Research article

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Brewing with 100% green malt – process development and key quality indicators

Celina A. Dugulin,[1] Luisa M. Acuña Muñoz,[2] Jasper Buyse,[2] Gert De Rouck,[2] Irina Bolat[3] and David J. Cook[1]*

Brewing with undried, germinated (green) malt has the potential to lower energy and water usage in the malting and brewing chain. However, doing so introduces technical and biochemical (flavour) challenges. Beers were brewed using 100% green malt (n = 3) or kilned pilsner malt (n = 3), prepared from the same batch in each case, utilising the pilot brewery at KU Leuven (2.5 hL). Three further pairs of beers were brewed whereby the green malt was pre-steeped under deaerated water for 1 hour; this procedure was previously shown to lower LOX activity in green malt. Six green malt beers were brewed with acceptable specifications in terms of pH, alcohol content, foam stability and colour. No significant taints or obvious defects were detected in green malt beers. Increased S-methyl methionine levels were measured in worts and beers made from green malt, however DMS concentrations in the finished beers did not differ significantly from the reference beers. Furthermore, the results demonstrated promising indicators for flavour stability, such as reduced TBI, lower residual FAN and trihydroxy fatty acid (THFA) levels in brews using untreated green malt. Using re-steep water in green malt brewing (for reasons of water economy), however, increased THFA levels, possibly because oxygen uptake was not adequately controlled at this step. Whilst further process optimisations are undoubtedly required, it is shown that an acceptable lager style beer could be brewed to a specification not dissimilar to that of a kilned malt control beer, using 100% green malt with intact rootlets. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

Keywords: green malt; brewing; sustainable brewing; lipoxygenase; DMS control; beer colour

Introduction

The malting and brewing industry is constantly aiming to improve its carbon footprint. Furthermore, governments of several countries have implemented taxes on carbon emissions and energy consumption based on the carbon footprint. Therefore, the reduction of energy and CO_2 emissions has become an economic imperative and several technologies have the potential to mitigate the carbon footprint of malt production (e.g. biomass CHP, hydrogen power).

Amongst these options, omitting the kilning process, the most dominant consumer of energy within the malting process *(1–4)*, would deliver substantial reductions in energy and water utilisation. However, the usage of well germinated, undried (green) malt for the brewing process involves technical challenges, mostly due to the high moisture content of green malt. Moreover, kilning aids the reduction of lipoxygenase activity *(5–8)*, regulates the S-methyl methionine levels *(9–11)*, enables rootlet removal and gives the characteristic colour and flavours to malt. Furthermore, green malt 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. On the other hand, green malt is rich in diastase enzymes, with great capacity, for example, to convert the starch of unmalted adjuncts into fermentable sugars *(12, 13)*. Additionally, the extra enzymatic potential of green malt could potentially be suitable for mashing in less time. The total heat load of the malt and thus of the future mash, wort and beer made of green malt is also significantly lower, implying a decrease in Maillard reactions and Strecker aldehyde formation. Current thinking suggests that these factors should favour an improvement in beer flavour stability *(14–17)*. Furthermore, higher heat loads during brewing have been associated with a decrease in free amino acid (FAN) assimilation during fermentation *(18)*. Thus, reducing heat load might improve FAN assimilation and thereby lower residual FAN levels after fermentation, leading to an improved beer flavour stability. Lastly, unlike kilned malt, green malt does not contain DMSO *(19)*, which can be reduced to DMS by yeast during fermentation.

Previous researchers *(12, 13, 20)* have reported that wort and beer of acceptable quality could be produced from green malt, provided a suitable mill was used. Unfortunately, no detailed brewing protocol or assessment of the resulting beer flavour or its' stability were published in these papers which date back to the 1960's. Additionally, the beer style used (stout), could potentially have masked flavour defects *(13)*. In particular, lipoxygenase activity should be controlled in order to avoid an increase in the staling potential of the final beer *(5, 8)*, as well as impaired foam stability *(21, 22)*. If oxygen is present, LOX enzymes can oxidise

* Correspondence to: David J. Cook, International Centre for Brewing Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, UK. Email: david.cook@nottingham.ac.uk

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

[2] KU Leuven, Bioengineering Technology TC, Ghent Technology Campuses, Gebroeders De Smetstraat, Gent 9000

[3] Boortmalt NV, Zandvoort 1, Haven 350, Bus 1, Antwerp 2030, Belgium

unsaturated fatty acids to form hydroperoxy fatty acids, which can be transformed via several enzymatic pathways *(23)* to mono-, di-and trihydroxy fatty acids. Subsequently, the latter can be degraded non-enzymatically to flavour active carbonyls, e.g. *trans*-2-nonenal or hexanal, that are known beer staling compounds *(8, 24, 25)*. Recent laboratory scale trials *(26)* indicated that re-steeping of green malt in combination with a LOX hostile mashing environment (63°C, pH 5.2 and oxygen free) could help to control LOX activity and the *trans*-2-nonenal potential of green malt. Alternatively, LOX-less *(22, 27)* or Null-LOX barley *(28)* varieties are now available and might thus be suitable when brewing with green malt.

A further quality concern when brewing with green malt is the high dimethyl sulphide (DMS) potential. DMS contributes a cooked corn characteristic to lager beers and levels should be carefully regulated according to the brand's particular style. The flavour threshold of DMS is around 30 μg/L. In some beers evident DMS is a negative quality factor, so levels should be controlled below threshold. In other beers, DMS adds to the complexity of the sulphur character and is a key part of the flavour profile. However, it should always be regulated closely so as not to imbalance lager beers. Previous research *(9, 11)* reported that wort of green malt contained high concentrations of the DMS-precursor S-methyl methionine; however, DMS levels in beer made of green malt were not higher than beers made of pale malt. Although such study outcomes are promising, the control of S-methyl methionine (SMM) levels remain a key focus when brewing with green malt.

The main objectives of the present study were to evaluate the technical feasibility of pilot-scale brewing using germinated green malt and to facilitate a comparison between key quality parameters of beers made from green malt and from kilned malts prepared from the same batches of green malt. Particular attention is paid to trihydroxy fatty acid (THFA) levels which can result from LOX activity, as well as DMS and S-methyl methionine levels. The data reported will help to define the future challenges and potential benefits of implementing beer production using green malt.

Material and methods

The French malting barley variety Etincel was sourced from Boortmalt, Antwerp. Samples (green malt and the corresponding kilned pilsner style malt) were collected at the equivalent timepoints (final day of germination and off-kiln respectively) during six industrial malting cycles. The green malt, which had a moisture content of 40.7 ± 1.1% was not microbiologically stable and could not be stored. Therefore, the brewing trials using green malt were started at the earliest possible time point, about 1.5-2 hours after malt collection. No further information of the commercial malting procedure is available.

Wort production and fermentation

Beers were prepared using 100% green malt (n = 3), green malt re-steeped before mashing (n = 3) or the corresponding reference kilned malt (pilsner malt, n = 6), utilising the 5 hL pilot brewing plant at KU Leuven, Technology Campus Ghent (Figure 1), brewing at 50% total capacity (2.5 hL). A thick mash was produced using a grist:liquor ratio of 1:2.2. Samples were collected throughout the brewing process and compared with wort and beer samples from brews produced using conventional pale lager malt, brewed under the same conditions (other than the amount of brewing liquor; temperature, calcium and lactic acid additions were adjusted to compensate for the higher moisture content in green malt).

Wort production using green malt

Milled green malt (68.9 kg, 40% moisture; wet disc mill, Hydromill, Meura, Belgium) was mixed with 70.4 kg (85°C) of deaerated, reversed osmosis brewing water enriched with 109 mg/L Ca^{2+} in the form of $CaCl_2$ (calcium chloride dehydrate, Merck KGaA, Darmstadt, Germany). CO_2 was injected in the mill inlet, increasing the protection against oxidation. Mashing conditions were selected to minimise lipoxygenase activity: pH 5.2 (1.4 mL/hL lactic acid; pH adjustment with 30% (v/v) lactic acid from 90% (v/v) (S)-lactic acid, Merck KGaA, Darmstadt Germany), mashing in at 63°C under oxygen limited conditions. The following mashing protocol was applied: 63°C (30 min), 72°C (15 min) 78°C (1 min) – temperature rise 3°C/min. Wort was filtered using a membrane assisted thin bed filter (Meura 2001, Meura, Belgium); with a weakworts cut-off point of 1.5°P. At onset of boiling, the sweet wort was adjusted to 13°P. Additionally, $ZnCl_2$ was added to give free Zn^{2+} ions at 0.2 mg/L. Wort was boiled for 60 min (atmospheric boiling) and hopping applied in pellet form: first hop – Magnum (13.0% (w/w) α- acids; 50.5 g/hL); late hop – Tettnanger (3.0% (w/w) α-acids; 100 g/hl) and Saaz (2.5% (w/w) α-acids; 120 g/hL) aiming for 29 mg iso-α-acids/L in the final beer. Wort clarification was performed by decantation in the combination vessel (wort settling) with a duration of 15 min. Samples for analysis in each batch were collected at the onset of mashing, end of mashing, mash filtration, first wort collection, onset of boiling, end of boiling, end of clarification and end of cooling (pitching wort; after wort aeration).

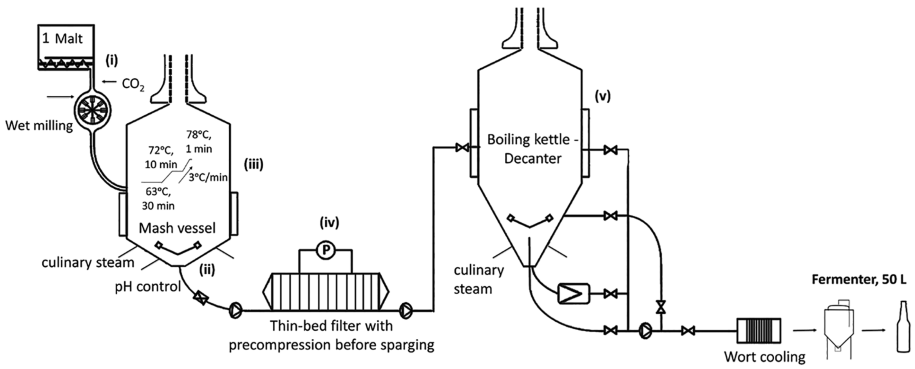

Figure 1. Process outline of the KU Leuven 5hL pilot brewery; Points i-v indicate critical points when brewing with green malt. *i) wet milling; ii) mash agitation; iii) mash conversion vessel; iv) membrane mash filter; v) kettle-decanter*

Wort production – re-steeping of green malt before mashing

A total of 68.9 kg of green malt was re-steeped (1 h, re-immersed in water after germination) in 70.4 kg water (deaerated, reversed osmosis brewing water enriched with 1.4 mL/hL lactic acid and 109 mg/L Ca^{2+} in the form of $CaCl_2$). Afterwards, the green malt was separated from the brewing liquor using a fine-meshed net. In order to remain water efficient, the water used for re-steeping was reused for mashing. The used re-steep water was heated to 85°C using a mobile immersion heater prior to use. Subsequently, the same brewing parameters were applied.

Wort production using reference (kilned, pilsner style malt)

For the kilned pilsner style malt, the same brewing parameters (apart from the brewing liquor) were applied. Pilsner malt: 44 kg of malt was used and mixed with 96.6 kg (69°C) of deaerated water containing 80 mg/L Ca^{2+} ($CaCl_2$) and lactic acid 1.0 mL/hL.

Fermentation, filtration and bottling

All worts were pitched with 10^7 yeast cells/mL (S-O4, Fermentis, top-fermenting). Fermentation was performed in a cylindroconical vessel (50 L) at 24°C. After fermentation, beer was submitted to 14 days of maturation at 0°C in 50 L kegs. Maturated beer was filtered using a plate filter (BECOPAD Eaton 350). All the batches received carbonation up to 5.6 g CO_2 per litre. Beer samples were bottled using a six-head counter pressure filler with double pre-evacuation with intermediate CO_2 rinsing and over-foaming with hot water injection before capping (Monobloc, CIME, Italy). Bottled beers were stored at 0°C prior to analysis.

Malt, wort and beer analysis: Standard analyses

The moisture content of malt samples was measured by mass loss on drying according to Analytica EBC method 4.2. Wort specific gravity and density, as well as alcohol content of the beer were analysed using an Anton Paar Alcolyser with a DMA 5000 density measurement device (Anton Paar Benelux, Gentbrugge, Belgium). Extract yield was calculated according to Analytica EBC Method 4.4. The CO_2 content of beers were measured by the Haffmans inpack TPO/CO_2 meter (Haffmanns c-TPO) and foam stability using the NIBEM-T Meter (Haffmans, Venlo, Netherlands). Standard wort and beer analyses were carried out according to the following EBC-methods using a spectrophotometer (Varian Cary 100, Agilent Technologies Inc., Australia): Beer colour: EBC method: 9.1, FAN (free amino nitrogen): 9.10 (for FAN determination of wort: 8.10); total polyphenols: 9.11 and flavanoid content: 9.12. Cold haze (analysis of the turbidity of beer kept for a minimum of 24 h at 0°C) and permanent haze (analysis of turbidity of beer kept for 24 h at 20°C) were determined using the Haffmans VOS ROTA 90 Turbidity meter, 90° light scatter. The thiobarbituric acid–index (TBI) of wort and beer was determined according to the method described by Thalacker and Böβendörfer *(29)* and expressed as the TBI for 100 mL of wort. Determination of proanthocyanidins was performed by measuring the red coloured cyanidin complex formed with 5% HCl (v/v)/n-butanol using the method according to Bate-Smith *(30)*.

Determination of DMS and S-methyl methionine in wort and beer

Headspace SPME GC-PFPD was used to quantitatively determine DMS and also indirectly S-methyl methionine (SMM) in wort and beer using the Thermo Finnigan TraceGC Ultra system (Interscience, Louvain-la-Neuve, Belgium). The GC system was equipped with a CTC CombiPAL autosampler, a S/SL injector with narrow bore glass inlet liner, an RTX-1 fused silica capillary column (30 m × 0.32 mm i.d., 3 μm film thickness, Restek), and a pulsed flame photometric detector (PFPD 5380, OI Analytical, Texas, USA) operating in sulphur mode. Helium was used as carrier gas (1.2 mL/min). The inlet temperature was set at 250°C and injection was carried out in the split mode (split ratio 10:1). The oven temperature was kept at 35°C for 3 min, then raised to 250°C at 5°C/min and held at 250°C for 5 min. The PFPD was set at 250°C and 560 V with air 1 and air 2 at 10 mL/min and hydrogen at 12.5 mL/min. Data processing was performed using Chromcard 2.3.2 (Thermo Electron Corporation, Milan, Italy) and WinPulse 32 2.0 (OI Analytical). After sample preparation, the vial was pre-equilibrated for two minutes at 30°C. The SPME needle was conditioned for 2 min at 300°C and then inserted through the septum. The Carboxen™/Polydimethylsiloxane (CAR/PDMS light blue) fiber (Stableflex, 85 μm, Supelco, Bellefonte, USA) was exposed to the headspace for 15 min, agitating at 250 rpm. The SPME fibre was thermally desorbed into the injection port of the GC for 3 min and subsequently post-conditioned for 2 min at 300°C. The quantification of the DMS content in the sample (wort, beer) is based on a calibration curve with standards of a known concentration of DMS (0.1-10 μg/L) and EMS (1 μg/L) as internal standard. The ratio of the area of the DMS to the surface of the EMS peak is correlated with the ratio of the DMS/EMS concentration. If necessary, samples were diluted by an appropriate dilution factor to allow for quantification within the linear range of the calibration curve.

The indirect quantification of the DMS precursor, S-methyl methionine, was based on the original method proposed by White and Wainwright *(10)* following a modified protocol by De Rouck et al. *(31)*, without the utilisation of NaOH to avoid possible side formations of oxidised products (DMSO and $DMSO_2$). The sample was prepared and placed at 100°C for 160 min. Due to this thermal treatment, the non-volatile SMM in the sample is converted to DMS. The difference between the content of DMS in the vial subjected to thermal treatment and the content of DMS in the non-heated vial is taken as the SMM concentration in the unknown sample and expressed as DMS equivalents.

Determination of trihydroxy fatty acids in grain, wort and beer

Gas chromatographic analysis of trihydroxy fatty acids (THFA) in beer samples was based on the procedures of Moeller-Hergt et al. *(32)* and Wackerbauer and Meyna *(33)*. Extraction of THFA in malt samples was conducted by using 50.0 ± 0.05 g of malt with 390 mL RO water, 10 mL of Brewtan (6 g/L) and 1 mL lactic acid (9% v/v), preheated to 70°C. The mix was mashed for 10 min at 70°C. Afterwards, the weight of the content of the beaker was adjusted to 450 ± 0.2 g by addition of reversed osmosis water and filtered on ice using filter paper (Whatman, grade 2555 ½ prepleated 320 mm, Sigma-Aldrich, UK). The first 20 mL of the filtrate was transferred to a small glass bottle and immediately frozen until further liquid-liquid extraction. The following liquid-liquid extraction was performed on a 5 mL aliquot of the (extracted) malt or wort sample, using 16 mL diethyl ether

(extra pure, Fisher Scientific). The mixture was shaken for 3 minutes and centrifuged at 9344 x g for 5 minutes (Hettich 320R, Germany). The upper layer was transferred to a glass vial using a glass syringe. Subsequently, the diethyl ether layer was evaporated using nitrogen. The liquid-liquid extraction was repeated three times (on the same 5 mL aliquot). After the final evaporation, 500 μL of the internal standard, heneicosan (36.5 mg/L; 98%, Sigma-Aldrich) diluted in hexane (anhydrous, 95%, Sigma-Aldrich), was added to the glass vial and evaporated. For the derivatisation, 300 μL of the silylation reagent (Silyl-991, Machery-Nagel) and 100 μL piridine (98%, Sigma-Aldrich) were added. The samples were subsequently heated at 90°C for 1 h using a laboratory block heater (digital heat block, VWR). The liquid was transferred into HPLC vials and kept at -20°C until GC analysis. The equipment used was a GC-FID (ThermoQuest Trace GC 2000; Interscience, Louvain-la-Nueve, Belgium) equipped with a fused silica analytical capillary column (CP-Sil 5 CB Low BLEED/MS; 50 x 0.25 mm i.d., 0.25 μm and a cyano-phenyl-methyl deactivated retention gap (2.5 x 0.53 mm i.d., Varian, Netherlands). Samples (2 μL) were manually injected using a Hamilton syringe (10 μL, Model 701 N Syringe). The oven temperature was kept at 40°C for 5 min, then raised to 290°C at 6°C/min and held at 290°C for 20 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Data processing was performed by Chromcard software 1.07.

Statistical analysis

All samples were analysed in at least three biological replicates with 2-4 technical replicates. The statistical significance of the data obtained was established with analysis of variance (ANOVA), a p-value below 0.05 was considered as statistically significant.

Results and discussion

Brewing performance and technical challenges

The commercially produced green malt was used as 100% of the grist in pilot scale brewing (2.5 hL). Six paired trials were conducted whereby beers were brewed first from a batch of green malt and subsequently from the kilned malt prepared from the green malt. Each pair were sampled from a different batch, albeit produced using the same barley variety and industrial malting process. Beers were produced under the same brewing conditions, other than the amount of brewing liquor. To account for the higher moisture content (40%) in green malt, less water (as described in Materials and methods section) was needed at the onset of mashing. Thus, a more water efficient process was achieved by brewing with green malt. A summary of the brewing performance of green malt (n = 3), re-steeped (prior to mashing) green malt (n = 3) and the corresponding reference pilsner malt (n = 6) is shown in Table 1.

The pilot brewery at KU Leuven (Figure 1) is equipped with a wet milling system (i), suitable for milling green malt. CO_2 was injected in the malt bin and the mill inlet, increasing the protection against oxidation, thus potentially favouring lipoxygenase control. The water flow during wet milling (considering the amount of water already in the grain) and the gap distance setting of the mill (19 kilned malt, 12 green malt, equipment specific units, Hydromill, Meura) were adjusted. Inappropriate setting of the disc gap (too fine or too coarse) led to blocking of the mash filter (iv) when brewing with green malt - as a result the brews had to be stopped and discarded. The filtration process in the pilot brewing trials of green malt wort was found to be considerably slower than that of pale kilned malt worts (Table 1). In part, this might reflect a need for further optimisation of the milled particle size distribution using the wet disc mill, but also relates to the thickness of the mash. Additionally, the mash stirring device (ii) employed was not a conventional agitator, but a homogeniser allowing low shear, ideal for kilned malt. However, it appears not to be optimal for mixing green malt mashes. The homogeniser, which sits in the bottom of the mash kettle, could not cope with the thickness of the mash of green malt, therefore only 50% of the total mash kettle capacity could be used and the brews had to be scaled down to 2.5 hL. Temperature and pH control (iii) at the onset of mashing were difficult due to the sub-optimal mixing. To allow for a lipoxygenase hostile *(23, 34–36)*mashing temperature and pH, the mash-in water volume needed to be reduced to allow for the higher water content of the green malt, with the liquor needing to contain more lactic acid and be heated to a higher temperature. Whilst these adjustments were calculated and applied, the pH and temperature proved very difficult to control accurately, which may have been due to insufficient mixing. Additionally, milling of kilned malt already causes friction which can increase the temperature and, in terms of pH, the composition of the steep water used in the malting process was unknown. Filling of the mash filter (iv) took about twice as long (4.0-12.0 min) in all six green malt brews compared to the reference brews (3.7 – 5.4 min; Table 1). Total filtration time increased in all six green malt brews. This could probably be improved in future by optimisation of the milling process, use of a mash vessel equipped with a more suitable type of low shear

Table 1. Brewing performance of green malt, re-steeped green malt and the corresponding reference kilned (pilsner) malt

Brew Nr.	1		2		3		4		5		6	
	GM	KM	GM	KM	GM	KM	GM*	KM	GM*	KM	GM*	KM
Mash filter (MF) filling time (min)	8.0	3.7	9.6	5.4	11.3	4.4	12.0	5.0	5.0	4.0	4.0	4.0
MF filtration time before sparging (min)	32.0	20.2	27.7	20.7	50.9	22.4	26.0	22.0	19.0	28.0	39.0	39.0
MF sparging and final compression time (min)	29.9	77.6	121.9	99.6	145.2	59.4	75.1	99.6	78.8	101.9	89.4	66.8
Boiling time (min)	60	60	60	60	60	60	60	60	60	60	60	60
Total wort volume (L)	190	220	180	240	200	199	190	230	200	220	180	184
Brewhouse yield (%)	61.8	72.5	55.5	80.3	44	67	64.2	73.9	65.8	69.2	57.3	62.1

GM = green malt; KM = kilned malt;
* indicates re-steeped green malt

stirring device, and fine tuning of the liquor to grist ratio. In general, the green malt brews had low flow rates and, as a consequence, sparging times took longer than for the reference brews (Table 1). Poor sparging rate could be attributed to the spongy and cohesive structure of the green malt 'cake', not allowing sparging water to sufficiently wash out the remaining sugars. Thus, brewing yield was lower in green malt brews than kilned malt brews.

In future trials, an optimised milling system is advised, in combination with a mash vessel equipped with a 'normal' mash agitator instead of the low shear homogeniser, used in the pilot brewery. Additionally, filtration and sparging operations need to be adjusted to cope with the structure of the green malt 'cake'. However, milling optimisation could potentially improve the composition of the grist, thus filterability and sparging rate. There were no technical issues during the boiling (v), clarification and cooling operations of the six green malt brews.

Wort characteristics

The characteristics of the cold pitching wort are shown in Table 2. Worts prepared from untreated green malt are compared to worts prepared from the kilnedmalt; similarly re-steeped green malt worts are compared to their corresponding reference brews.

EBC colour of the worts prepared from untreated green malt and re-steeped green malt were significantly lower compared to worts prepared from the kilned malt control however, a satisfactory yellow colour was still attained. This supports previous findings reported by MacWilliam et al. *(12)*. Kilned malt imparts characteristic colour compounds to beer, formed mainly via Maillard reactions initiated between reducing sugars and aminocompounds during kilning. Nevertheless, the yellow colour in green malt wort might originate from natural yellow pigments, such as polyphenols or the water-soluble vitamin riboflavin. Riboflavin is a yellow colouring matter, present in malt (1.2 – 5.0 μg/g) *(37)*. The precise origins of the colour contributed by green malt should be further investigated.

Contrary to expectations, the free amino nitrogen content of worts prepared from green malt (n = 3) were significantly lower compared to levels of their corresponding reference worts (Table 2). However, the reported minimum level (140 mg/L *(38)*, as nutrition for the yeast during fermentation, were easily achieved in all worts. Green malt is known to have a higher proteolytic activity than kilned malt. As shown in previous research, proteases seem to be protected in very thick mashing conditions even when mashing in at an elevated temperature of 63°C *(39)*. Thus, the decreased FAN levels could be interpreted as being a result of proteolytic inhibitors present in green malt. FAN levels measured in worts prepared from re-steeped green malt, on the other hand, did not differ significantly from the relevant control worts. When re-steeping green malt those inhibitors might have been removed, or proteolytic activity increased through some mechanism. Certainly, this observation requires further investigation.

Significantly lower concentrations of polyphenols (311.9 ± 33.6 mg/L) were measured in worts prepared from untreated green malt compared to their reference (379.0 ± 47.1 mg/L). These results further support the idea that the kilning step increases total polyphenol levels *(40)*, as well as polyphenol solubilisation *(41)*. However, polyphenol levels differed greatly between the individual brews, presumably due to the difficulties that occurred during

Table 2. Pitching wort characteristics prepared from green malt, re-steeped green malt or the corresponding reference kilned (pilsner) malt

			re-steeping trials	
	GM	KM	GM	KM
pH***	5.4 ± 0.2 ^{a}	5.2 ± 0.1 ^{b}	5.4 ± 0.1 ^{a}	5.2 ± 0.1 ^{b}
Colour (EBC)***	8.1 ± 1.9 ^{a}	10.9 ± 1.3 ^{b}	7.2 ± 0.5 ^{c}	10.9 ± 1.4 ^{d}
Density (g/cm^3)$^{n.s.}$	1.0478	1.0439	1.0500	1.0481
Original extract (° Plato)$^{n.s.}$	12.4 ± 0.5	11.8 ± 1.3	12.9 ± 0.3	12.4 ± 1.3
FAN (mg/L)***	220.9 ± 41.2 ^{a}	287.5 ± 35.8 ^{b}	269.5 ± 19.9 ^{c}	259.7 ± 47.8 ^{c}
Total polyphenols (mg/L)***	311.9 ± 33.6 ^{a}	379.0 ± 47.1 ^{b}	372.3 ± 36.7 ^{b,c}	363.0 ± 43.2 ^{c}
Flavanoids ((+)-catechin eq. mg/L)***	50.3 ± 3.7 ^{a}	54.6 ± 1.7 ^{a}	75.0 ± 2.5 ^{b}	66.1 ± 5.4 ^{c}
Proanthocyanidins (mg/L)***	59.0 ± 7.2 ^{a}	74.6 ± 20.1 ^{b}	71.4 ± 13.6 ^{c}	84.5 ± 19.0 ^{d}
Thiobarbituric acid index***	15.4 ± 1.5 ^{a}	45.1 ± 4.7 ^{b}	20.6 ± 1.5 ^{c}	51.2 ± 7.9 ^{d}
DMS (μg/L)***	106.1 ± 41.9 ^{a}	97.4± 22.3 ^{a}	139.0 ± 27.9 ^{b}	56.9 ± 27.9 ^{c}
SMM (mg/L)† ***	0.54; 0.23; 0.38 ^{a}	0.61; 0.05; 0.24 ^{b}	0.26; 0.46; 0.26 ^{a,b}	0.13; 0.05; 0.09 ^{c}
THFA (mg/L) ***	3.8 ± 1.5 ^{a}	7.8 ± 0.9 ^{b}	7.4 ± 0.5 ^{b}	6.0 ± 0.9 ^{c}

† Indirect determination of SMM from (Total DMS – DMS), expressed as DMS equivalents
Superscripts a-d represent the ANOVA post-hoc groupings. In each row treatments differed significantly from one another if they have a different ANOVA group letter.
Asterisks represent the P-value significance
* $P<0.05$;
** $P<0.01$;
*** $P<0.001$.
Data are the mean ± SD of 2-3 technical replicate measurements on each of 3 replicate brews, Statistics: One-Way ANOVA with Fisher's LSD post-hoc test. GM = green malt; KM = kilned malt

the sparging of green malt, affecting retention of polyphenols. Hence, a more technically consistent process is necessary to gain further information on the factors which determine total polyphenol levels in green malt wort. Flavanoid levels in wort did not differ significantly whether they were prepared from green malt (50.3 ± 3.7 mg/L) or kilned malt (54.6 ± 1.7 mg/L). Proanthocyanidins, the main haze active polyphenols, were significantly reduced in worts prepared from green malt (59.0 ± 7.2 mg/L) compared to the controls (74.6 ± 20.1 mg/L), which is consistent with the observations made by MacWilliam et al. *(12)*, who reported much lower anthocyanogen contents in green malt wort. Re-steeping, on the other hand, appeared to affect polyphenol solubilisation. The total polyphenol concentration of the re-steeped GM brews did not differ from the control brews (Table 2), while flavanoid levels were elevated (75.0 ± 2.5 mg/L). Additionally, re-steeping increased proanthocyanidin levels (71.4 ± 13.6 mg/L), compared to worts prepared from untreated green malt. These results, which potentially favour beer colloidal stability, are discussed in the following sections.

The thiobarbituric acid index (TB-Index) is used as an indicator for evaluating heat load during wort production and determines the 5-hydroxymethylfurfural (5-HMF) potential of wort and beer. The omission of the kilning process dramatically decreased the heat load of the malt, which resulted in a decreased TBI level in the wort (Table 2). Hence, the significantly lower TBI of green malt wort (15.4 ± 1.53) and re-steeped green malt wort (20.6 ± 1.5), compared to the corresponding reference worts (45.1 ±4.7 and 51.2 ± 7.9, respectively), could potentially benefit the flavour stability of the beer *(14, 16)*. Reducing the total mash filtration times of green malt brews in subsequent trials could further decrease the total heat load applied and thus the TBI.

DMS and S-methyl methionine – determination in wort

DMS and (indirectly) S-methyl methionine levels (SMM) were measured in all worts. Data were compared with worts prepared from their kilned malt control, prepared from the same green malt. Green malt is rich in the DMS-precursor SMM *(11)*, therefore overall DMS levels were expected to be higher compared to the control. S-methyl methionine levels were determined throughout the brewing process of the three untreated green malt samples and compared to the reference brews. Figure 2 illustrates the DMS and SMM levels from the onset of mashing to the pitching wort. It is noticeable that in all three brews, the SMM levels were 2-3 times higher (7.3 ± 1.3 mg/L) at mashing-in compared to the respective reference brew (3.0 ± 0.4 mg/L). SMM is being transformed into free volatile DMS for both kilned malt and green malt brews. As expected, DMS levels were rising (prior to boiling), while the amount of SMM was declining; the individual measured concentrations varied substantially between the different brews. This appeared to arise from variations between biological malt replicates (variation in SMM levels already at onset of mashing). Errors due to sampling and analysis were likely smaller, as wort samples were taken at the same timepoint in each case and immediately put on ice prior to analysis.

For example, the first and second brew exhibited significantly higher DMS levels until the onset of boiling in the green malt brews. In the second brew, the DMS concentration was even double that of the kilned malt brews. In contrast, the third brew showed higher DMS levels in the kilned malt wort. Although the analysis did not reveal a clear uniform pattern on DMS levels, overall the results show that a major part of the precursor is converted during mashing and filtration, and not solely during wort boiling (100°C, 60 min). As shown in previous studies *(19, 42)* during malt

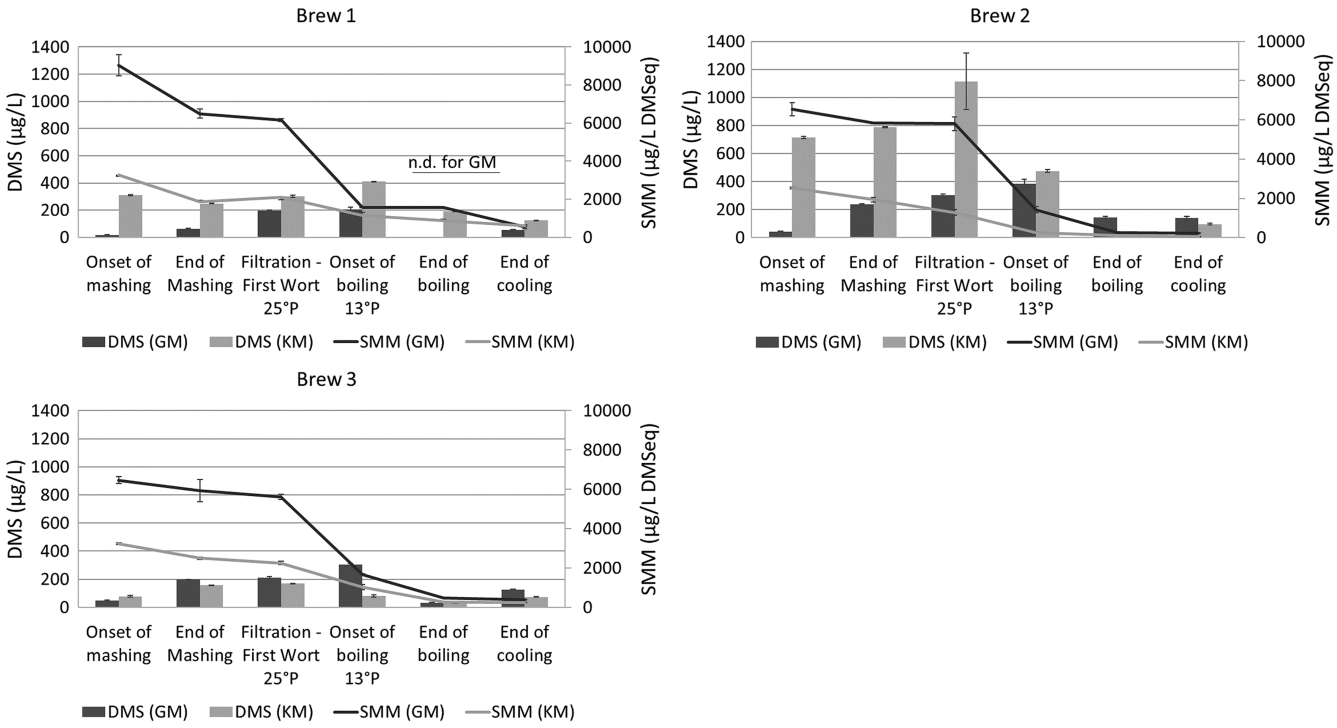

Figure 2. DMS and S-methyl methionine (expressed as DMS equivalents, µg/L) monitored in three individual brewing processes using green malt and the corresponding reference (pale) kilned malt. Data are the mean ± SD

kilning, SMM decomposes (pH-dependent) at temperatures above 70°C to DMS and L-homoserine. Any remaining DMS was satisfactorily evaporated during boiling, leaving worts of green malt brews with higher S-methyl methionine levels, but acceptable DMS concentrations. Additionally, DMS and SMM levels were determined in the wort prepared from re-steeped green malt and the corresponding reference malt, again resulting in acceptable DMS levels (Table 2). On average, all six brews using green malt as the raw material resulted in elevated SMM level, but acceptable DMS levels (122.6 ± 36.1 μg/L DMS v 77.15 ± 30.6 μg/L DMS). It appears that DMS levels in pitching wort can be controlled even when using green malt, given sufficient removal of DMS via evaporation during wort boiling.

Trihydroxy fatty acids – determination in wort

The malts used for the preparation of the beers were analysed for trihydroxy fatty acids (THFA) levels. Clearly, green malt has a higher lipoxygenase (LOX) activity compared to kilned malt *(26)*; a major threat for beer flavour and stability. The determined contents of THFA in the malts used for this study were significantly lower in kilned malt (39.6 ± 9.9 mg/kg, dry basis) compared to green malt (68.3 ± 4.5 mg/kg, d.b.). Interestingly, however, the THFA concentration measured at the onset of mashing was significantly lower in all three brews using green malt (n = 3, Figure 3). This implies a rapid breakdown of THFA to degradation products during wet milling and entry to the mash vessel. Similarly, significantly lower THFA levels were detected in all three worts of green malt (3.8 ± 1.5 mg/L) compared to their kilned malt reference (7.8 ± 0.9 mg/L). There was a clear THFA increase across mashing in kilned malt brews (Figure 3), whereas in the green malt brews, levels were more or less stable throughout the brewhouse. The formation of trihydroxy fatty acids from hydroperoxy fatty acids can occur through several enzymatic pathways *(23)*. Theoretically, lipoxygenase should have been blocked by the exclusion of oxygen and the high mash-in temperature of 63°C. However, membranebound lipase in malt is proven to be very thermostable (up to 67°C), and retains activity during most of the mashing process *(43)*. Thus, future studies should be directed to the oxidation of unsaturated fatty acids to further elucidate why THFA increased when brewing with kilned malt, but contrary to expectations, not when using green malt. Previous research from our group *(26)* had indicated that re-steeping of green malt in water for an hour was an effective means to reduce the LOX activity of green malt by around 50%. Accordingly, it was decided to assess the quality impact of this putative process at pilot scale, including the re-use of re-steep water as mashing liquor in the green malt brewing process in order to minimise overall water usage in the chain. Contrary to expectations, brewing with re-steeped malt almost doubled (7.4 ± 0.5 mg/L) THFA levels compared to untreated green malt (3.8 ± 1.5 mg/L) and it did significantly differ from its kilned malt control (6.0 ± 0.9 mg/L; Table 2). This suggests that not all appropriate mashing conditions were fulfilled to control unwanted LOX reactions. Possibly, by re-heating the steep water and not deaerating it prior to mashing, oxygen pick-up may have occurred. By keeping lipoxygenase hostile mashing parameters (63°C, pH 5.2 and oxygenfree), LOX-related reactions can be kept under control. However, considering that temperature and pH control were challenging in green malt brews due to the noted incompatibility of the mash homogeniser, these findings suggest that oxygen exclusion is a key criterion to avoid THFA formation.

Overall, the main conclusion of this part of the study was that the re-steeping procedure did not have a significant impact on the flavour stability indicators which it was designed to improve. Alternatively, it can be concluded that the LOX activity was

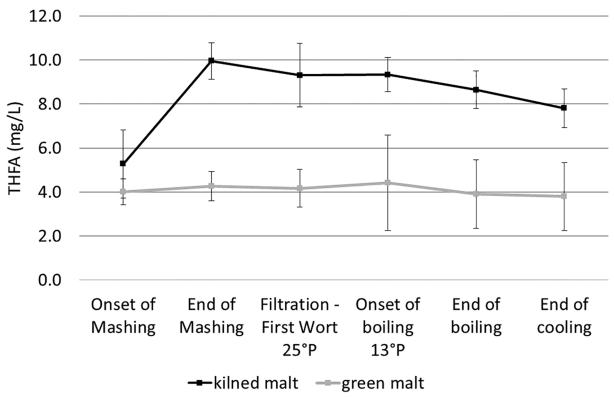

Figure 3. Trihydroxy fatty acid (THFA, mg/L) monitored in three individual brewing processes using green malt or its corresponding reference (pilsner) kilned malt. Data are the mean ± SD of 3 biological and 2 technical replicate measurements.

Table 3. Fermentation performance and beer characteristics prepared from green malt or the corresponding reference kilned (pilsner) malt

			re-steeping trials	
	GM	KM	GM	KM
pH	4.2 ± 0.0	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.0
Alcohol by volume % (v/v)	5.5 ± 0.4	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.2
Density (g/cm^3)	1.0054 ± 0.0018	1.0065 ± 0.0015	1.0059 ± 0.0020	1.0067 ± 0.0001
Specific gravity	1.0072 ± 0.0019	1.0083 ± 0.0015	1.0077± 0.0020	1.0085 ± 0.0001
Original gravity (°P)	12.1 ± 0.3	12.6 ± 0.5	12.7 ± 0.3	12.6 ± 0.3
Real extract % (w/w)	3.8 ± 0.3	4.1 ± 0.4	4.0 ± 0.5	4.2 ± 0.0
Real degree of fermentation (RDF)	69.7 ± 3.5	68.6 ± 1.9	69.8 ± 2.9	68.3 ± 0.5
Calories (kJ/100mL)	182.9 ± 4.9	189.9 ± 8.7	192.4 ± 5.2	191.0 ± 4.0

Data are the mean ± SD of 2-3 technical replicate measurements, GM = green malt; KM = kilned malt. Statistics: One-Way ANOVA with Fisher's LSD post-hoc test. GM = green malt; KM = kilned malt. There were no significant differences between treatments for the parameters reported.

sufficiently controlled in the original green malt brewing process, such that the potential advantage in LOX activity reduction offered by re-steeping was not realised.

Fermentation performance

Fermentation progression was similar across kilned malt and green malt worts and reached stationary phase three days after pitching. The pH dropped from 5.4 ± 0.1 to 4.3 ± 0.1 in green malt brews, and 5.2 ± 0.1 to 4.4 ± 0.1 in kilned malt brews. Final pH in the beer did not significantly differ across treatments, and all beers achieved typical finished beer pH values (4.2-4.5; Table 3). The kilned malt control fermentations reached an alcohol level of 5.4-5.7% v/v, which was more consistent than the green malt fermentations of 5.0 – 5.9% v/v (Table 3). However, statistically, all beers brewed were of similar alcohol content and degrees of fermentation, which did not significantly differ among the malts used. As illustrated in Figure 4, the FAN content of worts and beers prepared from untreated green malt (n = 3) were lower compared to levels of their corresponding reference wort/beer. Across fermentation, a higher proportion of FAN uptake (ranging between 70 – 82%) was observed relative to the corresponding kilned malt trials (52 – 66% FAN uptake), resulting in lower residual FAN in green malt beers compared to control beers. Previous studies suggested that higher heat loads in wort production led to lower FAN uptake, suggesting that heat related compounds reduce the assimilability of FAN by yeast *(18)*. However, when brewing with re-steeped green malt the tendency was towards the opposite effect (ranging between 53-64% FAN uptake; Figure 4). High levels of FAN in the wort, as found in the worts of kilned malt or re-steeped green malt, resulted in higher residual FAN in the final beer. High residual FAN in beer can result in elevated levels of Strecker aldehydes and consequently contribute to beer staling *(18, 44)*. Based on the comparison of the FAN levels of the worts and the final beers, the consumption by yeast of free amino nitrogen, as measured by the ninhydrin assay, can differ greatly. Measurement of the amino acid profile from both kilned malt and green malt pitching worts are required to further understand and explain the assimilability of the FAN.

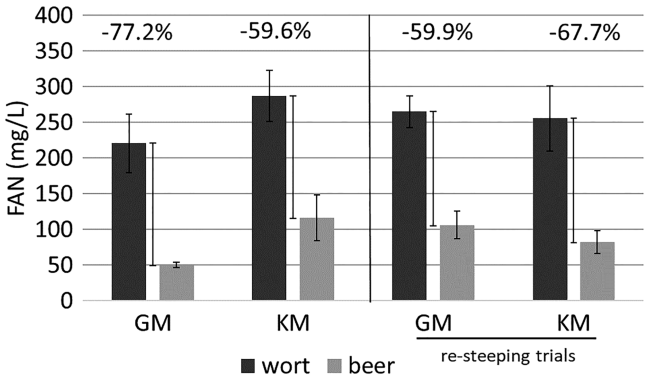

Figure 4. FAN levels (mg/L) in worts and beers prepared from green malt, re-steeped green malt and their corresponding reference malt. The average percentage uptake (%) from pitching wort to bottled beer is indicated. Data are the mean ± SD of 3 biological and 3 technical replicate measurements; GM = green malt, KM = kilned malt.

Characteristics of finished beers

The characteristics of the finished beers are presented in Table 4. All beers showed acceptable foam stability and low haze (chilled and permanent) formation. Haze formation in beer is caused mainly by interactions between haze active polypeptides and

Table 4. Beer characteristics prepared from green malt, re-steeped green malt or the corresponding reference kilned (pilsner) malt

			re-steeping trials	
	GM	KM	GM	KM
Colour (EBC)***	7.3 ± 1.2 ^{a}	9.5 ± 2.1 ^{b}	5.3 ± 0.4 ^{c}	8.4 ± 1.7 ^{d}
CO_2 (g/L)	5.8 ± 0.3	5.9 ± 0.1	6.3 ± 0.5	6.4 ± 0.3
NIBEM foam stability (sec.) $^{n.s.}$	176; 196; 115	139; 131; 119	178; 146; 151	141; 154; 164
Chill haze (EBC 90°scatter) $^{n.s.}$	1.32; 11.76; 7.72	6.21; 13.53; 1.86	6.3; 1.74; 2.97	6.52; 7.84; 4.61
Permanent haze (EBC 90°scatter) $^{n.s.}$	1.14; 7.94; 5.61	4.85; 10.65; 1.34	3.67; 1.33; 2.17	3.6; 4.93; 2.84
FAN (mg/L)***	50.3 ± 4.0 ^{a}	116.2 ± 32.2 ^{b}	106.3 ± 19.1 ^{b}	82.5 ± 15.8 ^{c}
Total polyphenols (mg/L) $^{n.s.}$	234.9 ± 31.7	250.9 ± 46.5	251.2 ± 7.7	268.5 ± 12.8
Flavanoids ((+)-catechin eq. mg/L)***	63.6 ± 5.1 ^{a}	60.1 ± 12.5 ^{a}	70.7 ± 3.3 ^{b}	73.4 ± 4.0 ^{b}
Proanthocyanidins (mg/L)**	39.4 ± 5.7 ^{a}	44.9 ± 5.3 ^{a}	34.6 ± 2.9 ^{b}	33.4 ± 2.8 ^{b}
DMS (μg/L) $^{n.s.}$	23.8 ± 9.9	24.3 ± 11.0	10.9 ± 2.7	12.7 ± 3.0
SMM (μg/L)†, ***	136.4 ± 37.1 ^{a}	44.1 ± 13.0 ^{b}	104.4 ± 45.5 ^{c}	13.5 ± 6.6 ^{d}
Thiobarbituric acid index***	10.6 ± 0.9 ^{a}	33.6 ± 6.4 ^{b}	15.5 ± 0.8 ^{c}	40.4 ± 5.1 ^{d}

† Indirect determination of SMM from (Total DMS – DMS), expressed as DMS equivalents

Superscripts a-d represent the ANOVA post-hoc groupings. In each row treatments differed significantly from one another if they have a different ANOVA group letter.

Asterisks represent the P-value significance

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$.

Data are the mean ±SD of 2-3 technical replicate measurements, Statistics: One-Way ANOVA with Fisher's LSD post-hoc test. GM = green malt; KM = kilned malt

polyphenols *(45–48)*. Polyphenols and flavanoid levels did not differ in beers prepared from green malt relative to the control beers (Table 4). The natural hazeactive polyphenols in beer are mainly proanthocyanidins, because of their size and potential to cross-link haze active proteins/peptides. However, in contrast to the lower proanthocyanidin levels reported in untreated green malt wort, there were no substantial differences noted in the fresh beers.

Unsurprisingly, the colour in the kilned malt control beers was higher than in the green malt beers. However, an acceptable colour was still achieved (Figure 5). Interestingly, the beers prepared from re-steeped green malt were significantly lower in colour than the beers prepared from green malt 'as is'. Potentially, the natural yellow colour pigments in malt (as discussed previously) could have been washed out during re-steeping. This theory would support our previous suggestion, that the colour of 'green malt beers' results from natural colour pigments, such as polyphenols and riboflavin.

The TBI levels decreased from the wort to the final beers, presumably due to the reducing power of yeast, reducing aldehydes to alcohols. Nevertheless, the untreated green malt (10.6 ± 0.9) and re-steeped green malt (15.5 ± 0.8) beers still had a significantly lower TBI in the beer compared to the reference brews (33.6 ± 6.4; 40.4 ±5.1, respectively), potentially benefitting beer flavour stability.

DMS and SMM levels were measured in all beers. Analysis of finished beers revealed DMS levels of 23.8 ± 9.9 μg/L on average (n = 3) in beers prepared from green malt and levels of 10.9 ± 2.7 μg/L in beers prepared from re-steeped green malt, which did not differ significantly from the controls (Table 4). SMM levels in all green malt beers (untreated and re-steeped) remained higher than those for kilned malt beers, although fermentation significantly reduced SMM levels. This confirms previous findings by White and Wainwright *(11)*. However, the remaining SMM could potentially be decomposed to DMS during pasteurisation, which is detrimental to final beer flavour. Therefore, further research was conducted to evaluate the impact of in-pack pasteurisation on the finished beers.

Figure 5. SurGreen (left) made of 100% green malt, in comparison to the reference beer (right) brewed with 100% pilsner malt [Colour figure can be viewed at wileyonlinelibrary.com]

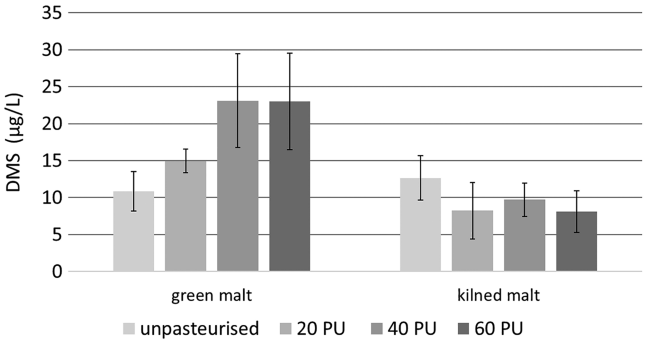

Figure 6. Influence of pasteurisation on DMS and SMM levels. Data are the mean ± SD of 3 biological and 2 technical replicate measurements.

Influence of pasteurisation on DMS formation

Further research was undertaken to evaluate the potential quality implications of the elevated precursor levels during beer pasteurisation. In a parallel experiment, beers (n = 3) were pasteurised to different degrees (20, 40, 60 Pasteurisation units; PU) so that the impact of elevated SMM in green malt beers could be ascertained. Typical process values for beer pasteurisation are about 14-15 PU, depending on beer style, alcohol content and the degree of contamination *(38)*. Hence, these data (Figure 6) suggest, that pasteurisation is not a major concern when brewing with green malt, provided that the initial DMS concentration is in an acceptable range.

Analysis of re-steeping water

Because the re-steeping water was used for mashing (to minimise overall water usage in the chain), it was likewise analysed for selected parameters. Due to the turbidity of the re-steeping water, it was difficult to express the pale-yellow colour of the re-steeping water in numbers. However, these findings support the view that the colour of beer is not only influenced by Maillard products, but also by other water-soluble compounds in the grain. In the re-steeping water, polyphenols (23.8 ± 7.2 mg/L) and flavanoids (5.2 ± 1.5 mg/L) were detected, but no proanthocyanidins. Additionally, FAN (31.7 ± 7.2 mg/L), low levels of DMS (5.9 ± 3.7 μg/L), and a surprisingly high concentration of SMM (407.4 ± 81.3 μg/L) were found. No THFA were detected in the re-steeping water. Heating of the re-steeping water to reach the required temperature for the onset of mashing did not influence the analytical results significantly.

Conclusion

The aim of this study was to evaluate the feasibility of brewing with 100% green malt with intact rootlets and to determine the quality of wort and beer made from green malt as compared to kilned malt brews processed from the same batch of malt. Even though further technological and process optimisations are undoubtedly required, this work confirms that an acceptable potable beer can be brewed using 100% green malt. No significant taints or obvious defects were detected in any of the beers prepared from green malt (untreated or re-steeped) compared to the reference brews.

The beers were tasted informally by expert tasters at both KU Leuven and the University of Nottingham, as well as a selection of visitors to our poster at the EBC Congress in Antwerp in 2019. The absence of any noted defects amongst 30-40 regular beer consumers is the basis for our conclusion that the green malt beers were 'acceptable' sensorially. Nevertheless, we fully appreciate that more detailed sensory evaluation of the organoleptic properties of green malt beers is required to evaluate their unique flavour profile and further understand how this might be complemented with the use of other grist materials to generate a more conventional kilned malt flavour in finished beers.

Since most breweries are configured to brew with kilned pale malt, adaptations are required when utilising green malt with a moisture content of more than 40%. Technical adaptations and milling optimisation are inevitable in order to avoid technical difficulties and reduced brewing yields due to poor sparging efficiency. In the present research, the thickness of the mash (1:2.2) obtained at the beginning of the process proved problematic for the low shear homogeniser used in this study. Also, the complex structure of the spent grains bed formed during mash filtration ('spongy, cohesive structure'), increased the likelihood of blockages with extended filtration and sparging periods. An optimised brewhouse process for wet milling, in combination with a normal (low shear) stirring device instead of the low shear homogeniser, used in the pilot brewery, is advised.

Increased SMM levels were measured in worts made from green malt, however DMS concentrations in the pitching wort were within an acceptable range. A further decline in SMM levels occurred across all fermentations. Tests carried out on the final beers, confirmed that DMS levels in beers made of green malt did not differ significantly from their reference brews. The presented data suggest that pasteurisation is not a major concern when brewing with green malt, provided that the initial DMS concentration is in an acceptable range. The finished beer specification was acceptable in terms of colour, pH, alcohol content and foam stability. The TBI was significantly lower in worts and beers prepared from green malt. It was interesting to note that the free amino nitrogen in green malt beer was considerably lower compared to kilned malt beers. Both of these factors should - in theory - be beneficial for the flavour stability of the aged beer.

Even though re-steeping seemed a promising technique by which to reduce LOX activity in green malt at laboratory scale, the results presented here suggest that it was unnecessary. LOX was adequately controlled in the pilot plant process by wet milling in deaerated liquor under CO_2 and mashing-in at 63°C, pH 5.2 under oxygen-limited conditions. Significantly lower trihydroxy fatty acid levels were determined in worts prepared from untreated green malt, compared to the reference wort. Furthermore, our results demonstrate that brewing with green malt need not be limited to the use of LOX-free barley varieties, although the latter may be beneficial for breweries, where strict LOX-hostile conditions cannot be applied or who wish to avoid additional costs (and health and safety considerations) of CO_2 injection.

Brewing with green malt is a disruptive technology and the process needs to be further optimised before it could be implemented widely in present day breweries. Due to the microbiological instability of green malt, it is either necessary to prepare malt/wort extract or to process rapidly by having a brewery and maltings co-located (transport of the high moisture commodity is not feasible at scale). However, the prospect of being able to prepare wort and beer of acceptable quality from 100% green malt, serves as a continuous incentive for future research.

Author contributions

Celina Dugulin: PhD student. Conducted research and formal analysis in this manuscript. Writing – original draft.
Luisa María Acuña Muñoz: Masters student. Conducted research and formal analysis.
Jasper Buyse: Brewmaster. Developed the brewing methodology protocols for using green malt in the brewery at KU Leuven, Technology Campus Ghent. Trained Celina Dugulin and Luisa María Acuña Muñoz in relevant procedures in the brewery.
Irina Bolat: Funding acquisition, conceptualisation, paper review and editing.
Gert De Rouck: Funding acquisition, supervision of PhD, conceptualisation and writing – review and editing.
David Cook: Funding acquisition, supervision of PhD, conceptualisation and writing – review and editing.

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Conflict of interest

The authors declare there are no conflicts of interest.

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