The Influence of Heavy Metal Ions on Beer Flavour Stability

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ABSTRACT

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In this study, the importance of iron and copper ions and their radical formation via the respective Fenton and Haber-Weiss reactions was confirmed. Of the other heavy metals present in the brewing process in relevant concentrations, the impact of manganese ions on beer flavour stability has been elucidated. In contrast to iron and copper, manganese ions are not removed from wort or beer to any great extent during the process. Additionally, manganese shows a similar radical-promoting effect to that seen with iron and copper. Its reactivity, and typically higher concentration than the other two metals in beer, appear to make manganese an especially potent pro-oxidant in beer. The results of the investigation clearly indicate that there are other heavy metals influencing the stale flavour characteristics of beer, in addition to the well-known metals, iron and copper. In contrast to the aforementioned ions, manganese does not enter the product by being leached out of a tank or from piping materials, but rather comes from the cereals employed in brewing. This finding, concerning the importance of manganese as a redox system in beer staling, can serve as a basis for a different approach in the choice of raw materials.

Key words: chemiluminescence, copper, flavour stability, heavy metal ions, iron, manganese, radical reactions.

INTRODUCTION

Flavour changes occur inevitably in beer during ageing and their nature depends on the type of beer and the storage conditions. An important type of change during beer staling, is caused by aroma active carbonyl compounds, which can be formed by radical reactions[9].

Since the investigations by Fenton[6] published in the year 1894, it has been known that iron ions can catalytically promote oxidative reactions. In 1934, Haber and Weiss[8] found final proof for the formation of radicals in aqueous solutions of bivalent iron and copper ions together with hydrogen peroxide and described the strongly oxidative character of these radicals (Figs. 1 and 2). In the Fenton reaction, iron(II)-ions are oxidized to iron(III) by hydrogen peroxide, forming a hydroxyl radical and a hydroxyl ion. The iron(III) eventually reacts with a further molecule of hydrogen peroxide generating two protons and a superoxide radical. These superoxide radicals react with copper(II)-ions to copper(I) and oxygen in the Haber-Weiss scheme. The copper(I)-ion generated is capable of splitting a hydrogen peroxide molecule into a hydroxyl ion and a hydroxyl radical. The formed radicals from both Fenton and Haber-Weiss schemes are extremely reactive and may give rise to radical chain reactions.

According to Bamforth and Parsons[2], hydroxyl radicals are the most important intermediates in the formation of aged flavour compounds in beer. The use of radical scavengers could improve beer flavour stability. In a later study, Bamforth[1] further ascertained this thesis by the finding that an addition of peroxides and heavy metal ions to beer led to a very rapid development of stale flavour and that peroxides catalyzed by copper ions according to the Haber-Weiss reaction gave rise to the formation of hydroxyl radicals.

Today, it is generally accepted that molecular oxygen is relatively stable and needs to be activated before developing its damaging impact in bottled beer[20]. The degradation of hydrogen peroxide can be considered the last step of this activation, while heavy metals are catalyzing this degradation[16]. Metals can also catalyze the formation of other radicals in beer without the influence of oxygen (e.g. in the formation of fatty acid radicals[18]). Heavy metal ions are therefore of decisive importance for beer ageing.

Iron and copper ions are known to have a negative influence on beer flavour stability. Even concentrations of copper below 50 ppb are reported to cause damage in the final product. The origin of these two metals in beer (Table I) from raw materials, brewing equipment, diatomaceous earth etc. has been well investigated and their losses during the brewing process to spent grains, trub, yeast and so on are also widely known[7,10,11,14,15,17,19,21].

Investigations to date concerning manganese have been focused on its importance as a yeast nutrient. Certain amounts of manganese are absorbed by yeast from the substrate (a decrease of 20–60 ppb during fermentation)[14,17]. Donhauser reports positive effects of higher manganese content in wort on subsequent fermentations[5]. The content of this metal in wort and beer is mainly influenced by the cereal raw materials (wort from wheat malt contains more manganese than wort from barley malt) and during the brewing process, no significant changes have been reported[4,5,14,17].

Even though manganese, iron and copper have related chemical properties as redox systems (Fig. 3), to date, there has been no comparative investigation concerning their effects on beer flavour stability.

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$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\circ} + OH^{-}$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + O_2^{\circ -} + 2\,H^{+}$$

Net:

$$2\,H_2O_2 \xrightarrow{Fe} OH^{\circ} + OH^{-} + O_2^{\circ -} + 2\,H^{+}$$

Fig. 1. Fenton reaction.

$$Cu^{2+} + O_2^{\cdot -} \longrightarrow Cu^{+} + O_2$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + OH^{\cdot} + OH^{-}$$

Net:

$$H_2O_2 + O_2^{\cdot -} \xrightarrow{Cu} OH^{\cdot} + OH^{-} + O_2$$

Fig. 2. Haber-Weiss reaction.

MATERIALS AND METHODS

In pilot plant experiments, heavy metals were added at different steps of the brewing process (kettle-full wort, cold wort and bottled beer). Heavy metal concentrations were monitored by atomic absorption spectrometry with induction-coupled plasma (AAS-ICP). Samples were taken from kettle-full wort, cold wort, beer in maturation and bottled beer. Flavour stability characteristics were assessed both by chemiluminescence determination of radical reactions and by using an expert taste panel.

Sensory analysis

The sensory evaluations were carried out in accordance with MEBAK methods[3], using a specialized taste panel for aged flavour with seven members. Some figures do not show the full extent of the scale from 1 to 5. Aging of the beer samples was at 28°C.

Chemiluminescence measurement

According to the chemiluminescence analysis proposed by Kaneda et al.[12], 10 mL of the beer sample, after being degassed by supersonication, was placed in a stainless steel dish (glass dish) and chemiluminescence was measured at 60°C for up to 20 h. Beer was stored at 0°C before being analysed. To record the radiated photons of the sample, a Tohoku Electronic Industrial Co. Ltd. Type CLA-2100 device was applied.

Measurements were carried out on three different batch series of beer. It was not possible to compare the figures quantitatively between the different batch series because the levels of sulphite in the beers were never exactly the same and it is well-known that the chemiluminescence measurement is very easily influenced by these reducing substances. Qualitatively, all base beer batches gave the same results, that means the sequence of beer stability (given by chemiluminescence analysis lag times) was identical. The data presented has thus been taken from one single series of trials representative for the entire set of data.

Table I. Concentrations of Fe, Cu and Mn in malt, wort and beer.

	Fe	Cu	Mn
Malt (mg/100g dm)	0.1–6.0[a] 2.5–3.2[b]	0.3–0.7[a] 0.2–0.3[b]	1.4–1.5[a] 0.8–1.2[b]
Wort (µg/L)	100–270[a] 300–500[b]	20–400[a] 50–100[b]	120–140[a] 70–150[b]
Beer (µg/L)			
Typical	< 200[a] < 20[b]	< 200[a] 20–40[b]	< 200[a] 80–120[b]
Max.	2000[a] 300[b]	1200[b]	350[b]

[a] Range of typical values according to Krüger and Anger.[13]
[b] Preliminary analysis results in brewery.

$$M^{(n+1)+} + LH \rightarrow M^{n+} + L^{\cdot} + H^{+}$$

$$M = \text{Metal (Fe, Cu, Mn, Ni, Co)}$$

$$LH = \text{unsaturated fatty acids}$$

Fig. 3. Direct formation of lipid radicals by metals.

Measurement of sulphur dioxide in beer

For every sample measured by chemiluminescence, the sulphite content was determined. For this measurement the distillation method according to Analytica-EBC (Section 9, Method 9.25.1) was combined with the sulphite test from Merck (Darmstadt, Germany). The SO_2 purged by the nitrogen gas stream was guided through a pH-neutral solution of Ellman's reagent. At a neutral pH-value, sulphite ions react with Ellman's reagent forming an organic thiosulphate, which can be detected photometrically.

Measurement of dissolved oxygen in bottled beer

The dissolved oxygen in the bottled beer was determined using a Micrologger 3650/113.S model from Orbisphere Laboratories (Geneva, Switzerland).

Measurement of heavy metal ions concentration

Heavy metal ions concentrations were monitored instrumentally using atomic absorption spectrometry with induction-coupled plasma (AAS-ICP). Samples were taken from kettle-full wort, cold wort, beer in maturation and bottled beer. Calibration was carried out by the addition of standards to the sample.

Hazy samples of wort and beer in maturation were clarified by centrifugation for 10 min at 5000 revolutions per min. Beer samples were degassed by ultrasonication for 10 min.

Trials with addition of heavy metals to the bottle

For the addition of heavy metals, distilled water was brought to pH 3 by adding H_2SO_4. Sulfates of Fe (II), Cu

Table II. Addition of heavy metals to beer: experiment 1.

Added metal (µg of metal/L = ppb)	Measured concentration (Standard deviation) Fe	Cu	Mn
Reference beer	26 (1.7)	32 (2.7)	96 (2.6)
30 ppb Fe	54 (6.5)	…	…
60 ppb Fe	66 (21.8)	…	…
90 ppb Fe	76 (3.8)	…	…
30 ppb Cu	…	60 (2.8)	…
60 ppb Cu	…	92 (0.5)	…
90 ppb Cu	…	117 (5.3)	…
30 ppb Mn	…	…	128 (2.7)
60 ppb Mn	…	…	161 (4.0)
90 ppb Mn	…	…	186 (1.4)
20 ppb Fe, Cu and Mn	61 (8.1)	49 (2.2)	118 (1.4)
40 ppb Fe, Cu and Mn	82 (6.4)	69 (2.0)	139 (2.6)
60 ppb Fe, Cu and Mn	98 (4.8)	88 (1.7)	158 (2.2)

and Mn were dissolved in this solution and then added to the empty bottles before bottling (maximum 300 µL per 333 mL bottle). The resulting concentrations are shown in Table II.

Trials with addition of heavy metals to kettle-full and cold wort from the pilot plant

For the addition of heavy metals, distilled water was brought to pH 3 by adding H_2SO_4. Sulphates of Fe (II), Cu and Mn were dissolved in this solution and then added to the wort copper or inline directly after the wort cooler (maximum 200 mL per 500 L batch). The resulting concentrations are shown in Table III.

Process parameters of the pilot plant brews

Mashing: Mashing was carried out during a time of 90 min, starting at a mashing-in temperature of 45°C, heating rates of 1°C per min and rests of 30 min at 56°C, 20 min at 65°C and 15 min at 72°C and mash transfer was at 78°C. The pH was adjusted to 5.5–5.6 and the mashing in ratio was 3.18 L water to 1 kg of malt. Two row spring barley malt of the "Scarlett" variety was employed.

Lautering was carried out with a lauter tun. Boiling time was 60 min at atmospheric pressure, light-stable tetra isomerised kettle extract was used. The pH of knock-out wort was adjusted to pH 5.2–5.3. The original extract of the cold wort was 13.4°P.

The initial fermentation temperature was 11°C, and the temperature was allowed to increase to 15°C at an apparent extract of 6°P. After the total diacetyl content was below 0.07 mg/L the green beer was cooled to a maturation temperature of –1°C. All brews were maturated for 4 days.

The beer was DE-filtered and the extract adjusted to 10°P. The heavy metal ions examined in this study were not detectable in the correction water. Total oxygen content of the bottled beers was in the range of 11 ppb, with a standard deviation of 4.5.

RESULTS AND DISCUSSION

General comments on the interpretation of chemiluminescence curves

The principle of flavour stability assessment using chemiluminescence analysis is the measurement of extremely small light intensities emitted by radical reactions in the beer. The sample is exposed to elevated reaction temperatures and is thus subjected to an accelerated deterioration. During the course of the analysis, which typically takes a couple of hours, the emitted photons are registered by a very sensitive photo electron multiplier as chemiluminescence counts per measuring interval. This measuring interval can be in the range of seconds to minutes and indicates the time during which the shutter of the photo electron multiplier is kept open and the photon counts are summed. The result of this analysis is a chemiluminescence light intensity plot over reaction time. Light intensity is regarded as a measure of the amount of radical reactions per time unit.

The general shape of chemiluminescence curves can be described as an initial increase of the signal during the warm-up phase of the chilled sample to reaction temperature, followed by an immediate decrease to the low light emission rates during the first phase of the aging reactions. This first phase, with low reaction rates, ends as soon as the radical scavengers contained in the beer have been consumed. After depletion of radical scavengers, the curve reaches a turning point and light emission intensity increases. Later, the curve reaches a maximum value, after which the reaction rate decreases again. The interpretation of such curves may either use the turning point or the maximum value as a reference. Both indicate the potential resistance of a sample against radical-mediated oxidation. The longer the time, the higher is the resistance and the better is the potential flavour stability. Both the time to reach the turning point, and the time to reach the maximum, are generally referred to as "lag times", although only the time to reach the turning point represents a "lag time" in the true sense of the meaning. However, we have found that the time to reach the maximum can be determined with higher precision, which is why the "lag time" in this work refers to the time to reach the maximum light intensity. Both versions of "lag-time" gave the same results qualitatively.

Table III. Addition of heavy metals to beer: series 2.

Added metal (ppb)	Measured concentration (Standard deviation) Fe	Cu	Mn
Without	18 (5.7)	36 (2.3)	132 (0.5)
200 ppb Fe	**173 (23.1)**	34 (2.1)	133 (0.6)
200 ppb Cu	14 (2.5)	**203 (12.2)**	129 (0.6)
200 ppb Mn	19 (5.4)	36 (2.7)	**330 (3.2)**

Addition of heavy metals to beer

In Fig. 4, the results of the first series of chemiluminescence measurements are shown. As there were no differences between the reference beer and the beer with the addition of iron, the measurement was repeated using a glass dish instead of the standard stainless steel dish supplied with the instrument (Fig. 5). The differences were clear when both figures were compared. It was assumed that iron was leaching out of the stainless steel dish during the measurement.

The experiments revealed that the impact of heavy metals on the promotion of radical reactions in beer increase from iron over manganese to copper. However,

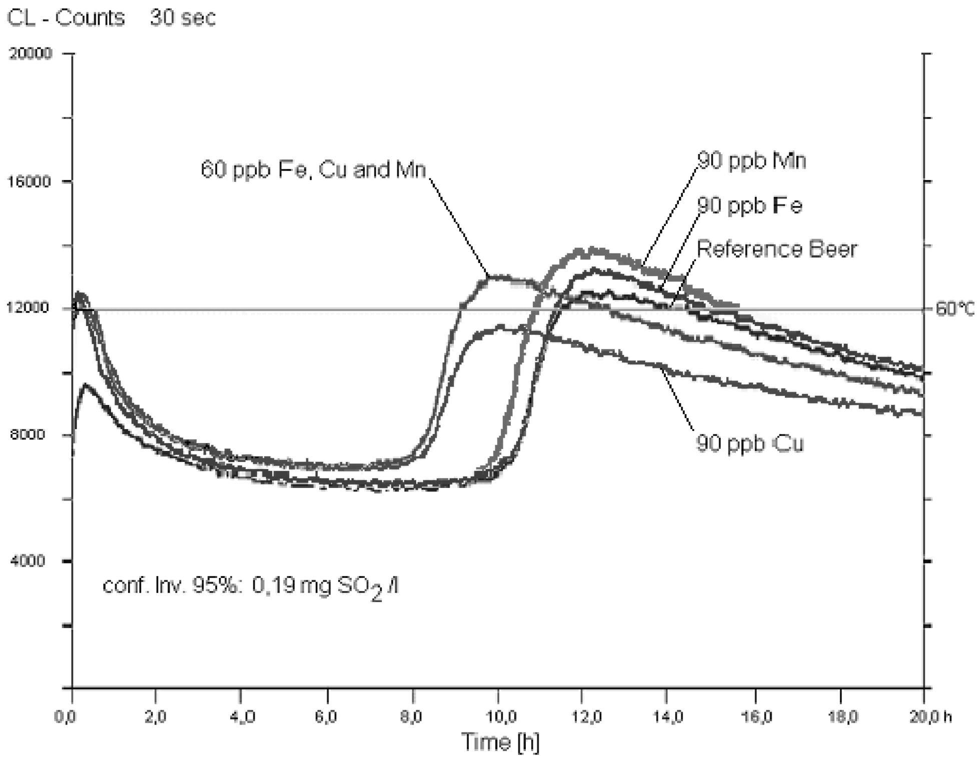

Fig. 4. Results of chemiluminescence measurement: addition of heavy metals to bottled beer (Measurements in a stainless steel dish): series 1.

certain losses of iron have to be taken into account. Probably due to overfoaming during bottling, the addition of iron was less successful than the addition of manganese (Table II) and only a concentration increase of 50 ppb of iron, compared to 90 ppb of manganese, was found in the beer. The results illustrated in Fig. 6 showed no significant differences concerning flavour stability between the different beers. For the beers with the addition of iron, tasters detected a metallic off-taste. The conclusion was that the added amounts were too low to reproducibly detect statistically relevant differences in flavour stability by sensory analysis. This low reproducibility was further illustrated by the relative positioning of the tasting results after four weeks of aging, which were somewhat better than the surrounding values. As a consequence, a second series of trials was initiated with higher metal ion concentrations.

Table III shows the added and the resulting concentrations in the four different beers evaluated in Series 2. Again it was observed that it was more difficult to add iron ions to the beer in a reproducible manner.

In Fig. 7, the results of chemiluminescence measurement are illustrated. The graphic shows that the catalyzing effect of heavy metals on radical reactions in beer increases from manganese over iron to copper. The damaging effect of heavy metals on beer flavour stability was confirmed by sensory analysis. Fig. 8 shows a stronger deterioration of the beers with addition of heavy metals than the reference beer after two weeks of storage. However, the order of magnitude of their catalyzing effect detected by chemiluminescence could not be confirmed by the taste panel. In this figure, "oxidation" refers to the state of staling, while "flavour stability" is the result of a comparison between samples stored at 28°C versus samples stored at 5°C.

Addition of heavy metals to kettle-full and cold wort

As literature values indicate, the three metals iron, copper and manganese occur in an approximate relation of 6:1:2 (Fe:Cu:Mn) in malt, kettle-full and cold wort[13] and our own measurements in Polar's Los Cortijos plant confirmed this relation (see Table I), and this was also applied in this series of trials.

Five different pilot plant brews were carried out and the results compared:

1. Reference beer, without addition of heavy metals
2. Beer with 900 ppb of a mix of Fe, Cu and Mn (6:1:2) salts added to kettle-full wort
3. Beer with 600 ppb Fe(II) salt added to cold wort
4. Beer, with 100 ppb Cu salt added to cold wort
5. Beer, with 200 ppb Mn salt added to cold wort

All other parameters were maintained unaltered. The metal concentrations in wort and beer were monitored at the steps kettle-full wort, cold wort, beer in maturation and bottled beer. The results of brews 1, 2 and 5 are shown in Figs. 9–11. To compensate for the differences in extract, which are part of any normal brewing process, such as evaporation or dilution steps, the results are given in µg metal/g extract. The range of extract concentration varied from knock-out wort to bottled beer between 13°P and 10°P.

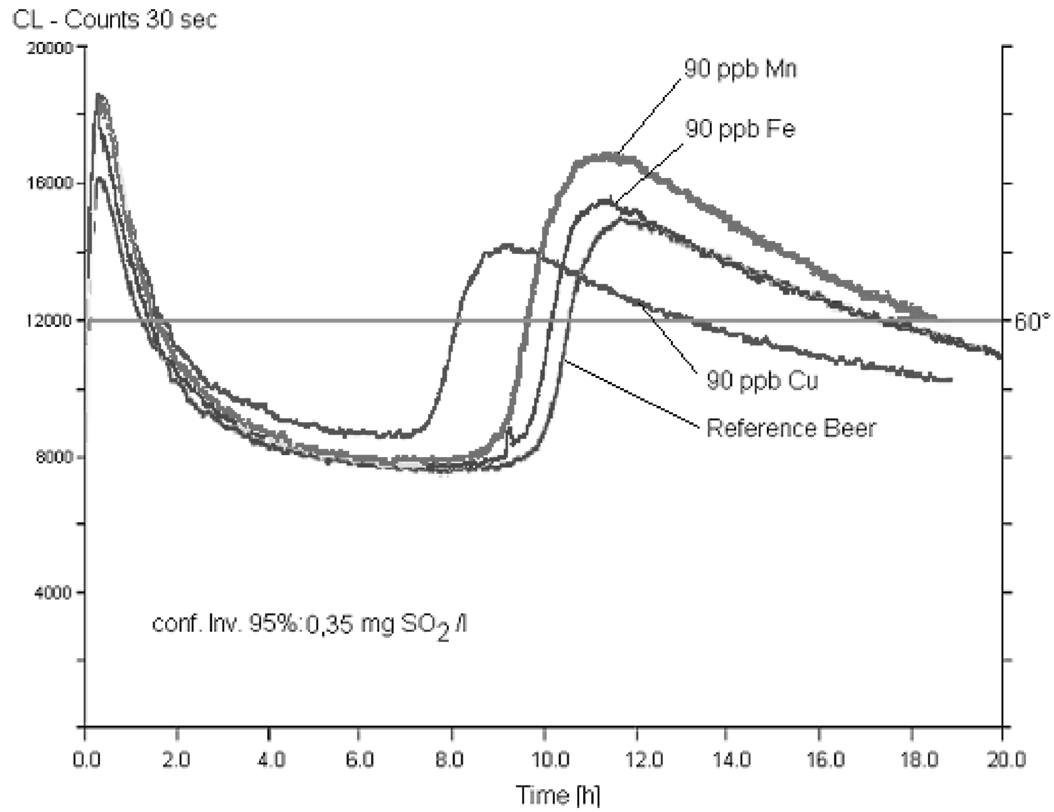

Fig. 5. Results of chemiluminescence measurement: addition of heavy metals to bottled beer (Measurements in a glass dish): series 1.

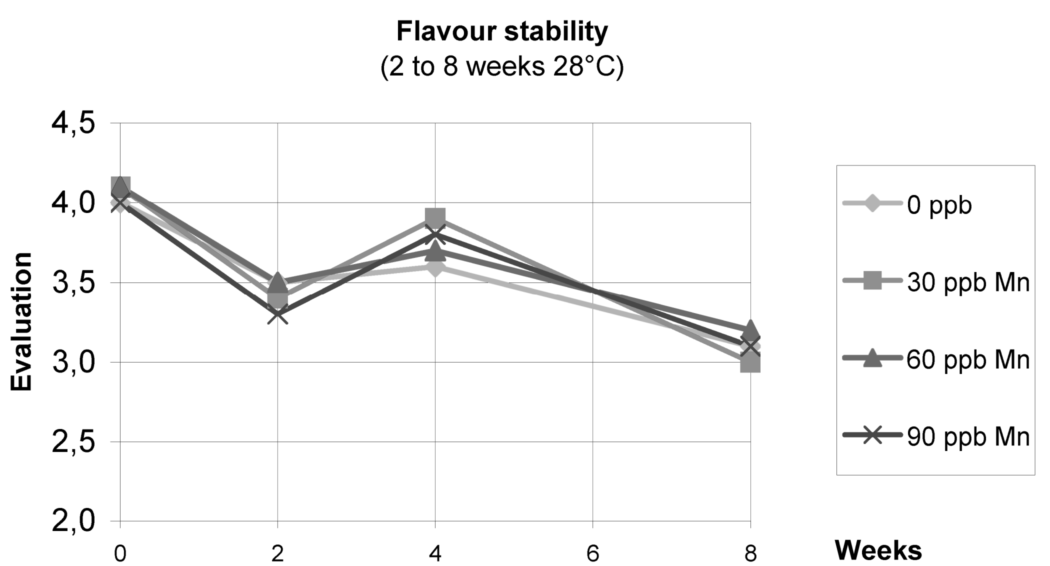

Fig. 6. Sensory analysis of beer with addition of manganese to bottled beer: series 1 (Standard deviations were below 0.4 units).

The absolute values of the metal content in the final beers are shown in Table IV. The extract content of the final beers was adjusted prior to filling. It can be observed that although high concentrations of iron were measured right before the beginning of fermentation, this did not lead to a higher concentration of iron in the final beer. During fermentation, the iron content was strongly diminished. For copper, a reduction of concentration was observed during wort boiling and wort treatment, as well as during fermentation. Only copper addition to the cold wort led to a slightly higher content in the final beer. In contrast, the manganese content declined somewhat

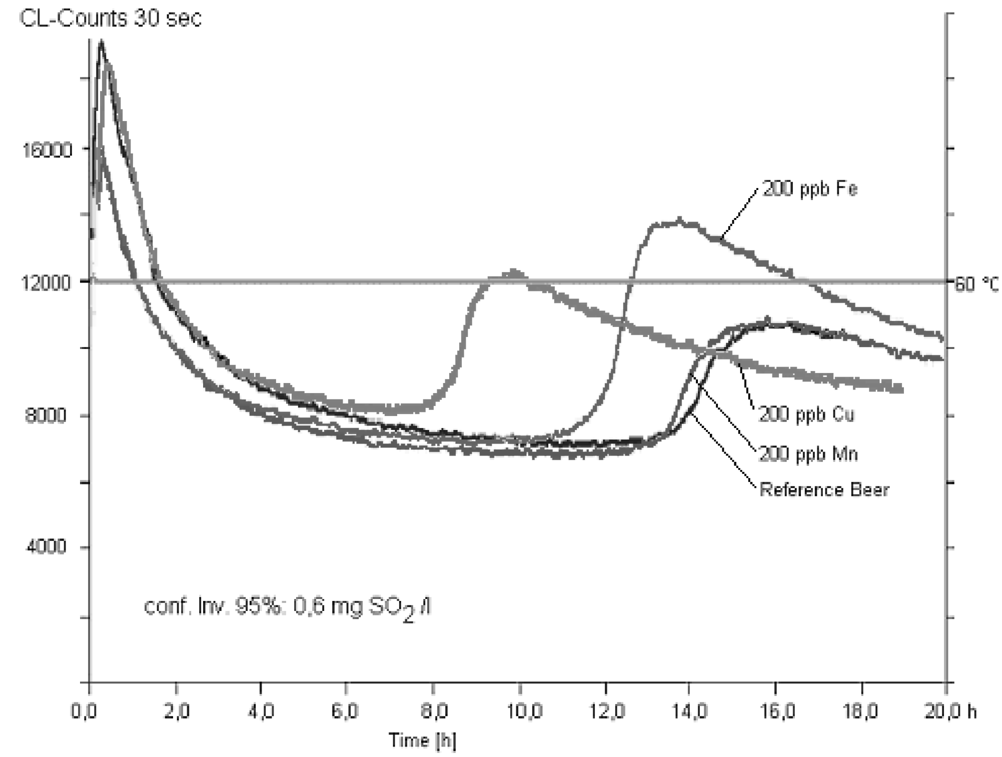

Fig. 7. Results of chemiluminescence measurement: Addition of heavy metals to bottled beer.

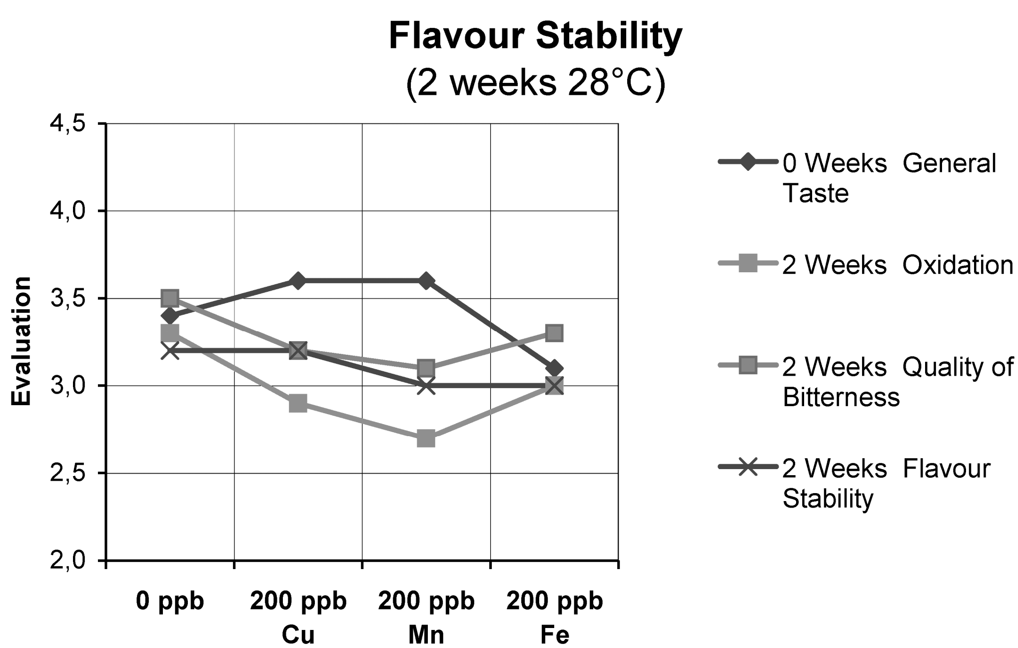

Fig. 8. Sensory analysis of beer with addition of Fe, Cu or Mn to the bottle (Standard deviations were below 0.3 units).

during the brewing process, but a higher content in both kettle-full as well as in cold wort lead to a higher content in the beer. This represents an important difference to iron and to copper.

The comparison of the relation of these metals in wort prior fermentation: 6:1:2 (Fe:Cu:Mn) with the relation found in yeast dry matter according to Lentini et al.[14]: 15:3:1 (Fe:Cu:Mn) reveals that the cellular uptake of manganese is lower than that of the other two metals, even though the amount provided by wort is relatively high. This coincides with our finding that manganese is diminished far less during fermentation than the other two metals.

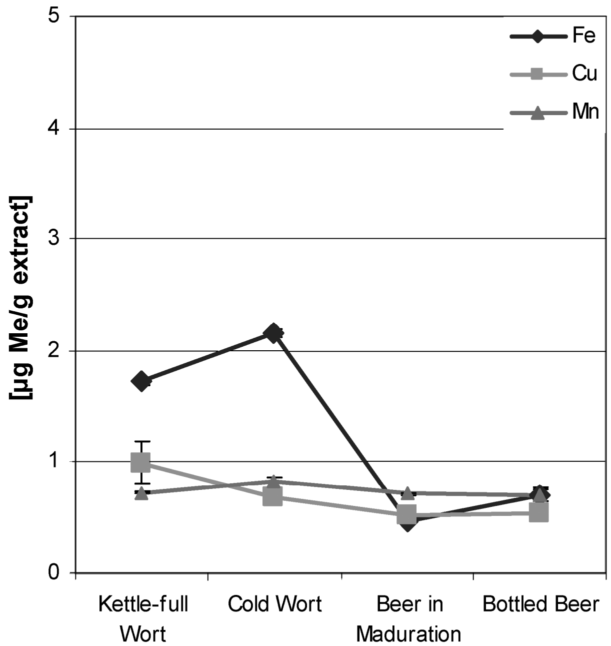

Fig. 9. Monitoring of metal content. Reference beer: without addition of metals.

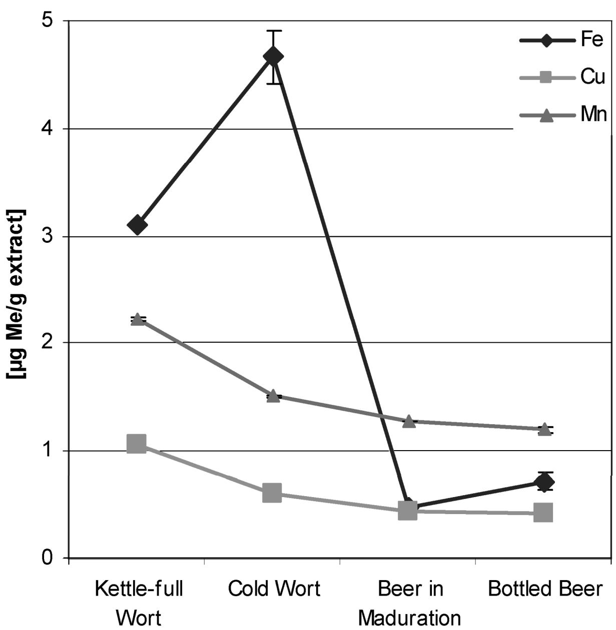

Fig. 10. Monitoring of metal content. Beer with addition of 600 ppb Fe, 100 ppb Cu and 200 ppb Mn to kettle-full wort.

The metal ion concentrations in bottled beer are of fundamental importance for the interpretation of the findings in sensory analysis as well as in chemiluminescence determinations. In summary, it is noteworthy that the iron content remains in the range of the reference beer in all cases, which suggests that the addition of this metal had no impact on the content in the final beer. An addition of 100 ppb of copper to cold wort increased the final beer content only by a quarter of the added amount. The only important increase, of almost half the added amount, was registered in the case of manganese.

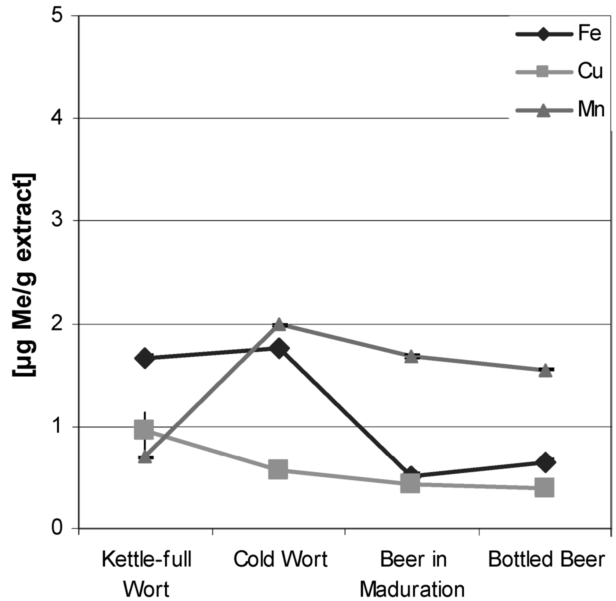

Fig. 11. Monitoring of metal content. Beer with addition of 200 ppb Mn to cold wort.

Table IV. Final concentration of heavy metals in beer (average of five bottles (ppb)).

	Measured concentration in bottled beer (Standard deviation)		
Added metal	**Fe**	**Cu**	**Mn**
Without addition	76 (8.5)	52 (3.6)	72 (5.4)
900 ppb Fe, Cu and Mn to kettle-full wort	76 (8.3)	44 (3.6)	123 (2.1)
600 ppb Fe to cold wort	71 (7.4)	55 (1.6)	73 (1.1)
100 ppb Cu to cold wort	59 (6.5)	76 (1.6)	60 (0.8)
200 ppb Mn to cold wort	67 (3.3)	40 (1.3)	159 (0.9)

During fermentation, no obvious difference between fermentation behaviour of the different brews was observed. Positive effects of manganese addition as reported in literature[5] could not be confirmed.

Directly after bottling, all beers had a very low oxygen content of approximately 10 µg/L and sulphite concentrations in the same range.

The results of sensory analysis after four weeks of storage at 28°C are not shown in a separate figure but can be summarized as follows:

- Beer with the addition of iron or copper to cold wort showed similar flavour stability to the reference beer.
- For beer with addition of manganese to cold wort, an accelerated deterioration was observed and a sherry aroma appeared after four weeks of storage.
- Beer with the addition of a mixture of iron, copper and manganese to kettle full wort was evaluated as strongly oxidised after two weeks of aging at 28°C.

The results of chemiluminescence measurements (Fig. 12) coincided well with sensory analysis and especially with the detected levels of heavy metals in the respective beers. It was observed that the beer with added manganese to cold wort and the beer with the addition of all metals to kettle-full wort both showed a faster oxidation than the other trials. The higher manganese content (see

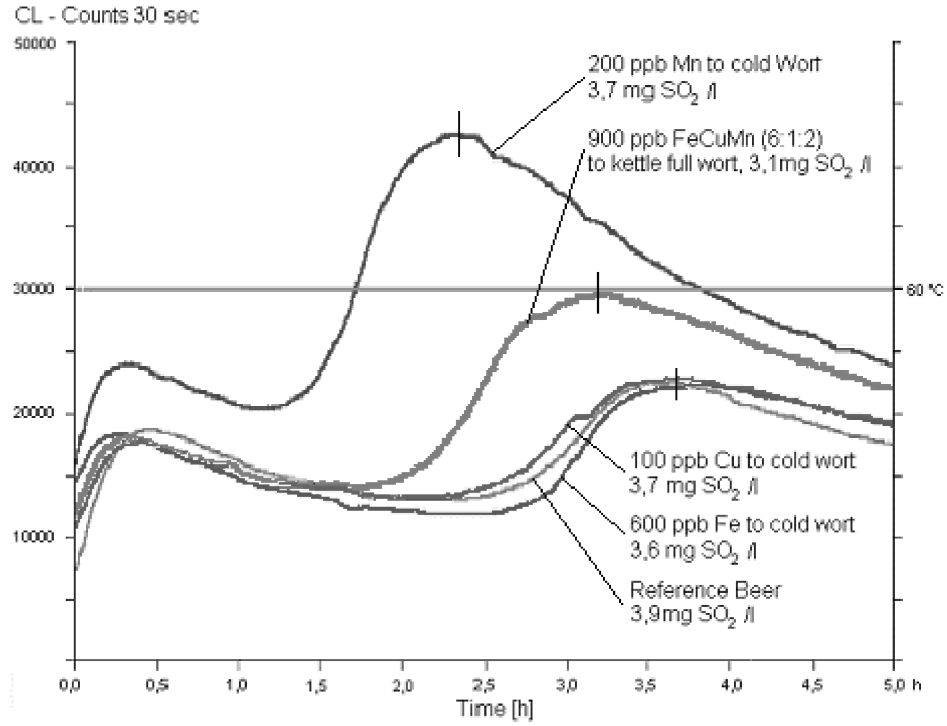

Fig. 12. Results of chemiluminescence measurement. Addition of heavy metals to kettle-full and cold wort.

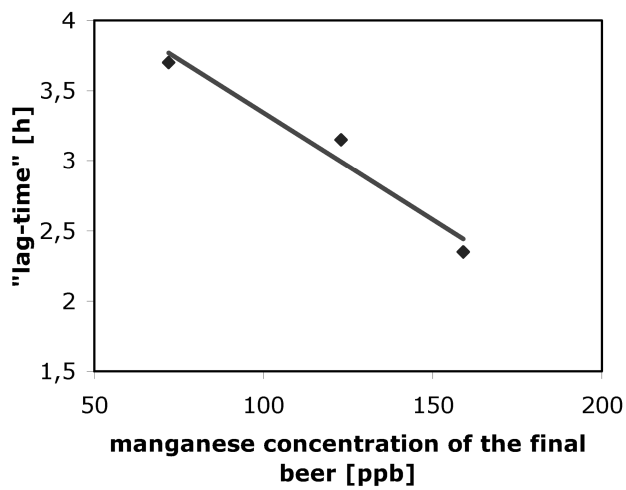

Fig. 13. Correlation of "Lag-Time" and concentration of manganese (left: reference beer; right: beer with addition of 200 ppb of manganese to cold wort; centre: beer with addition of 900 ppb of heavy metals (Fe:Cu:Mn 6:1:2) to kettle full wort). "Lag-Time" of beer with addition of all three metals (centre) to kettle full wort correlates in the same way with the manganese concentration as in the other two trials.

Table IV) in the final beer lead to an earlier increase of radical reactions in the beer, corresponding to a shorter "lag time". Fig. 13 shows an excellent correlation between manganese content of the final beer and the corresponding "lag-times".

As already mentioned, the copper added to kettle full wort was removed during wort boiling and iron was entirely removed during fermentation. This suggests that both heavy metals can only play a role in radical formation during wort boiling, and in the case of iron additionally during the early stages of fermentation. Based on the excellent correlation of manganese content in beer with chemiluminescence "lag-time", it can be assumed that iron and copper, coming from the raw material side, have relatively little influence on flavour stability of the final beer. The shorter "lag-time" of the beer with addition of all three metals to kettle full wort can be explained by the higher manganese content.

CONCLUSIONS

The results of this investigation can be summarized as follows:

- The effect of added heavy metals to beer on resistance against radical reactions decreases from Cu > Fe > Mn.
- Additions of manganese in amounts as small as 90 ppb shorten the "lag-time" in chemiluminescence measurement.
- The copper content was mainly reduced during wort boiling and trub removal.
- High losses of iron were observed during fermentation.
- Higher manganese content in wort always led to a higher manganese content in the beer.
- There was no negative influence due to higher concentrations of iron and copper ions in kettle-full wort, up to threefold of normal, on the final beers' chemiluminescence behaviour (resistance against radical reactions).
- The use of a metal plate for chemiluminescence measurement has an influence on the result, probably influencing the iron content of beer.

Even though the impact of a certain amount of manganese on flavour stability is not as strong as in the case of iron and copper, manganese typically occurs in higher concentrations in the final beer than the other heavy metals due to its stability during the brewing process. This suggests manganese is an important factor in beer oxidation. Since the manganese content of the final beer is determined by malt, further investigations will focus on whether the barley type or the malting and mashing parameters influence its concentration in the final beer.

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