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Investigating the evolution of free staling aldehydes throughout the wort production process

During aging, beer undergoes a variety of unfavourable chemical reactions, which lead to inevitable flavour deterioration. Especially in light lagers and Pilsner styles, many of the off-flavours are associated with the presence of the so-called staling aldehydes. However, the formation of these compounds is not fully understood until now. From the perspective of the brewing process, aldehydes may arise as a result of e.g. fatty acids oxidation, Strecker degradation of amino acids, Maillard reactions, etc. Aldehydes may also be released from the non-volatile precursor forms, the so-called bound state aldehydes. Therefore, to unravel another puzzle piece of beer flavour instability, we have monitored the free staling aldehydes throughout the process of wort production. Levels of 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, hexanal, furfural, methional, phenylacetaldehyde and *trans*-2-nonenal were quantified. High levels of aldehydes determined in malt and mashing-in samples indicate the malt as the major source of staling aldehydes introduced into the brewing process. Furthermore, a decrease in the levels of free aldehydes was observed over the entire process, with exception of furfural. The highest decrease was observed during boiling. On the other hand furfural increased, not only during boiling, but also during wort clarification. Finally, a relative increase in aldehydes was observed during sparging. In summary, this study identifies the most critical steps during wort production in relation to free aldehyde formation.

Descriptors: staling aldehydes, flavour instability, wort production

1 Introduction

Flavour characteristics of beer are an important – if not the most important – criterion for evaluation of beer quality. Yet, with time, beer changes its flavour – the fresh attributes, as e.g. overall bitterness quality or fruity aromas decline, whereas staling-related off-flavours appear (e.g. sherry, caramel, cardboard flavours) [1]. One of the major contributors to beer staling are aldehyde compounds [2], whose concentrations elevate during beer aging [3, 4]. Staling aldehydes are carbonyl compounds of relatively low molecular masses and high volatility. A very specific characteristic of these compounds is their low threshold level that can be perceived in the ppb, or even sub-ppb range, e.g. *trans*-2-nonenal [2, 5]. In general, aldehydes may arise via two mechanisms: *de novo* formation and the release from bound-state adduct forms. Several chemical pathways have been proposed for the *de novo* formation of aldehydes, of which the most relevant are oxidation of unsaturated fatty acids, Maillard reactions and Strecker degradation of amino acids [5–9]. The latter is often subcategorised under Maillard reactions, which include e.g. reactions of α-dicarbonyls, α-unsaturated carbonyls or Amadori compounds with amino acids [5]. Strecker aldehydes may also be formed as a result of direct oxidative degradation of amino acids [10]. On the other hand, aldehydes can also be converted into non-volatile adduct forms with e.g. bisulfite, cysteine, or other amino acids (imine formation), which, over time, can split up again and thereby release the free aldehydes [5, 11].

Beer flavour stability may be influenced by a number of factors, however, generally, it is discussed in the context of the influence of the raw materials and the brewing process. Malt, the brewing raw material used in large quantities, contains a variety of staling precursors e.g. amino acids, lipids, but also aldehydes as such [12–15]. On the other hand, malt is a more or less rich source of beer endogenous antioxidants, which inhibit the rate of oxidation reactions [16]. From the perspective of the brewing process, the most important factors influencing final beer flavour stability are: exposure of wort/beer to oxygen, amount of heat-load applied, wort/beer pH, and contact with transition metal ions [10, 17, 18]. Nonetheless, the impact of applied technologies during each step of wort production is obviously also important. For instance, milling regime, mashing-in pH and temperature have been reported as

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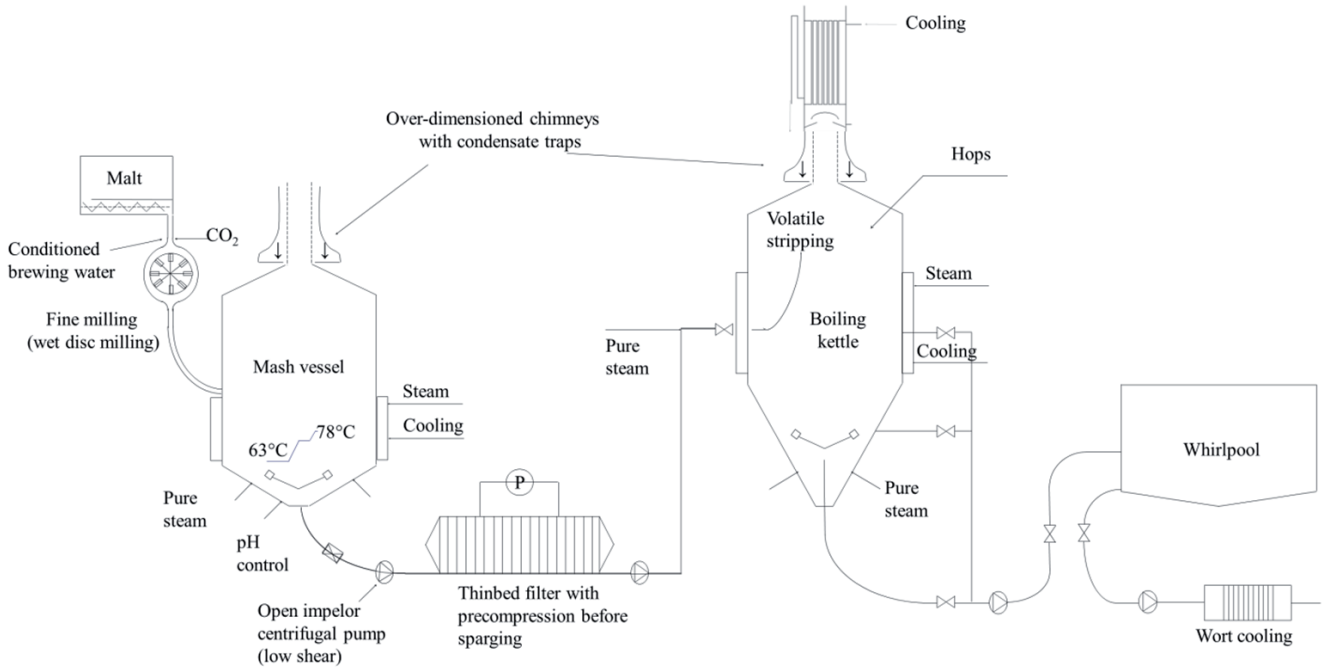

Fig. 1 Scheme of the pilot brewing plant

important control-point parameters for fatty acid oxidation [19]. Lipoxygenases present in malt – enzymes catalysing oxidative degradation of unsaturated fatty acids – markedly decrease in their activity when mashing-in at pH of 5.2 and a temperature of 63 °C is applied [17, 19, 20]. Moreover, quick and effective performance of mash filtration has been reported to positively correlate with improved beer flavour stability [18, 19]. During wort boiling, aldehydes evaporate, however, due to the application of substantial heat-load, new ones are also formed via e.g. Strecker degradation of amino acids or Maillard reactions [21–23]. On the other hand, lipid oxidation has been reported to hardly proceed during wort boiling [23]. Performance of wort clarification is also relevant, as the wort is still exposed to heat-load and the separated hot-trub contains significant amount of aldehyde precursors (e.g. lipids or aldehydes bound to insoluble trub particles) [19, 22, 24].

All in all, the performance of the brewing process is decisive for the wort composition and overall beer quality, including flavour stability. The chemistry of many staling precursors e.g. amino acids, sugars, or lipids, is relatively well (but far from completely) understood. However, to date, our knowledge on the overall change in the levels of aldehydes throughout the brewing process is insufficient. More specifically, detailed comparison of the consecutive processing steps on the change in aldehydes is still missing to draw a clear picture. Therefore, to better understand the relevance of the brewing process on flavour instability, from the perspective of free aldehyde behaviour, evolution of free aldehydes has been monitored over the entire wort production process.

2 Methodology

2.1 Wort production

Wort was produced in our 5 hL pilot brewing plant under oxygen limited conditions (for the brewery scheme see Fig. 1). Thick mashing was applied by using malt water ratio of 1 : 2.2 (w/w) – fine milled Pilsner malt (87 kg; wet disc mill, Meura, Belgium) was mixed with 1.91 hL of deaerated, reversed osmosis brewing water enriched with 80 ppm Ca^{2+} in the form of $CaCl_2$ (calcium chloride dehydrate, Merck KGaA, Darmstadt, Germany). The pH of the mash was adjusted to pH 5.3 with 30 % (v/v) lactic acid (from 90 % v/v (S)-Lactic acid, Merck KGaA, Darmstadt, Germany). Following mashing protocol was applied: 63 °C (30 min); 72 °C (15 min); 78 °C (1 min) – temp. rise 1 °C / min. Wort was filtered using a membrane assisted thin bed filter (Meura 2001, Meura, Belgium); sparging rate 2.33 L/kg with last running 1.5 °P. At onset of boiling, the sweet wort was adjusted to 13 °P. Wort was boiled for 60 min. 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 at 29 mg iso-α-acids/L in final beer. Wort clarification was performed using whirlpool. Samples for the analysis were collected at onset of mashing, end of mashing, mash filtration – first wort collection, mash filtration – sparging, onset of boiling, end of boiling, end of clarification and end of cooling (before wort aeration).

2.2 Extraction of aldehydes from malt

Under anaerobic conditions, 1 g of fine milled malt was mixed with 99 mL of N_2 flushed Milli-Q water. The sample was mixed vigorously for 15 min at ambient temperature. After sedimentation, 10 mL of solution was transferred to a 20 mL amber glass vial, capped and subjected to aldehyde quantification.

2.3 Sample preparation of wort prior aldehyde quantification

In order to prevent oxidation, wort samples' preparation was carried out under anaerobic conditions. The residue of mashing-in and end of mashing samples was separated by 10 min centrifugation at 4,000 rpm at –4 °C. All wort samples were diluted 10 times with

N_2 flushed Milli-Q water. An aliquot of 4 mL of the obtained mixture was transferred to a 20 mL amber glass vial and capped.

2.4 HS-SPME-GC-MS determination of aldehydes

The concentrations of 2-methylpropanal (2MP), 2-methylbutanal (2MB), 3-methylbutanal (3MB), hexanal (HEX), furfural (FUR), phenylacetaldehyde (PHE), methional (MET) and *trans*-2-nonenal (T2N) were quantified by headspace-solid phase microextraction with on-fibre derivatisation of carbonyl compounds, followed by gas chromatography coupled with mass spectrometry (HS-SPME-GC-MS). Samples were placed on a cooling tray at 5 °C. First, a sample was transferred by CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) from the cooling tray to the agitator. Subsequently, the sample was spiked with a stable isotope-labelled internal standard – 20 µg/L of 2MB-d_{10} (MercaChem, Nijmegen, the Netherlands) and 20 µg/L of benzaldehyde-d_6 (Sigma Aldrich Co., St. Louis, MO, USA) combined in ethanol absolute (Merck KGaA, Darmstadt, Germany). A 65 µm polydimethylsiloxane/divinylbenzene (Stableflex/SS SPME Fibre Assembly, Supleco Analytical, Bellefonte, PA, USA) SPME fibre assembled on the CombiPAL was first subjected to a bake-out conditioning (according to supplier's recommendations), then exposed to the headspace of 10 mL of aqueous o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) (Sigma Aldrich Co., St. Louis, MO, USA) solution (1 g/L). Derivatisation agent stock solution (PFBHA) was freshly prepared in a transparent 20 mL vial and placed in the agitator tray at 30 °C. The fibre was loaded with PFBHA for 10 min during its exposure to the headspace while being shaken at 250 rpm in cycles of 5 s shaking and 2 s rest. Subsequently, the fibre was exposed to the sample's headspace, extracting aldehydes for 30 min, while being shaken in cycles of 5 s and 2 s rest. Pentafluorobenzyloximes (PFBOs), formed as a result of interaction of the aldehydes with PFBHA, were thermally desorbed from the solid phase by introduction of the fibre into the injector of a focus gas chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA). The injector was equipped with a narrow bore glass inlet liner with a volume of 0.5 mL. The fibre was kept in the inlet for 3 min at 250 °C. Inlet was set to split mode with a split flow of 10 mL/min and split ratio of 12. The GC oven program applied was: 2 min at 50 °C; an increase of 6 °C/min up to 250 °C; and 5 min at 250 °C. Helium was used as a carrier gas and its flow rate was set on 0.8 mL/min. The column applied was Rtx-1 Crossbond 100 % dimethyl polysiloxane capillary column (40-m length, 0.18-mm i.d., 0.20-µm film thickness, Restek Corporation, Bellafonte, PA, USA). Transfer line between the gas chromatograph and the mass spectrometer was kept at 260 °C. For the components' detection an ISQ Single Quadrupole was used (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a chemical ionisation source type operated at 185 °C. Methane was used as a reagent gas and its flow rate was 1.5 mL/min. Electron lens was set to 1.5 V, the electron energy to 70 eV, the emission current to 50 µA, and the detector grain to 3.00×10^5. One characteristic ion per PFBO with a negative charge was used for identification and quantification in selected ion monitoring mode. Selected ion monitoring of the fragments was at m/z 247 (2MP); 261 (2MB and 3MB); 275 (HEX); 271 (FUR); 279 (MET); 295 (PHE); 315 (T2N); 270 (2MB-d_{10}); and 287 (benzaldehyde-d_6). Data were processed with XCalibur™ (Thermo Electron Corporation, Waltham, MA, USA) and quantified based on the external calibration line prepared from authentic reference compounds (N_2 flushed Milli-Q water was used as a matrix for calibration). Method was validated in model solutions with recovery rates of 100 % ± 5 % and standard deviations between 3 % to 8 % depending on the aldehyde. Quantification parameters of the method are presented in table 1. All reported results in this publication are the mean values of three independent measurements.

Table 1 **HS-SPME-GC-MS quantification parameters – range, correlation coefficient r^2, limit of detection (LOD), limit of quantification (LOQ)**

Aldehyde	Range [µg/L]	r^2	LOD	LOQ
2MP	0–271.5	0.9989	1.9	5.6
2MB	0–185.7	0.9977	0.2	0.8
3MB	0–265.1	0.9971	0.5	1.6
HEX	0–19.0	0.9919	0.1	0.2
FUR	0–1,494.2	0.9995	5.6	16.9
MET	0–201.1	0.9955	1.0	3.1
PHE	0–283.5	0.9979	2.6	7.7
T2N	0–1.9	0.9991	<0.1	<0.1

2.5 TB-Index

Thiobarbituric acid index (TB-Index) is a traditionally used indicator for evaluating heat load during wort production. Determination was carried out according to the procedure described by *Thalacker* and *Bößendörfer* [25]. An aliquot of 1.00 mL of wort sample was mixed with 9.00 mL of water and 5.00 mL of 0.02 M thiobarbituric acid (Merck KGaA, Darmstadt, Germany) in 90 % acetic acid (Sigma Aldrich Co., St. Louis, MO, USA). After homogenisation, the mixture was placed at 70 °C for 70 min, followed by cooling to 20 °C. Blank samples were prepared by mixing 1.00 mL of corresponding wort sample, 9.00 mL of water and 5.00 mL of 90 % acetic acid, without subjecting to heating. Samples were analysed by spectrophotometer (Varian Cary 100, Agilent Technologies Inc, Australia) at a measuring absorbance of 448 nm. TB-Index was calculated from the following formula: $TBI = (A_{sample} - A_{blank}) \times 100$; and expressed as TBI for 100 mL of wort.

2.6 Statistical analysis

All samples were analysed in three replicates. Mean values and standard deviations were calculated for all the intermediate points. Statistical significance was analysed by one-way analysis of variance (ANOVA) and *P*-value below 0.05 was considered as statistically significant.

3 Results and Discussion

Malt contains various compounds, originating from barley or formed during the malting process, that can play a significant role in relation to beer flavour quality and stability. *Jaskula* et al. [15] showed that the rate of beer ageing is positively correlated with FAN, Kolbach Index, heat-load (TB-Index) and free aldehyde content of the malt. Furthermore, a higher FAN content of the malt, usually coinciding

Table 2 **Free aldehyde concentration in malt [µg/kg] and mashing-in sample [µg/kg of malt]. Data represent mean value of three repetitions and standard deviation**

		2MP	2MB	3MB	HEX	FUR	MET	PHE	T2N
Pilsner malt	[µg/kg]	1,507.8	1,330.0	3,177.5	641.2	321.4	221.5	1,343.2	289.9
	SD	± 255.5	± 233.3	± 463.1	± 79.4	± 45.2	± 35.6	± 126.0	± 43.9
Mashing-in sample*	[µg/kg]	909.1	754.6	1,904.8	150.8	511.2	350.0	515.2	10.2
	SD	± 76.5	± 34.0	± 119.9	± 4.7	± 2.3	± 62.4	± 49.9	± 1.8

*Aldehyde concentration recalculated to 1 kg of malt

Table 3 **Minimum and maximum concentrations of aldehydes [µg/L] determined during wort production process. Data represent mean value of three repetitions and standard deviation**

		2MP	2MB	3MB	HEX	FUR	MET	PHE	T2N
Min. conc.	[µg/L]	33.4	6.9	65.0	7.7	208.5	83.5	117.2	0.4
	SD	± 1.7	± 0.3	± 1.6	± 0.4	± 1.0	± 1.7	± 5.6	± 0.0
Max. conc.	[µg/L]	471.2	343.0	865.8	71.3	414.6	159.1	234.2	4.6
	SD	± 37.1	± 18.4	± 57.0	± 5.9	± 23.8	± 28.3	± 22.7	± 0.8

with a higher aldehyde content, results in less flavour-stable beers [15, 26]. Therefore, first of all, in this study, we determined the content of free aldehydes in malt. The investigated aldehyde markers can be classified into Strecker degradation aldehydes (2-methylpropanal, 2- and 3-methylbutanal, methional, and phenylacetaldehyde), aldehydes formed during Maillard reactions (furfural) and lipid oxidation aldehydes (hexanal and *trans*-2-nonenal). As illustrated in table 2, malt contains considerably high levels of free aldehydes, especially Strecker degradation aldehydes are present in high concentrations. These results are also in accordance with aldehydes' levels measured in malt and reported in several other studies [12, 13, 27, 28]. Furthermore, the aldehyde content determined in malt was compared with the corresponding levels measured in samples taken at the onset of mashing (upon recalculation of the aldehydes' concentrations in the mashing-in samples back to the dry malt). Concentrations of the majority of marker aldehydes in mashing-in samples were at least 40 % lower than the corresponding aldehydes' levels determined in malt. FUR and MET showed a contrary relation, as their levels quantified in mashing-in samples were at least 50 % higher, compared to the levels found in malt. Furthermore, there was a very strong relation between the levels of aldehydes quantified in malt and mashing-in samples, as the correlation coefficient *r* was 0.94 for these samples. Nevertheless, the lower concentrations of aldehydes measured in mashing-in samples might be influenced by thick mash extraction and/or explained by a variable degree of evaporation depending on the specific nature of the compound. Moreover, aldehydes might possibly bind to other compounds (e.g. cysteine) forming non-volatile adducts [5, 11]. In particular, it appears that thick mashing lowers the extraction of HEX and especially T2N (see Table 2), likely due to the highly hydrophobic nature of the fatty acid oxidation aldehydes.

Considering the behaviour of free aldehydes during wort production, in general, quantified levels of free aldehydes varied markedly, depending on the sampling point and the nature of the analysed compound. To demonstrate the overall variation in the levels of aldehydes during the wort production process, table 3 covers the highest and lowest concentrations of aldehydes found. Apparently, the highest aldehyde levels, except for FUR were observed in the mashing-in samples, which suggests that malt is the major source of aldehydes introduced into the process.

With regard to the evolution of free aldehydes during wort production, obtained data revealed similar patterns for lipid oxidation products (HEX and T2N) and Strecker degradation aldehydes (2MP, 2MB, 3MB, MET and PHE), but not for Maillard reactions product (FUR).

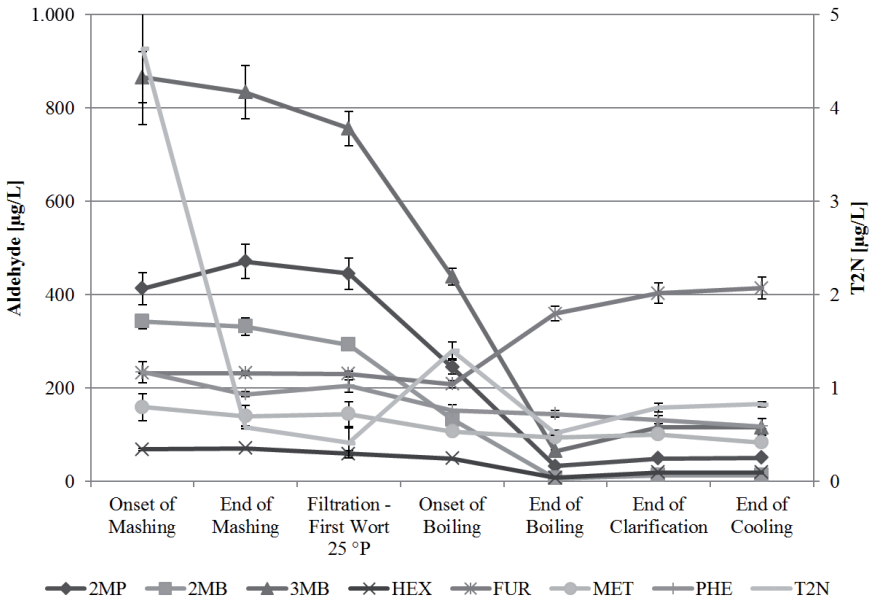

Fig. 2 **Change in aldehyde concentrations during the brewing process**

Table 4 Relative change in the levels of aldehydes during the entire brewing process and at different processing steps

	Change (%) of levels of aldehydes							
	2MP	**2MB**	**3MB**	**HEX**	**FUR**	**MET**	**PHE**	**T2N**
Mashing[1]	14 % N.S.	– 3 % N.S.	– 4 % N.S.	4 % N.S.	0 % N.S.	– 12 % N.S.	– 20 % p < 0.05	– 87 % p < 0.01
Mash filter conditioning[2]	– 6 % N.S.	– 12 % p < 0.05	– 9 % N.S.	– 15 % p < 0.05	0 % N.S.	3 % N.S.	9 % N.S.	– 29 % N.S.
Filtration[3]	– 45 % p < 0.01	– 55 % p < 0.01	– 42 % p < 0.01	– 20 % p < 0.05	– 10 % p < 0.05	– 26 % p < 0.05	– 26 % p < 0.01	240 % p < 0.01
Boiling[4]	– 86 % p < 0.01	– 95 % p < 0.01	– 85 % p < 0.01	– 84 % p < 0.01	72 % p < 0.01	– 12 % p < 0.05	– 5 % N.S.	– 63 % p < 0.01
Clarification[5]	48 % p < 0.01	93 % p < 0.01	79 % p < 0.01	153 % p < 0.01	12 % p < 0.05	7 % N.S.	– 8 % N.S.	52 % p < 0.01
Cooling[6]	3 % N.S.	0 % N.S.	0 % N.S.	0 % N.S.	3 % N.S.	– 17 % p < 0.01	– 11 % N.S.	5 % N.S.
Entire process[7]	– 88 % p < 0.01	– 96 % p < 0.01	– 87 % p < 0.01	– 72 % p < 0.01	78 % p < 0.01	– 47 % p < 0.01	– 63 % p < 0.01	– 82 % p < 0.01

[1] relative difference between 'mashing-in' and 'end of mashing' samples;
[2] relative difference between 'end of mashing' and 'first wort collection' samples;
[3] relative difference between 'first wort collection' and 'onset of boiling' samples';
[4] relative difference between 'onset of boiling' and 'end of boiling' samples;
[5] relative difference between 'end of boiling' and 'end of clarification' samples;
[6] relative difference between 'end of clarification' and 'end of cooling' samples;
[7] relative difference between 'onset of mashing' and 'end of cooling' samples.

As presented in figure 2, over the entire wort production process, an overall decrease in lipid oxidation and Strecker degradation aldehydes was observed, and an increase in a Maillard reactions product – FUR. The most abundant aldehydes at the beginning of the brewing process were saturated Strecker degradation aldehydes (2MP, 2MB, 3MB), yet their levels decayed remarkably over the whole wort production process (with the highest reduction rates during the boiling step). Overall reduction rates for 2MP, 2MB and 3MB were the highest from all selected marker aldehydes (see Table 4) and were respectively: –88 %; –96 %; and –87 %. On the other hand, MET and PHE also decreased over the entire wort production process (–47 % and –63 %, respectively), however, their reduction rates were less pronounced (even during boiling). With regard to the lipid oxidation aldehydes HEX and T2N, their levels decreased during wort production by –72 % and –82 %, respectively. In contrast, levels of FUR increased by 78 % over the entire process.

Table 4 presents the relative changes in levels of aldehydes during each processing step, as well as over the entire wort production process. With regard to the relative change in aldehydes' levels, the critical steps identified during wort production were: mashing (only for T2N), mash filtration, wort boiling and wort clarification. During mashing, the most substantial change was observed for T2N, which decreased by 87 % (Table 4), though the levels of another lipid oxidation marker aldehyde (HEX) hardly changed. T2N is a product of oxidation of unsaturated fatty acids in malt resulting from autoxidation or enzymatic degradation by malt-originating lipoxygenases. On the one hand, we aimed at supressing the increase in T2N levels at mashing-in by using a pH of 5.2 in combination with a temperature of 63 °C [17, 19, 29]. As mentioned previously in this paper, this, however, cannot explain the extremely low levels of T2N measured at the stage of mashing-in (see Table 2), and rather lack of extraction of T2N from the malt. Yet, high hydrophobic character of T2N and thick

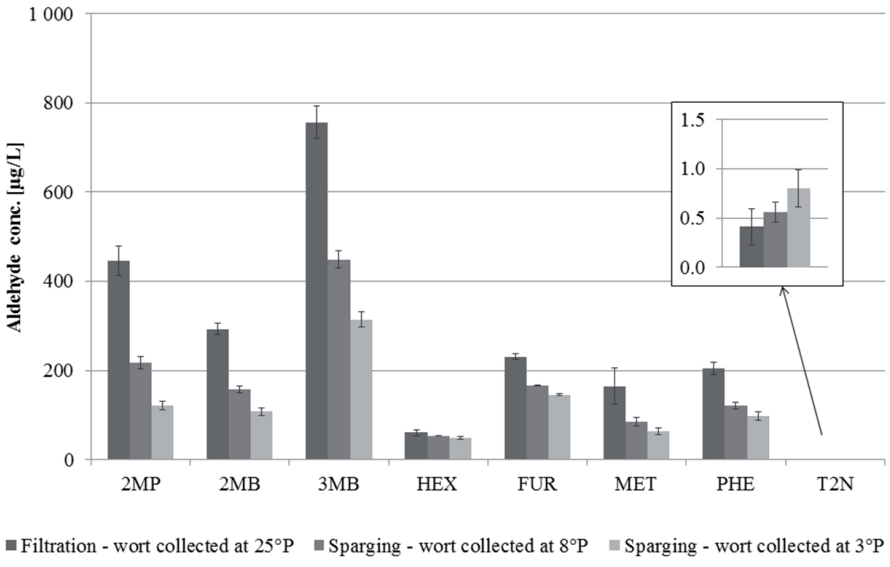

Fig. 3 Extraction of aldehydes during sparging

mashing-in conditions offer a reasonable explanation for low levels of T2N measured at mashing-in. Moreover, the observed high relative decrease of T2N during mashing (see Table 4) might be explained in a similar way, i.e. due to its high hydrophobicity and, therefore, interaction with proteins being massively coagulated during mashing.

Above all, the highest reduction in aldehydes' levels was observed during the mash filtration and wort boiling steps. Naturally, the first can be explained by the change in extract, as in the course of filtration and sparging, wort was diluted from 25 °P to 13 °P, resulting in lower aldehydes concentrations (except for T2N, see further). 2MP, 2MB and 3MB were reduced strongly during mash filtration. This must be due to their high volatility and possibly also adsorption to the spent grains.

Nonetheless, sparging revealed to be of particular importance in overall aldehyde extraction. As presented in figure 3, the absolute values of the levels of aldehydes were declining with the progress of sparging (except for T2N). However, upon extract normalisation to 25 °P (first wort), relative concentrations of all the marker aldehydes increased (see Fig. 4). This could indicate that by applying extensive sparging regimes relatively more aldehydes could be extracted. Though, it must be noted that sparging was carried out with acidified water (pH 3.5), which may have an effect on the overall release of aldehydes, e.g. from imine adducts [30], which, later on, could be stripped out during the boiling step. Furthermore, during sparging, interesting observation was made for HEX and T2N. Absolute levels of HEX quantified in first wort (25 °P) and sparged worts (8 °P and 3 °P) were of similar magnitude, whereas the levels of T2N increased with the progress of sparging (Fig. 3). Upon extract normalisation (to 25 °P), this observation was even more evident (Fig. 4). This might be explained by the remaining lipoxygenase activity within the layer of spent grains, as approximately two thirds of the lipoxygenase activity has been found to be present in the non-extractable malt material [31]. These membrane-bound enzymes are considered more heat-stable and, therefore, could remain active in the spent grains during lautering and sparging [31, 32].

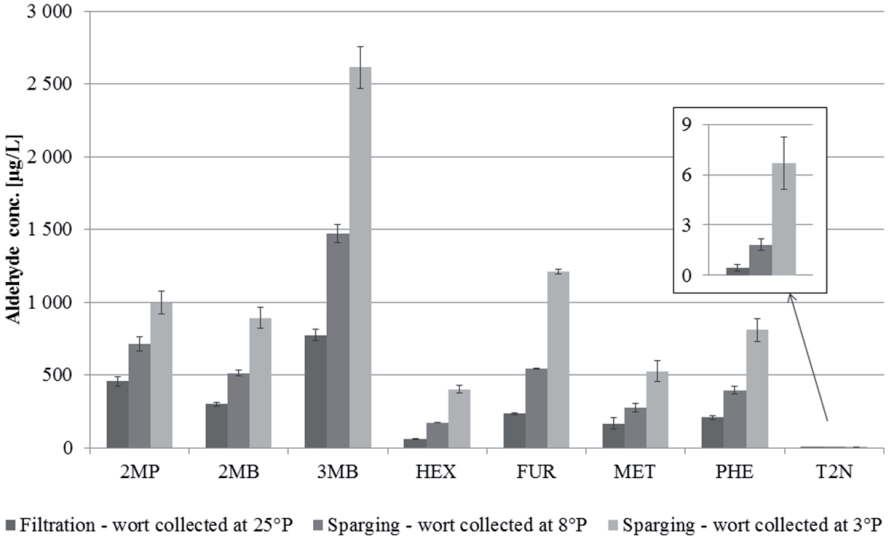

Fig. 4 Extraction of aldehydes during sparging upon extract normalisation to 25 °P

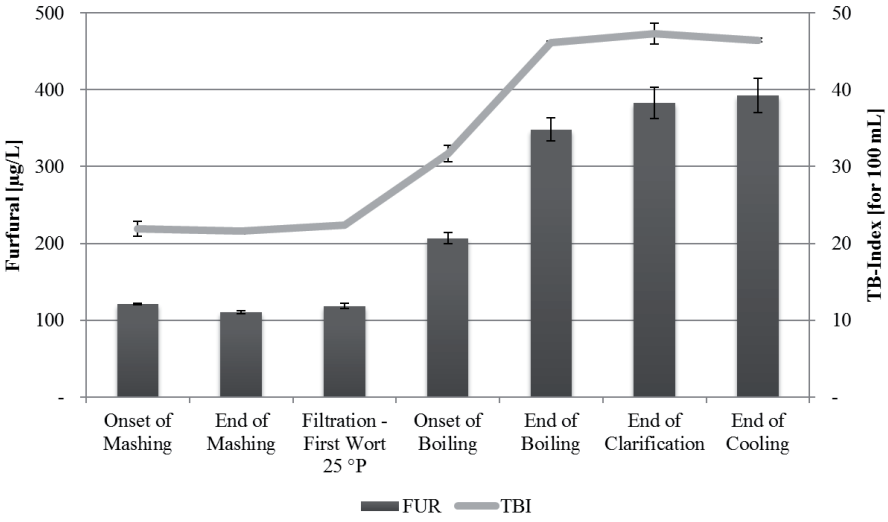

Fig. 5 Change in levels of FUR and TB-Index during wort production process upon extract normalization to 13 °P

Table 5 Literature boiling points for marker aldehydes. Values indicate the temperature [°C] at which the liquid phase is in equilibrium with the vapour at a pressure of 760 mmHg, if it is available. If the boiling point is available for different pressure, the value in the superscript indicates the pressure [mmHg]

Aldehyde	Boiling Point [°C]
2MP	64.5 [33]
2MB	90–92 [34]
3MB	92.5 [33]
HEX	131 [33]
FUR	161.7 [33]
MET	62^{11} [33]
PHE	195 [33]
T2N	101^{16}, 89^{12} [33]

During wort boiling aldehydes, due to their volatile character, evaporate. On the other hand, according to *De Schutter* et al. [23] new aldehydes can be formed and/or released, e.g. formation rates of FUR and 5-hydroxymethylfurfural increase as a function of boiling time, Strecker degradation proceeds at pseudo-zero-order rate, while lipid oxidation practically would not proceed. Therefore, the overall concentration of aldehydes during wort boiling is the balance between the ones being formed/released with the ones being

evaporated. In our study (see Table 4), high reduction in aldehyde levels was observed for the saturated Strecker aldehydes 2MP, 2MB and 3MB, which was –86 %, –95 % and –85 %, respectively. Less evident reduction was observed for MET (–12 %) and PHE (–5 %). Presumably, this can be explained by varying degrees of evaporation for these compounds (Table 5 pools the literature boiling points for marker aldehydes) – 2MP, 2MB and 3MB, due to their low molecular weight, are more volatile than PHE, comprising an aromatic structure, and MET, containing a reactive sulphur group. With regard to lipid oxidation products, a significant decrease in HEX and T2N was observed, thus the evaporation rate of these compounds was higher than their rate of potential formation. This suggests that lipid oxidation would hardly proceed during wort boiling, which is in line with De Schutter et al. [23]. Interesting behaviour was observed for the Maillard reactions' product FUR, whose levels increased markedly during wort boiling and continued to increase over wort clarification. This could be explained by low volatility of FUR and its production by applying heat-load. With regard to the entire brewing process, as presented in figure 5, FUR showed a perfect correlation with TB-Index, upon extract normalisation to 13 °P (correlation coefficient *r* equalled to 1.00). Thus, both TB-Index and FUR can be regarded as good heat-load indicators with regard to brewing process. Interestingly, during wort clarification, levels of aldehydes significantly increased, except for MET and PHE. Wort cooling, had a negligible effect on the change in levels of free aldehydes.

4 Conclusions

In conclusion, high levels of aldehydes determined in malt and mashing-in samples point to malt as the major source of staling aldehydes introduced into the wort production process. From the perspective of brewing technology, processing steps of mashing (only for fatty acid oxidation products), mash filtration with particular emphasis on sparging, wort boiling and wort clarification were identified as the critical steps in relation to flavour instability.

In general, aldehydes decrease in the course of the overall wort production process, with the exception of FUR, presumably due to its production by applying heat and its lower volatility. Higher relative extraction of aldehydes was observed with the progress of sparging and especially T2N appears to increase at this stage. During wort boiling the highest decrease in levels of aldehydes was observed, except for FUR. However, increased concentrations of aldehydes were observed during wort clarification (especially for HEX and T2N). In turn, FUR increased in levels during wort boiling and wort clarification and correlated strongly with TB-Index.

In order to obtain a more profound insight in the behaviour of aldehydes during the brewing process and beer aging, future research should include the analysis of both bound-state and free aldehydes, as well as the precursors for their formation.

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