Research article

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# Formation and degradation of 3-deoxyglucosone as a key intermediate for ageing indicators during wort boiling

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**The metabolite 3-deoxyglucosone (3-DG) is formed by carbohydrate caramelisation or the Maillard reaction. 3-DG is a precursor in the Strecker reaction forming beer ageing compounds, such as 2-methylbutanal or 3-methylbutanal. Although 3-DG is known as intermediate, recent studies have focused on 3-DG in beer. Foremost, the thermal load during wort boiling provides the best conditions for 3-DG formation and degradation, however, the reactivity of the dicarbonyl during the boiling process has not yet been explained. As a key intermediate, 3-deoxyglucosone could be a critical indicator for beer ageing stability. The 3-DG formation and reactivity during wort production depends on its precursor reactants (amino acids and glucose). The concentration in wort of these substances was varied using two malts with different malt modification along with two different mashing programmes. 3-Deoxyglucosone reactivity was observed by analysing dehydratisation to HMF (HPLC-UV), interconversion to 3-deoxygalactosone (3-DGal, HPLC-UV) and selected Strecker aldehydes (GC-SPME-MS). This study shows that wort boiling is the most important process in 3-DG formation as it contributes 47% of the final content compared with malting (28%) and mashing (25%). With degradation reactions, 3-DG is mainly interconverted to 3-DGal and, contrary to the literature, it could not be confirmed that enhanced 3-deoxyglucosone content affects Strecker reactions. The interconversion reaction during wort boiling determines the dicarbonyl potential of beer and influences the ageing stability. © 2021 The Authors. *Journal of the Institute of Brewing* published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling.**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Keywords:** 3-deoxyglucosone; wort boiling; 3-deoxygalactosone; Maillard reaction; brewing

## Introduction

Wort boiling is a key process step in beer production. The thermal load provides the best conditions for forming precursor substances. Typical compounds are dicarbonyls, which form aroma active substances in several chemical reactions. Specifically, the high content of low molecular sugars (glucose or fructose as precursor reactants) induces the best conditions for the Maillard reaction *(1)*. These carbohydrates can react through the Maillard reaction or caramelisation in enolisation and dehydration reactions to α-dicarbonyl compounds *(2, 3)*, with 3-deoxyglucosone (3-DG) the major compound in beer and wort *(4–6)*. Figure 1 summarises possible formation and degradation reactions of 3-DG during the wort boiling process.

Low molecular sugars such as glucose (Glc) can react with amino acids to form Amadori rearrangement products such as fructosyllysine (FL) that are precursor compounds for 3-DG or other dicarbonyl compounds *(2)*. After formation, 3-DG becomes a reactive compound and can be degraded in several consecutive reactions. Well known pathways include dehydration through formation of 3,4-dideoxyglucosone (3,4-DGE) to 5-(hydroxymethyl) furfural (HMF) *(5)*, the formation of Strecker aldehydes *(2, 7)* and the interconversion of 3-deoxyglucosone to 3-deoxygalactosone (3-DGal) *(4, 5)*. 3-DGal can react similarly to 3-DG *(5, 8)*. Beside these reactions, 3-DG can react in several pathways within the Maillard reaction, including the formation of melanoidins *(9)* or pyrraline by reacting with lysine *(10)*.

Regarding the formation of aroma active compounds, the Strecker degradation of amino acids is a dominant pathway that forms important aroma active compounds. Typical beer ageing flavours result from degrading valine (forming 2-methylpropanal), leucine (3-methylbutanal), isoleucine (2-methylbutanal) and phenylalanine (phenyl acetaldehyde) *(11)*. The free formed aldehydes during wort production cannot be transferred directly to beer, as yeast reduces them during fermentation. However, there are several masking reactions where aldehydes are first bound and then released in the final beer during the ageing process *(12)*. Possible reactions are the formation of bisulphite adducts *(13)*, imine adducts *(14)* or 2-substituted thiazolidine-4-carboxylic acids (cysteine adducts) *(15)*. It is reported that 85% of Strecker aldehydes formed during mashing and boiling are transferred to beer through masked reactions *(16)*. Therefore, the formation of ageing indicators in wort boiling are significant for final beer quality and sensory stability.

Until now, the reactivity of 3-DG to Strecker degradation has been described in several model reactions using single amino acids and dicarbonyl compounds *(17–22)*. Regarding the Strecker r-

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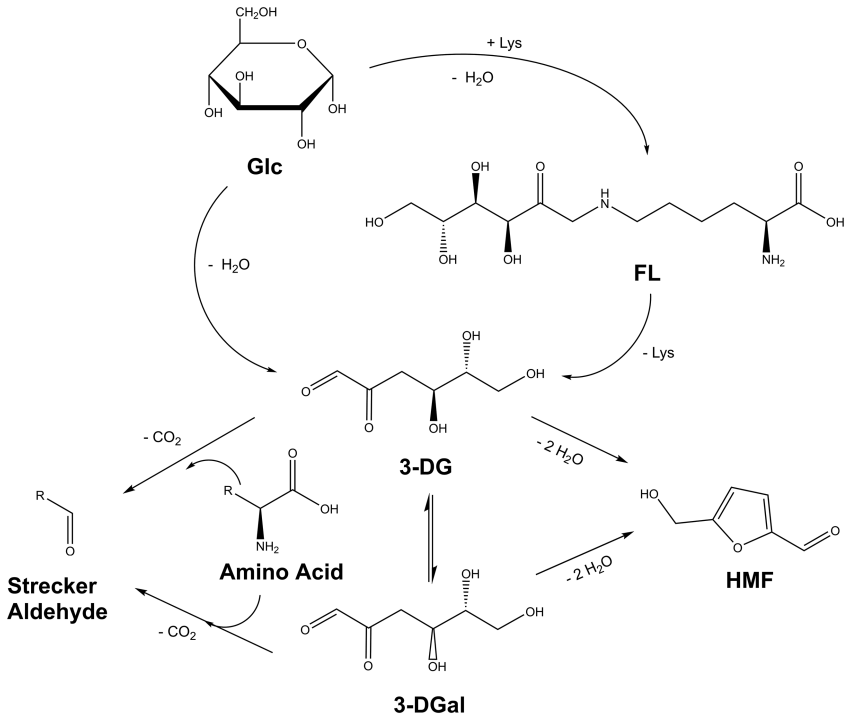


**Figure 1**. 3-DG metabolism during wort boiling

eactions, dicarbonyl is described as a reactive precursor compound *(22, 23)*. Within these experiments it was shown that small cleavage products of 3-DG, such as methylglyoxal or glyoxal, have an even higher reactivity towards Strecker degradation *(24, 25)*. Akagawa et al. showed the reaction to accelerate at higher pH values and at higher oxygen levels in model reactions *(18)*. Although the dicarbonyl potential for reacting in Strecker degradation exists *(6)*, until now, the 3-DG reactivity is described only in model reactions and not in complex food matrices *(22)*.

In beer production, there is limited insight into 3-deoxyglucosone formation. In the malting process a high amount of 3-DG (3.5–6.6 mg/100 g dry weight) is formed *(5, 26)*. Bravo et al. reported the concentration of 3-DG *(6)* during mashing (13 mg/L) and wort boiling (29 mg/L). During the boiling process, it was shown that the 3-DG content increased rapidly. The occurrence of the interconverted 3-DGal was first described by Bravo et al. *(6)*, but the interconversion of 3-DG and 3-DGal has not been reported in wort production. Recent studies have focused on beer with 3-deoxyglucosone a the major dicarbonyl compound, quantified in a range of 18–54 mg/L *(4–6)*. However, the formation and degradation of 3-DG as a key intermediate in wort production has not been investigated.

In this study, the hypothesis is that a varied initial 3-DG content and the potential of available reactants (amino acids, sugars) caused by different malt modification levels and intensity of mashing procedures influences the concentration of the dicarbonyl at the end of the wort boiling and subsequent reactions during the process. The reactivity of 3-DG could affect the final beer ageing stability in two ways. Firstly, the content of 3-deoxyglucosone and 3-deoxygalactosone provides the dicarbonyl potential in beer and induces ageing by improving the formation of beer ageing compounds. Secondly, the Strecker aldehydes exist in masked form in wort and beer and are released in the final beer, consequently accelerating beer ageing. Therefore, the aim of the study was to investigate the formation and selected degradation reactions of 3-DG as a key intermediate for ageing indicators during the wort boiling process by varying the initial content of 3-DG and its precursor reactants.

## Material and methods

### Chemicals

5-Hydroxymethylfurfural (HMF), all L-amino acids, [13C, 15N] labelled amino acids, L-lysin-6-13C-dihydro chloride, potassium dihydrogen phosphate, methanol (LC-MS-grade), o-phenylenediamine (OPD), calcium chloride dihydrate, glucose ammonium acetate, maltose, fructose, sucrose, hydrochloric acid, 4-fluorobenzaldehyde, citric acid, disodium hydrogen phosphate dihydrate, 3-methylcrotonaldehyde, acetic acid, magnesium sulphate, 2-methylbutyraldehyde (2MB), 3-methylbutyraldehyde (3MB), isobutyraldehyde (2MP), pheylacetaldehyde (PA), and water (LC-MS-grade) were obtained from Merck (Darmstadt, Germany). Acetonitrile for liquid chromatography mass spectrometry (LC-MS) analysis was purchased from VWR (Darmstadt, Germany). 3-DG (>95%) was from Apollo Scientific Ltd. (Cheshire, UK). 3-deoxygalactosone (>90%) was obtained from Carbosynth Ltd. (Berkshire, UK). The Chair of Food Chemistry of TU Dresden (Dresden, Germany) provided $N^{\varepsilon}$-fructosyllysine (79.6%). Before use, water for analysis was purified using a micropore water purification system (Thermo Fisher Scientific Inc., Waltham, USA). Distilled water was used for the mashing trials.

### Wort production and boiling

According to Nobis et al. *(26)*, two malts were produced by high (M1) and low (M2) proteolytic modification levels (assessed as soluble nitrogen) to vary the initial concentration of precursor



  

reactants of 3-DG. The desired specification of soluble nitrogen for brewing of barley malts is 650–750 mg/100 g dry weight (according to Congress mashing procedure) *(27)*. The observed levels were 733 mg/100 g dry weight (M1) and 684 mg/100 g dry weight (M2) analysed using MEBAK (R-205.11.030 [2016-03]) *(28)*. The definition of high (M1 – upper limit of specification) and low (M2 - lower limit of specification) malt modification level was as the required specification and is not comparable to the previous study by Nobis et al. *(26)*. To influence the enzymatic hydrolysis during mashing (substrate/wort production), each malt was further mashed using two different laboratory mashing procedures ('45' at 45°C and '63' at 63°C). Laboratory procedure 45 impacts the activity of proteolytic and cytolytic enzymes resulting in higher concentration of low molecular proteolytic reactants, mainly amino acids. Procedure 63 focusses on amylolytic enzymes and is the reference procedure. Malt (50 g) was ground with a DLFU disk mill from Bühler (Braunschweig, Germany) at a disk gap of 0.2 mm. Afterwards, 200 mL of tempered water was added and the suspension was mashed using procedure 45 (20 min: 45°C; 10 min: 45°C–63°C; 30 min: 63°C; 6 min: 63°C–73°C; 30 min: 73°C; 5 min: 73°C–78°C; 2 min: 78°C) and procedure 63 (30 min: 63°C; 6 min: 63°C–73°C; 30 min: 73°C; 5 min: 73°C–78°C; 2 min: 78°C). Table 1 presents an overview of the variations produced in the study. Subsequently, all mashes were filtered through a laboratory folded paper filter (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). After filtration, the laboratory worts were adjusted to 11.5% mass with water according to the wort extract content of pale lager beer. Extract content was measured according to MEBAK guidelines method 2.9.6.2 *(29)*. In all, four laboratory worts with different (assumed) levels of reactants (45M1 > 45M2 > 63M1 > 63M2) were produced.

For the boiling trials, each variation (6 x 8 mL) was boiled in a water bath for 60 min in 15 mL sealed glass tubes with screw caps. Individual tubes were sampled every 10 min and the tubes immediately cooled down with water. For each analysis 0.5 mL (amino acids, dicarbonyls, HMF, sugars, FL) and 5 mL (Strecker aldehydes) were sampled and frozen. Transfer procedures of all wort samples were standardised. Each wort was incubated with and without spiking of 16 mg/L of 3-DG to investigate the influence of increased 3-DG content before boiling. All experiments were performed in triplicate.

### Quantitation of Strecker aldehydes

Selected Strecker aldehydes (2MP, 2MB, 3MB, and PA) were determined using gas chromatography headspace solid phase microextraction mass spectrometry (GC-HS-SPME-MS) technique based on Saison et al. *(30)* as published by Lehnhardt et al. *(31)*. Methylcrotonaldehyde (2MP, 2MB and 3MB) and 4-fluorobenzaldehyde (PA) were used as internal standards. The calibration range of all Strecker aldehydes was 1–100 µg/L. Wort sample (1 mL) was mixed with 0.15 mL internal standard and 3.85 mL phosphate buffer (pH 5.5, 50 mM), placed in a 20 mL headspace vial and incubated at 40°C. The fibre was injected splitless at 270°C. The GC-system included Trace 1300 GC, TriPlus RSH autosampler and ISD QD mass spectrometer (Thermo Fisher Scientific Inc. Waltham, USA).

### Quantitation of 3-deoxyglucosone (3-DG) and 3-deoxygalactosone (3-DGal)

Derivatisation and HPLC conditions were as previously performed by Degen et al. *(4)* and Nobis et al. *(26)*. A Kinetex column (2.6 µm phenyl-hexyl, 100 Å, 150 x 2,1 mm) was used. Solvent A was 0.075% acetic acid in water and solvent B was 20% solvent A in methanol. The gradient mode was as follows - 0 min, 90% A; 15 min, 55% A; 16 min, 25% A; 19 min, 25% A; 19.5 min, 90% A; 28 min, 90% A. The flow rate was 0.2 mL/min, the column oven temperature was 30°C, the injection volume was 5 µL and detection wavelength was 312 nm. Wort samples were measured directly after derivatisation and membrane filtration (0.45 µm).

### Quantitation of 5-hydroxymethylfurfural

5-Hydroxymethylfurfural (HMF) was quantified by HPLC analytics according to the method published by Rufian-Henares et al. *(32)*. A Kinetex column (2.6 µm C18, 100 Å, 150 x 2,1 mm) was used. Solvent A was 2% acetonitrile in water and solvent B was methanol. The gradient mode was - 0 min, 100% A; 7 min, 100% A; 7.5 min, 20% A; 10.5 min, 20% A; 11 min, 100% A; 16 min, 100% A. The flow rate was 0.2 mL/min, the column oven temperature was 32°C, the injection volume was 15 µL and detection wavelength 280 nm. The HPLC system consisted of the UltiMate 3000 Autosampler, an UltiMate 3000 pump module, an UltiMate 3000 column compartment and the UltiMate 3000 Diode Array Detector (Thermo Fisher Scientific Inc. Waltham, USA). Data evaluation was performed by Chromeleon 6.80 Software from Thermo Fisher Scientific Inc.

### Quantitation of low molecular weight sugars

Fermentable sugars (glucose, fructose, maltose, and sucrose) were determined by high-performance anion exchange chromatography pulsed amperometric detection (HPAEC-PAD). Eluent A (250 mM NaOH) and eluent B (water) were mixed in gradient mode (0 min, 90% B; 20 min, 90% B; 21 min, 20% B; 45 min, 20% B;

**Table 1.** Overview of laboratory mashes

| Variation | Malt modification level | Mashing procedure |
|---|---|---|
| 45M1 | low malt modification level (M1: 684 mg/100 g dry weight soluble nitrogen concentration) | additional rest at 45°C to increase amino acid formation |
| 45M2 | high malt modification level (M2: 733 mg/100 g dry weight soluble nitrogen concentration) | additional rest at 45°C to increase amino acid formation |
| 63M1 | low malt modification level (M1: 684 mg/100 g dry weight soluble nitrogen concentration) | reference procedure |
| 63M2 | high malt modification level (M2: 733 mg/100 g dry weight soluble nitrogen concentration) | reference procedure |



  

46 min, 5% B; 48 min, 5% B; 49 min, 90% B; 60 min, 90% B) at a flow rate of 0.25 mL/min. A Dionex CarboPack PA10 analytical column (2 x 250 mm) and Dionex CarboPack PA10 guard column (2 x 50 mm) were used (Thermo Fisher Scientific Inc. Waltham, USA). The HPAEC system consisted of an ICS AS/AP autosampler, an ICS 5000 DP pump module, an ICS 5000 DC column compartment, and an ICS 5000 PAD detector (Thermo Fisher Scientific Inc. Waltham, USA).

### Quantitation of amino acids

Nineteen amino acids were determined according to Sonntag et al. *(33)* by high-performance liquid chromatography tandem mass spectrometry (HPLC MS/MS) in the multiple reaction monitoring (MRM) mode and as reported by Nobis et al. *(26)*.

### Quantitation of fructosyllysine

Determination of fructosyllysine (FL) was performed as reported by Nobis et al. *(26)*. The HPLC Agilent 1200 series system (Agilent, Waldbronn) consisted of a HiP-ALS SL autosampler, a 1200 series bin pump module, a 1200 series degasser and a 1100 series column oven coupled to a Triple Quad 4500 MS (SCIEX, Darmstadt). The ion spray voltage was set to 5500 V, the curtain gas pressure was set to 35 psi, the nebuliser gas pressure was 55 psi and the heater gas pressure was 65 psi. The turbogas temperature was set to 450°C. The analysis was conducted using 13C-labelled lysine as an internal standard.

### Model experiments with 3-deoxyglucosone

Investigations of 3-DG degradation and interconversion were performed by model incubations (2 mL) with phosphate buffer (50 mM) at different pH values (5.0, 5.2, 5.4, 5.6, 5.8 and 6.0). The first experiment was performed by varying the initial concentration of 3-DG at levels of 10, 20, 30, 40, 50 and 60 mg/L at pH value 5.6, which represents a typical wort pH. The second experiment was performed at different pH values (5.0, 5.2, 5.4, 5.6, 5.8 and 6.0) with an initial concentration of 30 mg/L 3-DG. Both variations were further conducted in the presence and absence of glycine at a concentration of 14 mg/L to evaluate the influence of the Maillard reaction. All samples were boiled for 60 min in closed systems (plastic tubes) and analysed in triplicate.

### Statistical analysis

Statistical analysis was performed using the software JMP® Pro 12 (SAS Institute GmBH, Heidelberg, Germany).

## Results and discussion

### Sugars and amino acids in the wort boiling process

The levels of low molecular sugars and amino acids in the worts (45M1 > 45M2 > 63M1 > 63M2) were investigated. Notably as glucose is a well known precursor of 3-deoxyglucosone *(2)*, with all variations there was no significant degradation of glucose during wort boiling. Nevertheless, formation of 3-DG by glucose degradation could take place as formation and degradation of glucose occur simultaneously. The malt modification levels, and mashing procedures slightly affected the resulting glucose concentration in wort. Mashing procedure 45 showed an initial glucose concentration of 5.8 ± 0.3 g/L for both malt modification levels. Mashing procedure 63 resulted in glucose concentrations of 6.3 ± 0.7 g/L (M1) and 4.6 ± 0.2 g/L (M2). In this approach a higher malt modification resulted in a higher initial glucose concentration (precursor reactant) in wort at the beginning of wort boiling. The more intensive malting procedure supported the amylolytic enzymatic activity *(34)*, thereby inducing an accelerated starch degradation during the mashing.

The second group of potential precursors for 3-DG formation in the Maillard reaction are amino acids, which were analysed during the boiling process. Table 2 shows the difference between the start and end of the boiling process of amino acids in all experimental approaches (45M1, 45M2, 63M1 and 63M2). Differences in amino acids indicate their reactivity during wort boiling. Additionally, 3-DG was spiked (16 mg/l) with each variation to investigate the influence of an increased concentration of the dicarbonyl on amino acid reactivity. Leucine (Leu), isoleucince (Ile), valine (Val), and phenylalanine (Phe) are common precursor compounds for Strecker reactions. Lysine is also shown as it was the only amino acid that was degraded constantly with all variations and is known as a key amino acid in the Maillard reaction *(2)*. The concentration of all measured amino acids and glucose during wort boiling are reported in the Supporting Information.

Comparing the initial level of amino acids (45M1: 2372 mg/L; 45M2: 3278 mg/L; 63M1: 2102 mg/L; 63M2: 1839 mg/L), it was confirmed that the mashing procedure which included a 45°C step increased the free amino acids. The enhanced proteolytic

**Table 2.** Changes (60 – 0 minutes) during wort boiling in selected amino acids, 3-DGal and HMF

| Mashing procedure | Malt modification | 3-DG addition (mg/L) | Δ leucine (mg/L) | Δ isoleucine (mg/L) | Δ valine (mg/L) | Δ phenylalanine (mg/L) | Δ lysine (mg/L) | Δ ∑ amino acids (mg/L) | Δ 3-DGal (mg/L) | Δ HMF (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 45 | M1 | 0 | 63.7 | 58.2 | 39.8 | 42.7 | -113.8 | 416 | 4.1 | 0.3 |
| | | 16 | 39.8 | 48.7 | 26.2 | 27.8 | -131.4 | 364 | 4.7 | 0.4 |
| | M2 | 0 | 16.5 | 28.3 | 10.7 | 14.7 | -203.1 | -177 | 2.7 | 0.2 |
| | | 16 | -6.3 | 26.8 | 2.7 | -1.5 | -206.7 | -799 | 3.4 | 0.3 |
| 63 | M1 | 0 | -14.7 | 10.5 | 7.0 | 7.1 | -177.3 | -287 | 3.1 | 0.3 |
| | | 16 | 0.9 | 5.4 | 2.6 | 3.8 | -182.3 | -354 | 3.6 | 0.4 |
| | M2 | 0 | -11.2 | -11.8 | -7.9 | -7.8 | -154.8 | -294 | 2.0 | 0.2 |
| | | 16 | -4.5 | -0.4 | -7.7 | -7.9 | -153.8 | -384 | 2.5 | 0.2 |

enzyme activity at 45°C results in higher amounts of the reactants *(35)*. Regarding the effect of malt modification (assessed by soluble nitrogen), there was no consistent trend in the sum of amino acids in the initial wort. The higher malt modification level (M1) has a lower initial sum of amino acids than M2 at the mashing procedure with enhanced proteolytic activity (procedure 45) and this effect is reversed at the reference mashing procedure (procedure 63). Generally, higher malt modification induces higher soluble nitrogen *(36)*, peptide *(37)* or amino acid content *(26)* in malt. Therefore, presumably 45M2 has a higher content of peptides in malt that are degraded during mashing, resulting in a higher initial content of amino acids during wort boiling. 45M1 has a higher amount of amino acids in malt *(26)* which are more greatly degraded during mashing and therefore reduced at the beginning of boiling.

Besides 45M1, all variations showed a decrease in total amino acids during wort boiling. Presumably, the increase in 45M1 is caused by higher levels of Amadori products (FL in Figure 2), which are degraded and then released as amino acids by dicarbonyl formation *(2)* during wort boiling. The 3-DG spiked samples demonstrated a trend for the inhibited formation or enhanced degradation of amino acids (Supporting Information and Table 2). This overall effect indicates enhanced Strecker degradation *(7)* or alternative Maillard reaction pathways, such as the formation of advanced glycation end products by a higher initial 3-DG concentration.

As important precursor substances and reaction partners, amino acids such as lysine were extensively degraded in all variations. This was slightly increased by 3-deoxyglucosone addition and could be caused by pyrraline formation *(2)*. Selected precursors (leucine, isoleucine, valine and phenylalanine) for known Strecker aldehydes showed no significant degradation in type 45 mashing samples. 3-DG addition partly affected the formation of the amino acids during wort boiling by decreasing formation. Only variation 63M2 showed a consistent degradation of leucine, isoleucine, valine and phenylalanine during wort boiling. Generally, there was no indication of the influence of 3-DG towards Strecker degradation of the selected amino acids. Although the amino acids (Table 2) showed a trend in formation in most experiments, Strecker reactions occur as formation and degradation proceed simultaneously.

## Formation of fructosyllysine and 3-deoxyglucosone during wort boiling

Following the reaction order, fructosyllysine was investigated as a direct precursor of 3-DG. In a complex wort matrix, caramelisation or the Maillard reaction does not only occur in wort boiling as it is already initiated in malting *(26)* and during mashing. Therefore, the initial concentration of the compounds varies during wort boiling due to malt modification and mashing. The formation and degradation of FL and 3-DG during wort boiling indicate the progress of the Maillard reaction and are directly linked to the 3-deoxyglucosone concentration in final wort. Figure 2 shows the concentration of FL and 3-DG during wort boiling. FL is mainly degraded, and 3-DG is formed in all variations without spiking (45M1, 45M2, 63M1, and 63M2). Recently it was shown that both compounds are formed simultaneously and the Maillard reaction is initiated in malt production *(5)*. Therefore, as demonstrated, the Maillard reaction goes further, focusing on the formation of dicarbonyls *(38)*.

The mashing procedure with the additional step of 45°C rest induces a higher FL initial concentration. Assumably, the enhanced lysine content reacts more strongly supported by a longer mashing procedure (and more reaction time) to FL. A higher malt modification level also results in a higher initial FL concentration. A more intensive malting process leads to higher contents of the Amadori compound in malt *(26)* and causes an increase in the initial FL concentration in wort. By including the recently published concentration of FL in the same malts *(26)*, it can be calculated that 37% at M1 and 50% at M2 of initial FL is already accounted from

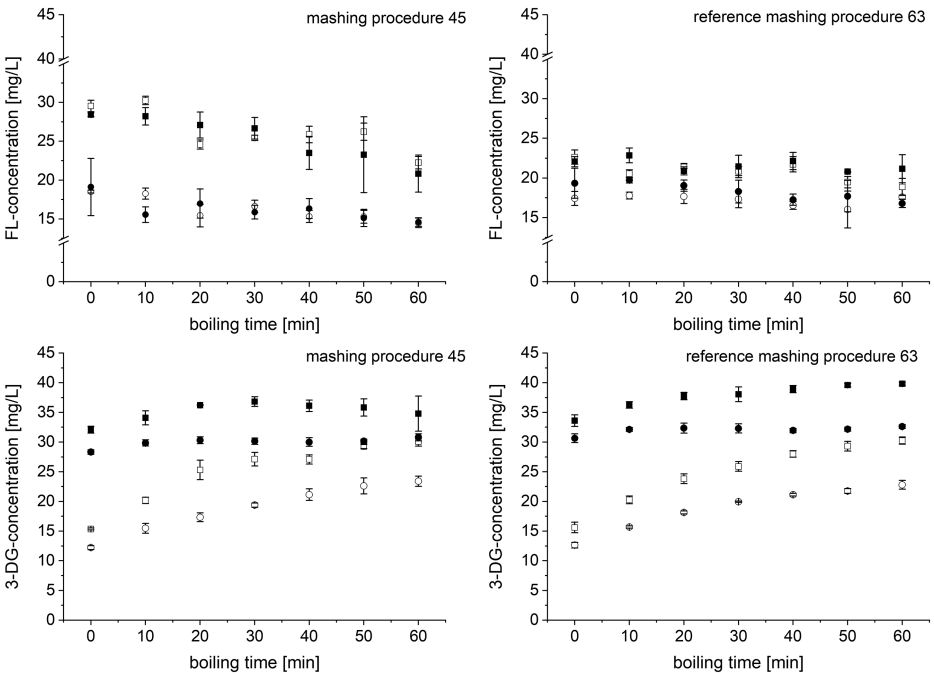


**Figure 2.** Concentration of 3-DG and FL during wort boiling at a high (M1, □) and low (M2, ○) malt modification level; white: without 3-DG; black: with 16 mg/L 3-DG; n = 3



  

the malt. Therefore, the FL formation during mashing could be low, through degradation during mashing. During boiling, FL is degraded in the following order: 45M1 > 45M2 ≥ 63M1 > 63M2. Accordingly, the increased concentration of the reactants (lysine and glucose) form higher amounts of FL during mashing, which is subsequently degraded during boiling. 3-DG spiking has no influence on FL degradation.

Fructosyllysine degradation results in the formation of 3-deoxyglucosone (Figure 2). The course of the curve of 3-DG concentration is comparable at all variations without spiking. Initially, the dicarbonyl is strongly formed for up to 20 minutes, but the rate of formation decreases during boiling. The relative formation of 3-DG is approximately 90% and independent of the variations (45M1, 45M2, 63M1 and 63M2). Unexpectedly, the results indicate that the mashing procedure does not affect the initial 3-DG concentration. In contrast, the level of malt modification shows a higher influence on the initial concentration of 3-deoxyglucosone. A greater modification level increases the initial 3-DG content, probably caused by a higher potential of the dicarbonyl due to the more intensive malting procedure *(26)*.

The 3-DG potential is the sum of the compound in malt, its precursor reactants (sugars and amino acids) and the enzyme potential for reactant formation during mashing. By evaluating the impact of the consecutive steps of wort production and comparing with the concentration of the same malt *(26)*, it could be calculated that independent of the variation, 28% of final 3-DG was formed during malting, 25% during mashing and 47% during wort boiling. Therefore, wort boiling mostly influences 3-DG formation in wort. The level of malt modification influences only the absolute content of 3-DG. Final concentrations are 30.0 ± 0.7 mg/L at 45M1 and 23.4 ± 0.8 mg/L at 45M2. These concentrations are comparable to Bravo et al. with 29.2 mg/L of 3-deoxyglucosone in the final wort *(6)*. Varying the initial concentration of 3-DG, the relative formation of the dicarbonyl decreased rapidly to 10%. The course stays more linear. Therefore, presumably the 3-DG degradation is catalysed in spiked samples, indicating that the Maillard reaction goes further to the final stage.

### Degradation of 3-deoxyglucosone

The first reaction in the interconversion of 3-deoxyglucosone, where 3-deoxygalactosone is formed by dehydration and rehydration *(8)*. The concentration of 3-DGal at all sampling points is shown at Supplementary Information. The dicarbonyl compound is formed linearly in all variations. The mashing procedure with the additional rest at 45°C results in a higher initial concentration (45M1: 0.67 ± 0.02 mg/L; 45M2: 0.67 ± 0.05 mg/L) than the reference procedure (63M1: 0.50 ± 0.10 mg/L; 63M2: 0.34 ± 0.04 mg/L). Therefore, the interconversion occurs during mashing and is accelerated by a more intensive thermal load by an additional rest in the mashing procedure. At the end of wort boiling, the concentration of 3-DGal is the following order: 45M1 (4.72 ± 0.21 mg/L) > 65M1 (3.61 ± 0.31 mg/L) > 45M2 (3.39 ± 0.15 mg/L) > 65M2 (2.37 ± 0.12 mg/L). The absolute formation of 3-DGal is enhanced by the increased initial concentration of 3-DG at the beginning of wort boiling (Figure 2). Wort boiling results in approximately 85% of the 3-DGal content in the final wort. Samples spiked with 3-DG show a higher rate of formation during boiling (Table 2) and a higher final content of 3-DGal (Supplementary Information). The concentration of 3-deoxygalactosone was enhanced by 13% at the more intensive malt modification level and surprisingly by 20% in the M2 variation. Presumably, 3-DG has more reaction partners in M1 (e.g., amino acids) and other pathways are also stimulated. Generally, it could be shown that the enhanced initial concentration of 3-DG accelerates 3-DGal formation although only a minor amount of added 3-deoxyglucosone (about 4%) contributes through interconversion to 3-deoxygalactosone. The calculation of interconversion rate was based on the difference of the increased 3-DGal formation in spiked and non-spiked samples (-Supplementary Information). Presumably, spiked 3-DG is partly stable, forming the intermediate 3,4-dideoxyglucosone-3-ene (3,4-DGE) *(5)* or reacts in other pathways such as the formation of HMF.

The second degradation reaction was the dehydration of 3-DG to HMF. The concentration of HMF at each sampling point is shown in the Supplementary Information. The thermal load of wort boiling forms HMF constantly at all variations between 0.33 and 0.51 mg/L. These levels are comparable to reported values where HMF was quantified up to 1 mg/L *(39)*. The mashing procedure and malt modification show no influence on the final content of HMF. Except for 63M2, the final HMF concentration is increased by adding 3-DG (Table 2), suggesting that the dicarbonyl partly reacts to HMF during wort boiling. The relative increase varies between 27–48%, but the absolute increase is surprisingly low at 0.09 mg/L, reflecting a conversion rate of 0.7% of spiked 3-DG. Regarding the absolute formation, the interconversion of 3-DG to 3-DGal is more important than its degradation to HMF.

The third pathway to be investigated was the formation of Strecker aldehydes. The concentration of all Strecker aldehydes are reported in the Supplementary Information and Figure 3 shows the final concentration of 2MB, 2MP, 3MB and PA. Strecker aldehydes are formed during boiling. Contrary to De Schutter et al. *(40)*, the formation is non-linear, as in our study a temporary maxima appear during wort boiling. Surprisingly, the mashing procedure showed no significant influence on the formation of Strecker aldehydes, although the amino acid concentration rapidly increased with the additional 45°C mashing step. Therefore, some of the aldehydes are already bound before and released during wort boiling. Specifically, kilning provides the best conditions for aldehyde formation and masking reactions such as imine or 2-substituted thiazolidine-4-carboxylic acid formation**.** It can be assumed, that an increased malt modification level enhances the final concentration of Strecker aldehydes. Here, the initial concentrations of quantified aldehydes are already increased (-Supplementary Information). It is proposed that the level of malt modification enhances the overall potential for Strecker aldehyde formation. Although Hofmann et al. in model studies suggested 3-DG as a precursor compound in Strecker reactions *(41)*, spiked samples showed no significant increase in the final content of Strecker aldehydes in all variations. Therefore, 3-DG is either partly stable under wort boiling conditions or undergoes further consecutive reactions such as the formation of advanced glycation end products, fragmentation to methylglyoxal or protein modification *(2)*. Further Strecker aldehydes can be formed and be partly bound during wort boiling.

### Interconversion of 3-deoxyglucosone in model solution

The interconversion of 3-DGal was the most critical reaction of the observed degradation reactions. Additionally, the wort boiling results indicate stability of 3-deoxyglucosone during wort boiling. Therefore, the interconversion of 3-DG in a wort model system was investigated by varying the pH value and the initial concentration of 3-DG with and without glycine. A variation in pH over the



  

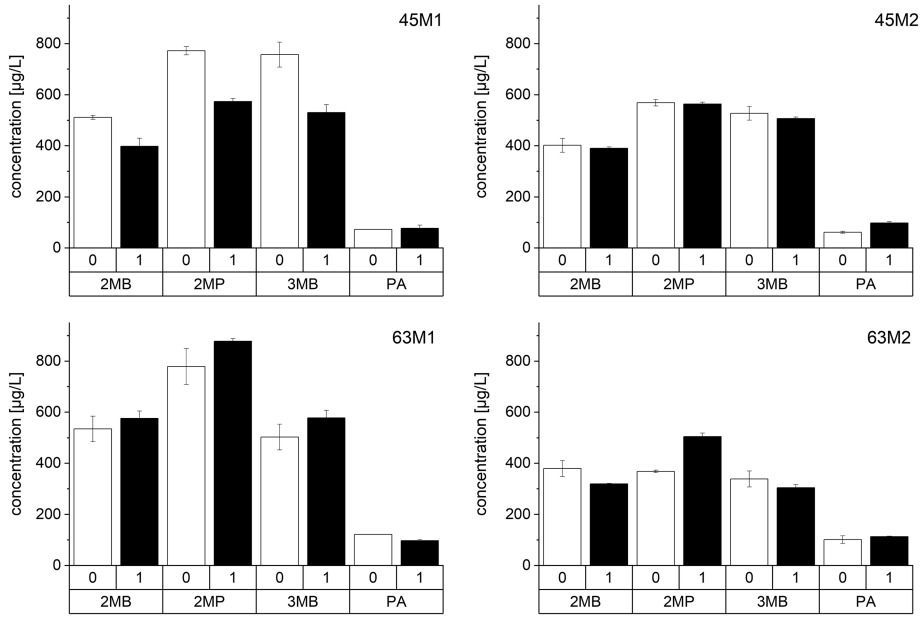


**Figure 3**. Final concentration of selected Strecker aldehydes during wort boiling; white bars (0): without 3-DG; black bars (1): with 16 mg/L 3-DG; n = 3

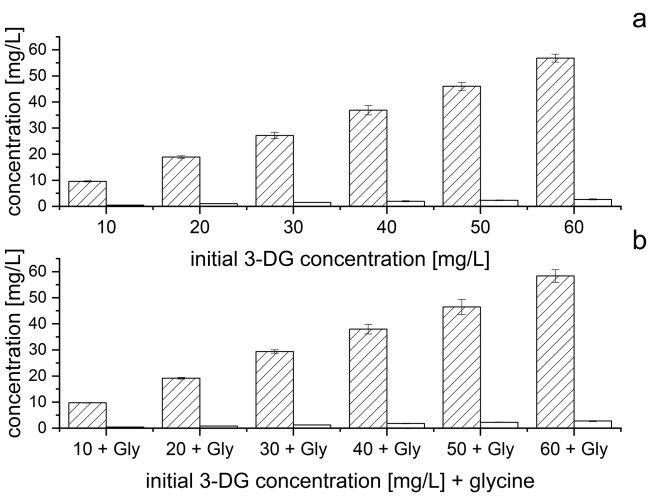


**Figure 4**. Dicarbonyl concentration after 3-DG incubation in wort boiling model systems at a pH 5.6; (a) varied initial concentration of 3-DG; (b) varied initial concentration of 3-DG + glycine; cross-striped bars: 3-DG; white bars; 3-DGal; n = 3

range of 5–6 does not affect the interconversion of 3-DG (data not shown). Figure 4 shows the concentration of 3-DG and 3-DGal after incubation with 3-DG degraded in all variations. The absolute amount of the degraded dicarbonyl is increased at higher initial concentrations. Therefore, the reaction potential is improved by an increased initial content of 3-DG. Regarding the relative degradation rate, there is no difference between the approaches. The reduction rate was about 17% in all variations. Therefore, most of 3-deoxyglucosone is stable under wort boiling conditions. The addition of glycine showed no significant difference in the degradation rate.

Further, the interconversion reaction was observed in the model incubations. The enhanced initial concentration of 3-DG results in a higher content of 3-DGal. Contrary to these results, the relative interconversion rate is independent of the initial 3-DG concentration with 25% of degraded 3-deoxyglucosone converted to 3-deoxygalactosone. Assumably, 3-DG undergoes further reaction such as polymerisation or dehydration to 3,4-DGE *(5)*. The addition of glycine did not affect the interconversion reaction. The 3-DGal formation in the model experiment explains the formation of 3-DGal in the wort boiling experiments and the increasing effect of 3-DGal formation by spiking experiments.

## Conclusions

This study investigated the formation and selected degradation reactions of 3-deoxyglucosone during wort boiling. It was shown that a higher content of amino acids (precursor reactant) caused by an additional proteolytic rest in the mashing procedure had no significant effect on 3-DG formation during wort boiling. However, higher levels of malt modification enhance 3-DG formation. The study highlighted that close to 50% of the final 3-DG concentration in wort is formed during wort boiling and is the most important process of 3-DG formation in beer production. Besides the increase in dicarbonyl, the Amadori compound fructosyllysine decreased, indicating the Maillard reaction goes further to the advanced phase. In addition, the study investigated the degradation reaction to 5-hydroxymethylfurfural, the interconversion to 3-deoxygalactosone and the formation of selected Strecker aldehydes to evaluate the influence of 3-DG reactivity on the sensory quality of beer. HMF was formed during boiling, but the compound had a low conversion rate of 0.7% to 3-DG. Although the interconversion to 3-DGal was formed more strongly, only 4% of 3-DG was interconverted. Investigations of this phenomenon with model reactions showed that the dicarbonyl is stable under wort boiling conditions. Only 16% of 3-DG was degraded, whereby 25% of the degraded 3-DG was interconverted to 3-DGal. Comparing all the investigated degradation reactions, interconversion is the most important. Although 3-DG is known as a Strecker precursor compound *(22)*, an enhanced initial concentration of the dicarbonyl did not increase the final concentration of Strecker aldehydes. It can be assumed that additionally formed ageing compounds are partly bound or that 3-DG undergoes further reactions



  

such as AGE formation, cleavage reactions or protein modification. Generally, the study showed that wort boiling generates the main dicarbonyl potential by forming 3-deoxyglucosone as a key intermediate for ageing indicators. Therefore, the results indicate that wort boiling strongly influences the ageing stability in beer through the reactivity of 3-DG.

## Author contributions

Arndt Nobis: conceptualisation, visualisation, investigation, writing (original draft).
Stefan Wendl: formal analysis, investigation.
Martina Gastl: conceptualisation, writing (review and editing), supervision, funding acquisition.
Thomas Becker: writing (review and editing), supervision

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## Conflict of Interest

The authors declare there are no conflicts of interest.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.



  