

# On the contribution of malt quality and the malting process to the formation of beer staling aldehydes: a review

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Despite decades of extensive research, beer flavour instability remains a challenge for both brewing and malting industries. Malt impacts the brewing process as well as the quality of the final beer. It also affects the stability of beer flavour, as it delivers to the brewing process various compounds with the potential to compromise the desired flavour characteristics of beer. These include staling aldehydes and their precursors, such as amino acids, reducing sugars,  $\alpha$ -dicarbonyls and bound-state aldehydes. In general, the content of these compounds depends on barley variety and quality, the malting regime and final malt quality. Malt that represents a low potential for beer staling, *i.e.* that has low values of Kolbach Index, heat load, colour, LOX activity, Strecker aldehydes, transition metal ions and high antioxidative activity, leads to beer with enhanced flavour stability. However, the consistent production of malt with the desired quality remains challenging. Approaches to achieve this include adjustment of steeping and germination conditions, allowing control of grain modification and thus, the reservoir of aldehydes precursors. Also, the application of alternative kilning technologies may reduce the applied heat load, responsible for the formation of staling aldehydes and triggering development of the oxidising free radical species. This review provides an evaluation of current knowledge on the contribution of the malting process and malt quality to the formation of beer staling aldehydes. © 2021 The Authors. *Journal of the Institute of Brewing* published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling.

**Keywords:** malt quality; malting process; staling aldehydes; beer flavour instability

## Introduction

### Contribution of free and bound aldehydes to beer flavour instability

The microbiological, colloidal, foam, colour and flavour stability of beer are considered to be critical quality parameters influencing drinkability and brand acceptance by the consumer (1,2). Moreover, according to the European law, beer – as a food product – needs to retain its properties until the ‘best before’ date, when stored properly (3). Unfortunately, various chemical reactions take place in the closed beer package, resulting in a change to the sensorial perception of beer over time, which starts almost instantly upon packaging. The most significant changes are the increase in off-flavours (e.g. cardboard associated with *trans*-2-nonenal) and the loss of pleasant flavour attributes (such as bitterness or ester character due to degradation of iso- $\alpha$ -acids and acetate esters, respectively) (4–11). Losses in esters also reduce their well known masking effect, thereby leading to an even more pronounced perception of off-flavours (8,9,12). Similarly, the synergistic effect caused by the sum of the intensities of beer ageing indicators allows the perception of off-flavours even when their concentrations in beer do not exceed their individual flavour thresholds (8–10). Exposure to high temperatures, light, vibrations during transport and/or contact with oxygen, as well as certain transition metal ions, accelerate the rate of beer staling (13–17). Unravelling the chemistry behind these changes, and thus learning how to control the

rate at which flavour change develops, is the key to prolonging beer freshness.

Measures adopted to improve beer flavour stability are considered most effective when applied downstream and close to the packaged product (18). However, the fact that staling precursors are developed upstream in the raw materials and brewhouse operations, means that brewers with ‘best in class’ flavour stability control measures are currently looking to these upstream stages to better understand the source of major staling precursors. One area of active research is to focus on the raw materials. Malt, as a major

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brewing ingredient, delivers to the brewing process various compounds, which can contribute to beer staling – *i.e.* amino acids, proteins, enzymes, reducing sugars and staling aldehydes (19–28). The content of these compounds in the malt is influenced by factors such as barley variety and malting process, which also directly affect malt quality. Therefore, this review discusses current knowledge on the impact of malt quality and malting process conditions on beer flavour deterioration with regard to beer staling aldehydes.

### Free aldehydes

In the late 1960s, the search for potential beer staling markers pointed to aldehydes as a class of compounds of paramount importance, as their increase coincides with the appearance of off-flavours during beer ageing (29). Moreover, free aldehydes show flavour-active properties and very low flavour thresholds, for example, *trans*-2-nonenal can be perceived at 0.03 µg/L, methional at 4.2 µg/L and 2-methylbutanal at 45 µg/L, when spiked individually to a lager beer (8). Further studies led to the identification of the most relevant aldehydes resulting from various chemical pathways, which are indicators of lager beer staling – the so-called ‘marker aldehydes’ (30). The most frequently reported are hexanal, *trans*-2-nonenal, furfural, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde (9,19,30–33).

These aldehydes may arise through *de novo* formation and/or due to their release from a bound-state. Various authors (9,34–45) have thoroughly discussed the possible reactions of *de novo* formation during malting and brewing. Marker aldehydes (see section ‘The most relevant formation pathways of marker aldehydes in relation to malt’) can arise through the following mechanisms: (1) lipid and fatty acid oxidation (auto- and enzymatic oxidation); (2) Maillard reactions; (3) Strecker degradation (Strecker degradation of amino acids in a strict sense, Strecker-like reactions, direct Strecker aldehyde formation from Amadori compounds, direct oxidation of amino acids); (4) oxidative degradation of isohumulones; (5) aldol condensation of short chain aldehydes; (6) oxidation of higher alcohols; (7) secondary oxidation of long chain aldehydes and (8) secretion by fermenting yeast.

### Bound-state aldehydes

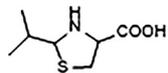
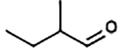
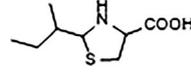
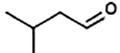
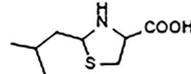
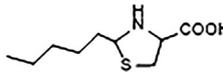
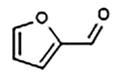
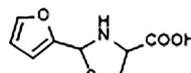
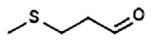
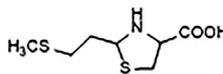
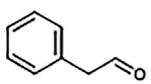
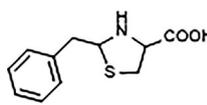
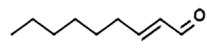
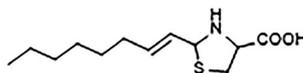
Aldehydes can also be present in the so called ‘bound-state’ forms. Free aldehydes are prone to binding due to the high electronegativity of the double bonded oxygen atom. Therefore, the electron deficient carbonyl carbon is likely to be attacked by nucleophiles (46,47). This may result in binding with other compounds, such as bisulphites (38,39), cysteine (48,49) or other amino acids (40). During beer storage and under specific conditions inside the beer package (pH, storage temperature, vibrations during transport), those adducts may dissociate, releasing free aldehydes, thus causing an increase in off-flavours (9,35,50). Unravelling the chemistry behind bound-state aldehydes is crucial in order to better understand beer staling.

The topic of aldehyde-adduct formation (the so-called ‘binding’) is complex since numerous chemical pathways are possible. Beer staling aldehydes are of different origin, chemical structures and properties, and they may react with various nucleophiles at different rates and under different reaction conditions (such as pH value, temperature, presence of oxygen). The binding behaviour of

selected beer staling aldehydes was thoroughly studied by Baert et al. (48,49). The authors demonstrated that the nucleophilic addition of cysteine or bisulphite to the carbonyl group of an aldehyde is affected by the electrophilic character of an individual aldehyde (see structures in Table 1). Accordingly, the electrophilicity of the carbon atom of the carbonyl group is influenced by the nature of the R group of the aldehyde (RCHO). In particular, the R group can be aliphatic (either saturated or unsaturated) or aromatic and because of this, the electrophilic character of aldehydes will vary. Thus, aldehydes substituted with an aromatic R group (e.g. phenylacetaldehyde) are less prone to binding to nucleophilic compounds compared to saturated aliphatic aldehydes (e.g. 2-methylpropanal) because the conjugated system of the aromatic substituent is decreasing the electrophilicity of the carbon atom of the carbonyl group. For the same reason,  $\alpha$ ,  $\beta$ -unsaturated aliphatic aldehydes such as *trans*-2-nonenal are less prone to binding nucleophilic compounds compared to saturated aliphatic aldehydes. Moreover, for saturated aliphatic aldehydes, the well known inductive effect should be taken into account when comparing their reactivity towards nucleophilic compounds. For instance, the inductive effect caused by the methyl group present at the 2-position of the R group, as is the case for 2-methylpropanal and 2-methylbutanal, reduces the readiness for binding nucleophiles to some extent (51). Baert et al. (49) and Bustillo Trueba et al. (52) reported on the influence of the pH of beer (4.4), wort (5.2) and malt (6.0) on the binding behaviour of aldehydes. A general trend of lower affinity to binding at lower pH, regardless of the nucleophile (cysteine or bisulphite) could be seen. This is because the more acidic pH (4.4) enhances protonation of the carbonyl oxygen causing enolisation, which reduces the aldehyde readiness towards binding. Cysteine and bisulphite reactivity was hardly affected by the pH of the model solution (4.4–6.0), due to the relatively high  $pK_a$  values of the cysteine amino group ( $pK_a=10.8$ ) and the sulfhydryl group ( $pK_a=8.3$ ) (51).

Regarding the release of flavouractive free aldehydes, until now, indirect methods under extreme conditions were applied to measure the dissociation of bound-state aldehydes. In 1983, Baker et al. (38) presented the release of carbonyl compounds from their corresponding bisulphite adducts. In 1990, Drost et al. (53) introduced the concept of ‘nonenal potential’ as an indicator of the possible release of *trans*-2-nonenal from a bound-state form. In 2015, Baert et al. (49) used 4-vinylpyridine as an aldehyde ‘releasing agent’, to demonstrate that the bound aldehydes are present in fresh beers. Only recently the development of analytical methodologies has allowed a direct determination of cysteinylated aldehyde adducts in model solutions (52), and somewhat later, in malt, brewing and beer samples (28). Bustillo Trueba et al. (52) conducted a detailed study investigating the chemical behaviour of cysteinylated aldehydes in model solutions. The results showed that the degradation rate of an adduct depends on the 2-substitution pattern (*i.e.* the nature of the R group) of the thiazolidine ring and on the pH value of the medium, e.g. at malt pH (6.0) decomposition of cysteine adduct was slower than at beer pH (4.4). Under the acidic conditions, the nitrogen atom may be protonated, leading to destabilisation of the thiazolidine ring and ring opening. Sensitivity to pH was also previously reported for bisulphite adducts, as Kaneda et al. (39) demonstrated that carbonyl compounds are present in a bound-state when  $SO_3^-$  is in the nucleophilic form (pH range 3–6). Conversely, imine adducts are more stable at higher pH (since an increase of pH raises Schiff base concentration), whereas a lower pH, similarly to the exposure to heat, promotes dissociation of the complex (40). Therefore, at the pH of malt (6.0), bound-state

**Table 1.** Molecular structures of free aldehydes and their corresponding cysteinylated forms, after Bustillo Trueba et al. (52)

Aldehyde	Molecular Structure	Cysteinylated Aldehyde	Molecular Structure
2-methylpropanal		2-isopropylthiazolidine-4-carboxylic acid	
2-methylbutanal		2-(sec-butyl) thiazolidine-4-carboxylic acid	
3-methylbutanal		2-isobutylthiazolidine-4-carboxylic acid	
hexanal		2-pentylthiazolidine-4-carboxylic acid	
furfural		2-(furan-2-yl) thiazolidine-4-carboxylic acid	
methional		2-(2-(methylthio)ethyl) thiazolidine-4-carboxylic acid	
phenylacetaldehyde		2-benzylthiazolidine-4-carboxylic acid	
<i>trans</i> -2-nonenal		(E)-2-(oct-1-en-1-yl)thiazolidine-4-carboxylic acid	

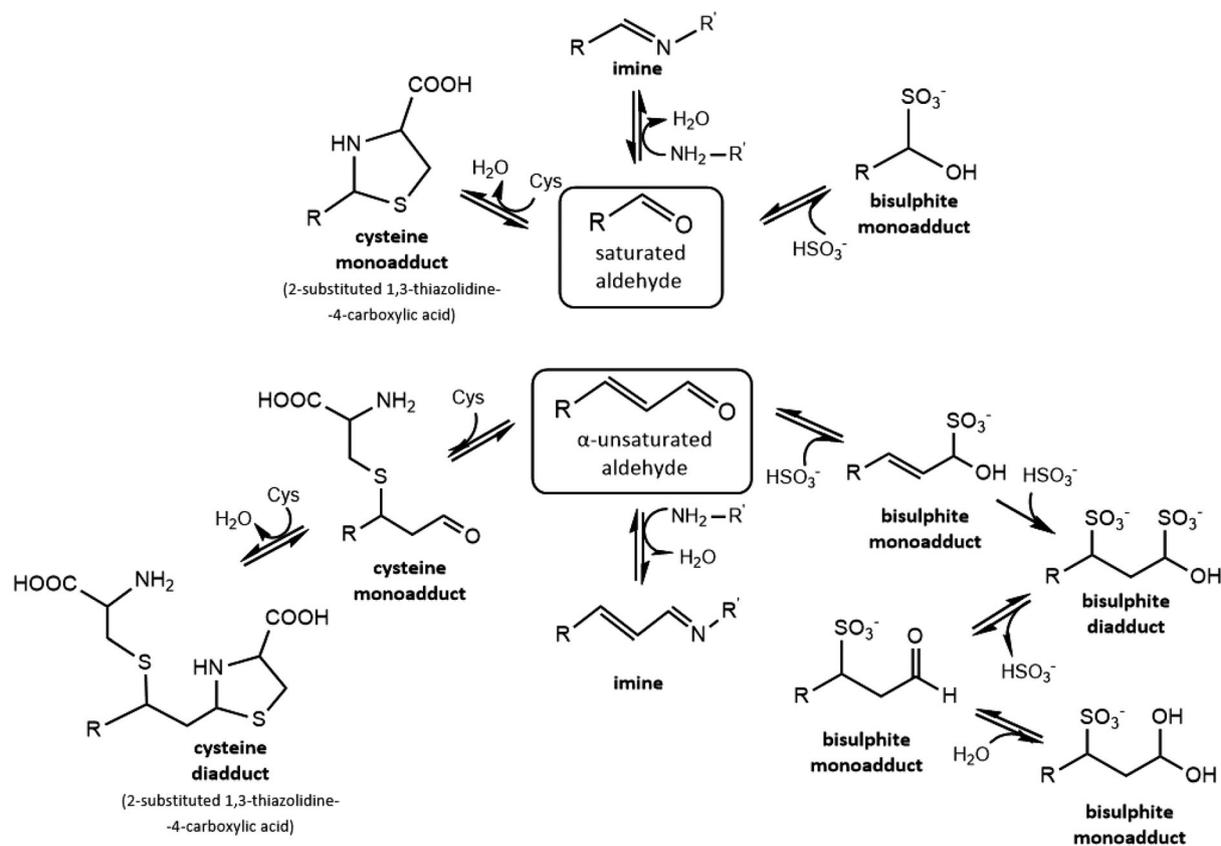
aldehydes may be formed more easily than in beer and their stability may also be higher. An overview of possible interactions between saturated and unsaturated aldehydes and cysteine, an amine or bisulphite respectively, is shown in Figure 1.

### The significance of free and bound-state aldehydes in beer ageing

The debate as to what extent bound-state forms may be responsible for beer staling is still ongoing. Regarding *de novo* formation, it has been suggested that aldehydes 'reappear' during beer ageing. Wietstock et al. (45) demonstrated that supplementation of fresh beer with leucine, isoleucine and phenylalanine in the presence of oxygen, leads to higher concentrations of the corresponding Strecker aldehydes (3-methylbutanal, 2-methylbutanal and phenylacetaldehyde, respectively) upon beer ageing for 30 weeks at 20°C. This indicates that *de novo* formation of Strecker aldehydes may indeed occur in a closed beer package and is enhanced by the presence of oxygen. Similar outcomes were

obtained by Gibson et al. (54), who added amino acids to fresh beer and observed an increase in Strecker aldehydes after forced ageing. Furthermore, Rangel-Aldao et al. (55) reported on the relevance of  $\alpha$ -dicarbonyls (intermediate products of Maillard reactions) to aldehyde formation while storing beer at elevated temperatures (28°C). The authors determined lower levels of furfural and 5-hydroxymethyl furfural (5-HMF) in beers with the addition of an  $\alpha$ -dicarbonyl trapping reagent. This is in agreement with Rakete et al. (44), who indicated that Maillard reactions resulting in the formation of furfural, occur to some extent during forced ageing of beer (two weeks at 50°C) since intermediates necessary for the reaction are present in beer.

On the other hand, it has been suggested that the conditions in a closed beer package do not promote *de novo* formation. For example, Lermusieau et al. (40) compared the content of *trans*-2-nonenal in oxygen free and oxygen receiving ageing beers. The results indicated that the increase in *trans*-2-nonenal over time is not caused by lipid oxidation in the beer package, but it is due to the release of its free form from a bound-state. Moreover,



**Figure 1.** An overview of the possible interactions between saturated (e.g. methional) and  $\alpha$ -unsaturated (e.g. *trans*-2-nonenal) aldehydes and cysteine, an amine or bisulphite, after Baert et al. (49).

Maillard reactions leading to *de novo* formation of e.g. furfural are favoured at conditions where pH is higher than typical beer pH values (for example, in malt) (46).

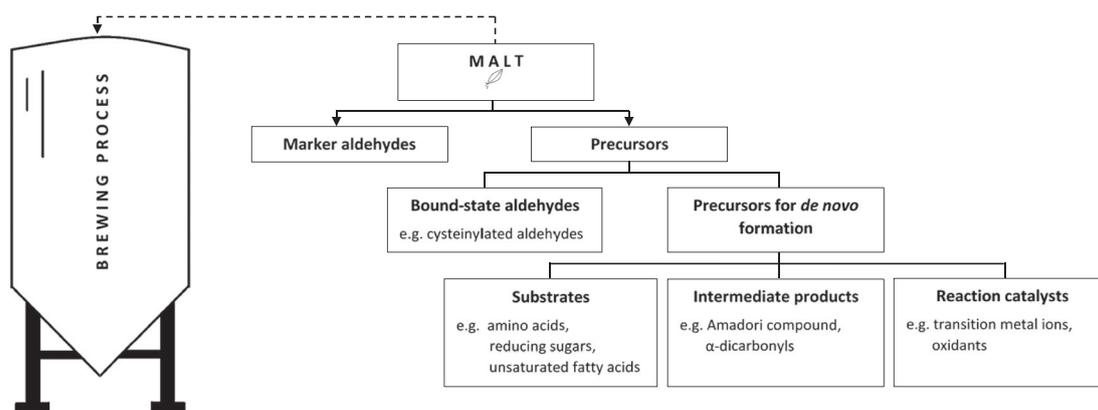
To date, free aldehydes originating from both potential pathways – *de novo* formation and release from a bound-state form – are considered to be contributors to beer flavour deterioration. Suda et al. (42) reported that 85% of Strecker aldehydes determined in aged beers are derived from the wort, whereas 15% originate from *de novo* formation in packaged beer. Furthermore, both free and bound-state aldehydes, might be delivered to the brewing process with the raw materials and/or could be formed during beer production (28). Formation of imine adducts may occur during malting (56,57) and brewing (50), whereas bisulphite adducts might be formed during fermentation or downstream (38,53,58). In summary, the above studies (28,38,42,50,53,56-58) point to the relevance of the malting and brewing process in the formation of bound-state aldehydes, as well as to malt as an essential source of beer staling compounds and their precursors.

### The most relevant formation pathways of marker aldehydes in relation to malt

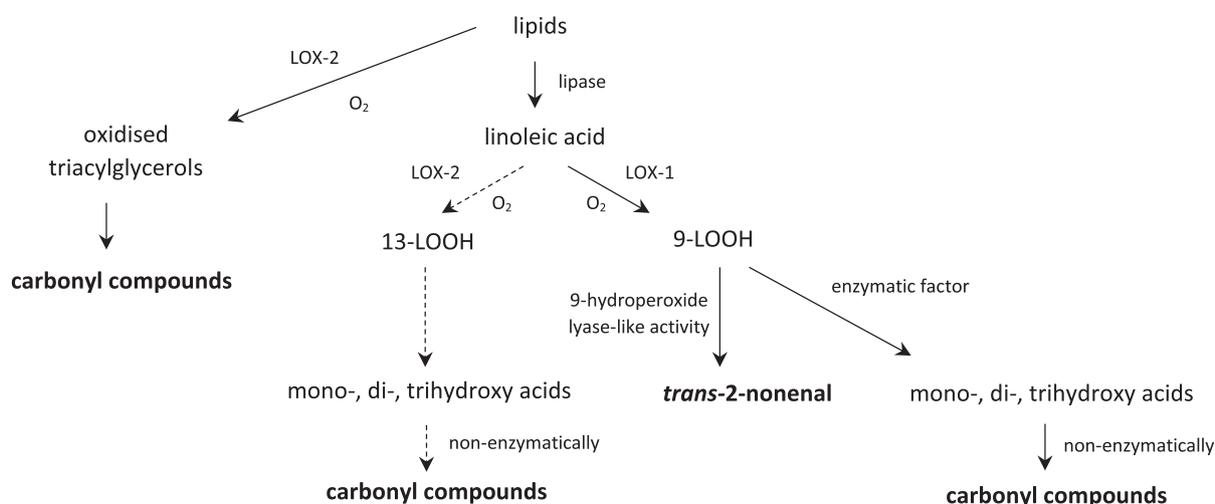
Malt provides aldehydes to the brewing process directly but also offers a variety of their precursors (Figure 2). The formation pathways of marker aldehydes are complex and consist of numerous steps, which strongly depend on the reaction conditions (e.g. pH, temperature, presence of substrates) and can

lead to various intermediates and final products. This section focuses on reactions taking place in malt (or analogous conditions) and leading to the formation of marker aldehydes, namely: hexanal, *trans*-2-nonenal, furfural, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde. These compounds represent the end products of typical formation reactions, e.g. oxidation of unsaturated fatty acids, Maillard reactions and Strecker degradation of amino acids.

**Hexanal and *trans*-2-nonenal.** The main chemical pathway leading to the formation of hexanal and *trans*-2-nonenal is the oxidation of unsaturated fatty acids via autoxidation or catalysed by enzymes. In the enzymatic pathway (Figure 3), linoleic acid (C18:2) and linolenic acid (C18:3), representing up to 60 and 10% of the total fatty acids in malt (59), are released in the presence of water from triacylglycerols by lipase (pH optimum 6.8) (60). The resulting free fatty acids are oxidised by lipoxygenase (LOX-1 and LOX-2, pH optimum 6.5) (61) to hydroperoxy fatty acids. LOX-1 yields 9-hydroperoxyoctadeca-10,12-dienoic acid (9-LOOH), whereas LOX-2 produces 13-hydroperoxyoctadeca-9,11-dienoic acid (13-LOOH). During malt kilning, most of the LOX activity is destroyed as both enzymes are heat-sensitive. However, LOX-1 is more heat resistant than LOX-2, thus the formation of 9-LOOH proceeds at a higher rate (62). Subsequently, 9- and 13-LOOH are subject to enzymatic degradation to mono-, di- and trihydroxy fatty acids followed by non-enzymatic breakdown resulting in carbonyl compounds.



**Figure 2.** Compounds contributed by malt to the brewing process, which may potentially affect beer flavour stability.



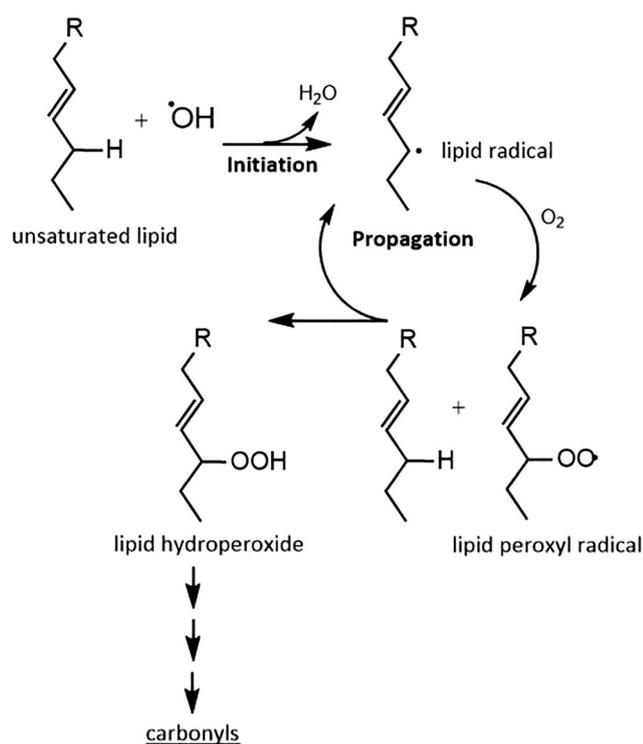
**Figure 3.** An overview of enzymatic oxidation leading to the formation of hexanal and *trans*-2-nonenal, according to Vanderhaegen et al. (1). The continuous line refers to reactions at a high rate, whereas the dashed line relate to pathways that proceed at a slower rate.

The pathway of 9-LOOH leads to *trans*-2-nonenal, whereas 13-LOOH yields hexanal (1). Another possible oxidation pathway of linoleic and linolenic acid esterified in triacylglycerol is by LOX-2, also leads to the formation of carbonyl compounds (1,59).

Regarding autoxidation, in the cascade of reactions, unsaturated fatty acids can be oxidised by reactive oxygen species and via lipid peroxy radicals into lipid hydroperoxides (9-LOOH and 13-LOOH) (Figure 4) (64). Again, various compounds may be formed from these precursors in enzymatic and non-enzymatic reactions leading to hexanal and *trans*-2-nonenal. The rate of autoxidation is enhanced by high temperatures and the presence of oxidants, e.g. transition metal ions (iron and copper) (9). Malt, among other brewing raw materials, is rich in these ions, delivering up to 97.5% of iron and 94.3% of copper to the process (65). Another critical reaction from the perspective of malting is the secondary autoxidation of unsaturated aldehydes. For example, *trans*-2-nonenal can be autoxidised into shorter chain aldehydes, such as hexanal (66).

**Furfural.** Furfural is one of the many products of Maillard reactions – a complex reaction chain initiated by an amine, amino acid,

peptide or protein reacting with a pentose reducing sugar (Figure 5) (9,67). The reaction is initiated by nucleophilic addition of an amino group to the reducing end of an open chain of sugar, leading to N-glycosylamine (Schiff base) formation (68). This intermediate undergoes Amadori rearrangement resulting in the formation of 1-amino-1-deoxyketose (Amadori compound), which undergoes enolisation and, depending on the pH, forms specific isomers. In the next stage, the amine is released and  $\alpha$ -dicarbonyls are formed – 3-deoxyosone (pH<5), and 1- or 4-deoxyosone (pH>7). Upon dehydration of 3-deoxyosone followed by cyclisation of the intermediate  $\alpha$ -dicarbonyl, and final dehydration, furfural is formed from pentose. The generated  $\alpha$ -dicarbonyls can also act as a reactant in Strecker degradation of amino acids. The kinetics of Maillard reactions are strongly dependent on the nature and proportion of reactants, temperature, time, pH and water activity (69–71). For example, pH value affects the reactivity of amino group ( $pK_a$  values around 9 or higher) and the proportion of open chain to closed chain forms of sugars (more aldose forms are present at higher pH). Also, a moderate water activity is required, allowing almost a subsequent addition and elimination of water molecule (see Figure 5). As Maillard reactions are mostly associated



**Figure 4.** An overview of the oxidation cascade of unsaturated fatty acids, initiated by reactive oxygen species, from Bamforth and Cook (63).

with the exposure of malt to high temperatures, these chemical pathways have been studied extensively with regard to dark speciality malts (72–74) and pale malts (75).

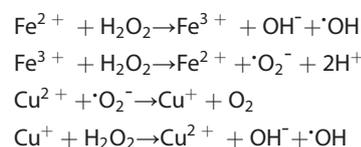
**2-Methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde and methional.** Strecker aldehydes may arise via several pathways. One of them is the Strecker degradation in a strict sense (Figure 6), which is a reaction between an amino acid and an  $\alpha$ -dicarbonyl. In the case of beer staling marker aldehydes, amino acids act as precursors (valine is a precursor of 2-methylpropanal, isoleucine of 2-methylbutanal, leucine of 3-methylbutanal, methionine of methional and phenylalanine of phenylacetaldehyde (9)), whereas a variety of  $\alpha$ -dicarbonyls are derived, among others, from Maillard reactions (76). The Strecker degradation in a strict sense is initiated by nucleophilic addition of the unprotonated amino group to the carbonyl group resulting in the formation of a hemiaminal. This unstable intermediate undergoes dehydration and subsequent irreversible decarboxylation forming an imine zwitterion. Water addition leads to an unstable amino alcohol, which breaks down into an  $\alpha$ -ketoamine and an aldehyde (1,9,76,77).

Alternatively, Strecker aldehydes may arise via Strecker-like reactions – between an amino acid and an  $\alpha,\beta$ -unsaturated carbonyl compound (e.g. *trans*-2-nonenal, furfural) (77) – or by direct oxidative degradation of amino acids (78). The latter was confirmed to occur in beer (45), however, further investigation is required with regard to barley, malt and model solutions of  $\text{pH} \approx 6$ .

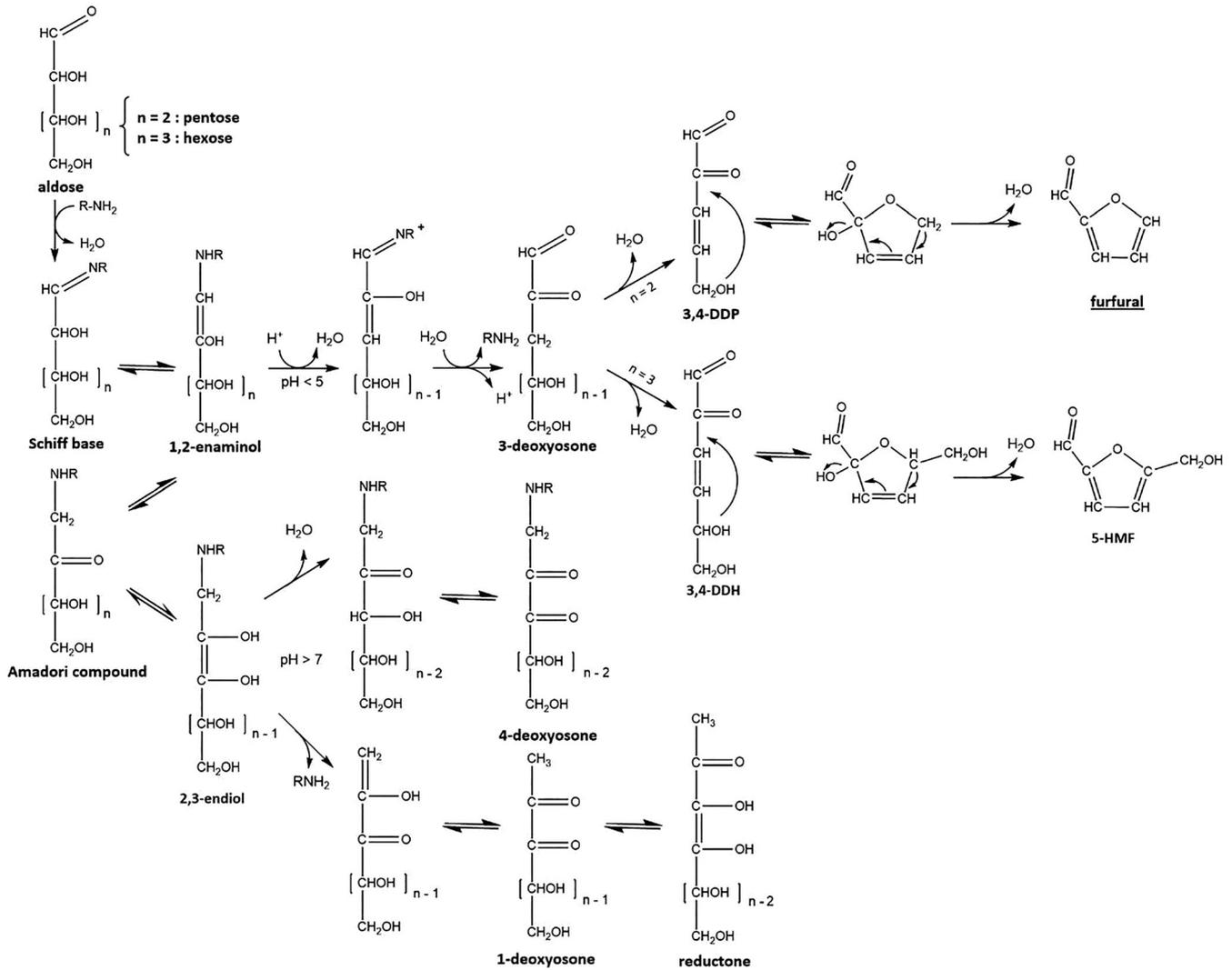
Another possibility is the reaction of Amadori compounds (derived from Maillard reactions) with amino acids (76). This pathway is likely to take place in malt, even though it was studied only in model solutions (with and without the presence of transition metal ions) (79,80). Firstly, compared to wort and beer, the pH of malt is more favourable to the formation of 1-amino-1-deoxyketoses

(Amadori compounds) and, secondly, during germination the proteolytic activity of malt increases, leading to the release of free amino acids from more complex structures. Therefore, in malting, conditions are more favourable for the formation of substrates for this particular formation pathway of Strecker aldehydes.

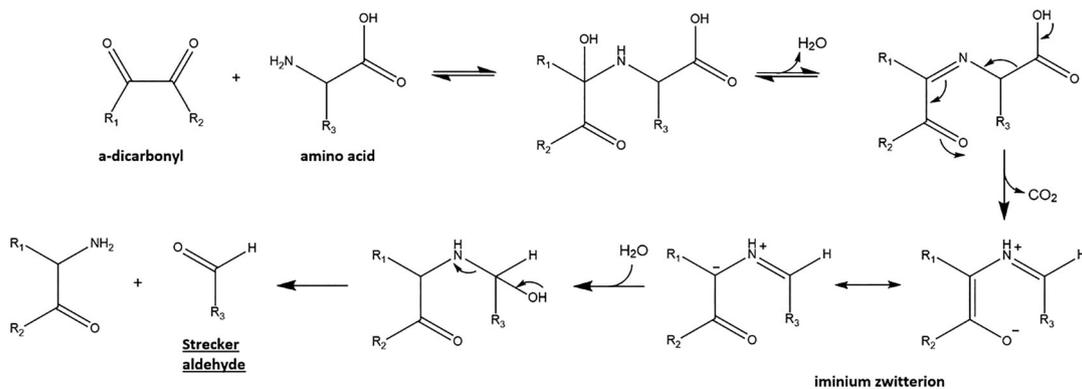
**Important precursors, intermediates and catalysts for *de novo* formation of aldehydes.** In the *de novo* formation of marker aldehydes, their precursors, intermediate products and catalysts play an essential role, the most relevant of which are presented in Table 2. The function of precursors, intermediates and some catalysts of marker aldehyde formation have been described in the above sections. Catalysts are crucial, especially from the perspective of oxidation reactions. In particular, the transition metal ions are important as well as anti- and prooxidants. Transition metal ions such as iron, copper and manganese are known to accelerate the rate of radical reactions *i.e.* the Fenton and Haber-Weiss reactions, which occur as follows (81):



The reaction chain starts with oxidation of  $\text{Fe}^{2+}$  by hydrogen peroxide to  $\text{Fe}^{3+}$ , resulting in the formation of a hydroxyl radical ( $\cdot\text{OH}$ ) and hydroxyl anion ( $\text{OH}^-$ ). Next,  $\text{Fe}^{3+}$  reacts with another molecule of hydrogen peroxide forming a superoxide radical ( $\cdot\text{O}_2^-$ ), two protons and reduced iron ( $\text{Fe}^{2+}$ ). The superoxide radical reacts with  $\text{Cu}^{2+}$  leading to oxygen and  $\text{Cu}^+$ . Finally, the generated  $\text{Cu}^+$  reacts with hydrogen peroxide forming a hydroxyl radical and hydroxyl anion, and oxidising copper to its original  $\text{Cu}^{2+}$  form. Free radical species (in particular the hydroxyl radical) are exceptionally reactive, which can lead to non-enzymatic oxidation of lipids resulting in hexanal and *trans*-2-nonenal (9), as well the direct oxidation of amino acids to Strecker aldehydes (78). As noted above, malt, is rich in these ions, contributing up to 97.5% of iron and 94.3% of copper to the brewing process (65). The extent of these reactions can be diminished by antioxidants, due to their reducing power, their radical scavenging and metal chelating properties, as well as by the antioxidative enzymes such as peroxidase, catalase and superoxide dismutase (43,82–84). Malt is a natural source of antioxidants e.g. flavan-3-ols, phenolic acids and ferulic acid, which are delivered to the brewing process (1,82). The antioxidant properties of barley and malt are mostly associated with phenolic compounds such as phenolic acids, flavonoids, proanthocyanidins and tannins (85,86), as well as some of the Maillard reaction products (MRPs), in particular, melanoidins and reductones (87). Phenolic compounds that are ultimately found in beer originate to a large extent from malt (e.g. pale malt delivers around 80–85% of polyphenols to beer, whereas dark speciality malt delivers approx. 95%), whereas hops contribute only a minor fraction of the total beer polyphenols (88–90). Melanoidins and reductones (intermediates of Maillard reactions) are present in all types of malt, however, in particular in dark speciality malts as they are mostly formed during the roasting process through intensive heating. As antioxidants, melanoidins, are superoxide scavengers and can interact with peroxide and hydroxyl radicals (91). However, Hashimoto et al. (92) reported that these compounds also may catalyse the oxidation of higher alcohols to carbonyl compounds, thereby impairing beer flavour stability. Similarly, reductones act as radicals



**Figure 5.** An overview of Maillard reactions leading to the formation of furfural, after Baert et al. (9). Reaction begins with pentose ( $n=2$ ) or hexose ( $n=3$ ), and yields  $\alpha$ -dicarbonyls (3-, 4-, and 1-deoxyosones) and some heterocyclic compounds (furfural and 5-hydroxymethylfurfural (5-HMF)). 3,4-DDP - 3,4-dideoxypentosulose-3-ene; 3,4-DDH - 3,4-dideoxyhexosulose-3-ene.



**Figure 6.** An overview of the Strecker degradation in a strict sense leading to the formation of 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional and phenylacetaldehyde, according to Baert et al. (9).

**Table 2.** Significant precursors and catalysts for the *de novo* formation pathways of beer staling aldehydes, based on (9,34,42,44,45)

Aldehydes	Precursors	Catalyst
<b>Fatty acid oxidation products</b>		
hexanal	- lipids and unsaturated fatty acids	- enzymes (e.g. lipase, lipoxygenase and hydroperoxide lyase)
<i>trans</i> -2-nonenal	- 9-LOOH, 13-LOOH - mono-, di- and trihydroxy fatty acids	- oxygen - high temperature - reactive oxygen species (ROS) (for autoxidation) - transition metal ions (Cu <sup>2+</sup> , Fe <sup>2+</sup> ) (for autoxidation)
<b>Maillard reactions products</b>		
furfural	- amines, amino acids, peptides, proteins - reducing sugars (e.g. xylose, fructose) - $\alpha$ -dicarbonyls	- high temperature - high pH in the first stage of reaction, followed by pH<5 for conversion of 1,2-enaminol
<b>Strecker degradation products</b>		
2-methylpropanal	- corresponding amino acids: valine, isoleucine,	- high temperature
2-methylbutanal	leucine, methionine, phenylalanine	- transition metal ions (Cu <sup>2+</sup> , Fe <sup>2+</sup> )
3-methylbutanal	- $\alpha$ -dicarbonyls	- reactive oxygen species (ROS)
methional	- $\alpha$ , $\beta$ -unsaturated aldehydes	- oxygen
phenylacetaldehyde	- Amadori compounds	

scavengers (87), but also they intensify Fenton and Haber-Weiss reactions by reduction of transition metal ions (e.g. Fe<sup>3+</sup> to Fe<sup>2+</sup>) (93). In accordance with these, are the results of Hoff et al. (94) who reported higher iron content and higher radical intensities in dark worts compared to pale malt worts. Regarding the anti-oxidative enzymes, barley contains superoxide dismutase, catalase, peroxidase, as well as ascorbate peroxidase (95–97). However, peroxidase acts as an antioxidative enzyme by removing hydrogen peroxide, it also oxidises barley phenolic compounds, thereby reducing the antioxidative potential of malt (98). Other, pro-oxidative enzymes are well known for their participation in lipid degradation, such as lipase, lipoxygenase and the hydroperoxidereactive enzyme system (hydroperoxide lyase and hydroperoxide isomerase), as well as polyphenol oxidase, which catalyses oxidation of phenolic compounds (95,98,99). Both the barley cultivar and malting process influence the above residual enzymatic activities found in the finished malt (82).

## Contribution of malt to beer flavour instability

To date, most research regarding beer staling has focused on the combined effects of the brewing process and beer storage conditions on the chemistry of beer ageing. However in 2004, Bamforth (41) stated: ‘... the scenario for malt in the context of flavour instability is so under-researched that it is impossible to be categorical either for or against its significance’. Since this time, the importance of malt quality in the context of beer flavour and flavour instability has been increasingly recognised (19–22,24,25,27,28,100–104). For beer staling aldehydes, according to De Clippeleer et al. (27), these compounds are primarily derived from malt, rather than from degradation of hop bitter acids, hence the latter are of less importance. In support of this, Ditrych et al. (20) evaluated levels of staling aldehydes across the wort production process and reported the highest levels of staling aldehydes in mashing-in samples. Thus, malt can be seen as the main brewing raw material delivering aldehydes to the brewing process.

Dong et al. (105) identified various flavouractive compounds in pale malt, among them marker aldehydes including 2-methylpropanal, acetaldehyde, 3-methylbutanal, 2-methylbutanal, hexanal, *trans*-2-nonenal and benzaldehyde. Reported quantities of marker aldehydes in pale malts are presented in Table 3 (19,106–109). The aldehyde profile of the different malt samples appeared to be quite similar; 3-methylbutanal was found in the highest concentration, followed by 2-methylpropanal and 2-methylbutanal, whereas *trans*-2-nonenal and methional were the lowest. Variations in aldehyde content among these malts can be explained by different barley cultivars, crop years and applied malting technologies (infrastructure and malting parameters), as well as the different analytical approaches adopted (e.g. SIFT-MS, HS-SPME GC-MS). Further, similar compounds were identified in caramalt and dark malts, which in comparison to pale malts contained higher amounts of aldehydes (110–112). For example, Yahya et al. (73) determined around 9,000  $\mu\text{g}/\text{kg}$  and 2,100  $\mu\text{g}/\text{kg}$  of furfural in black malt and crystal malt, respectively, while Gibson et al. (54) reported a three-fold higher concentration of 2-methylpropanal and 2-methylbutanal in worts produced with dark malts in comparison to their pale malt derived counterparts. Moreover, Gastl et al. (21) by applying air recirculation during germination and relatively long kilning cycle (36h) with kilning-off at 85°C, obtained malt which resulted in beers of a high acceptance score. The authors suggested that the combination of low Kolbach Index of malt and a low amount of Strecker aldehydes and hexanal in wort, as well as low heat load in wort can result in enhanced beer flavour stability.

Regarding the relationship between final beer flavour stability and malt, Bustillo Trueba et al. (28), showed that malt is rich in bound-state aldehydes, namely cysteinylated aldehydes, which could contribute to the increase of free forms during beer ageing, assuming these compounds ‘survive’ the brewing process. Also, it has been reported that brewing with malt, low in Kolbach Index (104) and low in free amino nitrogen (19,100) results in a lower rate of beer staling. Moreover, a high heat load, in the form of Maillard intermediates reacting with thiobarbituric acid (TBI), measured in

**Table 3.** Levels of marker aldehydes determined in pale malts that varied in barley cultivars, harvest year, malting technology (19,106–109) and their flavour description (33). 1 - (107) GC-FID on the extract of water vapour distillation; 2 - (106) SIFT-MS on the unground malt grains and HS-SMPE-GC-MS on malt extract; 3 - (19) HS-SMPE-GC-MS on malt extract; 4 - (108) HS-SMPE-GC-FID on malt extract with the addition of NaCl; 5 - (109) HS-SMPE-GC-MS on malt extract

Aldehyde	Concentration range [ $\mu\text{g}/\text{kg}$ dry mass]	Flavour description
<b>Fatty acid oxidation products</b>		
hexanal	173 – 1,010 <sup>2</sup> 495 – 1,123 <sup>3</sup> 449 – 1,669 <sup>5</sup>	bitter, winey
<i>trans</i> -2-nonenal	29 – 74 <sup>2</sup> 17 – 46 <sup>3</sup> 9 – 39 <sup>4</sup> 210 – 580 <sup>5</sup>	cardboard, papery
<b>Strecker degradation products</b>		
2-methylpropanal	612 – 2,311 <sup>2</sup> 722 – 3,480 <sup>3</sup> 1,128 – 3,469 <sup>5</sup>	grainy, fruity
2-methylbutanal	467 – 1,119 <sup>2</sup> 612 – 2,411 <sup>3</sup> 980 – 3,279 <sup>5</sup>	almond, malty
3-methylbutanal	1,741 – 2,585 <sup>1</sup> 1,213 – 4,271 <sup>2</sup> 3,053 – 4,215 <sup>3</sup> 2,834 – 8,218 <sup>5</sup>	malty, cherry, almond
methional	224 – 566 <sup>2</sup> 383 – 1,014 <sup>3</sup> 319 – 5,105 <sup>5</sup>	cooked potatoes
phenylacetaldehyde	400 – 853 <sup>2</sup> 198 – 1,014 <sup>3</sup> 319 – 5,105 <sup>5</sup>	flowery
<b>Maillard reactions products</b>		
furfural	185 – 477 <sup>1</sup> 285 – 412 <sup>2</sup> 89 – 651 <sup>3</sup> 391 – 1,116 <sup>5</sup>	caramel, bready

malt, in unboiled wort and fresh beer was found to be inversely related to the sensory score for beer freshness while examining forced-aged beers (21). The authors concluded that a low TBI is a basis for better beer flavour stability. Furthermore, this parameter measured in malt correlates with boiled and unboiled Congress wort colour (21). The above observations are in agreement with Furukawa Suárez et al. (22), who reported that the addition of speciality malts (e.g. caramel malt), which are characterised by higher EBC colour and heat load values, increases the content of beer staling aldehydes in forced-aged beers. In addition to the above, the antioxidant properties of malt appear to impact flavour stability as high radical scavenging activity (positively correlated with phenolic content) contributes to prolonged beer freshness (24). When evaluating the staling degree of forced-aged beers (forced-aged for six days at 50°C, followed by one day at 0°C), which were brewed with or without a reduced content of phenolic compounds, Mikyška et al. (23) reported a higher stale flavour intensity in the beer variant with a diminished phenolic content. The authors interpreted this as malt polyphenols having a positive impact on the staling degree of forced-aged beers. Though, the phenolic content was reduced by the addition of PVPP, the assumption was not tested on purified malt polyphenols. As noted before, besides polyphenols also Maillard reaction products (MRPs) are the

well known antioxidants present in malt. However, it has been demonstrated that brewing with the addition of dark speciality malts may result in a decreased oxidative stability of wort and beer, though dark malts are known for their high content of MRPs (22,113). These outcomes are in line with other studies (93,114) reporting that MRPs or intermediates such as reductones with an enediol structure, may accelerate the Fenton reaction, thereby yielding higher levels of reactive oxygen species (ROS) during beer ageing. Higher levels of ROS will in turn accelerate degradation of amino acids into Strecker aldehydes in darker beers (45,78). Finally, using electron spin resonance (ESR) spectroscopy, Kunz et al. (103), observed higher oxidative stability in beer brewed with 25%, 50%, 75% and 90% raw barley, in comparison to a 100% malt beer. Thus, it appears that the use of unmalted barley, which is not exposed to heat load, compared to conventional malt, imparts prolonged beer flavour stability.

Therefore, malt can be seen as a key factor considering free aldehydes as such, as well as aldehyde precursors (e.g. bound-state aldehydes, amino acids, reducing sugars, etc.) and intermediate products (e.g. Schiff bases,  $\alpha$ -dicarbonyls, etc.), since free aldehydes are largely removed during brewing (20) and the fermentation process (115). In particular, aldehydes precursors and intermediate products may affect beer staling.

In addition to brewing trials, various barley breeding experiments have been performed with the main focus on elimination of proanthocyanidins (116) and lipoxygenase activity (both LOX-1 and LOX-2 enzymes) (117–120), which are recognised as factors negatively influencing beer stability. Since a beer brewed with malted proanthocyanidin-free barley received a lower score in the sensory evaluation after one month of natural ageing than its proanthocyanidin control (116), it is possible that the content of low molecular weight polyphenols was insufficient to slow down oxidation reactions, resulting in an increase in off-flavours. A different approach - brewing with malted null-LOX barley - showed a positive influence on beer flavour stability. The latter was assessed by the *trans*-2-nonenal content and through sensory evaluation of forced-aged beers (117). In another study conducted by Hirota et al. (118), low levels of *trans*-2-nonenal were determined in both forced-aged beers, when null-LOX malt was used as the main ingredient and when applied in combination with a LOX-normal pale malt. Moreover, in the sensory evaluation conducted by trained panellists, null-LOX forced-aged beer obtained lower off-flavour and total staleness score. More detailed analysis, by the same research group, compared malt quality parameters between LOX and null-LOX malts (120). The authors reported no differences in general characteristics, except for LOX activity (16.1 vs 1.3 U/g). In forced-aged beers, *trans*-2-nonenal was detected in the range of 0.09–0.12 µg/L and 0.35–0.36 µg/L, when brewed with null-LOX and LOX-normal malt, respectively. In addition, Hirota et al. (120) suggested that the application of null-LOX malt would reduce the energy cost required to inactivate LOX in the brewhouse. Hoki et al. (119) indicated that modifying the malting schedule (e.g. decreasing germination temperature, increasing kilning temperature and/or prolonging kilning time) could reduce LOX-activity during malting, without the need of using null-LOX barley. Nevertheless, these adjustments would affect other malt quality parameters crucial for brewers, primarily malt colour. Therefore, the use of null-LOX barley may be more suitable to decrease *trans*-2-nonenal concentrations in malt and in aged beer. Additional brewing trials (119) with 74% of malted LOX-1-less barley variety 'Satuiku 2 go' and 26% adjuncts (starch, corn, rice) showed that fresh beer contained only 0.03 µg/L of *trans*-2-nonenal, whereas after one month at 30°C, aged beer and its control (beer brewed with LOX-normal malt) contained 0.11 and 0.16 µg/L, respectively. Considering 0.03 µg/L as an accepted flavour threshold value of *trans*-2-nonenal (8), brewing with LOX-1-less barley does not allow the concentration of this off-flavour to be maintained below sensory perception levels. Nevertheless, it does decrease the content of *trans*-2-nonenal to levels similar to those formed via the nonenzymatic reaction of autoxidation (9).

## Evolution of marker aldehydes and their precursors during the malting process

The (bio)chemical composition of malt depends on the grain to be malted (121,122) and the malting process (21,123,124). In this review, particular attention is drawn to malted barley, as it is the primary cereal used for brewing. In brief, the malting process consists of grain sorting and cleaning, steeping, germination and kilning. The main goals of malting are activation and formation of enzymes, partial degradation of endosperm matrix polymers (mainly proteins and β-glucans), improvement of grain friability and formation of colour as well as characteristic malt flavours. During malting, barley undergoes physical and chemical modifications

when exposed to the following key factors for varying lengths of time: water, oxygen and high temperature (123). By adapting the malting regime, the quality parameters of pale malt can be modified, for example, lower germination temperatures tend to decrease soluble nitrogen and increase extract yield (125). Moreover, flavour, colour and the reducing power of malt can be influenced by the kilning regime or by roasting (126). The latter process allows production of speciality malts such as crystal malt, caramel malt and chocolate malt, with flavour profiles that are well defined (73,74,87,105,126–130).

The importance of the malting process in relation to beer flavour instability is still poorly understood, even in the case of pale malts that represent the predominant grist material used across the brewing industry. Current available data on the influence of crucial malting parameters on the formation of free aldehydes and their precursors are summarised in Table 4, and in the following sections.

### Barley

#### Structure of barley grain in relation to marker aldehydes and their precursors.

Barley grain (*Hordeum vulgare*, *vulgare* L.) consists of approx. 65–68% of starch, 10–17% of protein, 3–9% of arabinoxylans, 4–9% of β-glucans, 2–3% of lipids, and 1.5–2.5% of minerals on a dry matter basis (132–134). The moisture content of stored barley and malt is low (approx. 12% and <5% for barley and malt, respectively), which prevents extensive chemical transformations inside the kernel and any microbial activity (123). Moreover, barley and malt kernels consist of a multi-layered structure, including husk, pericarp, testa, aleurone layer, embryo and endosperm (Figure 7). These are exposed during malting (albeit to differing extents) to water, air (oxygen) and heat, as well as to enzymatic, hormonal and microbial activity. Therefore, the formation of marker aldehydes can differ between grain structures. According to Fox et al. (135), of the kernel structures, the husk, aleurone, scutellum and embryo are primarily associated with barley and malt quality.

The husk, together with the pericarp, account for 7 to 14% of grain (dry matter basis), depending on the barley variety, grain size and growing environment (135,136). From the perspective of beer flavour instability, organic radicals which catalyse various oxidation reactions leading to marker aldehydes are mainly found in the husk, rather than in the flour fraction of malt (113). In addition, the husk provides flavours that are often described as 'husky' or 'grainy' (137). Lewis et al. (138) highlighted the importance of extracting malt polyphenols from the husk during the brewing process since these are responsible for a grainy taste, astringency and may also act as pro- or antioxidants. The latter may enhance or reduce the rate of marker aldehyde formation. Van Waesberghe et al. (139) suggested that beer flavour instability is significantly affected by husk components extracted during mashing and sparging, among them staling aldehydes, as well as their precursors and polyphenols. For example, beers brewed with malt containing husk showed a higher TBI than beers brewed using a corresponding 'hulled' malt (139). Though, the TBI is strongly correlated with the evolution of carbonyl compounds during beer ageing, 'de-husked beers' were more prone to staling, probably due to lack of husk polyphenols acting as antioxidants.

Another barley structure that may be important from the perspective of beer flavour instability is the aleurone layer, which makes up to 8 to 15% of the grain (dry matter) and contains

Malting stage	Influence		
	Positive	Negative	Ambivalent
<b>Barley selection</b>	<ul style="list-style-type: none"> <li>- Selection of barley with low lipoxygenase potential or null-LOX barley leads to decreased rate of enzymatic oxidation of unsaturated fatty acids (117–119).</li> <li>- Selection of winter barley allows reduction of the levels of polyunsaturated fatty acids (146).</li> <li>- Application of sonic waves improves the oxidative stability of wort by reduction of iron content in malt (143).</li> </ul>	<ul style="list-style-type: none"> <li>- Selection of barley with high nitrogen content increases the pool of aldehyde precursors (19,100,104,150–153).</li> <li>- The crop year, soil conditions and fertilisation may increase the nitrogen content (154,155) and levels of volatile compounds found in barley (106).</li> <li>- Frequent aeration during the wet phase and multiple air rests lead to higher LOX-activity (61).</li> <li>- Oxidation of steeping water with hydrogen peroxide or ozone results in an increased pool of aldehyde precursors (162).</li> </ul>	<ul style="list-style-type: none"> <li>- Winter barley has higher husk to endosperm ratio, which on the one hand results in a higher content of Maillard intermediates (139) and transition metal ions (65), however, on the other hand, it is rich in antioxidants (23,24,138,149).</li> <li>- Long steeping time enhances steeping degree and thus, results in high grain modification. This increases the pool of aldehyde precursors but also increases antioxidants content (147).</li> <li>- Re-steeping of green malt in acidified solution increases the content of Maillard precursors but also decreases the quantity of Strecker aldehydes (163,164).</li> <li>- High germination temperature leads to higher grain modification and increased pool of aldehyde precursors (145,165). However, it also results in a lower content of some staling aldehydes (107).</li> </ul>
<b>Steeping</b>	<ul style="list-style-type: none"> <li>- Application of lower germination temperatures leads towards lower LOX activity (62,63).</li> <li>- Maintenance of low moisture content of green malt results in reduced levels of marker aldehydes present in malt (107).</li> <li>- Grain asphyxiation or air recirculation during germination may suppress oxidation of unsaturated fatty acids and reduce LOX activity (21,61).</li> <li>- Acidification of green malt by lactic acid bacteria may indirectly reduce the content of aldehydes found in malt (174,175).</li> </ul>	<ul style="list-style-type: none"> <li>- Microbial contamination with <i>Fusarium</i> leads to higher levels of aldehydes found in malt (172,173).</li> <li>- Excessive treatment of green malt with gibberellic acid may increase the pool of aldehyde precursors (176,177).</li> </ul>	<ul style="list-style-type: none"> <li>- Exposure of malt to high kilning-off temperature for a long(er) time reduces LOX activity (61,168,182); however, the formation of Strecker aldehydes and furfural is intensified (74,116).</li> </ul>
<b>Kilning</b>	<ul style="list-style-type: none"> <li>- Moderate kilning-off temperature (up to 80°C) increases the polyphenol content in the finished malt (183).</li> <li>- Application of alternative drying methods may allow reduction of the heat load and grain inhomogeneity (difference between bottom, middle and top layer of a kiln bed) (186–188).</li> </ul>	<ul style="list-style-type: none"> <li>- High kilning temperature leads to a higher rate of Strecker aldehydes and furfural formation (74).</li> <li>- Inhomogeneity of malt caused by kilning grain in a thick layer (difference between bottom, middle and top layer of a kiln bed) (56).</li> <li>- High kilning off temperature enhances radical formation (113).</li> <li>- Application of sulphuring during kilning increases the content of soluble nitrogen (184).</li> <li>- Higher kilning temperatures increase malt colour and TBI, as well as the content of Maillard reactions</li> </ul>	

(Continues)

**Table 4. (Continued)**

Malting stage	Influence	
	Positive	Negative
<b>Storage</b>	- Prolonged storage time decreases LOX activity (181).	(e.g. reductones) and reactive oxygen species what may lead to increased levels of Strecker aldehydes (22,45,93,114). - Humid conditions increase levels of Strecker aldehydes and their precursors in malt (94,131).

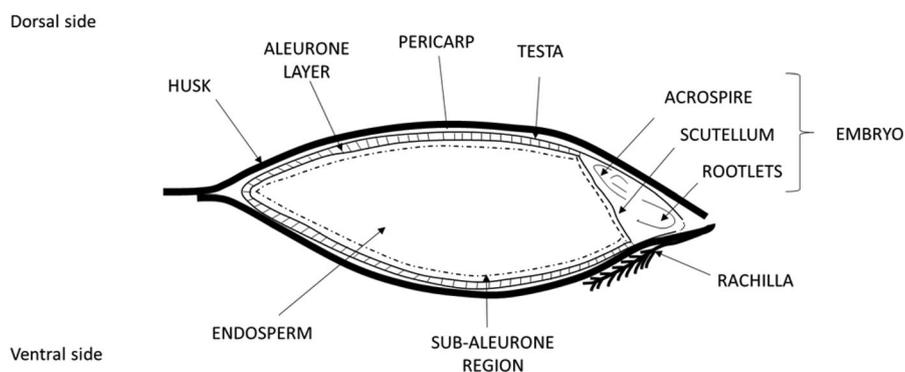
protein, lipid, glucan and xylan (123,135,138). Moreover, it is also rich in transition metal ions, such as iron, which may catalyse the formation of carbonyl compounds both in malt and wort (140). During germination, the aleurone synthesise and secrete enzymes that play a crucial role in the growth of the embryo and grain modification, including lipoxygenases, xylanase, peroxidases, polyphenol oxidases, dehydrogenases, esterases, phosphatases, phytases, proteases, lipases,  $\beta$ -glucanases, proteinases, peptidases,  $\alpha$ -amylase, limit dextrinase and  $\alpha$ -glucosidase (135,138). These enzymes catalyse degradation of polymers (e.g. proteins, starch) releasing low molecular-weight compounds, which can act as precursors for marker aldehydes (e.g. free amino acids required for Strecker degradation or Maillard reactions). Moreover, some enzymes (e.g. LOX-1 and LOX-2) are directly involved in the aldehyde formation pathways, since they are implicated in the formation of lipid degradation aldehydes. Furthermore, the aleurone layer is rich in ferulic acid; a well-known antioxidant (141).

The barley endosperm accounts for 75 to 80% of the grain dry matter. The endosperm cell wall consists of  $\beta$ -glucans (approx. 75%) and arabinoxylans (approx. 20%) (135,141). The remainder mostly consists of small and large starch granules embedded in a protein matrix (123,142). Therefore, the endosperm is a reservoir for storage compounds and is important from the perspective of the brewer, since the extract yield depends on the starch content. However, the relationship between endosperm components and beer flavour instability is still not well established.

The embryo is a living tissue, which accounts for 3 to 5% of the grain dry matter (123). It contains sugars, amino acids and lipids, which are mostly used to support the initial development of the embryo, prior to breakdown of the endosperm reserves (135). The embryo stimulates the development of hormonal and enzymatic pathways and is the starting point for transformation of the grain to a plant, through the growth of the acrospire and rootlets (123). The acrospire developed from the embryo during germination contains relatively high levels of lipoxygenase enzymes, soluble protein, free amino acids and dimethyl sulfide precursors (DMS-P), which are important from the perspective of beer staling especially with regard to hexanal, *trans*-2-nonenal and Strecker aldehydes (143). Brewing trials with the addition of 5 and 15% of acrospire material to the wort showed a significant increase in Strecker degradation aldehydes, hexanal and furfural measured in pitching wort, as well as a decline in the overall beer flavour stability, compared to the control brewing trials without the addition of the acrospire material (143).

#### The influence of barley variety and growing conditions.

Herb et al. (144) stated that the barley variety and the growth environment are equally important contributors to beer flavour. However, not much is known about the direct influence of either of these parameters on beer flavour instability. It is well established that barley variety and growing conditions influence the content of lipids, and thus precursors of hexanal and *trans*-2-nonenal (145). Spring barley contains higher levels of polyunsaturated fatty acids (including linoleic acid) than its winter counterpart (146). Consequently, the use of a winter barley variety might (to a certain extent) improve the quality of the final product (malt and/or beer), however, this still requires confirmation. Furthermore, the crop year, variety and application of fertiliser containing zinc can influence the content of antioxidants (mostly phenolic compounds) (147), which suppress aldehyde formation from radical reactions involving fatty acids, amino acids and higher alcohols (148). Also, winter barley presents higher antioxidant activity than spring



**Figure 7.** The barley grain structure, based on Briggs (123).

barley, due to the thicker husk (149). Various authors have stated that high availability of nitrogen in the soil (mostly due to extensive fertilisation) increases the content of proteins in the barley grain (150–153), which may become a substrate pool for aldehyde formation. Moreover, the nitrogen content in barley is related to various malt quality parameters, for example, Kolbach Index, free amino nitrogen, soluble nitrogen and colour (153). Also, it is known that the weather conditions during barley grain development in the field, play an essential role, as drought leads to elevated levels of proteins. Fortunately, it can be compensated partly by the selection of suitable barley varieties and application of an optimised irrigation system (154,155). Further, barley variety and crop year influence levels of volatile compounds (among them marker aldehydes) present in barley and later in malt (106). Svoboda et al. (108) detected *trans*-2-nonenal in the range from 0.28 to 3.06  $\mu\text{g}/\text{kg}$  of barley, when comparing 21 barley varieties. In addition, Cramer et al. (156) identified 3-methylbutanal, 2-methylbutanal, hexanal, 2-hexenal, 2-heptenal, 2-nonenal as key odorants in barley. The highest concentration was detected for hexanal ranging from 46 to 1,269  $\mu\text{g}/\text{L}$  of malt extract, depending on the barley variety.

### Steeping

The main aims of steeping are to increase grain water activity, as well as to wash away dust and germination inhibitors, and to improve friability by loosening of the grain structure (123). Steeping is performed by periodic submersion of kernels in water, according to the steeping regime (time, temperature, number of wet/dry phases and water aeration). Various enzymes which catalyse reactions yielding aldehyde precursors (such as lipoxygenase and proteases) are activated when the grain reaches a minimum of 32% moisture (123). This can lead to an increase in the activity of transaminase, peptidase and protease, along with the rise in water content of the grain, resulting in a subsequent increase in amino acids (157). Also, with an increase in the activity of saccharolytic enzymes, the levels of sugars such as maltotriose and sucrose decrease (158), while the concentrations of fructose and glucose increase concomitantly (159). Furthermore, during steeping the total polyphenol content decreases by around 6–7% (160), thus the pool of compounds, which may act as inhibitors of catalysts for *de novo* formation of aldehydes declines. The steeping regime also influences enzymatic activity during germination. For example, longer steeping results in higher LOX activity (102) and increased overall antioxidant activity of finished malt (the latter measured as the amount of free radicals, which can be eliminated by antioxidants present in the sample) (147). Moreover, LOX activity is also

enhanced by applying aeration throughout the wet phase of steeping, and when the steeping schedule includes more than one air rest (62).

In order to improve the potential impact of steeping on beer flavour stability, Müller et al. (161) proposed implementation of vibration at a frequency of 180–200 Hz, creating sonic waves. This led to a reduction in iron of approx. 30% in malt, thus improving oxidative beer flavour stability measured by electron spin resonance (ESR). An additional benefit of this treatment was improved grain washing, which enhanced water uptake and increased the homogeneity of the finished malt. Ma et al. (162) reported that oxidation of steep water by hydrogen peroxide or ozone resulted in an increase in free amino nitrogen,  $\beta$ -glucan and diastatic power. This could potentially improve the efficiency of malting as such; however, it would also deliver more aldehyde precursors to the brewing process. Mauch et al. (163,164) suggested that re-steeping of green malt under acid conditions, which should limit malting losses, could also increase the amount of beer staling markers present in fresh and aged beers. Such a rise was expected, especially in the case of furfural derived from Maillard reactions, as under acidic conditions the formation of its precursor (3-deoxyosone) is predominant over 1- and 4-deoxyosone formation (9). In contrast, in aged beers brewed with acidified malt, the authors measured lower levels of oxygen indicators (2-methylbutanal, 3-methylbutanal), probably due to a pH effect, as under acidic conditions, amino acids are protonated, and therefore less reactive towards Strecker degradation.

### Germination

During germination, grain modification takes place. This is characterised by the breakdown of cell walls, proteins, lipids and starch as a consequence of enzyme formation and activation. The process is performed in humid and aerobic conditions at temperatures ranging from 16 to 20°C for 4 to 5 days (123). Germination rate and chemical changes are temperature dependent; when higher temperatures are applied, more intense grain modification is achieved, resulting in an elevated pool of staling related compounds (145,165). Analysis of ‘non-conventional’ malting, with germination at ‘low’ (12°C) or ‘high’ (18°C) temperatures and various moisture contents, showed the influence of these parameters on aldehyde formation (107). Essentially, higher germination temperatures enhanced reduction of aldehydes to alcohols and resulted in malt with lower concentrations of 3-methylbutanal, furfural and hexanal. Furthermore, a lower moisture content in

green malt led to reduced concentrations of these compounds, especially 3-methylbutanal (107).

The lipoxygenase enzymes, LOX-1 and LOX-2 play an important role in the formation of marker aldehyde precursors (9,166). LOX activity increases approximately four-fold during germination and its rate depends mostly on the germination temperature and presence of oxygen (61). Lower germination temperatures can lead to reduced LOX activity in the finished malt (62). Also, grain asphyxiation (reduction of oxygen content in the air passing through the grain bed) may have a similar effect (61). In order to reduce LOX activity in malt, Baxter (61) proposed acidification of steeping water or asphyxiation of grain during germination, since both of the treatments resulted in a 3-fold decrease of LOX activity. This is in line with outcomes reported by Gastl et al. (21), suggesting the use of recirculated air enriched in CO<sub>2</sub> during germination to suppress oxidation of unsaturated fatty acids. This system enabled production of malt with a lower concentration of hexanal. In the case when LOX activity is high and oxygen is present in the system, enzymatic oxidation and auto-oxidation take place resulting in a decrease during germination and following it kilning, in the levels of triglycerides and a subsequent increase in free fatty acids, intermediates of *trans*-2-nonenal and hexanal, respectively (146,167,168). This is most likely due to both LOX activity and lipid metabolism of the grain. The highest concentrations of free fatty acids were found at the end of germination (169). Similarly, because of intensive proteolysis during germination, the quantity of amino acids increases (170). Frank et al. (159) observed a significant increase in amino acids, including valine (precursor of 2-methylpropanal). Similarly, the content of simple sugars (158) and polyphenols (171) becomes elevated.

The volatile fraction of malt also can be influenced through microbial contamination of green malt by *Fusarium poae* (172) and *Fusarium graminearum* (173). Chen et al. (173) reported a significant increase in the concentrations of 2-methylbutanal, pentanal, hexanal and *trans*-2-nonenal in contaminated malts. The authors suggested that these compounds were from the mycelium since the same aldehydes were detected in the sporulated mycelium itself. Therefore, contamination by *Fusarium* may directly deteriorate beer flavour and potentially its flavour stability. Introduction of lactic acid bacteria during germination may improve the biological stability of malt, since it can prevent or reduce contamination by *Fusarium* species (174). It also affects aspects of brewing performance, such as filtration time, which can result in a decrease in total heat load, and ultimately a lower aldehyde content in beer (175).

From a technological perspective, the addition of gibberellic acid is a common practice, applied to enhance enzyme formation. This results in shorter germination times and more extensive grain modification (lower  $\beta$ -glucan content, higher Kolbach Index and higher friability of the finished malt) (123). However, careful dosage is essential, as overdosing will result in extensive rootlet formation, extract yield losses, as well as high sugar and soluble nitrogen levels. This can further lead to the development of abnormal colour of the final malt and might indirectly contribute to beer flavour instability as more aldehydes precursors are generated (176,177).

## Kilning

During kilning, the grain is dried gradually by a flow of warm air (from 55–90°C for pale malts) in order to stop biochemical reactions, ensure product stability during storage, as well as to develop desired colour and flavour characteristics. The kilning

regime (processing time, temperature, humidity and airflow) affects the physical and (bio)chemical properties of malt, e.g. moisture content, growth of the embryo, enzymatic activity and aroma composition (73,178). In particular, aroma compounds (such as those conferring biscuit, toast, nutty, caramel flavours) and colour are highly impacted, leading to a broad range of commercially available malts such as Vienna or Crystal (179). Kilning is also a critical step of the malting process regarding beer flavour instability, mostly due to the applied heat load and the decrease in moisture content, which accelerate the formation of marker compounds (21). With regard to Strecker aldehydes, in the last stage of Strecker degradation, the presence of water is required for conversion of an iminium ion into an unstable amino alcohol (9). Therefore, Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal) are formed in humid conditions regardless of the temperature (tested range 105 to 180°C), whereas in dry conditions formation only occurs at very high temperatures (above 130°C) (74). As opposed to Strecker degradation aldehydes, the formation of furfural also takes place under dry conditions (74), because it requires dehydration of its direct precursor (3,4-dideoxypentosulose-3-ene). During kilning, minor changes to the lipid content (and therefore formation of fatty acid oxidation products) are observed with a decrease in humidity. In addition, grain modification terminates and the activity of lipases is significantly reduced (146,166). In general, with an increase in drying temperature and a decrease in water activity inside the grain, the overall enzymatic activity gradually declines. For example, lipoxygenase activity in the finished pale malt is reduced to about 5% of the initial value (166). Enzymatic inactivation takes place gradually as the conditions in the bottom, middle and top layer of the kilning bed differ. This is caused by the introduction of warm air from the bottom and by the stationary position of the grain (*i.e.* no turning of the kernels) during the process. Hence the bottom layer dries faster and enzyme inactivation occurs quicker than in the top layer (56). Therefore, the moment of inactivation mostly depends on the position of a kernel in the bed and the particular properties of the enzyme under consideration (56,61,145,166,168,180,181). As an example, Baxter (61) stated that lipoxygenase activity in green malt is relatively stable; however, a temperature increase up to 65°C reduces its activity by 70–90% depending on the time of grain exposure to the high temperature. Kilning-off at 85–90°C reduces this activity to 2%. Generally, higher processing temperature and longer kilning times result in a significant reduction of LOX activity in malt, which is positive from the perspective of beer flavour deterioration (168,182). Nevertheless, increased heat load also affects other malt quality parameters such as the aroma profile and, therefore could have an undesirable effect on beer flavour (119).

Kilning also influences antioxidants, which reduce the rate of oxidation reactions. The total polyphenol content increases during kilning (especially in the first phase of kilning) regardless of the barley variety (159,182). A marked increase was identified for (+)-catechin and ferulic acid (160). Inns et al. (183) showed that the content of ferulic acid increased until a temperature of 80°C was reached, then ferulic acid esterase was deactivated and, as a consequence, the enzymatic release of free phenols from their bound forms was suppressed. In general, high temperatures (as applied during kilning), enhance radical formation, which can also directly or indirectly affect beer flavour instability (113).

Some older malthouses with directly fired kilns apply sulphuring during kilning in order to control the formation of carcinogenic nitrosamines (184). This treatment however may increase the content of soluble nitrogen, leading to an increased pool of aldehyde precursors.

Huang et al. (128) sought to optimise a kilning regime as a function of selected indicators of beer quality and its flavour instability (LOX activity, *trans*-2-nonenal, hexanal, methional, phenylacetaldehyde and furfural content, heat load and wort sensory score). The authors selected kilning temperature, kilning time and a withering time of 86.35°C, 3.19 h and 14.00 h, respectively. According to the authors, malting barley following the proposed regime can result in a high quality pale malt with a low beer staling potential. A totally different approach is the application of alternative water removal methods such as freeze drying, already widely used in the food industry for drying coffee, spices, meats, food ingredients and other high value solid phase food products (185). Malt dried in this way, in comparison to its conventionally kilned counterpart, yields a higher amyolytic activity, higher extract yield, lower colour, as well as higher viscosity and turbidity of filtered wort (186). However, to date, the effect of this treatment on marker aldehydes has not been reported. A further option is drying with electromagnetic waves, although a recent publication in this field focused on the effects related to enzyme survival and energy efficiency rather than on beer staling compounds (187). Yet another proposed green malt drying technique is by vacuum oven drying (188). This is designed for a continuous operation whereby the green malt is transported through separate drying zones, which allows a more homogeneous malt to be produced. In order to facilitate moisture removal, the machine is equipped with a vacuum chamber to reduce vapour pressure. It would be interesting to investigate marker aldehyde formation during this process since two critical factors (temperature and the lack of homogeneity in conventional kilning caused by the thick bed) are obviously of reduced significance.

### Storage

To the best of our knowledge, studies regarding the evolution of beer staling aldehydes during storage in industrial conditions have not been performed, however, some laboratory scale experiments have (94,181). Hoff et al. (94) investigated the influence of storage time (up to 12 months), temperature (10 and 20°C) and humidity (water activity of 0.231 and 0.432) on oxidative stability (measured as a radical content in malt) and volatile profile (determined in sweet wort) of pilsner malt. The authors observed that the radical content measured in pilsner malt was positively correlated with water activity and that this parameter increased when the sample was stored at higher temperatures. Regarding the volatile profile, during the first six months of storage, pilsner malt was unaffected by storage temperature and water activity. However, longer storage (12 months) at 20°C led to the loss of some Strecker aldehydes, in particular 2-methylbutanal and 3-methylbutanal. Contrary to this, the content of phenylacetaldehyde, the other Strecker aldehyde, increased over the storage time, regardless of the temperature that the sample was exposed to. In another study conducted by Kaukovirta-Norja et al. (181), the authors investigated the influence of storage time (seven months at 5°C) of pale malt on LOX-activity. The authors observed a 25% decline in LOX-activity after a period of seven months. The reduction occurred linearly as a function of time.

## Conclusions

Despite extensive research on beer flavour instability, off-flavours appearing over time in a closed beer package remain a challenge for the brewing and malting industries. Malt delivers various compounds to the brewing process, many of which could potentially affect beer ageing. Among them are the so-called beer staling aldehydes, precursors for *de novo* formation of aldehydes and bound-state aldehydes. Marker aldehydes for beer flavour instability can arise in malt, wort and beer due to Maillard reactions, Strecker degradation, oxidation of amino acids, and (enzymatic) oxidation of unsaturated fatty acids. However, they may also be released from a bound-state, although it is acknowledged that the chemistry and the behaviour of bound-state aldehydes in a complex matrix such as malt require more detailed investigation. It is well established that malt quality influences not only the brewing performance and the flavour of the final beer but is also crucial from the perspective of beer staling. For example, malt characterised by low Kolbach Index, heat load, colour, LOX activity, Strecker aldehydes, transition metal ions content and high antioxidant activity, can lead to more flavour stable beer. In particular, the malting regime plays a crucial role, as it determines the overall malt quality but also affects the formation of aldehydes, as well as their intermediates and precursors. Selection of barley variety, together with the adequate adjustment of steeping and germination conditions, allows control over grain modification and thus the reservoir of aldehyde precursors. These compounds may undergo aldehyde formation pathways during malting, but also during brewing and in the final beer package. Kilning is the most critical stage of the malting process from the perspective of marker aldehydes, as high temperature enhances reaction rates, and oxygen triggers the formation of radicals, which leads to intensification of autoxidation. This results in the rapid formation of beer staling compounds already present in malt. However, the influence of post-kilning maltcooling is not well studied. Various proposals of the potential technological improvements have been suggested aimed at malt with a low beer staling potential. For example, the application of vibrations during steeping, microbiological management during germination or alternative drying techniques during kilning. It is anticipated that further investigations into malt properties and the handling of barley during malting will lead to a better understanding of the origins of the beer staling process, and ultimately may lead to enhanced beer flavour stability.

## Author Contributions

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## Conflicts of Interest

The authors declare there are no conflicts of interest.

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