S. Coghe, G. Derdelinckx and F. R. Delvaux

Effect of non-enzymatic browning on flavour, colour and antioxidative activity of dark specialty malts – A review

Non-enzymatic browning reactions such as Maillard reactions, caramelisation and pyrolysis, have a considerable impact on the properties of dark specialty malts. The mechanisms and chemical reactions leading to non-enzymatic browning are briefly described. While pyrolysis and caramelisation are restricted to roasting processes, Maillard reactions can occur during the production of all types of malt due to milder reaction conditions. As the Maillard reaction is the most important mechanism of non-enzymatic browning in malt, factors affecting the rate of this reaction including temperature, time, water activity, concentration and type of reactants, pH and occurrence of sulphur compounds are considered in detail. This review specifically focuses on flavour, colour and antioxidative activity. These major dark malt characteristics result from browning products, which pass into dark beer thereby influencing beer colour, flavour and flavour stability. Current knowledge on key compounds responsible for flavour (Strecker aldehydes and heterocyclic compounds), colour (LMW chromophores, melanoidins) and antioxidative activity (reductones and melanoidins) and elements in the chemical structure, which may contribute to these properties, are reviewed. Furthermore, testing procedures for colour determination, instrumental or sensory evaluation of flavour and assessment of antioxidative activity as well as the relationships between the different characteristics are described.

(Descriptors: Maillard reaction, caramelisation, pyrolysis, melanoidins, reducing power)

1 Introduction

The standard malting process used for the production of pilsner malt is a three-step process involving steeping, germination and kilning (1). By altering the drying phase in a kiln or in a roasting cylinder, a wide assortment of dark specialty malts can be produced ranging in colour from pale yellow over amber and brown to nearly black (i.e. from 5 to 1600 EBC units). Depending on the production process, dark malts can be categorized into four groups: colour malts, caramel malts, roasted malts and roasted barleys (2). While colour malts are produced in a standard kiln, the three other dark specialty malt groups, caramel malts, roasted malts and roasted barleys are dried at higher temperatures in a roasting drum using green malt, pilsner malt or roasted barley as substrate.

The brewing and distilling industries basically use dark malts as a source of colour, flavour and fermentable carbohydrate. In contrast to caramels, dark malts and their extracts can be regarded as natural (non E numbered) colourants and flavourants. Therefore, they gain more and more interest in the manufacturing of confectionery products, breakfast cereals, pre-digested foods and non-alcoholic beverages (3).

Non-enzymatic browning reactions have a considerable impact on the characteristics and quality of thermally processed malt. One of the obvious negative consequences is the loss of nutritive value. This loss is attributed to decrease of digestibility, destruction and/or biological inactivation of amino acids, inhibition of proteolytic and glycolitic enzymes and interaction with metal ions (4;5;6) but also by Maillard linking of proteins (7). Furthermore, while some in vitro studies have revealed harmful effects of Maillard reaction products including mutagenic, carcinogenic and cytotoxic effects (8;9), other studies revealed desmutagenic activity by certain Maillard compounds (10;11). With regard to fermentation, it was observed that high levels of Maillard products in wort lowered the attenuation (12). Nevertheless, non-enzymatic browning has the largest effect on the colour, flavour and antioxidative activity of malt (13). Each of these characteristics as well as the relation between the typical malt features will be discussed in detail. In the first part of this review, the different types of non-enzymatic browning as well as the factors with a clear effect on the intensity of the Maillard reaction will be considered.

Authors: Dipl.-Ing. Stefan Coghe, Prof. Dr. Dipl.-Ing. Guy Derdelinckx, Prof. Dr. Dipl.-Ing. Freddy Delvaux, Centre for Malting and Brewing Science, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Leuven, Belgium

2 Non-enzymatic browning reactions

Dark specialty malts received variable thermal treatments during their production process. When the desired malt colour is achieved, malts are rapidly cooled and browning reactions are stopped. Therefore, different intermediate products occur in malt. As the degree of browning can vary considerably, dark specialty malts are excellent reference materials for studying non-enzymatic browning reactions in foods. The different mechanisms of non-enzymatic browning that can occur during the thermal processing of malt include Maillard reactions, caramelisation and pyrolysis.

2.1 Pyrolysis

Pyrolysis is scorching or burning of sugar molecules at very high temperatures (14). In this reaction, the thermal energy is sufficient to break carbon-carbon bonds and results in products with a strong, penetrating burnt flavour. As pyrolysis requires high temperatures (> 200 °C), it is only of importance during the

production of roasted malt and roasted barley. Roaster manufacturers use the term "carbonisation" for processes occurring at these temperatures (15).

2.2 Caramelisation

Caramelisation is less drastic than pyrolysis and produces, for the most part, favourable flavour compounds and brown pigments (16). This type of sugar conversion occurs at lower temperatures than pyrolysis (>120°C) and is catalysed by both, acid and alkaline conditions, with optimal activity at pH <3 or pH >9 (17). In acidic medium, dehydration of pentoses results in the formation of furfural, whereas hexoses yield 5-hydroxymethyl furfural. However, 2-acetyl-3-hydroxyfuran (isomaltol), 3-hydroxy-2-methyl-pyran-4-one (maltol), and hydroxy-acetylfuran can also be produced. The chemical structure of some of these compounds is represented in Figure 2. Treatment with alkali causes enolisation and even fragmentation of the sugar molecule, followed by additional secondary reactions. In food products, caramelisation is favoured in concentrated sugar solutions, such as syrups or the endosperm of caramel malts (14). Due to the severe heating intensity and unfavourable pH conditions, this type of non-enzymatic browning is probably limited to the production of dark caramel malts, roasted barley and roasted malt (13).

2.3 Maillard reaction

The Maillard reaction is a general term used to describe the complex series of reactions subsequent to the reaction between reducing sugars and amino compounds. It is thought that this reaction is the most important mechanisms of non-enzymatic browning during thermal processing of malt. In contrast to caramelisation, not only sugars but also amino compounds are involved. Thermal energy leads to dehydrations and the formation of oxygen containing ring structures. Thus, much of the chemistry is similar to acid/base catalysed caramelisation of monosaccharides. However, Maillard reactions proceed at a high rate under substantially milder conditions, as *N*-containing intermediates possess a catalyst within the molecule. The addition of nitrogen opens new pathways such as Schiff base chemistry and Strecker degradation. The participation of nitrogen in Maillard reactions also allows for the production of nitrogen-containing compounds. As these reactions already commence from 50°C in the pH range of 4 – 7, they occur during the production of all types of malt (14).

In pilsner malt production, browning reactions are rather unwanted. Maillard reaction products have a negative effect on the quality and do not match with the hoppy character of pilsner beer. On the other hand, browning reactions are desired in dark malt production, as these reactions are responsible for the typical malt characteristics flavour, colour and antioxidative activity (13). For both malt groups, the intensity of the Maillard reaction can be controlled using different strategies (18). As the Maillard reaction is initiated by the interaction between amino compounds and reducing sugars, the release of these substrates during steeping, germination and the initial stages of kilning and roasting is a determinant factor for the development of typical malt characteristics during the subsequent heating phase.

On the sugar side, the reactants are essentially the monosaccharides glucose and fructose and the disaccharide maltose as well as, to a smaller extent, reducing pentoses. Sugar linked glycosidically in glycoproteins, glycolipids, flavonoid compounds or disaccharides participate in the Maillard reaction only after cleavage of the glycosidic bond (19). On the side of the amino component, both primary amines as well as secondary amino acids (proline) are of importance for malt. In the case of proteins, the μ-amino groups of lysine react predominantly. However, α-amino groups of terminal amino acids can also play a role.

The chemistry underlying the Maillard reaction is very complex and encompasses not one reaction pathway but a whole network of various reactions, which were reviewed by several authors (5;16;19;20;21;22). Since Maillard published his findings on non-enzymatic browning, a number of theories and mechanisms appeared in the literature and the fist attempt to integrate them was made by an American chemist named John Hodge (23). The comprehensive reaction scheme of Hodge is frequently quoted and still widely accepted today. It has been developed and elaborated by food technologists ever since, so the understanding of the reaction is advancing steadily.

A. Initial reactions

The Maillard reaction is initiated by a condensation reaction between the carbonyl group of a reducing sugar (aldose form) and the free amino group of a protein, peptide, amino acid or amine (Fig. 1). The subsequent elimination of water results in the formation of an *N*-substituted aldosylamine, which is quickly transformed into a 1-amino-1-deoxyketose via the Amadori rearrangement.

Depending on the pH of the system, Amadori compounds can undergo different types of enolisation reactions with the subsequent formation of different deoxyosones. These deoxyosones contain two vicinal carbonyl groups and are therefore also termed α-dicarbonyl compounds. At pH ≤7, Amadori compounds mainly undergo 1,2-enolisations to 3-deoxyosones, whereas higher pH values (pH ≥7) promote the degradation of Amadori compounds through 2,3 enolisation, leading to the formation of 1-deoxyosones. On cyclisation and water elimination, the 3-deoxyosones are transformed into furfural derivatives (Fig. 2). Hexoses give rise to hydroxymethylfurfural (HMF), whereas furfural is ob-

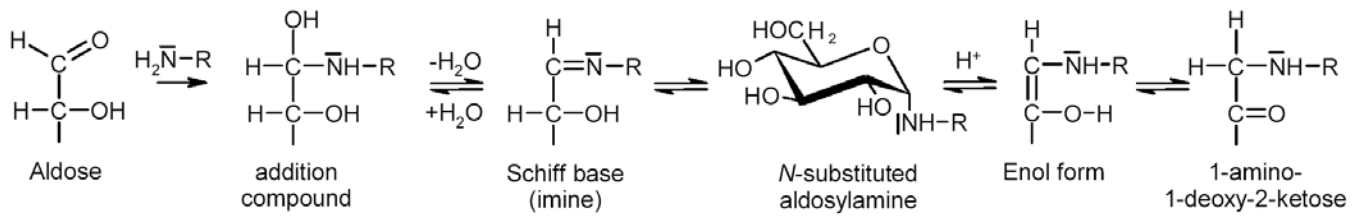

Fig. 1 The initial phase of the Maillard reaction leads to the formation of *N*-substituted aldosylamines and 1-amino-1-deoxy-2ketoses (Amadori compounds)

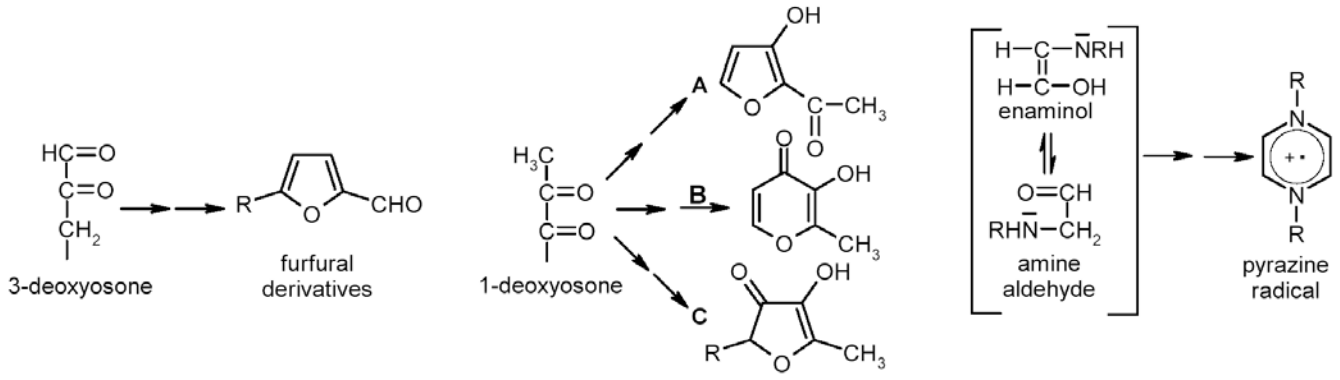

Fig. 2 3-deoxyosones and 1-deoxyosones are reactive Maillard reaction intermediates that after cyclisation yield furfural derivates and reductone ethers such as isomaltol (A), maltol (B) and furanones (C) (R= H, CH_3 or CH_2OH). Sugar cleavage products can give rise to the formation of pyrazine radicals

tained from pentoses. Typical dehydration products of 1-deoxyosones are reductone ethers such as maltol (2-methyl-3-hydroxy-γ-pyrone), isomaltol (3-hydroxy-2-acetylfuran) and furanones (R=H, R= CH_3, CH_2OH). These compounds are important aroma substances that also exert antioxidative activity.

Apart from cyclisation, deoxyosones can also undergo cleavage reactions. Retro-aldol type reactions are the most important reactions leading to the cleavage of deoxyosones but also of intact sugars (24), especially at higher pH values (25). Some of the obtained cleavage products, such as short chain (C_2-C_4) α-dicarbonyl and α-hydroxycarbonyl compounds, are considerably more reactive towards browning than other Maillard reaction intermediates such as Amadori compounds or deoxyosonones (24;26; 27;28) and were reported to substantially accelerate the Maillard reaction (19). Fragmentation products also play a considerable role in aroma formation. Electron spin resonance spectra have provided evidence for the formation of a flavour-active pyrazine radical from C_2 cleavage products (27) (Fig. 2).

Deoxyosones and smaller α-dicarbonyl compounds can react with amino acids according to the Strecker degradation (Fig. 3). The reaction is initiated by the formation of an addition compound, followed by a decarboxylation of the carboxyl group of the amino acid. Amino acids are degraded to the corresponding aldehydes with one carbon atom less, whereas the dicarbonyl compounds are transformed into aminoketones. The Strecker degradation occurs at higher concentrations of free amino acids and under more drastic reaction conditions, such as higher temperatures or pressures. Furthermore, the formation of aldehydes showed a linear pH dependence (29). This reaction can be of high importance for the flavour of thermally processed food products. The aldehydes formed, often termed Strecker aldehydes, are often flavour-active. Furthermore, two aminoketone molecules can condensate and yield pyrazine derivatives, which are the most important and widespread group of flavour substances in food chemistry (30).

B. Final stage of the Maillard reaction

As stated earlier, intermediate Maillard reaction compounds are highly reactive and take part in further reactions. Carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. Subsequently, in an advanced stage, a range of further reactions can take place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations, which ultimately, in a final stage, lead to the formation of unsaturated, brown nitrogenous polymers and co-polymers, known as melanoidins (22). As the formation of intermediates greatly differs by reactant and reaction conditions, the browned products undoubtedly differ in chemical structure, degree of polymerisation and other properties (20). However, few of the properties of these polymers have been characterised.

3 Conditions influencing Maillard reactions

The Maillard reaction is notoriously difficult to control. The most important factors affecting the rate and extent of the Maillard reaction are temperature, time, water activity (a_w), concentration and nature of reactants, pH and the presence of sulphur compounds (5;31;32). By manipulating these variables, the balance of the various chemical pathways making up the Maillard reaction changes (33). This results in a modification of the profile of the reaction products and affects the important malt attributes flavour, colour and antioxidative activity.

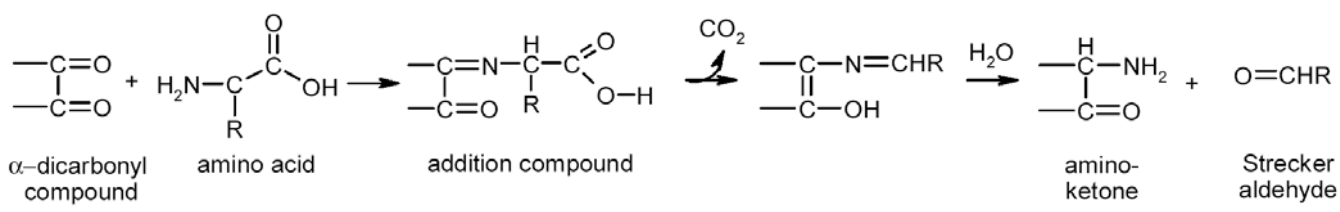

Fig. 3 Strecker reaction between α-dicarbonyl compounds and amino acids leads to the formation of aminoketones and aldehydes

3.1 Time, temperature

The time-temperature profile used has a considerable effect on the rate of the Maillard reaction and the specific compounds formed. However, the effect of time and temperature is very complex and has been the subject of many kinetic studies (22). Increasing the temperature shifts the reactions more toward advanced Maillard reaction products because of the higher activation energies required for these reactions (20). The rate can be more than 10 % higher for 1 °C in the range from 60 °C to 100 °C.

3.2 Moisture content

Water has an important effect on the Maillard reaction. It exerts its influence by controlling the liquid phase viscosity and by dissolution, concentration or dilution of the reactants (34;35). Due to an increased mobility of reactants, the reaction rate increases exponentially with increasing moisture content up to a maximum in the intermediate moisture range (36). At higher moisture levels, the rate of Maillard reaction decreases because of a dilution effect in the aqueous phase. Furthermore, since water is produced in the reaction (dehydration of sugars), a high moisture level leads to a decreased rate of reaction at high moisture levels (37). Therefore, it is generally accepted that maximal browning occurs between a_w 0.65 and 0.70 (20).

3.3 pH

The pH has a major influence on the importance of many pathways followed during the Maillard reaction. Therefore, the initial pH of the product and the buffering capacity of the system influence the rate and direction of the reaction and the profile of reaction products (38;39). The rate of browning is low at acid pH values and increases with increasing pH (32;40), to a maximum at pH 10 (41). At low pH values, amino compounds are protonated and lack an unshared electron pair. These are therefore less reactive with carbonyl compounds in the initial stage of the Maillard reaction (Fig. 1). The decrease in reaction rate at high pH values may be due to a deficiency of H^+ ions, which are required to catalyse the Amadori rearrangements. As indicated earlier, the enolisation of Amadori compounds, the cleavage of intact sugars or deoxyosones and the formation of Strecker aldehydes are also influenced by the pH value.

3.4 Reactants

The concentration and type of reagents are of importance for the rate of the Maillard reaction. Low molecular weight compounds tend to be more reactive than compounds of higher molecular weight as a result of greater steric hindrance in the latter (5). With regard to sugars, aldopentoses are usually more reactive than aldohexoses, whereas monosaccharides are more reactive than di- or oligosaccharides (42). Furthermore, aldoses appear to be more reactive than ketoses, probably due to the more sterically hindered carbonyl group of ketoses (5).

The nature of the amino compounds also affects the rate of Maillard browning. Under some conditions, it has been reported that the three basic amino acids, as well as the hydroxy amino acids serine and threonine, had the highest reactivity with α-dicarbonyl compounds, while the nonpolar and acidic amino acids had the lowest reactivity (43). Using other conditions, amino acids could be categorized into three groups, depending on the amount of colour formed during the Maillard reaction (41). The first group, which gave the most intense Maillard browning, included lysine, glycine, tryptophan and tyrosine, while alanine, valine, leucine, isoleucine, phenylalanine, proline, methionine, asparagine and glutamine were intermediate browning-producing amino acids. The seven remaining amino acids, including two basic amino acids (histidine and arginine), the two acidic amino acids (aspartic acid and glutamic acid), the two hydroxy amino acids (serine and threonine) and the thiol-containing amino acid cysteine, all belonged to the low browning group. A similar reactivity order was observed by Ajandouz and Puigserver (40). It was deducted that the basic side chain not necessarily gives rise to much browning as lysine and arginine were found to belong to the amino acid groups with the highest and lowest non-enzymatic browning, respectively. Nevertheless, it seems that acidic, SH-containing and hydroxy amino acids contribute very little to the Maillard reaction (41;44;45).

Not only the nature of sugars and amino acids, but also the molar ratio of both reactants has a significant impact on the rate of the Maillard reaction. In general, browning appears to be maximal when sugar is in excess (46). However, for some model systems and at certain pH values, colour formation was the highest with excess of the amino acid (32).

3.5 Sulphur compounds

Sulphur dioxide, sulphites and thiols are known to be inhibitors of the Maillard reaction (47;48). In malting, it has indeed been observed that sulphur dioxide in the gases reduced the colour of malt (1). Although the chemical basis for this effect is not completely understood, sulphur dioxide and sulphites appear to react with reducing sugars and carbonyl products of the Maillard reaction to produce sulfonic acid derivatives, which have a diminished tendency to brown.

4 Flavour

The Maillard reaction is the main non-enzymatic mechanism for the formation of flavours during thermal processing of food such as roasting of meat, baking of bread, roasting of coffee and curing or roasting of malt. The universality of flavour development in food arises from the occurrence of identical carbohydrate and amino compound building blocks. Therefore, dark malt can have flavour notes common to other food products such as caramel, toffee, chocolate or coffee. On the other hand, different forms and concentrations of these building blocks also result in the formation of unique flavours (14). As a result, the flavour of malted barley is quite distinct from malted wheat, even though the starting material and processing conditions are quite similar. Flavourants contributing most to the flavour of thermally processed malt are intermediate Maillard reaction products of low molecular weight (49). The end products of the Maillard reaction, the brown polymeric melanoidins, are not flavour-active (50). Strecker aldehydes and heterocyclic compounds are the most important flavour-active Maillard compounds (51). Nevertheless, some volatile phenolic compounds can also contribute to the flavour of thermally treated malt (52).

It has been observed that Strecker aldehydes of certain amino acids, in particular of leucine, phenylalanine and methionine are important contributors to the flavour of several thermally processed foods (53). The degradation of valine, isoleucine and leucine leads to the formation of Strecker aldehydes with a typical malt flavour (54). Among the Maillard-type flavours, heterocyclic compounds undoubtedly make the largest contribution to the overall flavour of dark malts. Heterocyclic compounds are satu-

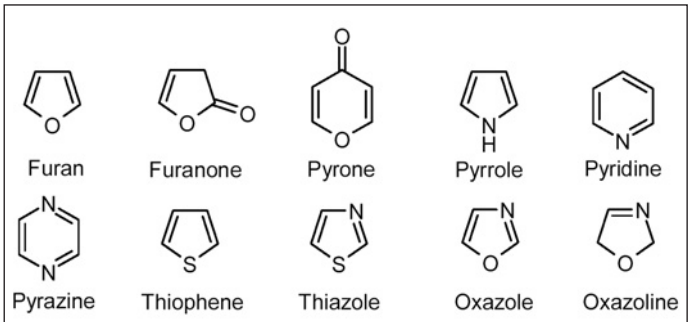

Fig. 4 Structural formulas of heterocyclic flavour compounds

rated or unsaturated ring structures from carbohydrates, containing nitrogen, oxygen or sulphur atoms in the carbon ring (Fig. 4). The role of several types of heterocyclic compounds in food flavour has been reviewed by Maga (55 – 61). Flavour-active oxygen heterocyclic compounds include furans, furanones and γ-pyrones (57;62). The participation of nitrogen in the Maillard reaction enables the production of flavour-active nitrogen-containing heterocyclic compounds such as pyrroles, pyridines and pyrazines (58;59;61). Sulphur heterocyclic compounds (thiophenes) are formed from sulphur containing amino acids (55). Some heterocycles containing both sulphur and nitrogen (thiazoles) or oxygen and nitrogen (oxazoles and oxazolines) are also reported to be flavour-active (56;60). Apart from the compounds specified thus far, other volatile Maillard compounds such as esters, acids, ketones, lactones, alcohols, alkenes, alkanes, amines and mercaptans can, positively or negatively, contribute to food flavour (19).

Using Gas chromatography-mass spectrometry, dark specialty malts were found to contain a large number heterocyclic Maillard reaction products (18;62;63;64). In colour malts and in lightly coloured caramel malts, oxygen heterocyclic components predominate while nitrogen containing heterocycles contribute most to the flavour of dark caramel malts and roasted malts (30;65;66;67).

4.1 Flavour analysis

Flavour can be assessed by instrumental (68) as well as by sensory analysis (69). Chromatography-based instrumental analysis generally starts with an extraction or isolation step. It is necessary to extract and/or isolate the flavour complex as completely as possible with minimal chemical changes. This may be achieved by several methods, including solvent extraction, adsorption, steam distillation, vacuum distillation and headspace vapour collection. The acquired samples may be further concentrated by means of adsorption-desorption, solvent recovery, freeze concentration or pervaporation. In a next step, flavour compounds are usually separated by gas, liquid or gel permeation chromatography. Finally, separated compounds may be identified by the determination of the retention index or by infrared spectroscopy, mass spectrometry or nuclear magnetic resonance. In flavour analysis, the organoleptic contribution of the separated compounds is frequently described using a sniff port (olfactometry). Flavour is also evaluated with non-chromatography-based instruments, for instance with recently developed electronic noses (70).

In a broad context, sensory evaluation can be defined as a scientific discipline used to measure, analyze and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing. Sensory evaluation is increasingly becoming an integral part of flavour analysis. Although sensory assessment is rather subjective in nature, it is widely accepted because current detectors do not exhibit the same sensitivity and selectivity as the human olfactory system (71). Testing procedures include descriptive analyses, difference tests, preference tests (hedonic measurements) and intensity measurements (69). The results of many of the descriptive and affective sensory methods can be studied by tabling the data, including the score of each judge for each sample, the means, the ranges and the deviations from the mean. Some of the variability in results is attributable to the samples themselves and may be a combination of differences in the raw materials and in the method of preparation. Sources of error in the judging include variability in the performance of one judge, variability on duplicate samples as well as variability among several judges on the same sample.

The data obtained by instrumental and sensory analysis can be further evaluated by statistical analysis (69). The original experiment should have been planned with statistical analysis in mind. It is difficult and sometimes impossible to apply statistics to a completed experiment not appropriately planned. Various methods of testing the significance of differences among means may be used. For this purpose, analysis of variance can be a very useful tool. Regression analysis can be performed to study the relationship between two or more variables. A technique frequently applied to interpret data obtained by analytical or sensory analysis is principal component analysis (PCA). PCA is a method for reducing a multivariate data set into a low-dimensional space, in order to increase the interpretability. A new set of noncorrelated variables or principal components (PCs) is constructed form a linear combination of the original variables, which may or may not be intercorrelated. In general, only two PCs, explaining most of the variability, are plotted. The obtained PC maps can give an indication of the degree of correlation between flavour compounds or attributes. Compounds or attributes that are close together in the PC space are positively correlated with each other, whereas negative or inverse correlations can be identified in the opposite quadrant.

4.2 Identification of key flavourants

During the first period of flavour research, analytical investigations were performed under the assumption that all volatiles occurring in food contribute to its flavour (72). In this view, the analytical procedure was restricted to the identification of the volatiles appearing as peaks in a GC/MS chromatogram. Later, it became clear that not all volatiles contribute to the overall flavour of a food product or model system. More important than the absolute level of a specific flavour compound is the ratio between the absolute level and the level at which perception is possible. In this context, the term "flavour threshold" can be defined as the lowest concentration of a pure compound perceivable by 50 % of the members of a taste panel (69). The definition can be extended for the concentration of a flavour compound allowing either its detection (detection flavour threshold) or identification (recognition flavour threshold). Flavour units (flavour activity values) are obtained by dividing the concentration of a substance by its flavour threshold. Compounds present in levels of 1 – 2 flavour units are generally weakly detectable, whereas at higher levels, a character should be easily noted (73).

Components possessing a high flavour activity value are usually essential for the overall flavour. Exceptionally, it was observed that the flavour of some compounds was suppressed despite the high flavour activity values. Conversely, in some cases, compounds with rather low flavour activity values were found to be important contributors to the flavour profile (72). The former

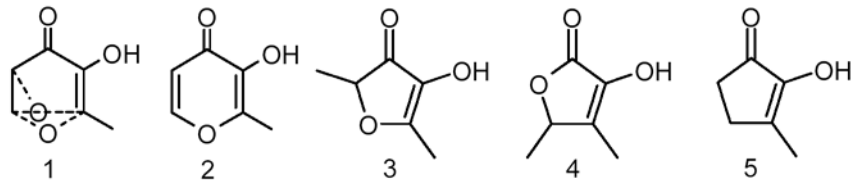

Fig. 5 Structural representation (1) and flavour compounds evoking caramel-like, sweet or nutty flavour impressions: maltol (2), furaneol (3), sotolon (4) and cyclotene (5). After Hodge et al., 1972 (77)

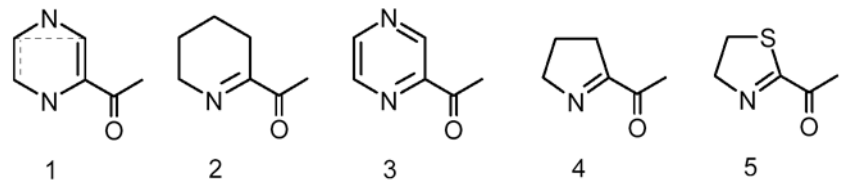

Fig. 6 Structural representation (1) and flavour compounds evoking bread-like or roasted flavour impressions: 2-acetyltetrahydropyridine (2), 2-acetylpyridine (3), 2-acetyl-1-pyrroline (4) and 2-acetylthiazonline (5). After Hodge et al., 1972 (77)

observation might be ascribed to flavour masking, changes in volatility or flavour binding, the latter to a synergetic effect. It's quite obvious that compounds present in concentrations largely exceeding their threshold value can mask the flavour impression evoked by another compound of which the concentration is just above its threshold. The volatility of a flavour compound is influenced by the overall composition of the food matrix (74). Higher amounts of fats and oils, for example, lower the volatility of hydrophobic flavourants. The reaction of flavour compounds with other matrix compounds can lower its concentration and hence the flavour may be suppressed. The synergetic effect can be explained by the possibility that different compounds present in concentrations below their respective flavour thresholds may cumulatively interact with a receptor site in the olfactory system to generate a definite response by the taster (73).

The knowledge that not all of the volatiles occurring in food contribute to its flavour was the reason for changing the methodology of analysis. To bridge the gap between analytical chemistry and sensory evaluation, a straightforward technique, the so-called flavour dilution analysis (alternatively described by aroma extract or odour dilution analysis), has recently been developed (72). For this purpose, an extract obtained from the food is diluted, usually as series of 1:1 or 1:2 dilutions. The headspace of each dilution is analysed by gas chromatography-olfactometry (GCO), allowing the detection of potent flavourants. For the separate flavourants, the result is expressed in terms of flavour dilution (FD) factors. This FD factor is the ratio of the concentration of the flavour compound in the initial extract to its concentration in the most dilute extract in which the flavour is still detectable by GCO. Consequently, the FD factor is a relative measure, which is proportional to the flavour units of the flavour compound in air. This type of assay offers the possibility to rank food components according to their relative flavour impact and has proved to be a powerful technique for the identification of key flavour compounds.

Barley malt can contribute both desirable and undesirable flavours to wort and beer. A broad spectrum of malt flavours can be acquired by varying barley variety and malting parameters, particularly degree of modification and kilning or roasting temperature profiles (14). Not all of the nearly 250 volatile components identified in dark malt considerably contribute to the overall flavour. A limited number of key flavour compounds were identified by a comparison of the relative aroma values of several flavourants (13) and by the application of flavour dilution analysis (3;75).

4.3 Relation between chemical structure and flavour

The perception of odour and taste evoked by a flavour molecule is influenced by the geometry and the presence of specific functional groups (16). Furthermore, chirality is also reported to have a considerable effect on the flavour impression (76). It has been postulated that the stereo-chemical arrangement of some structural elements significantly correlates with the sensory perception of heterocyclic compounds (77). A planar arrangement of carbonyl groups, enolic hydroxyl groups and methyl groups on oxygen heterocyclic compounds appears to elicit caramel-like, sweet or nutty flavours (Fig. 5). In this structure, the OH-group acts as a proton donor, whereas the CO-group functions as proton acceptor (16). Variations in flavour quality are caused by the hydrophobic part of the molecule. On the other hand, bread-like and roasted flavours might result from planar, unsaturated heterocyclic compounds with one or two nitrogen atoms in the ring structure and with an acetyl group in the 2 position (Fig. 6).

The threshold value also appears to depend on chemical structure, the number of side chains, the position of the side chains and the presence of functional groups (78). It has been reported that nitrogen heterocyclic compounds with few and short side chains have relatively high threshold values. When the substitutes on an aromatic ring structure are longer and/or numerous, the threshold value are often markedly lower. Some side chains such as methoxygroups are highly flavour-active and cause a drop in the threshold value.

5 Colour

At present, malt colour is the most important analytical property for the characterisation and differentiation of dark specialty malts. As barley only contains very low concentrations of naturally occurring pigmented substances, malt colour mainly develops during the malting process. For dark specialty malts, the Maillard reaction is the major source of colour formation. The colours produced range from pale yellow to very dark brown, depending on the extent of the reactions (79). Components contributing most to the colour of dark malt include coloured LMW chromophores and macromolecular melanoidins.

5.1 Chromophores

Flavour units and flavour dilution factors were already introduced to estimate the impact of a specific flavour compound. A compa-

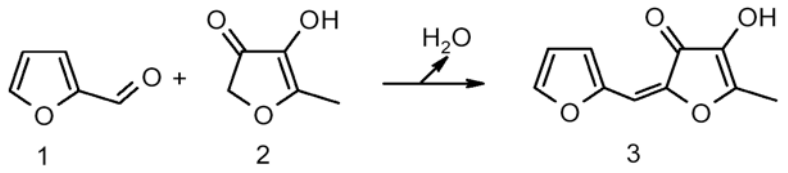

Fig. 7 The yellow coloured 2-[2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (3) is formed from the condensation reaction of furfural (1) and 4-hydroxy-5-methyl-2*H*-furan-3-one (2). After Hofmann, 1998 (81)

rable parameter, the colour dilution or CD factor, was developed to evaluate the colour contribution of LMW Maillard reaction products (80). To achieve colour dilution analysis (CDA), an aliquot of a browned solution is separated by HPLC into different fractions and the effluents of peaks are separately collected. These fractions are made up with water to the original volume and are then diluted stepwise (1:1). Triangular tests are performed until the visual detection threshold is reached. The dilution at which a colour difference between the diluted fraction and two blanks (water) can just be visually detected is defined as the colour dilution factor (81;82). As this CD factor is a measure of the colour activity (ratio of the concentration to the visual detection threshold), it allows the ranking of the isolated fractions according to their colour intensities (80). Further separation of the fractions with high CD factors, followed by HPLC purification and structure determination of the most intense colourants by means of 1H-, 13C- and 15N-NMR and LC/MS led to the identification of several key chromophores (83). In these studies, it was frequently observed that only a limited number of chromophores were responsible for a significant part of the total colour, suggesting that the variety of different key chromophores might be small in the Maillard reaction.

Several model experiments have been performed to provide more detailed information on the formation of LMW chromophores, indicating condensation reactions between methylene-active intermediates and carbonyl compounds as a general key reaction type in non-enzymatic browning (80). In active methylenes, hydrogen atoms are especially reactive. Extra resonance stabilisation of the enolate anion makes these hydrogen atoms more acidic, allowing a faster deprotonation.

Figure 7 gives an example of the formation of a yellow chromophore from the condensation of the methylene-active 3(2H)-furanone with furfural. The currently identified Maillard chromophores are mostly composed of two or three rings. These structures often contain oxygen or nitrogen and are frequently linked by a –CH= group (84;85). It has been claimed that the colour is due either to the chromophore of the methine-bridged compounds or to derived delocalised radicals (85). It has been reported that the most important intermediates in colour formation are 3-deoxyosuloses and 3,4-dideoxyosulos-3-enes (86). Several other carbohydrate degradation products such as, glyoxal, methylglyoxal, hydroxy-2-propanone, 3-hydroxy-2-butanone and glycolaldehyde have later been identified as major browning precursors (87).

5.2 Melanoidins

It is frequently agreed upon that most of the coloured compounds formed by the Maillard reaction are high molecular weight polymers, the so-called melanoidins (84). Nevertheless, more work has been done on the characterisation of LMW coloured compounds. Whether these LMW coloured products represent some melanoidin substructures or not is questionable (85). Owing to their disordered structures, melanoidins are particularly difficult to study and little information is available regarding their formation and constitution (19;21;88).

Melanoidins are reported to possess molecular masses of up to 100,000 Da (88;89) and a C:N ratio that varies with the conditions of preparation (50;90). It has been observed that melanoidins from roasted malt are of considerable higher molecular weight than melanoidins of other malt types (91). Current thinking is that melanoidins are likely to comprise a complex mixture of closely related structures with very similar charge:mass ratios (92). The formation or separation of melanoidins of uniform structure and mass is an essential prerequisite to their successful characterisation (92). Although a number of chromatographical and electrophoretical attempts have been undertaken to isolate and purify melanoidins from real food products such as coffee (93), malt (94) and dark beer (50), it was not yet possible to isolate and characterise a pure melanoidin. Structural elucidation is preferably performed on model system melanoidins. Only in recent years, techniques have become available to better allow the structural characterisation of these brown polymers. Identification of degradation products formed by oxidation/reduction (95) or alkaline/acid hydrolysis (96) can assist in drawing conclusions on the initial melanoidin structure.

There are currently three main proposals for the structure of melanoidins (97). Melanoidins might consist of repeating units of advanced Maillard reaction products such as furans and/or pyrroles (98). Figure 8 represents two types of potential polymeric substructures. Researchers have also postulated that melanoidins may result from the polymerisation of other sugar degradation products (90;99). Polymerisations of early-stage Maillard compounds (3-deoxyosuloses) via Aldol type condensations could lead to the formation of a basic melanoidin skeleton (Fig. 9). Carbohydrate side chains consisting of saccharides with intact glycosylic bonds may be attached to the melanoidin skeleton. These structures are probably branched via amino compounds (97). In a third proposal, melanoidins are formed by the cross-

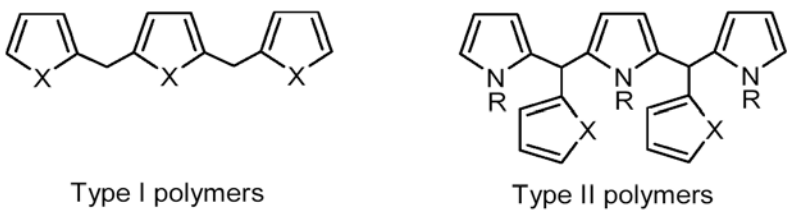

Fig. 8 Two types of polymeric melanoidin substructures consisting of repeating units of advanced Maillard reaction products (X= NR or O) (R = alkyl or amino acid residue). After Tressl et al., 1998 (98)

linking of LMW chromophores and HMW colourless proteins (79;100). This might be possible via the ε-amino group of lysine or arginine (Fig. 10).

As starting materials and reaction conditions have a strong influence on the elemental composition and the structure of melanoidins (99;101), it can be assumed that the above-mentioned structural proposals each partially supplement each other or that different structures coexist in real food systems. In order to increase the knowledge with regard to the structure and functions of melanoidins, the European Union has established a research network, designated COST Action 919 "Melanoidins in food and health" (92).

Extensive studies on model melanoidins have been performed to characterise the chemical species responsible for the typical brown colour (102;103;104). The determination of the chemical nature of chromophores has been hindered mainly by difficulties during purification of the pigments (102) and the fact that the colour of melanoidins may result from more than one chromophore group. Numerous studies have, therefore, been performed to break melanoidins and to characterise coloured substructures in the fragmentation products produced. Neither chemical (97;105;106) nor microbial degradation procedures (107;108;109) have succeeded in generating chemically defined chromophore substructures. This was only achieved recently, after complete enzymatic digestion of the melanoidin skeleton by proteolytic enzymes (100).

5.3 Colour determination

The degree of browning in model systems or food products is usually measured via the absorbance at 420 nm (22). For malt, colour is generally analysed using two standardized and internationally accepted methods (110). Malt colour can be evaluated spectrophotometrically by determining the absorbance of Con-

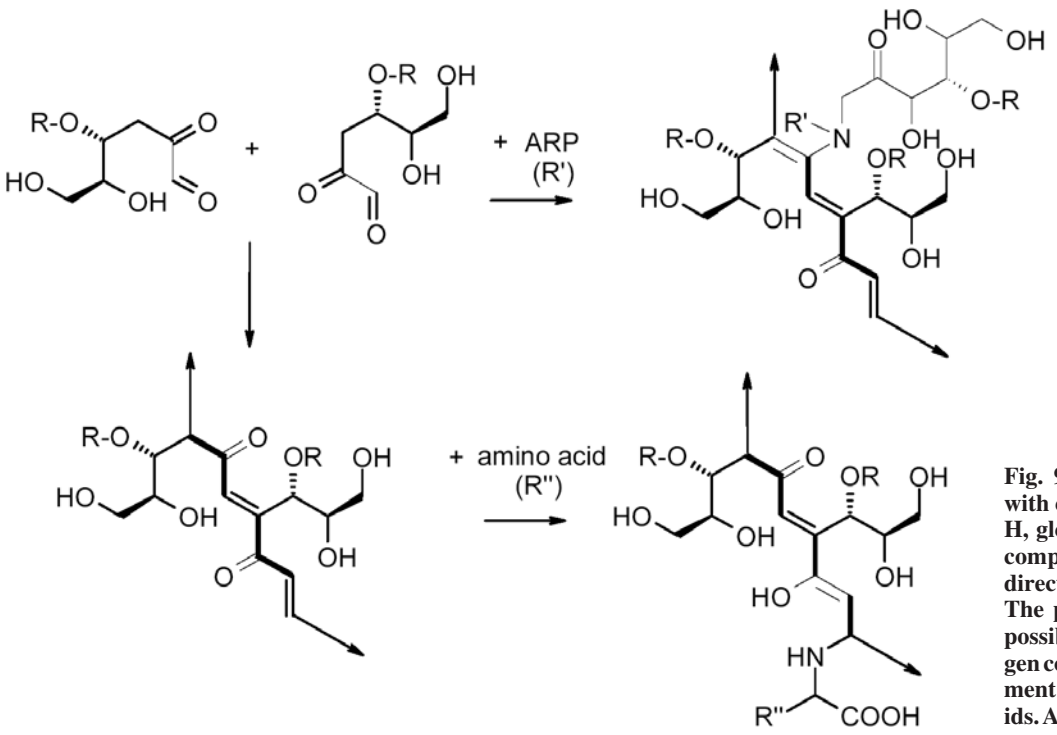

Fig. 9 Basic melanoidin skeleton with carbohydrate side chains (R = H, glc, $(glc)_n$), branched via amino compounds. Arrows indicate the direction of further polymerisation. The proposed structure opens the possibility of incorporating nitrogen containing Amadori rearrangement products (ARP) or amino acids. After Cammerer et al., 2002 (97)

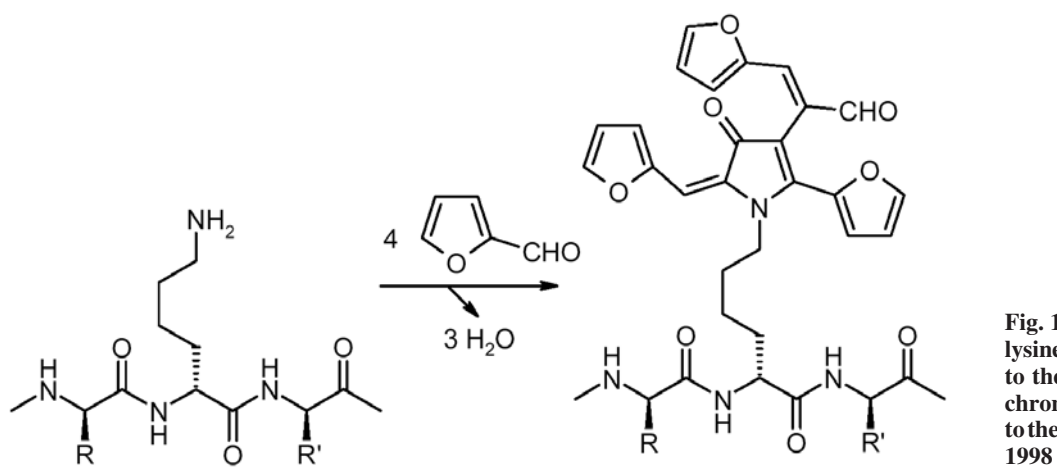

Fig. 10 Reaction of furfural with a lysine side-chain of proteins can lead to the formation of a red-coloured chromophoric substructure, linked to the protein chain. After Hofmann, 1998 (100)

gress wort at a wavelength of 430 nm. The second method is based on the direct visual comparison of the colour of Congress wort with coloured glasses, ranging in intensity from 2 to 27 EBC units.

However, neither the spectrophotometric nor the direct visual method are entirely satisfactory (111;112) partially because both methods do not differentiate between colour shades. Therefore, a new method based on tristimulus measurement can give additional information about malt colour. With this alternative approach, the whole visible spectrum, rather than a single wavelength, is considered. The transmission spectrum of Congress wort is converted into individual intensities of red (X), green (Y) and blue (Z) by the application of colour matching functions. These tristimulus values are further transformed into the parameters L* a* and b*, which represent colour in the CIE L*a*b* colour space (Fig. 11). The L* or lightness parameter corresponds to the overall lightness or darkness of the wort sample. This parameter can have any value between 0 (black or opaque) and 100 (white or clear). The a* and b* values represent the red/green and the yellow/blue colour shade respectively. Recently, repeatability coefficients of variation for L*, a* and b* and reproducibility coefficients of variation for L* and b* were judged acceptable by the ASBC subcommittee on beer colour using tristimulus analysis (113). With this analysis, it was observed that wort made with roasted malt was always darker than wort of the same EBC colour but prepared with other dark specialty malts (colour malt or caramel malt) (2). These findings were confirmed by visual inspection and by the evaluation of the complete absorption spectra of 20 EBC wort samples (12;91).

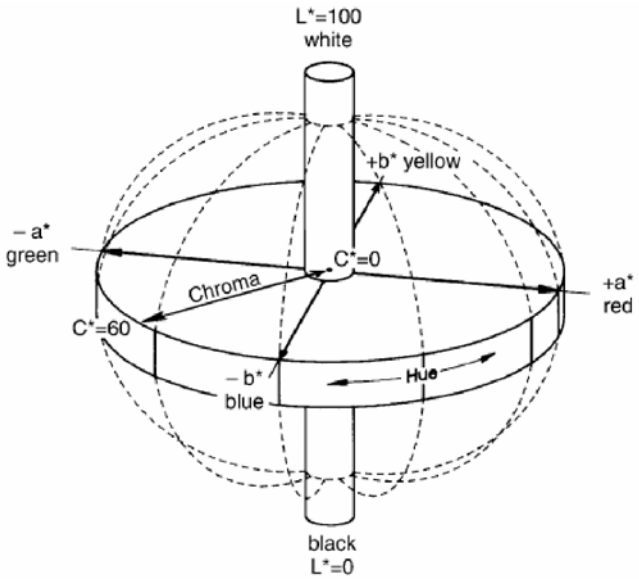

Fig. 11 Attributes of the CIE L*a*b* colour space. After Sharpe et al., 1992 (111)

6 Antioxidative activity

Antioxidative activity or "reducing power" is one of the most essential and least understood properties of Maillard reaction products. The strict chemical definition of "reducing power" is given by the capacity of a certain substance to donate electrons or hydrogen to another substance (114). In the brewing industry however, "reducing power" is used as a synonym for antioxidative activity, referring to the action of any compound capable of delaying, retarding or preventing oxidation processes caused by activated or radical oxygen species (115). Antioxidants exert their activity via different mechanisms such as decreasing molecular oxygen levels, reducing substrates (transfer hydrogen or electrons), scavenging free radicals, chelating pro-oxidant catalytic metal ions or decomposing peroxides (116;117).

Due to these different possible mechanisms, there has been considerable controversy on how to define and measure antioxidative activity in food and biological sciences. Researchers use a great multiplicity of methods for antioxidant testing. Nevertheless, a single antioxidative activity test can impossibly include all antioxidative mechanisms. As there are no approved, standardised methods to evaluate antioxidants, data obtained by different researchers are extremely difficult to compare and interpret (118).

6.1 Malt antioxidants

The recent trend towards minimising the use of "artificial" antioxidants in the brewing industry, has encouraged the investigation of naturally occurring antioxidants (119). It is thought that the natural (endogenous) antioxidants found in malt play a significant role in malting and brewing as inhibitors of beer oxidation. These components are reported to inhibit lipoxygenase activity during malting and mashing and limit auto-oxidation during brewing and beer storage (120;121;122). Malt contains a range of endogenous antioxidants originating either from barley (mainly polyphenols) or from the malting process (mainly Maillard reaction products) (120). The antioxidative activity of malt therefore varies with barley variety and with malt colour. Due to intense Maillard reactions, dark specialty malts contain more antioxidants than pale malts (2). Consequently, beers brewed with dark malts normally have a longer shelf life than pale beers (121). The existence of at least two types of Maillard reaction related antioxidants has been suggested (2). Redox reducing antioxidants are produced constantly during curing or roasting and develop linearly with malt colour. In contrast, fast-acting anti-radical antioxidants seem to develop in the initial stages of the Maillard reaction. As a result, these antioxidants were already found in considerable levels in low coloured malts.

6.2 Antioxidative activity of Maillard reaction products

The antioxidative activity of Maillard reaction products was first reported by Franzke and Iwainsky (123). Later, antioxidative activity of Maillard compounds has been ascertained in both, model systems (124;125;126) as well as in real food products such as beer (13) and coffee (127;128). Maillard reaction products exert antioxidative activity via various mechanisms. These compounds have been found to scavenge several active forms of oxygen including hydroxyl radicals, superoxides, peroxyl radicals and peroxides (129;130;131). Furthermore, in vitro studies have demonstrated that Maillard compounds also act as reducing agents (129;130;132) and as metal chelators (124;131;133;134). It has been recognized that antioxidative activity significantly depends on the polarity of the reaction mixture. Maillard reaction products appeared to be highly antioxidative in hydrophilic but less effective in lipophilic solutions (135). In addition, some studies indicate that, under certain conditions, Maillard reaction products can even exhibit prooxidative activity, meaning that they have the ability to promote or increase the rate of oxidation (129;136;137).

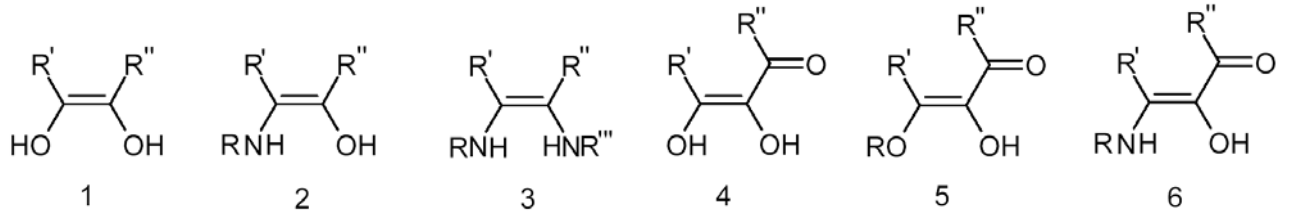

Fig. 12 Structures responsible for antioxidative activity: enediol (1), enaminols (2), enediamine (3), reductone (4), reductone ether (5), aminoreductone (6)

Reductones and melanoidins are considered to be the main Maillard reaction products with antioxidative activity (116;138). Similar to the coloured compounds, Maillard antioxidants can be of both, low as well as high molecular weight (122). Some free radicals (139) and volatile heterocyclic compounds (140) formed by the Maillard reaction are also reported to possess antioxidative activity.

Actually, the term reductone originates from malting and brewing science. It has been known for some time that beer is stabilized against oxidative changes through substances formed by Maillard reactions during malt kilning or roasting. Without at first knowing their structure, these protective compounds were called reductones (19). Initially, it was thought that reductones could be formed only by caramelisation (122). Later, it became clear that the Maillard reaction can also lead to these compounds, but only via the 2,3-enediol pathway (114). Like reductones, HMW melanoidins possess considerable antioxidative activity. In contrast to polyphenols, reductones and melanoidins are able to reduce DCI, a classic redox colourant, in less than 5 minutes (141). Therefore, these antioxidants are frequently termed "fast reducing agents".

6.3 Structural features responsible for antioxidative activity

Structural features responsible for the antioxidative activity of LMW Maillard reaction products are represented in Figure 12. A reductone is characterized by an enediol group, flanked by a carbonyl group (16). Reductones are fairly stable at pH <6 as resonance-stabilized monoanions. The enediol group can be oxidised to carbonyls, thereby conferring reducing power (116). Further heating leads to the formation of coloured melanoidins. The simplest reductone (triose reductone, Fig. 12 molecule 4, R′ = R′ = H) can be prepared from sugars, but the conditions required (heating in alkaline or acid solution) have no relevance to foodstuffs (19). The enediol group with a carbonyl group in the vicinity can be found in many heterocyclic compounds formed by caramelisation and Maillard reactions. Other chemical groups of importance to the overall antioxidative activity are enaminols and enediamines. In food products, it is now known that reductone ethers and amino reductones, which are structurally comparable with ascorbic acid (vitamin C), act as antioxidants (122).

Studies on low molecular weight Maillard reaction products may help to shed light on the structural features responsible for antioxidative activity of melanoidins. It is assumed that reductone groups, enamines, heterocyclic substructures and stable free radicals are responsible for antioxidative activity of melanoidins but there is no experimental evidence for this supposition (19;142). It is difficult to draw meaningful conclusions from the studies on this topic, partly because each study has used different precursors heated under different sets of conditions as well as different methods for assessing antioxidant activity, but also because little information is available concerning the structures of melanoidins. Nevertheless, it is obvious that the nature of the starting materials, as well as the reaction conditions, have a profound effect on the antioxidative activity (124;129;133).

7 Relation between flavour, colour and antioxidative activity

The typical features of the Maillard reaction should not be seen apart. While some Maillard reaction products possess only colour, flavour or antioxidative activity, other compounds undoubtedly possess more than one of these characteristics. Various coloured or flavour-active compounds probably also have antioxidative activity, whereas some LMW flavour compounds can also be coloured.

7.1 Relation between antioxidative activity and flavour

It has been known for some time that several flavour-active, heterocyclic compounds exhibit antioxidative activity. Sulfur-containing heterocyclic compounds (143), pyrroles (144), γ-pyrones (62;145), furans (145;146) as well as thiazoles, oxazoles and furanones (140) formed in Maillard model systems all possessed antioxidative activity. Antioxidative activity of flavour-active γ-pyrones such as maltol and 5-hydroxymaltol can be expected from the chemical structure, as these compounds are clear examples of reductone ethers. For other heterocyclic compounds antioxidative activity might be ascribed to the ring structure being able to scavenge free radicals (140). The degree of unsaturation of the heterocyclic ring, as well as the substituent type, have variable effects on the antioxidative capacity of heterocyclic compounds (146;147). For example, addition of a formyl group to a pyrrole ring or of methyl groups to thiophene remarkably enhanced antioxidative activity (146). Conversely, addition of formyl groups to thiophene or of diverse functional groups to furan has been found to decrease antioxidative activity (146).

7.2 Relation between antioxidative activity and colour

Colour formation and the development of antioxidative activity by Maillard reactions are usually linked (148). It has been observed that these attributes are positively and linearly correlated in Maillard model systems. The relationship between colour formation and antioxidant activity was also confirmed in foodstuffs such as coffee (127;128) and malt (13;116;138;149). However, in food products, this relationship was no longer linear over a broad colour range. Moreover, the correlation appears to depend on the degree of heating and the type of assay used to determine antioxidative activity. For dark specialty malts, antioxidative activity was generally found to increase with malt colour (2;13;116;149). However, when measuring ABTS radical scavenging, antioxida-

tive activity only correlated with malt colour up to a colour of 400 EBC units. Above this limit, no further increase in total antioxidant activity was observed (138). Charring of malt could even cause a significant drop in antioxidant potential as roasting proceeded (116). Similar results were obtained for coffee. Antiradical activity in aqueous extracts of roasted coffee was highest in medium roasted samples (127). The antiradical activity of coffee melanoidins consistently decreased as the intensity of roasting increased, while the ability to prevent linoleic acid peroxidation was higher in melanoidins from dark roasted samples (150). It has to be noted that chlorogenic acids, which can be thermally degraded during roasting, contribute to a significant part of the antioxidative activity of coffee beans. The loss in antioxidant capacity during the advanced phases of the roasting process can be attributable to the pyrolysis of coffee components, including melanoidins and phenols.

Although he absolute antioxidative activity of dark malts usually increases with malt colour, the reducing power per unit of colour was found to be the highest in pale malts (2;121). This may suggest that the compounds causing colour are not fully responsible for the reducing power (149). It may therefore be possible that alteration in process temperatures, times or rate of temperature rise allow separate development of colour and antioxidative activity. Moll also stated that there was no direct association between reductones and colour as reductones are formed prior to colour (122). If this is true, it should be possible to separate these characteristics. In a preliminary study on this topic, it has indeed been observed that fractionation by means of membrane filtration can produce a significant increase in relative antioxidative activity.

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