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The impact of wort production on the flavour quality and stability of pale lager beer

Fine milling in combination with thinbed mash filters has been introduced into the brewing industry more than 20 years ago. Although clearest worts can be obtained at highest gravity, in combination with high extract yield and improved starch conversion, a majority of brewers still use the conventional coarse milling in combination with lautertuns. Brewers seem still afraid having a negative impact of fine milling on wort and beer quality especially with regard to over extraction of polyphenols, proteins and oxidative enzymes. A lack of detailed data comparing both wort production methods could be a reason. In this study, the faster wort filtration as well as higher extract yield has been confirmed when using fine milling and a thin bed filter. A higher concentration of fermentable sugar led to this increased yield and not an increased level of proteins and polyphenols. On the contrary, fine milling in combination with thicker mash and thin bed filtration results in a lower level of haze sensitive protein in the pitching wort in comparison to coarse grist and the use of a lautertun. No striking differences have been found comparing analyses of fresh beer. Also during forced ageing of the beers at 30 °C, no difference in the formation of flavour negative compounds or degradation of bitterness has been noticed. Both wort production methods result in highly comparable beers with equal flavour stability.

Descriptors: milling, mashing, mash filter, wort production, flavour stability

1 Introduction

Driven by the objective to increase throughput and to reduce wort production costs in the brewing industry, many innovations have been introduced, especially with regard to the cost efficient mash filtration and extract recovery. Among them, a combination of fine milling and thinbed filtration as an alternative for coarse milling and traditional lautertuns is available [29]. Using an automated thinbed mash filter, including membranes for filter cake compression, clearest and highest gravity worts, as well as higher extract yields have been obtained in pilot operations. Soon after their introduction, the same results were reached under industrial conditions [4, 71].

In thinbed filters the depth of the vertical filter bed is approximately 4 cm, whereas in lautertuns a horizontal filter bed of 25-50 cm (depending on the malt load and the milling system) is obtained [23, 40]. In contrast with coarse milling, whereby the husks preferably remain intact, the husks in case of thinbed filtration have to be finely milled. Another point of difference is that when using thinbed filters, the filter cake produced during heavy wort filtration is compressed prior to sparging by an expandable membrane and again compressed after sparging. In lautertun operations however all is done to avoid compression of the filter cake or compensate by knifing of the filter bed. Knifing can result in an increased risk of turbidity, polyphenols and O_2-ingression which affect lauter performance and wort quality. Compression of the filter cake in thinbed filter operations prior to sparging allows the highest extract yields at the expense of lowest sparge rates (<2.5 hl/100 kg instead of <4 hl/100 kg in case of lautertun operations) [4, 51] or even very low sparge rates of 1.5 hl/100kg with optimized industrial conditions [28]. With fine milling, preferably under anti-oxidative conditions, starch free worts can be obtained with short infusion mashing and even after mashing-off and mash filtration at 95 °C [20].

Although a growing number of brewers nowadays have invested in thinbed filter brew lines, a majority still adopt the traditional combination of coarse milling and lautertuns [4, 51, 52]. Compared with traditional brewhouses, the higher extract yield frequently has been reported when using fine milling and thin bed filters [4, 51, 52, 55, 67] in combination with an increased starch conversion [55]. The total filtration cycle time is reduced by 60 min according to industrial results [4, 71]. Concerning the impact of milling on beer flavour quality and beer flavour stability, highly comparable results have been reported, but detailed analytical results have not been presented [19, 51].

Another important topic is the oxygen management in case of thinbed filter operations since excess of oxygen negatively affects lauter performance in practice [8, 12, 50, 63, 68, 70]. Indeed, excess oxygen leads to oxidative gelation and the formation of disulfide bounds that negatively affect mash filtration. The effect of O_2-entrainment can be corrected by the addition of anti-oxidants, such as SO_2 or gallotannins [1, 2, 8]. Anti-oxidative operations during

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wort production are preset conditions for improved flavour stability of final beer [1, 2, 6, 30, 33, 50, 56, 63, 65, 70]. However neutral findings were also found when oxygen entrainment was minimized at the early stages of mashing regarding flavour stability of beer [50]. Because of lowest mash rates and sparge rates, thinbed filters are best fit for cost efficient high gravity brewing [4, 29, 51]. Another exploitable issue is that with fine milling and thinbed filters, clearest worts can be obtained independent of whether husk material is present, as long as there is sufficient coagulated high molecular weight (HW) protein material to quickly form a filter bed [3, 11, 23, 24, 71]. With clearest worts, lowest levels of non-oxidized lipids can be obtained [4, 29, 51]. Lipids, fatty acids and also oxidized fatty acids (trihydroxy acids) negatively affect foam stability [38, 39]. Turbidity of pitching wort and accompanying unsaturated fatty acids affect ester formation during fermentation. Especially the middle chain fatty acid esters are influenced by the fatty acid concentration [26, 47]. When trub potential of the filtered wort is too low with a mash filter (<2 ml/l Imhoff), it is easy to manage by eliminating turbid wort recirculation. A critical point with regard to thinbed filters is that loading is crucial for efficient extract recovery by sparging whereas with lautertuns the load flexibility is somewhat higher [4]. A flexibility of 30 % of the malt loading however is possible with a membrane assisted thin bed filter. On the other hand, a lautertun also performs best at a distinct load. Brew sizes exceeding these loadings will affect the operation of the lautertun in reduced extraction efficiency, an extended run off time, and also higher turbidity, because of the low knifing of the chopping device or a combination of all [13]. Beside the standard flexibility on the nominal throw, it is technically possible to get increased load flexibility with a mash filter by a blind plate [4].

Among brewers and scientists, there is still a debate about the impact of fine milling on the final beer quality. For many brewers, fine milling of the complete husk fraction as well as of the intact and viable seedling of pale lager malt, is a big step to take [71]. Fine milling results in higher extraction levels of all malt compounds if mash is free of clots by using appropriate mixing systems. The positive effect with regard to higher yield and starch conversion is already mentioned, but there's also the increased release and extraction of risk-compounds for beer quality, e.g. proteins and polyphenols [4], pentosans [45] and glucans [36], DMS precursor [21], and lipoxygenase (LOX) [19]. However, compensations, even within the context of clean-label technology, are possible.

Polyphenol extraction can be controlled by sparging with acidified water [15, 27]. Proteolysis can be limited by higher mashing-in temperature [30] and, especially in case of anti-oxidative mashing, proteins get coagulated during mashing and retained in the filter bed during lautering [14, 43, 61]. A more complete protein precipitation is found in thick mashes [44]. Reactions between haze active proteins and polyphenols are at their best at high and balanced concentration and when pH is near 5 [14, 61]. The combined effect of fine milling, thicker mash, thinbed filtration, and mashing at decreased pH should lead to the efficient removal of high molecular weight proteins and polyphenols.

Beta glucans indeed are more easily extracted in case of fine milling at the expense of a higher viscosity and poorer lauter performance. However, the solubilization of glucans and pentosans is limited in thicker mashes (mash rate of 1:2.5) [36, 45]. Malt modification has a greater impact on the lauter performance than milling or mash rate [37]. Microflora management during malting can have a positive effect on mash filtration and extract yields. The use of starter cultures during malting has been reviewed quite recently [32]. It leads to lower levels of β-glucans and arabinoxylans in malt and an improved lauter performance [9, 41, 46, 57]. However, there is the negative effect of excessive shear forces on industrial mash filtration due to badly designed stirrers, pumps, and pipeline layout. Short and high temperature mashing schemes and state of the art engineering of pipe lines, stirring, and transfer operations will definitely result in a decreased solubilization of glucans, arabinoxylans, and β-glucan gel formation resulting in an improved lauter performance [27, 35, 45, 63].

Pale lager malts are characterized by residual DMS precursor (DMSP), mainly present in the seedling. It has been observed that with fine milling slightly more DMSP is found than with coarse milling. However, this increased extraction did not result in a higher DMS-level after boiling and in the final beer [21].

LOX-activity, if still present in malt, risks being involved in the enzymatic oxidation of fatty acids during malting and mashing-in as well as on the production of precursors of stale flavour aldehydes [78, 79]. More LOX is extracted by fine milling [19], but when the grist is hydrated and mashed-in at pH 5.2, the LOX activity decreases already by 50 % [79]. Extracted LOX is also rapidly inactivated at 63 °C [34]. It was found that upon mashing, only one third of bound LOX is released, whereas LOX, bound in the spent grains is apparently more heat stable with more bound LOX in the spent grains after coarse milling compared with fine milling [1, 2, 80]. The lowest levels of hydroxy fatty acids, resulting from LOX activity, were found for the combination of mashing-in at 60 - 63 °C and under CO_2 atmosphere (anti-oxidative wort production) [16, 21, 77]. Addition of anti-oxidants to the brewing liquor also limits LOX activity [2, 42, 72, 73]. A completely different approach whereby milling and mashing has no effect on the LOX-reaction at mashing-in is to brew with low-LOX or LOX-free malt (barleys characterized by a zero to low LOX-peak-activity towards the end of germination, prior to drying) [22, 31, 62].

In this paper a detailed study of wort and beer characteristics is presented, comparing coarse milling-lautertun operations to fine milling-thinbed filter operations using the optimal conditions for both wort production methods. Beers brewed on pilot scale have been aged and evaluated both sensoryially and analytically.

2 Materials and methods

2.1 Wort production

Conventional wort production – coarse milling

From the same batch of malt, three Pilsner beers have been prepared in our pilot scale brewery of 2 hL (brewing line 2) under the following conditions: 40 kg coarse milled Pilsner malt (2-roller

mill) (Pfungstädter composition: husks: 30 %; coarse grist: 16 %; fine grist I: 20 %; fine grist II: 20 %; flour: 9 %; fine flour: 5 %) is mixed with 1.4 hL de-aerated reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}); mashing-in: 64 °C, pH of 5.3 (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; sparging with 3.5 l/kg brewing water until combined sweet wort has an extract content of 11,5 °P; extract of last runnings 2 °P; wort boiling: 60 min atmospheric boiling by use of an boiling kettle with internal boiler (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 (filling in 6 min, rest of 20 min, emptying in 20 min, temperature of 95 °C); after cooling and aeration, the wort (original gravity: 12 °P) has been pitched with 10^7 yeast cells/ml (inoculum: dry yeast, strain W 34/70 (Fermentis), hydration 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 cylindroconical tanks; maturation: 10 days at -0.5 °C; beer filtration: kieselguhr/cellulose sheets (pore size 1 μm); 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).

Conventional wort production – fine milling

From the same batch of malt as before, three Pilsner beers have been prepared in the second pilot brewery of KAHO Sint-Lieven (brewing line 1; 5 hL scale). The following conditions were used: 87 kg fine milled Pilsner malt (disc milling under water, Meura) is mixed with 1.91 hL de-aerated 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 5.3; 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 (Meura 2001); sparging with 2.5 l/kg water (extract of last runnings 1,5 °P and 1° P after final compression); extract of the combined sweet wort is 14.5 °P; at the onset of boiling, the sweet wort is mixed with brewing water to obtain an equal extract content of 11.5 °P. The diluted wort is transferred to the boiling kettle of brew line 2; all further steps (boiling, iso-α-acid addition, whirlpool, wort cooling, aeration, pitching, fermentation, maturation, beer filtration, saturation and filling) were carried out as described under 'Conventional wort production – coarse milling'.

2.2 Analyses

Standard analysis of wort and beer samples

The alcohol content, extract, original gravity, final attenuation of beers have been measured by an AntonPaar beer alcolyser, in combination with a DMA 5000 density meter (Anton Paar Benelux, Gentbrugge, Belgium). Oxygen levels have been measured with an Intap4000 portable DO-meter (Mettler Toledo, Elscolab). Carbon dioxide levels were determined on the basis of temperature and pressure measurements. Standard wort and beer analyses were carried out according to EBC-methods: FAN (free amino nitrogen): 9.10; total polyphenols: 9.11; flavanoid content: 9.12; foam stability, using the NIBEM-T Meter (Haffmans, Venlo, The Netherlands): 9.42. Cold haze: analysis of turbidity of beer kept for a minimum of 24 h at 0 °C (Haffmans VOS ROTA 90 Turbidity meter, 90 ° light scatter, Venlo, The Netherlands). Forced haze: beer for 5 days at 60 °C-1 day 0 °C. Measurement of haze at 0 °C and 20 °C at an angle of 90 ° and 25 °. Beer colour: IOB method 9.1. Determination of proanthocyanidins: method according to Bate-Smith (1973) [7]. High molecular weight soluble protein: Bio-Rad protein assay, according to Bradford [10]. Sensitive protein: IOB method 9.37. TB-index (thiobarbituric acid): method according to Thalacker and Böβendörfer (2005) [66]. TRAP (total reactive antioxidant potential): method according to Araki et al. (1999) [5]. SO_2 measured using the enzyme kit of Boehringer Manheim 10725854035, r-biopharm, Germany.

Gas chromatographic analysis of trihydroxy fatty acids and some fatty acids

Determination of trihydroxy fatty acids in wort and beer samples was based on the published procedures of Möller-Hergt et al. (2001) [54] and Wackerbauer and Meyna (2001) [76]. Equipment: GC-FID (Thermo Quest CE Trace 2000 (Interscience, Louvain-la-Neuve, Belgium)) 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.

GC-MS determination of aldehydes

Volatile aldehydes in wort and beer have been determined according to Vesely et al. (2003) [75], using headspace-solid phase micro-extraction (HS-SPME) with on-fibre PFBOA (o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) derivatisation and capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ™ II) GC/MS system (Interscience, Louvain-la-Neuve, Belgium)). The DSQ™ II was coupled to a Thermo Trace GC Ultra (Interscience, Louvain-la-Neuve, Belgium) equipped with a CTC-PAL autosampler, a heated split/splitless injector (250 °C) with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused-silica capillary column (40 m × 0.18 mm i.d., 0.2 μm film thickness, Restek, Interscience, Louvain-la-Neuve, Belgium). Data processing was performed by the XCalibur™ data system (Thermo Electron Corporation, Austin, TX, USA).

GC-MS determination of esters and higher alcohols

Extraction of volatile esters and higher alcohols from beer have been done by headspace-solid phase micro-extraction (HS-SPME) for 30 min at 40 °C using a 65 μm PDMS-DVB fiber coating. Components were separated and detected by capillary gas chromatography/mass spectrometry (CGC/MS) (Ion Trap – ITQ) CGC/MS system (Interscience, Louvain-la-Neuve, Belgium) operating in the electron impact mode). The ITQ was coupled to a ThermoFinnigan Trace GC (Interscience, Louvain-la-Neuve,

Belgium) equipped with a CTC-PAL autosampler, a heated split/splitless injector with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused-silica capillary column (40 m × 0.18 mm i.d., 0.2 μm film thickness, Restek, Interscience, Louvain-la-Neuve, Belgium). Helium was the carrier gas at a flow rate of 0.8 mL/min. The inlet temperature was 230 °C and the injection occurred in the split mode (split ratio 1/12). The oven temperature has been held at 40 °C for 3 min, then raised to 200 °C at 6 °C/min, followed by an increase to 250 °C at 15 °C/min and finally held at 250 °C for 3 min. Processing of the chromatographic data was performed by the Xcalibur ™ data system (Thermo Electron Corporation, Austin, TX, USA). For each component, a calibration curve was made in order to quantify the component in beer.

UPLC determination of iso-α-acids

UPLC separation of iso-α-acids has been performed on an Acquity UPLC (Waters, Milford, USA), consisting of a PDA detector, column heater, sample manager, binary solvent delivery system and an Acquity UPLC HSS C18 1.8μm column (2.1 i.d. × 150 mm; Waters, USA). Data reprocessing by Empower 2 software. Chromatographic conditions: eluent A: milli-Q water adjusted to pH 2.80 with H_3PO_4 (85 %, Merck, Darmstadt, Germany); eluent B: HPLC-grade CH_3CN (Novasol, Belgium). Elution: isocratic using 52 % (v/v) solvent B and 48 % (v/v) solvent A. Analysis time: 12 min. Flow rate: 0.5 mL.min-1. Column temperature: 35 °C. UV detection: 270 nm (iso-α-acids). The trans/cis iso-α-acids ratio (T/C-ratio) is related to the measured concentrations of trans- and cis-isocohumulone and trans- and cis-isohumulone.

HPLC determination of sugars

Prior to HPLC analysis, proteins have been removed from the wort and beer samples by precipitation with Carrez-reagent. Therefore, 10 mL of beer is mixed with 200 μL of Carrez-1 (106 g $K_4Fe(CN)_6.3H_2O$ dissolved in 1,000 mL demineralized water) and 200 μL of Carrez-2 (220 g $Zn(CH_3COO)_2.2H_2O$ and 30 mL of glacial acetic acid, made up to 1,000 mL with demineralized water). After centrifugation at 11,000 g for 5 minutes, the samples are ready for. The HPLC sugar profiles of the beers were obtained using a Nucleodur NH_2 RP 100-5 column (250 × 4 mm i.d.; Macherey-Nagel, Düren, Germany), kept at ambient temperature. A mixture of water/acetonitrile (37/63; v/v) has been used as mobile phase. Calibration has been performed using a mixture of glucose and maltose (VWR, Belgium) at concentration levels of 250 mg/L, 500 mg/L 1,000 mg/L and 10,000 mg/L for each component. Relative response factors, compared to maltose, have been determined for maltotriose up to maltoheptaose and used for quantification.

UPLC determination of amino acids

The Acquity Ultra Performance LC (UPLC) separation system has been used to quantify individual free amino acids in wort and beer. The UPLC system uses relatively new technology to achieve excellent separation of over 20 amino acids in less than 10 minutes between injections. Before derivatization of amino acids in the beer samples, the proteins were removed. Therefore, 20 μl Carrez I reagent (106 g potassium ferrocyanide trihydrate ($K_4Fe(CN)_6.3H_2O$ in 1,000 ml water) and 20 μl Carrez II reagent (220 g zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) and 30 ml acetic acid is made-up to 1,000ml with mQ-water) was added to 1 ml of previously degassed beer. After addition, the sample was mixed and centrifuged (in 2 ml eppendorf microtube for 5 min.). Sample derivatization has been done using the Waters AccQ•Tag Ultra Chemistry Package.

UPLC separation of amino acids has been performed on an Acquity UPLC (Waters, Milford, USA), consisting of a PDA detector, column heater, sample manager, binary solvent delivery system and an AccQ•Tag™ Ultra column (2.1 i.d. × 100 mm; Waters, USA). Data were reprocessed using the Empower 2 software. Chromatographic conditions were: AccQ•Tag Ultra Eluent A Concentrate (10 times diluted) (Waters, Milford, USA); AccQ•Tag Ultra Eluent B (Waters, Milford, USA). Elution: gradient elution according to Waters AccQ•Tag Ultra method. Analysis time: 9.5 min. Flow rate: 0.7 mL.min-1. Column temperature: 60 °C.

2.3 Statistical analysis

Statistical analysis was performed using Matlab 2011 “Statistics Toolbox”.

Results were considered statistically significant by means of a Wilcoxon rank-sum test at p-value < 0.05. Each brewing experiment is performed 3 times (n = 3). An analytical result is expressed as the mean value of those 3 experiments. If there are doubts about the suitability of the data for the requirements of a t-test, the distribution being non-normal or the samples taken are so small that one cannot tell if they are part of a normal distribution, the Wilcoxon rank-sum test (also called The Mann–Whitney U test) may be used instead. It is used to test the null hypothesis that two samples come from the same population (i.e. have the same median). This test can be used for a limited number of measurements.

3 RESULTS AND DISSCUSION

In order to evaluate the impact of fine milling-mash filter operations on final beer quality and -stability in comparison to coarse milling-lautertun operations, pilot brews have been made as described in ‘materials and methods’. Boiling, hot wort clarification and further processing is done under similar conditions and same density. However to start, mash rates are different for both systems. In case of thinbed filter operations a thick is required whereas mashes for lautertun operations are to be more diluted. State of the art brewing schemes have been applied in view of brewing beers with enhanced flavour stability. The latter starts with mashing-in conditions of 63 °C in combination with pH 5.2 and with a minimum of oxygen entrainment during milling and mashing-in according to literature [1, 2, 6, 25, 30, 33, 50, 56, 63, 65, 70, 74]. The pH of these trials was 5.3 and not 5.2 because of the risk of somewhat lower glucose levels (results to be published in JASBC, paper already accepted). In case of coarse milling it was technically impossible by the applied milling procedure to mill in CO_2 atmosphere, but brewing water was de-aerated. For the fine milling trials, the malt bin has been flushed with CO_2 before milling. Milling under water with a disc mill is used for fine milling resulting in a combined milling

and mashing-in whereby oxygen poor conditions were obtained. In case of the applied lautertun operations, the malt:water ration is limited to 1:3.5 whilst for thin bed filtration the mash can be much thicker and is only limited for sufficient enzyme conversion and stirring. This thicker mash already will naturally compensate for higher extraction of risk compounds as mentioned in the introduction.

3.1 Brewing performance

The filtration performance of both filtrations is presented in Figure 1 and Table 1. The density of first wort with fine milling is considerable higher compared with coarse milling due to the higher mash rate (1:2.2 for fine milling vs 1:3.5 for coarse milling). First wort filtration is faster in case of fine milling-mash filter due to the high mash rate (filtration time 12 min vs 30 min in case of coarse milling) and also sparging time is shorter in case of thin bed filter operation (54 min vs 80 min). Total filtration time is presented in Table 1 since transfer time, settling time, turbid wort circulation time needs to be taken in account. Total cycle time is much shorter with a mash filter and has been reported before partly due the fact that thin bed filtration starts during the transfer and not after transfer and settling time [4, 51]. The most important difference is found at the beginning of the filtration cycle. With a mash filter, the mash is separated during the mash transfer whereas with a lautertun separation only starts after mash transfer, settling of the husks, and recirculation of the first cloudy worts. With an optimized lautertun, as available on the market, the sparging cycles are comparable to those with thinbed filters. The volume of water needed for mashing and sparging is significantly lower with fine milling and mash filter and amounts to 4.7 l water /kg malt (Table 1). Thicker mashes need less energy for heating during mashing, lower sparge rates lead to smaller volumes of sweet wort and less energy will be needed for boiling.

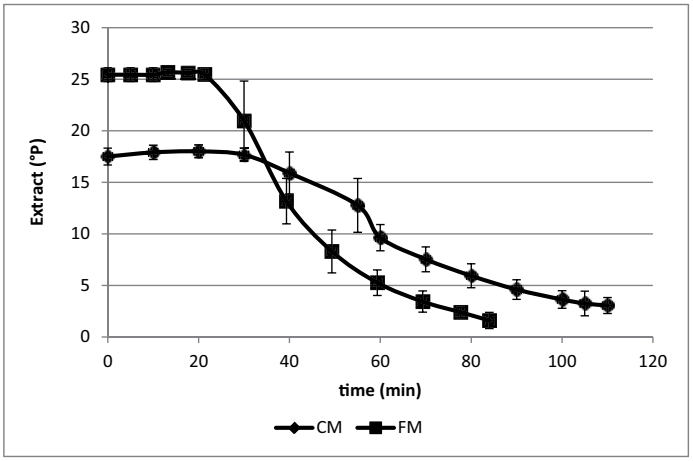


Fig. 1 **Extract content upon wort filtration. (FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun)(n = 3, error bars = standard deviation (S.D.))**

Table 1 **Filtration details for the coarse milling and fine milling experiment (FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun)**

	CM-lautertun	FM-mash filter
Filter surface (m^2)	0.266	2.56
Filter loading (kg/m^2)	150	34
Transfer time (min)	6	4
Settling time (min)	15	–
Turbid wort circulation (min)	5	–
First wort filtration (min)	30	12
1st compression (min)	–	8
Sparging (min)	80	54
Final compression (min)	–	10
TOTAL CYCLE TIME (min)	**136**	**88**
Brewing water (l/kg)	3.5	2.2
Sparging water (l/kg)	3.5	2.5
Total water (l/kg)	**7**	**4.7**

3.2 Comparison of pitching wort

Pitching worts of both brewing methods have been analysed via the analytical procedures described in 'materials and methods'. Mean values of 3 trials are presented in Table 2. A Wilcoxon rank-sum test at p-value < 0.05 is used in order to define significance. All results are recalculated to 12 °P in order to compare the composition of both worts. The extract content of the pitching wort after coarse milling-lautertun or after fine milling-thin bed filter was 12.39 ± 0.15 °P and 12.63 ± 0.40 °P respectively. No significant difference was found.

An increased level of proteins and polyphenols could be expected with fine milling. However, it was reported in earlier studies [14, 61] that higher levels of proteins and polyphenols resulted in an increased coagulation and flocculation of proteins together with polyphenols. Indeed, a significantly lower level of haze sensitive protein is found in the wort produced after fine milling (Table 2). The polyphenol concentration is also lower, but the difference is not significant at the 0.05 level.

Mashing-in at 63 °C does not cause any problem concerning the

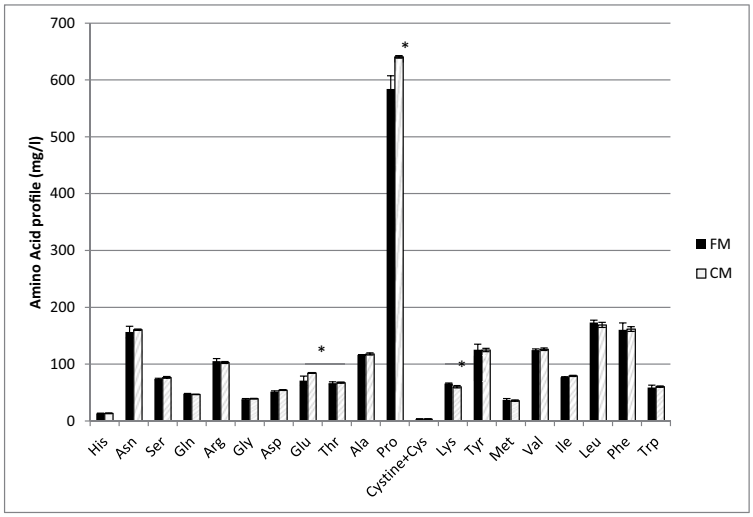


Fig. 2 **Amino acid profile of pitching wort (mg/l)(recalculated to 12°P) (n=3, error bars = S.D.). FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun. A significant difference at the α=0.05 level is indicated with***

His: histidine; Asn: asparagine; Ser: serine; Gln: glutamine; Arg: arginine; Gly: glycine; Thr: threonine; Ala: alanine; Pro: proline; Cys: cysteine; Lys: lysine; Met: methionine; Val: valine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: Tryptophan

Table 2 **Analyses of pitching wort (recalculated to 12 °P to compare results) (FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun) (mean value of 3 measurements ± S.D.). A significant difference at the α = 0.05 level is indicated with ***

		FM	CM	
HMW soluble proteins	mg/l	523 ± 60.88	536 ± 21.36	
Sensitive proteins	Δ haze EBC	41.82 ± 3.78	57.15 ± 10.97	*
Total polyphenols	mg/l	236.38 ± 8.88	226.05 ± 14.99	
Proanthocyanogens	mg/l	61.77 ± 4.04	60.14 ± 4.82	
Monomeric flavanoids	cat. Eq. (mg/l)	54.39 ± 3.16	48.73 ± 9.25	
Sum amino acids	mg/l	2110 ± 21	2186 ± 29.97	
FAN	mg/l	252 ± 11	221 ± 17.71	
Wort viscosity	mPas.s	1.89 ± 0.05	1.74 ± 0.06	*
Glucose	g/l	11.09 ± 1.03	8.64 ± 0.60	*
Maltose	g/l	68.05 ± 0.52	67.61 ± 2.28	
Maltotriose	g/l	16.28 ± 1.42	14.05 ± 0.79	*
Maltotetraose	g/l	4.49 ± 0.33	5.15 ± 1.04	
Maltopentaose	g/l	1.82 ± 0.06	2.77 ± 1.34	
Maltohexaose	g/l	2.25 ± 0.39	3.70 ± 0.95	
Maltoheptaose	g/l	1.11 ± 0.08	0.99 ± 0.09	
Sum DP1-3	g/l	95.42 ± 2.88	90.30 ± 0.25	*
Dihydroxy fatty acids	mg/l	4.42 ± 0.14	4.42 ± 0.14	
Trihydroxy fatty acids	mg/l	3.17 ± 0.05	3.07 ± 0.05	
Palmitinic acid	mg/l	0.15 ± 0.07	0.25 ± 0.07	
Linolic acid	mg/l	0.10 ± 0.01	0.10 ± 0.01	
Tr-9-octadecenoic acid	mg/l	0.28 ± 0.01	0.28 ± 0.01	
Octadecanoic acid	mg/l	0.24 ± 0.07	0.44 ± 0.07	
2-Methyl propanal	µg/l	21.5 ± 0.2	22.1 ± 0.1	
2-Methyl butanal	µg/l	17.5 ± 0.01	17.2 ± 0.63	
3-Methyl butanal	µg/l	50.1 ± 0.71	46.5 ± 4.06	
Hexanal	µg/l	2.6 ± 0.48	2.0 ± 0.28	
Furfural	µg/l	465 ± 7.65	409 ± 4.64	*
Methional	µg/l	345 ± 7.01	279 ± 34.5	*
Benzaldehyde	µg/l	4.89 ± 1.32	5.20 ± 1.04	
Phenylacetaldehyde	µg/l	295 ± 27.1	257 ± 18.3	
(E)-2-nonenal	µg/l	0.15 ± 0.01	0.11 ± 0.01	*
Sum aldehydes	µg/l	1202 ± 14.7	1038 ± 61.5	*

amino acid levels [2, 59, 64]. Most of the amino acids are already present in malt and a decreased mashing pH of 5.3 can result in higher amino acid levels. Thicker mash is also protecting proteases [64]. A somewhat increased FAN level is found in the fine milling trials (Table 2) due to the fine milling and the protection of protease by the thick mash, but this difference is not significant at the 0.05 level. A detailed profile of the amino acids is presented in Figure 2. Both brewing methods clearly lead to very comparable amino acid compositions under the chosen brewing conditions.

A higher extraction of β-glucans can be expected with fine milling, but on the other hand a decreased extraction is reported when mashes are more concentrated [36]. Wort viscosity is measured and a slightly, but significant increase of viscosity is measured in wort produced with fine milling. However, the observed increase doesn't create problems in view of wort filtration and also no difficulties are expected during beer filtration.

As reported previously by Mousia et al. (2004) [55] and Menger (2006) [52], fine milling in combination with thick mash results in a more efficient starch conversion. The levels of glucose and maltotriose indeed are significantly higher in the wort produced due to fine milling and thicker mashes. As apparent from Table 2, maltose level is not influenced by the wort production process. The level of fermentable sugar (sum of glucose, maltose and maltotriose) is significantly higher in wort produced with fine milling and thick mash and amounts to 95.4 g/l compared to 90.3 g/l with traditional coarse milling and thinner mash. The increased brew house yield can be ascribed to higher sugar levels but does not lead to higher concentrations of risk compounds such as proteins and polyphenols (see also Table 2).

No significant differences have been found in fatty acid composition and trihydroxy fatty acid levels. Apparently, the chosen mashing conditions do not result in an extra formation of di- and trihydroxy fatty acids regardless the way of milling. LOX reactions can be blocked by limiting O_2 entrainment during milling and mashing-in, lower mash pH and higher mashing-in temperature in case of fine milling as reported before [21]. In these trials using fine milling in combination with mashfilter, the first turbid worts have not been recycled which results in a sufficient and similar level of fatty acids in the pitching wort.

In the aldehydes composition minor differences have been observed. Fine milling results in significantly higher concentrations of free furfural, methional, nonenal and total aldehydes levels (Table 2). A better extraction and release of bound aldehydes due to fine milling could be the reason especially at a decreased pH of 5.3. This seems a negative aspect of fine milling, but yeast reduces free aldehydes during fermentation and it has been reported that no differences are found in aldehyde contents after fermentation when aldehydes were added after boiling suggesting that the presence of free aldehydes before fermentation might not be of major importance for beer flavour stability [60]. The content of higher alcohols might be higher and will be measured in the next section.

3.3 Comparison of fresh beers

The pitching worts have been fermented as described in the 'material and methods' section. After maturation, beer filtration and carbonation, beers are bottled and analysed. The results are presented in Table 3. The higher level of fermentable sugar in the pitching wort results in a somewhat higher apparent degree of fermentation (ADF) in case of fine milling. However, the difference is not significant. No differences are found in the amino

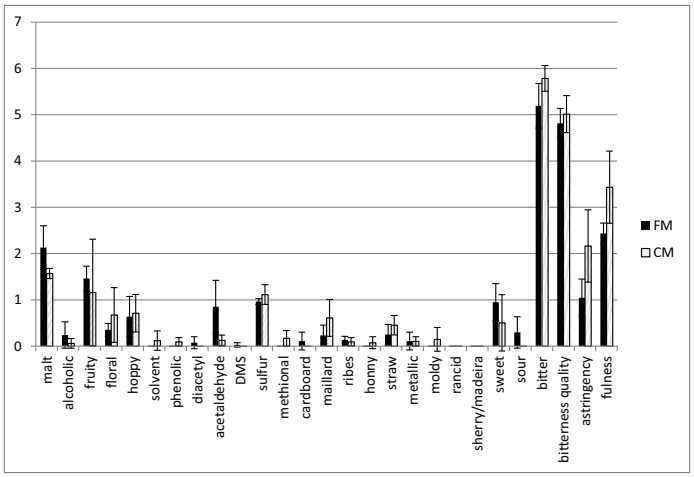


Fig. 3 **Sensorial evaluation of the fresh beers. Panel of 8 trained assessors. Scores between 0 and 8: 0=absent, 2=very weakly present, 4=weakly present, 6=clearly present, 8=strongly present**

FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun (n=3, error bars = S.D.)

acid uptake resulting in a comparable residual FAN level in the final beer. The foam stability of both beers is highly comparable although a significantly lower level of soluble protein in the beer produced after fine milling was noticed. This lower protein content can be explained by a slightly lower level of haze sensitive protein. This results in a slightly improved colloidal stability. Especially at the 90° measurement angle, the improved colloidal stability, for beers produced with fine milling, is significant. Extra stabilization however remains necessary. The bitterness and CO_2 content is within specs. The SO_2 content is rather low, but highly comparable and differences are not significant.

No differences in aldehyde composition are found in the fresh beers. Fine milling resulted in significantly higher levels of free aldehydes in the pitching wort, but, as already mentioned before, yeast is able to reduce these free aldehydes [60]. Table 3 further shows that no significant differences are found in the ester and higher alcohol profile of the fresh beers pointing to a similar fermentation process of the different worts.

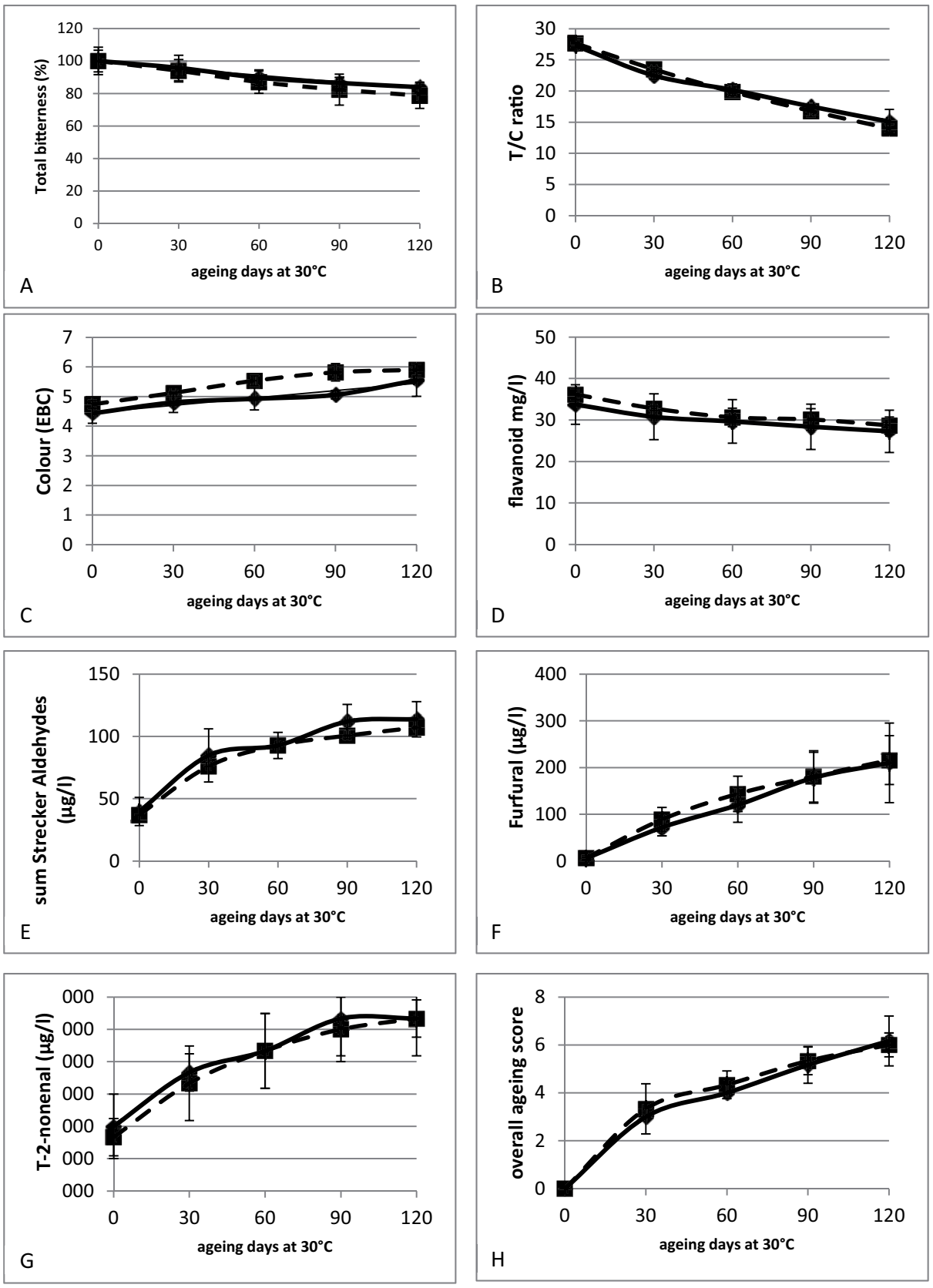
Descriptive sensory evaluation of the fresh beers was carried out by the trained panel of 8 panellists. The mean values of the results are presented in Figure 3. In conclusion, no significant differences in flavour profile are found between the 2 production methods of beer.

3.4 Comparison of beer ageing

Beers were aged at 30 °C for 120 days to evaluate the effect of both brewing methods on the eventual instability of beer. The results with regard to the decline of bitterness, T/C ratio, and flavonoids as well as the increase of aldehydes, colour, and overall ageing score by sensory evaluation are presented in Figure 4 (A-H). These sensory and analytical parameters are strongly correlated with beer ageing as reported in literature [48, 49]. The brewing method has no influence on the degradation of bittering principles (Fig. 4A) and the T/C ratio (Fig. 4B). As reported in several studies, iso-α-acids are not stable during beer ageing, due to oxidative deterioration [17, 18]. Cis-isohumulones are more stable than trans-isohumulones

Table 3 **Fresh beer analyses (FM: Fine milling – thin-bed filter, CM: Coarse milling – lautertun) (mean value of 3 measurements ± S.D.). A significant difference at the α=0.05 level is indicated with ***

		FM	CM	
Ethanol	v/v%	5.49 ± 0.37	5.30 ± 0.07	
Apparent extract	g/100g	1.85 ± 0.16	2.06 ± 0.15	
Original extract	g/100g	12.63 ± 0.40	12.39 ± 0.15	
ADF	%	85.35 ± 1.09	83.37 ± 2.04	
FAN	mg/l	78.31 ± 13.12	91.38 ± 5.76	
Sum Amino Acids	mg/l	873 ± 76	913 ± 15	
Foam stability	NIBEM-T (s)	245 ± 13	235 ± 6	
HMW soluble protein	mg/l	313 ± 12.7	361 ± 7.7	*
Haze sensitive protein	Δ haze (90°)	10.1 ± 0.9	13.0 ± 0.1	*
Total polyphenols	mg/l	164 ± 1.9	166 ± 1.2	
Proanthocyanogens	mg/l	24.6 ± 2.4	22.6 ± 2.9	
Monomeric flavanoids	cat. Eq. (mg/l)	35.3 ± 0.2	37.7 ± 1.2	
Beer colour	EBC	4.4 ± 0.3	4.7 ± 0.1	
Cold haze (0°C)	EBC	0.51 ± 0.12	0.70 ± 0.18	
TBI		25.1 ± 2.02	27.9 ± 0.77	
TRAP	mM Asc. Eq.	1.18 ± 0.14	1.22 ±0.03	
total iso-α-acids	mg/l	23.0 ± 1.41	25.0 ± 2.41	
SO_2	mg/l	5.3 ± 0.8	5.9 ± 1.0	
CO_2	g/l	5.4 ± 0.2	5.6 ± 0.2	
O_2 (D.O.)	µg/l	73.5 ± 6.2	70.3 ± 7.2	
Forced haze (90°)(0°C)	EBC	7.50 ± 3.12	13.7 ± 1.55	*
Forced haze (25°)(0°C)	EBC	9.74 ± 4.29	16.8 ± 1.80	
Forced haze (90°) (20°C)	EBC	1.41 ± 0.36	2.15 ± 0.61	*
Forced haze (25°) (20°C)	EBC	0.26 ± 0.12	0.55 ± 0.17	
2-Methyl propanal	µg/l	6.62 ± 1.66	7.14 ± 3.01	
2-Methyl butanal	µg/l	2.99 ± 1.08	3.35 ± 1.82	
3-Methyl butanal	µg/l	11.7 ± 2.63	12.1 ± 5.26	
Hexanal	µg/l	0.87 ± 0.50	0.71 ± 0.19	
Furfural	µg/l	5.71 ± 2.28	6.59 ± 5.30	
Methional	µg/l	7.45 ± 3.00	5.26 ± 1.56	
Benzaldehyde	µg/l	1.76 ± 1.48	1.78 ± 1.40	
Phenylacetaldehyde	µg/l	9.21 ± 2.25	7.51 ± 2.69	
t-2-Nonenal	µg/l	0.02 ± 0.01	0.02 ± 0.01	
Sum aldehydes	µg/l	46.3 ± 14.0	44.5 ± 20.0	
Ethylacetate	µg/l	13.6 ± 1.55	14.0 ± 1.58	
3-Methylbutanol	µg/l	31.5 ± 1.87	35.6 ± 5.30	
2-Methylbutanol	µg/l	10.3 ± 0.75	12.0 ± 1.93	
Iso amylacetate	µg/l	718 ± 112	802 ± 127	
Ethylhexanoate	µg/l	148 ± 22	161 ± 13.1	
Ethyloctanoate	µg/l	227 ± 21.6	240 ± 18.0	
Phenyl ethylacetate	µg/l	235 ± 27.5	242 ± 25.9	
Ethyldecanoate	µg/l	91 ± 6.8	103 ± 2.6	



: Fine milling – thin-bed filter, :Coarse milling - lautertun
(n=3, error bars = S.D.) (Sum Strecker aldehydes= 2-methyl propanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde) (aging scores between 0 and 8: 0=fresh, 2=very weakly aged, 4=weakly aged, 6=clearly aged, 8=strongly aged).

Fig. 4 (A-H): Evaluation of forced beer ageing at 30°C

(decrease of trans/cis ratio) which is in accordance with literature [17, 18]. For both beers a similar decline in T/C ratio is observed (Fig. 4B). The increase in colour is also found to be similar for both beers (Fig. 4C). Beers brewed after fine milling are somewhat lower in colour, also after ageing (Fig. 4C). Flavanoids are very sensitive to oxidation and can act as anti-oxidants [53, 58]. In the fresh beers, no difference in flavanoid content has been found and during ageing the same decrease has been noticed (Fig. 4D). Aldehydes play an important role in the ageing of beer and especially by their effect on the flavour and taste of aged beers [17, 60, 69]. No differences in the aldehyde level have been found in the fresh beer nor in beers during ageing. In both aged beers there is a similar increase of furfural, Strecker aldehydes (2-methyl propanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde) and trans-2-nonenal. Fine milling has at least an equal effect on the sensorial flavour stability of beer (Fig. 4H). The chosen mashing conditions resulted in similar ageing profiles regardless the way of milling and wort production in these trials. Despite of state of the art anti-oxidative wort production, beers are still ageing. It is reported that also malt has a very important impact on beer quality and flavour stability [17].

4 Conclusions

Membrane assisted thinbed filtration in combination with fine milling have been introduced more than 20 years ago in the brewing industry. Although it has been clearly demonstrated in industrial operations that with fine milling and membrane assisted thin bed filters highest extract yield and clearest wort can be obtained at highest gravity and in shortest time, a great number of breweries is still afraid of using this technology. This is probably due to a lack of detailed analyses of beers brewed after fine milling and membrane assisted mash filter. Both wort production systems requires other malt:water ratios which already results in compensations for fine milling of malt. In this paper it has been clearly demonstrated that wort, beer and ageing profiles are highly similar for conventionally brewed beers produced after fine milling and membrane assisted thinbed filter as well as after coarse milling and lautertuns under the chosen production conditions which depend on the pilot facilities. Higher yield after fine milling is due to an increased content of sugars and not to the extraction of risk compounds such as proteins and polyphenols. On the contrary, fine milling and thick mash result in wort and beer with a significantly lower level of sensitive protein without affecting the foam potential. With state of the art mashing conditions, fine milling and thin bed filter operations have no negative impact on the flavour quality and flavour stability of beer.

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