Making Sense of Flavor Change in Beer

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ABSTRACT

By critical evaluation of published literature and the application of reasoning based on the laws of chemistry and physics, the potential for addressing staling in beer has been assessed. The key factors that determine the flavor life of beer are oxygen content of the packaged product, the level of sulfur dioxide in the beer and the temperature encountered by the beer in storage. It is argued that upstream oxidation, while not unimportant, has perhaps been overplayed. It is important, however, that yeast has the opportunity to 'clean up' any upstream oxidation through its ability to reduce carbonyl species. Adducts between carbonyls and sulfur dioxide are not reduced by yeast, and therefore it seems that the preferred strategy would be one of minimizing SO₂ production by yeast, in particular as this would allow better conversion of acetaldehyde to alcohol. Acetaldehyde has a far greater binding capacity for SO₂ than have the staling aldehydes and its presence interferes with the ability of agents to bind staling substances.

Keywords: staling, oxygen, sulfur dioxide, carbonyl

THE FLAVOR CHANGES THAT OCCUR IN BEER

It seems obvious that for any logical approach to be made to "improving" the shelf life of a beer then the brewer should first identify the undesirable changes in flavor which occur in his/her beer and know what the chemistry is that underpins those changes. Alas, very few brewers have this knowledge. Indeed, few and far between are the published descriptions of flavor changes in beer. Most people refer either directly (or indirectly, via occasional 'cribs' of the original - for example, Fig 1) to the original diagram of Charles Dalgliesh [7]. Dare I question the rigor of the original experimentation that went in to the generation of that illustration? Nevertheless as a pattern of aroma change in beer, it has long since become a part of brewing dogma.

THE CHEMICAL SPECIES RESPONSIBLE

There is no question, however, of the significance of carbonyl compounds (i.e. those substances containing a C=O group in their molecular structure) in the aging of beer. Lustig [11] has named a selection of such compounds that he feels to be of particular significance as determinants of aged character (Table 1). It is likely that this is not an exhaustive list and various workers (see for example Walters [14]) stressed that beer contains a very large number of carbonyl species. Such substances have been associated with the papery or cardboard character most frequently considered as the main feature of aged beer. However flavor notes such as ribes ('blackcurrant buds') and metallic may...
Bitter Taste
Ribes Aroma
Sweet Aroma
Sweet Taste, Toffee-like Aroma and Flavor
Cardboard Flavor

FIGURE 1
The changes in beer flavor with time in package - the Dalgliesh plot.

TABLE 1
The principle determinants of staling as described by Lustig.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methylbutanal</td>
<td>2-furfural</td>
</tr>
<tr>
<td>5-methyl-2-furfural</td>
<td>benzaldehyde</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>diethylsuccinate</td>
</tr>
<tr>
<td>ethylphenylacetate</td>
<td>2-acetylfuran</td>
</tr>
<tr>
<td>2-propionylfuran</td>
<td>γ-nonalactone</td>
</tr>
</tbody>
</table>

TABLE 2
Cleaning up aged character in beer.

<table>
<thead>
<tr>
<th></th>
<th>Stale aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh lager</td>
<td>1.0</td>
</tr>
<tr>
<td>Lager after aging</td>
<td>3.0</td>
</tr>
<tr>
<td>Aged lager + semicarbazide</td>
<td>1.5</td>
</tr>
</tbody>
</table>

1 ml of 100mM semicarbazide-hydrochloride was added to 50 ml beer, either fresh or after aging for 18h at 60°C. Aroma (not taste! – semicarbazide is a poison!) was scored on scale of 1 (fresh) to 4 (extremely stale). Beers were evaluated (blindfolded) immediately.

THE PATHWAYS BY WHICH CARBONYL SPECIES ARE FORMED

Unsurprisingly there have been a host of routes proposed by which carbonyl compounds may arise in beer, of which the most frequently cited are:

- Oxidation of unsaturated fatty acids
- Degradation of iso-α-acids
- Strecker degradation of amino acids
- Melanoidin-catalyzed oxidation of higher alcohols

Moreover, aldol condensation reactions are invoked as an opportunity for different carbonyl compounds to interact to form new, ever more flavor-active ones.

Two essential observations are necessary at this point. First, most of these alleged precursors of aging substances are familiar and necessary components of beer. For instance, eliminating bitter compounds or even higher alcohols is not a practical option.

Secondly, the extent to which these various reactions need to take place in order to generate sufficient carbonyls to be detectable is extremely limited. For example, it has been calculated that, assuming the unsaturated fatty acid linoleic acid to be a precursor of staling carbonyls, only 0.00002% of it would need to be oxidized in order to give a readily detectable cardboard note [2].

The prospects are not good for a brewer being able to prevent a myriad of reactions, each of which needs to occur to only a very limited extent in order to generate readily detectable levels of carbonyl species.

LIPID OXIDATION

Of all the purported pathways leading to staling aldehydes, that which has attracted most attention is the oxidation of unsaturated fatty acids. There has been a degree of controversy concerning whether the non-enzymic autoxidation of unsaturated fatty acids or the enzymic- (lipoxygenase-) catalyzed route is the more significant. The lipoxygenase-route invokes the concept of...

Carbonyl compounds tend to be very flavor-active (i.e. they have low flavor thresholds). Furthermore, it might be anticipated from the broad range of structures of these molecules that they have a diversity of origins. The prospects of being able to minimize the extent to which this spread of molecules is produced are not good. Of greater promise would be any strategy involving the elimination of the staling compounds based on their common feature, namely their carbonyl group. Table 2 summarizes an experiment that demonstrates how carbonyl-binding compounds can virtually instantaneously eradicate stale aroma from beer. The agent employed here (semicarbazide) is of course not a material which could be used commercially in beer. However, sulfur dioxide can act in a similar way (see later).
damaging reactions occurring upstream in the process, viz the early stages of mashing, before this heat-sensitive enzyme is destroyed. The argument is that the products of lipoxygenase action (hydroperoxides) proceed through the process and into the finished beer, there to yield staling carbonyls as they break down with time.

Theoretical analysis, based on extant literature, suggests that oxygen entering into a mash is much more likely to be consumed in reactions other than that catalyzed by lipoxygenase. It is argued that the last of these leads to staling of the ensuing beer. Indeed, it has been suggested that oxidation of polyphenols lowers the endogenous antioxidant potential which would otherwise go forward to protect the beer. Another view is that substances such as the melanoids are converted to oxidized forms, which can proceed to potentiate conversion of higher alcohols to staling carbonyls. Yet nobody is completely satisfied that all, or even any, of these is relevant to beer staling.

OXYGEN CONSUMPTION IN THE BREWHOUSE

There is no doubt that some oxygen is consumed during mashing. It seems that a large proportion reacts with polyphenols, rather less with sulfhydryl groups and, some would have it, a portion with unsaturated fatty acids. It is argued that the last of these leads to staling of the ensuing beer. While it may be fairly argued that the yardstick for quantifying improvement in flavor stability is a notorious variable, and often a badly specified one (viz. organoleptic judgement of aged character), on the basis of this data it can be concluded that the benefits of minimizing oxygen uptake in wort production are marginal, certainly when compared with the importance of minimizing oxygen levels in the finished product. It is most probable that the earliest claims for the benefit of minimizing oxygen ingress in wort production were made at a time when straightforward precautions (e.g. bottom filling of vessels) were often not taken. And so now there are few opportunities for order-of-magnitude improvements over ‘control’ brews in which good practice has become endemic.

Perhaps another of the problems is that the extent to which oxygen is actually taken into wort has been over-estimated: rather less O₂ can access the wort in a production scale brewhouse than in a well-mixed beaker on a laboratory bench.

According to Fick’s Law, the rate of uptake of oxygen into a wort is given by $K_a(C^* - C)$

where $K$ is a constant, $a$ is the ratio of the cross-sectional area at the surface of the wort to the volume of that wort, $C^*$ is the concentration of oxygen in the interface between the atmosphere and the wort and $C$ is the concentration of oxygen already present in the wort.

In turn, according to Henry’s Law, the concentration of oxygen in the interface is given by $K_1 \cdot pO_2$

Where $K_1$ is another constant and $pO_2$ is the partial pressure of oxygen in the atmosphere.

Leaving aside the precise values for these constants and, indeed, for the amounts of oxygen present, I need only highlight the relationship between oxygen uptake rates and the surface area/volume ratio.

For a one-liter beaker with a 10cm wide neck, the surface area will be 78.55cm². Thus the ratio of surface area to volume is 78.55/1000 = 0.0785.

For a hypothetical open mashing vessel 600 cm across, the surface area will be 282,780 cm². Assuming it to be 240 cm deep, then the volume of wort contained may be 57,000,000 cm³. Thus the surface area to volume ratio is 0.005.

Thus the capacity for oxygen uptake is 15-fold greater in the beaker (as might be used in a laboratory experiment) than in the commercial vessel. And this assumes that the latter is open: in reality the access point for air may be nothing more than an open manhole.

I suggest that there is limited scope for dramatic improvements arising from minimizing still further the extent to which air is ‘driven’ in to a mash.

The benefits of restricting oxygen uptake in the brewhouse.

<table>
<thead>
<tr>
<th>Brewery</th>
<th>Precautions</th>
<th>Decrease in staling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Gentle bottom fill – minimum agitation – gentle transfer</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>Gentle bottom fill – deaerated brewing water – CO₂ purge of grist</td>
<td>15</td>
</tr>
</tbody>
</table>

First Table showing the benefits of restricting oxygen uptake in the brewhouse.

There can be no such divergence of opinion about the importance of minimizing oxygen levels in the final container. It has repeatedly been demonstrated that high air levels in final package greatly reduce shelf life. Modern fillers are designed to achieve very low O₂ levels.

**But why does beer filled to very tight oxygen specifications still go stale?**

There are at least three possible reasons:
a. even at oxygen levels as low as 100 ppb, there is enough oxygen to cause the production of staling substances

b. the production of these staling substances is due to substances which 'carry' the oxidizing potential into the beer, such substances having become oxidized earlier in the process: it is they which react with the stale compound precursors in the beer, rather than oxygen per se

c. the staling compounds are already present in the freshly packaged beer, only registering as a flavor defect with time because they progressively become 'unmasked'.

How much oxygen is needed to cause a staling reaction?

Without a definitive understanding of all the chemical reactions that might be involved in furnishing carbonyl compounds to beer, we can only make calculations using the available data. Let us assume that the principle precursors of staling carbonyls in beer are the unsaturated fatty acids, e.g. linoleic acid. A beer might contain 0.15 \( \mu \text{M} \) linoleic acid\(^{11}\). We know that for oxygen to become able to oxidize linoleic acid it must be activated to a radical form such as superoxide and that this activation can be due to metal ions such as iron or copper. Let us assume that our beer contains 0.1 ppm oxygen (3 \( \times 10^{-9} \text{M} \)) and 0.01 ppm iron (0.18 \( \times 10^{-6} \text{M} \)) and has a pH of 4.1.

The relevant reactions with their rate constants are:

\[
\text{O}_2 + \text{Fe}^{3+} \rightarrow \text{O}_2^- + \text{Fe}^{2+} \quad k_1 = 1.3 \times 10^6 \text{ M}^{-1} \text{s}^{-1}
\]

(At pH 4.1, \( \text{O}_2^- \) will be 83% protonated, as \( \text{O}_2\text{H}^- \))

\[
\text{O}_2\text{H}^- + \text{linoleic acid} \rightarrow \text{hydroperoxide (stale compound precursor)}
\]

\[
k_2 = 1.18 \times 10^3 \text{ M}^{-1} \text{s}^{-1}
\]

Rate of activation of oxygen to superoxide =

\[
k_1 (1.3 \times 10^6) \times 3 \times 10^9 \times 0.18 \times 10^{-6}
\]

\[
= 7.8 \times 10^6 \text{ M}^{-1} \text{s}^{-1}
\]

That is, there is the potential for all of the oxygen (3 \( \mu \text{M} \)) to be converted to superoxide in 3/0.7 = 4.3 seconds.

Making the gross assumption that it is thus converted, and that the superoxide formed (which will be 83%, i.e. 2.49 \( \mu \text{M} \), in the protonated form), then the rate of potential linoleic acid oxidation will be \( k_2 \times 2.49 \times 10^6 \times 0.15 \times 10^6 = 0.44 \times 10^{-9} \text{ M}^{-1} \text{s}^{-1} \).

That is the 150 nM linoleic acid would be whittled away in 341 seconds.

Trans-2-nonenal, a principle end product of linoleic acid oxidation, has a flavor threshold of 0.1 ppb. It is usually assumed that a flavor substance is detectable when present at 5 flavor units (1 f.u. = concentration/threshold). 0.5 ppb equates to 3.6 \( \times 10^{-12} \text{ M} \).

Therefore to produce a detectable nonenal character in beer we would need to see only:

\[
\frac{3.6 \times 10^{-12} \times 100}{150 \times 10^{-9}} = 0.0024\% \text{ oxidation of the available linoleic acid}
\]

A great many assumptions went into this lengthy calculation. Let us say that we have overplayed three of the stages 10-fold. That would still mean that just 2.4% of the trace of linoleic acid found in beer would need to be oxidized to give a stale character.

In short, even at 0.1 ppb oxygen there is ample scope for oxidative damage.

But what if the stale compounds are already present?

There is considerable evidence that this is, indeed, the case. The carbonyl group in many aldehydes and some ketones reacts with bisulfite (sulfur dioxide) to form adducts\(^{151}\) (Fig 2) in a manner analogous to that described earlier for semicarbazide (see Table 2). These adducts have a greatly reduced flavor potency. The rationale is that \( \text{SO}_2 \) produced in fermentation (or added for those markets where this is permissible) binds to these carbonyls and renders them flavorless. This would apply to carbonyl substance emerging from raw materials, those developing in wort and beer production and those which arise as a result of deterioration in the packaged beer. It has been proposed that the decrease in concentration of \( \text{SO}_2 \) that is observed in stored beer shifts the equilibrium toward release of carbonyl substances, with an attendant development in aged character. The ‘trick’ therefore would be to avoid \( \text{SO}_2 \) decline.

To what extent can we comprehend this as a rational solution to flavor instability, particularly in markets such as USA where there is a legal maximum of 10 ppm on total \( \text{SO}_2 \)?

Again, we can approach the problem using some broad-brush calculations. These are based on the equation\(^{60}\) that represents the equilibrium depicted in Fig 2:

\[
K = \frac{[S] [X-x]}{[x]}
\]

where:

\( K \) is the apparent equilibrium constant for the dissociation of acarbonyl-bisulfite complex

\( S \) is the concentration of free bisulfite

\( X \) is the total concentration of carbonyl compound in the beer (free and bound)

\( x \) is the concentration of carbonyl-bisulfite complex

\[
\text{FIGURE 2}
\]

Adduct formation in beer.
For any individual carbonyl compound (or group of carbonyl compounds), it is possible to calculate the amount which will be bound up in the complex with bisulfite from the re-arranged equation:

\[ [x] = \frac{[X]}{K + [S]} \]

It will be noted that \( \text{SO}_2 \) (actually, bisulfite because \( \text{SO}_2 \) is 98% in the form of \( \text{HSO}_3^- \) at beer pH) is relatively non-specific in its tendency to react with compounds with aldehyde groups. Hence it will react, for instance, with reducing sugars. The published information on equilibrium constants \( K \) for carbonyl-bisulfite complexes is limited, but for most compounds studied, \( K \) is in the range 1 to \( 5 \times 10^{-4} \) at pH 4.0, though it is lower for sugars (e.g. for glucose it is \( 6.4 \times 10^{-1} \)) and higher for acetaldehyde \( (1.4 \times 10^{-6}) \). The extent to which bisulfite is available for binding those carbonyls which cause aged character in beer will be closely linked to the concentration of competing aldehyde groups, as is depicted in Fig 2.

Let us simplify matters to a hypothetical three component system, one that contains acetaldehyde, glucose and ‘total staling aldehydes’. In the absence of available information, let us assume that the \( K \) for the reaction of bisulfite with this last group is \( 5 \times 10^{-4} \). Taking values of 10 ppm (0.23 mM) for the level of total acetaldehyde in a beer, 0.01% (w/v) glucose (i.e. 0.5 mM) and 200 ppb total carbonyls (a value taken from ref 12).

Then for the beer containing 10 ppm (0.156mM) bisulfite:

\[ K \text{ for the Acetaldehyde-bisulfite equilibrium is } 1.4 \times 10^{-6} \]

Therefore concentration of adduct would be calculated to be:

\[ 0.23 \times 10^{-3} \times \frac{0.156 \times 10^{-3}}{1.4 \times 10^{-6} + 0.156 \times 10^{-3}} \approx 0.23 \text{ mM} \]

\[ i.e. \text{ essentially all of the acetaldehyde would be bound as the bisulfite complex.} \]

\[ K \text{ for the glucose-bisulfite equilibrium is } 0.64 \]

Therefore concentration of adduct would be:

\[ 0.5 \times 10^{-3} \times \frac{0.156 \times 10^{-3}}{0.64 + 0.156 \times 10^{-3}} \approx 0.12 \mu M \]

Thus, the glucose would only bind a tiny proportion of the available bisulfite – and this would apply even if its level were increased 100-fold (and that would be a very sweet beer indeed!)

Assuming a ‘net’ molecular weight for the group of staling aldehydes of 150, then they are present in our hypothetical beer at around 1 \( \mu M \).

Assuming \( K \) is \( 5 \times 10^{-4} \) for the interaction of these carbonyls with bisulfite, then the adduct concentration will be:

\[ 1 \times 10^{-6} \times \frac{0.156 \times 10^{-3}}{5 \times 10^{-4} + 0.156 \times 10^{-3}} = 0.24 \mu M \]

That is, if all of the bisulfite were available to this population of aldehydes, then only one quarter would be bound in the adduct form. But it isn’t all available – and in fact the much higher concentration of acetaldehyde will ‘squeeze’ these carbonyls out. We would predict that a beer containing 10 ppm bisulfite, but no acetaldehyde, will display less stale character than one containing an equimolar concentration of acetaldehyde. However the concentration of bisulfite would need to be increased several fold in order to bind all of the carbonyls. Various assumptions have been made in these calculations, but it is a reasonably safe to infer that the tendency for a beer to display stale character is intimately linked to its content of \( \text{SO}_2 \).

One strategy pursued has been the amplification of \( \text{SO}_2 \) production through the use of a genetically-modified yeast [10]. The problem here will remain one of the desirable and legal limits on the amount of sulfites in beer.

The alternate approach would be the exact converse: minimize \( \text{SO}_2 \) production by yeast. Sulfur dioxide is a natural product of yeast metabolism and its tendency to bind acetaldehyde limits the ability of the alcohol dehydrogenase in yeast to produce ethanol. If \( \text{SO}_2 \) production in fermentation were eliminated, this would facilitate the reduction of acetaldehyde (and other carbonyl substances) by yeast. If allowable and desirable, \( \text{SO}_2 \) could then be employed in the finished product to scavenge carbonyls developing in the finished product. It would need to be recognized that such a dramatic modification to yeast metabolism would have implications for the balance of the remaining sulfur-containing volatiles in beer.

Sulfur dioxide is progressively lost from beer

The other problem confronted by the brewer is how to retain bisulfite in the beer. It is lost in a first order reaction at rates which differ (for as yet unexplained reasons) from beer to beer [10]. Just about the only approach which can be assured to minimize the loss of bisulfite is to maintain the temperature of the beer as low as possible. Thus Lett demonstrated that the half-life of \( \text{SO}_2 \) in beer held at 40°C was 27 days, whereas it was 3 years in beer held at 0°C.

Keep beer cold

Lowering the temperature, of course, retards the rate of all chemical reactions, including those leading to the formation of the staling substances in beer just as much as those processes which are removing the bisulfite that is masking those substances. A properly packaged beer stored at 0°C may not show staling in a year, whereas the same beer held at 50°C (not impossible in some climates) will show clear deterioration in a week or two. Perhaps the single most important thing a brewer can do, therefore, to repel the staling of beer is to maintain it at the lowest practical temperature from the point of production and pack-
ing through to the point of consumption.
In fact, heat tends to have a disproportionate effect on the development of staling carbonyls in beer. There have been various reports of compounds developing on warm storage (e.g. of the type used in rapid aging tests) which don’t ordinarily develop within realistic timescales of ‘normal’ storage. Perhaps Walters’ data\textsuperscript{1151} is most informative in this regard.

So what is to be done?

It would be easy to infer from the above discussion that a brewer might never achieve mastery over flavor instability in beer. This is unduly pessimistic. From a personal perspective on the current published literature on the science of flavor deterioration in beer and in the light of calculations made in this and a previous paper\textsuperscript{131}, figure 3 gives a strategy for confronting staling problems.

\textbf{NOTE:}

Since the initial writing of this paper, Johannesen et al (Johannesen, P. F., Nyborg, M. & Hansen, J., Proceedings of the European Brewery Convention Congress, Cannes, 1999, 655-662) have reported the construction of lager strains of brewer’s yeast deficient in the production of sulfite. Entirely in the manner suggested above, such altered strains produced less acetaldehyde and gave beers with flavor lives to match those from control strains, provided SO\textsubscript{2} was added prior to packaging.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
Process stage & Barley & Malting & Grist & Milling & Mashing & Boiling & Fermentation & Downstream & Packaging \\
& & & & & & wort collection & & processing & distribution \\
\hline
High relevance & & & & & & & & & \\
low cost/risk & & & & & & & & & \\
\hline
High relevance & & & & & & & & & \\
high cost/risk & & & & & & & & & \\
\hline
Low relevance & Selection & & & & & & & & \\
low cost/risk & of barley & & & & & & & & \\
with low & & & & & & & & & \\
LOX potential & & & & & & & & & \\
\hline
Low relevance & Sparging & & & & & & & & \\
high cost/risk & of grist & & & & & & & & \\
with inert gas & & & & & & & & & \\
\hline
\end{tabular}
\end{table}

\textbf{FIGURE 3}

Strategies for dealing with staling beer.
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