Wietstock, P. C., Kunz, T. and Methner, F.-J.

Influence of Hopping Technology on Oxidative Stability and Staling-Related Carbonyls in Pale Lager Beer

Storage-induced deterioration of beer flavor is a major issue for many breweries and technological measures to diminish staling are limited. In this study, the effect of different hopping technologies on content of pro-oxidative iron ions, concomitant oxidative beer stability and staling-related carbonyls was investigated. In lab-scale brewing trials, hop dosages during mashing-in or at the onset of boil caused a decrease of pro-oxidative iron ions in dependency of the amount of hop CO_2 extract dosed. Though, the effectiveness was clearly limited by the 'accessibility' of iron ions. The amount of 'free' iron was found to be dependent on the initial malt bill, and the hops' efficiency in terms of reducing the iron content was higher the more iron was present and the more hop CO_2 extract was dosed. Pilot-scale brewing trials (120 L) were carried out in duplicate using different hopping technologies: hops dosed only at the onset of boil (reference), mash hopping, divided hop dosage, first wort hopping, and continuous hop dosage. Hop CO_2 extract was the sole hop product used. While standard beer quality parameters were unaffected from the hopping, the bitter substance yields suffered from later hop dosages or in particular from hop dosages during mashing. All brews with modified hoppings showed reduced iron contents of up to ~30 % and improved oxidative stabilities as compared to the reference brews with the exception of the first wort hopping brews where the oxidative stability as measured by electron spin resonance spectroscopy was worse. After storage (12 weeks, 28 °C), the staled beers' carbonyl contents were noticeably distinguishable and were lowered up to 66.9 % when modified hopping was applied. Sensory analysis of the fresh and aged beers was in accordance with the analytical data and revealed improved sensory properties for the beers produced by applying modified hopping regimes with the exception of the mash hopping brew from brewing series 2, whose sensory properties were rated lower. This study provides new findings with regards to the anti-staling characteristic of hops and its application by simple modifications of the hopping technology.

Descriptors: Hop dosage, hops, beer ageing, mash hopping, first wort hopping, continuous hopping, aldehydes, iron

1 Introduction

Hops are next to water, malt and yeast one of the four ingredients used for beer production. They can be dosed at various stages during wort production; though, in Germany, the purity law limits the usage of extracts from hops to the hot part of beer production, while pelletized hops, hop powders, or cone hops can also be applied in the cold part, e.g. during fermentation or maturation [1]. Mostly, hops are added during early stages of wort production to allow sufficient time for the isomerisation of hop α-acids to iso α-acids via an acyloin ring contraction which contribute to the beer's final bitterness [2]. Yet, in brewing-related research history, also the effects of adding hops at earlier stages of the brewing process were tested. *Kolbach* and *Wilharm* [3] examined in 1943 the effects on dosing hops to the mash and found high bitter substance losses and no effect on the coagulation of nitrogen in the wort. *Schur* and *Pfenninger* [4] confirmed the high losses but reported a 'finer' and 'more distinct' hop aroma in beers when mash hopping was applied. *Gresser* [5] on the contrary found no hop aroma nor did he detect higher oil contents. *Preis* and *Mitter* [6] produced beers by adding certain amounts of the total hop bill to the first wort and found improved sensory properties and an improved bitter substance yield but milder bitterness when compared to beers where hops were not added to the first wort. But also the hop dosage during wort boiling as well as hop dosage modifications were shown to have a great effect on the oxidative stability of beer as investigations from *Wietstock* et al. [7], *Kunz* et al. [8], and *Mikyška* et al. [9] showed.

The high potential of hops and hop constituents in relation to not only providing bitterness and aroma but also featuring antioxidative and health beneficial properties was already discovered and studied intensively [7, 8, 10–18] making them also interesting for the pharmaceutical industry [19]. Particularly hop α-acids were shown to be capable of forming complexes with metal ions such as iron [8, 16, 20, 21], which, in turn, favors beer flavor stability because these metal ions are depleted as catalysts in the so-called Fenton reaction [16]. Hop α-acids were shown to possess a high antioxidative potential while iso-α-acids were less effective. The hop α-acids' metal chelation behavior was in fact reported to be

Authors

Dipl.-Ing. Philip C. Wietstock, Dipl.-Ing. Thomas Kunz, Prof. Dr.-Ing. Frank Jürgen Methner, Technische Universität Berlin, Department of Food Technology and Food Chemistry, Chair of Brewing Science, Berlin, Germany; corresponding author: philip.wietstock@tu-berlin.de

very advantageous in terms of beer quality since metal ions which promote oxidative reactions such as Cu or Fe are complexed while other vital metal ions, such as e.g. essential yeast nutrients (Zn) remained unaffected [8, 22]. *Ting* et al. [14] additionally explained the antioxidative nature of hop α- and β-acids by their ability to form stable phenoxy radicals and/or block oxidative intermediates of the Maillard reaction.

Hop additions at the end of boiling or in the whirlpool were consequently demonstrated to be advantageous with regards to beer flavor stability as unisomerized α-acids are 'supplied' again which then do not isomerize but deploy their antioxidant activity during wort cooling [8]. This can be disadvantageous because the bitter substance yields become low when adding hops late on, and therefore, more hops need to be added when still aiming to achieve the same bitterness in the beers. Yet, even though hops possess considerable amounts of metal ions [23, 24], their addition during the process still yields a reduction of certain metal ions, in particular iron ions which, in turn, favors the beer's oxidative stability [8].

The effect of hop polyphenols is also discussed in literature and appears to be controversial. While there is great evidence that polyphenols possess the ability to attenuate oxidation reactions [13, 25, 26], the polyphenol's impact on oxidative beer flavor stability seems to be ambiguous [27] and is an ongoing debate. Mikyška et al. [9] performed brewing trials using hop extracts, sole hop pellets, or a combination of both, as single dosages or in divided dosages and found a good correlation between hop antioxidants and the appearance of carbonyl compounds during storage. This effect was mostly traced back to the antiradical activities of hop polyphenols which were imparted to the wort by the pellet dosages. Though, Wietstock et al. [7] reported no or only very little effect of hop polyphenols on the formation of harmful hydroxyl and ethoxy radicals in wort as measured by electron spin resonance spectroscopy, and the biggest antioxidative potential of hops was claimed to be derived from hop α-acids and hop β-acids. Other researchers see again hop polyphenols as the only source for the hops antioxidative capacity [13]. All these findings, however, appear to be a very much a matter of which method was used for determining the antioxidative capacity [28].

Ultimately, it seems therefore indispensable to verify analytical results from antioxidative measurements with storage trials in which ageing-related compounds are monitored and to verify analytical results with sensory analyses. Furthermore, asides from the debate which hop ingredient represents now the biggest antioxidative source, there is no doubt that dosing hops to beer reduces tremendously the formation of staling aldehydes as compared to an unhopped beer [7, 29, 30].

Various pathways have been suggested to be the origin of active off-flavor compounds in bottled beer. Amongst them, iso-α-acids were also identified as a potential source of flavor-active off-flavor compounds. *Hashimoto* and *Eshima* [31] reported the formation of staling aldehydes from the iso-α-acids' alkanoyl side chain by oxidative degradation. *De Clippeleer* et al. [32] anticipated the degradation of particularly *trans*-iso-α-acids as the source of carbonyls during beer storage first, but then found this pathway to be only of minor importance when performing brewing trials. This was confirmed by investigations from *Schmidt* et al. [33]. *Rakete* et al. [34] found evidence of the formation of flavor-active carboxylic acids from iso-α-acids by hydrolytic β-dicarbonyl cleavage under oxidative conditions and thus further clarified a mechanism originally proposed by *Williams* and *Wagner* [35]. Further reactions yielding beer deterioration comprise fatty-acid degradation, aldol condensation, oxidation of higher alcohols, and the Maillard reaction, all of which can occur at different reactions during beer production such as already during mashing, during wort boiling, or after bottling. For reviews of these pathways, see [36–38]. Additionally, free radicals such as the hydroxyl radical or hydroxyethyl radical were also shown to play a substantial role during ageing of beer [39–42]. They are formed via a mechanism involving activation of molecular oxygen as catalyzed by transition metal ions (Fe^{2+} and Cu^{+}) which can act as electron donors. Oxygen forms subsequently a series of active intermediates and reacts with transition metal ions again in the so-called Fenton and Haber-Weiss reaction thereby forming highly-reactive hydroxyl radicals. A direct formation of aldehydes by free radical attack was recently suggested by Wietstock and *Methner* [43]. Yet, independent from the substantial reactions yielding staling compounds, ongoing research imparts also the liberation of those compounds from a bound state during storage [44, 45]. Apart from that, it is evident that oxidative conditions trigger staling of beers whether it may be through a de novo formation or oxidation-promoted release of substances.

Every single step and event during beer production has undoubtedly a decisive influence on the final beer quality and its resistance against the appearance of ageing-related off-flavors. Oxidation and beer deterioration reactions occur already during early stages of beer production such as during mashing or during wort boiling. Diminishing oxidative reactions by complexing or removing pro-oxidative metal ions, e.g. Cu and Fe, at these early stages can therefore be advantageous for the final beer quality as antioxidants are preserved and/or oxidation reactions occur to a lower extent. One goal of this study was therefore to distinctively remove iron ions at the early stages of wort production, ultimately aiming at producing beers with an increased oxidative stability and diminished or delayed formation of staling off-flavors. The present study therefore aimed to scrutinize the hop dosage's influence at different points of addition during wort production, while taking the bitter substance yield into account. Hop CO_2 extract was used because it represents a hop product which is often applied in the industry. This study provides new insights in the hop acids' ability and mode of diminishing or delaying staling of beer during storage. Outcomes from this study therefore point to the importance to rethink the hopping technology applied in industry and adapt it with the goal of a higher efficiency, such as not only providing bitterness but also utilizing the hops antioxidant properties.

2 Materials and methods

2.1 Chemicals

Benzaldehyde, 2-furfural, iron II sulfate hepta hydrate, 2-methylbutanal, 3-methylbutanal, methional, pentanal, phenylacetalde-

hyde, and α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN) were purchased from Sigma Aldrich Inc., Steinheim, Germany. Sodium carbonate and sodium sulfate were obtained from Merck KGaA, Darmstadt, Germany. Diethylether and anhydrous ethanol were purchased from VWR international GmbH, Darmstadt, Germany. All chemicals were of analytical grade or higher. All aqueous solutions were made with double-distilled water and prepared freshly every day. The international calibration standards of iso-α-acids (ICS-I3) or a standardized calibration extract for α- and β-acids (ICE-3) were purchased from Labor Veritas AG, Switzerland. Purified α-acids (86.4 % purity), hop CO_2 extracts, or hop pellets were supplied courtesy from Hopsteiner.

2.2 Standard wort and beer analysis

Extract (2.9.2.3), alcohol (2.9.6.3), color (2.12.2), pH (2.13), foam stability (2.18.2), total nitrogen (2.6.1.1), free amino nitrogen (2.6.4.1.1), total polyphenols (2.16.1), bitter units (2.17.1), endogenous antioxidative potential (EAP) and radical levels (T_{600}) (2.15.3) were analyzed according to MEBAK [45]. The numbers in parentheses indicate the method used.

2.3 Determination of hop acid concentrations by HPLC

Hop acids were quantitated according to [47]. Chromatographic determination was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Böblingen, Germany) at a constant temperature of 40 °C and a flow rate of 1.2 mL/min with a 5 µL injection volume. Two mobile phases were used. Mobile phase A was 100 % methanol, mobile phase B was 55 % methanol, 44 % water and 1 % phosphoric acid. The elution began isocratically with 50 % of mobile phase B for the first 12 min followed by a gradient descent to 20 % of mobile phase B over the next 3 min, which was then held for 10 min. A Purosphere Star™ LC-18 5 µm C18 silica column was used for separation. Absorbance was measured at 270 nm and 314 nm. As reference for the iso-α-acids, an international calibration extract (ICS-I3) was used, whereas for α- and β-acids, a standardized hop extract (ICE-3) was deployed.

2.4 Determination of SO_2 levels in beers

Sulphur dioxide concentrations of beer were analyzed by a continuous flow analyzer (CFA -Skalar; Kat.-Nr.: 593-998) according to an optimized method using a new Teflon membrane [48]. Prior to measurement, beer samples were degassed by ultrasonic treatment for 5 minutes and filtered through a black band filter (Schleicher & Schuell, Dassel, Germany) during which the first 10 mL were discarded.

2.5 Determination of metal ion concentrations in worts and beers.

Metal ion concentration was measured using an iCAP 6200 inductively coupled plasma-optical emission spectroscopy (ICP-OES) system fitted with a CID 86 detector and argon as the carrier gas. The following parameters were used for the measurements: RF power: 1150 W; argon gas flow rates: auxiliary 0.5 L/min, nebulizer 0.5 L/min; sample flow rate: 4.0 mL/min. The analytical wavelengths used for the determination of iron were 239.5 and 259.9 nm. A six-point calibration curve was used to quantify the test samples' concentrations. The calibration was done matrix-matched ranging from 0–1 mg/L or 0–250 µg/L in wort or beer, respectively, to deplete influences of the samples' organic matrices. All calibration curves showed good linearity ($R^2 > 0.99$).

2.6 Quantitation of aldehydes by solvent-assisted flavor evaporation (SAFE)-GC/MS

Solvent assisted flavour evaporation (SAFE) according to *Engel, Bahr* and *Schieberle* [49] and high resolution gas chromatography (HRGC) coupled to mass spectrometry (MS) analysis was used to measure staling aldehyde concentration in aged beers. An aliquot (100 mL) of beer was passed through a folded paper filter and spiked with 1 µg of pentanal as an internal standard. The sample was extracted twice with 150 mL diethyl ether. To remove the non-volatile material, the unified extracts were distilled under high vacuum by means of a SAFE apparatus. The distillate was washed twice with a 0.5 M Na_2CO_3 solution and water, dried over Na_2SO_4, and concentrated to 5 mL using a Vigreux column. A sample (1 µL) of this concentrated distillate was applied via a cold injection system (Gerstel, Mülheim, Germany) in 10:1 split mode to a gas chromatograph (6890, Agilent Technologies, Waldbronn, Germany) fitted with a capillary column (VF-5 MS, 60 m x 0.25 mm, 0.25 µm film, Varian, Darmstadt, Germany). The following temperature program was used for HRGC: after 12 min at 35 °C, the oven temperature was raised to 150 °C at a rate of 12 °C/min and then to 250 °C at 30 °C/min where it was held for 5 min. The flow rate of the helium carrier gas was 0.6 mL/min. The MS analysis was performed by an MSD 5973 mass spectrometer (Agilent Technologies, Waldbronn, Germany). Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV using selected ion monitoring. Carbonyl concentrations were calculated by using a single point internal standard procedure. An internal response factor of the reference compounds in relation to the internal standard was determined, and was then used to calculate the amount of the compounds in the sample. Table 1 depicts the retention times and target ions of all compounds used for quantitation.

Table 1 **Retention Times and *m/z* Ratios Used for Quantitating Carbonyl Compounds by GC/MS. A Varian VF-5 MS column (60 m x 0.25 mm, 0.25 µm) was used for analyte separation. All aldehydes were quantitated by using commercially available reference compounds**

Substance	t_R [min][a]	m/z
3-methylbutanal	10.15	58
2-methylbutanal	10.63	58
pentanal (IS)	12.96	58
2-furfural	19.05	96
methional	21.50	104
benzaldehyd	23.03	106
phenylacetaldehyd	24.79	91
ethyl nicotinate	27.61	106
γ-nonalacton	29.55	85

[a] t_R = retention time

2.7 Sensory analysis

Sensory analysis of fresh and stored beers was conducted according to DLG (Deutsche Landwirtschafts-Gesellschaft e.V.) in multiple sessions with 10–12 trained panelists. The beers' attributes odor, taste, palate fullness, freshness and quality of bitterness are rated on a scale of 1 to 5, where 5 represents the highest rating and 1 the lowest rating. When ratings of 3 or lower are assigned to a sample, then it is declared 'not vendible' and the downgrading has to be justified with a detailed description of the off-notes. In order to get an improved comparability of the samples, a 'DLG score' was calculated based on the area that the final rating would make in a spider web diagram with these five attributes. Following the DLG standards, the ratings of the attributes 'purity of odor', 'purity of taste', and 'quality of bitterness' were included twice, and the attributes 'palate fullness' and 'freshness' were included single for the calculation.

2.8 Effects and hop bitter substance yields of hop dos ages during mashing-in

Worts were produced in lab-scale by mixing well 70 g of fine grist (100 % Pilsner malt) with 300 mL of double-distilled water. Mashing was conducted under continuous stirring using a laboratory masher (Bender & Hohbein, Bruchsal, Germany) and applying the following temperature program: mashing-in at 62 °C, heating up to 66 °C at 1 °C/min, 30 min rest, heating to 72 °C, 20 min rest, heating to 78 °C. After reaching 78 °C, the mash was immediately filtered using paper filters and the mashing-beakers and stirrers were rinsed with 100 mL of double-distilled water (T = 78 °C) which was added on top of the filters. The first 100 mL of wort were collected and were also poured back on top of the filters. Along with mashing-in the malt, 80 mg of hop CO_2 extract (Hallertauer Perle, c(α-acid) = 44.8 % wt./wt.) or hop pellets type 45 (Hallertauer Perle, c(α-acid) = 8.8 % wt./wt.) were added. 200 mL of collected wort were then boiled for 60 min under reflux and 200 mg/L of the same hop CO_2 extract were added at the start of boil. Sampling was done from the wort after filtration and from the boiled wort. Aliquots were taken and were immediately cooled using an ice bath. Hop acid concentrations from the worts were subsequently quantified by HPLC. The trials were conducted in triplicate.

2.9 Effects of hop CO_2 extract additions during mashing or during start of boil

The mashing and separation procedure in these trials was similar to the previous trial. First, the effects of adding hops during mashing-in were assessed and 0, 44.6, 89.3, 133.9, 223.2, 446.4 mg/L of hop CO_2 extract (Hallertauer Perle, c(α-acid) = 44.8 % wt./wt.) were added directly during mashing-in. In a separate trial, the mash's iron concentration was artificially increased by adding 200 µg/L of Fe^{2+} to the mashing-in liquor, and the hop dosage was limited to 0, 223.2, and 446.4 mg/L of hop CO_2 extract (Hallertauer Perle, c(α-acid) = 44.8 % wt./wt.) dosed during mashing-in. The sweet worts after filtration were collected and the iron concentrations from all worts were determined on the same day by ICP-OES.

In addition to dosing hops during the mashing-in step, the effects of adding hop CO_2 extract at the start of boil on iron concentration of sweet wort were assessed. In this trial, the same mashing procedure as described before was used again but this time in total, 3 L of unboiled wort were produced, divided into three 1 L aliquots and 0 µg/L Fe^{2+}, 100 µg/L Fe^{2+}, or 200 µg/L Fe^{2+} were added, respectively. The worts were then divided again in 100 mL aliquots and brought to a boil under reflux. Each aliquot was then mixed with 0, 44.6, 89.3, 133.9, 223.2, 446.4 mg/L of hop CO_2 extract (Hallertauer Perle, c(α-acid) = 44.8 % wt./wt.), respectively. After 5 minutes incubation at > 98 °C, the worts were immediately cooled in an ice bath and the iron concentration from all wort samples was determined on the same day by ICP-OES. All trials were done in triplicate.

2.10 Influence of malt type and hop dosage on iron ion concentration of wort

The effect of using 100 % Pilsner malt, 100 % Munich malt type I, or a mixture of both malts in equal amounts on iron concentration in wort was assessed in a lab-scale trial. Mashing and filtration was done again as described previously but this time, 1 L of each initial malt bill was produced in triplicate. After bringing the wort to a boil, 208.8 mg/L of hop CO_2 extract (Hallertauer Magnum, c(α-acid) = 47.9 % wt./wt.) were added and the worts were boiled under reflux for 60 minutes. Samples were taken from the worts after boiling and were immediately cooled prior to directly determining the iron concentrations using ICP-OES. All trials were done in triplicate.

2.11 Production of beers using different hop dosages in semi-technical scale

Five different beers were produced on a 1.2 hL scale in duplicate. The malt bill consisted of 90 % Pilsner malt and 10 % Munich malt type I and an infusion mash procedure similar to that of the previous trials was used for sweet wort production. The mash solids were separated from the sweet wort using a lauter tun. Table 2 depicts the different hop dosages, masses and points of addition. The amounts of hop α-acids added were designed to obtain an identical bitterness in the beers. The isomerization yield was assumed to be 33.33 % when dosing hops at the onset of boil. A yield of 5 % was expected from adding hops to the mash and the addition at the onset of boil was accordingly lowered. For the divided hop dosage, an additional amount of 10 % was given

Table 2 **Hop dosages used for beer production in semi-technical scale. All values are in mg/L. Please consult the materials and methods section for information about the design of the brews**

Brew	Abbrev.	Hop α-acid additions in [mg/L]					
		mash-in	first wort	start of boil	30 min boil	end of boil	whirlpool
Reference	REF	–	–	90	–	–	–
Mash hopping	MAH	60	–	81	–	–	–
Divided hop dosage	DIV	–	–	45	27	18	9
First wort hopping	FWH	–	45	–	27	18	–
Continuous hop dosage	CON	–	–	45	10 * 4,5 in 5 min intervals		

in the whirlpool to compensate a potential diminished isomerization yield caused by the later hop additions. For the first wort hopping and for the continuous hop dosage, no corrections as related to the quantity of hops dosed were made, and the total amounts added were similar to the reference (hops dosed only at the onset of boil). The hop product used was hop CO_2 extract (Hallertauer Magnum, c(α-acid) = 47.9 % wt./wt.). The wort was boiled under atmospheric pressure for 60 minutes, followed by a 15 min whirlpool rest. After subsequently cooling to pitching temperature of 14 °C using a plate heat exchanger, the worts were fermented in open vessels using the bottom-fermenting yeast strain W34/70 (Fermentis, Marcq en Baroeul, France) in cylindroconical tanks until a residual apparent extract of 3.5 % wt./wt. was reached. The green beers were subsequently stored at room temperature for 1 day, transferred to kegs and were maturated at 0–2 °C until filtered using membrane candle filters (5 µm/1 µm/0.45 µm; Donaldson, Haan, Germany). The beers were then bottled in 0.5 L bottles using a four organ filler (JS Maschinen GmbH, Bergen, Germany). The oxygen levels in the bottled beers were controlled regularly and did not exceed 50 µg/L as measured using a DIGOX 6.1 apparatus (Dr. Thiedig, Berlin, Germany). The pitching worts were sampled and kept frozen at –18 °C until analyzed. The beers were analyzed directly after filling and after 12 weeks storage at 28 °C in the dark.

3 Results and discussion

3.1 Effects and hop bitter substance yields of hop dosages during mashing-in

In general, the yield of hop bitter substances during wort boiling is considerably low and was shown to be dependent on many factors such as e.g. losses with trub during wort boiling, pH, temperature, etc. [50]. There is only little published of how the bitter substance yield is to be expected when applying a hop dosage during mashing. Therefore, as a preliminary trial, lab-bench trials were performed in which hops were added during mashing-in.

Table 3 depicts the results from lab-bench brewing trials. Hop iso-*α*-acid utilization was determined in both the filtered mashes and the worts after boiling. It is apparent from the data that the hop bitter substance yields were very low when adding hops during mashing-in. Adding pellets instead of hop CO_2 extract showed little higher yields for iso-*α*-acids which maybe traced back to the lower *α*-acid masses added at the onset of mashing. The hop

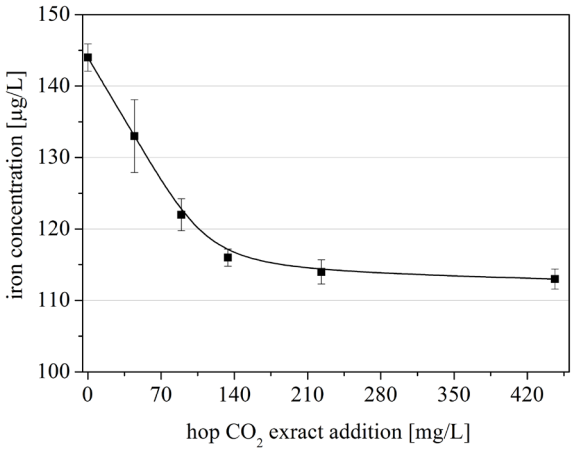

Fig. 1 Effect of hop CO_2 extract additions on iron concentration during mashing. Iron concentration was determined from filtered samples. Mean values are presented. Error bars represent ± 1 standard deviation. N = 3

α-acid utilization rates during wort boiling were in a normal range and showed no clear dependency as to what hop product was added during mashing-in. The little higher yields at end of boiling observed in the trials where hop CO_2 extract or hop pellets were added during mashing-in may also be derived from residual *α*-acids which 'survived' the filtration step and were therefore still present at the onset of boil.

Regarding the total yield, clearly, adding hops during mashing results in a worse yield of bitter substances as opposed to when adding hops only at the onset of boil. The higher total yield when adding pellets instead of hop CO_2 extract may also be explained by the difference of total *α*-acids added during mashing and boiling (hop pellets, 107.2 mg/L vs. hop CO_2-extract, 179.2 mg/L).

As shown in figure 1, adding hop CO_2 extract during mashing at amounts of 44.6 to 133.9 mg/L yielded a significant reduction of 144 µg/L of iron initially present in the mash until at maximum, a concentration of 114 µg/L (21 % reduction) was reached. This level was not further exceeded even when additions were increased up to 446.4 mg/L of hop CO_2 extract.

Recent studies [16] reported that hop α-acids are good iron chelators and the effect as seen in figure 1 can therefore most probably traced back to the α-acids' ability to bind iron thereby forming amorphous complexes which then precipitate and are then lost during the spent grain removal. It is apparent that still only ca. 21 % of the total iron was removed and it was therefore assumed that the residual 79 % of the iron was firmly bound in complexes

Table 3 Hop α-acids, iso-α-acids, and bitter acid yields of hop pellet or hop CO_2 extract additions during lab-scale mashing and wort boiling. Mean values ± 1 standard deviation are presented. N = 3

Hop product added during mashing-in	mashing				wort boiling				total yield[a] [%]
	α-acids added [mg/L]	iso-α-acids detected [mg/L]	α-acids detected [mg/L]	yield[a] [%]	α-acids added [mg/L]	iso-α-acids detected [mg/L]	α-acids detected [mg/L]	yield[a] [%]	
no hops added	–	–	–	–	89.6	33.9 ± 1.3	4.3 ± 0.3	37.8 ± 1.5	[37.8]
hop CO_2-extract	89.6	1.5 ± 0.2	0.3 ± 0.2	1.7 ± 0.2	89.6	36.8 ± 1.4	3.6 ± 0.1	41.1 ± 1.6	20.5
hop pellets (type 45)	17.6	1.3 ± 0.3	< 0.1	7.4 ± 1.7	89.6	37.6 ± 2.2	3.6 ± 0.2	42.0 ± 2.5	35.1

[a] Yields are calculated based on the sum of iso-α-acids at the end of boiling divided by the amount of α-acids added during mashing and/or wort boiling

Table 4 Effect of hop CO_2 extract additions and Fe^{2+} additions during mashing on free amino nitrogen content, bitter units and final iron concentration in sweet wort. Mean values ± 1 standard deviation are presented. N = 3

Hop CO_2 extract	Free amino nitrogen [mg/L]		Bitter units [BE]		Iron concentration [µg/L]	
	0 µg/L Fe^{2+}	200 µg/L Fe^{2+}	0 µg/L Fe^{2+}	200 µg/L Fe^{2+}	0 µg/L Fe^{2+}	200 µg/L Fe^{2+}
No addition	198.4 ± 7.2	197.0 ± 3.3	< 1	< 1	185.4 ± 8.2	179.8 ± 12.2
223.2 mg/L	196.5 ± 5.7	177.6 ± 5.8	6.0 ± 1.2	5.7 ± 0.6	124.9 ± 9.9	122.4 ± 5.8
446.4 mg/L	195.7 ± 1.7	177.2 ± 6.3	10.5 ± 0.4	10.0 ± 0.7	121.5 ± 11.2	119.8 ± 8.2

with other mash or wort constituents thus being not accessible for the α-acids' action.

In a separate trial, it was therefore assessed if an additional dosage of 'free' iron to the mash, added as iron(II) sulfate hepta hydrate, results in a higher relative depletion of iron. A supplementary addition of iron yielded no further increase in the iron concentration as measured by ICP-OES, and the addition of α-acids, added as hop CO_2 extract, showed no enhanced effectiveness (see Tab. 4). In accordance with the results from figure 1, it is likely that there are various constituents with free binding sites for iron present in the mash which immediately reacted with iron and made it non-accessible for the reaction with α-acids. *Svendsen* and *Lund* [51] reported that there are free binding sites for metal ions available in beer and it is most likely that there are even more binding sites in mash because there are more constituents in solution as opposed to beer. Accordingly, it can be anticipated that the additional iron was immediately bound and then removed with the spent grain.

From the free amino nitrogen data (Tab. 4), it can be observed that α-acid additions together with Fe^{2+} additions showed in fact an effect and provoked a distinct decrease of the mash FAN while no decline was seen when neither Fe^{2+} nor α-acids were added. Similar to α-acids, proteins or amino acids together with polyphenols form complexes with iron [52, 53] and it is therefore conceivable that α-acids together with mash nitrogen constituents and iron formed amorphous coagulates which subsequently precipitated. Hop α-acids appear to play a central role in these reactions as the effect was not observed when only Fe^{2+} was added. It is also interesting, though, that the effect was only seen when additional iron was brought into the mash. Schur and Pfenninger [4] reported no effect on the nitrogen composition in wort when adding hops to the mash; however, in their study also no additional iron was added.

The bitter units in trials were again very low and bitter substance yields ranged from 5–6 % which is in accordance with the previous findings (Tab. 3) and with literature data [3–5]. The extracts of the mashes were in the range of 13.95 to 14.04 % wt./wt. with deviations of at maximum 0.03 %, and differences in original gravity can thus not be accountable for the observations.

3.2 Influence of malt type and hop dosage on iron ion concentration of wort

Recent investigations demonstrated the hop dosage's strong influence on the iron concentration in wort and beer [8]. When aiming at a high efficiency of the hop dosage with regards to reaching a high precipitation of iron, it is crucial to know the maximum iron precipitation possible and at which amount of hops added this maximum is reached. An experiment was therefore designed in which a base wort's iron concentration was artificially increased by adding extra iron, and subsequently, various amounts of hop CO_2 extract were added to the wort at the start of boil.

In contrast to the trials where hops were dosed to the mash, in these trials, the iron additions yielded also a proportional increase of iron being detected. The recovery rates for iron were 55 % and 60 % for 100 µg/L and 200 µg/L of iron being added, respectively (Fig. 2).

The reduced iron concentrations in the worts after hop addition were clearly a function of the amount of hop CO_2 extract added at the onset of boil and the relative iron depletion was more pronounced, the more iron was present. At an addition of 200.9 mg/L of hop CO_2 extract, a maximum decrease of only 9 % was seen when no iron was added, and 33 % and 47 % when 100 and 200 µg/L iron ions were added, respectively. The response of the iron concentration plotted over the amount of hop CO_2 extract added followed an exponential descent and after an addition of 100.4 mg/L CO_2 extract (= 45.0 mg/L α-acids), > 90 % of the maximum iron depletion possible was already achieved in all trials while higher additions had only minor effects. Higher initial iron concentrations also resulted in little higher iron concentrations in the worts even at high hop additions which can be anticipated by a transition of 'free' iron ions into strongly bound forms which are not prone to be complexed by the hop constituents, in particular α-acids, anymore.

There is evidence that the malt type used has an influence on the wort's and beer's metal ion composition and concentrations [54,

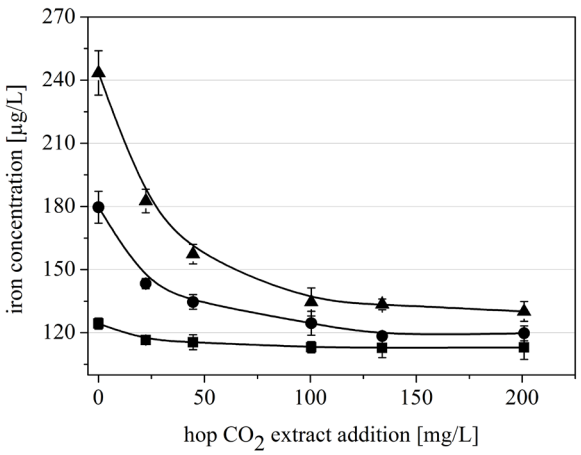

Fig. 2 Effect of hop CO_2 extract additions and Fe^{2+} additions on final iron concentration in wort. A base wort (■) was spiked with 100 µg/L Fe^{2+} (●) or 200 µg/L Fe^{2+} (▲) and brought to a boil. After 5 min, 0–200.4 mg/L hop CO_2 extract was added. Mean values are presented. Error bars represent ± 1 standard deviation. N = 3

Table 5 **Extract, pH value, and color of worts produced with 100 % Pilsner malt, an equal mixture of Pilsner malt and Munich malt type I, and 100 % Munich malt type I. Mean values ± 1 standard deviation are presented. N = 3**

		100 % Pilsner malt	50 % Pilsner malt 50 % Munich malt type I	100 % Munich malt type I
extract	[%-wt./wt.]	11.80 ± 0.06	11.87 ± 0.05	11.81 ± 0.03
pH-value	[–]	5.70 ± 0.02	5.41 ± 0.03	5.23 ± 0.02
color	[°EBC]	7.3 ± 0.3	18.6 ± 0.8	29.1 ± 0.4

55]. As a consequence from the previous trials' outcomes (cp. Fig. 2), it is conceivable that the hop dosage's influence and effectiveness also may change with the initial malt bill used and the wort composition. To test this hypothesis, trials were conducted in which worts were produced by using different initial malt bills: 100 % Pilsner malt; 50 % Pilsner malt, 50 % Munich malt type I; 100 % Munich malt type I. Hop CO_2 extract was then added at the onset of boil and the worts were incubated for 5 min prior to cooling them rapidly, and subsequent analysis.

Table 5 depicts the extracts, pH-values, and colors of the individual wort samples without hops added. While wort extracts were not influenced by initial malt bill used, the color increased when using higher proportions of Munich malt and the pH dropped which may be explained by the acidifying properties of certain Maillard reaction products [50].

As demonstrated in figure 3, the initial malt bill had a significant effect on the initial iron concentrations of the worts and increased with higher proportions of Munich malt (50 % Munich malt, 84 % increase; 100 % Munich malt, 202 % increase) (Fig. 3).

The hop dosage clearly caused a lowering of the iron concentration and its extent was strongly dependent on the malt bill used. The iron content decreased by 19 %, 29 %, and 59 % when 100 % Pilsner malt, an equal mixture of both malts, or 100 % Munich malt type I were used, respectively. It is not clear, though, if iron is present in a more 'vulnerable' form when using higher proportions of Munich malt type I, or if the iron reduction due to the hop dosage was more pronounced because a higher quantity of iron was present. Though, these data summed up imply that the direct binding capacity of iron in wort is much lower in comparison to mash. As a consequence, the proportional 'free' iron ion content with increasing munich malt additions is higher, and iron is then more 'vulnerable' to the hop acids' attack and complex formation.

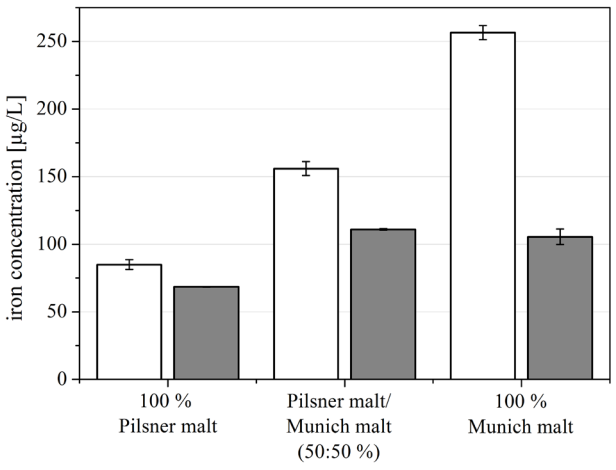

Fig. 3 **Effect of initial malt bill on iron concentration in sweet wort and iron depletion by hop CO_2 extract additions. Without hops added (□) and with 208.8 mg/L hop CO_2 extract added (■). Mean values are presented. Error bars represent ± 1 standard deviation. N = 3**

Taking all these data together thus far, the principal conclusions from these experiments are: first, an addition of ca. 45–60 mg/L hop α-acids can be considered as sufficient in terms of achieving a maximum iron precipitation during mashing or wort boiling at these experimental conditions such as e.g. the malt bill used, etc.; but secondly and more importantly, the hop dosage dependent iron removal is greatly dependent on the mash or wort matrix and the availability or 'vulnerability' of iron ions. The hop dosage should therefore be adapted to the individual wort matrix when aiming at a maximal iron precipitation at the onset of wort production.

3.3 Pilot-scale brewing trials

Based on these findings from the previous trials, brewing trials were designed and conducted in the institutes' pilot plant. The brews' hop dosages were chosen in a way to represent a high variety of points of addition and aiming at an increased oxidative stability of the final beers. In addition to the dosage points from the preliminary trials, first wort hopping and a continuous hop dosage were applied. Table 6 (see next page) depicts the beer analysis data from the duplicate brews.

The parameters original gravity, apparent extract, alcohol, apparent final degree of attenuation, color, pH, total nitrogen, free amino nitrogen, total polyphenols, and foam showed little variations within the treatments but no clear dependency on the type of hop dosage used. All values were in acceptable range for lager beers [56].

The hop bitter acid concentrations also displayed dissimilarities between the duplicate brews but an apparent influence of the hopping method used was observed. While α-acid and β-acid levens were both very low due to their limited solubility in beer [57], the total iso-α-acid concentration was generally lower than the reference when applying a divided hop dosage (DIV), first wort hopping (FWH), or a continuous hop dosage (CON). The iso-α-acid concentrations of the mash hopping (MAH) brews were dissimilar in both brews and was higher than the reference brew in the first brew and lower in the second brew. Concomitant to the iso-α-acid concentrations, the bitter units showed the same pattern and were 15–31 % lower as compared to the reference for the DIV, FWH, and CON brew in the first brews, and 4–17 % lower in the second brews. The brews where hops were dosed to the mash were either 4 % higher or 4 % lower than the reference. Though, when taking the total amount of hops used into consideration and looking at

Table 6 **Beer analytical data from beers as produced by applying different hopping technologies. REF, 100 % of hop CO_2 extract dosed at the onset of boil; MAH, mash hopping; DIV, divided hop dosage; FWH, first wort hopping; CON, continuous hop dosage**

		Brew series 1					Brew series 2				
		REF	MAH	DIV	FWH	CON	REF	MAH	DIV	FWH	CON
Original gravity	[%-wt./wt.]	11.27	11.41	10.94	11.36	11.12	11.14	10.89	11.11	11.21	11.08
Apparent extract	[%-wt./wt.]	1.83	1.90	2.01	2.06	1.96	2.31	2.03	1.99	1.97	1.95
Alcohol	[%-vol.]	4.99	5.03	4.71	4.92	4.84	4.67	4.68	4.82	4.88	4.82
Apparent degree of attenuation	[%]	83.7	83.3	81.6	81.8	82.4	79.2	81.4	82.1	82.4	82.4
Color	[EBC]	6.8	6.7	7.1	6.8	6.4	6.9	7.0	7.4	8.0	7.4
pH-value	-	4.40	4.37	4.31	4.41	4.35	4.30	4.33	4.35	4.37	4.33
Total N (12 %)	[mg/L]	738	667	671	631	694	717	733	770	808	724
FAN (12 %)	[mg/L]	79	82	58	81	72	73	75	89	90	72
NIBEM 30	[s]	231	229	226	213	215	243	239	222	215	220
Polyphenols (12 %)	[mg/L]	149	154	141	145	144	152	149	156	157	145
Hop iso-α-acids	[mg/L]	24.4	26.2	18.0	20.6	21.6	22.9	20.3	16.9	19.5	21.3
Hop α-acids	[mg/L]	< 1	1.0	< 1	2.3	< 1	< 1	< 1	< 1	1.1	< 1
Hop β-acids	[mg/L]	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Bitter units	[BU]	26	27	18	22	22	23	22	19	22	22
Bitter substance yield	[%]	29.9	19.1	18.2	24.4	24.4	25.6	15.6	19.2	24.4	24.4
SO_2	[mg/L]	4.2	3.9	3.5	3.8	3.5	4.7	3.2	4.1	3.4	3.2
Iron	[µg/L]	77	56	56	69	51	74	56	53	64	54
EAP-value	[min]	211	200	185	191	186	231	181	216	156	171
T_{600}-value [$*10^6$]	[–]	0.85	0.65	0.66	0.99	0.61	0.64	0.64	0.67	1.13	0.83

the overall bitter substance yield, the picture was different and the reference brews had the highest bitter substance yield from all trials. The mash hopping was the lowest followed by the divided hop dosage, while the first wort hop dosage and the continuous hop dosage displayed similar hop bitter substance yields of 24.4 % in both brewing series. Clearly, the total bitter substance yields suffered from later hop additions and in particular when adding hops during mashing.

The beers' SO_2 content ranged between 3.2 and 4.4 mg/L and were unaffected again by the hop dosages. These fluctuations are most likely dependent on the fermentation performance and yeast viability and not influenced by the hop dosage.

The beer's iron concentrations were clearly affected again by the hop dosage and were lowered by up to ~30 % when e.g. adding 50 % of the total hops at beginning of boiling and the remainder to be added continuously (CON). The mash hopping, the divided hop dosage, and the continuous hop dosage resulted in the lowest iron concentrations, followed by the first wort hop dosage. The reference brews were highest in iron throughout both brewing series. In consideration to the findings from the previous trials and literature data [8, 16], it is evident that the hop dosage's effect on the beers' iron concentrations can be traced back to the hop constituents' iron complexing properties.

The relation between beer constituents, the 'lag-time' or EAP-value and the radical concentration after a certain time measurement (T-value) is complex. While the EAP-value usually correlates well with substances that quench activated oxygen species such as e.g. the beer's SO_2 content, the T-value or radical concentration after a certain time is mostly influenced by substances that suppress or promote radical formation such as e.g. complexing agents or transition metals, respectively [39, 52, 58, 59].

In accordance with literature, the ESR measurements were affected strongly by the sample's SO_2 content, and it is therefore difficult to interpret the impact of the hop dosage as potential effects are most probably coped over by the samples' SO_2 content. To minimize the SO_2 influence on the measurement, the sample's SO_2 content can be artificially increased and the EAP-value is then divided by its SO_2 content giving the Beverage Antioxidative index (BAX) as an indicator for the sample's oxidative stability [60]. According to the method's functional principle, applied to this study, dividing the samples' EAP-value by their SO_2 contents gives an approximation of the BAX-values ranging from 50.2 to 53.1 $min*mg^{-1}*L$ in brewing series 1 and 45.9 to 56.6 $min*mg^{-1}*L$ in brewing series 2. With the exception of the FWH brew from brewing series 2, the BAX-value was always lowest for the reference brews, and the hop dosage modifications consequently yielded evidently an increase of the oxidative stability of the beers matrices. Thus, a lower 'SO_2-consumption rate' during storage can be expected by the brews produced using modified hop dosages in comparison to the reference brews.

The modified hop dosages also resulted in distinctly lower T_{600}-values (a measure for free radical levels) which can be ascribed to their effect on the beer's iron concentrations. The high T_{600}-values

Table 7 **Carbonyl contents of beers as produced by using different hopping technologies after prolonged storage of 12 weeks at 28 °C and from the fresh reference beers. REF, 100 % of hop CO_2 extract dosed at the onset of boil; MAH, mash hopping; DIV, divided hop dosage; FWH, first wort hopping; CON, continuous hop dosage**

	Carbonyl concentrations in µg/L[a]											
	Brew series 1						Brew series 2					
	REF (fresh)	REF	MAH	DIV	FWH	CON	REF (fresh)	REF	MAH	DIV	FWH	CON
3-methylbutanal	5.0	14.0	4.0	9.4	13.0	5.5	3.9	9.6	2.4	4.3	6.5	5.8
2-methylbutanal	2.9	9.3	3.4	5.7	8	4.7	2.4	6.2	1.9	3.4	5.1	4.8
2-furfural	1.7	53.0	18.0	12.0	18.0	14.0	2.0	44.0	14.9	12.8	18.7	16.0
methional	2.3	9.5	2.3	7.2	5.9	6.5	2.5	8.8	3.8	4.3	5.8	4.7
benzaldehyde	< 1.0	1.4	< 1.0	< 1.0	1.3	< 1.0	< 1.0	1.3	1.0	1.3	1.1	1.1
phenylethanal	2.6	5.3	2.4	3.1	4.8	3.3	1.9	3.9	3.3	4.1	4.0	3.5
ethyl nicotinate	3.2	7.4	6.2	3.6	9.3	3.7	2.8	5.6	9.1	4.9	6.7	4.5
γ-nonalacton	6.1	16.1	14.6	13.3	17.3	15.0	5.8	15.5	12.8	11.1	9.9	13.0
∑ aldehydes	23.8	116	50.9	54.3	77.6	52.7	21.3	94.3	49.2	46.2	57.8	53.4

[a] Carbonyl concentrations were calculated by using a single point internal standard procedure

of the brews where hops were dosed in the first wort (FWH) made an exception and there is no satisfactory explanation for this observation.

The described effects can be most probably traced back to the chelation and/or early removal of pro-oxidative metal ions due to the modified hop dosages. Kunz et al. [59] also found that higher iron values and higher contents of hop α-acids in beer promote deterioration reactions or improve the oxidative beer stability, respectively.

The beers' staling aldehyde concentrations were also determined and are depicted in table 7. From the fresh beers, only the reference brews were measured. After storage at 28 °C in the dark, the aldehyde concentrations of all beers were analyzed again. The reference brews exhibited the highest concentrations of all aldehydes measured after 12 weeks storage at 28 °C with the exception of benzaldehyde where concentrations were alike. The aldehyde levels as detected in the second brews were generally little lower than those in the first brews. Interestingly, the MAH beers from both brews displayed very low total aldehyde concentrations, and only the DIV beers from brewing series 2 were lower than the MAH beers' aldehyde concentrations from brewing series 2. This may be explained by the suppression of oxidative reactions already during the early process steps of wort production. Furfural, which is typically a good indicator for a sample's exposure to heat [61, 62], was also influenced by the hop dosage. However, recent findings from [63] also found a direct interaction between hop constituents and the formation of furfural in model solutions at wort boiling conditions thus confirming the observations from this study.

Summing up all aldehydes measured and comparing the brews within the two brewing series revealed a reduction of 43.9–66.9 % and 48.7–60.9 % as compared to the reference from brew series 1

Table 8 **Sensory analysis of fresh beers and of aged beers (28 °C, 12 weeks) as produced by using different hopping technologies. REF, 100 % of hop CO_2 extract dosed at the onset of boil; MAH, mash hopping; DIV, divided hop dosage; FWH, first wort hopping; CON, continuous hop dosage**

		Fresh beers					Aged beers (28 °C, 12 weeks)				
		REF	MAH	DIV	FWH	CON	REF	MAH	DIV	FWH	CON
Brew series 1	Purity of odor	4.6	5.0	4.8	5.0	5.0	3.2	4.3	4.2	4.1	4.2
	Purity of taste	4.5	5.0	4.0	4.7	4.6	3.2	4.4	4.5	4.3	4.3
	Palate fullness	4.6	4.6	4.0	5.0	4.8	4.4	4.5	4.2	4.5	3.6
	Freshness	4.6	5.0	4.4	4.4	5.0	4.3	4.7	4.5	4.6	4.4
	Quality of bitterness	4.0	4.4	4.0	4.4	4.7	3.2	4.5	4.3	4.0	4.6
	DLG scores[a]	39.5	46.5	36.8	44.8	46.5	24.2	39.6	37.6	35.9	36.6
Brew series 2	Purity of odor	4.3	4.5	4.2	4.6	4.8	3.7	3.3	4.4	4.3	4.3
	Purity of taste	4.5	4.6	4.5	4.6	4.5	3.8	3.1	4.1	4.1	4.4
	Palate fullness	4.4	4.5	4.5	4.7	4.6	4.0	4.0	4.0	4.3	4.0
	Freshness	4.6	4.4	4.6	4.4	4.6	4.3	4.1	4.4	4.6	4.6
	Quality of bitterness	4.4	4.7	4.8	4.4	4.7	4.0	4.0	3.9	3.9	4.0
	DLG scores[a]	39.2	41.7	40.4	41.4	43.6	30.5	25.9	34.8	35.3	36.3

[a] For calculation of DLG scores consult the materials and methods section

and 2, respectively. Even though the individual concentrations did not exceed their respective odor or flavor thresholds, the hop dosage's effectiveness in diminishing their concentrations was still apparent and the modified hopping technologies used were clearly superior in comparison to the brews where hops was solely dosed at the beginning of wort boiling.

Based on previous studies and literature data, the hops' strong reducing effect may be traced back to the following factors: Firstly, the hop constituents' reactions with iron consequently counteract the ferrous iron's catalytic actions in the Fenton and Haber-Weiss reaction systems thereby diminishing oxidative reactions during wort boiling and in beer. Wietstock and Methner [43] proposed a pathway for the formation of Strecker aldehydes from their parental amino acids by hydroxyl and hydroxyethyl radical attack, and this pathway is accordingly blocked or diminished when radical formation is lowered. This is supported by findings from Wietstock and *Shellhammer* [16] who demonstrated that particularly hop α-acids are capable of abating hydroxyl radical formation by complexing iron ions. Hop α-acids can also react with copper ions thus counteracting radical formation while other metal ions were shown to be unaffected [22] which may add additional effectiveness in counteracting the radical-provoked formation of staling aldehydes.

Secondly, but not less importantly, hop constituents may react with intermediates in the Maillard reaction or block or inhibit oxidative pathways of the Maillard reaction as recently suggested in [63]. This assumption is supported by the observation that the formation of furfural was also affected by the used hopping technology. Furfural can be formed in the Maillard reaction from pentose sugars such as xylose [64, 65] but was also shown by Rakete et al. [66] to be derived through an oxidation-mediated pathway from hexose sugars such as maltose via 3-desoxypentoson as reactive intermediate. Here again, the hop constituents may be capable of blocking or diminishing this oxidative pathway because of their reactions with transition metals.

Complementary to the aldehyde analysis, a sensory analysis of the fresh and aged beers was carried out. The data from the sensorial analysis is depicted in table 8. While no off-flavors were detected in all fresh beers, after storage, the reference beer from the brewing series 1 was clearly down-rated and off-flavor impressions were described as 'oxidized' as noticeably detected by all panelists. The beers as produced using the modified hopping technologies were also down-rated but all of them were still considered to be 'vendible' (rating > 3).

The fresh beers form brewing series 2 were rated between 4.2 and 4.8 implying that no defects were present. The reference beer was down-rated again after prolonged storage and was marked again with the descriptor 'oxidized' by all panelists. The beer which was produced using mash hopping got also ratings of < 3. This cannot be explained by the experimental data because the aldehydes measured were low, and also no other defects were observed. All other beers (DIV, FWH, CON) were rated lower than the fresh beers but again were still considered 'vendible'.

In terms of the 'DLG scores' and when comparing fresh and aged samples, it is obvious that REF beers from both brew series showed the most pronounced decrease of the overall sensory attributes (with the exception of MAH from brew series 2) upon ageing whereas the modified hoppings, and in particular the DIV beers, were more resistant against the appearance of staling characteristics.

4 Conclusions

Taking all these data together, clearly, the modified hop dosages were superior in suppressing staling as compared to a beer produced with hops dosed solely at the beginning of wort boiling. The hops effectiveness can be partly traced back but is not necessarily limited to the hop α- and β-acids' ability to suppress oxidative reactions during wort production which, in turn, abates the formation of staling aldehydes. The amount of hops dosed and the points of addition used clearly need to be adapted to the wort matrix to achieve a high efficiency. At this, the impact from the malt used and the availability or 'vulnerability' of metal ions for the interaction with hop α-acids appeared to be from great importance. *Lüers* [67] mentioned in 1950 that the addition of hops to the mash is a 'substantial waste'. This seems to be valid as related to the hop bitter acid utilization; yet, when taking the results from this study into consideration, this statement has to be qualified again as the positive effects on beer flavor stability were not known. Still, high losses have to be taken into account when adding hops to the mash. The continuous hop dosage was found to be best compromise between hop bitter substance yield and improved oxidative stability because only little lower bitter substance yields were found in comparison to the reference brews while the beers were clearly superior as related to their oxidative stability.

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