

# The flavor instability of beer

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Perhaps the biggest remaining quality challenge for brewers is the achievement of flavor stability. The factors determining flavor robustness in beer are extremely complex.

There is no universally accepted terminology for the flavor changes that occur in beer and certainly no surety that any two beers will age in exactly the same way, to give the same flavor notes in identical proportions. “Gently”-flavored lagers and strong ales are not predisposed to the selfsame flavor changes. If we are to generalize in pursuit of simplification, then one of the first changes is a perceptible decline in bitterness, and the beer may be perceived as harsh. There will also be a decline in fruity/estery and floral notes. Some beers will develop a ribes (blackcurrant buds, tomcat urine) aroma and most beers are claimed to develop a wet paper or cardboard character. Bready, sweet, toffee-like, honey, earthy, straw, hay, woody, winery and sherry-like are all notes that have been reported (Drost et al., 1971; Meilgaard, 1972; Dalgliesh, 1977; Whitear, 1981).

There is a difference between the flavor perception of beer aged “naturally” (remembering that this may vary in regions with extremes in climate) and in beer which has been “forced” aged. Thus Kaneda et al. (1995) say that a beer aged at 25°C develops primarily caramel-like characteristics, whereas at 30°C or 37°C the cardboard notes are emphasized.

To add to the complexity of the situation, it is not entirely clear that beers displaying a pronounced age character necessarily meet with disfavor in the marketplace. Thus Guinard and co-workers (2001) showed that when judged under branded conditions, imported beers were found by an expert panel to display stale characteristics, but they were nonetheless preferred to domestic beers. When the beers were not brand-identified, there was an equal preference for the imported and domestic beers. This highlights that branding may be at least as significant as inherent flavor characteristics in consideration of beer choice. Stephenson and Bamforth (2002) also demonstrated that branding was

of major significance in beer, however when a given brand is identified there is a preference for the fresh version of that beer.

Achieving flavor stability is a major challenge, especially as what happens to the beer in between packaging and consumption is often out of the control of the brewer. It has even been suggested that the aged character should be maximized in beer before it leaves the brewery, on the basis that no further flavor change will occur (Torline et al., 1999). This chapter makes the fundamental assumption, however, that most brewers do desire to minimize flavor change and that their beers should be inherently fresh. The basic credo is that all packagings of a given brand of beer should taste identical, such that a consumer knows precisely how a beer will taste when purchased.

In theory, any perceptible change in flavor that renders a beer different from that expected for the beer in question amounts to flavor instability. For the most part discussion is of carbonyl compounds. It was Hashimoto (1966) who first reported on the substantial increase in the level of carbonyl compounds in ageing beer. Thereafter, Palamand and Hardwick (1969) first described the development of E-2-nonenal (cardboard-like aroma), which above all other compounds is the one most frequently referred to in the context of staling. However, many other compounds may change in their amount, taking them either above or below their flavor threshold and thus registering as a change in perceived flavor. As many as 600–700 substances contributing to the flavor of beer can be detected by the human taste and olfactory system, some at extremely low concentrations. Table 3.1 lists some of the compounds that have been associated with flavor deterioration in beer. There is a tremendous diversity here, albeit a preponderance of carbonyl compounds, so small wonder that agents which bind carbonyls can strip the aged character from beer (Hashimoto, 1972b; Bamforth, 2000).

**Table 3.1**  
Compounds formed during beer storage

Class	Compounds
Aldehydes	Acetaldehyde E-2-Nonenal E-2-Octenal E,E-2,4-Decadienal E,E-2,6-Nonadienal 2-Methylbutanal 3-Methylbutanal Benzaldehyde 2-Phenylacetaldehyde 3-(Methylthio) propionaldehyde
Ketones	E- $\beta$ -Damascenone diacetyl 3-Methyl-2-butanone 4-Methyl-2-butanone 4-Methyl-2-pentanone 2,3-Pentanedione

(Continued)

**Table 3.1**  
(Continued)

Class	Compounds
Cyclic acetals	2,4,5-Trimethyl-1,3-dioxolane 2-Isopropyl-4,5-dimethyl-1,3-dioxolane 2-Isobutyryl-4,5-dimethyl-1,3-dioxolane 2-Sec butyl-4,5-dimethyl-1,3-dioxolane
Heterocyclic compounds	Furfural 5-Hydroxymethylfurfural 5-Methylfurfural 2-Acetylfuran 2-Acetyl-5-methylfuran 2-Propionylfuran Furan Furfuryl alcohol Furfuryl ethyl ether 2-Ethoxymethyl-5-furfural 2-Ethyoxo-2,5-dihydrofuran Maltol Dihydro-5,5-dimethyl-2(3H)-furanone 5,5-Dimethyl-2(5H)-furanone 2-Acetylpyrazine 2-Methoxypyrazine 2,6-Dimethylpyrazine Trimethylpyrazine Tetramethylpyrazine
Ethyl esters	Ethyl-3-methylbutyrate Ethyl-2-methylbutyrate Ethyl-2-methylpropionate Ethylnicotinate Diethyl succinate Ethyl lactate Ethyl phenylacetate Ethyl formate Ethyl cinnamate
Lactones	$\gamma$ -Nonalactone $\gamma$ -Hexalactone
S-compounds	Dimethyl trisulphide 3-Methyl-3-mercaptopbutylformate

From Vanderhaegen et al. (2006).

There is substantial literature on the flavor instability of beer. Alas, much of it is of dubious value and can display serious deficiencies, particularly for its lack of robustness in sensory techniques (Meilgaard, 2001) and an over-reliance on chemical and instrumental data. Ultimately, the only test of what is and what is not relevant for the enhancement of beer shelf life is whether flavor stability can be unequivocally demonstrated to occur when perceived organoleptically.

The challenges of studying such a complex phenomenon and the attendant seeming inconsistencies in the results obtained, have led to considerable disagreement in the literature on the relative importance of various chemical changes and process stages to flavor instability. The apparent failure of brewers to achieve the degree of flavor robustness they want in the finished product, despite the major advances made in control of parameters such as oxygen levels in the final package, has led them to search further back in the process for the reasons and, they hope, the solutions. Thus, there has been great focus on the brew house in recent years and even some suggestions that flavor instability is built into the system as early as the malt house. The apparent improvements that have been made by focusing on malting and wort production appear to be relatively minor.

Another major shortcoming with research on flavor stability is the aforementioned over-emphasis on E-2-nonenal. As long ago as 1981, van Eerde and Strating showed that whilst this compound increases within days in beers aged at 40°C, there was no equivalent increase in 4 months of storage at 20°C. Narziss et al. (1980, 1999), Foster et al. (2001), Schieberle and Komarek (2003) and Vesely et al. (2003) reported similar findings.

### Factors impacting the shelf life of beer

The extent to which a foodstuff such as beer will age in the marketplace can be described by the formula given by Singh and Cadwallader (2003).

$$rQ = \varphi(C_i, E_j) \quad (3.1)$$

where

$rQ$  = rate of quality deterioration

$C_i$  = compositional factors (e.g. content of reactive species, catalysts, inhibitors, pH, etc.)

$E_j$  = environmental factors (e.g. temperature, light, mechanical stress)

$\varphi$  = proportionality constant

Many factors contribute to the ageing of beer and can be basically divided into *intrinsic* factors (i.e. compositional ones) and *extrinsic* factors (i.e. events and conditions outwith the beer but to which the beer is exposed). Although the formula makes no attempt to weigh the various parameters, it is clear that a change in any one of them will impact flavor stability.

Of all the intrinsic factors, the one most extensively studied is oxygen.

### Oxygen

Oxygen accounts for 21% of the gases in dry air and is thus plentifully available to react with wort and beer if air is allowed access. The concentration that will dissolve in wort and beer is dependent upon:

- The partial pressure of oxygen above the liquid: higher pressures (and proportion of oxygen in the gas phase) give a higher oxygen concentration in solution

**Table 3.2**  
Solubility of oxygen in worts of increasing strength

Gravity (Plato)	Solubility of oxygen (ppm) at 1 atmosphere pressure and 10°C	Solubility of oxygen (ppm) at 1 atmosphere pressure and 20°C
Deionized water	10.9	8.9
9	8.7	7.1
12	8.3	6.8
15	8.0	6.6
18	7.8	6.4

- The temperature: higher temperatures afford less oxygen in solution
- The quantity of other materials dissolved in the liquid, which in turn relates to the strength of the wort and beer: more competing solutes lead to less oxygen in solution.

The oxygen concentration in de-ionized water is 0.34 mM (10.9 ppm) at 10°C and 0.28 mM (8.9 ppm) at 20°C. Because of the high concentration of dissolved materials in wort, solubility of oxygen is less at successively higher strengths (Table 3.2). For worts at lower atmospheric pressures (higher altitudes) the concentration of dissolved oxygen will be proportionately less. At a given atmospheric pressure, the solubility of oxygen in beer will also be less than in pure water, but rather greater than in wort.

Oxygen is much more soluble (seven to eightfold) in organic solvents than in water. This has seldom been taken into consideration when considering oxidation in brewing systems: the oxygen concentration in a localized lipid environment (e.g. mash and trub particles) may be somewhat greater than in the bulk aqueous phase.

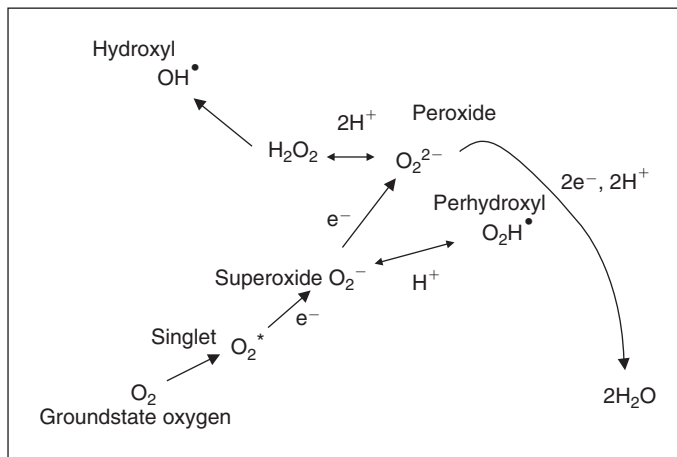
### Reactive Oxygen Species

It was Bamforth and Parsons (1985) who first drew attention to the role of active oxygen species rather than ground-state oxygen in potentiating flavor damage in beer.

The principle basis for the toxicity of oxygen is via its conversion to reactive “free radical” forms, or reactive oxygen species (ROS) as they are often now collectively termed, because not all damaging species produced from oxygen are radicals.

A free radical is a species with an independent existence containing one or more unpaired electrons. Free radicals can be formed either by loss or acquisition of electrons from non-radicals.

An input of energy to the oxygen molecule can “flip” the spin of one of the outer orbital electrons in oxygen, generating the highly reactive *singlet oxygen*.

**Figure 3.1**

The activation of oxygen through the addition of electrons.

Addition of one extra electron to ground-state oxygen forms the *superoxide* radical,  $O_2^-$ . As superoxide needs to gain only one electron to complete its outermost orbital, it is more reactive than ground state oxygen. One further electron leads to *peroxide*,  $O_2^{2-}$ , which is not a radical, but is more reactive than ground state oxygen. Essentially the two atoms in the ground state oxygen are held together by two bonds, in superoxide by one-and-a-half bonds and in peroxide by only one bond. This makes the bond in hydrogen peroxide relatively fissile and the input of energy (e.g. as light) effects a splitting.

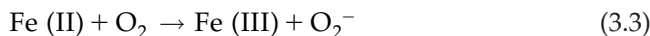


The *hydroxyl* radical produced is immensely reactive.

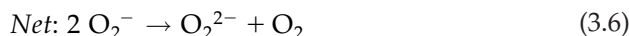
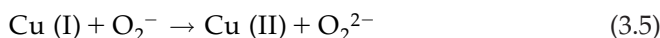
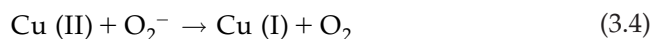
A summary of the ROS is given in Figure 3.1.

### Transition metal ions

With the exception of zinc, the metals in row one of the d-block of the Periodic Table possess unpaired electrons (i.e. they too are radicals). Iron (II), then can donate an electron to oxygen, with the attendant formation of iron (III) and superoxide.



Copper can both receive electrons from and donate electrons to superoxide.

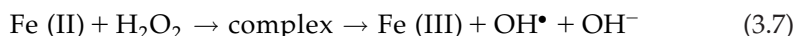


In other words the copper is acting as a catalyst – it ultimately remains unchanged and is available to continue this dismutation reaction, provided it has access to superoxide.

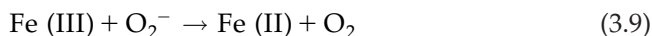
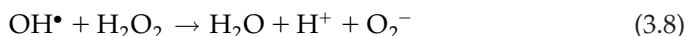
Of the other metals in this category that are likely to be found in brewing systems, manganese can enter into this type of radical reaction, but zinc cannot.

### Fenton reaction

It's over 100 years since Fenton first demonstrated the reaction



Furthermore, these reactions occur



Analogous reactions occur with copper.

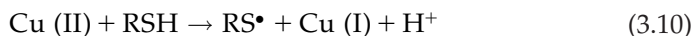
And so it can be seen from reactions of this type (Equations 3.2–3.8) that metal ions such as iron and copper are effective in stimulating the formation and multiple inter-conversions of radicals from oxygen.

### Sources of hydrogen peroxide

Whilst hydrogen peroxide can be produced by the metal ion-catalyzed activation of oxygen, it might also be noted that it can be produced by the oxidation during mashing of sulfhydryl groups in malt proteins (Muller, 1997). The proteins cross-link through the disulfide bridges formed (thereby forming complexes that retard wort separation), and simultaneously hydrogen peroxide is produced.

### Sulfur compounds

Sulfur is of further relevance in the context of radical damage. Just as oxygen can be activated into more potent forms, so too can the next element in its group, sulfur. For instance



Indeed, the co-presence of –SH and oxygen can lead to



Superoxide and hydroxyl also react with RSH to form  $\text{RS}^\bullet$ .

These various sulfur radicals are extremely reactive, too, and can promulgate the formation of oxygen radical species if they encounter more oxygen.

In a complex “soup” such as wort or beer, then, there are a myriad of opportunities for radicals to form. There will be many more than those identified here.

### Radicals produce radicals

The reaction shown in Equation 3.11 is just one example of a radical producing another through reaction with a non-radical species. Another example is the formation of a 1-hydroxyethyl radical from ethanol, through the reaction of the hydroxyl radical with ethanol.

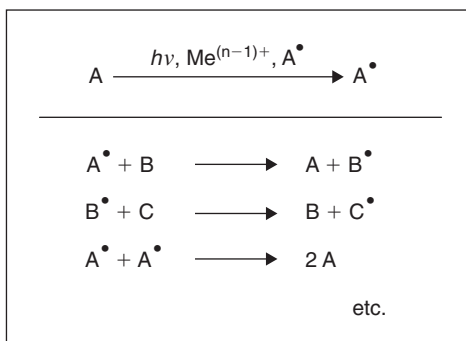


The hydroxyethyl radical is probably one of the most abundant in beer, owing to the high concentration of ethanol present (Andersen et al., 2000). These authors imply that this is the radical primarily detected in electron spin resonance spectroscopy (esr) measurements on beer and suggest that this radical reacts with ground state oxygen to generate perhydroxyl, which is the species that is primarily causing the damage in the beer. The hydroxyethyl radical will also degrade to produce acetaldehyde (Andersen and Skibsted, 1998).

Thus these radical products can react with other species (and with one another). In other words, once a radical has been produced it can dissipate its energy in the formation of other radicals, which in turn pass on their energy through reactions with other species; viz, a chain reaction is set in motion, sometimes known as *propagation* (Figure 3.2). It is only when two radicals interact to form a non-radical species that the chain is terminated.

The extent to which these various reactions occur is a function of

- the rate of formation of the very first radicals, which in turn depends upon the concentration of the species destined to become a radical (e.g. oxygen) and the concentration of activating species (noting that these activating species may not always be in forms where they are “active” – e.g. a metal ion such as copper may not be capable of converting peroxide to hydroxyl if it is sequestered



**Figure 3.2**  
Radical propagation.



by a chelating agent such as an amino acid or if it is hidden away within an insoluble particle. The state and availability of metal ions is referred to as their *speciation*.)

- the concentration of other species capable of reacting with the first radicals, in turn, to become radicals themselves
- prevailing local conditions (e.g. pH, temperature)
- the relative rate constants for the various reactions under these conditions
- the presence of any catalytic species (including enzymes).

Clearly there is a vast complexity of reactions and interactions that can occur, particularly in a complex aqueous milieu such as wort and beer. All we can hope to do is to assess the probability of individual reactions occurring.

Let us take a very simple scenario: the opportunity for oxygen to be “activated” in beer.

Let us assume that we have a concentration of oxygen in beer of 0.3ppm, which might be considered a typical achievement for many brewers for beer in final pack (although some of course aspire to lower oxygen concentrations than this). This is equivalent to a concentration of oxygen of  $9.4\mu\text{M}$ . Furthermore, let us suppose that free (unchelated, non-bound) iron is present in the beer at just 0.01 ppm ( $0.18\mu\text{M}$ ).

The rate of a chemical reaction is a function of the concentration of the reactants multiplied by a rate constant: faster reactions have higher rate constants.

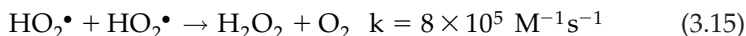
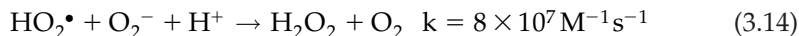
The rate constant for the reaction of oxygen with iron (II) is  $1.3 \times 10^6 \text{M}^{-1} \text{s}^{-1}$ . And so, with the prevailing concentrations of iron and oxygen, the rate of activation of oxygen to superoxide in reaction 3.3 (see earlier) would be

$$\begin{aligned} \text{rate} &= (1.3 \times 10^6) \times (9.4 \times 10^{-6}) \times (0.18 \times 10^{-6}) \\ &= 2.2 \times 10^{-6} \text{M}^{-1} \text{s}^{-1} \end{aligned}$$

The superoxide radicals formed may have various fates. The likelihood of these various fates depends on which molecules they encounter, which in turn depends on the concentrations of these molecules in solution. Thus in a beer, the greatest likelihood is that the first molecule encountered will be one of water, the next most likely being one of ethanol, and so on. Because more than a single superoxide radical will be produced in a given locale (i.e. there will be a high localized concentration), there is also a high probability that these radicals will react together. In fact this happens far more rapidly if one or both of adjacent superoxides are protonated to form the perhydroxyl radical.



The  $\text{pK}_a$  of this equilibrium is 4.8. Thus at beer pH the majority of the superoxide will be in the perhydroxyl form, enabling these reactions to occur.



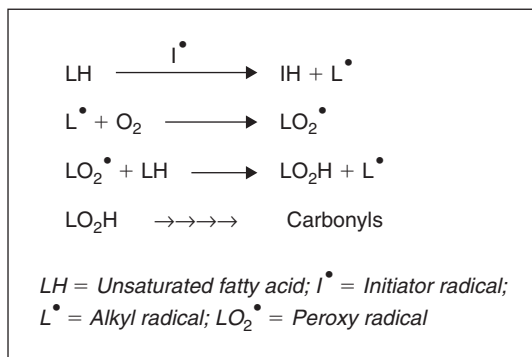
The hydrogen peroxide formed is even more reactive than is superoxide (perhydroxyl), and in turn it could react with the iron in the beer, according to the Fenton reaction (see Equation 3.7) for which the rate constant is  $76\text{M}^{-1}\text{s}^{-1}$ .

Consequently, we arrive at the hydroxyl radical, one of the most reactive species known, and one capable of reacting with a myriad of species, including ethanol, to produce the hydroxyethyl radical (Equation 3.12). The rate constant for the reaction of hydroxyl with ethanol is  $7.2 \times 10^8\text{M}^{-1}\text{s}^{-1}$  (at pH 7).

Hydroxyl and perhydroxyl (and also singlet oxygen) are capable of reacting with an unsaturated fatty acid, such as linoleic acid, and thereby setting in motion the chain reaction suggested by many to lead to stale flavor development (Figure 3.3). The authors do not have to hand the rate constant for the reaction of hydroxyl with linoleic acid, but it is likely to be of the same order of magnitude as for the reaction with lecithin, viz.  $10^8\text{M}^{-1}\text{s}^{-1}$ . The rate constant for the reaction of perhydroxyl with linoleic acid is five orders of magnitude lower ( $1.18 \times 10^3\text{M}^{-1}\text{s}^{-1}$ ). Other radicals capable of triggering the peroxidation of unsaturated fatty acids include  $\text{RS}^\bullet$  (see above).

The reader by now will have realized the complexity of the situation – and should not lose sight of the fact that this is only the tip of the iceberg! The flux of species through the myriad of reactions will depend on the rate constants and concentration of the various species. For instance, the rate constant for the dismutation reaction of superoxide/perhydroxyl is almost five orders of magnitude greater than that for the reaction of perhydroxyl with linoleic acid. It is only if the linoleic acid concentration is substantially higher than the local concentration of perhydroxyl that the latter reaction would be favored. Many suppositions must enter into a discussion of a highly simplified scenario such as this, but it is evident that the rate-limiting step (the one with the lowest rate constant) is for the conversion of peroxide to hydroxyl (Fenton reaction).

Oxidation of unsaturated fatty acids is the only route by which staling materials (principally carbonyls) arise in beer via radical reactions (see later). It is also important to stress that unsaturated fatty acids and iso- $\alpha$ -acids, etc., do not need to be degraded to any great extent in these radical reactions in order to cause a flavor change in beer: the carbonyl staling products have very low



**Figure 3.3**

Autocatalytic oxidation of unsaturated fatty acids.

flavor thresholds. Such carbonyls can have flavor thresholds of 0.1 ppb or less. Although the concentrations of the reactants in the various reactions we have discussed above are relatively low and, despite the proportionately low rate constants of reactions such as the Fenton reaction, only a very limited amount of oxidation needs to take place to generate perceptible stale character (see Bamforth (1999) for a calculation).

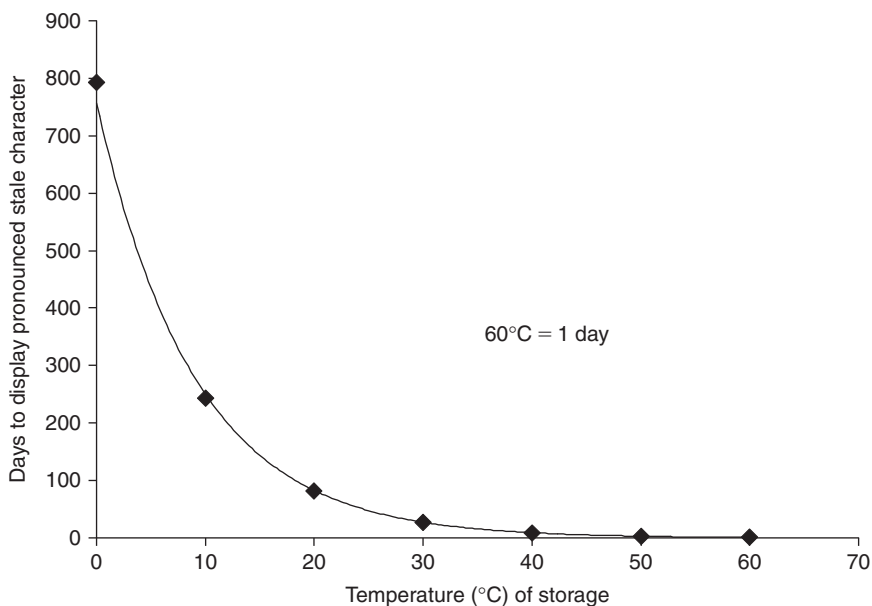
### The impact of temperature

Apart from oxygen, the most dramatic impact on flavor stability of any parameter, on the route from barley to a beer in the customer's hand, is the temperature of storage of the beer. According to Arrhenius' Law

$$k_{t+10} = 2 \sim 3k_t \quad (3.16)$$

where  $k_t$  is the rate of a chemical reaction at a given temperature and  $k_{t+10}$  is the rate of that reaction when the temperature is raised by 10°C. If the temperature of the beer is raised by 10°C, the rate of reactions, including those responsible for staling, is increased two to threefold.

This huge impact of temperature is illustrated in Figure 3.4, in which a factor of 3 in Equation 3.16 is used. There is a strong likelihood that this is valid, because accelerated ageing regimes are generally founded on a supposition that 1 day at 60°C equates to 4 weeks at 30°C and the shape of the plot bears this out. Perusal of Figure 3.4 reveals that if beer can be held at, say, 10°C as



**Figure 3.4**

The impact of temperature on flavor deterioration in beer (from Bamforth 2004b).

opposed to room temperature then this will “buy” about 5 months of shelf life. Equally, increasing the temperature of storage from 20°C to 35°C (e.g. beer being held in winter as opposed to summer in a garage in Davis, California) has a vast impact on the development of stale character. It is only too apparent how critical the logistics of distribution are and how any attempt to bring the temperature down post-packaging is highly advisable.

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## The chemistry of flavor change in beer

Many flavor active compounds present in uninfected beer are capable of changing their levels during storage in the final package.

Compounds may

- (a) decrease in level leading to flavor deterioration by loss of a desirable character
- (b) increase in level leading to a flavor deterioration by an increase in an undesirable character.

In turn, category b compounds may arise

- (i) because they are produced *de novo* in a chemical reaction
- (ii) by the release of pre-formed material that is bound up in the beer with a “holding agent” that prevents their flavor from being expressed
- (iii) because conditions have changed in a beer which makes the likelihood of either type of change [(i) or (ii)] more likely, for example a change in redox conditions.

The relevant chemistry underpinning the changes described in (a), (b), (i), (ii) and (iii) is as follows, remembering that, while all of these reactions are feasible and at some time or another have been proposed as contributors to flavor change, they may have varying degrees of actual relevance.

In passing we might note that there has been an over-emphasis on the compound E-2-nonenal in the literature. To imply that a solitary compound is primarily responsible for ageing is naive.

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## Enzymic oxidation of unsaturated fatty acids

Lipoxygenase (LOX) catalyses the oxidation of polyunsaturated fatty acids, notably linoleic acid, to hydroperoxides (Doderer et al., 1992). In turn these are substrates for hydroperoxide isomerase (Schwarz and Pyler, 1984; Zimmerman and Vick, 1970) and hydroperoxide lyase (Kuroda et al., 2003). An ensuing sequence of non-enzymic reactions leads to the production of unsaturated carbonyl compounds, including E-2-nonenal (formerly known as *trans*-2-nonenal). It is argued that hydroperoxides produced upstream in malting and brewing survive into the finished beer and progressively decay to release stale character. LOX is produced in the barley embryo during germination (Boivin et al., 1996), during which hydroperoxides are also produced (Bamforth et al., 1993). However, the latter are not measurable after kilning and their fate is unknown. LOX is a very heat-sensitive enzyme and is substantially destroyed during

kilning (Bamforth et al., 1991), especially during more stringent ale regimes. It will survive mashing at lower temperatures, but is rapidly destroyed at 65°C (Boivin et al., 1996). It has been argued that if this enzyme has any relevance whatsoever in mashing, then it can only be at the point of initial striking of malt with brewing water, at which point alone there seems to be sufficient substrate and enzyme for the enzyme to act (Biawa and Bamforth, 2002). Taking mashing pH from 5.5 to 5.0 halves lipoxygenase activity (Doderer et al., 1991). Barleys lacking lipoxygenase have been developed and are claimed to result in beers more resistant to staling (Hirota et al., 2005).

### Non-enzymic oxidation of unsaturated fatty acids

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Linoleic acid is susceptible to oxidation even in the absence of enzymes. The reaction is autocatalytic and needs only a small amount of initial “trigger” to start the cascade of radical reactions. The first agents needed to start the chain reaction may typically be oxygen radicals such as hydroxyl and perhydroxyl (the protonated form of superoxide that is the prevalent at beer pH (Kaneda et al., 1997)). Linoleic acid, oxygen and activating metal ions such as iron may be at relatively minuscule levels in beer, yet still sufficient to allow the staling sequence to occur (Bamforth, 1999; see earlier discussion). Other workers (e.g. Lermusieau et al., 1999; Noel et al., 1999a,b) have proposed that nonenal production does not occur in the finished beer, but rather that 30% occurs during mashing and 70% during wort boiling.

### Oxidation of iso- $\alpha$ -acids

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Unhopped beers seldom develop an oxidized flavor, which suggests a likely role for the iso- $\alpha$ -acids as precursors of staling compounds (Hashimoto et al., 1979). In model systems it has been shown that volatile carbonyls (alkenals and alkadienals with chain lengths of between 6 and 12 carbon atoms) can be produced from a solution of bitter substances, higher alcohols and melanoidins. The *trans* isomers are more prone to degradation than are the *cis* isomers (De Cooman et al., 2000; Araki et al., 2002). Reduced side-chain iso- $\alpha$ -acids do not give staling carbonyls (Hashimoto, 1988).

### Oxidation of higher alcohols

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Alcohols in beer can be converted to their equivalent aldehydes through the mediation of melanoidins, with the oxidized carbonyl groups on the latter acting as electron acceptors (Hashimoto, 1972a). Devreux et al. (1981) suggest that the reaction is inhibited by polyphenols and requires light, so is of secondary significance in beer. Meanwhile, Irwin et al. (1991) argue that the efficiency of conversion is so small as to make the pathway irrelevant.

### Strecker degradation of amino acids

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Amino acids can react with  $\alpha$ -dicarbonyl compounds, such as the intermediates in browning reactions. The amino acid is converted into an aldehyde with one fewer carbon atom (Blockmans et al., 1975; Hashimoto and Kuroiwa, 1975). Polyphenols may have a catalytic role (Blockmans et al., 1979).

### Aldol condensations

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In the aldol condensation, separate aldehydes or ketones react to form larger carbonyl species. This is a plausible route through which E-2-nonenal may be produced, by a reaction between acetaldehyde and heptanal (Hashimoto and Kuroiwa, 1975). Proline may act as a catalyst.

### Acetal formation

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Cyclic acetals can be formed by the condensation of 2,3-butanediol with carbonyls such as acetaldehyde (Peppard and Halsey, 1982).

### Binding of carbonyls by sulfur dioxide

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Sulfite is capable of forming addition complexes with carbonyl containing compounds, the resultant "adducts" display no perceptible flavor at the concentrations likely to be found in beer (Barker et al., 1983). It has been suggested that carbonyls produced upstream bind to the sulfite produced by yeast, thereby carrying through into the finished beer, to be progressively released as SO<sub>2</sub> is consumed in other (as yet unknown) reactions (Ilett and Simpson, 1995). It has been suggested that the greater significance of sulfite for protecting against staling is through its role as an antioxidant (Kaneda et al., 1994). In this regard, Dufour et al. (1999) indicate that SO<sub>2</sub>-carbonyl binding actually occurs through the C=C of the unsaturated aldehyde, rather than at the carbonyl group and, as such, is non-reversible.

### Binding of carbonyls by amino groups in proteinaceous species

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A similar scenario is understood to occur with carbonyl compounds entering into reversible Schiff base formation with amino groups, including proteinaceous species in the grist (Lermusieau et al., 1999).

### Reduction of carbonyl compounds by yeast

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Yeast is capable of reducing carbonyl compounds (Peppard and Halsey, 1981). These of course include the well appreciated reactions leading from acetaldehyde

to ethanol and diacetyl to acetoin and butanediol. But many other aldehydes and ketones produced upstream will be reduced, leading to the belief by some that upstream production of such carbonyls is unimportant. Various enzymes may be involved in this reduction (Collin et al., 1991; Debourg et al., 1993, 1994; Laurent et al., 1995).

### Release of flavor active compounds by enzymes from yeast

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Yeast is known to release a range of enzymes with potential impact on product quality. Included amongst these are the glycosidases, the substrates for which include complexes of carbohydrate with several significant hop aroma components (Biendl et al., 2003). If these enzymes remain in beer (e.g. if beer is not pasteurized) then conceptually there may be a progressive change in hop character over time. Chevance et al. (2002) showed  $\beta$ -glucosidase enhanced the release of (E)- $\beta$ -damascenone in beer.

### Oxygen radical scavenging by polyphenols and melanoidins

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Oxygen radicals will react with a diversity of species and trigger a cascade of ensuing radical events in the process stream and in beer. Radical scavengers, which halt this cascade by trapping radicals without forming fresh radicals, may include polyphenols (Owades and Jakovac, 1966) and melanoidins (Hayase et al., 1986). Polyphenols may not only scavenge superoxide (Yuting et al., 1990) and hydroxyl (Husain et al., 1987) and the peroxy radicals formed in the autocatalytic oxidation of unsaturated fatty acids (Torel et al., 1986), but may also protect against staling by chelating metal ions and inhibiting LOX (Boivin et al., 1975). Again we find conflicting evidence in the literature. Andersen et al. (2000) could find no impact of polyphenols on free radical scavenging in wort and beer, whereas Kaneda et al. (1995) and McMurrugh et al. (1996) suggested that up to 60% of the reducing power in beer comes from this source. Regarding Maillard reaction products, Andersen et al. (2000) found them to be pro-oxidants, whereas Bright et al. (1999) and Coghe et al. (2003) described the benefit to flavor stability of these materials, especially from more roasted malts.

### Hydrogen peroxide removal by peroxidases

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A key player in the process of oxidation in the brew house and finished beer is hydrogen peroxide. It is detectable in beer and implicated in radical formation. It is also produced during mashing, and during the cross-linking of thiol groups in gel proteins (Muller, 1997). In turn, the peroxide is removed by a reaction with polyphenols in reactions catalyzed by the peroxidases (Clarkson et al., 1982). Thus the risk of radical generation in mashing depends substantially

on the relative ability of peroxidases to out-compete non-enzymic systems that trigger the formation of hydroxyl from peroxide in the Haber-Weiss reaction.

### **Vicinal diketone release in beer from incompletely eliminated precursors**

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If acetolactate and acetoxybutyrate are not completely converted during fermentation and maturation to diacetyl and pentanedione, thereby allowing yeast to consume them, then they will survive into beer and progressively degrade in the final package (Inoue and Yamamoto, 1971).

### **Sulfur compounds**

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Reducing agents, such as the amino acid cysteine, will progressively release dimethyl sulfide from dimethyl sulfoxide in final package (Bamforth, 1985). Residual yeast (in naturally conditioned products) will also do this and will reduce sulfur dioxide to hydrogen sulfide (Walker and Simpson, 1994). Compounds responsible for the ribes character, 3-methyl-3-mercaptopbutyl formate (Schieberle, 1991) and 4-mercapto-4-methyl-penta-2-one (Tressl et al., 1980) are also produced on storage. Peppard (1978) and Gijs et al. (2002) have reported the development of DMTS in beer from various precursors.

### **Changes in ester levels**

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Stenroos (1973) and Neven et al. (1997) reported a decrease in the level of iso-amyl acetate during the storage of beer. However, a range of other esters (see Table 3.1) increase in quantity during storage (Bohnan, 1985b; Gijs et al., 2002; Lustig et al., 1993; Miedaner et al., 1991; Williams and Wagner, 1978).

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## **An evaluation of processes from barley to beer in the context of flavor instability**

Table 3.3 lists the potential impact of all stages from field to package on flavor instability.

### **Some critical comments**

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One of the biggest challenges in any discussion of flavor instability is the acute shortage of good sensory data to support many of the claims that have been made, particularly for oxygen control upstream. This is illustrated in Figure 3.5. Most commonly, the intensity of stale flavor is reported on a scale of the



**Table 3.3**

Process impacts on flavor instability (derived from Bamforth, 2004a)

Raw material or process stage	Parameter	Hypothesis
Barley selection	Potential for staling precursors	Barleys differ in their propensity to develop lipoxygenase (LOX)
	2-Row vs. 6 row	6-Row has higher enzyme potential, more polyphenol potential
	Winter vs. spring	Winter has more polyphenol potential
	Low proantho-cyanidin varieties	Less antioxidant potential through lower polyphenol levels?
	Lipoxygenase-null varieties	Absence of lipoxygenase so no contribution of enzyme-catalyzed lipid oxidation
Steeping and germination	Development of redox enzymes and the extent to which they can act	Factors promoting modification also encourage LOX in terms of levels and extent to which it produces hydroperoxides
		Peroxidases (POD) also increase in level
		Developing sugar and amino acids as melanoidin precursors
Kilning	Destruction of LOX	Higher kilning temperatures destroy LOX
	Destruction of hydroperoxides	Hydroperoxides are also lost on kilning – but where do they go?
	Production of melanoidins	Melanoidins and intermediates leading to them may have antioxidant properties. However they may also promote the oxidation of higher alcohols
Malt storage	Loss of LOX	LOX levels diminish on storage, thereby leaving less oxidation potential in the grist
Milling	Hammer vs. roller	Finer milling leads to greater extraction of all components – including LOX and lipid
	Wet vs. dry	Greater opportunity for leaching of undesirables in wet system – also commencement of LOX reaction
	Embryo preservation	Milling procedures that avoid embryo damage will leave lipid and LOX in an unextractable form and they will go into the spent grains
Mashing-in	Oxygen	It is the point at which milled grist is mixed with water that the LOX risk is greatest because it is here that substrate concentrations are high enough for LOX and it is not yet destroyed

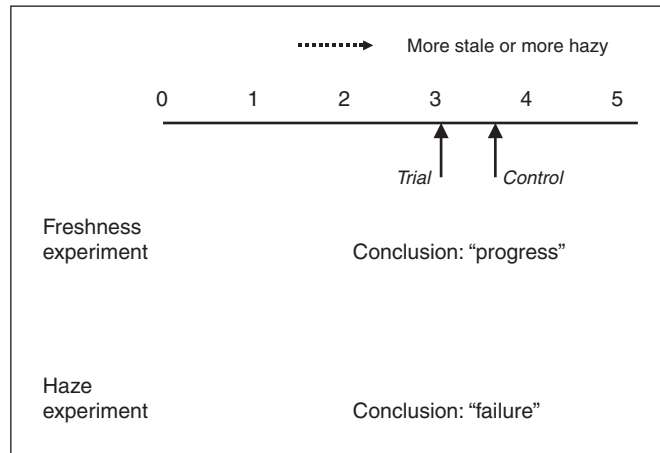
(Continued)

**Table 3.3**  
(Continued)

Raw material or process stage	Parameter	Hypothesis
Mashing	Temperature regime	Low mashing temperatures allow more LOX survival. Heat damage during decoction mashing
	Number of vessels	Increased risk of oxygen pick up with more vessels – for example in decoction mashing
	Oxygen	Oxygen as substrate for LOX, but also reacting non-enzymically with –SH groups in proteins to make peroxides, which act as substrates for POD. Also oxygen radical formation
	pH	Lower mashing pH reduces rate of LOX. However superoxide radical more damaging in protonated forms at lower pH
	Vessel fabric	Leaching of Cu from copper vessels. Cu promotes oxygen radical formation
Solid adjuncts	Antioxidant potential	Roasted adjuncts contain melanoidins that may act as radical scavengers
	Lipid level	Some cereals, for example rice, have high lipid potential unless polished
Wort separation	System	Modern mash filter affords less turbid worts and reduced lipid level. Brighter worts allow increased levels of SO <sub>2</sub> production by yeast
	Collection of weaker worts	Increased extraction of lipid, but also more tannoids (potential anti-oxidants)
Kettle	Duration	Risk of thermal damage in prolonged boiling (and in whirlpool stand)
	Kettle design	Rolling boils to ensure volatilization of undesirable flavors
	Energy saving approaches	Thermal damage, for example in high temperature/high pressure wort boiling
Liquid adjuncts	Selection and level of use	Sugars and syrups devoid of staling precursors so their use “thins out” stale potential
Hopping	Choice of material	Most polyphenols in whole hops, least in extracts
		Reduced iso- $\alpha$ -acids do not degrade to unsaturated carbonyls
		Cis isomers less prone to degradation – selection of hop products with high cis ratio
Hot wort clarification	Removal of trub	Trub impacts yeast performance (vigor – ability of yeast to eliminate carbonyls and to produce sulfur dioxide). Also risk of thermal damage if whirlpool stand is prolonged

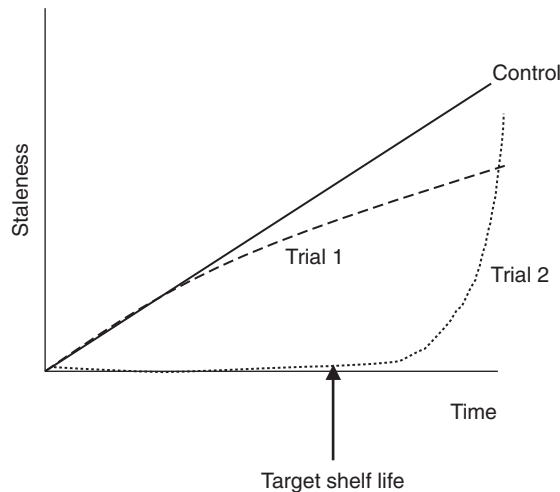
**Table 3.3**  
(Continued)

Raw material or process stage	Parameter	Hypothesis
Cooling and oxygenation	Introduction of oxygen	Oxygen “melding” into wort; O <sub>2</sub> impacts cold break formation. Impact on vigor of yeast performance (see hot wort clarification)
Yeast selection	Yeast characteristics (strain, pitching rate, health)	Strains differ in ability to produce SO <sub>2</sub>
		Healthy yeast and good pitching rates for efficient removal of carbonyls, including VDKs and acetaldehyde
Cold wort handling pre-fermentation	Removal of cold break	Brighter worts lead to more SO <sub>2</sub> production by yeast
Primary fermentation	Removal of undesirable flavor components	See yeast selection. If fermentation is out of spec then there will be increased survival of VDK and acetaldehyde and insufficient production of SO <sub>2</sub>
Secondary fermentation/warm conditioning	Flavor refinement	Final mopping of VDK’s (ensure precursors also fully eliminated)
Cold conditioning	Buffer stock	Beer at lowest practical temp is at its most stable
	Oxygen stripping	Opportunity here to eliminate last traces of oxygen prior to packaging
Filtration and stabilization	Clarification with minimum O <sub>2</sub> and metal pick up	Filtration presents risk of oxygen and iron pick-up. Some argue for avoidance of PVPP because it removes polyphenol antioxidants
Packaging	Lowest oxygen pick-up	The biggest threat to high oxygen levels and therefore staling in beer
Final beer composition	pH	Higher the pH on the scale 4–4.5, the less the level of the most damaging oxygen species
	O <sub>2</sub>	The lower the better
	SO <sub>2</sub>	Antioxidant and binds staling agents
	Precursors (e.g. acetolactate, DMSO)	If they are present in beer they will potentiate change in final product
	“Carbonyl potential”	The less “bound-up” carbonyl the better
Warehousing	Temperature, time	All chemical reactions proceed much more quickly as temperature increases
Transportation	Temperature	As for warehousing, agitation also promotes breakdown
	Agitation	
	Distance logistics	
Package labeling	Born on or best before dates	Make customer aware of the risks



**Figure 3.5**

Different expectations for flavor stability and colloidal stability trials (from Bamforth, 2004b).



**Figure 3.6**

Better to gauge flavor stability in terms of time to onset of stale flavor (from Bamforth, 2004b).

type whereby zero indicates no stale character and five signifies intense staling. The researchers may do their trials and then report, say, that the control beer (no precautions) has a staleness value of 3.7 and the trial (with precautions – e.g. inert gas blanketing of brew house vessels) has a value of 3.1. Ergo, the treatment is claimed to be beneficial in moving towards improved flavor stability. Leaving aside the frequent absence of statistical treatment of the data (reliability of tasting protocol, numbers of replications of the trials) and taking the information in good faith and at face value, should we be impressed? Say that the trial was not on flavor stability, but rather on haze stability. Would an

improvement in a haze value from 3.7 to 3.1 impress anyone? After all, the beer would still be hazy. In common with haze, we fervently suggest that the criterion should not be intensity of stale character, but rather *time to development of stale character* (Figure 3.6). At the end of the ageing time period, the beer from trial 2 shows worse staling than the beer from trial 1. But the time “bought” in terms of weeks to the onset of staling is vastly improved.

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