Review

The chemistry of beer aging – a critical review

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Abstract

Currently, the main quality problem of beer is the change of its chemical composition during storage, which alters the sensory properties. A variety of flavours may arise, depending on the beer type and the storage conditions. In contrast to some wines, beer aging is usually considered negative for flavour quality. The main focus of research on beer aging has been the study of the card-board-flavoured component (*E*)-2-nonenal and its formation by lipid oxidation. Other stale flavours are less described, but may be at least as important for the overall sensory impression of aged beer. Their origin has been increasingly investigated in recent years. This review summarizes current knowledge about the chemical origin of various aging flavours and the reaction mechanisms responsible for their formation. Furthermore, the relationship between the production process and beer flavour stability is discussed.
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1. Introduction

As for other food products, also for beer, several quality aspects may be subject to changes during storage. Beer shelf-life is mostly determined by its microbiological, colloidal, foam, colour and flavour stabilities. In the past, the appearance of hazes and the growth of beer spoilage micro-organisms were considered as the main trouble-causing phenomena. However, with progress in the field of brewing chemistry and technology, these problems are now largely under good control. Most of the interest has shifted to factors affecting the changes in beer aroma and taste, as beer flavour is regarded as the most important quality parameter of the product. However, bearing in mind that *de gustibus et colouribus non est disputandum*, consumers do not necessarily dislike the flavour of an aged beer. Indeed, a study (Stephenson & Bamforth, 2002) with consumer trials pointed out that aging flavours are not always regarded as off-flavours. More important for appreciation of a beer were the expectations consum-

ers have in recognizing the flavour of just the particular brand of beer that they generally drink. To meet the consumers expectations, the flavour of a certain beer brand must be constant. However, as the expected flavour is normally the flavour of the particular fresh beer, as a result of beer aging, such flavour may change, and the expected flavour is lost. This should mainly be considered as the most important reason that beer staling is undesirable.

Starting from the 1960s, several studies have focussed on the chemical aspects of beer staling. Notwithstanding 30–40 years of research, beer aging remains difficult to control. With the increasing export of beer, due to market globalisation, shelf-life problems may become extremely important issues for some breweries. Beer aging is a very complex phenomenon. This overview on the chemistry of beer aging intends to illustrate the complexity of the aging reactions.

2. Sensory changes in beer during storage

The literature on beer staling reveals only few reports dealing with the actual sensory changes during beer

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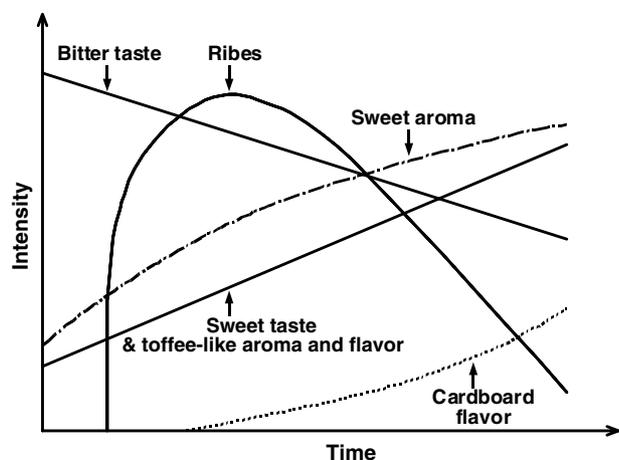
Fig. 1. Sensory changes during beer aging according to Dalgliesh (1977).

storage. Dalgliesh (1977) described the changes in the most detail. However, the Dalgliesh plot (Fig. 1) is a generalization of the sensory evolution during beer storage and is by no means applicable to every beer. A constant decrease in bitterness is observed during aging. This is partly due to sensory masking by an increasing sweet taste. In contrast to an initial acceleration of sweet aroma development, the formation of caramel, burnt-sugar and toffee-like aromas (also called leathery) coincides with the sweet taste increase. Furthermore, a very rapid formation of what is described as *ribes* flavour is observed. The term *ribes* refers to the characteristic odour of blackcurrant leaves (*Ribes nigrum*). Afterwards, the intensity of the *ribes* flavour decreases. According to Dalgliesh (1977), cardboard flavour develops after the *ribes* aroma. On the other hand, according to Meilgaard (1972), cardboard flavour constantly increases to reach a maximum, followed by a decrease. Besides these general findings, other reported changes in flavour are harsh after-bitter and astringent notes in taste (Lewis, Pangborn, & Tanno, 1974) and wine- and whiskey-like notes in strongly aged beer (Drost, Van Eerde, Hoekstra, & Strating, 1971). Positive flavour attributes of beer, such as fruity/estery and floral aroma tend to decrease in intensity. For the overall impression, the decrease of positive flavours may be just as important as development of stale flavours (Bamforth, 1999b; Whitear, Carr, Crabb, & Jacques, 1979).

Often beer staling is presented as just being related to cardboard flavour development. While, in some cases, and especially in lager beers, cardboard flavour is the major manifestation of beer staling, this can not be generalized. Aging flavours vary between beer types and certainly, for speciality beers, other stale flavours are often more prominent. Whitear (1981) reported aging notes of a strong ale as burnt, alcoholic, caramel, liquorice and astringent flavours, whereas cardboard and metallic flavours were not found. Moreover, strong ini-

tial burnt flavours in dark beers may mask the development of aging flavours and result in a better flavour stability of this beer type. However, as will be explained further on, other factors probably also account for this observation.

Contact of beer with oxygen causes a rapid deterioration of the flavour and the type of flavour changes depends on the oxygen content of bottled beer. For instance, there is a close correlation between the *ribes* odour and headspace air, and this flavour can be avoided in the absence of excessive contact with air (Clapperton, 1976). Furthermore, it is found that beer staling still occurs at oxygen levels as low as possible (Bamforth, 1999b), which suggests that beer staling is partly a non-oxidative process.

Apart from oxygen concentration, storage temperature affects the aging characteristics of beer, by affecting the many chemical reactions involved. The reaction rate increase for a certain temperature increase depends on the reaction activation energy. This activation energy differs with the reaction type, which means that the rates of different reactions do not equally increase with increasing temperature. Consequently, beer storage at different temperatures does not generate the same relative level increase of staling compounds. Some sensory studies confirm this prediction. According to Furusho et al. (1999), cardboard flavour shows different time courses during lager beer storage at 20 and 30 °C. In the early phase of beer aging, this results in a sensory pattern with relatively more cardboard character when beer is stored at 30 °C compared to 20 °C. This agrees with the findings of Kaneda, Kobayashi, Furusho, Sahara, and Koshino (1995b) that lager beer aged at 25 °C tends to develop a predominantly caramel character whereas, at 30 or 37 °C, more cardboard notes are dominant.

From these examples, it follows that the Dalgliesh plot (Fig. 1) is a simplification of the sensory changes during storage. The nature of flavour changes is complex and mainly depends on the beer type, the oxygen concentration and the storage temperature.

3. Chemical changes in beer during storage

3.1. General

Flavour deterioration is the result of both formation and degradation reactions. Formation of molecules, at concentrations above their respective flavour threshold leads, to new noticeable effects, while degradation of molecules to concentrations below the flavour threshold may cause loss of initial fresh beer flavours. Furthermore, interactions between different aroma volatiles may enhance or suppress the flavour impact of the molecules (Meilgaard, 1975a).

3.2. Volatile compounds

3.2.1. Analysis

With the introduction of gas chromatography in the 1960s, it became possible to study the changes in beer volatiles during storage. In the late 1960s, several studies (Ahrenst-Larsen & Levin Hansen, 1963; Engan, 1969; Jamieson, Chen, & Van Gheluwe, 1969; Trachman & Saletan, 1969; von Szilvinyi & Püspök, 1969) reported the formation of staling-related compounds. Table 1 shows a classification of the volatiles currently known as being related to concentration changes during beer aging.

In recent years, new techniques, such as aroma extraction dilution analysis (AEDA), have been developed to evaluate the relevance of detected volatiles to odour perception in foods. (Belitz & Grosch, 1999). Using this method, several staling compounds have been identified in beer (Gijs, Chevance, Jerkovic, & Collin, 2002; Schieberle, 1991; Schieberle & Komarek, 2002). In this technique, a flavour extract of beer is sequentially diluted and each dilution is analyzed by GC–O (gas chromatography/olfactometry) by a small number of judges. The extraction method is very important, as it is essential to ensure that extracts with an odour representative of the original product are obtained. The flavour dilution (FD) of an odorant corresponds to the maximum dilution at which that odorant can be perceived by at least one of the judges. Consequently, the FD factors give an estimation of the importance of volatiles for the perceived flavour of a beer sample. The method should be regarded as a first step in the screening for staling compounds and not to obtain conclusive results about the relevance of flavour compounds.
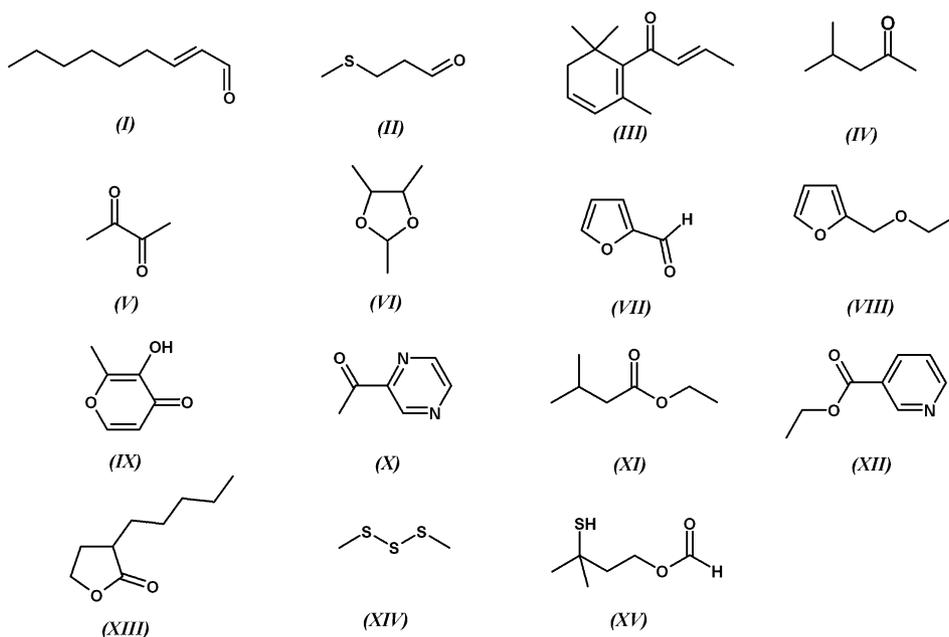
3.2.2. Carbonyl compounds

From the start of research on staling compounds, carbonyls attracted most attention. Such compounds were known to cause flavour changes in food products such as milk, butter, vegetables and oils. Hashimoto (1966) was the first to report a remarkable increase in the level of volatile carbonyls in beer during storage, in parallel with the development of stale flavours. Acetaldehyde was one of the first compounds for which a concentration increase was observed in aged beer (Engan, 1969) and further research (Meilgaard, Elizondo, & Moya, 1970; Meilgaard & Moya, 1970; Palamand & Hardwick, 1969) on alkanals and alkenals revealed their high flavour potency in beer. In that context, Palamand and Hardwick (1969) first described (E)-2-nonenal as a molecule, which on addition to beer, induces a cardboard flavour similar to such flavour in aged beer. A year later, the identification, in heated acidified beer, of (E)-2-nonenal, by Jamieson and Van Gheluwe (1970), as the molecule responsible for cardboard flavour, was considered a breakthrough in beer flavour research. In the following years, other studies (Drost et al., 1971; Meilgaard, Ayma, & Ruano, 1971; Wohleb, Jennings, & Lewis, 1972) confirmed the results, but all referred to heated and acidified (pH 2) beer. Such extreme storage conditions were initially used to obtain detectable levels, as research on beer carbonyls is complicated due the extremely low levels at which many of these compounds occur. However, it is questionable whether the results are representative of real storage conditions. In general, it remains important that steps in the analytical procedure are avoided, which might alter or form compounds of interest.

Direct analysis by gas chromatography of either a headspace or a solvent extract of non-treated beer is not applicable because other, more abundant, volatiles frequently obscure the carbonyl compound peaks. Wang and Siebert (1974) first developed a method to follow the (E)-2-nonenal concentration increase under more normal storage conditions (6 days at 38 °C). The technique was based on extraction of beer with dichloromethane, followed by derivatisation of (E)-2-nonenal with 2,4-dinitrophenylhydrazine (DNPH) under acidic conditions. The treated beer extract was subjected to separation and concentration steps by means of column and thin-layer chromatography, and finally analysed by high performance liquid chromatography. With this method, there was a concentration increase in levels of (E)-2-nonenal to levels above the flavour threshold of 0.1 μg/L. In other studies (Greenhoff & Wheeler, 1981a; Greenhoff & Wheeler, 1981b; Hashimoto & Eshima, 1977; Jamieson & Chen, 1972; Stenroos, Wang, Siebert, & Meilgaard, 1976) on aldehydes in beer, similar analysis techniques, based on carbonyl 2,4-dinitrophenylhydrazone formation, were used, and although isolation techniques were usually different, they confirmed the increase of (E)-2-nonenal and other linear C_4–C_{10} alkenals and alkanals in beer during storage. Due to the growing importance of (E)-2-nonenal and other carbonyls in beer, various techniques have been proposed to measure their concentrations in beer. Many methods remain based on derivatisation of the carbonyls in order to decrease the interference caused by the beer matrix. Derivatisation agents, such as o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) (Grönqvist, Siirilä, Virtanen, Home, & Pajunen, 1993; Ojala, Kotiaho, Siirilä, & Sihvonen, 1994; Angelino et al., 1999) or hydroxylamine hydrochloride (Barker, Pipasts, & Gracey, 1989) have been applied to liquid–liquid extracts of beer and the derivatives were eventually analysed using GC–MS or GC–ECD. These procedures remain laborious and time-consuming. More recent methods, using solid-phase micro-extraction (Vesely, Lusk, Basarova, Seabrooks, & Ryder, 2003) or stir bar sorptive extraction (Ochiai, Sasamoto, Daishima, Heiden, & Hoffmann, 2003) with on-site PFBOA derivatisation of carbonyls, have been developed. Other techniques include the extraction of

Table 1
Overview of the currently known volatile compounds formed during storage of beer

Chemical class	Compounds
Linear aldehydes	• Acetaldehyde
	• (E)-2-octenal/(E)-2-nonenal (I)/(E,E)-2,6-nonadienal/(E,E)-2,4-decadienal
Strecker aldehydes	• 2-Methyl-butanal/3-methyl-butanal/2-phenylacetaldehyde/benzaldehyde/3-(methylthio)propionaldehyde (II)
Ketones	• (E)-β-damascenone (III)
	• 3-Methyl-2-butanone/4-methyl-2-butanone/4-methyl-2-pentanone (IV)
	• Diacetyl (V)/2,3-pentanedione
Cyclic acetals	• 2,4,5-Trimethyl-1,3-dioxolane (VI)/2-isopropyl-4,5-dimethyl-1,3-dioxolane/2-isobutyl-4,5-dimethyl-1,3-dioxolane/2-sec butyl-4,5-dimethyl-1,3-dioxolane
Heterocyclic compounds	• Furfural (VII)/5-hydroxymethyl-furfural/5-methyl-furfural/2-acetyl-furan/2-acetyl-5-methyl-furan/2-propionylfuran/furan/furfuryl alcohol
	• Furfuryl ethyl ether (VIII)/2-ethoxymethyl-5-furfural/2-ethoxy-2,5-dihydrofuran
	• Maltol (IX)
	• Dihydro-5,5-dimethyl-2(3H)-furanon/5,5-dimethyl-2(5H)-furanon
	• 2-Acetylpyrazine (X)/2-methoxypyrazine/2,6-dimethylpyrazine/trimethylpyrazine/tetramethylpyrazine
Ethyl esters	• Ethyl 3-methyl-butyrate (XI)/ethyl 2-methyl-butyrate/ethyl 2-methyl-propionate
	• Ethyl nicotinate (XII)/diethyl succinate/ethyl lactate/ethyl phenylacetate/ethyl formate/ethyl cinnamate
Lactones	• γ-Nonalactone (XIII)/γ-hexalactone
S-compounds	• Dimethyl trisulfide (XIV)
	• 3-Methyl-3-mercaptobutylformate (XV)

carbonyls by vacuum distillation, followed by GC–MS analysis (Lermusieau, Noel, Liegeois, & Collin, 1999) or steam distillation, solid phase extraction and isocratic HPLC–UV detection (Santos et al., 2003).

Since the Jamieson and Van Gheluwe (1970) publication, expectations were high that (E)-2-nonenal could be considered as *the* molecule responsible for beer staling and that methods to reduce its concentration would finally solve the problem of beer staling. However, its in-

crease during storage is by no means ubiquitous. According to Van Eerde and Strating (1981), (E)-2-nonenal increased at 40 °C in several beers within a few days to levels above its threshold whereas, at 20 °C this was not found, even after 4 months of storage. A similar result was obtained by Narziss, Miedaner, and Graf (1985). Moreover, recent publications (Foster, Samp, & Patino, 2001; Narziss, Miedaner, & Lustig, 1999; Schieberle & Komarek, 2002; Vesely et al., 2003)

mention no significant increases in (*E*)-2-nonenal concentration during beer aging. In contrast, other authors (Lermusieau et al., 1999; Liegeois, Meurens, Badot, & Collin, 2002; Santos et al., 2003) continue to report its formation and observations that it occurs independently of the oxygen concentration in a bottled beer (Narziss et al., 1985; Noel et al., 1999; Walters, Heasman, & Hughes, 1997b).

Despite such seemingly contradictory reports, there are indications that some carbonyl compounds are important in flavour staling. This statement can be illustrated by Hashimoto's demonstration (Hashimoto, 1981) that carbonyl scavengers, such as hydroxylamine, immediately diminish certain aspects of the aging flavour in beer.

Other linear aldehydes have flavour properties similar to those of (*E*)-2-nonenal (Meilgaard, 1975b). The involvement of these linear C_4–C_{10} alkanals, alkenals and alkedienals in beer aging has been studied to a lesser extent. In a study of Greenhoff and Wheeler (1981a, 1981b), the levels of all linear C_4–C_{10} 2-alkenals increased. In particular, longer chain 2-alkenals, starting from 2-heptenal, surpassed their threshold during beer storage. Only the shorter chain linear alkanals; butanal, pentanal and hexanal, were significantly formed. Harayama, Hayase, and Kato (1994) reported that the alkedienals, (*E,Z*)-2,6-nonadienal and (*E,E*)-2,4-decadienal take part in flavour staling.

Other aldehydes formed during beer storage are the so-called Strecker aldehydes: 2-methyl-propanal (Wheeler, Pragnell, & Pierce, 1971; Bohmann, 1985b; Vesely et al., 2003), 2-methyl-butanal (Miedaner, Narziss, & Eichhorn, 1991; Vesely et al., 2003), 3-methyl-butanal (Miedaner et al., 1991; Vesely et al., 2003; Wheeler et al., 1971), benzaldehyde (Miedaner et al., 1991; Wheeler et al., 1971), phenylacetaldehyde (Miedaner et al., 1991; Vesely et al., 2003) and methional (Gijs et al., 2002; Vesely et al., 2003). Generally, their concentrations increase more at elevated oxygen concentrations (Bohmann, 1985a; Miedaner et al., 1991; Narziss et al., 1985). AEDA of aged beer revealed that methional (cooked potato-like) (Gijs et al., 2002; Schieberle & Komarek, 2002) and phenylacetaldehyde (sweet, honey-like) (Schieberle & Komarek, 2002) are relevant for the sensory profile of aged beer. The other Strecker aldehydes do not seem important for stale flavour formation, but can be considered as suitable markers for beer oxidation (Narziss, Miedaner, & Eichhorn, 1999a, 1999b).

For ketones, an AEDA study revealed that a carotenoid-derived compound,β-damascenone (rhubarb, red fruits, strawberry) affects beer flavour during aging (Chevance, Guyot-Declerck, Dupont, & Collin, 2002; Gijs et al., 2002). Carotenoid-derived flavour components had already been suspected to be staling components by Strating and Van Eerde (1973). Other ketones whose concentrations increase with beer age are 3-methyl-butan-2-one and 4-methylpentan-2-one (Hashimoto & Kuroiwa, 1975; Lustig, Miedaner, Narziss, & W., 1993) and the vicinal diketones; diacetyl and 2,3-pentanedione. This is more pronounced at higher oxygen levels and diacetyl may even surpass its flavour threshold (Wheeler et al., 1971).

3.2.3. Cyclic acetals

Particularly when beer is in contact with oxygen, the cyclic acetals, 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, increase during storage (Vanderhaegen et al., 2003b). A flavour threshold of 900 µg/l and a maximum concentration in beer of around 100 µg/l were reported for 2,4,5-trimethyl-1,3-dioxolane (Peppard & Halsey, 1982).

3.2.4. Heterocyclic compounds

Heterocyclic compounds, some with carbonyl functions, represent a large group of compounds subject to concentration changes during beer aging. The following furans are formed (Lustig et al., 1993; Madigan, Perez, & Clements, 1998; Varmuza, Steiner, Glinsner, & Klein, 2002): furfural, 5-hydroxymethyl-furfural (HMF), 5-methyl-furfural, 2-acetyl-furan, 2-acetyl-5-methyl-furan, 2-propionylfuran, furan and furfuryl alcohol. Although they generally remain far below their flavour thresholds, they are mentioned as sensitive indicators of beer flavour deterioration (Bernstein & Laufer, 1977; Brenner & Khan, 1976; Shimizu et al., 2001b). Furfural and HMF levels may increase with time at an approximately linear rate, which varies logarithmically with the storage temperature (Madigan et al., 1998). Oxygen seems without effect. Interestingly, a close correlation is found between their increase and the sensory scores for stale flavour. Therefore, these compounds can be used as indicators of heat-induced flavour damages to beer. Recently, we reported that furfuryl ethyl ether can also function as such an indicator (Vanderhaegen et al., 2004a). Furthermore, this furanic ether may increase to levels above the flavour threshold (6 µg/l) during storage, inducing a solvent-like stale flavour in the beer (Vanderhaegen et al., 2003b).

According to Lustig et al. (1993), the following furanones also appear during beer aging: dihydro-5,5-dimethyl-2(3H)-furanone, 5,5-dimethyl-2(5H)-furanone, dihydro-2(3H)-furanone, 3-methyl-2(5H)-furanone and 5-methyl-2(5H)-furanone. Furanones generally have burnt flavours, but no data are available on their importance for beer staling.

Pyrazines form another group of heterocyclic molecules subject to changes during storage. According to Qureshi, Burger, and Prentice (1979), the concentrations

of some pyrazines decrease very rapidly (pyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine) and some even completely disappear (2-acetylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine). The concentrations of other pyrazines appreciably increase, e.g., 2,6-dimethylpyrazine, trimethylpyrazine and tetra-methylpyrazine. This is somewhat contradictory to the AEDA results of Gijs et al. (2002) who considered 2-acetylpyrazine (sweet, candy floss, caramel), 2-methoxy-pyrazine (cereal roasted) and also maltol (caramel, roasted) relevant for the sensory profile of aged beer (5 days, 40 °C).

3.2.5. Esters

Volatile esters introduce fruity flavour notes and are considered highly positive flavour attributes of fresh beer. Isoamyl acetate, produced by yeast, e.g., gives a banana-like flavour. However, during storage, the concentration of this ester can decrease to levels below its threshold level (Neven, Delvaux, & Derdelinckx, 1997; Stenroos, 1973) which results in a diminished fruity flavour of beer.

In contrast, certain volatile esters (ethyl 3-methyl-butyrate, ethyl 2-methyl-butyrate, ethyl 2-methyl-propionate, ethyl nicotinate, diethyl succinate, ethyl lactate, ethyl phenylacetate, ethyl formate, ethyl furo-ate and ethyl cinnamate) are synthesized during beer aging (Bohmann, 1985b; Gijs et al., 2002; Lustig et al., 1993; Miedaner et al., 1991; Williams & Wagner, 1978). Williams and Wagner (1978) related the formation of ethyl 3-methyl-butyrate and 2-methyl-butyrate to the development of winy flavours. The importance of these molecules for the flavour of aged beer was also recently reported using AEDE experiments (Schieberle & Komarek, 2002) and Gijs et al. (2002) confirmed this also for ethyl cinnamate (fruity, sweet).

Finally, lactones or cyclic esters, such as γ-hexalac-tone and γ-nonalactone (peach, fruity) tend to increase in concentration (Eichhorn, Komori, Mieda-ner, & Narziss, 1989) and the latter molecule is considered important for the flavour of aged beer (Gijs et al., 2002).

3.2.6. Sulfur compounds

Sulfur compounds generally have an extremely low flavour threshold in beer and small concentration changes may have noticeable effects on flavour. Di-methyl trisulfide (fresh-onion-like) may increase to above its flavour threshold of 0.1 μg/l (Gijs et al., 2002; Gijs, Perpete, Timmermans, & Collin, 2000; Williams & Gracey, 1982). An AEDA experiment revealed that also 3-methyl-3-mercaptobutyl formate (catty, *ribes*) (Schieberle, 1991) is involved. Another *ribes* flavour-linked molecule in aged beer is 4-merca-pto-4-methyl-penta-2-one (Tressl, Bahri, & Kossa, 1980).

3.3. Non-volatile compounds

Non-volatile compounds in beer can be important for taste and mouthfeel. Changes in concentration may therefore induce important sensory alterations.

Iso-α-acids, the main bitterness substances in beer, are particularly sensitive to degradation during storage (De Cooman, Aerts, Overmeire, & De Keukeleire, 2000; King & Duineveld, 1999; Walters et al., 1997b), which results in a decrease in sensory bitterness. The iso-α-acids comprise six major components: the *trans*- and *cis*-isomers of isocohumulone, isohumulone and isoadhumulone. The *trans*-isomers are much more sensitive to degradation than the *cis*-isomers. The concentration ratio *trans/cis* isomer was proposed as a good marker for the flavour deterioration of beer (Araki, Takashio, & Shinotsuka, 2002).

Apart from iso-α-acids, polyphenols are some of the more readily oxidized beer constituents (Kaneda, Kano, Osawa, Kawakishi, & Kamimura, 1990). McMurrough, Madigan, Kelly, and Smyth (1996) measured decreases of the flavanols (+)-catechin, (−)-epicatechin, prodel-phinidin B3 and procyanidin B3 concentration during storage at 37 °C. The loss was highest during the first four to five weeks but continued at a decreased rate throughout prolonged periods. Dimeric flavanols disappeared more rapidly than monomers. In contrast, after a lag period of about 5 weeks, the levels of tannoids began to increase (McMurrough, Madigan, & Kelly, 1997) and the changes in the polyphenol contents were associated with the appearance of harsh/astringent tastes.

There are only few reports on beer storage-related changes in amino acids. In general, a slight decrease is observed of some individual amino-acids (Basarova, Sa-vel, Janousek, & Cizkova, 1999) and glutamine has been proposed as a staling marker (Hill, Lustig, & Sawatzki, 1998).

3.4. Chemical origin of beer flavour deterioration

A closer look at the changes in chemical constituents of aging beer reveals the enormous complexity of the beer staling phenomenon. Early on, it was assumed that mainly (E)-2-nonenal was responsible for sensory changes, but now it is evident that a myriad of flavour compounds is responsible. A stale flavour is considered the result of formation and degradation reactions. With AEDA some new compounds have already been discovered although it remains necessary to study their flavour-affecting properties, using spiking experiments and sensory evaluations. An overview of the chemical reactions involved in beer staling may help to better understand the beer-aging phenomenon.

4. Reaction mechanisms of aging processes in beer

4.1. General mechanisms

Chemically, beer can be considered as a water-ethanol solution with a pH of around 4.2 in which hundreds of different molecules are dissolved. These originate from the raw materials (water, malt, hops, adjuncts) and the wort production, fermentation and maturation processes. However, the constituents of freshly bottled beer are not in chemical equilibrium. Thermodynamically, a bottle of beer is a closed system and will thus strive to reach a status of minimal energy and maximal entropy. Consequently, molecules are subjected to many reactions during storage, which eventually determine the type of the aging characteristics of beer.

Although many conversions are thermodynamically possible, their relevance to beer aging is mainly determined by the reaction rates under practical storage conditions. The reaction rate is a function of substrate concentrations and rate constants, which differ between reaction types and which are temperature-dependent. In practice, reaction rates increase with higher substrate concentrations and storage temperatures.

4.2. Reactive oxygen species in stored beer

Oxygen, in particular, causes a rapid deterioration of beer flavour, meaning that oxygen must initiate some very important aging reactions. The importance of reactive oxygen species (ROS) in beer staling was first indicated by Bamforth and Parsons (1985). In recent years, studies using electron spin resonance (ESR) with spin trapping reagents (Andersen & Skibsted, 1998; Kaneda, Kano, Koshino, & Ohyanishiguchi, 1992; Kaneda et al., 1988; Uchida & Ono, 1996; Uchida & Ono, 1999) and chemiluminescence (CL) (Kaneda, Kano, Osawa, Kawakishi, & Koshino, 1991) analysis made it possible to unravel the initial oxygen-dependent reactions (Fig. 2).

Oxygen in the ground state ($^3O_2$) is quite stable and will not easily react with organic molecules. In the presence of ferrous iron (Fe^{2+}) in beer, oxygen can capture an electron and form the superoxide anion (O_2^-) and Fe^{3+}. Copper ions probably have the same behaviour and Cu^+ is oxidized to Cu^{2+} (Kaneda, Kobayashi, Takashio, Tamaki, & Shinotsuka, 1999). It is believed that Cu^+/Cu^{2+} and Fe^{2+}/Fe^{3+} ions are part of a mixed function oxidation system in which polyphenols, sugars, isohumulones and alcohols might act as electron donors (Kaneda et al., 1992). The superoxide anion can be protonated to form the perhydroxyl radical (OOH·), which has much higher reactivity. The pKa of this reaction is 4.8, which means that, at the pH of beer, the majority of the superoxide will be in the perhydroxyl form. The superoxide anion can also be reduced by Fe^{2+} or Cu^+ to the peroxide anion (O_2^{2-}). In beer, this anion is readily protonated to hydrogen peroxide (H_2O_2). Hydroxyl radicals (OH·) can then be produced from H_2O_2 or the superoxide anion O^{2-} by metal-induced reactions, such as the Fenton and the Haber–Weiss reaction.

The reactivity of the oxygen species increases with their reduction status (superoxide anion < perhydroxyl radical < hydroxyl radical). The concentration of free radicals during the aging of beer increases with increasing iron/copper ion concentrations, with increasing oxygen concentrations or with higher storage temperatures (Kaneda et al., 1992; Kaneda, Kano, Osawa, Kawakishi, & Kamada, 1989). Furthermore, the free radicals are not always generated just after the start of the aging

Reaction A: Fenton reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH· + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2· + 2H^+$$
$$\text{Net: } 2H_2O_2 \quad OH· + OH^- + O_2· + 2H^+$$

Reaction B: Haber-Weiss reaction

$$Fe^{3+} + O^{2-} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH· + OH^-$$
$$\text{Net: } O^{2-} + H_2O_2 \rightarrow O_2 + OH· + OH^-$$
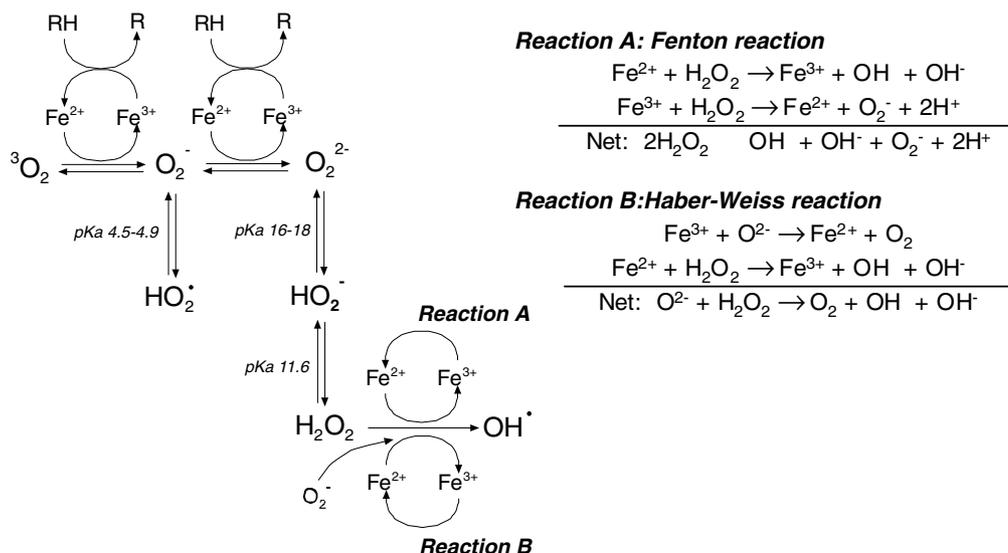
Fig. 2. Reactions producing reactive oxygen species (ROS) in beer (Kaneda et al., 1999).

process, but can be formed after a definite time period, called the "lag time" of free-radical generation (Uchida & Ono, 1996; Uchida, Suga, & Ono, 1996). The "lag-time" seems related to the endogenous antioxidant activity of beer and can be used as an objective tool for its evaluation.

Hydroxyl radicals are one of the most reactive species that have been identified. Therefore, it was suggested that they non-selectively react with ethanol in beer because it is the second most abundant compound of beer and a good radical scavenger. The findings of Andersen and Skibsted (1998), which revealed the 1-hydroxyethyl radical as quantitatively the most important radical in beer, support this. The 1-hydroxyethyl radical arises in the reaction of ethanol with the hydroxyl radical. Generally, the reactive oxygen species (O_2^-, HOO$^\cdot$, H_2O_2 and HO$^\cdot$) react with all kinds of organic molecules in beer, such as polyphenols, isohumulones and alcohols, resulting in various changes in the sensory profile of beer.

4.3. Aging reactions producing carbonyl compounds

4.3.1. Importance of carbonyl mechanisms

Soon after the importance of carbonyl compounds for beer staling was revealed, pathways for their formation were suggested. From the beginning, reaction mechanisms leading to (E)-2-nonenal have been the focus of this research. Many routes have been studied in beer model systems and it therefore remains difficult to tell to what extent a particular reaction mechanism is relevant under normal storage conditions.

4.3.2. Oxidation of higher alcohols

The most important alcohols in beer are ethanol, 2-methyl-propanol, 2-methyl-butanol, 3-methyl-butanol and 2-phenyl-ethanol. Various researchers have reported that the concentrations of the corresponding aldehydes increase during beer aging, in particular when oxygen was present (see above).

Hashimoto (1972) studied the increased formation of aldehydes due to exposure of beer to higher oxygen levels. High temperatures, low pH and the supplementation of additional higher alcohols to beer led to higher concentrations of aldehydes. Moreover, direct oxidation of alcohols by molecular oxygen was not possible in beer model systems, unless melanoidins were present. A reaction mechanism was proposed in which alcohols transfer electrons to reactive carbonyl groups of melanoidins. Molecular oxygen accelerates the oxidation of the alcohols, probably because the melanoidins are transformed in such a way that the reactive carbonyl groups are involved in the electron-transfer system.

Devreux, Blockmans, and vande Meersche (1981) doubted the importance of this pathway as they observed the requirements of light and inhibition by low

concentrations of polyphenols. Furthermore, the reactivity of alcohols decreases with their molecular weight. Irwin, Barker, and Pipasts (1991) found this pathway irrelevant in the formation of (E)-2-nonenal because of the very low efficiency (0.2%) of 2-nonenol to nonenal conversion in model systems.

Nonetheless, it was recently reported that the 1-hydroxyethyl radical is quantitatively the most important radical in stale beer due to hydroxyl radicals reacting with ethanol (Andersen & Skibsted, 1998). A main degradation product of the radical is acetaldehyde (Fig. 3). Even though ethanol is more abundant in beer than any other organic molecule, ROS may react in a similar manner with the main higher alcohols. From this perspective, the formation of ROS and their reaction with alcohols can be regarded as a generalization of the reaction mechanism proposed by Hashimoto (1972).

4.3.3. Strecker degradation of amino acids

Amino acids in stored beer may be a source of aldehydes. Blockmans, Devreux, and Masschelein (1975) observed an increased formation of 2-methyl-propanal and 3-methyl-butanal when either valine or leucine were added to beer and oxygen was present. The reaction was catalysed by Fe and Cu ions. This was explained by a Strecker reaction between amino acids and α-dicarbonyl compounds (Fig. 4). The reaction involves transamination, followed by decarboxylation of the subsequent α-ketoacid, resulting in an aldehyde with one carbon atom less than the amino acid.

Additional α-dicarbonyl compounds in beer are possibly formed by the Maillard reaction, the oxidation of reductones or the oxidation of polyphenols. Thum, Miedaner, Narziss, and Black (1995) mention that Strecker degradation is only important at strongly increased amino acids contents, but not at the amino acid concentrations normally present in beer (±1 g/l).

4.3.4. Aldol condensation

Hashimoto and Kuroiwa (1975) suggested that aldol condensation of carbonyl compounds is possible under the mild conditions existing in beer during storage. For example, (E)-2-nonenal was formed by aldol condensation of acetaldehyde with heptanal in a model beer stored for 20 days at 50 °C and containing 20 mmol/l of proline (Fig. 5). In these reactions, the amino acids may be the basic catalysts through the formation of an imine intermediate. This pathway can produce carbonyl compounds with lower flavour thresholds from carbonyls present in beer which are less flavour active, and which can be formed by other pathways. Although the aldol condensation pathway seems plausible, it is not clear whether the amounts of reaction products are sufficiently high to reach threshold concentrations under normal beer storage conditions (Bamforth, 1999b).
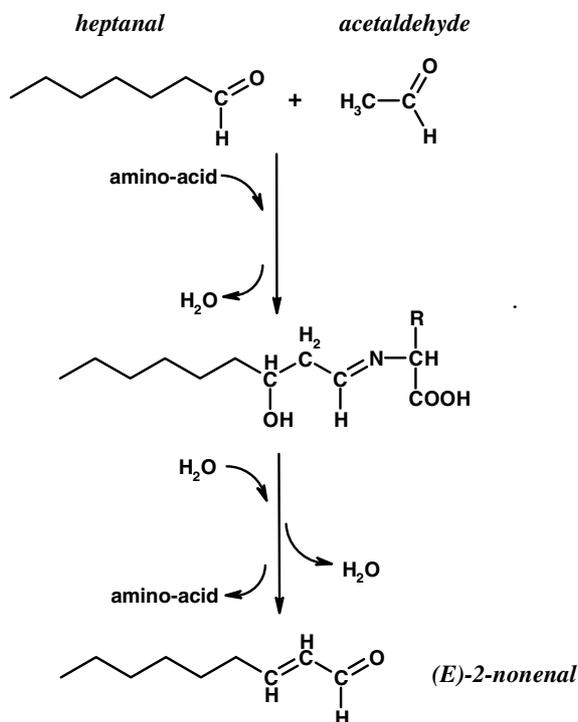
Fig. 3. Reaction of ethanol with the hydroxyl radical in beer according to Andersen and Skibsted (1998).

Strecker degradation of amino acids

Formation of dicarbonyl compounds

Fig. 4. Formation of aldehydes in beer by Strecker degradation of amino acids (Thum et al., 1995).

4.3.5. Degradation of hop bitter acids

The degradation of hop bitter acids (iso-α-acids, α-acids and β-acids) not only decreases sensory bitterness, but also results in the formation of products. There are indications that some of them are involved in the appearance of aging flavours. Indeed, Hashimoto and Eshima (1979) reported that beer brewed without hops hardly develops a typical stale flavour, even after a long

Fig. 5. Formation of (E)-2-nonenal in beer by aldol condensation of acetaldehyde and heptanal according to Hashimoto and Kuroiwa (1975).

R_a = CH(CH$_3$)$_2$

R_b = CH$_2$CH(CH$_3$)$_2$

R_c = CH(CH$_3$)CH$_2$CH$_3$

Fig. 6. Most important hop bitter acids in beer.

shelf storage. The exact degradation mechanism for hop acids and the chemical structures of the volatiles formed, have not been completely elucidated. Fig. 6 presents the structure of the most important beer hop bitter acids.

Iso-α-acids quickly degrade in the presence of ROS (Kaneda et al., 1989), the *trans*-isomer being much more sensitive than the *cis*-isomer (De Cooman et al., 2000). However, recent research (Huvaere et al., 2003) indicates that electrons are released from iso-α-acids in the presence of suitable electron acceptors, which do not necessarily require the involvement of oxygen species. As a result, oxygen- and carbon-centred radicals are formed. These radicals are very reactive and lead to products of varying nature; however, all lack the tricarbonyl chromophore. The double bonds in the side-chains of the hop acids are less reactive toward oxidation than was commonly thought. It is now clear that iso-α-acids can be subject to oxidative-type degradation in the absence of molecular oxygen.

The reduced side-chain iso-α-acids, used to impart light resistance, have fewer structural positions sensitive to radical formation. Consequently, they show more resistance to oxidative breakdown.

According to Hashimoto and Eshima (1979), volatile degradation products of iso-α-acids in beer model systems are carbonyl compounds with various chain lengths, such as C_3 to C_{11} 2-alkanones, C_2 to C_{10} alkanals, C_4 to C_7 2-alkenals and C_6 to C_7 2,4-alkedienals. In an earlier study (Hashimoto & Kuroiwa, 1975), ace-

tone, 2-methyl-propanal, 3-methyl-butan-2-one, 4-methyl-pentan-2-one and 2-methyl-3-buten-2-ol were also identified as oxidation products. Moreover, Williams and Wagner (1979) showed that degradation of the carbonyl side-chain of α-acids and β-acids releases 2-methyl-propionic acid, 2-methyl-butyric acid and 3-methyl-butyric acid. As will be explained later, these acids are precursors in the formation of staling esters.

4.3.6. Oxidation of unsaturated fatty acids

4.3.6.1. General mechanisms and intermediates. From the start of research on beer staling, the oxidation of unsaturated fatty acids received more attention than any other reaction. Soon after the cardboard flavour of beer was linked to (E)-2-nonenal formation, it was suggested that its formation and that of other saturated and unsaturated aldehydes was due to lipid oxidation (Dale & Pollock, 1977; Drost et al., 1971; Jamieson & Van Gheluwe, 1970; Tressl, Bahri, & Silwar, 1979). This was certainly related at that time to the extensive research and knowledge of the oxidative breakdown of lipids in foods, leading to carbonyl compounds and rancidity. In beer and wort, the only lipid substrates of significance are linoleic acid (C18:2) and linolenic (C18:3) acid, arising from malted barley. These acids are mainly released from triacylglycerols by the activity of barley and malt lipases (Baxter, 1984). During malting, slight changes in fatty acids and lipid composition occur. Hydrolysis

of triacylglycerols to fatty acids occurs mainly during mashing. Malt lipase remains active through much of the mashing process (Schwarz, Stanley, & Solberg, 2002).

At present, there is strong evidence that lipid oxidation does not occur in beer after bottling. (*E*)-2-nonenal is released from other precursors, in a non-oxidative process, as the oxygen concentration of bottled beer does not influence the (*E*)-2-nonenal release (Lermusieau et al., 1999; Noel et al., 1999).

Oxidation intermediates of linoleic acid, trihydroxy fatty acids, have been investigated as possible precursors (Drost et al., 1971; Graveland, Pesman, & Van Eerde, 1972). However, they convert to (*E*)-2-nonenal only in acidified beer (pH 2), thus excluding them as plausible precursors in beer (Stenroos et al., 1976).

Generally, it is now agreed that, during wort production, enzymatic and non-enzymatic oxidation, mainly of linoleic acid, generates a "(*E*)-2-nonenal potential" initiating the (*E*)-2-nonenal formation during beer storage. Drost, van den Berg, Freijee, van der Velde, and Hollemans (1990) defined the nonenal potential as the potential of wort to release (*E*)-2-nonenal after its treatment for 2 h at 100 °C at pH 4.0, under an argon atmosphere.

Noël and Collin (1995) found strong evidence that (*E*)-2-nonenal in wort forms Schiff's bases (imines) with amino acids or proteins which pass into beer. During storage, (*E*)-2-nonenal is then released and this is enhanced at low pH (Lermusieau et al., 1999). "Free" (*E*)-2-nonenal in wort is reduced to nonenol by yeast fermentation and nonenol is not significantly re-oxidized during beer storage (Irwin et al., 1991).

There has been some debate about whether (*E*)-2-nonenal is similarly released from non-volatile bisulfite adducts during storage. The sulfite formed by yeast during fermentation would form reversible adducts with (*E*)-2-nonenal (Barker, Gracey, Irwin, Pipasts, & Leiska, 1983). Sulfite can add on to the carbonyl function and on to the double bound of (*E*)-2-nonenal. As during storage, the sulfite concentration gradually decreases, the (*E*)-2-nonenal would be released. However, Dufour, Leus, Baxter, and Hayman (1999) recently showed that, while bisulfite addition to the carbonyl function is reversible, bisulfite addition to the double bond in (*E*)-2-nonenal is irreversible. Due to the irreversible nature of the bisulfite addition to the double bond and the stability of such adducts, (*E*)-2-nonenal cannot be released from these non-volatile species. This supports the observations of other authors (Kaneda, Takashio, Osawa, Kawakishi, & Tamaki, 1996; Lermusieau et al., 1999), describing a minimal formation of reversible (*E*)-2-nonenal-bisulfite adducts during fermentation.

The increase of the cardboard-flavoured compound (*E*)-2-nonenal in aging beer is thus probably linked to oxidation processes earlier in the production process, mainly in the brewhouse. Mashing and wort boiling

are both important for the oxidation of linoleic acid and the subsequent release of (*E*)-2-nonenal. There are two possible oxidation routes: auto-oxidation or an enzymatic oxidation with lipoxygenases (LOX) only during mashing and malting. There is a great deal of controversy concerning the relative importance of both routes for (*E*)-2-nonenal release in finished beer (Bamforth, 1999a; Stephenson, Biawa, Miracle, & Bamforth, 2003). This is partly related to the use, in brewing research, of small-scale equipment in which the oxygen ingress is much larger than in industrial scale brewing. Recent studies (Lermusieau et al., 1999; Liegeois et al., 2002) using wort spiked with deuterated (*E*)-2-nonenal, revealed that 70% of the (*E*)-2-nonenal released during beer staling was initially produced during boiling, and the other 30% during mashing. However, these results do not exclude the possibility that some oxidation intermediates of fatty acids, such as hydroxy fatty acids and hydroperoxy fatty acids, produced by LOX during mashing, may be converted non-enzymatically to (*E*)-2-nonenal under the extreme conditions of wort boiling.

4.3.6.2. Auto-oxidation of fatty acids. Auto-oxidation of a fatty acid is initiated by the abstraction of a H-atom from the molecule by free radicals. As previously mentioned, hydroxyl radicals (HO$^{\bullet}$) are exceptionally reactive toward many molecules found in food. In the complex wort environment it is, however, debatable whether a hydroxyl radical can reach a fatty acid before it finds much more abundant molecules (e.g., sugars). Therefore, auto-oxidation is more likely to be initiated by the slower-reacting peroxy radicals (ROO$^{\bullet}$), abstracting the most weakly bound H-atom in the fatty acid. Besides the peroxy radicals that are produced in the pathway (hence the auto-catalytic character), perhydroxyl radicals (HOO$^{\bullet}$) may also abstract the H atoms. With linoleic acid, the methylene group at position 11 is activated, especially by the two neighbouring double bonds (Fig. 7). Hence, this is the initial site for H abstraction, leading to a pentadienyl radical, which is then stabilized by the formation of two hydroperoxides at positions 9 and 13 (9-LOOH and 13-LOOH), each retaining a conjugated diene system. The monoallylic groups in linoleic acid (positions 8 and 14) also react to a small extent and form four hydroperoxy acids (8-,10-, 12- and 14-LOOH), each isomer having two isolated double bonds. The proportion of these minor hydroperoxy acids is about 4% of the total.

The hydroperoxy acids can be further subject to non-enzymatic oxidation or degradation processes leading to a variety of compounds such as volatiles. Several reaction mechanisms have been suggested to explain their formation. Ohloff (1978) proposed an ionic mechanism for the formation of (*E*)-2-nonenal from 9-LOOH and hexanal from 13-LOOH in aqueous systems (Fig. 8). A heterolytic cleavage is initiated by protonation of the
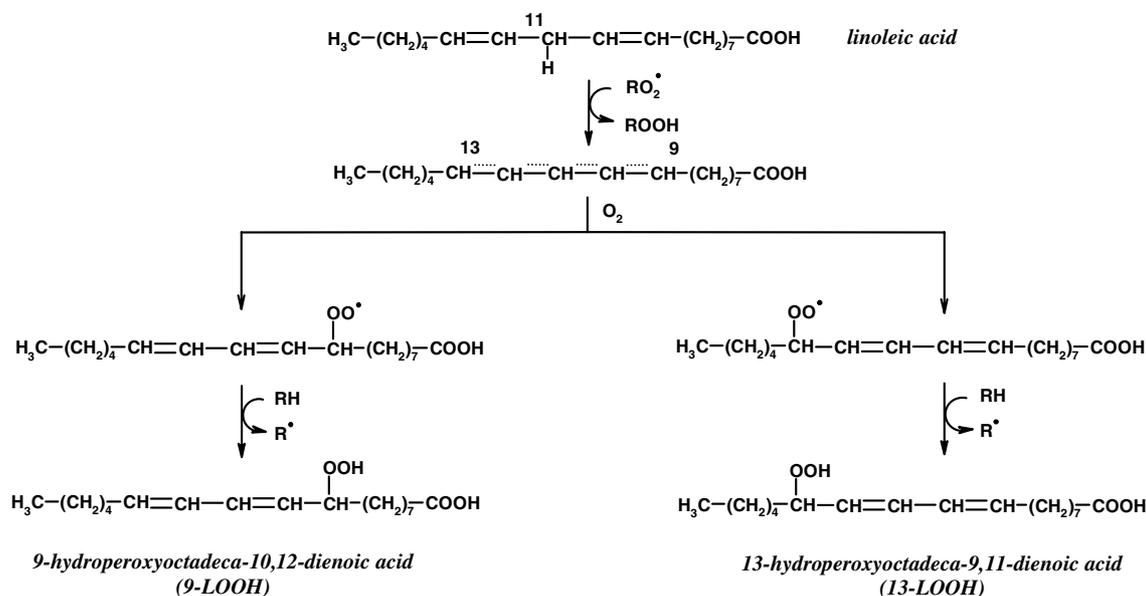
Fig. 7. Formation of the hydroperoxy fatty acids 9-LOOH and 13-LOOH by autoxidation of linoleic acid (Belitz and Grosch, 1999).
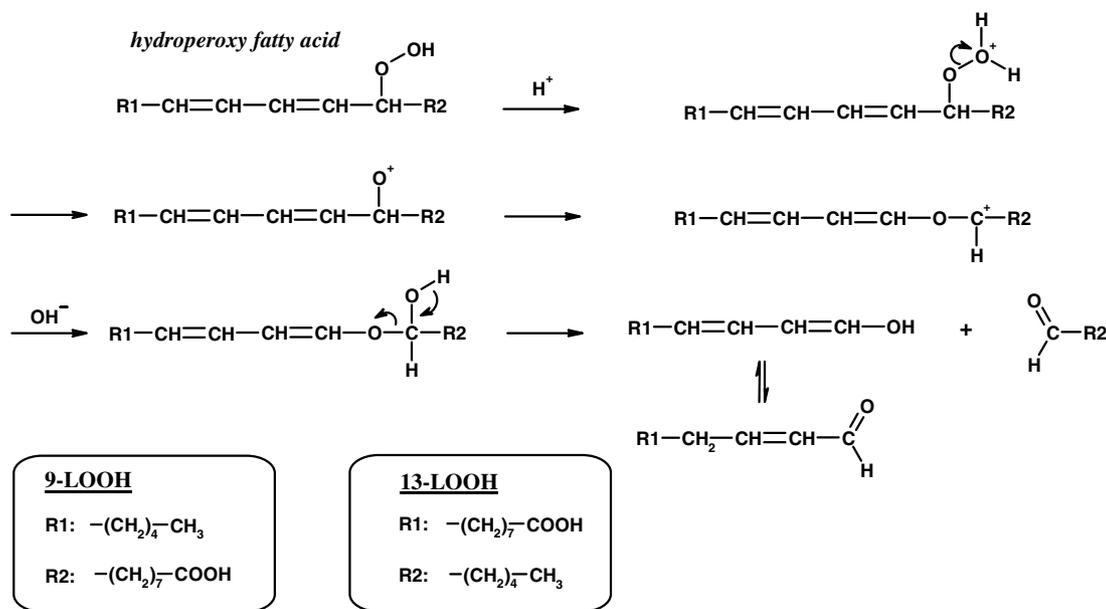

Fig. 8. Proton-catalysed cleavage of 9-LOOH and 13-LOOH hydroperoxy acids of linoleic acid according to Ohloff (1978).

hydroperoxide group. After elimination of a water molecule, the oxo-cation formed is subjected to an oxygen atom insertion reaction exclusively on the C–C linkage adjacent to the double bound. The carbenium ion is hydroxylated and then splits into an oxo-acid and an aldehyde (2-nonenal or hexanal).

On the other hand, in the oil phase of some foods, a β-cleavage of hydroperoxy acids is the predominant degradation reaction. It involves a homolytic cleavage with a formation of short-lived alkoxy radicals. The cleavage further away from the double bond is energetically preferred, since it yields resonance-stabilized compounds. In this way, 2,4-decadienal is formed by the degradation of 9-LOOH.

Newly formed unsaturated aldehydes are susceptible to further oxidation reactions, which in turn produce other carbonyl compounds.

4.3.6.3. Enzymatic breakdown of fatty acids. Germinated barley contains two lipoxygenase enzymes, namely LOX-1 and LOX-2 (Baxter, 1982). LOX-1 is present in raw barley and increases during germination, whereas LOX-2 only develops during germination (Yang & Schwarz, 1995). They can oxidize fatty acids with a *cis,cis*-1,4-pentadiene system, such as linoleic acid and linolenic acid, to their hydroperoxy acids. Linoleic acid is stereo- and regio-specifically oxidized to 9-LOOH by LOX-1 and to 13-LOOH by LOX-2 (Doderer, Kokkelink, van

der Veen, Valk, & Douma, 1991; Garbe, Hübke, & Tressl, 2003; Hugues et al., 1994). Both enzymes are very heat-sensitive, with LOX-1 being somewhat more heat-resistant than LOX-2. Therefore, during kilning, most LOX activity is destroyed and the remaining activity in malt is mainly due to LOX-1 (Yang & Schwarz, 1995). The remaining activity seems to be the main cause of fatty acid oxidation during mashing. This is in accordance with an observed concentration ratio of 9-LOOH/13-LOOH of 10/1 in wort during mashing at 52 °C (Walker, Hughes, & Simpson, 1996). LOX enzymes become completely inactivated at temperatures above 65 °C. Both enzymes exhibit a pH optimum at 6.5. LOX-1 has a broad pH range with the activity falling to 50% at pH 5. The pH-range of LOX-2 is much narrower and this enzyme is almost completely inactive at pH 5 (Doderer et al., 1991). A reduced formation of hydroperoxy fatty acids was observed at higher initial mashing temperatures (Kobayashi, Kaneda, Kano, & Koshino, 1993b) or when the pH was lowered from 5.5 to 5.0 (Kobayashi, Kaneda, Kano, & Koshino, 1993a). The hydroperoxy fatty acids are subject to further enzymatic or non-enzymatic breakdown (Kobayashi, Kaneda, Kano, & Koshino, 1994) and (E)-2-nonenal can be formed from 9-LOOH.

Mono-, di- and trihydroxy fatty acids accumulate during mashing and are possibly formed by enzymatic breakdown of hydroperoxy fatty acids (Kobayashi et al., 2000b). Recently, Kuroda, Kobayashi, Kaneda, Watari, and Takashio (2002) showed that, during mashing, linoleic is transformed to di- and trihydroxy acids by LOX-1 and an additional enzyme, which is more heat-stable than LOX-1. This enzymatic factor seems related to peroxygenase (POX), a member of the plant cytochrome P450-containing systems that use hydroperoxide fatty acid as a substrate and catalyze the hydroxylations without NADPH or molecular oxygen. In turn, the new hydroxy acids can be broken down non-enzymatically to various carbonyl compounds (Tressl et al., 1979), but considerable levels remain present in the finished beer (Kobayashi et al., 2000a). Furthermore, it was revealed that 9-LOOH is also transformed to (E)-2-nonenal by 9-hydroperoxide lyase-like activity during mashing (Kuroda, Furusho, Maeba, & Takashio, 2003).

During the germination of barley, a hydroperoxy acid isomerase appears, which catalyzes the transformation of hydroperoxy acids to ketols. The ketols can be converted non-enzymatically to mono-, di- and trihydroxy acids. Although hydroperoxy acid isomerase is found in malt, Schwarz and Pyler (1984) reported that it is tightly bound to the insoluble barley grist and is not released in the soluble fraction of the mash. Therefore, it can be assumed that this enzyme is not involved in hydroperoxy acid transformations during mashing.

Finally, linoleic and linolenic acid, esterified in triacylglycerol, can also be oxidized by LOX enzymes (Garbe et al., 2003; Holtman, VredenbregtHeistek, Schmitt, & Feussner, 1997), LOX-2 having a higher activity than LOX-1. The finding of esterified hydroxyfatty acids in triacylglycerols or phospholipids in barley and malt (Wackerbauer & Meyna, 2002; Wackerbauer, Meyna, & Marre, 2003) and concentration increases during storage gave evidence for lipid oxidation by LOX enzymes. These oxidized lipids are also likely precursors of
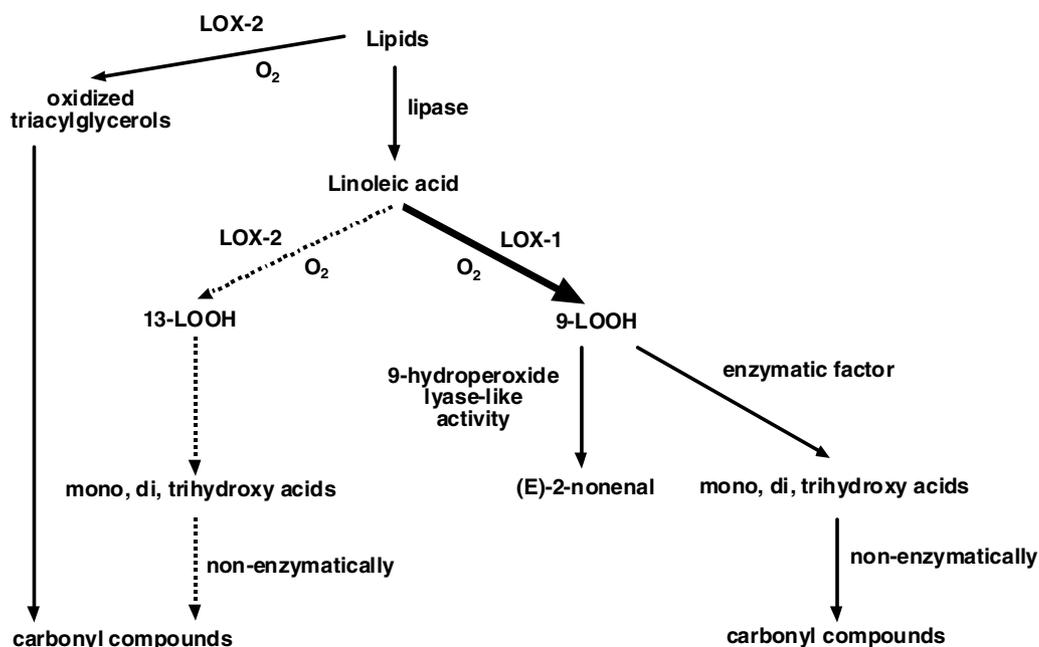

Fig. 9. Overview of the currently known enzymatic oxidation pathways of linoleic acid leading to carbonyl compounds.

carbonyl compounds in wort. The currently described enzymatic pathways for oxidation of lipids during beer production are summarized in Fig. 9.

4.3.7. Formation of (E)-β-damascenone

(E)-β-damascenone belongs to a class of carotenoid-derived carbonyl compounds. Potential precursors of damascenone in beer are allene triols and acetylene diols formed by degradation of neoxanthin, which is present in the basic ingredients of beer (Chevance et al., 2002). Moreover, it was proven that the appearance of (E)-β-damascenone in beer increased during aging when a β-glucosidase was added. The non-enzymatic release in beer was enhanced at low pH (Gijs et al., 2002). As the proposed precursors are linked to sugars, as observed in wines (Bureau, Baumes, & Razungles, 2000), (E)-β-damascenone might also result from a chemical hydrolysis of glycosides during beer aging. Consequently, glycosides may also be considered as important sources of flavours related to beer aging (Biendl, Kollmannsberger, & Nitz, 2003). This can be of great significance in the production of speciality beers, which are often characterized by the use fruits or herbs, rich in glycosides.

4.4. Acetalization of aldehydes

The cyclic acetals (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) originate from a condensation reaction (Fig. 10) between 2,3-butanediol (up to 280 mg/l in beer) and an aldehyde (acetaldehyde, isobutanal, 3-methyl-butanal and 2-methyl-butanal, respectively) (Peppard & Halsey, 1982). In beer, an equilibrium between 2,4,5-trimethyl-1,3-dioxolane, acetaldehyde and 2,3-butanediol is reached quite rapidly. As a result, the increase in the acetaldehyde concentration during aging causes the 2,4,5-trimethyl-1,3-dioxolane concentration to increase very similarly (Vanderhaegen et al., 2003b).

4.5. Maillard reaction

Many heterocyclic compounds found in aged beers are well known products of the Maillard reaction. The diverse and complex reactions between reducing sugars and proteins, peptides, amino acids or amines, as well as the numerous consecutive reactions, are all classified as Maillard reactions. In contrast to lipid oxidation, studies of Maillard reactions related to beer aging are scarce. Such limited interest may stem from observations that the currently known Maillard products in aged beer (e.g., furfural and 5-hydroxymethyl furfural), remain below their flavour threshold. On the other hand, the typical flavour of many food products is due to Maillard reactions. Studies with model systems containing a single type of sugar and amino acid revealed the formation of a myriad of Maillard compounds (Hofmann & Schieberle, 1997; Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995), suggesting that in food, including beer, an even greater variety of products can be formed. Probably, the list of Maillard products in the previous section is only a small reflection of the actual number of beer aging-related compounds. Some of them might merit more interest, as it was found recently that the Maillard reaction is responsible for the development of bready, sweet and wine-like flavour notes dur-
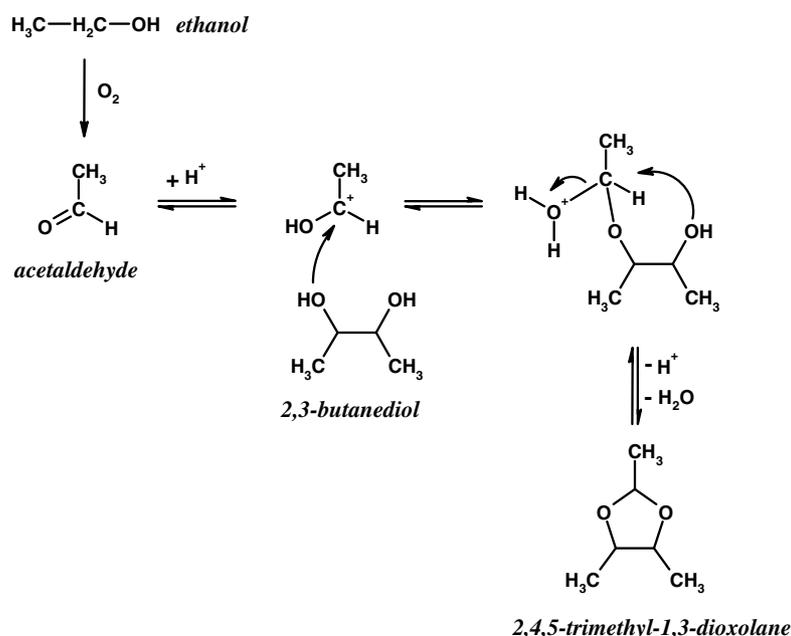

Fig. 10. Formation mechanism of 2,4,5-trimethyl-1,3-dioxolane in beer (Vanderhaegen, 2004).

ing beer staling. This was demonstrated through the use of the specific Maillard reaction inhibitor, aminoguanidine (Bravo et al., 2001b).

Quantitatively, one of the most important heterocyclic staling compound is 5-hydroxymethyl-furfural. Its formation by the Maillard reaction is shown in Fig. 11. The reaction starts with a Schiff's base formation between a carbonyl group of a hexose sugar and an amino group, leading to an imine. This undergoes an Amadori rearrangement to a more stable 1-amino-1-deoxyketose (also called *Amadori product*). However, at the pH of beer, the Amadori product is subject to enolization and subsequent release of an amine, which leads to 3-deoxy-2-hexosulose (3-DH). This reactive α-dicarbonyl compound can (among various secondary products) give rise to HMF. Starting from a pentose, furfural is formed. At this stage HMF, furfural and other compounds are merely intermediates of the Maillard reaction. They are subject to further reactions (condensation, dehydration, cyclisation, isomerisation,) producing brown pigments of high molecular weight, the melanoidins.

According to Rangel-Aldao et al. (2001), 3-DH is present in considerable quantities in fresh beer (230 μM) and degrades during storage at 28 °C. Furthermore, the concentration of a degradation intermediate (3-DDH) also decreases strongly (Bravo, Sanchez, Scherer, & Rangel-Aldao, 2001a). In contrast, other deoxyosones, 1-deoxy-2,3-hexodiulose (1-DH), 1,4-dideoxyhexosulose (1-DDH) and 1,4-dideoxy-2,3-pentodiulose (1-DDP) increase (Bravo et al., 2001b). 1-DH is formed by degradation of the Amadori product of hexoses, whereas 1-DDH and 1-DDP are probably formed by Strecker degradation of 1-DH and 1-deoxy-pentodiulose (1-DP).
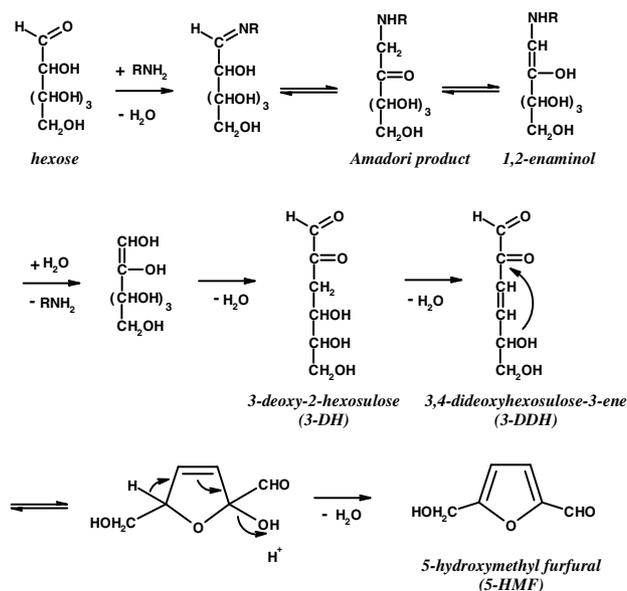
During storage, some Maillard intermediates may react with typical beer constituents to give staling compounds. Furfuryl ethyl ether arises in beer due to an acid-catalysed condensation reaction (Fig. 12) of furfuryl alcohol and ethanol (Vanderhaegen et al., 2004a). In the production of beer, furfuryl alcohol is formed by Maillard reaction mainly during malt kilning and wort boiling. Evidence was found that a Maillard reaction of maltose and α-(1,4)-oligoglucans is responsible. Reduction of furfural by yeast may further increase the furfuryl alcohol concentration during fermentation (Vanderhaegen et al., 2004b).

4.6. Synthesis and hydrolysis of volatile esters

Chemical condensation reactions between ethanol and beer organic acids occur at significant rates during beer storage. For example, 3-methyl-butyric acid and 2-methyl-butyric lead to ethyl 3-methyl-butyrate and ethyl 2-methyl-butyrate (Williams & Wagner, 1979). The precursor acids are produced by oxidation of hop α- and β-acids in beer as mentioned previously. In contrast, some esters, such as iso-amyl acetate, can be hydrolysed and their contribution to the flavour decreases. Chemical hydrolysis and esterification are acid-catalysed processes, but the activity of enzymes with esterase activity, sometimes detected in beer, can also affect the ester profile. Neven (1997) showed that some esterases are released by yeast into beer as a result

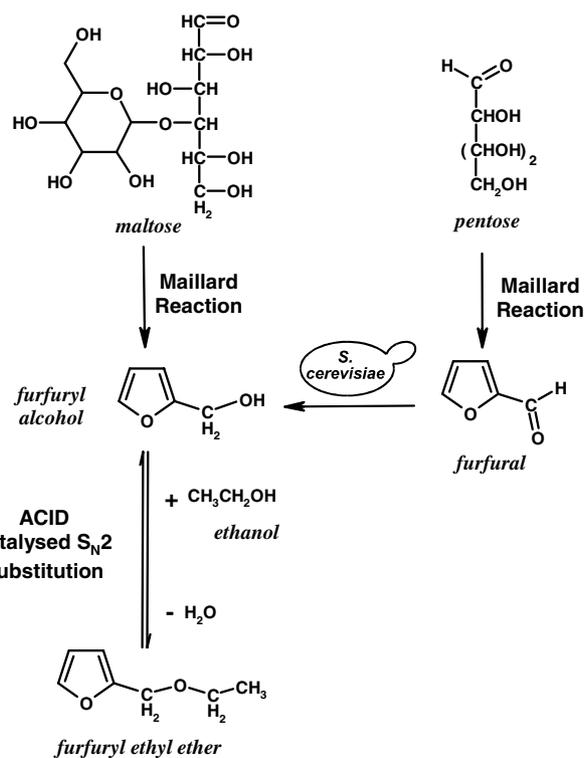Fig. 11. Formation of 5-hydroxymethyl furfural by Maillard reaction.

Fig. 12. Formation mechanism of furfuryl ethyl ether during beer storage and its precursor, furfuryl alcohol, during beer production.

of cell autolysis during fermentation and maturation. Such esterase activity is strain dependent and top-fermenting yeasts are more active than bottom fermenting yeasts. The optimal activity in beer is between 15 and 20 °C. Furthermore, the enzyme is largely inactivated by beer pasteurisation. Horsted, Dey, Holmberg, and Kielland-Brandt (1998) showed that an extracellular esterase of *Saccharomyces cerevisiae* had an optimal activity at pH 4–5 and identified *TIP1* as the structural gene. The activity of such esterases leads to biochemical aging processes in parallel with chemical aging reactions. Another effect of biochemical aging is due to proteases in beer which, by protein hydrolysis, cause less foam stability (Ormrod, Lalor, & Sharpe, 1991). Especially, bottle-refermented beers, in contact with an inactive yeast layer during storage, may become more susceptible to biochemical transformations (Vanderhaegen et al., 2003a).

4.7. Formation of dimethyltrisulfide

Various precursor molecules in beer may trigger the formation of dimethyltrisulfide (DMTS). According to Peppard (1978), the reaction between methanesulfenic acid and hydrogen sulfide leads to DMTS during beer storage. Methanesulfenic acid is formed by β-elimination from S-methylcysteine sulfoxide, introduced to beer from hops. Other DMTS precursors may be 3-methylthiopropionalehyde and its reduced form, 3-methylthiopropanol (Gijs & Collin, 2002; Gijs et al., 2000). The production of DMTS is enhanced at low pH (Gijs et al., 2002).

4.8. Degradation of polyphenols

Polyphenols in beer easily react with ROS and free radicals. The structural changes due to oxidation have not been completely elucidated. It is believed that simple polyphenols polymerize to high molecular weight species (tannins), either by acid catalysis, or by oxidative mechanisms (Gardner & McGuinness, 1977). Possibly, polyphenols are first oxidized to quinones or semi-quinone radicals, which interact with other phenolic compounds. Furthermore, polymerisation reactions also can be induced by acetaldehyde, formed by yeast or by ethanol oxidation, through the formation of ethyl bridges between flavanols (Delcour, Dondeyne, Trousdale, & Singleton, 1982; Saucier, Bourgeois, Vitry, Roux, & Glories, 1997). Apart from polymerisation, ring opening in oxidised phenols was proposed as an alternative degradation mechanism (Cilliers & Singleton, 1990). During beer storage, phenolic polymers interact with proteins and form insoluble complexes and hazes.

4.9. Oxidative versus non-oxidative beer aging

From the previous considerations, it becomes clear that oxygen triggers the release of free radicals, which can easily react with many beer constituents, leading to rapid changes in the flavour profile. Among these processes are the oxidation of alcohols, hop bitter compounds and polyphenols. Since oxygen is very detrimental for the flavour of beer, brewers have tried to minimize the oxygen pick-up in finished beer. Modern filling equipment can achieve total oxygen levels in the bottle of less than 0.1 mg/l. At such low oxygen levels, it is debatable whether the formation of reactive oxygen species (ROS) is the determining factor in the aging of these beers. Indeed, other molecules present in beer have enough reactivity to interact and form staling compounds. Beer staling is often regarded as only the result of oxidation, but non-oxidative processes may be just as important, especially at the low oxygen levels reached in modern breweries.

Non-oxidative reactions causing flavour deterioration are esterifications, etherifications, Maillard reactions, glycoside and ester hydrolysis. Even (*E*)-2-nonenal, a compound long suspected to be the main cause of oxidized flavour, paradoxically appears to arise by non-oxidative mechanisms in beer. This explains why beer staling is possible in the absence of oxygen. On the other hand, although some compounds result from oxidation reactions, it is at present not really clear which compound(s) is/are responsible for the oxidation off-flavour of beer.

In conclusion, some reactions have received considerably more attention than others, partly due to historical factors. However, it remains important to evaluate the relevance of specific reported reactions in the overall beer aging process. Such assessment has scarcely been done and it is currently not clear how important a specific aging reaction is for the changes in flavour perception of a particular beer.

Nonetheless, better knowledge of reaction mechanisms involved in staling phenomena, allows closer study of the effects on particular reactions of wort and beer production parameters.

5. Inhibiting and promoting effects on beer aging reactions

5.1. Types of reactions

Chemical and biochemical processes, which occur during beer storage, proceed simultaneously although at different rates. To what extent certain reactions take place depends on storage conditions and by competition and interaction of pathways. This also applies to reactions during the brewing process, which determine the precursor concentrations for staling reactions in the final beer. Several methods have been suggested to control, to some degree, the reactions responsible for flavour deterioration during beer storage.

5.2. Oxidative beer aging reactions

5.2.1. General

Especially in bottled beer, excessive amounts of oxygen may cause a rapid change in aroma and taste. In recent years, it became evident that levels of oxygen throughout the brewing process can also affect the beer shelf-life downstream. Minimizing the formation and activity of ROS (O_2^-, HOO', H_2O_2 and HO') in beer and wort, must be a first step for improving beer flavour stability.

Molecular oxygen itself is not very reactive but its initial concentration determines the level of ROS. In the activation of oxygen, transition metal ions (Cu^+ and Fe^{2+}) act as electron donors. Consequently, process and technological parameters should be adapted to minimize wort and beer oxygen pick-up and the copper and iron ion concentrations.

The activation of oxygen can be stimulated by pro-oxidant molecules, which are generally able to reduce metal ions. In this process the pro-oxidant itself may become a radical, which reacts with other constituents or degrades and may produce off-flavours. Actually, oxidative reactions in wort and beer must be regarded as a chain of redox agents involved in electron transfer reactions. On the other hand, the effects of oxygen can be inhibited by certain beer or wort components (anti-oxidants). Generally, the anti-oxidant activity is based on the capture of ROS and free radicals. The capture of metal ions with some chelating agents is another anti-oxidative approach.

In the past few years, the anti- or pro-oxidative activity of wort and beer has been investigated by various methods including the determination of:

(a) the capacity to reduce the iron-(II)-dipyridyl complex (Chapon, Louis, & Chapon, 1981);
(b) the ability to scavenge the radical cation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) in an aqueous phase (Araki et al., 1999);
(c) the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity (Kaneda, Kobayashi, Furusho, Sahara, & Koshino, 1995a, 1995b);
(d) chemiluminescence (CL), either directly or after reaction with the radical scavenger, 2-methyl-6-phenyl-3,7-dihydroimidazo(1,2-a)pyrazin-3-one (Kaneda, Kano, Kamimura, Kawaskishi, & Osawa, 1991; Kaneda, Kano, Kamimura, Osawa, & Kawakishi, 1990a, Kaneda, Kano, Kamimura, Osawa, & Kawakishi, 1990b; Kaneda, Kano, Osawa, Kawakishi, & Koshino, 1994);
(e) free radicals by electron spin resonance (ESR) (Kaneda et al., 1989; Kaneda et al., 1988);
(f) 2-thiobarbituric acid-reactive substances (Grigsby & Palamand, 1976);

(g) the redox potential (Buckee, Mom, Nye, & Hamond, 1997; Galic, Palic, & Cikovic, 1994; van Strien, 1987);
(h) the capacity to delay methyl linoleate oxidation in lipidic media and at high temperature, followed by gas chromatography (Boivin et al., 1993; Maillard & Berset, 1995);
(i) linoleic acid hydroperoxide in a Fenton-type reaction (Bright, Stewart, & Patino, 1999);
(j) the inhibition time of 2,2′-azobis(2-amidinopropane) dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid (Liegeois, Lermusieau, & Collin, 2000).

The major endogenous anti- or pro-oxidants in wort and beer are discussed below.

5.2.2. Sulfite

Conversion of sulfate by yeast (from water and raw materials) is the major endogenous source of sulfite in beer. A study (Andersen, Outtrup, & Skibsted, 2000) using the ESR lag phase method (e) highlighted sulfite as one of the most effective antioxidants in beer. Its presence postpones the formation of free radicals (mainly the 1-hydroxyethyl radical) measured by ESR spin trapping. The effectiveness of sulfite seems to be due to its two electron non-radical-producing reaction with peroxides.

5.2.3. Polyphenols

Polyphenolic compounds are important antioxidants in many systems. Generally, in beer, 70–80% of the polyphenol fraction originates from barley malt and another 20–30% from hop. Lower molecular weight polyphenols, in particular, are excellent anti-oxidants. With increasing molecular weight, the reducing power decreases (Buggey, 2001). Polyphenols can react with free radicals to produce phenoxy radicals (Fig. 13), which are relatively stable due to delocalization of the free radical over the aromatic ring. Some polyphenols are also anti-oxidants, by their ability to chelate transition metal ions. On the other hand, certain polyphenols behave as pro-oxidants due to their ability to transfer electrons to transition metal ions (Bamforth, 1999b).

There is some controversy concerning the relevance of polyphenols as anti-oxidants in beer and wort. ESR lag phase studies (Andersen et al., 2000; Andersen &
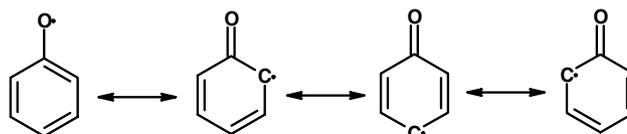

Fig. 13. Stabilization of phenoxy radical by delocalization.

Skibsted, 2001) showed no significant effect of polyphenols on the formation of free radicals in beer during storage or in wort during brewing. This was attributed to the extreme reactivity of hydroxyl radicals and their non-selective elimination through reaction with other prominent compounds of beer (ethanol) or wort (sugars). This avoids radical-scavenging with polyphenols present in relatively small concentrations. However, it is not clear whether this applies also to other radicals, such as, e.g., fatty acid oxidation radicals in wort.

In beer, polyphenols contribute up to 60% of the endogenous reducing power measured in the iron-(II)-dipyridyl test (a) and the DPPH test (c) (Kaneda et al., 1995a; McMurrough et al., 1996). Partial removal of the polyphenol fraction by polyvinylpolypyrrolidone (PVPP) treatment diminishes the reducing power by 9–38%, but does not make the beer more susceptible to oxidative damage (McMurrough et al., 1996). This was confirmed in sensory experiments by Mikyska, Hrabak, Haskova, and Srogl (2002). The PVPP-treated beers developed a less astringent character. Moreover, according to Walters et al. (1997b) and Walters, Heasman, and Hughes (1997a), (+)-catechin and ferulic acid reduce the formation of particular carbonyl compounds in beer at high oxygen levels, but not at low oxygen levels. Furthermore, ferulic acid levels determine whether it is pro-oxidant (low concentration) or anti-oxidant (high-concentration).

The main effect of polyphenols on flavour stability is probably situated in the mashing and wort boiling steps (Liegeois et al., 2000; Mikyska et al., 2002). In particular, polyphenols extracted from hop during wort boiling significantly contribute to the reducing power and effectively diminish the nonenal potential of wort (Lermusieau, Liegeois, & Collin, 2001). Sensory experiments (Mikyska et al., 2002) also confirm the positive effects of hop polyphenols, during brewing, on flavour stability.

5.2.4. Melanoidins and reductones

Malt kilning (up to 80 °C), malt roasting (110–250 °C) and wort boiling generate antioxidants through Maillard reactions (Boivin et al., 1993). These antioxidants include reductones and melanoidins. The reducing power of reductones is due to the endiol group (Fig. 14), which can generate carbonyls. Ascorbic acid (vitamin C)
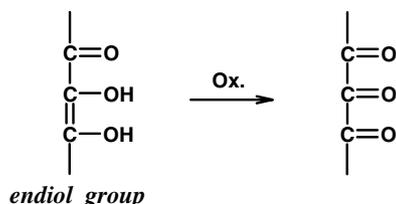
is a typical reductone, but it is not produced by Maillard reactions. However, in the production of beer, ascorbic acid is often used as an exogenous anti-oxidant. Recently, ESR studies questioned the relevance of ascorbic acid for flavour stability. On addition to beer rather a pro-oxidative activity was found due to the formation of more free radicals (Andersen et al., 2000).

A limited number of studies (Wijewickreme, Kitts, & Durance, 1997; Wijewickreme & Kitts, 1998b; Wijewickreme, Krejpcio, & Kitts, 1999) are related to the antioxidant properties of melanoidins and conclusions concerning the structural features responsible for anti-oxidative activity are difficult to draw. Moreover, melanoidins or its precursors may also present pro-oxidative properties as Hashimoto (1972) showed their involvement in the oxidation of alcohols to aldehydes during beer storage. The levels of antioxidants resulting from Maillard reactions and sugar caramelization are low in light malts, but significant in dark speciality malts (Bright et al., 1999; Coghe, Vanderhaegen, Pelgrims, Basteyns, & Delvaux, 2003; Griffiths & Maule, 1997). The higher reducing power of wort and beer produced from darker coloured malts may then contribute to a better flavour stability, often reported for such beers.

5.2.5. Chelating agents

Apart from polyphenols, various other compounds in wort and beer, including amino acids, phytic acid and melanoidins (Wijewickreme & Kitts, 1998a), may function as sequestration agents for metal ions. In wort and beer, an equilibrium exists between free and chelated metal ions. Depending on chelator type, bound metal ions have either less or more capability to promote oxygen radical formation (Bamforth, 1999b).

5.3. Enzymatic oxidation of fatty acids

Many strategies have been proposed for reducing the enzymatic oxidation of fatty acids. Lipoxygenase activity during mashing can be controlled by several technological parameters. LOX enzyme activity during mashing is influenced by the temperature regime and the wort pH. Mashing-in at high temperatures (>65 °C) effectively inhibits LOX enzymes (Kobayashi et al., 1993a). However, this condition is not very acceptable, as this temperature inactivates other indispensable malt enzymes: amylases, glucanases or proteases. Lowering the mash pH from 5.4 to 5.1 seems more efficient for reducing LOX activity (Kobayashi et al., 1993a). Lipoxygenase in wort also appears to be inhibited by polyphenols (Goupy, Hugues, Boivin, & Amiot, 1999).

Another approach consists of limiting the extraction into the mash of lipoxygenase enzymes. This is possible by selecting malts with low LOX contents or by reducing the LOX activity by kilning at intense regimes. Milling regimes that leave the embryo intact, were also

Fig. 14. Oxidation of a reductone.

Table 2
Strategies for dealing with beer staling according to Bamforth (2000b)

Process stage	Barley	Malting	Grist	Milling	Mashing & Wort collection	Boiling & clarification	Fermentation & conditioning	Downstream processing	Packaging & distribution
High relevance – low cost/risk						Avoidance of excessively prolonged heating	Strategies to enhance sulfur dioxide within legal limits	Oxygen minimization Use of sulfur dioxide Minimum pick-up of iron and copper	Minimum oxygen uptake in package
High relevance – high cost/risk									Refrigerated stockholding and transportation Stock rotation and logistics Oxygen-scavenger crown corks
Low relevance – low cost/risk		Suppression of embryo development – e.g., rootlet inhibitors Maximized kilning temperatures	Increased use of sugar adjuncts Use of coloured malts		Suppressing oxygen ingress High mashing-in temperatures Reduced mashing pH		Bright worts	Use of ascorbic acid Use of reduced iso-α-acids	
Low relevance – high cost/risk	Selection of barleys with low LOX potential		Sparging of grist with inert gas	Milling regimes which minimize embryo damage Anaerobic milling					

proposed (van Waesberghe, 1997). Later, Bamforth (1999a) suggested that the availability of oxygen in the mash is more likely to limit lipoxygenase activity than the availability of enzymes. This was confirmed by Kobayashi et al. (2000b). A prevention of oxygen ingress during mashing reduces the enzymatic oxidation of fatty acids.

5.4. Non-oxidative beer aging reactions

Non-oxidative reactions in stored beer are of very different natures. Consequently, the effects of production and storage parameters are highly variable. Nevertheless, several non-oxidative aging processes, such as the release of aldehydes from imines, esterification, etherification, ester hydrolysis, dimethyltrisulfide formation and glycoside hydrolysis, are all promoted at a low beer pH. A low pH may also enhance oxidative reactions by protonation of superoxide (O_2^-) radicals to the more reactive perhydroxyl radicals $(HOO^{\cdot})$. All this supports the sensory findings that beer ages faster at low pH (Kaneda, Takashio, Tomaki, & Osawa, 1997). Shimizu et al. (2001b) correlated the decrease in pH during fermentation with the cellular size of the pitching yeast. A higher pH was obtained with yeast large-cell sizes and the resulting beers showed better flavour stability (Shimizu, Araki, Kuroda, Takashio, & Shinotsuka, 2001a). Yeast also has an important role in decreasing the amount of staling compounds and its precursors. Yeast metabolism during the fermentation is mainly fermentative. This results in an excess of reduced coenzymes NADH and NADPH. To regenerate these coenzymes, they are used by several aldoketoreductases (Debourg, Laurent, Dupire, & Masschelein, 1993; Debourg, Verlinden, Van De Winkel, Masschelein, & Van Nedervelde, 1995; Van Iersel, Eppink, Van Berkel, Rombouts, & Abee, 1997; Van Nedervelde, Oudjama, Desmedt, & Debourg, 1999; Van Nedervelde, Verlinden, Philipp, & Debourg, 1997). This so-called ''yeast reducing power'' results in the reduction of wort aldehydes to alcohols during fermentation. Recently, an interesting yeast NADPH-dependent reductase was described (Sanchez et al., 2001), capable of reducing α-dicarbonyl intermediates of the Maillard reaction, such as 3-deoxy-2-hexosulose (3-DH). However, further investigations must demonstrate whether, under beer fermentation conditions, yeast can reduce such Maillard reaction dicarbonyls.

Maillard products such as furfural increased more during beer aging when malt was kilned at higher temperatures and the thermal load on wort was higher during production (Narziss, Back, Miedaner, & Lustig, 1999). The staling compound, furfuryl ethyl ether, showed the same behaviour (Vanderhaegen et al., 2004b).

On the other hand, Maillard reactions are inhibited by sulfite (Wedzicha & Kedward, 1995). Together with its ability to inhibit radical formation and to bind with carbonyl compounds, sulfite seems to be a very good inhibitor of beer staling.

6. Concluding remarks

Optimisation of the brewing process with respect to flavour stability requires a clear insight of the types of flavour changes during storage and the nature of the molecules involved. This may, however, vary between beer types (e.g., pilsner beers and speciality beers). Secondly, it is necessary to clarify the reaction pathways in beer leading to the staling compounds. Finally, the influence of the production process on the staling reactions must be made clear.

Knowledge of the aging phenomenon in a particular type of beer can be used to develop appropriate technological process improvements to control its particular flavour stability. Besides their relevance for flavour stability, the investment costs for suggested process modifications must be evaluated and a balance should be made between better and longer flavour stability and costs. Table 2, presented by Bamforth (2000a, 2000b), is helpful in summarizing such considerations. Although the strategies seem straightforward, practical experience may soon show that flavour stability is still hard to control. There might be one explanation for this: the knowledge of staling components is still incomplete, not only concerning the number and types of compounds, involved but also the reaction mechanisms.

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