invited paper

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# A Challenge in the Study of Flavour Instability

**There has been a substantial focus over many years on the potential impact of upstream ingress of oxygen on the ensuing flavour stability of the beer produced from the wort. There have been conflicting conclusions, ranging from those who believe the effect is substantial to those who believe that upstream oxidative events are of little significance for the shelf life of beer. Here we have made a theoretical analysis of the likeliest destination for oxygen in mashes, focusing on the kinetics of enzymic and non-enzymic reactions. We suggest that a focus on kinetic modelling of events throughout the process should be taken by researchers, recognizing that it is the rate with which staling reactions occurs that is of most significance as it speaks to the rate at which flavour change is observed in beer, the latter being of more significance than the extent to which it occurs.**

Descriptors: enzymes, flavour stability, mashing, oxygen, reaction rate

## 1 Introduction

There has been increasing attention over recent decades to the consumption of oxygen during sweet wort production [1, 2]. The reaction of oxygen with wort components leads to an increase in colour, reduced rates of wort separation, a reduction in polyphenol levels and of haze-forming polypeptides emerging in sweet wort, an increase in turbidity and a decrease in the level of measurable thiol substances [3]. It has also been claimed that oxidative reactions occurring in the mash are to the detriment of the flavour stability of the final beer [4], although there are those who refute this notion [1]. In this discussion paper we theorize on the extent to which upstream oxidation may or may not have significance and we propose the most worthwhile strategy with which to address future research into the flavour instability of beer.

## 2 Discussion

In respect of this issue, there have been various explanations for why upstream oxidation **may** lessen the shelf life of beer.

- There is an oxidation of precursors originating in the grist, with unsaturated fatty acids claimed by many to be of most significance [5]. Such oxidation may be enzyme-catalysed [lipoxygenase, 6] or non-enzymic, effected through reactive oxygen species [7]. It is argued that the staling molecules become attached to binding agents as adducts, notably amine groups in polypeptides [8], these adducts emerging into the finished beer and progressively releasing the staling compounds over time. Indeed, some argue that these oxidation and binding reactions occur in malting, hence the advent of lipoxygenase-free malts [9].
- There is a removal through oxidizing reactions of antioxidant molecules, notably polyphenols, thereby lessening the antioxidant potential of the finished beer [10].
- Oxygen is consumed in certain reactions in the mash with the production of oxidized intermediates and these are the agents that carry the oxidizing potential into the finished beer, where they exert their effect through oxidizing precursors in the production of off flavours [11].

Leaving aside the relative significance of each of these mechanisms, it is generally agreed that it makes sense to minimize oxygen uptake in sweet wort production. Even if there is no sizeable impact on the flavour stability of the finished beer, it is hard to argue that deliberate introduction of oxygen at this stage is in any way beneficial.

The amount of oxygen entering into a mash will depend on a number of factors [12]:

- The amount of oxygen dissolved in the water used to mash-in, which in turn will depend on the ionic composition of the water and on the temperature, with less oxygen being dissolved as the salt content and temperature increase.
- The amount of air trapped in the milled grist
- The opportunity for oxygen to get into the mash, which in turn will depend inter alia on the extent of agitation, the surface area of the air-liquid interface, the number of transfers and the robustness of pumps.
- The use of any blanketing strategies, for example mashing under an inert gas atmosphere.

Assuming, however, that despite all precautions there will be some degree of oxygen availability for reactions with mash components, the question is begged: what is the likeliest destiny for this oxygen?

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Accordingly, in this brief theoretical paper we compare some of the reactions, both enzyme-catalysed and non-enzymic, through which oxygen might be scavenged in mashes

## 2.1 Enzymes in mashing that consume oxygen

Table 1 lists the enzymes of which we are aware that are present in malt and which in theory might have a role to play in scavenging ground state oxygen.

*Bamforth* et al [16] presented an estimation of the significance of thiol oxidase in the consumption of thiol groups and therefore oxygen in mashing, based on a measured specific activity for the enzyme extract at pH 5.0 of 0.015 µmol cysteine oxidized per min per mg of protein when measured at 30 °C. At a protein concentration of approx. 4 mg/mL in a mash, the thiol oxidase potential would be of the order of 0.06 µmol/mL/min. Assuming that 65 % of the activity survives mashing at 65 °C and assuming that the reaction occurs two times faster for each rise in temperature of 10 degrees Celsius, then Bamforth et al [16] suggested a potential thiol oxidation rate of 0.5 µmol/mL/min in mashing. *Stephenson* et al [3] measured thiol levels in mashes on the order of 0.05 µmol/mL. Thus it was inferred that the enzyme potential exceeds the amount of substrate available and that it will not be the enzyme but rather the sunbstrate that will be in limiting quantities. It begs stressing that there is a decrease in the level of thiol oxidase in malt during post-kiln storage [16]. Indeed it was suggested that this event forms the scientific explanation for storing malt prior to mashing: the theory is that thiol oxidase oxidizes the amino acid cysteine (cys-SH), with the product cys-S-S-cys in turn oxidizing the sulfhydryl groups in gel proteins with the resultant cross-linking contributing to teig formation and the ensuing reduction in rates of lautering.

*Kanauchi* et al [15] made a similar evaluation for oxalate oxidase but concluded that this enzyme is far less relevant than thiol oxidase in scavenging oxygen from mashes, calculating that oxalate oxidase could remove all of the oxygen in 20 minutes at conversion temperature whereas the thiol oxidase would in theory eliminate oxygen instantly if there is sufficient of the other substrate.

Considering ascorbate oxidase [17], we measured a level of 5 units per g malt. One unit oxidizes 1 mM substrate (ascorbate or oxygen) per minute at 25 °C. This is measured in the assay at pH 7 but activity is only 40 % of this level at typical mashing pHs. The enzyme (there are two isoforms) is rather heat tolerant and 50 % will survive 30 minutes of mashing at 70 °C. Based on an approximation that the enzyme will work 10 x faster at conversion temperature (according to Arrhenius) and assuming a water to grist ratio of 3:1 then we can see that the ascorbate oxidase level in a mash is going to be capable of removing oxygen at a rate a little higher than 5 mM per minute. Compare this with an estimate that a mash might contain less than 0.1 mM oxygen. I.e. if there is sufficient ascorbic acid, then this enzyme alone will have a voracious appetite for oxygen. The $K_m$ for ascorbic acid of this enzyme is 0.35 mM for one isoform and 3.25 mM for the other. Taking the first value then a concentration of 5 mM ascorbic acid would saturate the enzyme and the latter would be functioning at its maximum rate.

*Kaukovirta-Norja* and colleagues [18] in studying lipoxygenase in model mashes showed that significant loss of oxygen occurred only if additional linoleic acid was introduced. This confirmed the prediction of *Biawa* and Bamforth [13] that the level of linoleic acid in a mash was insufficient to support activity of lipoxygenase.

## 2.2 Non-enzymic consumption of oxygen

Of course, oxygen may also enter into non-enzymic reactions in the mash. Amongst these the reactions likely to be of most relevance are of oxygen with

- Metal ions, notably iron, copper and manganese, thereby setting in train the production of reactive oxygen species [ROS, 19].
- Polyphenols [20]
- Thiols [11]

In a previous theoretical discourse on these types of issue, Bamforth [12] discussed the rate constants for various reactions involving metal ions in the production of ROS. Drawing attention to the limited reactivity of ground state oxygen, it seems most relevant to consider the production of species such as superoxide (especially its protonated from perhydroxyl), peroxide and hydroxyl as intermediates in the routes by which oxygen reacts with other molecules, including polyphenols and thiols. Metal ions such as iron, copper and (it is now realised) manganese have a role to play in these processes [7]. Alternatively, as we discussed at length earlier, it is the enzymes using ground state oxygen as

**Table 1 Oxygen consuming enzymes in mashing**

| Enzyme | $K_m$ values | pH range | Thermotolerance | Reference |
|---|---|---|---|---|
| Lipoxygenase | 0.13 mM for oxygen, 0.31 mM for linoleic acid | Km for oxygen decreases as the pH is lowered from 5.5. to 4.5 | Destruction within 5 minutes at 65 °C | 13, 14 |
| Oxalate oxidase | 0.46 mM for oxygen, 0.1 mM for oxalic acid | | 70–80 % survival for 2 h at 70 °C | 15 |
| Thiol oxidase | – | Max activity at pH 8.0. Approx. 10 % of this activity at mashing pH | 50 % survives 30 min at 80 °C | 16 |
| Ascorbate oxidase | 0.39 mM for oxygen, 0.35 mM for ascorbate (lower MW enzyme) | 50 % of maximum velocity at pH 5–5.5 | 40–50 % survives heating at 70 °C for 2 h | 17 |

a substrate that will be the other route by which ground state oxygen is eliminated.

### 2.3 The comparative significance of enzymic and non-enzymic consumption of oxygen in mashes

The question is begged, therefore, is it enzyme catalysis or non-enzymic reactions that primarily remove oxygen from mashes? Bamforth [12] calculated that the theoretical rate of the iron-catalysed conversion of oxygen to superoxide in a mash is 3.2 mM per second. Comparing this to the rate of oxygen consumption estimated for ascorbate oxidase (above) then we can see that the potential for the non-enzymic removal of oxygen is perhaps an order of magnitude greater than the enzymic approach. However by their very nature these calculations can only be an approximation. The very fact that we can demonstrate the very real benefit of ascorbate additions to a mash in terms of protecting against oxidation [17] indicates that this enzyme-catalysed reaction is capable of scavenging at least a proportion of the oxygen. The estimate for the iron-catalysed consumption of oxygen was based on an iron level of 0.1 mg/L, which is likely to be vastly higher than is present in the mash. Furthermore the estimate is based on the assumption that there is unrestricted access of the iron to oxygen, which is highly unlikely to be the case, for the majority of any iron in the mash is likely to be adsorbed within particles and/or chelated. The probablility, therefore, is that the non-enzymic consumption of oxygen is actually occurring at a substantially lower rate than that theorised previously [12].

### 2.4 Conclusions: A challenge

Which leads to a challenge to future researchers addressing the topic of flavour instability. As one of us has emphasized [21, 22] the assessment of flavour stability should not primarily be on the basis of the intensity of staling, but rather the time taken for the first indication that the flavour has changed. We might even misspell the word as “stay-bility”: the extent to which a beer will stay with the desired flavour; in other words how long can the flavour “stay” acceptable? We urge that researchers shift their focus from assessing the intensity of stale character, to one of gauging flavour stability on a basis of time. And in this vein, we suggest that there needs to be far more attention paid to the study of the rates of both non-enzymic and enzymic reactions occurring throughout the brewing process in relation to their impact on flavour instability. The key word is “rate”. We urge a kinetic strategy, with the development of models founded on the types of approach indicated in this paper, followed by experimentation to ascertain the extent of correspondence between measurement and prediction.

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