(iv) Beer Stability and Spoilage

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The Chemistry of Aging Beer

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Abstract

Packaged beer is a closed system that continuously will be submitted to alterations by chemical reactions. These alterations will lead to perceivable changes in flavor stability, foam stability and colloidal stability. In this chapter, the underlying mechanisms of the reactions leading to aging of beer are discussed. Radical reactions, often initiated by reactive oxygen species, have an important contribution to beer staling and form a continuous thread to the stability of beer. Oxidation of unsaturated fatty acids and Maillard reactions lead to hundreds of different compounds. Furthermore degradation of hop bitter acids and carotenoids, oxidation of polyphenols, hydrolysis of glycosides and esters or synthesis of esters, acetalization of aldehydes will all contribute to the aging of beer. When using antioxidants, attention has to be paid to the slower reacting oxygen species as H_2O_2, rather than the most reactive species as the hydroxyl radical.

List of Abbreviations

1-DH	1-deoxy-2,3-hexodiulose
3-DH	3-deoxy-2-hexosulose
$^3RF^\bullet$	Riboflavin radical
HMF	Hydroxymethylfurfural
MBT	3-methyl-2-butene-1-thiol
PVPP	Polyvinylpolypyrrolidone

Introduction

Every living creature alters his environment due to the continuous exchange of metabolites. A closed system will be highly influenced by this metabolism and will evolve to a state of thermodynamic non-equilibrium, far from the minimal Gibbs free energy. When this living entity is removed, a myriad of chemical reactions will slowly lead toward the chemical equilibrium of the system. This principle also counts for beer fermentation. Yeast in concentrations of 60 up to 120 million cells/ml will convert wort sugars into alcohol and CO_2, while producing secondary metabolites and converting components already present in the fermenting wort. Some of these compounds are present above their flavor threshold, while others are precursors of potent compounds arising during beer aging, altering the quality of the beer. When yeast is removed from beer before packaging, exogenous enzymes will still be present, influencing the chemical composition of the beer by enzymatic reactions. When finally the beer is pasteurized, the reactions will have a pure chemical origin, ultimately leading to the minimal enthalpy and maximal entropy. Some of these alterations will remain unnoticed, but others will lead to significant changes in beer flavor, haze and foam stability. While microbiological and colloidal stability is largely under control, flavor stability still remains a major concern to the brewer in the twenty-first century. More precisely it is the flavor instability or flavor deterioration that upsets a brewer. If the consumer cannot identify the flavor perception of the tasted beer with the expectation of that particular fresh brand, the beer will be called aged. However, the consumer will not necessarily have less appreciation for the aged beer, as flavor perception is a very personal sensation. Nevertheless flavor stability remains the engine of beer aging research. At first, with the discovery of (E)-2-nonenal (Jamieson and Van Gheluwe, 1970; Palamand and Hardwick, 1969), the primary focus was set on the oxidation of lipids. At present, it is clear that not only lipid oxidation is responsible for beer deterioration, as other reactions such as radical chain reactions, Maillard reactions, degradation reactions of polyphenols and hop bitter acids also play an important role. This chapter intends to obtain insight in the extreme complexity of beer-aging reactions, focussing on the reaction mechanisms and the chemical nature of compounds, rather than the flavor appreciation that is crucial to brewers.

Chemical Changes During Beer Aging

Pure chemically, beer has to be considered as a water–ethanol solution (ethanol content 3–12 vol. %) with a pH between 4 and 4.5 and several hundreds, or thousands of {AQ1}

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different compounds. The nature of these compounds can vary from very small volatiles as methanethiol to high-molecular, non-volatile proteins, polyphenols and melanoidins. As these compounds are not in chemical equilibrium, formation and degradation processes will take place during beer storage. Each reaction rate is determined by its reaction rate constant, its activation energy and the initial concentration of substrates, and largely depends on the storage temperature (the storage temperature has to be kept as low as possible) and the initial concentration of the reagents. Therefore, every different kind of beer will age in a typical way, determined by the raw materials, production parameters, packaging techniques and storage conditions. An important staling agent is oxygen, which is present in low levels in beer bottles. Although modern equipment allows no more than 20–50 ppb (μg/l) oxygen in the bottled beer, it is impossible to have the headspace entirely oxygen-free. In this section, first the role of oxygen on radical formation will be discussed, followed by two main chemical reaction pathways, lipid oxidation and Maillard reactions. Then individual beer staling reactions will be considered and finally some antioxidants and pro-oxidants will be highlighted.

Formation of reactive oxygen species

Oxygen in its ground state ($^3O_2$) is relatively non-reactive; it seems thus unlikely that oxygen immediately attacks beer constituents after beer is bottled. However, by energy, light or catalytic activity, activated forms of oxygen can be generated (reactive oxygen species), like singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydroperoxyl radical ($^{\bullet}OOH$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2). Reactive oxygen species, as the name indicates, have a strong activity which allows them to react with different kinds of components (Bamforth and Parsons, 1985; Halliwell and Gutteridge, 1984). Recently a general generation pathway (Figure 36.1) of reactive oxygen species in beer has been proposed (Kaneda *et al.*, 1999). First, $O_2^{\bullet -}$ will be produced from O_2 during storage of beer. This reaction will be catalyzed by the oxidation of Fe^{2+} ions or Cu^{+} ions to Fe^{3+} and Cu^{2+}, both present in beer in trace amounts. The pKa value of $O_2^{\bullet -}/^{\bullet}OOH$ is 4.7–4.9, the $^{\bullet}OOH$ radical being the most reactive species. Thus the low pH value of beer slightly favors the formation of $^{\bullet}OOH$, unless $O_2^{\bullet -}$ is further reduced to O_2^{2-}. Due to the high pKa values of O_2^{2-}/HO_2^{-} and HO_2^{-}/H_2O_2, predominantly H_2O_2 will be formed in beer. It is believed that the formation of hydrogen peroxide is a consequence of mixed function oxidation systems, which involve oxygen in its ground state, Fe^{3+} and Cu^{2+}, but also require electron donors, like ethanol (Chapon and Chapon, 1979), ascorbic acid (Brezova *et al.*, 2002), polyphenols, isohumulones and melanoidins (Irwin *et al.*, 1991; Morales, 2005). Next, $^{\bullet}OH$ is produced by metal-catalyzed reactions as the Fenton reaction and the Haber–Weiss reaction.

There seems to occur a so-called lag-time in beer before the $^{\bullet}OH$ radical is formed during storage. In that period endogenous antioxidants in beer consume the $O_2^{\bullet -}$, O_2^{2-} and H_2O_2 produced during the coupled oxidation reactions, before the $^{\bullet}OH$ radical can be formed (Uchida *et al.*, 1996). It was reported that an antagonistic action of pro-oxidants,

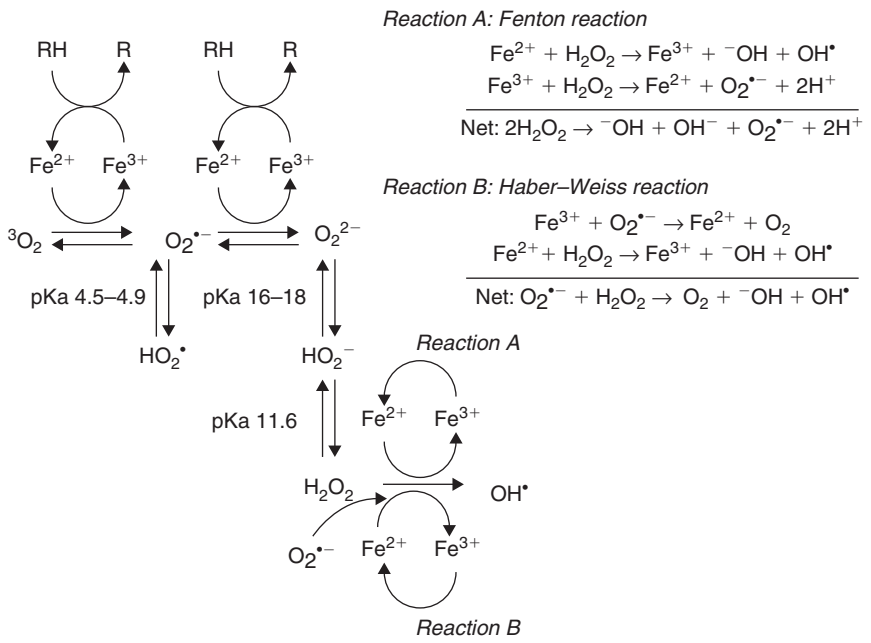

Figure 36.1 Creation of reactive oxygen species (Reprinted from Vanderhaegen *et al.*, 2006, Copyright (2006), with permission from Elsevier). Stable $^3O_2$ will be converted to O_2^{-} and O_2^{2-} by mixed function oxidation systems. Hydrogen peroxide in combination with Fe^{2+} or Cu^{+} will generate the OH-radical via the Fenton reaction or the Haber–Weiss reaction.

like iron and Maillard reaction products, shortens the lag-time (Uchida and Ono, 2000). Pro-oxidants are defined as compounds that are known to be able to produce H_2O_2 (like cysteine or sulfhydryl group containing proteins), or are known to be able to reduce metal ions to the oxidation states that are active for the Fenton reactions with peroxides as mentioned before (Brezova *et al.*, 2002).

Antioxidants are defined as compounds able to quench peroxides (like sulfite), or able to inactivate trace amounts of metals that otherwise may generate hydroxyl or alkoxyl radicals from the peroxides (Andersen *et al.*, 2000). After depletion of these antioxidants, $^{\bullet}OH$ radicals will accumulate and can cause damage to beer components. The $^{\bullet}OH$ radical displays a high reactivity toward several beer compounds as ethanol, sugars, isohumulones, polyphenols, alcohols, fatty acids, etc. This can initiate a series of reactions responsible for the production of carbonyl and phenolic radicals and ultimately lead to staling compounds in beer. This lack of specificity of the $^{\bullet}OH$ radical makes it almost impossible to quench this radical intermediate in beer by adding antioxidants that would react specifically with this radical. Hydrogen peroxide and organic hydroperoxides are the only reactive oxygen species that are stable enough to be trapped efficiently by antioxidants which normally only are present in micro-molecular concentrations (Andersen *et al.*, 2000). Because of the high reactivity of ethanol, being the most abundant compound in beer, this compound will be the primary reactant of $^{\bullet}OH$, resulting in the $EtO^{\bullet}$ radical. This radical will bind oxygen, resulting in acetaldehyde and the hydroperoxyl radical (Figure 36.2). The latter can be reduced to hydrogen peroxide, which can react again with metal ions to continue the radical chain reactions (Andersen and Skibsted, 1998). As it is impossible to know all of the variables necessary to prevent oxidative deterioration by reactive oxygen species, minimization of radical formation will be the best strategy. This can be accomplished by keeping the level of O_2 as low as possible, by lowering the temperature, and by introducing chain breakers or quenchers, generally referred to as antioxidants (Bamforth, 2001).

Oxidation of unsaturated fatty acids

Since the identification of (E)-2-nonenal as the volatile which is presumed to be responsible for the cardboard flavor of aged beer (Jamieson and Van Gheluwe, 1970, Palamand and Hardwick, 1969), this powerful odorant was embraced by all beer researchers as the principal cause of beer deterioration. These findings directed the beer staling research toward the formation of (E)-2-nonenal by the oxidation of unsaturated fatty acids (Dale *et al.*, 1977; Drost *et al.*, 1971; Stenroos *et al.*, 1976; Tressl *et al.*, 1979).

There are two pathways to the oxidation of unsaturated fatty acids: enzymatic oxidation (Drost *et al.*, 1971) and autoxidation (Lindsay, 1973). Enzymatic oxidation occurs during mashing. Due to the activity of both lipase and lipoxygenase, present in malt, linoleic and linolenic acid hydroperoxides are formed (Kobayashi *et al.*, 1993). The limitation of oxygen ingress and a mashing-in temperature at 65°C can decrease the production of hydroxy fatty acids during mashing (Kobayashi *et al.*, 2000b). Nevertheless, the levels of dihydroxyoctadecenoic acid and trihydroxyoctadecenoic acid in commercial beer vary between 0.6–1.6 ppm and 6–15 ppm, respectively (Kobayashi *et al.*, 2000a).

During the boiling process, the enzymes are inactivated due to the high temperature in the wort kettle. As the presence of oxygen cannot completely be excluded, reactive oxygen species are formed due to heat addition to wort. These reactive oxygen species attack susceptible double bonds of oleic, linoleic and linolenic acid, resulting in predominantly 9- and 13-hydroperoxides. Further breakdown of these hydroperoxides leads to the formation of (E)-2-nonenal and other aldehydes. As reduction of these compounds by yeast occurs during fermentation and maturation, it seems evident that yeast exerts a high influence on the overall flavor stability of beer (Wackerbauer *et al.*, 2003).

Despite all the beer-aging models, actual lipid oxidation does not seem to occur in bottled beer at normal storage temperatures, nor can nonenol oxidation account for the presence of (E)-2-nonenal (Lermusieau *et al.*, 1999). The total amount of (E)-2-nonenal in aged beer originates from autoxidation during wort boiling (Noël *et al.*, 1999), and enzymatic action during mashing (Liegeois *et al.*, 2002). During mashing and wort boiling, (E)-2-nonenal can form

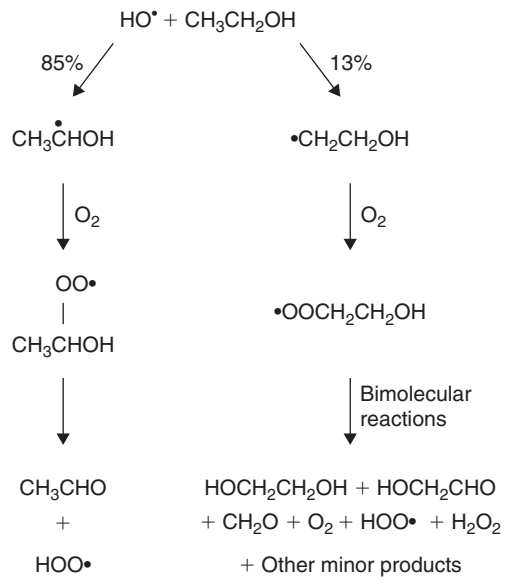

Figure 36.2 Radical reaction of OH with ethanol (Reprinted from Andersen and Skibsted, 1998, with permission from the American Chemical Society). Ethanol is the most abundant molecule in beer and the non-selective $^{\bullet}OH$-radical will quickly react predominantly with ethanol. Preferentially, this will lead to acetaldehyde and the perhydroxyl radical, which after reduction to H_2O_2 can regenerate OH via the Fenton or Haber–Weiss oxidation pathway.

an imine adduct with amino acids and proteins through the formation of Schiff's base. Hence the compound is protected from reduction to nonenol by yeast (Noël and Collin, 1995). During beer aging, (E)-2-nonenal can again gradually be released by acid hydrolysis of (E)-2-nonenal-adducts at beer pH (Lermusieau *et al.*, 1999).

Measures were taken to prevent or to limit oxygen ingress in the brewhouse, which will lead to a decreased oxidation of unsaturated fatty acids, but oxygen cannot completely be excluded during wort production and wort treatment in practical brewing (Drost *et al.*, 1990).

The Maillard reaction

The Maillard reaction predominantly occurs during wort production due to the high temperatures, but some of the reactions will continue during beer storage, even at low temperature.

Initial Reaction Initiated by the reaction of a reducing sugar and an amino-compound, the Maillard reactions develop as a highly complex labyrinth of pathways, which are at present not yet fully elucidated. The complexity of the Maillard reactions was first visualized 40 years after its discovery in a complex scheme of reactions and products (Hodge, 1953). The non-enzymatic browning reactions are ubiquitous in all processed food products; hence they will occur during the production of wort (Tressl, 1979) and continue during beer storage (Bravo *et al.*, 2002).

After mashing, the fermentable sugar content of wort consists of 10% glucose, 60–70% maltose (2 glucose-units linked together in a α-1,4 glycosidic linkage) and 20–30% maltotriose (3 units). Due to the high sugar level and the presence of amino compounds, the Maillard reaction will easily and rapidly occur during wort boiling. An overview of the Maillard reaction involving monomer formation is depicted in Figure 36.3.

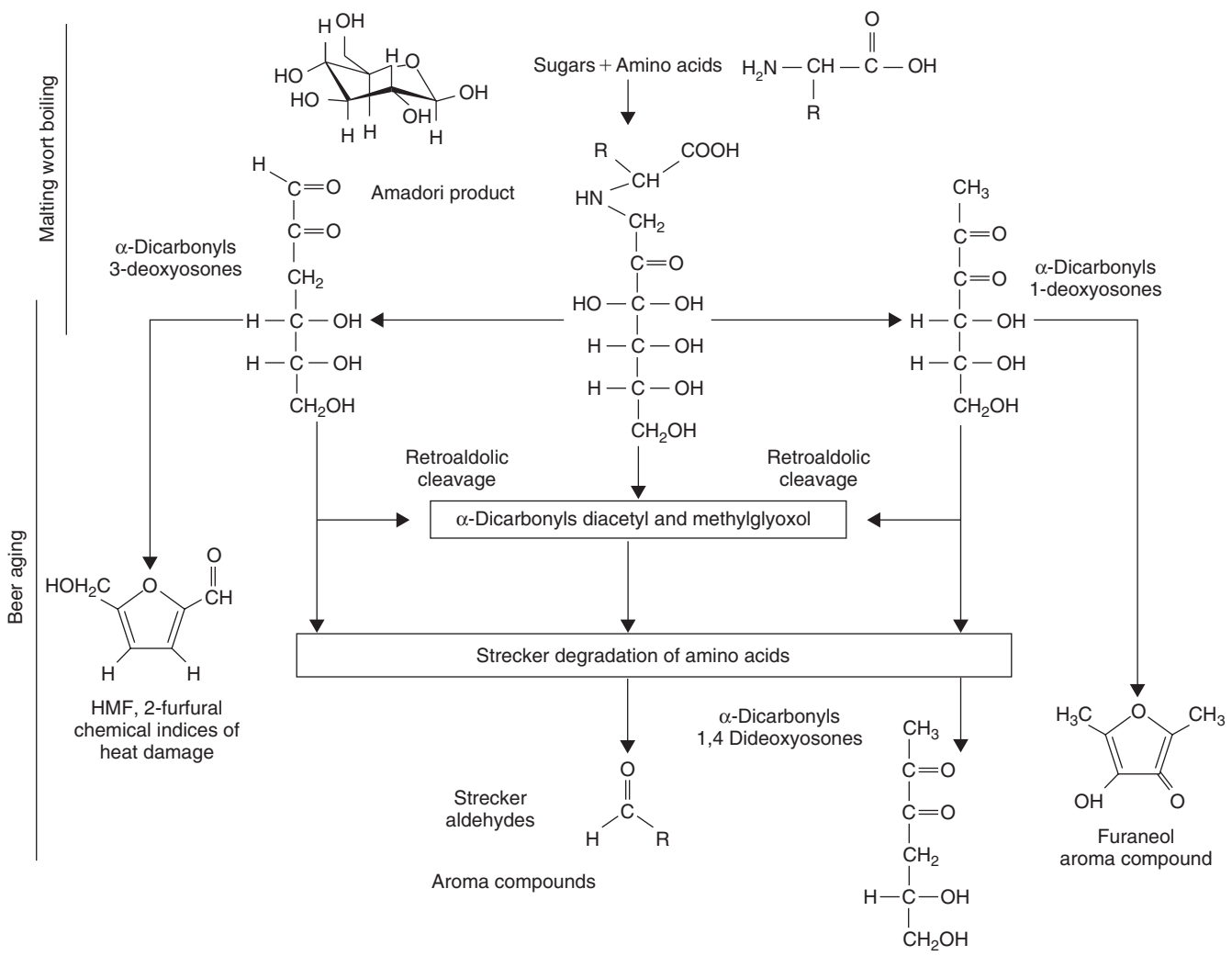

Figure 36.3 The Maillard reaction (Bravo *et al.*, 2002). The reaction of a reducing sugar and an amino acid leads to the formation of the Amadori product. After enolization, 1- and 3-deoxy dicarbonyl compounds can be formed, leading to the synthesis of furanones and furan compounds, respectively. Retro-aldolic cleavage of the dicarbonyl compounds or Strecker degradation give rise to other dicarbonyl compounds or Strecker aldehydes, respectively.

Quantitatively, 5-hydroxymethylfurfural (HMF, originating from hexose sugars) and furfural (from pentose sugars) are the most important Maillard monomers in wort. The reaction is initiated with the formation of a Schiff's base between the carbonyl group of the reducing sugar and an amino-compound (which acts as a nucleophile), resulting in an imine. This undergoes an Amadori-rearrangement to 1-amino-1-deoxyketose (Yaylayan and Huyghues-Despointes, 1994). This Amadori product is subject to enolization and subsequent release of the amino group; 2,3-enolization resulting in the formation of 1-deoxy-2,3-hexodiulose (1-DH) or 1,2-enolization in the formation of 3-deoxy-2-hexosulose (3-DH) (Hirsch *et al.*, 1995). At wort and beer pH, the latter reaction is favored, so quantitatively more 3-DH is formed. After subsequent dehydrations, finally HMF or furfural can be formed, while 1-DH predominantly generates furanones like furaneol.

The monomeric compounds are not the end products of the Maillard reaction. After further reactions (condensation, cyclization, dehydration, isomerization . . .), highly colored melanoidins are produced. Notwithstanding several models of melanoidin structures have been proposed so far, the majority of melanoidins is still lying in mysterious obscurity (Adams *et al.*, 2003; Cammerer *et al.*, 2002; Tressl *et al.*, 1998). Although melanoidins are known to act as antioxidant, some reports also indicate a pro-oxidant activity (Hashimoto, 1972). There are various mechanisms by which Maillard reaction products may act as antioxidants (Ames, 2001): oxygen scavengers, reactive oxygen scavengers (Morales, 2005), reducing agents, and metal chelating agents (Wijewickreme *et al.*, 1997). A strong correlation between malt color (and therefore beer color) and antioxidative ctivity is established. According to Coghe *et al.* (2003), at least two types of Maillard reaction related antioxidants are present in wort: redox-reducing antioxidants and radical scavenging antioxidants. Redox-reducing antioxidants develop linearly with malt color (accounting for the observed correlation between malt color and antioxidative activity). Fast-acting antiradical antioxidants however, seem to be associated with low-colored malts. No correlation has been found between browning intensity and efficiency for scavenging oxygen radicals in solution (Morales, 2005).

Glucose accounts for only 10% of the reducing sugars available in wort, while the concentration of maltose is about 60–70% of the fermentable sugars. Maltose, maltotriose and other malto-oligosaccharides are reducing sugars as well and can also react with amino compounds, yielding α-dicarbonyl compounds via a peeling-off mechanism, proposed by Hollnagel and Kroh (2000). In the case of maltose (Figure 36.4), a β-elimination of D-glucose occurs, resulting in 1-amino-1,4-dideoxysone. The addition of a water molecule and subsequent retro-Claisen condensation yields formic acid and 3-deoxypentosulose, the latter being a precursor of furfuryl alcohol (Vanderhaegen *et al.*, 2004). Furfural, after reduction by yeast, and furfuryl alcohol are precursor of 2-furfuryl ethyl ether, a well-known beer staling compound with a solvent-like flavor and harsh taste (Harayama *et al.*, 1995; Vanderhaegen *et al.*, 2004).

In next sections, the importance of α-dicarbonyl compounds, the Strecker degradation and aldol condensation will be discussed.

Alpha-Dicarbonyl Compounds Although the Maillard reaction is significantly accelerated at elevated temperatures like wort boiling, the reaction still occurs during beer storage at room temperature. The formation of 10 different α-dicarbonyl compounds was observed during beer storage: glyoxal, methylglyoxal (formed by a retro-aldolic cleavage of 1-DH or 3-DH according to Weenen (1998), 2,3-butanedione, pyruvate, pentanedione, 1,4-dideoxypentosulose (Strecker degradation product of 1-deoxy-2,3-pentodiulose), 1,4-dideoxyhexosulose (Strecker degradation product of 1-DH), 1-DH and 3-DH (Bravo *et al.*, 2002). The accumulation of these compounds indicates that the Strecker degradation, retro-aldolic cleavage and the degradation of Amadori-compounds still take place during beer aging at moderate temperatures. Due to the central role of α-dicarbonyl compounds in the proceeding of the Maillard reactions, blocking these intermediates could prevent typical aging compounds to develop. The use of α-dicarbonyl trapping reagents like aminoguanidine, also used in diabetes research (Edelstein and Brownlee, 1992), can block these intermediates and inhibit the dicarbonyl-mediated beer aging. When adding aminoguanidine to dicarbonyl compounds, it reacts irreversibly and rapidly to a permanent 3-aminotriazine, as depicted in Figure 36.5 (Hirsch *et al.*, 1992), which prevents any further reaction with the dicarbonyl moiety (Bravo *et al.*, 2001; Rangel-Aldao *et al.*, 2001a). It has been observed that the addition of 2 mM aminoguanidine to fresh beer retards the formation of HMF during storage and moreover, it has a positive effect on the flavor stability during natural aging (Rangel-Aldao *et al.*, 2001b).

Strecker Reaction In the 1970s it was found that an increased formation of 2-methylpropanal and 3-methylbutanal occurred in beer when valine and leucine were added, respectively (Blockmans *et al.*, 1975). This was explained by the Strecker reaction between amino acids and α-dicarbonyl compounds. The reaction of phenylalanine with methylglyoxal is shown in Figure 36.6 as an example. The Strecker degradation involves a transamination, followed by a decarboxylation and hydrolysis, resulting in an aldehyde with one carbon atom less than the amino acid, and an α-aminoketone (Hofmann *et al.*, 2000). Although the Strecker aldehydes (acetaldehyde (Ala), 2-methylpropanal (Val), 2-methylbutanal (Ile), 3-methylbutanal (Leu), methional (Met), phenylacetaldehyde (Phe) and benzaldehyde (Phe)) increase significantly during beer aging, the individual compounds rarely exceed their flavor threshold (Thum *et al.*, 1995). However, they are frequently monitored to get insight in overall quality parameters, brewing

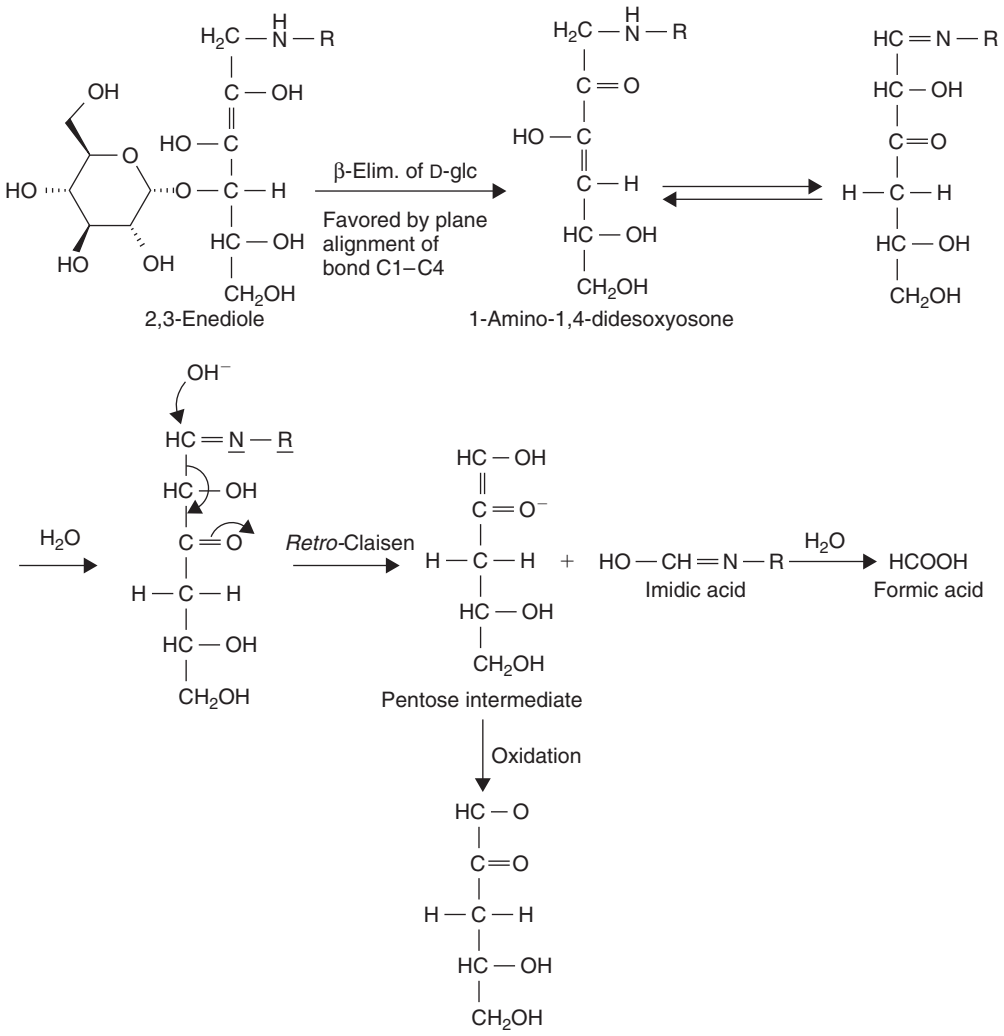

Figure 36.4 Formation of 3-deoxypentosulose from maltose (Reprinted from Hollnagel and Kroh, 2002, with permission of the American Chemical Society). After β-elimination of glucose, a 1-amino-1,4-didesoxyosone is produced, that after water addition and a retro-Claisen reaction leads to formic acid and a C-5 dicarbonyl compound.

technology and storage conditions (Methner *et al.*, 2003). Beside Strecker aldehydes, also α-aminoketones are formed in this reaction, which can further react with other aminoketones to alkylpyrazines, after subsequent cyclization and dehydration (Tressl, 1979). These alkylpyrazines are often characterized by a nutty, roasted flavor.

Recently a Strecker-type degradation of amino acids by oxidation products of unsaturated fatty acids, the 4,5-epoxy-2-alkenals, was observed by Hidalgo and Zamora (2004). Later, the proposed reaction mechanism was extended to hydroxyalkenals as well (Hidalgo *et al.*, 2005). The authors suggested a reaction mechanism, shown in Figure 36.7, where in the first step an imine is formed, followed by a decarboxylation and hydrolysis, yielding a Strecker aldehyde and an alkyl-pyridine. As the reaction proceeds evenly at 37°C and 60°C, the results suggest that heating is not required for this reaction to occur. Moreover, these Strecker-like reactions lead to pyrrole compounds, able to polymerize to brown pigmented melanoidin-like structures (Hidalgo *et al.*, 2003; Zamora *et al.*, 2000). Based on these observations, a merged reaction pathway between the Maillard reaction and lipid oxidation was proposed (Zamora and Hidalgo, 2005).

Aldol Condensation Hashimoto and Kuroiwa (1975) suggested that an aldol condensation in beer is possible under mild conditions during storage. They observed an aldol condensation between acetaldehyde and heptanal, after 20 days of incubation at 50°C, in the presence of proline (which is always present in beer, as it is not metabolized by yeast under normal fermentation conditions). In this reaction amino acids can act as catalysts by the formation of an imine intermediate. This pathway can convert compounds with a medium flavor threshold into compounds with a very low flavor threshold. The proposed reaction mechanism is shown in Figure 36.8.

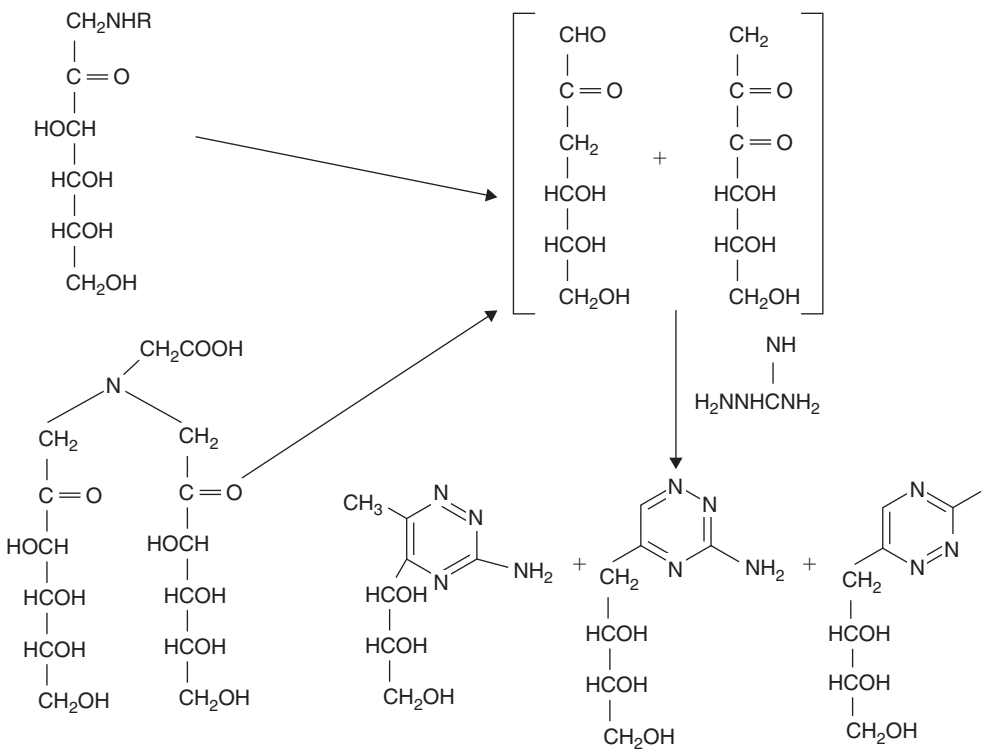

Figure 36.5 Formation of aminotriazine adducts after reaction of aminoguanidine with 1-DH and 3-DH (Reprinted from Hirsch *et al.*, 1995, with permission of Elsevier) Amadori compounds will react to either 1-DH or 3-DH. These dicarbonyl intermediates can be trapped by the reactive guanyl-group of aminoguanidine, which leads to the irreversible formation of triazines.

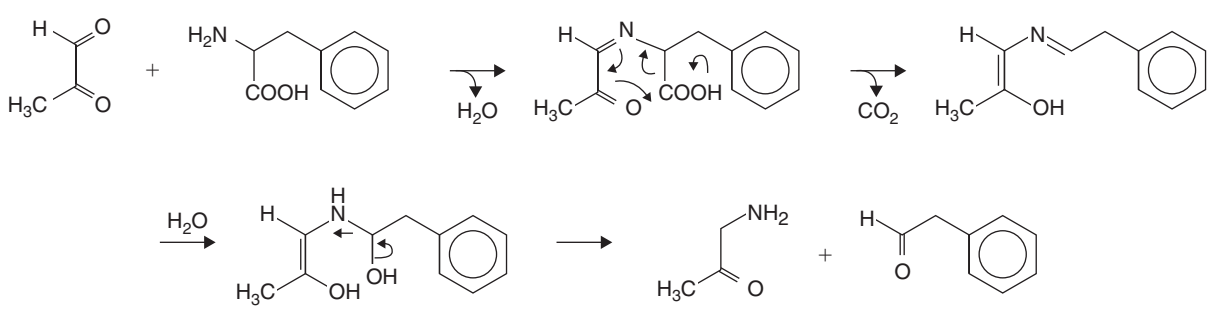

Figure 36.6 Strecker degradation of phenylalanine by methylglyoxal (Reprinted from Hofmann *et al.*, 2000, with permission from the American Chemical Society). After condensation of the α-dicarbonyl compound and the amino acid to an imine intermediate, decarboxylation occurs. Water addition to the intermediate releases the aminoketone and phenylacetaldehyde.

Degradation of Hop Bitter Acids Hop bitter acids (α-acids, β-acids and iso-α-acids) also undergo oxidative degradation during beer storage, resulting in alkanones, alkenals and alkadienals of varying length and structure (Hashimoto and Eshima, 1977). Particularly isohumulones are very susceptible to oxidative degradation due to the double bond and the carbonyl group of the isohexenoyl side chain. This oxidation of bitter acids is accompanied by a decrease in beer bitterness (Hashimoto and Eshima, 1979). More recently, it was demonstrated that the gradual decrease of beer bitterness intensity is largely due to the instability of *trans*-iso-α-acids. It was suggested that the ratio of *trans*/*cis*-iso-α-acids offers a reliable criterion to evaluate bitterness deterioration as a function of time (De Cooman *et al.*, 2000). The difference between *cis*-and *trans*-iso-α-acids is depicted in Figure 36.9. Oxidation of α-acids and iso-α-acids leads to complex polycyclic structures by intramolecular reactions, mostly dehydrations. Presumably, as long as functional groups remain in the oxidized bitter substances, oxidation will continue to proceed. Hop β-acids are highly unstable due to the highly susceptible unsaturated side chains and undergo autoxidation which starts already during hop storage. During the brewing process, β-acids will be oxidized almost completely (De Keukeleire, 1981a, b). To overcome the oxidative deterioration of hop bitter compounds, reduced isomerized

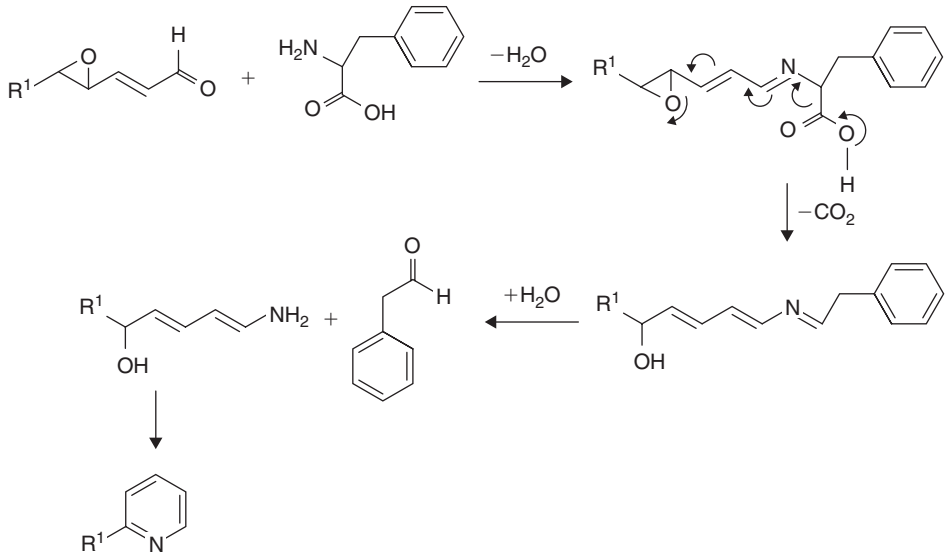

Figure 36.7 Strecker-type degradation of phenylalanine by a 4,5-epoxy-2-alkenal (Reprinted from Hidalgo and Zamora, 2004, with permission of the American Chemical Society). After the condensation of a 4,5-epoxy-2-alkenal and an amino acid to an imine, decarboxylation occurs. Subsequent hydrolysis of the imine compound releases the Strecker aldehyde and the unsaturated amine, which can further react to an alkylpyridine.

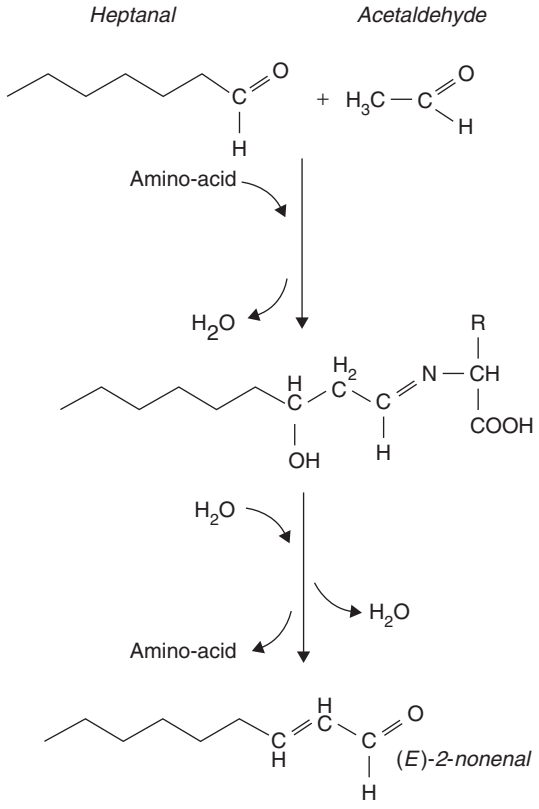

Figure 36.8 Aldol condensation of heptanal and acetaldehyde (Hashimoto and Kuroiwa, 1975). Aldehydes can react via an aldol condensation to unsaturated aldehydes. Amino acids function as catalysts by the formation of an imine intermediate to accelerate the reaction. After hydrolysis of the imine intermediate, the amino acid is recovered and the unsaturated aldehyde is released.

hop extracts are frequently used. Tetrahydroiso-α-acids are extremely resistant to oxidative deterioration and promote foam stability and bitterness consistency, while the light-stable dihydroiso-α-acids do not appear to be stable at all during storage (De Cooman *et al.*, 2001).

A well-known isohumulone degradation compound is 3-methyl-2-butene-1-thiol (MBT), responsible for the light-struck or skunky flavor in beer exposed to visible light. The light struck flavor compound is characterized by an extreme low flavor threshold, 7 ng/l (Irwin *et al.*, 1993). As isohumulones do not absorb light in the visible region, MBT production has to be triggered by a photo-sensitizer, like riboflavin. There exists a correlation between MBT formation and the riboflavin concentration in beer (Sakuma *et al.*, 1991). Riboflavin, present in beer in concentrations of 0,2–1,3 mg/l, absorbs visible light and can be excited to its triplet state ($^3RF^{\bullet}$), followed by an electron transfer from iso-α-acids to $^3RF^{\bullet}$ (Goldsmith *et al.*, 2005). This leads to the formation of the 2-methyl-2-butene radical, which reacts to MBT with H_2S (Huvaere *et al.*, 2005). The initiation of the radical reaction is depicted in Figure 36.10. As this reaction can occur in the absence of oxygen, it can have a major impact on beer quality. Therefore it is better to store beer in darkness and the use of brown beer bottles is preferred above green or white bottles.

Oxidation of Polyphenols Degradation of polyphenols may affect the bitter quality and astringency of beer, but also the beer color (Dadic, 1974). Upon oxidation or acid catalysis, polymerization reactions lead to complex polyphenols of high molecular weight. Possibly, the initiation of the

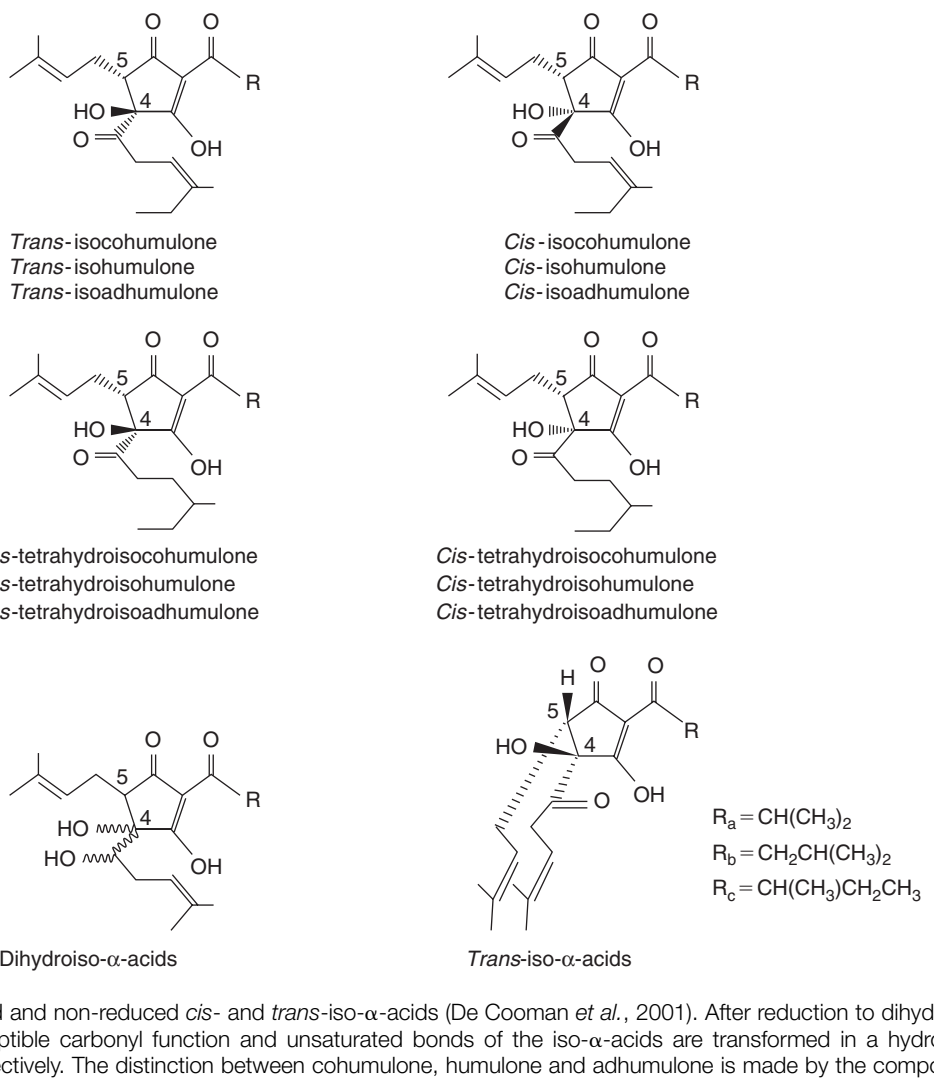

Figure 36.9 Reduced and non-reduced *cis*- and *trans*-iso-α-acids (De Cooman *et al.*, 2001). After reduction to dihydro- or tetrahydro-iso-α-acids, the susceptible carbonyl function and unsaturated bonds of the iso-α-acids are transformed in a hydroxyl function and saturated bonds, respectively. The distinction between cohumulone, humulone and adhumulone is made by the composition of the side chain (R).

polymerization reactions starts with the oxidation to quinone or semi-quinone radicals, which then further interact with other phenolic compounds (Gardner and McGuinness, 1977). During beer storage, the complex phenolic polymers, called tannoids, as well as oxidized monomeric phenols interact with proteins to form insoluble complexes and hazes, finally leading to colloidal instability.

Treatment of beer with recommended dosages of polyvinylpolypyrrolidone (PVPP) extends effectively the colloidal stability of beer without compromising flavor stability. Although partial removal of flavanoid polyphenols reduces the endogenous-reducing capacity, the resulting beers do not seem to be more susceptible to oxidative flavor damage (McMurrough *et al.*, 1996).

Carotenoid Degradation or Hydrolysis of Glycosides The most important member of the norisoprenoids in beer, β-damascenone, can be found in malt extract (Farley and Nursten, 1980), hops (Tressl *et al.*, 1978a, b), beer (Lermusieau *et al.*, 2001) and wine (Genovese *et al.*, 2005). This compound is characterized by a very low flavor threshold in water (20–90 ng/l) and can be found in naturally aged beers in a concentration up to 210 μg/l. Concerning the mechanism of formation of β-damascenone, acid hydrolysis might explain the increase of β-damascenone during beer aging (Chevance *et al.*, 2002). The hypothesis of acid hydrolysis is supported by the observed increase in β-damascenone concentration when lowering the beer pH (Gijs *et al.*, 2002). Potential precursors include the allene triols and acetylene diols arising from the degradation of neoxanthin (shown in Figure 36.11). Degradation of neoxanthin leads first to the Grasshopper ketone. Reduction of the Grasshopper ketone, followed by acid hydrolysis, ultimately leads to β-damascenone.

It was also found that β-glucosidase treatment of beer leads to an increase in the β-damascenone concentration, due to the hydrolysis of glycosylated β-damascenone precursors (Chevance *et al.*, 2002). Through degradation of

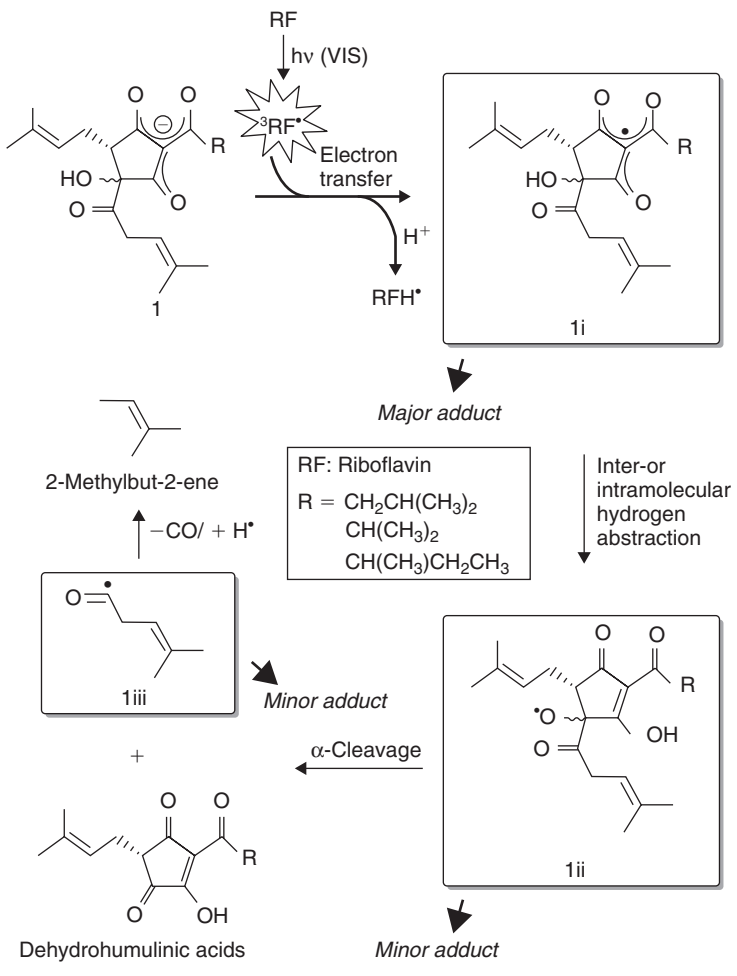

Figure 36.10 Reaction of triplet riboflavin with isohumulones (Reprinted from Huvaere *et al.*, 2005, with permission from the American Chemical Society). Excited riboflavin will extract an electron from bitter acids. This initiates intramolecular reactions, ultimately leading to the formation of the 2-methyl-2-butene radical. After reaction with H_2S, MBT is formed.

carotenoids or hydrolysis of glycosides, other important compounds, contributing to beer aging, might also be released.

Acetalization of Aldehydes A condensation reaction between 2,3-butanediol (concentration up to 280 mg/l in beer) and an aldehyde like acetaldehyde, isobutanal, 3-methylbutanal and 2-methylbutanal, leads to cyclic acetals as 2,4,5-trimethyl-1,3-dioxolane, 2-isopropyl-4,5-dimethyl-1,3-dioxolane, 2-isobutyl-4,5-dimethyl-1,3-dioxolane and 2-sec. butyl-4,5-dimethyl-1,3-dioxolane, respectively (Peppard and Halsey, 1982). The equilibrium between 2,4,5-trimethyl-1,3-dioxolane, acetaldehyde and 2,3-butanediol is reached rapidly in beer. Consequently, during beer aging, the concentration of 2,4,5-trimethyl-1,3-dioxolane will increase similarly to the increase of acetaldehyde (Vanderhaegen *et al.*, 2003).

Synthesis and Hydrolysis of Esters During beer aging, esters formed above their chemical equilibrium will hydrolyze at a characteristic rate. Acetate esters of higher alcohols will hydrolyze more rapidly than the corresponding ethyl esters, independent of the alcohol content of the beverage (Ramey and Ough, 1980). Isoamyl acetate is found to be hydrolyzed both chemically and enzymatically. The enzymatic hydrolysis depends greatly on pH, storage temperature and fermentation/maturation conditions and is performed by esterases released after cell-lysis during fermentation/maturation and bottle conditioning (for beers refermented in the bottle, a typical habit for Belgian specialty beers). The greatest decrease in positive fruity esters is observed in beers refermented in the bottle and in non-pasteurized beers. After pasteurization, the enzymatic hydrolysis is eliminated during beer aging (Neven *et al.*, 1997).

In contrast to hydrolysis, the esterification of ethanol with organic acids also occurs during beer aging. In fresh beer, the concentration of these ethyl esters is relatively low and well under the equilibrium concentration. It is therefore most likely that they were almost not formed by yeast during fermentation (Vanderhaegen *et al.*, 2003).

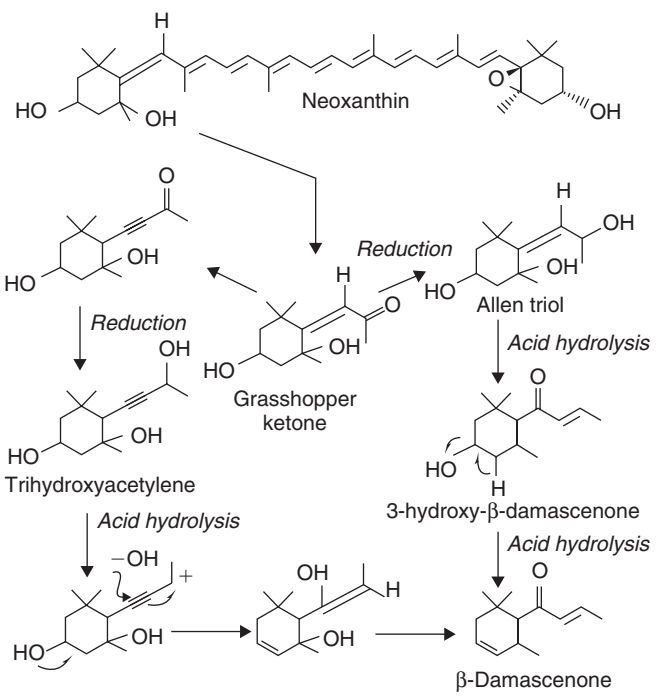

Figure 36.11 Formation of β-damascenone from neoxanthin (Reprinted from Chevance *et al.*, 2002, with permission from the American Chemical Society). Degradation of neoxanthin leads to the formation of the Grasshopper ketone, which after reduction and subsequent acid hydrolysis, gives rise to β-damascenone.

The precursor acids are found to be originating partly from hop, by oxidation of hop bitter acids to for example isovalerate and 2-methylbutyrate. After the conversion with ethanol to ethyl esters, these compounds impart a winey, brandy-like flavor to beer (Williams and Wagner, 1978, 1979). Precursors of ethyl ester formation can also be delivered by the Strecker degradation, which can also give rise to organic acids (Hofmann *et al.*, 2000). In addition to the hop acids and the Strecker acids, acids originating from yeast metabolism are present in beer, which may explain the increased concentration of diethyl succinate, ethyl pyruvate, ethyl lactate, ethyl nicotinate and ethyl phenylacetate in aged beer (Vanderhaegen *et al.*, 2003).

Antioxidants and Reducing Agents vs. Pro-oxidants A broad definition of an antioxidant is: a substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1989). However, the way antioxidants can exert their antioxidant activity differs between compounds. Moreover, most of the presumed antioxidants can have pro-oxidant capabilities as well, for example, ascorbic acid (Brezova *et al.*, 2002), sulfhydryl compounds, chelating agents, polyphenols, Maillard-reaction products (Bamforth, 2001). Maillard-reaction products as melanoidins are known to scavenge free and active oxygen, act as reducing agents and chelate metals (Ames, 2001). However, there have been reports of pro-oxidant activity as well (Hashimoto, 1972).

Phenolic compounds, like (+)-catechin, quercetin, 4-vinylguaiacol and rutin are known to quench superoxide radicals and to inhibit lipid peroxidation. Recently however, it was stated that at beer pH, the scavenging activity of the polyphenols and phenolic acids is very low comparing to sulfite (Nakamura *et al.*, 2003). Other phenolic compounds, as ferulic acid, often demonstrate poor quenching abilities but good peroxidation inhibition. However, some antioxidants can have pro-oxidant properties as well, like (+)-catechin in low concentrations (Walters *et al.*, 1997a). In beer (+)-catechin and ferulic acid also seem to specifically decrease the rate of the *cis*-isohumulone degradation, but they can exert a negative influence on beer colloidal (catechin) or flavor (ferulic acid, after conversion to 4-vinylguaiacol) properties (Walters *et al.*, 1997b).

Sulfite is a very potent inhibitor of flavor deterioration. It is naturally formed by yeast and is frequently used in the food industry. Sulfite stabilizes the flavor in two ways, as antioxidant and as carbonyl scavenger in aldehyde-bisulfite adducts.

Concerning the formation of reactive oxygen species, sulfite is found to be the only compound that is able to effectively retard the formation of radicals in beer during storage. Hydrogen peroxide and organic hydroperoxides are the only reactive oxygen species that are stable enough to be trapped efficiently by antioxidants which normally only are present in micromolecular concentrations (Andersen *et al.*, 2000). Moreover, sulfite reacts with H_2O_2 in an acid-base catalyzed reaction that does not involve radical intermediates. This action prevents the radicals to be produced via the Fenton and Haber–Weiss reactions (Hoffmann and Edwards, 1975).

Sulfite is known to form adducts to staling aldehydes. The strength of the aldehyde bisulfite complexes decreases with increasing chain length and the presence of double bonds. Based on these observations, a staling mechanism was proposed. When the concentration of acetaldehyde rises during beer storage, sulfite will be transferred from staling aldehydes to acetaldehyde, thereby releasing the staling aldehydes (Nyborg *et al.*, 1999).

Kaneda *et al.* (1996) observed the bisulfite adduct of acetaldehyde and other aldehydes, and concluded that the interaction between nonenal and bisulfite was too weak to form stable adducts during beer fermentation. Dufour *et al.* (1999) on the other hand, observed a permanent irreversible sulfite adduct to the double bond of unsaturated aldehydes. The stability of such adducts makes the mechanism of release of unsaturated aldehydes during beer aging relatively improbable. In Figure 36.12 different possible bisulfite adducts are shown. The sulfite addition to the carbonyl group is reversible, but the addition to the carbonyl double bond is irreversible and highly stable.

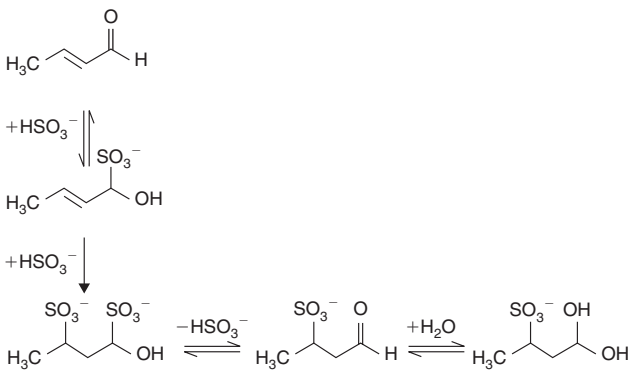

Figure 36.12 Proposed mechanism of bisulfite addition to (E)-2-nonenal (Dufour *et al.*, 1999). The addition of sulfite to the carbonyl group of (E)-2-nonenal is reversible. This is in contrast with the definitive addition of sulfite to the double bond of (E)-2-nonenal.

It is also believed that sulfite stabilizes intermediates of the Maillard reaction by forming adducts. For example, glyceraldehyde forms a stable hydroxysulfonate adduct, which renders this aldehyde non-reactive in processed food (Keller *et al.*, 1999). In conclusion, it can be stated that sulfite stabilizes the flavor as an antioxidant because of the good radical scavenging characteristics rather than its capacity to bind carbonyls (Kaneda *et al.*, 1994). Concerning the formation of reactive oxygen species, sulfite is found to be the only compound that is able to effectively retard the formation of radicals in beer during storage. Moreover, sulfite reacts with H_2O_2 in an acid-base catalyzed reaction that does not involve radical intermediates. This action prevents the radicals to be produced via the Fenton and Haber–Weiss reactions (Hoffmann and Edwards, 1975).

Yeast itself is also a very potent antioxidative creature. *Saccharomy cescerevisiae* is able to reduce a variety of carbonyls and seems to play a very important role in the reduction of beer staling compounds and their precursors (Shimizu *et al.*, 2002).

Conclusion

It is obvious that the knowledge of beer aging is far from complete, given the myriad of reactions that exert an influence on the composition and freshness of beer. Most of the reactions cannot be avoided, but they can at least be minimized. Therefore, excessive heat addition during wort production should be avoided, beer should be bottled with a minimum of oxygen and it should be stored at low temperatures and in the dark.

Summary Points

- Beer changes continuously toward its chemical equilibrium.
- Beer aging will lead to flavor deterioration and colloidal instability.
- Activated forms of oxygen play an important role in beer aging, initiating radical reactions.
- Trace metals function as catalysts during radical formation.
- Lipid oxidation leads to the formation of aldehydes.
- The Maillard reaction pathway is highly complicated and exerts a major influence on beer staling by dicarbonyl compounds and Strecker reactions.
- Degradation of hop bitter acids decreases bitterness but also creates flavor compounds.
- Oxidized polyphenols will form complexes with proteins and cause colloidal haze.
- Certain esters, present above their equilibrium and responsible for the fresh flavor will be hydrolyzed, while other esters will be synthesized chemically.
- Carotenoid degradation and hydrolysis of glycosides lead to the release of potent flavor compounds.
- Most of the used antioxidants are also known to exhibit pro-oxidant behavior in certain circumstances.
- Antioxidants should trap hydrogen peroxide or organic peroxides to efficiently protect beer from aging reactions.

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