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Yeast uptake of iron, copper and manganese and the subsequent impact on the flavor stability of beer

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Introduction

Brewers spend a significant amount of time and effort ensuring that their product meets the desired flavour profile. However, during the time it takes for the product to reach the consumer this profile can change, either through the loss of positive attributes or the development of stale characteristics. The mechanisms of flavour instability are the subject of significant current research and involve both oxidative and non-oxidative routes. Metal ions can catalyse the oxidative instability of beer by acting as pro-oxidants within the system. Molecular oxygen, itself relatively unreactive, can be activated by the action of transition metal ions which act as electron donors. The ability to optimise the brewing process in a way which limits flavour deterioration is hampered by the time required for these changes to take place. Therefore a need exists to accurately predict these flavour changes before they happen. One method that is suggested to accomplish this, and is used by many breweries, is the measurement of free radicals in beer using Electron Spin Resonance (ESR), also known as Electron Paramagnetic Resonance (EPR).

In the present research we applied the EPR forced aging assay to investigate the direct impact that these key metal ions have on the formation of Reactive Oxygen Species (ROS) in a lager beer. The most common metrics extracted from this assay are the lag-time and EPR signal intensity at a designated time point. These are used to describe the antioxidant potential and oxidative stability of the beer, respectively.

Metal ions may impact the flavor at any point in the process, but particularly in package, provided there is oxygen present. Although there are substantial amounts of all of these metals in malt and hops, a relatively small proportion is carried through to wort. Yeast is responsible for the removal of a proportion of metal ions during fermentation, depending on their bioavailability, whilst compromised yeast may also leak metals back into the system. Metal ion pickup may also occur subsequent to fermentation, such as during kieselguhr filtration. In this research we utilized small-scale fermentation systems to screen several brewing yeast strains, both *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, and demonstrate the difference between their ability to sequester metal ions during fermentation. We compare this data with the oxidative stability assessment of the resulting beers and postulate the relative importance of this sequestration on the metrics discussed above.

Methods

Oxidative stability assay

EPR was utilised to assess the oxidative stability of beers. The spin trap POBN was used, based on the concentration and EPR measurement parameters of Kunz *et al*. 2013, using an E-scan (Bruker, USA). The assays were completed at 60 ° C, for at least 450 minutes with 16 measurements per sample. Curves were fitted to the data based on a five parameter logistic curve, determined using GraphPad Prism 6 software. The T450 is the value of the curve after 450 minutes. The lag-time was determined to be the time at which the EPR intensity was 12 % that of the difference between the top and bottom asymptotes of the predicted curve.

Impact of metals on the oxidative stability assay

To investigate the impact of individual metal ions on the oxidative stability assay a standard 5.5 % v/v lager, 3 weeks old, was obtained. It had initial levels of 57 ppb copper, 26 ppb iron and 119 ppb. Ammonium iron(II) sulfate hexahydrate, Copper(II) sulphate pentahydrate and Manganese(II) chloride tetrahydrate solutions were added directly before the assay commenced to obtained elevated levels of the iron, copper and manganese, respectively.

Total metal quantification

Samples were acidified with the addition of HNO_3, to achieve a final acid concentration of 2 %. Analyses were carried using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500cx) using collision cell mode (He-gas).

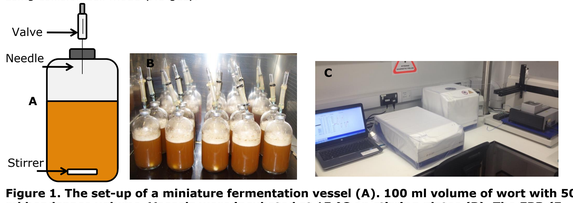

Figure 1. The set-up of a miniature fermentation vessel (A). 100 ml volume of wort with 50 ml headspace volume. Vessels were incubated at 15 °C on stirring plates (B). The EPR (E-Scan, Bruker) used for the oxidative stability assay is also shown (C).

Fermentations

A total of 13 industrial brewing yeast strains with varying responses to oxidative stresses (determined in a previous study) were selected, 11 *Saccharomyces pastorianus* and two *Saccharomyces cerevisiae*, to investigate the impact that yeast strain has on metal content and the oxidative stability of beer. Miniature (100 ml volume) fermentations were completed (Figure 1), using the same 1.060 OG (70/30 malt adjunct) 15 IBU wort. All strains were pitched at 1.5 x 10^7 cells per ml and fermented at 15 ° C, with each strain being completed in triplicate. Fermentations were monitored by the weight loss of the vessels and were ended when this plateaued.

Results

The impact of metal ions on the oxidative stability assay

Figure 2 shows the impact of additional metal ions on the lag time and T450 of a standard lager. The greatest impact on lag time was achieved with copper, followed by iron, with manganese having no significant impact. Manganese also didn't impact upon the T450 value, whereas iron increased it. Copper, however, reduced this value. As a result the method of lag time calculation, this reduction in T450 will have contributed to the greater reduction in lag time seen when copper is increased. Figure 3 shows the assay curves from which these metrics were derived.

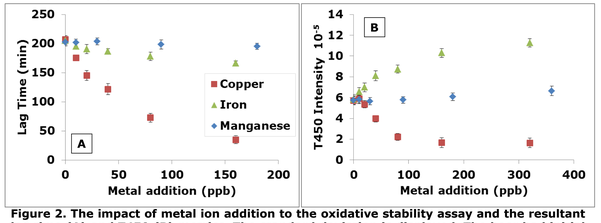

Figure 2. The impact of metal ion addition to the oxidative stability assay and the resultant lag time (A) and T450 (B) metrics. The standard deviation is displayed. The beer had initial concentrations of 57 ppb copper, 26 ppb iron and 119 ppb manganese, and the amount added in addition to these are shown.

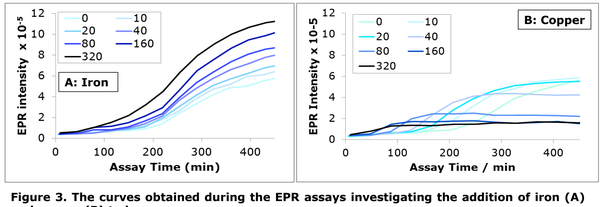

Figure 3. The curves obtained during the EPR assays investigating the addition of iron (A) and copper (B) to beer.

Fermentations

12 of the yeast strains fermented the wort satisfactorily, with only strain D failing to reach attenuation (Table 1). This strain was not analysed further. The impact that yeast has on the oxidative stability of beer is evident in Figure 4, where many different EPR curves have been generated from the same initial wort. Some produced a sigmoidal curve (those displayed in color) to which a lag time could be determined within the assay time (0-450 min). Several curves were not sigmoidal within the assay time and lag times could not be determined.

Iron and copper have been shown to impact the oxidative stability of beer, measured by ESR. The concentration of metal ions in the beer produced was measured, with the reduction in metal content attributed to the yeast. The wort initially contained 135 ppb manganese, 59 ppb copper and 85 ppb iron. The majority of the manganese remained in the beer, with only 14 – 26 % being removed from the system. Between 47 % and 85 % of iron and 40 % and 83 % of copper was removed, depending on the yeast strain. There was a strong correlation between the removal of these two metals by the yeast (R^2=0.92). There was limited correlation between the final concentration of either metal and the T450 value. The correlation between the lag time value and metal concentrations was also poor, although this may be linked to the low number of lag times determined (5 out of 12).

Table 1. The Strains used in this study, their species, the time taken to reach attenuation, the final ethanol concentration and the pH (the mean and standard deviation of triplicate samples is shown).

Strain ID	Species	Attenuation (days)	Ethanol (% v/v)	pH
A	*S. pastorianus*	5	7.7±0.1	3.9±0.0
B	*S. pastorianus*	5	7.7±0.1	3.8±0.0
C	*S. pastorianus*	7	7.5±0.1	3.8±0.0
E	*S. pastorianus*	7	7.8±0.1	3.7±0.0
F	*S. pastorianus*	6	7.4±0.4	3.9±0.1
G	*S. pastorianus*	8	7.9±0.1	3.8±0.0
H	*S. pastorianus*	5	8.0±0.3	3.9±0.0
I	*S. pastorianus*	5	8.1±0.0	3.9±0.0
J	*S. pastorianus*	8	7.5±0.1	3.7±0.1
K	*S. pastorianus*	6	7.5±0.1	3.9±0.0
L	*S. pastorianus*	5	7.5±0.1	3.9±0.0
M	*S. cerevisiae*	9	7.2±0.1	3.9±0.0

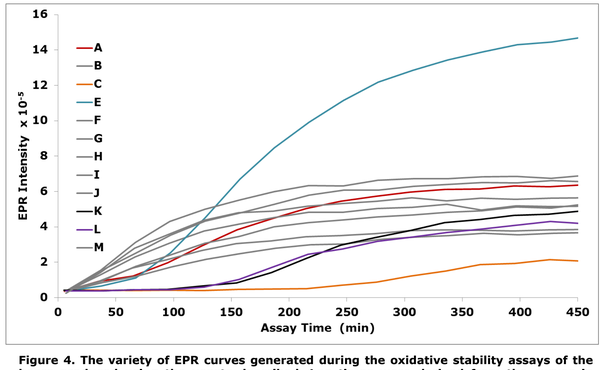

Figure 4. The variety of EPR curves generated during the oxidative stability assays of the beers produced using the yeasts described. Lag times were derived from the curves in colour and these values, along with the T450 (for all assays), can be found in Figure 5.

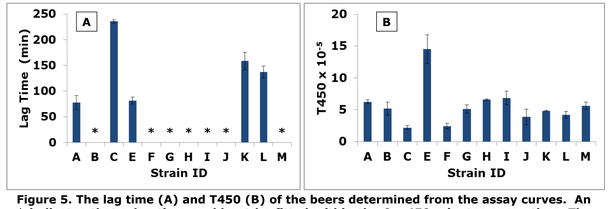

Figure 5. The lag time (A) and T450 (B) of the beers determined from the assay curves. An * indicates that a lag time could not be fitted within the 0 - 450 minutes assay time. The standard deviation is displayed.

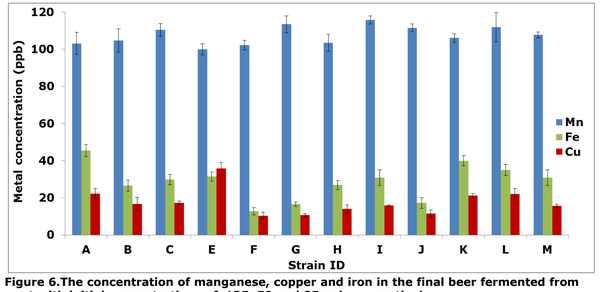

Figure 6.The concentration of manganese, copper and iron in the final beer fermented from wort with initial concentrations of 135, 59 and 85 ppb, respectively.

Conclusions

Metal ions have long been associated with reduced beer oxidative stability and increased staling (Kaneda *et al*. 1992). In this study we have clearly demonstrated that copper and iron impacted the oxidative stability assessed by EPR. They both reduced the lag time metric, thought to be an indicator of antioxidant potential. Iron also reduced the oxidative stability of the beer, indicated by a greater T450 intensity. These observations are in keeping with the theory that the two metals promote Fenton reactions, resulting in free radical generation. However, added copper reduced the T450 value in the beer trialled which would appear to be at odds with the current knowledge. Whether this is an anomaly of the beer, a facet of the assay or an intriguing observation in respect to beer flavour stability is unclear and is currently being explored further. Manganese was shown to have little impact on either EPR metric and, despite being present in much higher concentrations in beer, appears to be of less concern for brewers concerned about flavour stability.

The ability of yeast to remove metal, particularly iron has previously been linked to the production of more oxidatively stable beers (Berner and Arneborg, 2011). By varying the yeast strain we were able to demonstrate the important role it plays in determining the oxidative stability of the resulting beer. However, a clear correlation between the removal of metal and the lag-time or T450 values was not observed, suggesting that other yeast actions have more significant roles in the oxidative stability of beer, with SO_2 production (which was not measured in this study) likely to be a dominant factor.

References

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Acknowledgments

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