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Sufficient Formation and Removal of Dimethyl Sulfide (DMS) without Classic Wort Boiling

The formation and removal of dimethyl sulfide (DMS) was studied during a new method for wort production, without classic wort boiling. This was compared with classic brewing trials comprising one hour of wort boiling. The new wort production method consists of fine milling of malt under water to minimize LOX reactions, mashing-off at 95 °C, membrane assisted thin bed filtration, in-line injection of clean steam in the filtered wort and stripping of the wort while entering the combination vessel ('boiling' kettle/decantor), optional clean steam injection during filling of the combination vessel, and finally decantation of the hot trub in the same combination vessel. Oversized chimneys with condensate traps are installed on both the mash vessel and the combination vessel to promote removal and prevent re-entrance of unwanted volatiles, including DMS. Like in classic wort production, the contents of DMS precursor and DMS were sufficiently reduced when applying the new brewing method and DMS presented no flavour problem in the finished beer. The processing time of the innovative wort production method until the end of filling of the combination vessel and optional stripping of the wort was only 2.5 hours as compared to at least 3.5 hours for classic brewing using the same brewing line. Therefore, application of the proposed innovative wort production method allows for preparation of significantly more brews per day with sufficient removal of unwanted volatiles.

Descriptors: wort production, DMS, wort boiling

1 Introduction

In traditional brewing practice, to boil a wort and moreover to have sufficient evaporation and removal of unwanted volatiles, a high energy input is required. Therefore, several technological innovations have been introduced during the last decades to improve the efficiency of wort boiling. Already in the eighties, considerable attention was paid to the boiling process aiming at a reduced energy input (cost savings) by decreasing the heat load of the wort without compromising the removal of unwanted volatiles, in particular dimethyl sulfide (DMS). Several concepts were investigated to guarantee sufficient evaporation with lower demands of energy, such as high temperature wort boiling [39], sparging of wort with inert gas to facilitate evaporation [34], the use of a heat holding tank in combination with wort stripping [7, 40], continuous wort boiling [6, 9], application of thin film evaporation [50], forced convection [49], dynamic low-pressure boiling [8, 32], flash evaporation after gentle boiling [5, 22, 29, 44], boiling under vacuum [30], the use of new heating devices [4, 10, 14, 31], and a boiling system based on rectification [18].

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Tables and figures see Appendix

In particular for pale lagers, it has been shown multiple times that a high heat load is negative for the wort quality and the flavour stability of the final beer. The direct effect of heat load lies in the high levels of furfural and HMF (5-(hydroxymethyl)-2-furfural), oxidized fatty acids, and excessive production of carbonyl compounds, among which Strecker degradation aldehydes [1, 2, 12, 28, 36–38, 41, 45, 53, 55]. Also a decreased assimilability of free amino nitrogen (FAN) by the yeast is found as an indirect effect of heat load, whereby residual FAN levels in the beer may also result in the formation of Strecker degradation aldehydes during beer storage [11, 23].

In view of a reduced heat load and enhanced flavour stability, the biggest challenge in minimizing or even abolishing the wort boiling step, is the effective formation and evacuation of DMS, besides sufficient production of protein flocks. The flavour threshold value of DMS in beer is estimated at around 30 ppb [15]. Above this level, DMS may cause a 'cooked vegetable' note, which is mostly considered as an offending off-flavour. Nevertheless, in certain lagers up to 90 ppb of DMS has been reported, meaning that DMS will contribute significantly to the flavour of these beers [51].

The most important precursor of DMS is S-methylmethionine (SMM) coming from the malt [3, 13, 46, 54]. DMS can be formed from SMM at temperatures above 60 °C [3, 13, 46, 54]. In the entire volatilization process for DMS, this transformation reaction of SMM into DMS was found to be the rate limiting factor [33, 56]. Regarding the removal of volatiles, the engineering fundamentals of the boiling process and the evaporation efficiency in various boiling systems were explained lately [16, 17, 24, 42]. Insufficient evacuation of DMS and subsequent oxidation can lead to the

formation of dimethyl sulfoxide (DMSO) and dimethyl sulfone ($DMSO_2$). In particular DMSO may act as a new precursor for DMS since yeast DMSO reductase is able to reduce DMSO back into DMS during fermentation [21, 25].

To solve the problem of protein flocculation, trials have been performed using silica hydrogel based filtration of the wort [27]. Furthermore, DMS was removed in these trials through forced evaporation by spraying of the wort. Although promising results were obtained in this work, special attention to head retention must be paid. Most recently, another innovative approach for wort production, based on an integrated method of special heat treatments and efficient wort stripping, has been presented by our institute [1]. This novel approach comprises wet, oxygen-free fine milling of malt and mashing-off at 95 °C, during which early DMS formation and removal, as well as efficient protein coagulation, may already take place. Next, a membrane-assisted thin bed filter is used to retain the protein flocks whilst DMS formation continues. Injection of clean steam in the wort pipeline will strip off the DMS from the wort while entering the combination vessel ('boiling' kettle/decantor). Clean steam injection in the combination vessel during filling will guarantee further formation and volatilization of DMS and other unwanted volatiles. Once the wort filtration cycle is finished, no further wort boiling should be required.

In this paper, the focus is on the degradation of DMS precursor (SMM) and DMS evacuation when applying this highly innovative wort production method, in comparison with classic wort production as a reference.

2 Materials and methods

2.1 Classic wort production – coarse milling

Three Pilsner beers were prepared in our pilot scale brewery of 2 hL (brewing line 1) using the following conditions: 40 kg coarse milled Pilsner malt (2-roller mill) is mixed with 1.4 hL reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}); mashing-in: temperature of 64 °C, pH of 5.2 (pH adjustment with lactic acid 30 %; v/v); brewing scheme: 64 °C (30 min), 72 °C (20 min), 78 °C (1 min) (rise in temp. at 1 °C/min); wort filtration: lauter tun; wort boiling: 60 min atmospheric boiling (evaporation: about 5 %); at the end of boiling, 0.2 ppm Zn^{2+} ions were added, as well as iso-α-acids extract aiming at 25 ppm iso-α-acids in the finished beer (3.85 g iso-α-acids added/hL; utilisation: 65 %); wort clarification: open whirlpool; after cooling and aeration, the wort (original gravity: 12 °P) was pitched with 10^7 yeast cells/ml (inoculum: dry yeast, strain W 34/70 (Fermentis), was hydrated for 1 hour in sterile water with a volume of 10 times the weight of the dry yeast); primary fermentation: 8 days at 12 °C in cilindroconical tanks; maturation: 10 days at –0.5 °C; beer filtration: kieselguhr/cellulose sheets (pore size 1μm); CO_2 saturation up to 5.6 g/L; packaging: 6 head rotating counter pressure filler (monobloc, CIMEC, Italy) using double pre-evacuation with intermediate CO_2 rinsing and overfoaming with hot water injection before capping (final oxygen levels: below 50 ppb).

2.2 Classic wort production – fine milling

Three Pilsner beers were prepared in the second pilot brewery of KaHo St.-Lieven (brewing line 2; 5 hL scale), outlined in figure 1. This brewing installation is a prototype plant for innovative wort production, but also classic brewing trials can be performed in it. The following conditions were used: 84 kg fine milled Pilsner malt (wet disc mill, Meura) is mixed with 1.84 hL reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}) and approx. 400 ml lactic acid (30 %, v/v) (precise volume to be added is malt depending); mashing-in: temperature of 64 °C, pH of 5.2; brewing scheme: 64 °C (30 min), 72 °C (20 min), 78 °C (1 min) (rise in temp. at 1 °C/min); wort filtration: membrane assisted thin bed filter; wort boiling: 60 min atmospheric boiling using a double jacket for heating (evaporation: about 5 %); at the end of boiling, 0.2 ppm Zn^{2+} ions were added, as well as iso-α-acids extract aiming at 25 ppm iso-α-acids in the finished beer (3.85 g iso-α-acids added/hL; utilisation: 65 %); wort clarification: decantation in combination vessel; all further steps (wort cooling, aeration, pitching, fermentation, maturation, beer filtration, saturation and filling) were carried out as described under 'Classic wort production – coarse milling'.

2.3 Innovative wort production

Three Pilsner beers were prepared in the innovative pilot brewery of 5 hL (brewing line 2; see Figure 1), using the following conditions: 84 kg fine milled Pilsner malt (wet disc mill, Meura) is mixed with 1.84 hL reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}) and approx. 400 ml lactic acid (30 %, v/v) (precise volume to be added is malt depending); mashing-in: temperature of 64 °C, pH of 5.2; brewing scheme: 64 °C (30 min), 72 °C (20 min), 95 °C (15 min) (rise in temp. at 1 °C/min); wort filtration: membrane assisted thin bed filter; in line injection of clean steam in filtered wort and stripping of wort while entering the combination vessel; optional: injection of clean steam in the combination vessel during kettle filling; the following steam injection regimes were applied for the respective brews: Innovative Brew I: in-line steam injection of first wort in pipeline; Innovative Brew II: the same as brew I but with additional steam injection in pipeline during sparging; Innovative Brew III: the same as brew II but with additional steam injection in the combination vessel during kettle filling; at the end of kettle filling, 0.2 ppm Zn^{2+} ions were added, as well as iso-α-acids extract aiming at 25 ppm iso-α-acids in the finished beer (3.85 g iso-α-acids added/hL; utilisation: 65 %); wort clarification: decantation in combination vessel ; all further steps (wort cooling, aeration, pitching, fermentation, maturation, beer filtration, saturation and filling) were carried out as described under 'Classic wort production – coarse milling'.

2.4 Headspace SPME GC-PFPD analysis of DMS and DMS precursor in wort and beer

DMS and indirectly also DMS precursor were quantitatively determined in wort or beer samples by headspace SPME using a Thermo Finnigan TraceGC Ultra system (Interscience, Louvain-la-Neuve, Belgium), equipped with a CTC CombiPAL autosampler, a S/SL injector with narrow bore glass inlet liner, a 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) operated in the sulfur mode. Helium was used as carrier gas at 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 were processed with 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 2 min at 30 °C. The SPME needle was then inserted through the septum and the Carboxen™/Polydimethylsiloxane fiber (Stableflex, 85 µm, Supelco, USA), previously conditioned for 2 min at 300 °C, was exposed to the headspace for 15 min with agitation at 250 rpm. The fibre was thermally desorbed into the injection port of the GC for 3 min. Finally, the fibre was post-conditioned for 2 min at 300 °C. Quantification of the DMS level in unknown samples (wort or beer) was performed by external calibration of the equipment with known amounts of DMS (0.1, 0.5, 1, 2.5, 5, 10 ppb) and EMS (1 ppb, internal standard) dissolved in water. The ratio of the DMS peak area over the EMS peak area is used to correlate with the DMS/EMS concentration. If necessary, brewery samples were diluted by an appropriate dilution factor to allow for quantification within the linear range of the calibration curve.

2.5 Gas chromatographic analysis of trihydroxy fatty acids

Determination of trihydroxy fatty acids in beer samples was based on the published procedures of Möller-Hergt et al. [35] and Wackerbauer and Meyna [47]. Equipment: GC-FID (Thermo Quest CE Trace 2000 (Interscience, Benelux)) equipped with an AS 2000 autosampler (Interscience, Benelux), a cyano-phenyl-methyl deactivated retention gap (2.5 m × 0.53 mm i.d., Varian, Middelburg, The

Netherlands), and a fused silica analytical capillary column (CP-Sil 5 CB LOW BLEED/MS; 50 m × 0.25 mm i.d., 0.25 µm film thickness, Varian, Middelburg, The Netherlands). Data processing was performed by Chromcard software 1.0.7.

3 Results and discussion

3.1 Determination of DMS

Different types of detectors have been applied for the determination of the DMS content in wort and beer, such as the Flame Ionisation Detector (FID), the Sulfur Chemiluminescence Detector (SCD), the Flame Photometric Detector (FPD) and its Pulsed Flame variant (PFPD), the Atomic Emission Detector (AED), the Mass Spectrometer (MS), and the Sniffing Detector (GC-O) [43,46,48]. Currently, the best way to quantify DMS appears to be headspace solid-phase microextraction coupled with gas chromatography-pulsed flame photometric detection (HS-SPME-GC-PFPD) [19, 20, 52], using ethyl methyl sulfide (EMS) as internal standard. We therefore applied HS-SPME-GC-PFPD for quantification of DMS (and indirectly of DMS precursor) in our brewery samples (for SPME and GC-PFPD conditions, see Materials and Methods).

To evaluate the linearity of the method, a calibration curve was established for DMS dissolved in water. The results shown in figure 2 demonstrate that linearity is found within the range from 0 to at least 10 µg DMS/kg (ppb). As similar data on DMS quantification in brewery samples were obtained when using calibration curves made by standard addition of known amounts of DMS to wort or beer, for practical reasons, all quantifications of DMS during this comparative technological study were based on external calibration of the equipment with known amounts of DMS dissolved in water.

3.2 Determination of DMS precursor

It is not possible to quantify directly the precursor molecules of DMS, i.e. *S-methylmethionine* (SMM), via the above SPME-GC-PFPD methodology. For that purpose, the non-volatile DMS precursor needs to be converted into DMS and by measuring the DMS content of the sample before and after this treatment, the DMS precursor content can be derived.

Mostly, NaOH is used as reagent to convert DMS precursor into DMS, but it has been reported that this reaction may also lead to unwanted side formation of oxidized products such as DMSO and $DMSO_2$, thus resulting in incorrect quantitative determination of DMS precursor [13, 43]. Therefore, in this work, we aimed at efficient conversion of DMS precursor into DMS without the addition of any oxidizing agent, *i.e.* by merely heating the samples at 100°C for a sufficient time [46].

To evaluate the conversion efficiency of DMS precursor into DMS by heat, aqueous samples containing known amounts of SMM were heated at 100 °C in a drying oven for various time intervals. After the different exposure times at 100 °C, the DMS content in the samples was analyzed by the described SPME-GC-PFPD methodology (for the results, see Figure 3).

After 160 min of heating, a recovery of DMS of 97 % is noticed, pointing to almost full conversion of SMM into DMS. Even after 500 min of heat exposure, the DMS recovery still amounts to 98 %. These results demonstrate SMM transformation into DMS without significant (i.e. at maximum 2 % of by products may be formed based on the recovery) formation of by-products. Thus, both DMS and DMS precursor contents can be determined by the above described protocol for sample preparation and subsequent SPME-GC-PFPD analysis. For practical reasons, the DMS pre-cursor content of brewery samples will always be expressed in this paper as DMS equivalents in µg/kg since these values clearly reflect the potential of DMS formation from SMM.

3.3 The influence of milling on DMS formation and evaporation in classic brewing trials

In order to investigate the influence of milling of malt, *i.e.* course milling-lauter tun wort filtration versus fine milling-thin bed wort

filtration, on DMS formation and evaporation, three similar brews were made in each of the two pilot plants of KaHo St.-Lieven, *i.e.* brewing line 1 and 2, respectively, using the same raw materials and brewing parameters. Pilot plant 1 (scale: 2 hL) requires coarse milled malt on account of lauter tun wort filtration. In pilot plant 2 (scale: 5 hL), the malt is fine milled under water and a membrane assisted thin bed filter is used for wort filtration. In fact, this second brewing line has been designed to produce wort under innovative brewing conditions, but due to its flexible configuration, classic brewing trials can be performed here as well. To limit early fatty acid oxidation by lipoxygenase (LOX) activity [1], mashing-in was performed at 64 °C and pH 5.2 for all brews (for more details on the applied brewing schemes, see Materials and Methods, Classic wort production).

The amount of trihydroxy fatty acids (THFA) in the finished beers was measured as a marker for LOX activity during brewing. It has been reported previously by our institute that THFA values from 2.9 mg/L up to 11.6 mg/L can be found in commercial Pilsner beers [26]. Results shown in table 1 demonstrate relatively low levels of THFA in all finished experimental beers (approx. 3 mg/L), which is indicative for mashing-in under effective, anti-oxidative conditions. Thus, in this respect, fine wet milling of the malt under CO_2 atmosphere performs equally well as coarse milling when mashing-in conditions are such as to compensate for the increased risk of LOX reactions. The influence of milling and concomitant wort filtration technology on DMS formation and evaporation in these classic brewing trials was investigated by determining the contents of DMS precursor (measured as DMS equivalents) and free DMS at different stages of wort production and in the finished beers. The results of these analyses are depicted in figures 4 and 5, respectively.

As apparent from figure 4, a slightly increased content of DMS precursor was found in the first wort when the malt is fine milled (184.7 ppb ±21.5 ppb (fine milling) compared to 154.6 ppb ± 20.7 ppb (coarse milling)). This was rather expected since the DMS precursor is mainly located in the seedlings, which remain more intact in case of coarse milling of the malt. During the wort boiling phase, due to thermal degradation, the content of DMS precursor strongly decreases in both series of brewing trials, to arrive at similar and low values from the end of boiling till the finished beers. The observed evolution of the levels of DMS precursor during classic wort production is furthermore in accordance with literature [25].

Increased extraction of DMS precursor when performing fine milling is, however, not a problem in view of free DMS, as shown in figure 5. Despite fast formation of DMS during classic wort boiling, its removal poses no problem in both series of brewing trials since final concentrations of free DMS from the end of boiling were always clearly below the flavour threshold value of 30 ppb. Our findings on the evolution of free DMS levels during classic wort production are also in good agreement with previous reports [3,15,25].

The process times after the different stages in classic brewing in pilot plant 1 and 2, respectively (taking mashing-in as the starting point), are presented in table 2. Clearly, until the onset of wort cooling, the process time in pilot plant 2 (fine milling/thin bed filter) is about 50 minutes less compared with pilot plant 1 (coarse milling/lauter tun). This is because in case of using a membrane assisted thin bed filter, wort filtration is during transfer to the boiling kettle, whereas lauter tun filtration requires mash transfer from the mash vessel to the lauter tun and subsequent sedimentation of spent grains. Thin bed filtration is also generally faster than lauter tun filtration. Furthermore, in our new brewing installation (see Figure 1), transfer to a separate whirlpool is no more required since decantation of the wort and hot trub separation take place in the combination vessel.

3.4 DMS formation and evaporation during innovative brewing trials

The proposed innovative brewing method is performed in our new brewing plant 2 (5 hl scale, see Figure 1). First of all, this method comprises fine wet milling of malt under circumstances whereby LOX reactions are maximally prevented (milling under water and CO_2, at 64 °C and pH 5.2). Mashing-off is carried out at 95 °C for 15 min to enhance early degradation of DMS precursor and protein coagulation. During subsequent hot wort thin bed filtration, further degradation of DMS precursor is possible and oxidation of released DMS into DMSO is limited by pre-rinsing of the thin bed filter with CO_2. During transfer to the combination vessel, the filtered wort is injected with clean steam to strip off unwanted volatiles while entering the kettle, which also functions as a decantor in wort clarification. Optionally, during kettle filling, direct steam injectors in the combination vessel can be used to achieve extra stripping. Once the combination vessel is completely filled, there is no further wort boiling in this innovative brewing scheme.

Three different innovative brews, further indicated as Innov I, Innov II, and Innov III, were prepared according to the above described technology (for more experimental details, see Materials and Methods, Innovative wort production). The difference between these three brews is in the steam injection regime (see Table 3). An increased use of steam injection has been performed in order to evaluate the efficiency of DMS formation/removal and to determine the required degree of heat treatments. Therefore, similar to the classic brewing trials, the contents of DMS precursor (expressed as DMS_{eq}) and free DMS were measured at different stages of wort production and in the final beers (see Figures 6 and 7).

Results on the determination of the levels of trihydroxy fatty acids (THFA) in the final beers (approx. 3 ppm, see Table 1) again suggest that appropriate mashing-in conditions were selected to anticipate unwanted LOX reactions. The impact of mashing-off at 95 °C on the extent of transformation of DMS precursor becomes apparent from figure 6. Clearly, mashing-off at 95 °C for 15 min already results in a much lower amount of DMS precursor in the first worts of all innovative brews (approx. 80 ppb DMS_{eq}), compared to the classic brewing trials (approx. 180 ppb DMS_{eq}). The content of DMS precursor in the first worts of the three innovative brewing trials is also well comparable. During subsequent stages of innovative wort production, a further steady decrease in DMS precursor is observed to end up with low levels at the onset of wort cooling and in the finished beers, similar to classic brewing.

Figure 7 displays the evolution of free DMS during innovative brewing. In the brewing trial "Innov I", clean steam is injected only during transfer of the first wort to the combination vessel. Compared to the other brews, an elevated concentration of free DMS is found in this trial at the onset of wort cooling (120 ppb). The DMS level in the final beer, however, amounts to around 60 ppb, which was only slightly detectable on sensory evaluation. On the other hand, despite enhanced degradation of DMS precursor upon mashing-off at 95 °C, the initial DMS concentration in the first wort of the brew "Innov I" (before injection of clean steam) was very similar to the DMS concentration of first wort prepared by classic brewing (mashing-off at 78 °C). It therefore appears that, due to the oversized chimney of the mash tun of the new pilot brewery, already a lot of free DMS is evacuated very efficiently when mashing-off at 95 °C.

To promote further wort stripping, in-line clean steam injection was applied during both the transfer of first wort and sparging, *i.e.* additional second wort stripping, in the brewing trial "Innov II". Figure 7 demonstrates that in this experiment the removal of free DMS during wort transfer to the combination vessel was somewhat more effective, but, nevertheless, the concentration of DMS in the wort prior to cooling remained relatively high (approx. 100 ppb). In the final beer, however, the DMS level was in the range of its flavour threshold value and not detectable upon sensory evaluation. The problem of still relatively high DMS concentrations during innovative wort production may be related to the decreasing temperature during filling of the combination vessel. Indeed, at the entree of the combination vessel, there is a strong evaporation and concomitant decrease in temperature of the wort to around 85–90 °C. At that lower temperature, evaporation of DMS and removal of unwanted volatiles in general, will occur less efficiently. To verify this assumption, a third innovative brew (Innov III) was prepared with in-line steam injection during first and second wort transfer, whilst the temperature in the combination vessel was kept at 99.9 °C during the filling operation by in-kettle clean steam injection. As can be seen from figure 7, the amount of free DMS is now clearly much lower at the end of filling of the combination vessel and at the onset of wort cooling (approx. 50 ppb). Furthermore, the DMS level in the final beer derived from "Innov III" is as low as in classic brewing, *i.e.* less than 20 ppb, which is well below the flavour threshold value.

The process times after the different stages in innovative brewing are presented in table 2. Following mashing-in, only 165 min are required to produce wort ready for cooling. This is significantly less than the corresponding process times for classic wort production via coarse milling (285 min) and fine milling (235 min), respectively.

4 Conclusions

To circumvent classic wort boiling, an innovative wort production method including mashing-off at 95 °C, is presented in this work. At this high mashing-off temperature, early DMS formation and removal should be promoted, whilst proteins will form flocks which can be retained in a membrane assisted thin bed filter. In particular, the stripping need of the innovative wort production method with regard to DMS removal, was studied in this paper.

From the analytical point of view, a new method for determination of the level of DMS precursor (expressed as DMS equivalents), is proposed. It was found that simple heat treatment for a sufficient time at 100 °C results in almost complete transformation of SMM into DMS, without significant formation of unwanted by-products. This allows for a much more reliable determination of DMS precursor in brewery samples.

Brewing under classic conditions using fine milling in combination with a membrane assisted thin bed filter, resulted in a somewhat higher extraction of DMS precursor during mashing than coarse milling. However, at the end of wort boiling, no significant differences could be found in levels of DMS precursor and free DMS between fine milling-thin bed filter operations and coarse milling-lauter tun operations, respectively. In our pilot brewery installations, the total wort production time until the onset of wort cooling is, however, about 50 minutes less when applying fine milling in combination with thin bed filtration.

During the innovative wort production trials, a sufficient and well comparable decrease in DMS precursor was found. Based on measurements of DMS precursor and free DMS, the extra early produced DMS at the high mashing-off temperature of 95 °C must have been very well removed, due to the presence of the overdimensioned chimney on the mash vessel. However, to achieve further efficient formation and removal of free DMS, like in classic brewing, a high input of clean steam (both in-line and in-kettle) appeared to be required. Because of a reduction in temperature at the entrance of the filtered wort in the combination vessel, free DMS was not sufficiently removed during kettle filling, even when applying full wort stripping at transfer. However, when using extra steam injection during kettle filling, the DMS content of the collected wort was sufficiently reduced to end up in the finished beer well below the DMS flavour threshold value, without classic wort boiling. Moreover, the trihydroxy fatty acid content of all beers was relatively low, suggesting that LOX reactions were well blocked at mashing-in in all brewing trials, even when applying fine milling of the malt.

Because of relatively fast wort filtration and in particular the absence of the wort boiling stage, the processing time of the innovative wort production method is about 70 % compared to classic brewing. Consequently, significantly more brews per day may be produced in a cost-efficient way, since direct steam injection is also energetically more efficient than indirect heating.

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Received 30 December, 2009, accepted 20 February, 2010

Appendix

Table 1 Levels of trihydroxy fatty acids (mg/L) in finished beers

CB CM I	CB CM II	CB CM III	CB FM I	CB FM II	CB FM III	Innov I	Innov II	Innov III
2.9	3.0	2.9	3.0	3.1	2.9	3.0	3.1	2.9

CB classic brewing;
Innov innovative brewing;
CM coarse milling;
FM fine milling;
Roman numerals denote the different brewing trials

Table 2 Process times (min) after different stages in brewing (calculated from mashing-in)

	First wort	Onset boiling	End boiling	Start cooling
Classic brewing, coarse milling (Pilot plant 1)	90	200	260	285
Classic brewing, fine milling (Pilot plant 2)	70	155	215	235
Innovative brewing (Pilot plant 2)	90		150	165

Table 3 heat treatments by clean steam injection during innovative brewing in pilot plant 2

	Innov I	Innov II	Innov III
In-line steam injection during first wort filtration	wort temp.: 99 °C	wort temp.: 99 °C	wort temp.: 99.0 °C
In-line steam injection during sparging	none	wort temp.: 99 °C	wort temp.: 99.0 °C
In-kettle steam injection during filling of combination vessel	none	none	wort temp.: 99.9 °C

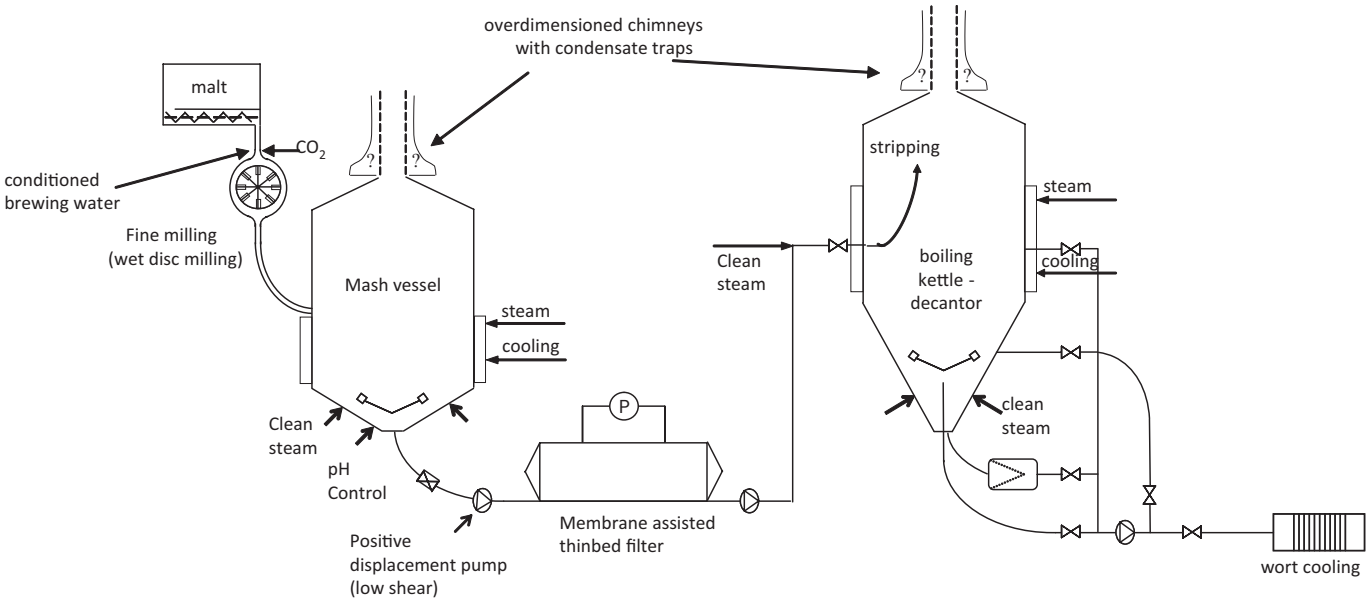

Fig. 1 Outline of innovative pilot brewing plant (brewing line 2; 5 hL scale) of KaHo St.-Lieven

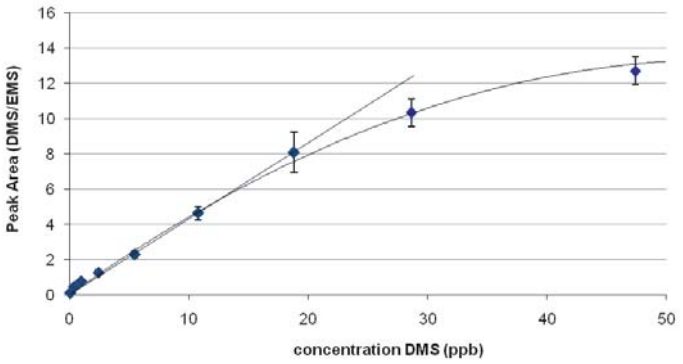

Fig. 2 **Evaluation of linearity of DMS determination method (n=3; error bars = +/- standard deviation (S.D.))**

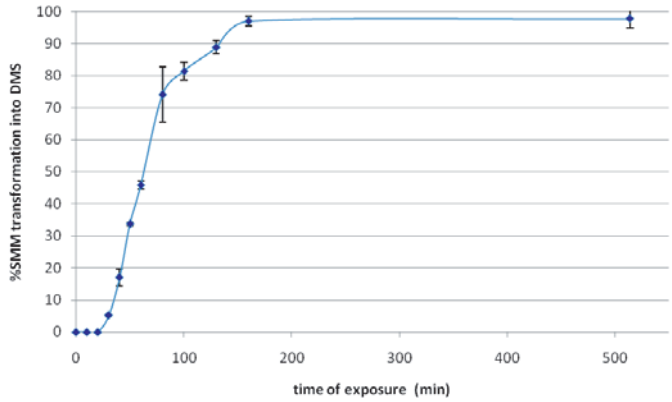

Fig. 3 **DMS formation from SMM at 100°C as a function of heating time (n=3; error bars = +/- S.D.)**

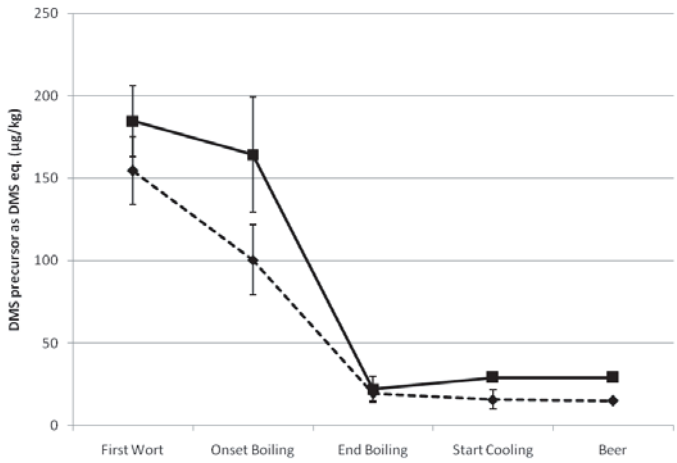

Fig. 4 **Evolution of DMS precursor, expressed as DMS equivalents in µg/kg, during classic brewing (n=6; error bars = +/- S.D.)**

(—◆— : course milling, brewing line 1;

—■— : fine milling, brewing line 2)

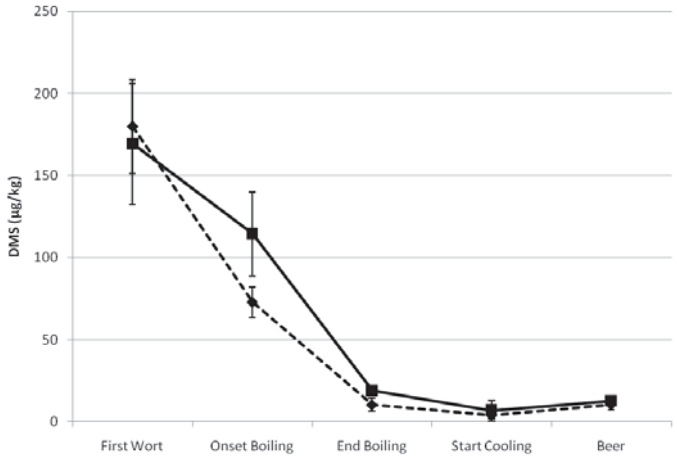

Fig. 5 Evolution of content of free DMS (µg/kg) during classic brewing (n=6; error bars = +/- S.D.)

(: course milling, brewing line 1;

: fine milling, brewing line 2)

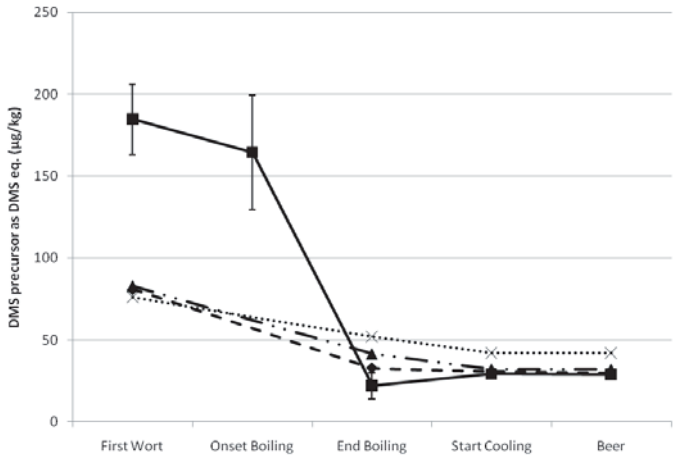

Fig. 6 Evolution of DMS precursor, expressed as DMS equivalents in µg/kg, during classic brewing and innovative brewing in brewing line 2 (n=6; error bars = +/- S.D.)

(: classic brewing;

: innovative brew I;

: innovative brew II;

: innovative brew III) (for stage(s) of clean steam injection when performing innovative brewing, see Materials and Methods, Innovative wort production)

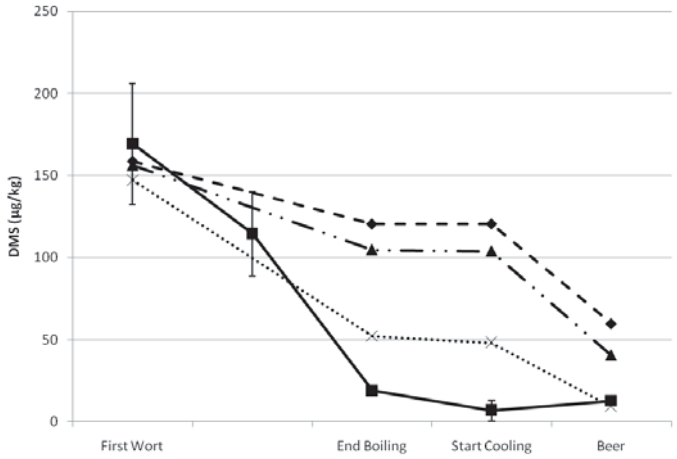

Fig. 7 Evolution of content of free DMS (µg/kg), during classic brewing and innovative brewing in brewing line 2 (n=6; error bars = +/- S.D.)

(: classic brewing;

: innovative brew I;

: innovative brew II;

: innovative brew III) (for stage(s) of clean steam injection when performing innovative brewing, see Materials and Methods, Innovative wort production)