Maciej Ditrych, Wouter De Sutter, Gert De Rouck, Guido Aerts and Mogens Larsen Andersen

Influence of high concentrations of aldehydes added at mashing-in on beer flavor stability: no evidence for direct contribution to aged beer aldehydes

Aldehydes are key markers of beer flavour stability, however, their origin during brewing and ageing remains debated, particularly with respect to *de novo* formation and release from bound-state forms. The present study investigated whether aldehydes present at the mashing stage contribute to the pool of free aldehydes in beer after fermentation and ageing. A mixture of selected aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde, furfural, hexanal, and *trans*-2-nonenal) was added at mashing-in at concentrations corresponding to 3-fold and 10-fold levels relative to those quantified in a reference mash. Beers were produced in pilot scale and aldehyde concentrations were analysed in fresh samples and after 90 days of forced ageing at 30 °C using HS-SPME-GC-MS. The addition of aldehydes at mashing-in did not result in increased concentrations of free aldehydes in fresh beer. After forced ageing, all variants exhibited comparable aldehyde levels, and no consistent dose–response relationship between initial and final aldehyde concentrations was observed. These findings indicate that aldehydes present at early stages of brewing do not persist as a direct source of free aldehydes in finished or aged beer. The results indicate that aldehyde levels in aged beer are primarily determined by reactions occurring during storage, particularly Strecker degradation and other thermally driven pathways, rather than by the initial concentration of free aldehydes in the mash.

Descriptors: Staling aldehydes, flavour instability, brewing, mashing, shelf-life

1 Introduction

Beer flavour stability is a key quality parameter that determines the sensory acceptance and shelf life of beer. During storage, beer undergoes a range of chemical changes that can lead to the development of stale flavours, often described as cardboard-like, honey-like, or worty [1-6]. These changes are influenced by multiple factors, including raw material composition, brewing conditions, oxygen exposure, and storage environment, making flavour stability a complex and multifactorial phenomenon [1,7-13].

https://doi.org/10.23763/BrSc26-06ditrych

Authors

Maciej Ditrych (ORCID ID: 0000-0001-8186-9346), Lodz University of Technology, Faculty of Biotechnology and Food Sciences, Institute of Fermentation Technology and Microbiology, Lodz, Poland; Wouter De Sutter, Gert De Rouck (ORCID ID: 0000-0001-9092-8205), Guido Aerts, KU Leuven, Faculty of Engineering Technology, Department of Microbial and Molecular Systems (M2S), Laboratory of Enzyme, Fermentation and Brewing Technology, Technology Campus Ghent, Ghent, Belgium; Mogens Larsen Andersen (ORCID ID: 0000-0003-4694-486X), University of Copenhagen, Faculty of Science, Department of Food Science, Ingredient and Dairy Technology, Frederiksberg, Copenhagen, Denmark; corresponding author: maciej.ditrych@p.lodz.pl

Among the various compounds associated with beer ageing, aldehydes are widely recognised as important markers of flavour instability. Compounds such as 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, furfural, hexanal, and *trans*-2-nonenal contribute to characteristic stale flavour notes, even at low concentrations. As a result, aldehydes have been frequently reported as indicators of beer freshness and as analytical targets in studies of flavour stability [3,6,14-17].

The origin of aldehydes in beer remains a subject of ongoing discussion. Two types of mechanisms are generally considered. On the one hand, aldehydes can be formed *de novo* during storage through chemical reactions such as Strecker degradation, Maillard reactions, and lipid oxidation [1,7,9]. *De novo* formation of aldehydes may also include direct oxidation reactions of amino acids [18-20]. On the other hand, a significant fraction of aldehydes in fresh beer exists as reversible bound forms, for example as adducts with cysteine or bisulfite and can be released during ageing as the equilibrium shifts towards the free aroma-active compounds [21-24]. The relative contributions of these two types of mechanisms are still not fully resolved.

Aldehydes are already present at earlier stages of brewing [25,26]. Malt contains both free aldehydes and their precursors [23,27-33] and aldehyde concentrations can increase during mashing and wort production [1,8,13,34]. These early-stage aldehydes may originate from thermal reactions, oxidative processes, and transformations

This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/

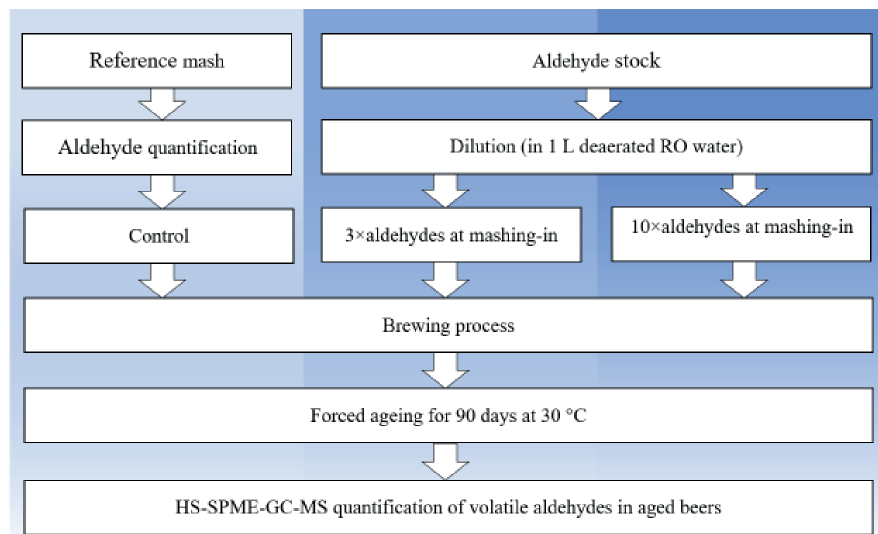

Fig. 1 Schematic representation of the experimental design. Aldehyde concentrations quantified in a reference mash sample collected at mashing-in were used to define target levels for spiking. A mixed aldehyde stock solution was prepared in absolute ethanol and diluted in deaerated reverse osmosis water to obtain 3-fold (3×) and 10-fold (10×) concentrations relative to the reference mash. The solutions were added at the onset of mashing-in. Three beer variants were produced, including a control without aldehyde addition, each in two biological replicates. After brewing, beers were subjected to forced ageing (90 days at 30 °C) and analysed for free aldehydes using HS-SPME-GC-MS

of amino acids and lipids. However, it remains unclear to what extent aldehydes present in malt and wort are carried through the brewing process and contribute to the aldehyde pool in finished and aged beer.

Despite extensive research on beer flavour instability, the role of aldehydes introduced at early stages of brewing remains insufficiently understood. In particular, it is unclear whether increasing the concentration of aldehydes at mashing-in leads to higher concentrations of free aldehydes in fresh beer or after ageing. This question is critical to understanding whether aldehydes present in malt and mash act as direct contributors to flavour instability or whether their influence is mediated indirectly through other precursors and/or processes.

Therefore, the aim of the present study was to evaluate the contribution of aldehydes present at mashing-in to the pool of free aldehydes in beer. A mixture of selected aldehydes, that are known to appear during beer ageing, was added at the onset of mashing at concentrations corresponding to threefold and tenfold levels relative to those naturally present in the mash. The resulting beers were analysed in fresh state and after forced ageing to assess whether changes in aldehyde concentrations at the mash stage influence the aldehyde profile of finished and aged beer.

2 Materials and Methods

2.1 Preparation of aldehyde stock solution and addition to mash

A mixed aldehyde stock solution was prepared to reflect the relative composition of aldehydes quantified in a reference mash sample collected at mashing-in using the same malt. The stock solution contained 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde, furfural, hexanal, and *trans*-2-nonenal (Sigma-Aldrich Co., St. Louis, MO, USA). The aldehydes were dissolved in absolute ethanol (≥99.5 %, Merck KGaA, Darmstadt, Germany) and mixed in proportions corresponding to their relative concentrations determined in the reference brewing sample collected at mashing-in. The stock solution was prepared volumetrically, taking into account the density and purity of each compound. The mixture was prepared in sealed amber glassware under oxygen-limited conditions, thoroughly mixed, and stored at −20 °C for a maximum of 7 days prior to use.

For brewing experiments, aliquots of the stock solution were diluted in 1 L of deaerated reverse osmosis water immediately prior to mashing-in. The diluted solutions were added directly to the mash at the onset of mashing. The amount of stock solution added was adjusted to obtain aldehyde concentrations corresponding to threefold (3×) and tenfold (10×) the levels quantified in the reference mash. The control variant received no aldehyde addition. The total addition volume was kept constant across all variants.

To minimise oxidative and evaporative losses, preparation and handling of aldehyde solutions were performed under oxygen-limited conditions using sealed vessels and the solutions were added immediately after preparation.

2.2 Wort and Beer Production

Unpasteurised top-fermented pale beers were produced in a pilot-scale brewing plant at KU Leuven, Technology Campus Ghent (Ghent, Belgium). Each batch was prepared using 44 kg of pilsner malt and a grain-to-liquor ratio of 1:2.2. The brewing process was carried out under oxygen-limited conditions. Prior to brewing, the installation was saturated with CO_2. During milling and mashing-in, a continuous CO_2 supply was maintained and terminated after mashing-in.

Malt was milled using a wet disc mill (Meura, Péruwelz, Belgium) and mixed with deaerated reverse osmosis water enriched with Ca^{2+} ions (80 mg/L) in the form of calcium chloride dihydrate (Merck KGaA, Darmstadt, Germany). At mashing-in, mash pH was adjusted to 5.2 using 30 % (v/v) lactic acid (from 90 % (v/v) (S)-lactic acid, Merck KGaA, Darmstadt, Germany). Aldehydes were added at this stage as aqueous solutions in deaerated reverse osmosis water to achieve concentrations corresponding to 3× and 10× the levels quantified in the reference mash (Figure 1). A control batch was made without aldehyde addition. The following mashing programme was applied: 63 °C (30 min), 72 °C (15 min), and 78 °C (1 min), with a temperature increase of 1 °C/min. Mash filtration was performed using a membrane-assisted thin bed filter (Meura 2001, Meura, Péruwelz, Belgium). Wort boiling was carried out for 60 min. Hop pellets were added during wort boiling according to a three-stage hopping regime, aiming at achieving 20 mg/L iso-α-acids in the final

Table 1 Analytical performance characteristics of the HS-SPME-GC-MS method for the determination of free aldehydes

Aldehyde	Investigated range (µg/L)	Linearity R^2	Limit of detection (LOD) (µg/L)	Limit of quantification (LOQ) (µg/L)	Accuracy (%)	Recovery (%)
2-Methylpropanal	2.41–94.07	0.9985	1.870	5.610	5.9 %	105.0 %
2-Methylbutanal	0.48–47.74	0.9999	0.250	0.749	3.4 %	100.7 %
3-Methylbutanal	0.95–68.15	0.9997	0.533	1.599	3.3 %	104.0 %
Hexanal	0.05–2.46	0.9982	0.059	0.178	8.2 %	102.1 %
Furfural	4.67–373.62	0.9992	5.648	16.945	6.0 %	99.9 %
Methional	0.95–49.16	0.9985	1.044	3.131	7.6 %	93.7 %
Phenylacetaldehyde	0.93–69.85	0.9981	2.568	7.703	9.5 %	94.1 %
trans-2-Nonenal	0.01–0.48	0.9996	0.005	0.015	5.4 %	98.7 %

beer. Bittering hops (Magnum, 13.0 % (w/w) α-acids) were added at the onset of boiling at a rate of 50 g/hL. Aroma hops were added at the end of boiling, consisting of Tettnanger (3.0 % (w/w) α-acids) at 100 g/hL and Saaz (2.5 % (w/w) α-acids) at 120 g/hL. After boiling, wort was clarified via whirlpool, cooled to 20 °C, and aerated with sterile filtered air (0.2 µm). Fermentation was conducted using Saccharomyces cerevisiae (Fermentis S-04, Fermentis Lesaffre, Lille, France) at a pitching rate of 80 g/hL. Prior to pitching, yeast was rehydrated in sterile reverse osmosis water (1:10 m/v).

Fermentation was carried out for 3 days at 20 °C in 50 L cylindroconical stainless steel fermenters under atmospheric pressure. Subsequently, beer was transferred to 50 L kegs and lagered for 14 days at 0 °C. After lagering, beer was filtered using cellulose sheets (BECOPAD 350, Eaton, Belgium), carbonated to 5.6 g CO_2/L, and bottled in 25 cL brown glass bottles using counter-pressure filling with double pre-evacuation. Oxygen-scavenging crown caps were applied. Dissolved oxygen after bottling was below 7 ± 5 µg/L.

2.3 Forced Ageing of Beer Samples

Fresh beer was stored at 0 °C in darkness. Forced ageing was conducted at 30 °C in darkness for 15, 30, 60, and 90 days. After each ageing period, samples were stored at 0 °C prior to analysis for a maximum of three months.

2.4 Standard Beer Analysis

Basic beer parameters, including alcohol content, density, apparent extract, real and original extract, and degree of fermentation, were determined using an Anton Paar DMA 5000 Alcolyser and Anton Paar Alcolyzer (Anton Paar, Graz, Austria). pH was measured using a P600 pH meter (Consort, Turnhout, Belgium). Dissolved and total package oxygen levels were determined using a Haffmans Inpack TPO/CO_2 Meter c-TPO (Pentair Haffmans, Venlo, the Netherlands).

2.5 HS-SPME-GC-MS Quantification of Volatile Aldehydes

Free aldehydes were quantified using headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC-MS) following derivatisation with o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA). The method was based on previously described procedures [25,35,36]. Prior to extraction, the SPME fibre was conditioned according to the manufacturer's instructions and exposed to a PFBHA solution (1 g/L in Milli-Q water) for 10 min. Subsequently, the fibre was exposed to the headspace of the sample for 30 min. After extraction, the fibre was thermally desorbed in the inlet of a gas chromatograph (Thermo Fisher Scientific Inc., Waltham, USA) for 3 min at 250 °C. Chromatographic separation was carried out using an Rtx-1 Crossbond 100 % dimethylpolysiloxane capillary column (40 m × 0.18 mm × 0.20 µm; Restek Corporation, Bellefonte, USA). The GC oven programme was as follows: 50 °C for 2 min, followed by a temperature increase of 6 °C/min to 250 °C, with a final hold of 5 min. Helium was used as carrier gas at a flow rate of 0.8 mL/min. The inlet operated in split mode (split flow 10 mL/min; split ratio 12). Detection was performed using a single quadrupole mass spectrometer with a chemical ionisation source operated at 185 °C. Methane was used as reagent gas (1.5 mL/min). Quantification was carried out in selected ion monitoring mode using one characteristic ion per pentafluorobenzyloxime derivative. External calibration was performed using aldehyde standards prepared in nitrogen-flushed Milli-Q water ($R^2 \geq 0.980$). Data acquisition and processing were performed using XCalibur™ software (Thermo Fisher Scientific Inc., Waltham, USA). Analytical performance parameters for the HS-SPME-GC-MS quantification of free aldehydes are presented in Table 1.

Regarding aldehyde quantification in malt sample, freeze-dried malt samples (1 g) were extracted with 99 mL of nitrogen-flushed Milli-Q water in 100 mL serum bottles sealed with crimp caps to minimise oxygen exposure and protected from light. The extraction was carried out under oxygen-limited conditions by stirring the mixture (250 rpm, 15 min, 20 °C), followed by sedimentation of solid particles. An aliquot of the supernatant (10 mL) was transferred to 20 mL amber glass GC vials, sealed with crimp caps, and subjected to HS-SPME-GC-MS analysis according to the optimised extraction conditions reported by Filipowska et al [29].

Mash and beer samples were prepared under oxygen-limited conditions prior to analysis. Mash samples were diluted tenfold with nitrogen-flushed Milli-Q water, whereas beer samples were

Table 2 **Concentration of selected free aldehydes in malt and corresponding mashing-in samples used as reference for the preparation of aldehyde mixtures. Data represent mean values and standard deviations for mash samples (n=3). Malt values are expressed on a dry matter basis**

Compound	Malt (µg/kg dm)	Mash at mashing-in (µg/L)
2-Methylpropanal	1131 ± 91	251.5 ± 2.0
2-Methylbutanal	1069 ± 50	179.9 ± 8.8
3-Methylbutanal	2592 ± 132	502.3 ± 33.2
Methional	202 ± 50	72.2 ± 14.8
Phenylacetaldehyde	963 ± 33	136.9 ± 38.9
Hexanal	615 ± 70	76.7 ± 1.3
trans-2-Nonenal	454 ± 42	11.2 ± 9.7
Furfural	402 ± 64	134.6 ± 23.1

Table 3 **Basic physicochemical characteristics of fresh beers produced with different aldehyde levels present at mashing-in (reference, 3×, and 10×). Values represent mean values of two biological replicates. Capital letters in superscripts indicate statistically significant differences between variants (one-way ANOVA followed by Tukey's test, $p < 0.05$)**

Parameter	Reference	3× aldehydes at mashing-in	10× aldehydes at mashing-in
Alcohol (% v/v)	5.36 ^{B}	5.34 ^{B}	5.24 ^{A}
Density (g/mL)	1.0082 ^{A}	1.0081 ^{A}	1.0090 ^{A}
Apparent extract (°P)	2.64 ^{A}	2.69 ^{A}	2.89 ^{A}
Real extract (°P)	4.69 ^{A}	4.41 ^{A}	4.45 ^{A}
Original extract (°P)	12.60 ^{A}	12.69 ^{A}	12.45 ^{A}
Apparent degree of fermentation (%)	78.95 ^{A}	78.74 ^{A}	77.14 ^{A}
Real degree of fermentation (%)	66.03 ^{A}	64.95 ^{A}	66.09 ^{A}
pH	4.26 ^{A}	4.22 ^{A}	4.32 ^{B}
Total package oxygen (µg/L)	9 ^{A,B}	2 ^{A}	11 ^{B}

analysed without dilution. Beer samples were degassed prior to analysis. Subsequently, 4 mL aliquots of each sample were transferred to 20 mL amber glass vials, sealed with PTFE/silicone septa, and subjected to HS-SPME-GC-MS analysis.

3 Results

The concentrations of selected free aldehydes in malt and corresponding mashing-in samples are presented in Table 2. Aldehydes were detected for all analysed compounds in both matrices. Due to differences in units (µg/kg dry matter for malt and µg/L for mash), the concentrations are not directly comparable. However, a similar distribution of compounds was observed, with 3-methylbutanal being the most abundant aldehyde in both malt and mash samples, followed by 2-methylpropanal and 2-methylbutanal. The remaining aldehydes, including methional, phenylacetaldehyde, hexanal, *trans*-2-nonenal, and furfural, were present at lower levels. The variations between mash replicates were low for most compounds. The aldehyde concentrations measured in the mashing-in samples were used as the reference for the preparation of aldehyde mixtures applied in the brewing trials.

Following the determination of aldehyde concentrations in malt and mashing-in samples, the resulting beers were analysed to evaluate both the consistency of the brewing process and the evolution of aldehydes in the finished product. The results are presented in three parts. First, the basic physicochemical characteristics of the beers were assessed to confirm process consistency. Second, the concentrations of selected free aldehydes were determined in fresh beers. Finally, the evolution of aldehydes during storage was investigated by analysing beers after forced ageing, with particular focus on the relationship between aldehyde levels at mashing-in and those measured after storage.

The basic physicochemical characteristics of the fresh beers are presented in Table 3. All variants showed very similar values for the analysed parameters. Alcohol content (5.2–5.4 % v/v), original extract (12.5–12.7 °P), and degrees of fermentation (77–79 % apparent; 65–66 % real) are typical of a standard-strength pale ale produced with top-fermenting yeast. The pH values (4.2–4.3) and low extract levels further confirm a well-attenuated beer. Overall, the degree of variation between the samples was low, pointing to good repeatability of the pilot-scale brewing trials. Only minor differences between variants were observed for selected parameters, but no consistent trend related to aldehyde addition at mashing-in was evident.

The concentrations of selected free aldehydes in fresh beers are summarised in Table 4. Overall, aldehydes were present at very low concentrations, with several compounds below the limit of quantification (LOQ) or limit of detection (LOD). In particular, 2-methylpropanal and methional were below the LOQ in all variants, while furfural and *trans*-2-nonenal were below the LOD. Among the quantifiable compounds, 3-methylbutanal was present at concentrations of 4.15–4.28 µg/L across the variants. 2-methylbutanal was detected at lower levels (1.07–1.32 µg/L), while hexanal was present at low concentrations ranging from 0.21 to 0.26 µg/L. Phenylacetaldehyde was below the LOQ in the control beer and detected in the spiked variants at concentrations of 8.22–8.42 µg/L. The concentrations of the quantified aldehydes were comparable across the control, 3×, and 10× variants, with no systematic differences observed between the treatments.

The concentrations of free aldehydes in beers after 90 days of forced ageing are presented in Figure 2. All analysed aldehydes increased substantially compared to fresh beer, with furfural reaching the highest concentrations (322–346 µg/L), followed by 2-methylpropanal, 3-methylbutanal and phenylacetaldehyde. No consistent differences between the experimental variants were observed. The concentrations of all analysed aldehydes were comparable across the reference, 3×, and 10× variants. This lack of differentiation was observed for all compound groups, including Strecker aldehydes

Table 4 **Concentrations of selected free aldehydes in fresh beers. Three variants were analysed: control beer (no aldehydes added) and beers spiked at mashing-in with aldehydes at 3-fold (3×) and 10-fold (10×) concentrations relative to levels quantified in the reference mash. Data represent mean values and from two biological replicates**

Compound	Control	3× aldehydes at mashing-in	10× aldehydes at mashing-in
2-Methylpropanal	<5.60*	<5.60*	<5.60*
2-Methylbutanal	1.32	1.10	1.07
3-Methylbutanal	4.28	4.15	4.22
Methional	<3.13*	<3.13*	<3.13*
Phenylacetaldehyde	<7.70*	8.42	8.22
Hexanal	0.21	0.23	0.26
trans-2-Nonenal	<0.02*	<0.02*	<0.02*
Furfural	<5.65**	<5.65**	<5.65**

* determination below quantification limit

** determination below detection limit

(2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde), lipid oxidation markers (hexanal, *trans*-2-nonenal) and Maillard-derived compounds (furfural). One-way ANOVA performed on the available observations revealed a significant effect of treatment only for 2-methylbutanal and hexanal, whereas no significant differences were observed for the remaining aldehydes. Tukey's HSD post-hoc test indicated a significant difference between the reference and 3× variant for 2-methylbutanal and between the reference and 10× variant for hexanal. However, no consistent dose-dependent pattern was observed across the analysed aldehydes. Despite the substantial increase in aldehyde concentrations during ageing, no dose-dependent relationship between aldehyde levels at mashing-in and those measured after storage was evident.

4 Discussion

The addition of aldehydes at mashing-in did not result in increased concentrations of free aldehydes in fresh beer (see Table 4). Similarly, after 90 days of forced ageing at 30 °C, comparable aldehyde levels were observed across all variants, regardless of the initial aldehyde concentrations introduced at the mashing stage. No consistent dose-dependent relationship between aldehyde levels at mashing-in and those measured after storage was observed (see Figure 2).

During wort production, several process steps contribute to the reduction and transformation of aldehydes, thereby limiting their persistence into finished beer. During mash separation and subsequent wort clarification, aldehyde concentrations may decrease due to dilution effects, as well as volatilisation and interactions with solid phases such as spent grains [23,25,34]. Volatile compounds, particularly Strecker aldehydes, may be partially removed, while adsorption to solids may further contribute to lowering their concentrations. Wort boiling represents an additional critical stage, in which aldehydes are both removed and formed. Owing to their volatility, certain aldehydes may evaporate during boiling, whereas thermal reactions, including Strecker degradation and Maillard-type reactions, can lead to the formation or release of new aldehydes [34]. At the same time, the behaviour of individual aldehydes depends on their physicochemical properties. For example, hydrophobic compounds such as *trans*-2-nonenal tend to partition into solid phases during mashing and wort separation, including binding with proteins and spent grains, which reduces their concentration in the liquid phase and limits their subsequent transfer into wort and beer.

Fermentation is one of the most important stages determining the fate of aldehydes in beer. Brewing yeast efficiently reduces aldehydes to their corresponding higher alcohols, which are generally less flavour-active [23,37,38]. Aldehyde reduction occurs through metabolic pathways involving the synthesis and catabolism of amino acids, including pyruvate metabolism and the Ehrlich pathway [39-42]. The process is catalysed by a range of enzymes, including alcohol dehydrogenases, NAD(P)H-dependent reductases and aldo-keto reductases, which differ in substrate specificity [43-47]. In addition to aldehydes, yeast can reduce α-dicarbonyl intermediates of Maillard reactions, thereby limiting the formation of Strecker aldehydes. This has been demonstrated by studies showing reduced dicarbonyl concentrations

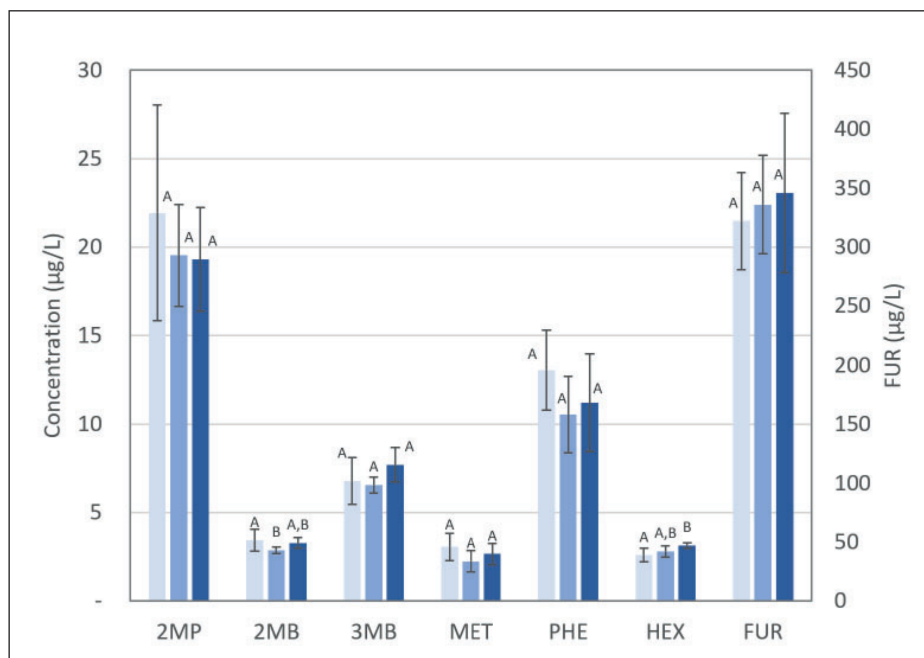

Fig. 2 **Concentrations of selected free aldehydes in beers after 90 days of forced ageing at 30 °C. Three variants were analysed: reference (no aldehydes added) (●) and samples spiked at mashing-in with aldehydes at 3-fold (●) and 10-fold (●) concentrations relative to the reference mash. Bars represent mean values (min n = 6) and error bars indicate standard deviation. Statistical differences between variants were evaluated using one-way ANOVA followed by Tukey's HSD test and are indicated by different capital letters ($p < 0.05$). Furfural (FUR) is presented on a secondary y-axis (right-hand side) due to its substantially higher concentration**

and lower Strecker aldehyde levels following the introduction or overexpression of yeast reductases [48,49].

Experimental studies have shown that aldehydes added to wort are rapidly removed during fermentation, with the most pronounced reduction occurring during the early stages of yeast activity [37,38,44,50]. Recent studies have further shown that yeast metabolism can profoundly alter the volatile composition of wort through the selective transformation and utilisation of malt-derived aroma compounds and their precursors [51]. Linear and Strecker aldehydes are particularly susceptible to yeast reduction, while elevated fermentation temperatures generally accelerate the process through increased enzymatic activity [38,44]. Although yeast strain, pitching rate, fermentation temperature, and re-pitching practices can influence the rate of aldehyde reduction, final aldehyde concentrations after fermentation tend to converge across different brewing conditions [47,52,53]. Early hypotheses suggested that residual aldehydes may be protected from yeast metabolism through binding to wort constituents. However, recent studies have not confirmed the presence of cysteine- or bisulphite-bound aldehydes in fermentation samples, indicating that such adducts are unlikely to transfer aldehydes from wort into the finished beer [23,24].

Although sulfite concentrations were not determined in the beers analysed in the present study, a comparable brewing trial [17] employing the same malt, brewing protocol and yeast strain yielded sulfite concentrations below the limit of detection (<0.6 mg/L). While the role of sulfite-bound aldehydes cannot be assessed without direct quantification of sulfite adducts, such low sulfite concentrations suggest only limited (if any) antioxidant protection during storage. Consequently, all beer variants were likely subjected to broadly similar oxidative conditions. An exception was the lower total package oxygen (TPO) level measured in the 3× variant. However, despite this difference, no corresponding reduction in aldehyde concentrations was observed after forced ageing. Furthermore, all TPO values were low and indicative of good packaging practice, suggesting that the observed variation was unlikely to have had a meaningful impact on the ageing behaviour of the beers.

The accumulation of aldehydes during beer ageing is generally attributed to two main mechanisms: *de novo* formation and the release of aldehydes from bound-state adducts. The latter assumes that aldehydes formed during wort production may be retained in non-volatile forms and subsequently liberated under specific conditions, such as elevated temperature or changes in pH [9,23,54,55]. This concept is supported by isotope-labelling studies, which have shown that a substantial fraction of Strecker aldehydes detected in aged beer originates from precursors already present in wort, indicating the presence of a reservoir of bound aldehydes [50]. Early investigations suggested bisulphite-bound aldehydes as potential contributors to beer ageing [21,22], however, more recent analytical studies have not confirmed their presence in malt, wort, or beer [24]. Similarly, imine formation between aldehydes and amino acids appears to be of limited relevance under brewing conditions. In contrast, aldehyde binding to cysteine has been more consistently observed, with evidence supporting the formation of cysteinylated adducts and their reversible behaviour depending on matrix conditions [23,56]. Recent work by Maia and Cook [57] further demonstrated that staling aldehydes may exist in dynamic equilibria between free and matrix-associated states, with both aldehyde structure and beer composition influencing their binding behaviour. The authors observed substantial differences in aldehyde binding and displacement among commercial lager beers, highlighting the importance of beer matrix interactions in determining aldehyde availability during ageing. These findings indicate that, although bound-state aldehydes exist, their chemical nature and contribution to flavour instability remain complex and not fully resolved.

In parallel, aldehydes can be formed *de novo* during storage through chemical pathways such as Strecker degradation (and direct oxidation of amino acids), Maillard reactions and lipid oxidation. Isotope-labelling experiments using amino acids have provided direct evidence for the formation of Strecker aldehydes in the beer matrix during storage, confirming that new aldehydes can arise independently of those present at earlier stages [24]. Although both mechanisms contribute to the aldehyde pool, current evidence suggests that *de novo* formation becomes increasingly important during ageing [23,24,58-60].

Within this mechanistic framework, the results of the present study indicate that aldehydes introduced at mashing-in do not contribute directly to the pool of free aldehydes in aged beer. Despite a substantial increase in aldehyde concentrations at the mash stage, no corresponding increase was observed after forced ageing, and no consistent dose–response relationship was detected. Similar observations were recently reported by Lehnhardt et al. [61], who monitored aroma-relevant aldehydes throughout wort production in 21 breweries and observed a continuous decrease in aldehyde concentrations during brewing. Despite substantial differences in brewhouse configurations and aldehyde concentrations at early process stages, these differences were not directly reflected in the aldehyde profile of the final beer. Nevertheless, the absence of direct carryover does not exclude the relevance of malt-derived aldehydes for flavour stability. Malt is a major source of precursors, including amino acids, reducing sugars, and lipid-derived compounds, which participate in reactions such as Strecker degradation, Maillard chemistry and lipid oxidation during brewing and storage [11].

Therefore, the results of this study indicate that direct manipulation of free aldehyde concentrations at mashing-in is unlikely to be an effective strategy for controlling aldehyde levels in finished or aged beer. Instead, the findings support a system-level perspective in which aldehyde formation is governed primarily by precursor composition, redox conditions, and reaction pathways occurring during fermentation and storage. In this context, approaches aimed at improving flavour stability may benefit from focusing on the control of precursor availability, oxidative processes, and fermentation performance rather than on the concentration of free aldehydes at early stages of brewing.

5 Conclusions

The present study demonstrates that increasing aldehyde concentrations at mashing-in does not result in elevated levels of free

aldehydes in fresh or aged beer. No dose-dependent relationship was observed between aldehyde levels introduced at the mash stage and those measured after 90 days of forced ageing. These findings indicate that aldehydes present during mashing do not directly contribute to the aldehyde pool in finished beer. Instead, aldehyde concentrations appear to be governed by a combination of transformation, binding, and de novo formation processes occurring during fermentation and storage. Consequently, manipulation of free aldehyde levels at early stages of brewing is unlikely to provide an effective strategy for controlling flavour stability. Future research should therefore focus on the role of precursor compounds, redox conditions, and bound-state aldehyde equilibria in determining beer flavour stability.

Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Maciej Ditrych: conceptualisation, methodology, investigation, formal analysis, visualisation, writing – original draft.

Wouter De Sutter: brewing trials.

Gert De Rouck: conceptualisation, methodology, resources.

Guido Aerts: methodology, writing – review & editing.

Mogens Andersen: methodology, writing – review & editing.

Use of AI

AI-assisted tools were used to refine language, including grammar and readability, of the text written by the authors. The scientific content, data analysis, and conclusions are solely the responsibility of the authors.

6 References

1. Vanderhaegen, B.; Neven, H.; Verachtert, H.; Derdelinckx, G.: The chemistry of beer aging – a critical review, Food Chemistry, **95** (2006), pp. 357–381. https://doi.org/10.1016/j.foodchem.2005.01.006
2. Saison, D.; De Schutter, D. P.; Uyttenhove, B.; Delvaux, F.; Delvaux, F. R.: Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds, Food Chemistry, **114** (2009), pp. 1206–1215. https://doi.org/10.1016/j.foodchem.2008.10.078
3. Malfliet, S.; Van Opstaele, F.; De Clippeleer, J.; Syryn, E.; Goiris, K.; De Cooman, L.; Aerts, G.: Flavour instability of pale lager beers: Determination of analytical markers in relation to sensory ageing, Journal of the Institute of Brewing, **114** (2008), pp. 180–192. https://doi.org/10.1002/j.2050-0416.2008.tb00324.x
4. Zufall, C.; Racioppi, G.; Gasparri, M.; Franquiz, J.: Flavour stability and aging characteristics of light-stable beers, Proceedings of the 30th European Brewing Convention Congress, Prague, Fachverlag Hans Carl, Nürnberg, Germany, Contribution **68**, 2005.
5. Dalgliesh, C. E.: Flavour stability [of beer], Proceedings of the 16th European Brewing Convention Congress, Amsterdam, DSW Dordrecht, the Netherlands, 1977.
6. Jaskula-Goiris, B.; De Causmaecker, B.; De Rouck, G.; De Cooman, L.; Aerts, G.: Detailed multivariate modeling of beer staling in commercial pale lagers, BrewingScience, **64** (2011), pp. 119–139.
7. Bamforth, C. W.: The science and understanding of the flavour stability of beer: a critical assessment, Brauwelt International, **17** (1999), pp. 98–111.
8. Bamforth, C. W.: A critical control point analysis for flavor stability of beer, Technical Quarterly of the Master Brewers Association of the Americas, **41** (2004), pp. 97–103.
9. Baert, J.; De Clippeleer, J.; Hughes, P. S.; De Cooman, L.; Aerts, G.: On the origin of free and bound staling aldehydes in beer, Journal of Agricultural and Food Chemistry, **60** (2012), pp. 11449–11472. https://doi.org/10.1021/jf303670z
10. Lehnhardt, F.; Gastl, M.; Becker, T.: Forced into aging: analytical prediction of the flavor-stability of lager beer – a review, Critical Reviews in Food Science and Nutrition, **59** (2019), pp. 2642–2653. https://doi.org/10.1080/10408398.2018.1462761
11. Filipowska, W.; Jaskula-Goiris, B.; Ditrych, M.; Bustillo Trueba, P.; De Rouck, G.; Aerts, G.; Powell, C.; Cook, D.; De Cooman, L.: On the contribution of malt quality and the malting process to the formation of beer staling aldehydes: A review, Journal of the Institute of Brewing, **127** (2021), pp. 107–126. https://doi.org/10.1002/jib.644
12. Mertens, T.; Kunz, T.; Gibson, B. R.: Transition metals in brewing and their role in wort and beer oxidative stability: a review, Journal of the Institute of Brewing, **128** (2022), pp. 77–95. https://doi.org/10.1002/jib.699
13. Ditrych, M.; Aerts, G.; Andersen, M. L.: Technological strategies for controlling aldehyde formation in beer: a review of brewing-related flavour instability, BrewingScience, **78** (2025), pp. 77-102. https://doi.org/10.23763/BrSc25-10ditrych
14. Čejka, P.; Čulík, J.; Horák, T.; Jurková, M.; Olšovská, J.: Use of chemical indicators of beer aging for ex-post checking of storage conditions and prediction of the sensory stability of beer, Journal of Agricultural and Food Chemistry, **61** (2013), pp. 12670–12675. https://doi.org/10.1021/jf403361h
15. Liu, J.; Li, Q.; Dong, J.; Chen, J.; Gu, G.: Multivariate modeling of aging in bottled lager beer by principal component analysis and multiple regression methods, Journal of Agricultural and Food Chemistry, **56** (2008), pp. 7106–7112. https://doi.org/10.1021/jf800879v
16. Ortiz, R. M.: Analysis of selected aldehydes in packaged beer by solid-phase microextraction (SPME)-gas chromatography (GC)-negative chemical ionization mass spectrometry (NCIMS), Journal of the American Society of Brewing Chemists, **73** (2015), pp. 266–274. https://doi.org/10.1094/ASBCJ-2015-0430-01
17. Ditrych, M.; Filipowska, W.; Soszka, A.; Buyse, J.; Hofmann, S.; Jensen, S.; Jaskula-Goiris, B.; De Rouck, G.; Aerts, G.; Andersen, M. L.; De Cooman, L.: Modelling of beer sensory staleness based on flavour instability parameters, Journal of the Institute of Brewing, **130** (2024), pp. 47–66. http://doi.org/10.58430/jib.v130i1.45
18. Wietstock, P. C.; Methner, F. J.: Formation of aldehydes by direct oxidative degradation of amino acids via hydroxyl and ethoxy radical attack in buffered model solutions, BrewingScience, **66** (2013), pp. 104–113.
19. Wietstock, P. C.; Kunz, T.; Methner, F. J.: Relevance of oxygen for the formation of Strecker aldehydes during beer production and storage, Journal of Agricultural and Food Chemistry, **64** (2016), pp. 8035–8044. https://doi.org/10.1021/acs.jafc.6b03502

20. Wietstock, P. C.; Baldus, M.; Oehlschlaeger, M.; Methner, F. J.: Hop constituents suppress the formation of 3-methylbutanal and 2-furfural in wort-like model solutions, Journal of the American Society of Brewing Chemists, **75** (2017), pp. 41–51. https://doi.org/10.1094/ASBCJ-2017-2001-01
21. Kaneda, H.; Osawa, T.; Kawakishi, S.; Munekata, M.; Koshino, S.: Contribution of carbonyl-bisulfite adducts to beer stability, Journal of Agricultural and Food Chemistry, **42** (1994), pp. 2428–2432. https://doi.org/10.1021/jf00047a012
22. Kaneda, H.; Takashio, M.; Osawa, T.; Kawakishi, S.; Koshino, S.; Tamaki, T.: Analysis of aldehyde-bisulfites in beer by HPLC fluorescence detection with post column derivatization, Journal of Food Science, **61** (1996), pp. 105–108. https://doi.org/10.1111/j.1365-2621.1996.tb14736.x
23. Bustillo Trueba, P.; Jaskula-Goiris, B.; Ditrych, M.; Filipowska, W.; De Brabanter, J.; De Rouck, G.; Aerts, G.; De Cooman, L.; De Clippeleer, J.: Monitoring the evolution of free and cysteinylated aldehydes from malt to fresh and forced aged beer, Food Research International, **140** (2021), 110049. https://doi.org/10.1016/j.foodres.2020.110049
24. Bustillo Trueba, P.: Beer Flavour Instability: Unravelling Formation and/or Release of Staling Aldehydes, PhD Thesis, KU Leuven, Leuven, Belgium, 2020.
25. Ditrych, M.; Filipowska, W.; De Rouck, G.; Jaskula-Goiris, B.; Aerts, G.; Andersen, M. L.; De Cooman, L.: Investigating the evolution of free staling aldehydes throughout the wort production process, BrewingScience, **72** (2019), pp. 10–17. https://doi.org/10.23763/BrSc18-21ditrych
26. Ditrych, M.; Mertens, T.; Filipowska, W.; Soszka, A.; Jaskula-Goiris, B.; De Rouck, G.; Andersen, M. L.: Effect of mashing-in pH on the biochemical composition and staling properties of the sweet wort, Journal of the American Society of Brewing Chemists, **82** (2024), no. 3, pp. 238–251. https://doi.org/10.1080/03610470.2024.2319928
27. De Clippeleer, J.; Van Opstaele, F.; Vercammen, J.; Francis, G. J.; De Cooman, L.; Aerts, G.: Real-time profiling of volatile malt aldehydes using selected ion flow tube mass spectrometry, LCGC North America, **28** (2010), pp. 386–395.
28. Dugulin, C. A.; Clegg, S. C.; De Rouck, G.; Cook, D. J.: Overcoming technical barriers to brewing with green (non-kilned) malt: A feasibility study, Journal of the Institute of Brewing, **126** (2020), pp. 24–34. https://doi.org/10.1002/jib.602
29. Filipowska, W.; Jaskula-Goiris, B.; Ditrych, M.; Schlich, J.; De Rouck, G.; Aerts, G.; De Cooman, L.: Determination of optimal sample preparation for aldehyde extraction from pale malts and their quantification via headspace solid-phase microextraction followed by gas chromatography and mass spectrometry, Journal of Chromatography A, **1612** (2020), article 460647. http://doi.org/10.1016/j.chroma.2019.460647
30. Filipowska, W.; Bolat, I.; De Rouck, G.; Bauwens, J.; Cook, D.; De Cooman, L.: Formation of staling aldehydes in different grain bed layers in an industrial scale maltings, Journal of the Institute of Brewing, **129** (2023), pp. 276–306. https://doi.org/10.58430/jib.v129i4.34
31. Dong, L.; Piao, Y.; Zhang, X.; Zhao, C.; Hou, Y.; Shi, Z.: Analysis of volatile compounds from a malting process using headspace solid-phase micro-extraction and GC-MS, Food Research International, **51** (2013), pp. 783–789. https://doi.org/10.1016/j.foodres.2013.01.052
32. Herrmann, M.; Gastl, M.; Thiele, F.; Back, W.: Malt volatile compounds (Part I), BrewingScience, **60** (2007), pp. 110–117.
33. Svoboda, Z.; Mikulíková, R.; Běláková, S.; Benešová, K.; Márová, I.; Nesvadba, Z.: Optimization of modern analytical SPME and SPDE methods for determination of trans-2-nonenal in barley, malt and beer, Chromatographia, **73** (2011), pp. 157–161. https://doi.org/10.1007/s10337-011-1958-x
34. De Schutter, D. P.; Saison, D.; Delvaux, F.; Derdelinckx, G.; Rock, J. M.; Neven, H.; Delvaux, F. R.: Release and evaporation of volatiles during boiling of unhopped wort, Journal of Agricultural and Food Chemistry, **56** (2008), pp. 5172–5180. https://doi.org/10.1021/jf800610x
35. De Clippeleer, J.: Flavour stability of pale lager beer. Chemical-analytical characterisation of critical factors related to wort production and hopping, PhD Thesis, KU Leuven, Leuven, Belgium, 2013.
36. Baert, J.: Unravelling the Role of Free and Bound-State Aldehydes in Beer Flavour Instability, PhD Thesis, KU Leuven, Leuven, Belgium, 2015.
37. Saison, D.; De Schutter, D. P.; Vanbeneden, N.; Daenen, L.; Delvaux, F.; Delvaux, F. R.: Decrease of aged beer aroma by the reducing activity of brewing yeast, Journal of Agricultural and Food Chemistry, **58** (2010), pp. 3107–3115. http://doi.org/10.1021/jf9037387
38. Gibson, B.; Aumala, V.; Heiniö, R. L.; Mikkelson, A.; Honkapää, K.: Differential evolution of Strecker and non-Strecker aldehydes during aging of pale and dark beers, Journal of Cereal Science, **83** (2018), pp. 130–138. https://doi.org/10.1016/j.jcs.2018.08.009
39. Äyräpää, T.: Formation of higher alcohols from 14C-labelled valine and leucine, Journal of the Institute of Brewing, **73** (1967), pp. 17–30. https://doi.org/10.1002/j.2050-0416.1967.tb03012.x
40. Äyräpää, T.: The formation of phenylethanol from 14C-labelled phenylalanine, Journal of the Institute of Brewing, **71** (1965), pp. 341–347. https://doi.org/10.1002/j.2050-0416.1965.tb02068.x
41. Äyräpää, T.: Formation of higher alcohols from amino acids derived from yeast proteins, Journal of the Institute of Brewing, **73** (1967), pp. 30–33. https://doi.org/10.1002/j.2050-0416.1967.tb03013.x
42. Äyräpää, T.: Formation of higher alcohols by various yeasts, Journal of the Institute of Brewing, **74** (1968), pp. 169–178. https://doi.org/10.1002/j.2050-0416.1968.tb03111.x
43. Dufour, J. P.: Influence of industrial brewing and fermentation working conditions on beer sulphur dioxide level and flavour stability, Proceedings of the 23rd European Brewing Convention Congress, Lisbon, IRL Press, Oxford, UK, 1991, pp. 209–216.
44. Debourg, A.; Laurent, M.; Goossens, E.; Borremans, E.; Vandewinkel, L.; Masschelein, C. A.: Wort aldehyde reduction potential in free and immobilized yeast systems, Journal of the American Society of Brewing Chemists, **52** (1994), pp. 100–106. https://doi.org/10.1094/ASBCJ-52-0100
45. Debourg, A.; Laurent, M.; Dupire, S.; Masschelein, C. A.: The specific role and interaction of yeast enzymatic systems in the removal of flavour-potent wort carbonyls during fermentation, Proceedings of the 24th European Brewing Convention Congress, Oslo, IRL Press, Oxford, UK, 1993, pp. 437–444.
46. Laurent, M.; Geldorf, B.; van Nedervelde, L.; Dupire, S.; Debourg, A.: Characterisation of the aldoketoreductase yeast enzymatic system involved in the removal of wort carbonyls during fermentation, Proceedings of the 25th European Brewing Convention Congress, Brussels, IRL Press, Oxford, UK, 1995.
47. Perpète, P.; Collin, S.: Fate of the worty flavours in a cold contact fermentation, Food Chemistry, **66** (1999), pp. 359–363. https://doi.org/10.1016/S0308-8146(99)00085-0

48. Sanchez, B.; Galindo-Castro, I.; Penttilä, M.; Rangel-Aldao, R.: Development of brewers' yeast with increased carbonyl reducing activity, Proceedings of the 28th European Brewing Convention Congress, Budapest, Fachverlag Hans Carl, Nürnberg, Germany, 2001, pp. 47, 1–9.
49. Vehkomäki, M. L.; Vidgren, V.; Vilpola, A.; Virtanen, H.; Sanchez, B.; Galindo-Castro, I.; Penttilä, M.; Rangel-Aldao, R.: Development of brewers' yeast with increased carbonyl reducing activity, Proceedings of the 28th European Brewing Convention Congress, Budapest, Fachverlag Hans Carl, Germany, 2001.
50. Suda, T.; Yasuda, Y.; Imai, T.; Ogawa, Y.: Mechanisms for the development of Strecker aldehydes during beer aging, Proceedings of the 31st European Brewing Convention Congress, Venice, Fachverlag Hans Carl, Nürnberg, Germany, pp. 103, 1–7, 2007.
51. Féchir, M.; Janssens, P.; Dorignac, E.; Montandon G.: Explorative Study on the Interactions Between Specialty Malts and Lager Yeast Strains: Impact on Beer Aroma and Volatile Composition, Journal of the American Society of Brewing Chemists, 84 (2026), pp. 50–64 https://doi.org/10.1080/03610470.2025.2583823
52. Perpète, P.; Collin, S.: Evidence of Strecker aldehyde excretion by yeast in cold contact fermentations, Journal of Agricultural and Food Chemistry, **48** (2000), pp. 2384–2386. https://doi.org/10.1021/jf000071h
53. Perpète, P.; Collin, S.: How to improve the enzymatic worty flavour reduction in a cold contact fermentation, Food Chemistry, **70** (2000), pp. 457–462. https://doi.org/10.1016/S0308-8146(00)00111-4
54. Baert, J.; De Clippeleer, J.; De Cooman, L.; Aerts, G.: Exploring the binding behavior of beer staling aldehydes in model systems, Journal of the American Society of Brewing Chemists, **73** (2015), pp. 100–108. https://doi.org/10.1094/ASBCJ-2015-0109-01
55. Baert, J.; De Clippeleer, J.; Jaskula-Goiris, B.; Van Opstaele, F.; De Rouck, G.; Aerts, G.; De Cooman, L.: Further elucidation of beer flavor instability: the potential role of cysteine-bound aldehydes, Journal of the American Society of Brewing Chemists, **73** (2015), pp. 243–252. https://doi.org/10.1094/ASBCJ-2015-0531-01
56. Bustillo Trueba, P.; De Clippeleer, J.; Van Der Eycken, E.; Guevara Romero, J. S.; De Rouck, G.; Aerts, G.; De Cooman, L.: Influence of pH on the stability of 2-substituted 1,3-thiazolidine-4-carboxylic acids in model solutions, Journal of the American Society of Brewing Chemists, **76** (2019), pp. 272–280. https://doi.org/10.1080/03610470.2018.1546094
57. Maia, C.; Cook, D.: Binding of Staling Aldehydes to the Beer Matrix: Insights into the Equilibria Through Shelf-Life Which Drive Beer Flavor Instability, Journal of the American Society of Brewing Chemists, **84** (2026), pp. 96–107. https://doi.org/10.1080/03610470.2025.2585548
58. Nobis, A.; Kwasnicki, M.; Lehnhardt, F.; Hellwig, M.; Henle, T.; Becker, T.; Gastl, M.: A comprehensive evaluation of flavor instability of beer (part 2): The influence of de novo formation of aging aldehydes, Foods, **10** (2021), article 2668. https://doi.org/10.3390/foods10112668
59. Nobis, A.; Lehnhardt, F.; Gebauer, M.; Becker, T.; Gastl, M.: The influence of proteolytic malt modification on the aging potential of final wort, Foods, **10** (2021), article 2320. https://doi.org/10.3390/foods10102320
60. Lehnhardt, F.; Nobis, A.; Skornia, A.; Becker, T.; Gastl, M.: A comprehensive evaluation of flavor instability of beer (part 1): Influence of release of bound state aldehydes, Foods, **10** (2021), article 2432. https://doi.org/10.3390/foods10102432
61. Lehnhardt F.; Schneiderbanger H.; Messner C.; Gastl M.: Impact of Brew House Parameters on Beer Aging: Mapping the German Brewery Landscape. J. Am. Soc. Brew. Chem. **84** (1) (2026), 84–95. https://doi.org/10.1080/03610470.2025.2524658

Received 4 May 2026, accepted 17 June 2026