https://doi.org/10.1007/s11947-016-1849-0.

7. Müller C, Kleinwächter M, Selmar D, Methner FJ. 2013. The influence of elevated steeping temperatures on the resulting malt homogeneity and malt quality. BrewingScience 66:114-122.

8. Huang X, Han Y, Yu S. 2004. The effect of steeping conditions on Arapiles malting. Monatsschrift für Brauwissenschaft 57:13-15.

9. Jones D, Lelyveld TP, Mavrofidis SD, Kingman S, Miles N. 2002. Microwave heating applications in enviromnental engineering - a review. Resources, Conservation & Recycling 34:75-90. https://doi.org/10.1016/S0921-3449(01)00088-X.

10. Davies N. 2010. Cutting the Carbon Footprint of Malting by 75%--Achievable or Just Hot Air? Tech Q Master Brew Assoc Am 47:23-28. https://doi.org/10.1094/tq-47-4-0902-01.

11. Duff SR. 1963. Use of green malt in brewing. Journal of the Institute of Brewing 69:249-252. https://doi.org/10.1002/j.2050-0416.1963.tb01925.x.

12. MacWilliam IC, Hudson JR, Whitear AL. 1963. Wort from green malt and unmalted cereals. Journal of the Institute of Brewing 69:303-308. https://doi.org/10.1002/j.2050-0416.1963.tb01930.x.

13. Cook AH, Hudson JR. 1964. New Possibilities in Grists for Brewing. Proceedings Annual meeting - American Society of Brewing Chemists 22:29-33. https://doi.org/10.1080/00960845.1964.12006733.

14. Moir M. 1992. The 1990 laurence bishop silver medal lecture*the desideratum for flavour control. Journal of the Institute of Brewing 98:215-220. https://doi.org/10.1002/j.2050-0416.1992.tb01107.x.

15. Leclercq G. 2020. Einsatz von Grünmalz - der Enzymkick. Brauwelt 19:498-500.

16. MacWilliam IC. 1972. Effect of kilning on malt starch and on the dextrin content of resulting worts and beers. Journal of the Institute of Brewing 78:76-81. https://doi.org/10.1002/j.2050-0416.1972.tb03433.x.

17. Briggs DE. 1998. The biochemistry of malting, 163, 192. Malts and Malting. Blackie Academic & Professional, London, UK.

18. Yahya H, Linforth RST, Cook DJ. 2014. Flavour generation during commercial barley and malt roasting operations: A time course study. Food Chemistry 145:378-387. https://doi.org/10.1016/j.foodchem.2013.08.046.

19. Huang S, Yu J, Yin H, Lu J, Dong J, Li X, Hu S, Liu J. 2016. Optimization of kilning progress for equilibrating multiple parameters that strictly affect malt flavour and sensory evaluation. Journal of the Institute of Brewing 122:706-713. https://doi.org/10.1002/jib.369.

20. Woonton BW, Jacobsen JV, Sherkat F, Stuart IM. 2005. Changes in Germination and Malting Quality During Storage of Barley. Journal of the Institute of Brewing 111:33-41. https://doi.org/10.1002/j.2050-0416.2005.tb00646.x.

21. Briggs DE, Woods J. 1993. Dormancy in malting barley: studies on drying, storage, biochemistry and physiology. HGCA Project Report (United Kingdom).

22. Guiga W, Boivin P, Ouarnier N, Fournier F, Fick M. 2008. Quantification of the inhibitory effect of steep effluents on barley germination. Process Biochemistry 43:311-319. https://doi.org/10.1016/j.procbio.2007.12.001.

23. EUREKA SWAN Project. 2006. EUREKA SWAN - Reduction of water use in the EU Malting Industry. http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=12449 (last accessed June, 2020)

24. Briggs DE. 1986. Accelerating Malting: A Review of Some Lessons of the Past from the United Kingdom. Journal of the American Society of Brewing Chemists 45:1-6. https://doi.org/10.1094/ASBCJ-45-0001.

25. Brookes PA, Lovett DA, Macwilliam IC. 1976. The steeping of barley. A review of the metabolic consequences of water uptake, and their practical implications. Journal of the Institute of Brewing 82:14-26. https://doi.org/10.1002/j.2050-0416.1976.tb03716.x.

26. Turner HM, Elmore L, Walling J, Lachowiec J, Mangel D, Fischer A, Sherman J. 2019. Effect of Steeping Regime on Barley Malt Quality and Its Impacts on Breeding Program Selection. Journal of the American Society of Brewing Chemists 77:267-281. https://doi.org/10.1080/03610470.2019.1629794.

27. Johnston JHS. 1954. Physical factors affecting the kilning of barley and malt. Journal of the Institute of Brewing 60:318-340. https://doi.org/10.1002/j.2050-0416.1954.tb02759.x.

28. Hertrich J. 2013. Topics in Brewing: Malting. Tech Q Master Brew Assoc Am 50:131-141.

29. Mallett J. 2014. From barley to malt. Malt: A practical guide from field to brewhouse. Brewers Publications, Boulder, Colorado, USA.

30. Rennie H, Ball K. 1979. The influence of malt storage on wort separation. Journal of the Institute of Brewing 85:247-249. https://doi.org/10.1002/j.2050-0416.1979.tb03917.x.

31. Kunze W. 2014. Technology; Brewing and Malting, VLB Berlin,Berlin, Germany.

32. Bamforth CW, Roza JR, Kanauchi M. 2009. Storage of Malt, Thiol Oxidase, and Brewhouse Performance. Journal of the American Society of Brewing Chemists 67:89-94. https://doi.org/10.1094/ASBCJ-2009-0219-01.

33. Doug S. 2010. Emissions, energy, water and malt. The carbon conundrum in the maltings. Brewer & Distiller International:38-41.

785 34. Mauthner F, Hubmann M, Christoph B, Fink C. 2014. Manufacture of malt and beer with low
786 temperature solar process heat. Energy Procedia 48:1188-1193.
787 https://doi.org/10.1016/j.egypro.2014.02.134.

788 35. Brudzynski A, Roginski H. 1969. Comparative studies of kilned and freeze-dried malts. Journal of the
789 Institute of Brewing 75:472-476. https://doi.org/10.1002/j.2050-0416.1969.tb06385.x.

790 36. Djekic I, Tomic N, Bourdoux S, Spilimbergo S, Smigic N, Udovicki B, Hofland G, Devlieghere F, Rajkovic
791 A. 2018. Comparison of three types of drying (supercritical CO2, air and freeze) on the quality of dried
792 apple – Quality index approach. LWT - Food Science and Technology 94:64-72.
793 https://doi.org/10.1016/j.lwt.2018.04.029.

794 37. Ratti C. 2001. Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering
795 49:311-319. https://doi.org/10.1016/S0260-8774(00)00228-4.

796 38. Hugues M, Boivin P, Gauillard F, Nicolas J, Thiry J, Richardforget F. 1994. Two lipoxygenases from
797 germinated barley - heat and kilning stability. Journal of Food Science 59:885-889.
798 https://doi.org/10.1111/j.1365-2621.1994.tb08150.x.

799 39. Yang G, Schwarz P. 1995. Activity of lipoxygenase isoenzymes during malting and mashing Journal of
800 the American Society of Brewing Chemists 53:45-49. https://doi.org/10.1094/ASBCJ-53-0045.

801 40. Kuroda H, Furusho S, Maeba H, Takashio M. 2003. Characterization of factors involved in the
802 production of 2(E)-nonenal during mashing. Bioscience, Biotechnology and Biochemistry 67:691-697.
803 https://doi.org/10.1271/bbb.67.691.

804 41. Yang G, Schwarz P, Vick BA. 1993. Purification and characterization of lipoxygenase isoenzymes in
805 germinating barley Cereal Chemistry 70:589-595.

806 42. De Buck A, Aerts G, Bonte S, Dupire S. 1995. Rapid evaluation of the two lipoxygenase isoenzyme
807 activities in malt. Proc Eur Brew Conv Cong, Brussels, IRL Press, Oxford, 209-216.

808 43. De Buck A, Aerts G, Bonte S, Dupire S, Van den Eynde E. 1997. Relation between lipoxygenase
809 extraction during brewing, reducing capacity of the wort and the organoleptical stability of beer. Proc
810 Eur Brew Conv Cong, Maastricht, IRL Press, Oxford, 333-340.

811 44. Ye H, Harasymow S, Zhang X-Q, Paynter B, Wu D, Jones M, Shu X, Li C. 2014. Sequence variation and
812 haplotypes of lipoxygenase gene LOX-1 in the Australian barley varieties. BMC Genetics 15:36-36.
813 https://doi.org/10.1186/1471-2156-15-36.

814 45. Doderer A, Kokkelink I, van der Veen S, Valk BE, Schram A, Douma AC. 1992. Purification and
815 characterization of two lipoxygenase isoenzymes from germinating barley. Biochimica et Biophysica Acta
816 1120:97-104. https://doi.org/10.1016/0167-4838(92)90429-H.

817 46. Baxter ED. 1982. Lipoxidases in Malting and Mashing. Journal of the Institute of Brewing 88:390-396.
818 https://doi.org/10.1002/j.2050-0416.1982.tb04130.x.

819 47. Anness BJ, Bamforth CW. 1982. Dimethyl sulphide - A review. Journal of the Institute of Brewing
820 88:244-252. https://doi.org/10.1002/j.2050-0416.1982.tb04101.x.

821 48. White FH, Wainwright T. 1976. Isolation and partial characterization of the dimethyl sulphide
822 precursor in green malt and its effect on beer dimethyl sulphide levels. Journal of the Institute of
823 Brewing 82:292-296. https://doi.org/10.1002/j.2050-0416.1976.tb03777.x.

824 49. White FH, Wainwright T. 1977. The presence of two dimethyl sulphide precursors in malt, their
825 control by malt kilning conditions, and their effect on beer DMS levels. Journal of the Institute of
826 Brewing 83:224-230. https://doi.org/10.1002/j.2050-0416.1977.tb03799.x.

827 50. Yang B, Schwarz P, Horsley R. 1998. Factors involved in the formation of two precursors of
828 dimethylsulfide during malting. Journal of the American Society of Brewing Chemists 56:85-92.
829 https://doi.org/10.1094/ASBCJ-56-0085.

830 51. White FH, Wainwright T. 1976. The measurement of dimethyl sulphide precursor in malts, adjuncts,
831 worts and beer. Journal of the Institute of Brewing 82:46-48. https://doi.org/10.1002/j.2050-
832 0416.1976.tb03721.x.

833 52. Yang B, Schwarz PB. 1998. Application of Nitrogen-Purging of Malt Extracts to Measure Two
834 Dimethylsulfide Precursors by Headspace Gas Chromatography. Journal of the American Society of
835 Brewing Chemists 56:81--84. https://doi.org/10.1094/ASBCJ-56-0081.

836 53. Bamforth CW, Martin HL. 1983. The degradation of β-glucan during malting and mashing: the role of
837 β-glucanase. Journal of the Institute of Brewing 89:303-307. https://doi.org/10.1002/j.2050-
838 0416.1983.tb04190.x.

839 54. Hämäläinen JJ, Reinikainen P. 2007. A simulation model for malt enzyme activities in Kilning. Journal
840 of the Institute of Brewing 113:159-167. https://doi.org/10.1002/j.2050-0416.2007.tb00273.x.

841 55. Barber MG, Jackson EA, Smith DB. 1994. Total and individual barley (1–3), (1–4)-β-D-glucanase
842 activities in some green and kilned malts. Journal of the Institute of Brewing 100:91-97.
843 https://doi.org/10.1002/j.2050-0416.1994.tb00812.x.

844 56. Dugulin CA, Clegg SC, De Rouck G, Cook DJ. 2020. Overcoming technical barriers to brewing with
845 green (non-kilned) malt: a feasibility study. Journal of the Institute of Brewing 126:24-34.
846 https://doi.org/10.1002/jib.602.

847 57. Sopanen T, Laurière C. 1989. Release and Activity of Bound beta-Amylase in a Germinating Barley
848 Grain. Plant Physiology 89:244-249. https://doi.org/10.1104/pp.89.1.244.

849 58. Schroeder SW, MacGregor AW. 1998. Synthesis of Limit Dextrinase in Germinated Barley Kernels and
850 Aleurone Tissues. Journal of the American Society of Brewing Chemists 56:32-37.
851 https://doi.org/10.1094/ASBCJ-56-0032.

852 59. Anness BJ, Bamforth CW, Wainwright T. 1979. The measurement of dimethyl sulphoxide in barley
853 and malt and its reduction to dimethyl sulphide by yeast. Journal of the Institute of Brewing 85:346-349.
854 https://doi.org/10.1002/j.2050-0416.1979.tb03940.x.

855 60. Gastl M, Spieleder E, Hermann M, Thiele F, Burberg F, Kogin A, Ikeda H, Back W, Narziss L. 2006. The
856 influence of malt quality and malting technology on the flavour stability of beer. Monatsschrift für
857 Brauwissenschaft 59:163-175.

858 61. De Clippeleer J, De Rouck G, De Cooman L, Aerts G. 2010. Influence of the hopping technology on the
859 storage-induced appearance of staling aldehydes in beer. Journal of the Institute of Brewing 116:381-
860 398. https://doi.org/10.1002/j.2050-0416.2010.tb00789.x.

861 62. Malfliet S, Van Opstaele F, de Clippeleer J, Syryn E, Goiris K, de Cooman L, Aerts G. 2008. Flavour
862 instability of pale lager beers: determination of analytical markers in relation to sensory ageing. Journal
863 of the Institute of Brewing 114:180-192. https://doi.org/10.1002/j.2050-0416.2008.tb00324.x.

864 63. Jaskula-Goiris B, De Causmaecker B, De Rouck G, Aerts G, De Cooman L. 2015. Influence of malt
865 specification on beer quality and flavour stability. Proceedings of the XV Chair De Clerck, Belgium, Gent.

64. Baert JJ, De Clippeleer J, Hughes PS, De Cooman L, Aerts G. 2012. On the origin of free and bound staling aldehydes in beer. Journal of Agricultural and Food Chemistry 60:11449-11472. https://doi.org/10.1021/jf303670z.

65. Vanderhaegen B, Neven H, Verachtert H, Derdelinckx G. 2006. The chemistry of beer aging - A critical review. Food Chemistry 95:357-381. https://doi.org/10.1016/j.foodchem.2005.01.006.

66. Drost BW, van den Berg R, Freijee FJM, van der Velde EG, Hollemans M. 1990. Flavor stability. Journal of the American Society of Brewing Chemists 48:124 - 131. https://doi.org/10.1094/ASBCJ-48-0124.

67. Trinci APJ. 1972. Culture turbidity as a measure of mould growth. Transactions of the British Mycological Society 58:467-IN414. https://doi.org/10.1016/S0007-1536(72)80095-0.

68. Briggs DE, Stevens R, Young TW, Hough JS. 1981. Malting and Brewing Science: Malt and Sweet Wort, Chapman & Hall,London.

69. Salama A-RA, El-Sahn MA, Mesallam AS, Shehata AME-T. 1997. The chemical composition, the nutritive value and the functional properties of malt sprout and its components (acrospires, rootlets and husks). Journal of the Science of Food and Agriculture 75:50-56. https://doi.org/10.1002/(SICI)1097-0010(199709)75:1<50::AID-JSFA833>3.0.CO;2-0.

70. Bonnely S, Peyrat-Maillard MN, Rondini L, Masy D, Berset C. 2000. Antioxidant activity of malt rootlet extracts. Journal of Agricultural and Food Chemistry 48:2785-2792. https://doi.org/10.1021/jf990793c.

71. Peyrat-Maillard MN, Bonnely S, Rondini L, Berset C. 2001. Effect of Vitamin E and Vitamin C on the Antioxidant Activity of Malt Rootlets Extracts. LWT - Food Science and Technology 34:176-182. https://doi.org/10.1006/fstl.2001.0752.

72. Meng D-J, Lu J, Fan W, Dong J-J, Zhang J, Kong W-B, Lin Y, Shan L-J. 2009. Optimization of extraction conditions for antioxidant phenolic compounds from malt rootlets using response surface methodology. Journal of Food Biochemistry 33:291-305. https://doi.org/10.1111/j.1745-4514.2009.00218.x.

73. Vriesekoop F, Krahl M, Hucker B, Menz G. 2012. 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly. Journal of the Institute of Brewing 118:335-345. https://doi.org/10.1002/jib.49.

74. Vaughan A, O'Sullivan T, Sinderen D. 2005. Enhancing the Microbiological Stability of Malt and Beer — A Review. Journal of the Institute of Brewing 111:355-371. https://doi.org/10.1002/j.2050-0416.2005.tb00221.x.

75. Flannigan B. 1996. The microflora of barley and malt, 83-125. In: Priest FG,Campbell I, eds. Brewing Microbiology. Springer US, Boston, MA.

76. Briggs DE. 1978. Weeds, pests and diseases in the growing crop, 339-368. In: Briggs DE, ed. Barley. Springer, Dordrecht, Netherlands.

77. Schwarz PB, Jones BL, Steffenson BJ. 2002. Enzymes associated with Fusarium infection of barley. Journal of the American Society of Brewing Chemists 60:130-134. https://doi.org/10.1094/ASBCJ-60-0130.

78. Sheneman JM, Hollenbeck CM. 1960. Microbial patterns in malt. Lactic acid bacteria. Proceedings Annual meeting - American Society of Brewing Chemists 18:22-27. https://doi.org/10.1080/00960845.1960.12006892.

907 79. Douglas PE, Flannigan B. 1988. A Microbiological Evaluation of Barley Malt Production. Journal of the
908 Institute of Brewing 94:85-88. https://doi.org/10.1002/j.2050-0416.1988.tb04562.x.

909 80. O'Sullivan TF, Walsh Y, O'Mahony A, Fitzgerald GF, Sinderen D. 1999. A Comparative Study of
910 Malthouse and Brewhouse Microflora. Journal of the Institute of Brewing 105:55-61.
911 https://doi.org/10.1002/j.2050-0416.1999.tb00006.x.

912 81. Petters HI, Flannigan B, Austin B. 1988. Quantitative and qualitative studies of the microflora of
913 barley malt production. Journal of Applied Bacteriology 65:279-297. https://doi.org/10.1111/j.1365-
914 2672.1988.tb01895.x.

915 82. Briggs DE, McGuinness G. 1993. Microbes on barley grains. Journal of the Institute of Brewing
916 99:249-255. https://doi.org/10.1002/j.2050-0416.1993.tb01168.x.

917 83. Flannigan B, Okagbue RN, Khalid R, Teoh CK. 1982. Mould flora of malt in production and storage.
918 Brewer & Distiller International 31.

919 84. Peterreins F, Van Waesberghe J. 2003. *Production of wort/malt extract or wort granulates/malt*
920 *extract granulates through stabilized green malt*. WO/2003/048294.

921 85. Justé A, Malfliet S, Lenaerts M, De Cooman L, Aerts G, Willems K, Lievens B. 2011. Microflora during
922 malting of Barley: Overview and Impact on malt quality. BrewingScience 64:22-31.

923 86. Scott PM. 1996. Mycotoxins transmitted into beer from contaminated grains during brewing. The
924 Journal of AOAC International 79:875-882.

925 87. Bokulich NA, Bamforth CW. 2013. The Microbiology of Malting and Brewing. Microbiology and
926 Molecular Biology Reviews 77:157-172. https://doi.org/10.1128/mmbr.00060-12.

927 88. Caplice E, Fitzgerald GF. 1999. Food fermentations: role of microorganisms in food production and
928 preservation. International Journal of Food Microbiology 50:131-149. https://doi.org/10.1016/S0168-
929 1605(99)00082-3.

930 89. Benthin S, Villadsen J. 1995. Different inhibition of Lactobacillus delbrueckii subsp. bulgaricus by D-
931 and L-lactic acid: effects on lag phase, growth rate and cell yield. Journal of Applied Bacteriology 78:647-
932 654. https://doi.org/10.1111/j.1365-2672.1995.tb03111.x.

933 90. Ross RP, Morgan S, Hill C. 2002. Preservation and fermentation: past, present and future.
934 International Journal of Food Microbiology 79:3-16. https://doi.org/10.1016/S0168-1605(02)00174-5.

935 91. Meura. Hydromill. https://www.meura.com/products/hydromill.html (last accessed July, 2020)

936 92. Dugulin CA, Acuña Muñoz LM, Buyse J, De Rouck G, Bolat I, Cook DJ. 2020. Brewing with 100% green
937 malt - process development and key quality indicators. Journal of the Institute of Brewing 126:343-353.
938 https://doi.org/10.1002/jib.620.

939 93. Garshol LM. 2020. Malt, 78. Historical Brewing Techniques: The Lost Art of Farmhouse Brewing.
940 Brewers Publications, Boulder, Colorado, USA.

941 94. Preece IA. 1948. Some aspects of stability of brewery malt α-amylase. Journal of the Institute of
942 Brewing 54:141-147. https://doi.org/10.1002/j.2050-0416.1948.tb01367.x.

943 95. Henson CA, Duke SH, Vinje MA. 2014. A Comparison of Barley Malt Amylolytic Enzyme
944 Thermostabilities and Wort Sugars Produced during Mashing. Journal of the American Society of
945 Brewing Chemists 72:51-65. https://doi.org/10.1094/ASBCJ-2014-0130-01.

96. Sissons M, Taylor M, Proudlove M. 1995. Barley Malt Limit Dextrinase: Its Extraction, Heat Stability, and Activity During Malting and Mashing. Journal of the American Society of Brewing Chemists 53:104-110. https://doi.org/10.1094/ASBCJ-53-0104.

97. Evans D, Wallace W, Lance R, McLeod LC. 1997. Measurement of beta-amylase in malting barley (Hordeum vulgare L.). Part 2. The effect of germination and kilning. Journal Of Cereal Science 26:241-250. https://doi.org/10.1006/jcrs.1997.0120.

98. Trummer J, Poreda A, Berski W. 2019. The effect of malting on green lentils and their suitability for wort production. Przemysł Fermentacyjny i Owocowo-Warzywny 63:14-18. https://doi.org/10.15199/64.2019.10.1.

99. Jin Y-L, Speers RA, Paulson AT, Stewart RJ. 2004. Effect of β-Glucans and Process Conditions on the Membrane Filtration Performance of Beer. Journal of the American Society of Brewing Chemists 62:117-124. https://doi.org/10.1094/ASBCJ-62-0117.

100. Bamforth CW, Martin HL. 1981. Beta-glucan and beta-glucan solubilase in malting and mashing. Journal of the Institute of Brewing Institute of Brewing (Great Britain) 87:365-371. https://doi.org/10.1002/j.2050-0416.1981.tb04052.x.

101. Bamforth CW. 1999. Beer Haze. Journal of the American Society of Brewing Chemists 57:81-90. https://doi.org/10.1094/ASBCJ-57-0081.

102. Speers RA, Jin Y-L, Paulson AT, Stewart RJ. 2003. Effects of β-Glucan, Shearing and Environmental Factors on the Turbidity of Wort and Beer. Journal of the Institute of Brewing 109:236-244. https://doi.org/10.1002/j.2050-0416.2003.tb00164.x.

103. Back JF, Oakenfull D, Smith MB. 1979. Increased thermal stability of proteins in the presence of sugars and polyols. Biochemistry 18:5191-5196. https://doi.org/10.1021/bi00590a025.

104. De Rouck G, Jaskula B, De Causmaecker B, Malfliet S, Van Opstaele F, De Clippeleer J, De Brabanter J, De Cooman L, Aerts G. 2013. The influence of very thick and fast mashing conditions on wort composition. Journal of the American Society of Brewing Chemists 71:1-14. https://doi.org/10.1094/ASBCJ-2013-0113-01.

105. O'Rourke T. 2015. Enzymes in Brewing. Brewer and Distiller International October:28-34.

106. Griffin OT, Collier JA, Shields PD. 1968. The development of liquid malt. Journal of the Institute of Brewing 74:154-163. https://doi.org/10.1002/j.2050-0416.1968.tb03109.x.

107. Wettstein D, Jende-Strid B, Ahrenst-Larsen B, Erdal K. 1980. Proanthocyanidin-free barley prevents the formation of beer haze. Tech Q Master Brew Assoc Am 17:16-23.

108. Curtis NS. 1966. Head and haze - A review. Journal of the Institute of Brewing 72:240-250. https://doi.org/10.1002/j.2050-0416.1966.tb02958.x.

109. Wackerbauer K, Meyna S. 2002. Freie und triglycerid-gebundene Hydroxyfettsaeuren in Gerste und Malz I. Einfluss von Sorte, Provenienz und Erntejahr. Monatsschrift für Brauwissenschaft 55:52-57.

110. Wackerbauer K, Meyna S. 2002. Freie und triglycerid-gebundene Hydroxyfettsaeuren in Gerste und Malz II. Die Entwicklung waehrend des Maelzungsprozesses. Monatsschrift für Brauwissenschaft 55:110-114.

111. Wackerbauer K, Meyna S, Marre S. 2003. Hydroxy fatty acids as indicators for ageing and the influence of oxygen in the brewhouse on the flavour stability of beer. Monatsschift für Brauwissenschaft 56:174-178.

987 112. Liégeois C, Meurens N, Badot C, Collin S. 2002. Release of deuterated (E)-2-nonenal during beer
988 aging from labeled precursors synthesized before boiling. Journal of Agricultural and Food Chemistry
989 50:7634-7638. https://doi.org/10.1021/jf020617v.

990 113. Kobayashi N, Kaneda H, Kuroda H, Watari J, Kurihara T, Shinotsuka K. 2000. Behavior of mono-, di-,
991 and trihydroxyoctadecenoic acids during mashing and methods of controlling their production. J Biosci
992 Bioeng 90:69-73. https://doi.org/10.1016/S1389-1723(00)80036-5.

993 114. Kuroda H, Kobayashi N, Kaneda H, Watari J, Takashio M. 2002. Characterization of Factors That
994 Transform Linoleic Acid into Di- and Trihydroxyoctadecenoic Acids in Mash. J Biosci Bioeng 93:73-77.
995 https://doi.org/10.1263/jbb.93.73.

996 115. Guido L, Boivin P, Benismail N, R. Gonçalves C, A. Barros A. 2005. An early development of the
997 nonenal potential in the malting process. European Food Research and Technology 220:200-206.
998 https://doi.org/10.1007/s00217-004-1079-y.

999 116. Kobayashi N, Kaneda H, Kano Y, Koshino S. 1993. The production of linoleic and linolenic acid
1000 hydroperoxides during mashing. Journal of Fermentation and Bioengineering 76:371-375.
1001 https://doi.org/10.1016/0922-338X(93)90024-3.

1002 117. Bamforth CW, Lentini A. 2009. The flavor instability of beer, 85-109. Beer: A Quality Perspective

1003 118. Kobayashi N, Kaneda H, Kano Y, Koshino S. 1994. Behavior of Lipid Hydroperoxides During Mashing.
1004 Journal of the American Society of Brewing Chemists 52:141-145. https://doi.org/10.1094/ASBCJ-52-
1005 0141.

1006 119. Kobayashi N, Kaneda H, Kuroda H, Kobayashi M, Kurihara T, Watari J, Shinotsuka K. 2000.
1007 Simultaneous Determination of Mono-, Di-, and Trihydroxyoctadecenoic Acids in Beer and Wort. Journal
1008 of the Institute of Brewing 106:107-110. https://doi.org/10.1002/j.2050-0416.2000.tb00046.x.

1009 120. Santos, Jr., Carneiro, Jr., Guido L, Almeida P, Rodrigues J, Barros AA. 2003. Determination of E-2-
1010 nonenal by high-performance liquid chromatography with UV detection - Assay for the evaluation of
1011 beer ageing. Journal of Chromatography A 985:395-402. https://doi.org/10.1016/S0021-9673(02)01396-
1012 1.

1013 121. Van Waesberghe J, Aerts G, De Cooman L. 2001. Flavour stability starts with malt and
1014 compensations in the brewhouse for LOX and aldehydes in malt. EBC symposium on Flavour and Flavour
1015 Stability, Nancy, France, Fachverlag Hans Carl, Nürnberg, Germany, 1-16.

1016 122. Meilgaard MC, Dalgliesh CE, Clapperton JF. 1979. Beer Flavour Terminology. Journal of the Institute
1017 of Brewing 85:38-42. https://doi.org/10.1002/j.2050-0416.1979.tb06826.x.

1018 123. Jamieson AM, Van Gheluwe JEA. 1970. Identifcation of a compound responsible for cardboard
1019 flavor in beer. Proceedings Annual meeting - American Society of Brewing Chemists 28:192-197.
1020 https://doi.org/10.1080/00960845.1970.12006980.

1021 124. Saison D, Vanbeneden N, De Schutter DP, Daenen L, Mertens T, Delvaux F, Delvaux FR. 2010.
1022 Characterisation of the flavour and the chemical composition of lager beer after ageing in varying
1023 conditions. BrewingScience 63:41--53.

1024 125. Coghe S, Martens E, D'Hollander H, Dirinck PJ, Delvaux FR. 2004. Sensory and Instrumental Flavour
1025 Analysis of Wort Brewed with Dark Specialty Malts. Journal of the Institute of Brewing 110:94-103.
1026 https://doi.org/10.1002/j.2050-0416.2004.tb00188.x.

1027 126. Anness BJ. 1984. Lipids of barley, malt and adjuncts. Journal of the Institute of Brewing 90:315-318.
1028 https://doi.org/10.1002/j.2050-0416.1984.tb04282.x.

127. Anness BJ, Reed RJR. 1985. Lipids in the brewery - A material balance. Journal of the Institute of Brewing 91:82-87. https://doi.org/10.1002/j.2050-0416.1985.tb04310.x.

128. Yabuuchi S. 1976. Occurrence of a New Lipoxygenase Isoenzyme in Germinating Barley Embryos. Agricultural and Biological Chemistry 40:1987-1992. https://doi.org/10.1080/00021369.1976.10862346.

129. Holtman WL, Vredenbregt-Heistek JC, Schmitt NF, Feussner I. 1997. Lipoxygenase-2 oxygenates storage lipids in embryos of germinating barley. European Journal of Biochemistry 248:452-458. https://doi.org/10.1111/j.1432-1033.1997.00452.x

130. Van Mechelen JR, Schuurink RC, Smits M, Graner A, Douma AC, Sedee NJ, Schmitt NF, Valk BE. 1999. Molecular characterization of two lipoxygenases from barley. Plant Molecular Biology 39:1283-1298. https://doi.org/10.1023/A:1006118003998.

131. Franke W, Frehse H. 1953. Zur Autoxydation der ungesättigten Fettsäuren. VI. Über die Lipoxydase der Gramineen, im besonderen der Gerste. Journal 295:333. https://doi.org/10.1515/bchm2.1953.295.1.333.

132. Fornaroli S, Petrussa E, Braidot E, Vianello A, Macrì F. 1999. Purification of a plasma membrane-bound lipoxygenase from soybean cotyledons. Plant Science 145:1-10. https://doi.org/10.1016/S0168-9452(99)00066-7.

133. Fenzl V, Schönberger ØL. 2018. Improved Method for Measuring Lipoxygenase Activity in Barley and Malt. Journal of the American Society of Brewing Chemists 76:24-28. https://doi.org/10.1080/03610470.2017.1402583.

134. Boudnitskaya EV, Borisova IG. 1972. Investigation of lipoxygenase functions in chloroplasts and mitochondria from Pisum sativum seedlings. FEBS Letters 24:359-362.

135. Braidot E, Petrussa E, Micolini S, Tubaro F, Vianello A, Macrì F. 2004. Biochemical and immunochemical evidences for the presence of lipoxygenase in plant mitochondria. Journal of Experimental Botany 55:1655-1662. https://doi.org/10.1093/jxb/erh197.

136. Schwarz PB, Pyler RE. 1984. Lipoxygenase and Hydroperoxide Isomerase Activity of Malting Barley. Journal of the American Society of Brewing Chemists 42:47-53. https://doi.org/10.1094/asbcj-42-0047.

137. Hirota N, Kuroda H, Takoi K, Kaneko T, Kaneda H, Yoshida I, Takashio M, Ito K, Takeda K. 2005. Development of novel barley with improved beer foam and flavor stability - The impact of lipoxygenase-1-less barley in the brewing industry. European Brewery Convention Congress, Prague.

138. Yu J, Huang S, Dong J, Fan W, Huang S, Liu J, Chang Z, Tian Y, Hao J, Hu SM. 2014. The influence of LOX-less barley malt on the flavour stability of wort and beer. Journal of the Institute of Brewing 120:93-98. https://doi.org/10.1002/jib.122.

139. Bamforth CW. 1999. Enzymic and Non-Enzymic Oxidation in the Brewhouse: A Theoretical Consideration. Journal of the Institute of Brewing 105:237-242. https://doi.org/10.1002/j.2050-0416.1999.tb00025.x.

140. Hirota N, Kaneko T, Kuroda H, Kaneda H, Takashio M, Ito K, Takeda K. 2005. Characterization of lipoxygenase-1 null mutants in barley. Theoretical and Applied Genetics 111:1580-1584. https://doi.org/10.1007/s00122-005-0088-y.

141. Skadhauge B, Knudsen S, Lok F, O O. 2005. Barley for production of flavour-stable beer. Proc Eur Brew Conv Cong, Prague, Fachverlag HansCarl, Nürnberg, 1-13.

142. Hoki T, Kanatani R, Saito W, Iimure T, Zhou TS, Takoi K, Tanigawa A, Kihara M, Ogushi K. 2018. Breeding of lipoxygenase-1-less malting barley variety 'Satuiku 2 go'. Journal of the Institute of Brewing 124:112-120. https://doi.org/10.1002/jib.483.

143. Hoki T, Saito W, Hirota N, Shirai M, Takoi K, Yoshida S, Shimase M, Saito T, Takaoka T, Makoto K, Yamada S. 2013. Breeding of Lipoxygenase-1-less Malting Barley Variety CDC PolarStar and Effect of Lipoxygenase-1-less Trait on Beer Quality at Pilot and Commercial Scale Brewing. BrewingScience 66:37-45.

144. Garbe LA, Barbosa de Almeida R, Nagel R, Wackerbauer K, Tressl R. 2006. Dual positional and stereospecificity of lipoxygenase isoenzymes from germinating barley (green malt): biotransformation of free and esterified linoleic acid. Journal of Agricultural and Food Chemistry 54:946-955. https://doi.org/10.1021/jf051993t.

145. Prasad A, Sedlářová M, Kale RS, Pospíšil P. 2017. Lipoxygenase in singlet oxygen generation as a response to wounding: in vivo imaging in Arabidopsis thaliana. Scientific Reports 7:9831. https://doi.org/10.1038/s41598-017-09758-1.

146. Rosahl S. 1996. Lipoxygenases in plants--their role in development and stress response. Zeitschrift für Naturforschung C 51:123-138. https://doi.org/10.1515/znc-1996-3-401.

147. Vahamidis P, Stefopoulou A, Kotoulas V, Lyra D, Dercas N, Economou G. 2017. Yield, grain size, protein content and water use efficiency of null-LOX malt barley in a semiarid Mediterranean agroecosystem. Field Crops Research 206:115-127. https://doi.org/10.1016/j.fcr.2017.02.022.

148. Anderson RJ, Clapperton JF, Crabb D, Hudson JR. 1975. Dimethyl sulphide as a feature of lager flavour. Journal of the Institute of Brewing 81:208-213. https://doi.org/10.1002/j.2050-0416.1975.tb03679.x.

149. Kavanagh TE, Derbyshire RC, Hildebrand RP, Clarke BJ, Meeker FJ. 1976. Dimethyl sulphide formation in malt—Effect of malting conditions. Journal of the Institute of Brewing 82:270-272. https://doi.org/10.1002/j.2050-0416.1976.tb03768.x.

150. Kavanagh TE, Steward SR, Hildebrand RP, Clarke BJ, Meeker FJ. 1975. Trace volatile constituents of beer dimethyl sulphide formation in malt. Journal of the Institute of Brewing 81:322-327. https://doi.org/10.1002/j.2050-0416.1975.tb03700.x.

151. Pimenta MJ, Kaneta T, Larondelle Y, Dohmae N, Kamiya Y. 1998. S-adenosyl-L-methionine:L-methionine S-methyltransferase from germinating barley. Purification and localization. Plant Physiology 118:431-438. https://doi.org/10.1104/pp.118.2.431.

152. Dickenson CJ. 1979. The relationship of dimethyl sulfide levels in malt, wort and beer. Journal of the Institute of Brewing 85:235-239. https://doi.org/10.1002/j.2050-0416.1979.tb03914.x.

153. Anness BJ. 1980. The reduction of dimethyl sulphoxide to dimethyl sulphide during fermentation. Journal of the Institute of Brewing 86:134-137. https://doi.org/10.1002/j.2050-0416.1980.tb03973.x.

154. Anness BJ. 1981. The determination of dimethyl sulphoxide in aqueous solution. Journal of the Science of Food and Agriculture 32:353-358. https://doi.org/10.1002/jsfa.2740320407.

155. White FH, Parsons R. 1975. The development of dimethyl sulphide in malting brewing and fermentation. Proc Eur Brew Conv Cong, Nice, France.

156. Bamforth CW. 2013. Flavor: Practical Guides for Beer Quality, American Society of Brewing Chemists,St Paul MN.

157. De Rouck G, Flores-González AG, Clippeleer J, De Cock J, De Cooman L, Aerts G. 2010. Sufficient formation and removal of dimethyl sulfide (DMS) without classic wort boiling. BrewingScience 63:31-40.

158. Knudsen S, Rils P, Skadhauge B, Bech LM, Olsen O. 2011. *Energy saving brewing method*. WO 2011/150933.

159. Dong L, Piao Y, Zhang X, Zhao C, Hou Y, Shi Z. 2013. Analysis of volatile compounds from a malting process using headspace solid-phase micro-extraction and GC–MS. Food Research International 51:783-789. https://doi.org/10.1016/j.foodres.2013.01.052.

160. Filipowska W, Jaskula-Goiris B, Ditrych M, Schlich J, De Rouck G, Aerts G, De Cooman L. 2020. Determination of optimal sample preparation for aldehyde extraction from pale malts and their quantification via headspace solid-phase microextraction followed by gas chromatography and mass spectrometry. Journal of Chromatography A 1612:460647. https://doi.org/10.1016/j.chroma.2019.460647.

161. Ditrych M, Filipowska W, De Rouck G, Jaskula-Goiris B, Aerts G, Andersen M, De Cooman L. 2019. Investigating the evolution of free staling aldehydes throughout the wort production process. BrewingScience 72:10-17. https://doi.org/10.23763/BrSc18-21ditrych.

162. Bettenhausen HM, Barr L, Broeckling CD, Chaparro JM, Holbrook C, Sedin D, Heuberger AL. 2018. Influence of malt source on beer chemistry, flavor, and flavor stability. Food Research International 113:487-504. https://doi.org/10.1016/j.foodres.2018.07.024.

163. De Clippeleer J, Van Opstaele F, Vercammen J, Francis GJ, De Cooman L, Aerts G. 2010. Real-time profiling of volatile malt aldehydes using selected ion flow tube mass spectrometry. LC GC North America 28:386-395.

164. Vesely P, Lusk L, Basarova G, Seabrooks J, Ryder D. 2003. Analysis of Aldehydes in Beer Using Solid-Phase Microextraction with On-Fiber Derivatization and Gas Chromatography/Mass Spectrometry. Journal of Agricultural and Food Chemistry 51:6941-6944. https://doi.org/10.1021/jf034410t.

165. Jaskula-Goiris B, De Causmaecker B, De Rouck G, De Cooman L, Aerts G. 2011. Detailed multivariate modeling of beer staling in commercial pale lagers. BrewingScience 64:119-139.

166. Herrmann M, Gastl M, Thiele F, Back W. 2007. Malt volatile compounds (Part I). BrewingScience 60:110-117.

167. Bamforth C. 1999. The science and understanding of the flavour stability of beer: A critical assessment. Brauwelt International 2:98-110.

168. Beal AD, Mottram DS. 1994. Compounds contributing to the characteristic aroma of malted barley. Journal of Agricultural and Food Chemistry 42:2880-2884. https://doi.org/10.1021/jf00048a043.

169. Kaukovirta-Norja A, Laakso S, Reinikainen P, Olkku J. 1998. The effect of kilning on the capability of malt to oxidise lipids. Journal of the Institute of Brewing 104:327-332. https://doi.org/10.1002/j.2050-0416.1998.tb01004.x.

170. Maule DR. 1966. The fate of humulone during wort boiling and cooling. Journal of the Institute of Brewing 72:285-290. https://doi.org/10.1002/j.2050-0416.1966.tb02967.x.

171. Debourg A, Laurent M, Goossens E, Borremans E, Van De Winkel L, Masschelein CA. 1994. Wort Aldehyde Reduction Potential in Free and Immobilized Yeast Systems. Journal of the American Society of Brewing Chemists 52:100-106. https://doi.org/10.1094/ASBCJ-52-0100.

172. Peppard TL, Halsey SA. 1981. Malt flavour - transformation of carbonyl compounds by yeast during fermentation. Journal of the Institute of Brewing 87:386-390. https://doi.org/10.1002/j.2050-0416.1981.tb04055.x.

173. Lermusieau G, Noël S, Liégeois C, Collin S. 1999. Nonoxidative mechanism for development of trans-2-nonenal in beer. Journal of the American Society of Brewing Chemists 57:29-33. https://doi.org/10.1094/ASBCJ-57-0029.

174. Kaneda H, Osawa T, Kawakishi S, Munekata M, Koshino S. 1994. Contribution of carbonyl-bisulfite adducts to beer stability. Journal of Agricultural and Food Chemistry 42:2428-2432. https://doi.org/10.1021/jf00047a012.

175. Baert JJ, De Clippeleer J, De Cooman L, Aerts G. 2015. Exploring the Binding Behavior of Beer Staling Aldehydes in Model Systems. Journal of the American Society of Brewing Chemists 73:100-108. https://doi.org/10.1094/ASBCJ-2015-0109-01.

176. Baert JJ, De Clippeleer J, Jaskula-Goiris B, Van Opstaele F, De Rouck G, Aerts G, De Cooman L. 2015. Further Elucidation of Beer Flavor Instability: The Potential Role of Cysteine-Bound Aldehydes. Journal of the American Society of Brewing Chemists 73:243-252. https://doi.org/10.1094/ASBCJ-2015-0531-01.

177. Baert JJ, De Clippeleer J, Bustillo Trueba P, Jaskula-Goiris B, De Rouck G, Aerts G, De Cooman L. 2018. Exploring Aldehyde Release in Beer by 4-Vinylpyridine and the Effect of Cysteine Addition on the Beer's Pool of Bound Aldehydes. Journal of the American Society of Brewing Chemists 76:257-271. https://doi.org/10.1080/03610470.2018.1518639.

178. Bustillo Trueba P, Jaskula-Goiris B, De Clippeleer J, Goiris K, Praet T, Sharma UK, Van der Eycken E, Sanders MG, Vincken JP, De Brabanter J, De Rouck G, Aerts G, De Cooman L. 2019. Validation of an ultra-high-performance liquid chromatography-mass spectrometry method for the quantification of cysteinylated aldehydes and application to malt and beer samples. Journal of Chromatography A 1604:460467. https://doi.org/10.1016/j.chroma.2019.460467.

179. Perpète P, Collin S. 2000. How to improve the enzymatic worty flavour reduction in a cold contact fermentation. Food Chemistry 70:457-462. https://doi.org/10.1016/S0308-8146(00)00111-4.

180. Saison D, De Schutter DP, Vanbeneden N, Daenen L, Delvaux F, Delvaux FR. 2010. Decrease of Aged Beer Aroma by the Reducing Activity of Brewing Yeast. Journal of Agricultural and Food Chemistry 58:3107-3115. https://doi.org/10.1021/jf9037387.

181. Bustillo Trueba P, De Clippeleer J, Van der Eycken E, Guevara Romero JS, De Rouck G, Aerts G, De Cooman L. 2018. Influence of pH on the Stability of 2-Substituted 1,3-Thiazolidine-4-Carboxylic Acids in Model Solutions. Journal of the American Society of Brewing Chemists 76:272-280. https://doi.org/10.1080/03610470.2018.1546094.

182. Cortés N, Kunz T, Methner F-J, Suárez AF, Hughes P. 2010. Development and correlation between the organic radical concentration in different malt types and oxidative beer stability. Journal of the American Society of Brewing Chemists 68:107-113. https://doi.org/10.1094/ASBCJ-2010-0412-01.

183. Kunz T, Strähmel A, Cortés N, Kroh LW, Methner F-J. 2013. Influence of Intermediate Maillard Reaction Products with Enediol Structure on the Oxidative Stability of Beverages. Journal of the American Society of Brewing Chemists 71:114-123. https://doi.org/10.1094/ASBCJ-2013-0429-01.

184. Furukawa Suárez A, Kunz T, Cortes Rodriguez N, Mackinlay J, Hughes PS, Methner F-J. 2011. Impact of colour adjustment on flavour stability of pale lager beers with a range of distinct colouring agents. Food Chemistry 125:850-859. https://doi.org/10.1016/j.foodchem.2010.08.070.

185. Kunz T, Müller C, Mato-Gonzales D, Methner FJ. 2012. The influence of unmalted barley on the oxidative stability of wort and beer. Journal of the Institute of Brewing 118:32-39. https://doi.org/10.1002/jib.6.

186. Özcan MM, Aljuhaimi F, Uslu N. 2018. Effect of malt process steps on bioactive properties and fatty acid composition of barley, green malt and malt grains. Journal of Food Science and Technology 55:226-232. https://doi.org/10.1007/s13197-017-2920-1.

187. Lund MN, Kramer AC, Andersen ML. 2015. Antioxidative Mechanisms of Sulfite and Protein-Derived Thiols during Early Stages of Metal Induced Oxidative Reactions in Beer. Journal of Agricultural and Food Chemistry 63:8254-8261. https://doi.org/10.1021/acs.jafc.5b02617.

188. Andersen ML, Skibsted LH. 1998. Electron Spin Resonance Spin Trapping Identification of Radicals Formed during Aerobic Forced Aging of Beer. Journal of Agricultural and Food Chemistry 46:1272-1275. https://doi.org/10.1021/jf9708608.

189. Hoff S, Lund MN, Petersen MA, Jespersen BM, Andersen ML. 2012. Influence of Malt Roasting on the Oxidative Stability of Sweet Wort. Journal of Agricultural and Food Chemistry 60:5652-5659. https://doi.org/10.1021/jf300749r.

190. Jenkins D, James S, Dehrmann F, Smart K, Cook D. 2018. Impacts of Copper, Iron, and Manganese Metal Ions on the EPR Assessment of Beer Oxidative Stability. Journal of the American Society of Brewing Chemists 76:50-57. https://doi.org/10.1080/03610470.2017.1402585.

191. Poreda A, Bijak M, Zdaniewicz M, Jakubowski M, Makarewicz M. 2015. Effect of wheat malt on the concentration of metal ions in wort and brewhouse by-products. Journal of the Institute of Brewing 121:224-230. https://doi.org/10.1002/jib.226.

192. Pagenstecher M, Maia C, Andersen ML. 2020. Retention of iron and copper during mashing of roasted malts. Journal of the American Society of Brewing Chemists Published online. https://doi.org/10.1080/03610470.2020.1795609.

193. De Rouck G, De Clippeleer J, Poiz S, De Cock J, van Waesberghe J, De Cooman L, Aerts G. 2007. Prolonged flavour stability by production of beer with low residual FAN using active dry yeast. Proc Eur Brew Conv Cong, Nürnberg, Fachverlag Hans Carl, pp.76:1-13.

194. Boyce B, Koch JC, Grinnell D, Zarnkow M. 2011. *Method and system for producing a malt beverage having a high degree of fermentation*. PCT/US2011/035759.

Tables

Table 1
Representative analysis of green malt and pale kilned malt

	green malt	kilned malt
MC[a] (%)	41.3*	3.7
Hot water extract [a] (l°/kg)	308	305
Cold water extract [a] (%)	19.4	19.5
Diastatic Power[a] (°L)	131	98
Nitrogen [a] (%)	1.59	1.55
SMM (mg/kg d.m.)	10-30 [b], 50-60[c], 12.6[d]	1.5–6.1 [b], 10-20 [c], 2.6[d]
DMSO (mg/kg d.m.)	0 [c], 0.28[e],	5.2-9.3 [c], 3.2-14.7 [f]
Total LOX activity (U/g d.m.)	27.5 [d], 12.6 [g], 25-33 [h]	1.6[d], 0.3 [g], 0.1-0.5 [h]
α-amylase activity (CU/g d.m.)	217[d]	211[d]
β-amylase activity (BU/g d.m.)	15.3[d]	9.7[d]

[a] MacWilliam et al. [16] green malt was freeze dried before analysis, *moisture content before freeze drying; [b] White and Wainwright [51], S-methyl methionine; [c] Yang et al. [50]; [d] Dugulin et al. [56] CU = Ceralpha Units; BU = Betamyl-3®Units; [e] Anness et al. [59]; [f] Yang et al. [52]; [g] E. D. Baxter [46]; [h] Yang and Schwarz [39]

Table 2

Representative numbers of microbes in/on barley, green malt and kilned malt [81]

	barley (dry)	green malt (5 days)	kilned malt
Nr. of aerobic heterotrophic bacteria/ kernel or ml	1.8×10^6	5.7×10^7	5.6×10^6
Nr. of lactobacilli/ kernel or ml	2.0×10^2	8.7×10^6	1.6×10^5
Nr. of filamentous fungi/kernel or ml	2.0×10^2	1.5×10^2	2.0×10^2
Nr. of yeasts/ kernel or mL	4.7×10^3	3.9×10^6	3.2×10^4

Table 3: Lipid derived aldehydes, alkenols, sulphur compounds and phenols from malts indicated as relative amounts in each malt, according to Moir and co-workers [14]. GM = green malt, KM = kilned malt

Lipid-derived aldehydes			Alkenols			Sulphur compounds			Phenols		
	GM	KM		GM	KM		GM	KM		GM	KM
hexanal	+++	+	3-hexen-1-ol	+++	++	dimethyl sulphide (DMS)	+++	+	4-vinylguaiacol	++	++
2,4-decadienal	+++	++	2-nonen-1-ol	+++		2-methylthioacetaldehyde	++	+	4-vinylphenol	+	
2-hexenal	++	+	1-penten-3-ol	++		methional	++	+	phenol		
heptanal	++		2-penten-1-ol	++		4-methylthio-2-butanone		+	o-cresol		
2,4 heptadienal	++		2-hepten-1-ol	++		3-methylthiohexanal			p-cresol		
2-octenal	++		2,4-decadien-1ol	++					2-ethylphenol		
nonanal	++		2-hexen-1-ol	+					4-ethylphenol		
2-nonenal	++	++	1-octen-3-ol	+					4-ethylguaiacol		
2,6- nonedienal	++		2-octen-1-ol	+					eugenol		
2-butenal	+								isoeugenol		
2-heptenal	+	++									
2,4-nonadienal	+	+									

Table 4

Representative analysis of worts prepared from green malt and pale kilned malt

	green malt	kilned malt
Specific gravity[a]	1028	1028
ph wort	5.75-6.0[a], 5.32 [b], 5.4 [e]	5.6[a], 5.12 [b], 5.2 [e]
Colour	8-10[a], 6.2-10 [e]	3[a], 9.6-12.2 [e]
Nitrogen (mg/100 ml) wort [a]	60-75	40
Amino nitrogen (% of total)[a]	40	33
Fermentability[a]	86	75
Carbohydrate recovery (%)[a]	99	98
Anthocyanogen (unit not defined)[a]	0.05-0.15	0.45
Attenuation limit corrected to 1055 in wort[b]	1011.6.	1011.0
DMS (µg/L)	150-236 [c], 100-500 [d], 50-150[e]	78-260 [c], 6-16 [d], 50-120[e]
DMS-P (µg/L)	822-1022 [c], 230-540 [e]	271-656 [c], 50-610 [e]

[a] MacWilliam et al. [12], [b] Duff SR [11], [c] White and Wainwright [49], [d] White and Wainwright [48], [e] Dugulin et al. [92]

Table 5

Selected analytical data for beers brewed from green malt and pale kilned malt

	green malt	kilned malt
pH	4.01[a], 4.2 [e]	3.98 [a], 4.4 [e]
Colour (EBC)	11 [b]*, 12[b$], 6.1-8.5 [e]	9 [b]*,15 [b$], 7.4-11.5 [e]
Specific gravity [b]	3.03*, 3.01[$]	4.38*, 4.04[$]
Head retention (half life, sec.) [b]	89*, 92**, 91[$]	98*, 81**, 106[$]
Limiting attenuation [a]	1011.8 – 1013.0	1011.5
Total carbohydrate [b]	13.3	16.7
Residual fermentable sugars (g/L) [b]	1.1	2.8
Non-fermentable carbohydrate (g/L) [b]	12.2	13.9
Nitrogen (g/L)	0.72-0.80[a], 0.54[b] *, 0.53[b $]	0.68 [a], 0.62[b]*, 0.65[b$]
DMS (µg/L)	31-38 [c], 30-70 [d], 15-30 [e]	61-84 [c], 50-95 [d], 15-30 [e]
DMS-P (µg/L) [e]	100-170	30-60

[a] Duff [11], limiting attenuation and nitrogen content corrected to an original gravity of 1055, [b] MacWilliam et al. [16] * = at bottling, **5 weeks after bottling, [$] draught, [c] White and Wainwright [49], [d] White and Wainwright [48], Dugulin et al. [92]

Figures

Figure 1: Growth of the barley kernel (A) barley after the first steep, (B) appearance of the first rootlet (chit malt) and onset of germination, (C) well-germinated green malt with rootlets

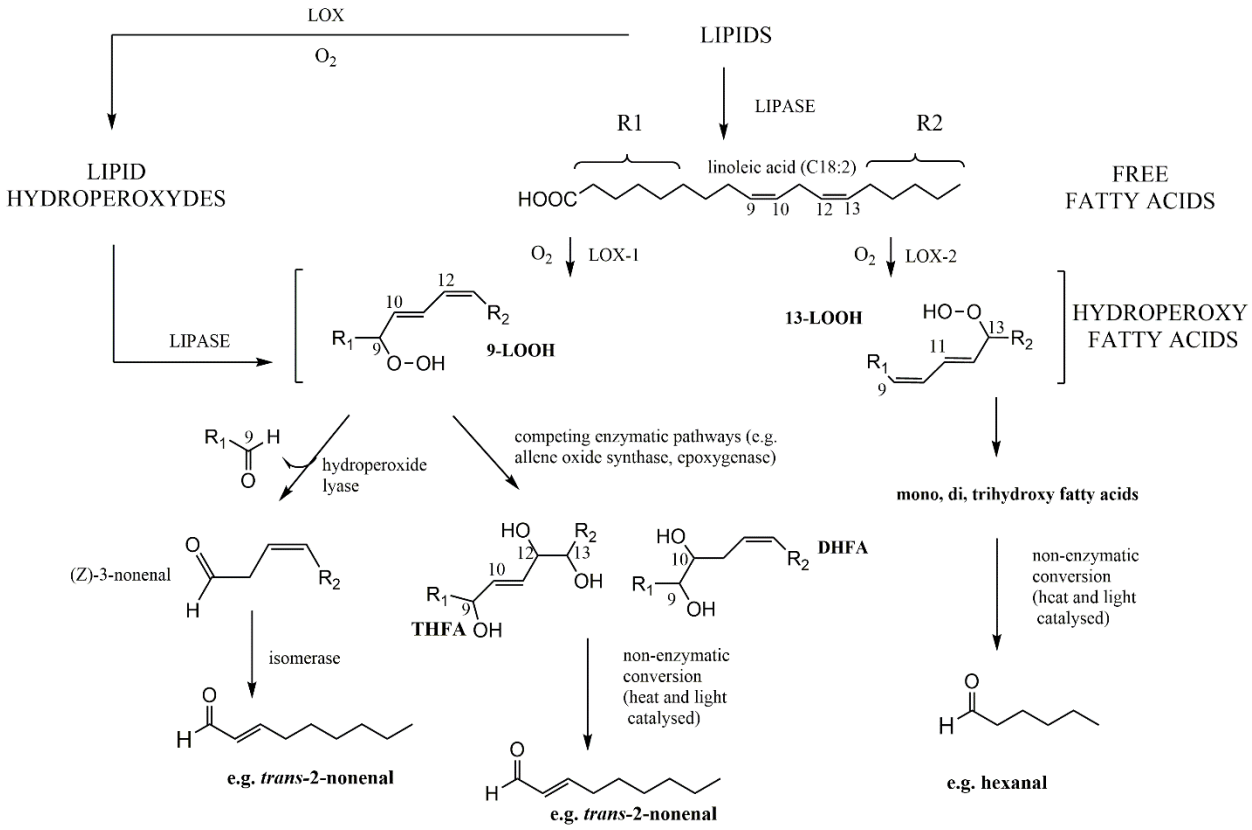

Figure 2: Suggested pathways for the formation of hydroperoxyl fatty acids through autooxidation and enzymatic activity of lipase and lipoxygenase according to Kobayashi et al. [116] and the subsequent formation of *trans*-2-nonenal and hexanal through some suggested pathways [64, 65, 114, 116]; THFA = trihydroxy fatty acids; DHFA = dihydroxy fatty acids