
12 The Maillard Reaction: Source of Flavour in Thermally Processed Foods

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12.1 Introduction

Since man first discovered fire, thermal treatment of foods has been one of the most common ways to prepare food. The use of heating improved the eating quality of food in terms of flavour and digestibility and it also became apparent that cooked food could be stored for longer time than the raw material. Cooked foods develop characteristic flavour and colour and the main reactions which take place are the breakdown of lipid, sugars, amino acids, carotenes, thiamine and other trace food components.

The Maillard reaction, which occurs between amino compounds and reducing sugars, has been recognised for over 60 years as one of the most important routes to flavour and browning in cooked foods [1]. This extremely complex reaction has been the subject of much research by food scientists seeking to identify compounds that provide the flavour and colour characteristics of heated foods (see reviews by Hodge [2], Hurrell [3], Mauron [4], Mottram [5] and Nursten [6, 7]). The reaction has implications in other areas of the food industry, including the deterioration of food during processing and storage (owing to the loss of essential amino acids and other nutrients) and the protective effect of the antioxidant properties of some Maillard reaction products [7]. In recent years the physiological significance of the reaction has been recognised in relation to *in vivo* glycation of proteins and the link to diabetic complications and cardiovascular and other diseases [7, 8]. The possibility of mutagenic compounds being formed in the Maillard reaction has also been recognised for many years and this was given particular attention in the 1980s when carcinogenic heterocyclic aromatic amines were isolated from well-grilled or charred steaks and were shown to derive from Maillard reactions involving amino acids, reducing sugars and creatinine [9]. In 2002 the Maillard reaction between the amino acid asparagine and reducing sugars was shown to be responsible for the formation of the suspect carcinogen acrylamide (2-propenamide) in fried and oven-cooked potato and cereal products at concentrations as high as 5 mg/kg [10, 11]. This illustrates the complexity of the reaction and its important place in food science.

The Maillard reaction is inextricably linked to the desirable flavour and colour characteristics of cooked foods and this review provides an insight into some of the chemistry associated with flavour generation in the reaction and the different aromas which are involved. The chemical pathways associated with the initial and intermediate stages of the Maillard reaction are presented and routes by which the important classes of aroma compounds may be formed from Maillard intermediates are discussed.

12.2 The Chemistry of the Maillard Reaction

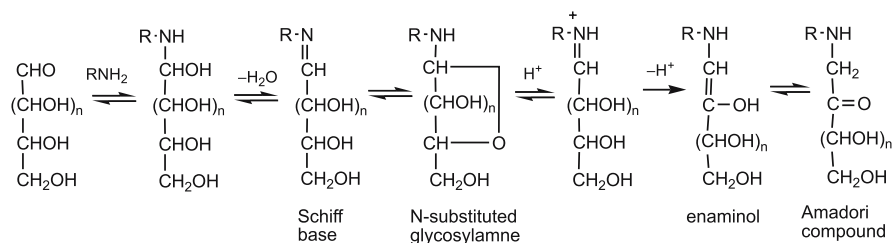
Thermal reactions between amino acids and carbonyl compounds were first observed by Strecker [12] in 1862, who described the formation of aldehydes through oxidative degradation of amino acids. Soon after this Schiff [13] started investigating the addition reactions between amino and carbonyl groups. However, it was a French scientist, Louis-Camille Maillard, who in 1912 first reported the formation of colour through the interaction of amino acids with glucose [14]. The chemical interpretation of the reaction had to wait another 40 years until Hodge in 1953 drew up a scheme to explain the essential steps in the complex reaction [15]. It is noteworthy that some 50 years later the Hodge scheme still provides the basis for our understanding of the reaction.

12.2.1 Stages in the Maillard Reaction

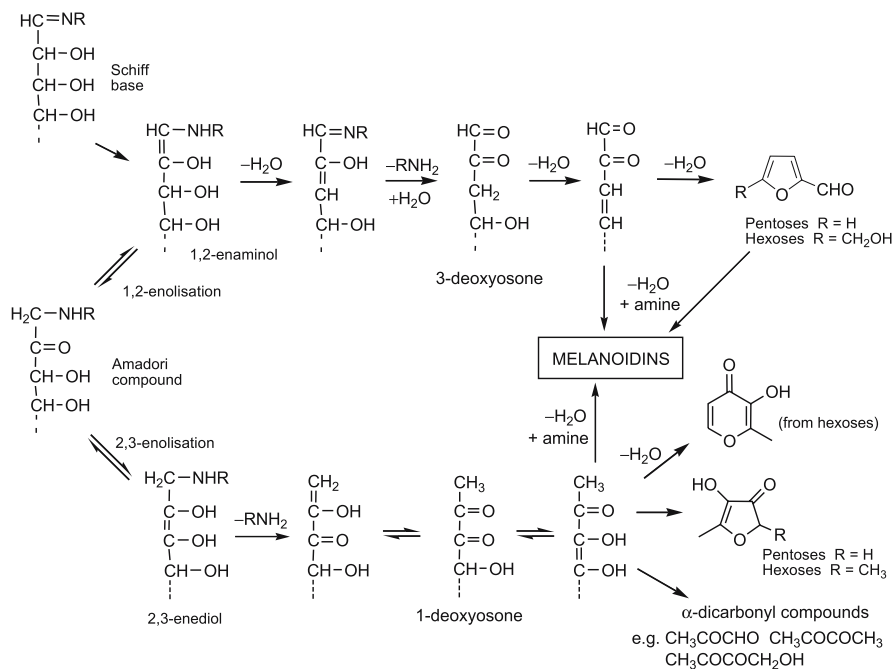
The chemical mechanisms involved in initial stages of the Maillard reaction have been studied in some detail and involve the condensation of the carbonyl group of the reducing sugar with the amino compound to give a glycosylamine. During thermal processing this breaks down to various sugar dehydration and degradation products. These compounds interact with other reactive components such as amines, amino acids, aldehydes, hydrogen sulphide and ammonia, and it is these interactions which provide the basis for the colours and aromas which characterise cooked foods.

The scheme devised by Hodge divides the Maillard reaction into three stages. The reaction is initiated by the condensation of the carbonyl group of a reducing sugar with an amino compound (Scheme 12.1), producing a Schiff base. If the sugar is an aldose, this cyclises to an N-substituted aldosylamine. Acid-catalysed rearrangement gives a 1,2-enaminol, which is in equilibrium with its keto tautomer, an N-substituted 1-amino-2-deoxyketose, known as an Amadori rearrangement product. Ketosugars, such as fructose, give Heyns rearrangement products by related pathways. It is also considered that the N-substituted aldosylamine can degrade to fission products via free radicals without forming the Amadori or Heyns rearrangement products [16].

The Amadori and Heyns rearrangement products are unstable above ambient temperature. They have various keto-enol tautomers, which undergo enolisation, deamination, dehydration and fragmentation steps giving rise to a collection of sugar dehydration and fragmentation products, containing one or more carbonyl groups, as well as furfurals, furanones and pyranones (Scheme 12.2). In this intermediate stage of the Maillard reaction the amino acid also undergoes deamination and decarboxylation through Strecker degradation (Sect. 12.2.2). The aldehydes, furfurals, furanones and other carbonyls produced at this stage may contribute to flavour characteristics associated with the Maillard reaction.



Scheme 12.1 Initial steps in the Maillard reaction showing the formation of an Amadori compound



Scheme 12.2 Intermediate stages of the Maillard reaction showing the formation of carbonyl compounds

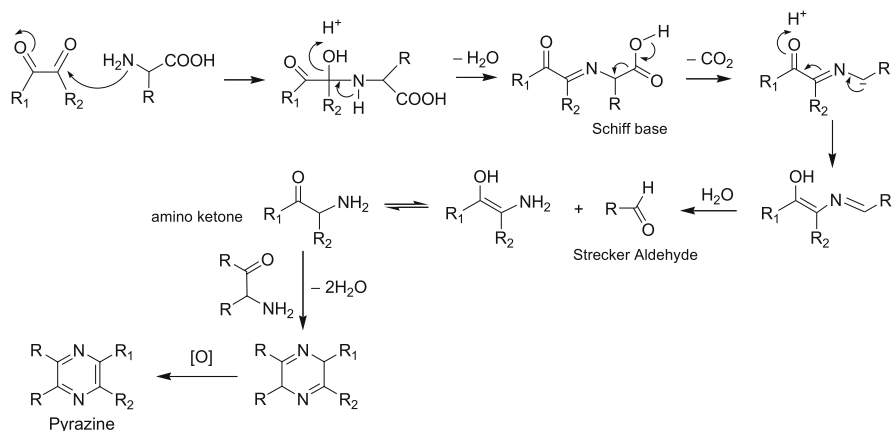
The products of the initial and intermediate stages of the Maillard reaction are colourless or pale yellow and Hodge attributed colour formation to the final stage of the reaction, where condensation between carbonyls (especially aldehydes) and amines occurs to give high molecular mass, coloured products known as melanoidins. These have been shown to contain heterocyclic ring systems, such as pyrroles, pyridines and imidazoles, but their detailed structures are unknown. The final stage of the reaction is of great importance for flavour formation when carbonyl compounds react with each other, as well as with amino compounds and amino acid degradation products, such as hydrogen sulphide and ammonia. It is these interactions that lead to the formation of flavour compounds, including important heterocyclics, such as pyrazines, pyrroles, furans, oxazoles, thiazoles and thiophenes.

12.2.2

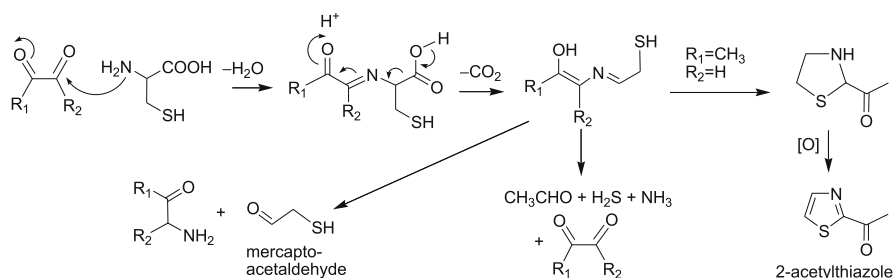
Strecker Degradation

An important reaction associated with the Maillard reaction is the Strecker degradation of amino acids [12, 17]. In the initial and intermediate stages of the Maillard reaction the mechanisms focus on the degradation of sugar, initiated or catalysed by amino compounds. Strecker degradation, on the other hand, can be seen as the degradation of α -amino acids initiated by carbonyl compounds. It is usually considered as the reaction between an amino acid and an α -dicarbonyl compound in which the amino acid is decarboxylated and deaminated, yielding an aldehyde, containing one less carbon atom than the original acid (termed a Strecker aldehyde), and an α -aminoketone (Scheme 12.3). However, the reaction need not be restricted to dicarbonyls. Any active carbonyl group which can form a Schiff base with the amino group of an amino acid should, under appropriate conditions, promote the decarboxylation and deamination of an amino acid. Thus, α -hydroxycarbonyls and deoxyosones, formed as Maillard intermediates, as well as dicarbonyls, can act as Strecker reagents and produce Strecker aldehydes. Other carbonyl compounds found in foods which could act as Strecker reagents include 2-enals, 2,4-decadienals and dehydroascorbic acid.

Strecker degradation is very important in flavour generation, as it provides routes by which nitrogen and sulphur can be introduced into heterocyclic compounds in the final stage of the Maillard reaction. The α -aminoketones are key precursors for heterocyclic compounds, such as pyrazines, oxazoles and thiazoles. In the case of alky pyrazines, the most direct and important route for their formation is thought to be via self-condensation of α -aminoketones, or condensation with other aminoketones [18]. If the amino acid is cysteine, Strecker degradation can lead to the production of hydrogen sulphide, ammonia and acetaldehyde, while methionine will yield methanethiol (Scheme 12.4). These compounds, together with carbonyl compounds produced in the Maillard reaction, provide intermediates for reactions giving rise to important aroma com-



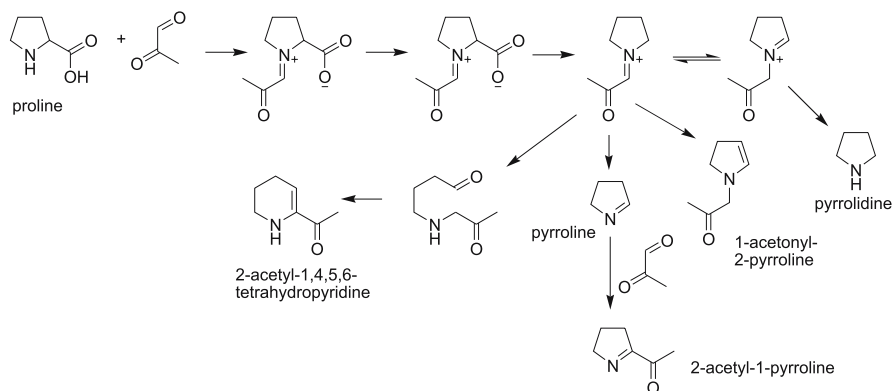
Scheme 12.3 Strecker degradation



Scheme 12.4 Strecker degradation of cysteine

pounds, including sulphur-containing compounds such as thiophenes, thiazoles, trithiolanes, thianes, thienothiophenes and furanthiols and disulphides.

Proline and hydroxyproline differ from the other amino acids in that they contain a secondary amino group in a pyrrolidine ring; therefore, they do not produce aminoketones and Strecker aldehydes in the reaction with dicarbonyls. However, nitrogen heterocyclics are produced, including 1-pyrroline, pyrrolidine, 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine (Scheme 12.5) [19].



Scheme 12.5 Routes to the formation of compounds with bread-like aromas from the reaction of proline with 2-oxopropanal

12.3

Classes of Aroma Compounds Formed in the Maillard Reaction

The aroma volatiles produced in the Maillard reaction have been classified into three groups by Nursten [6], and this provides a convenient way of viewing the origin of the complex mixture of volatile compounds derived from the Maillard reaction in foods:

1. "Simple" sugar dehydration/fragmentation products: furans, pyrones, cyclopentenones, carbonyl compounds, acids
2. "Simple" amino acid degradation products: aldehydes, sulphur compounds (e.g. hydrogen sulphide, methanethiol), nitrogen compounds (e.g. ammonia, amines)
3. Volatiles produced by further interactions: pyrroles, pyridines, pyrazines, imidazoles, oxazoles, thiazoles, thiophenes, dithiolanes, trithiolanes, dithianes, trithianes, furanthiols

The first group contains compounds produced in the early stages of the reaction by the breakdown of the Amadori or Heynes intermediates, and includes similar compounds to those found in the caramelisation of sugars. Many of these compounds possess aromas that could contribute to food flavour, but they are also important intermediates for other compounds. The second group comprises simple aldehydes, hydrogen sulphide or amino compounds that result from the Strecker degradation occurring between amino acids and dicarbonyl compounds.

The products in these two groups are capable of further reaction, and the subsequent stages of the Maillard reaction involve the interaction of furfurals, furanones and dicarbonyls with other reactive compounds such as amines, amino acids, hydrogen sulphide, thiols, ammonia, acetaldehyde and other aldehydes.

These reactions lead to many important classes of flavour compounds that comprise the third group of compounds in the classification.

12.3.1

Oxygen-Containing Compounds

Furans and pyrans with oxygenated substituents (furfurals, furanones, pyranones) occur in the volatiles of all heated foods, and are among the most abundant products of the Maillard reaction. Compounds such as furfural, 5-hydroxymethylfurfural, 2-acetylfuran, maltol and isomaltol generally impart caramel-like, sweet, fruity characteristics to foods. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone and its 5-methyl homologue, which have been found in many heated and non-heated foods, have aromas described as caramel-like, burnt pineapple-like, although at low concentrations the dimethyl derivative attains a strawberry-like note. These furanones are believed to be important contributors to the aroma of cooked meat in their own right and as precursors of other aroma compounds [20, 21]. The odour threshold values of furfurals and furanones are generally at the parts per million level [22]. Oxygenated furans may contribute to caramel-like, sweet aromas in heated foods; however, they are important intermediates to other flavour compounds, including thiophenes, furanthiols and other sulphur-containing compounds.

Aliphatic carbonyl compounds, such as diacetyl, which has a butter-like odour, also may contribute to the aromas derived from the Maillard reaction, and many of the Strecker aldehydes also have characteristic aromas (Table 12.1).

Table 12.1 Aldehydes and some other related intermediates formed in by Strecker degradation

Amino acid	Strecker aldehyde	Odour description
Valine	2-Methylpropanal	Green, overripe fruit
Leucine	3-Methylbutanal	Malty, fruity, toasted bread
Isoleucine	2-Methylbutanal	Fruity, sweet, roasted
Phenylalanine	Phenylacetaldehyde	Green, floral, hyacinths
Methionine	Methional, methanethiol, 2-propenal	Vegetable-like aromas
Proline	Pyrrolidine, 1-pyrroline. No <i>Strecker</i> aldehyde	Important intermediates for bread-like aromas
Cysteine	Mercaptoacetaldehyde, acetaldehyde, hydrogen sulphide, ammonia	Important intermediates for meat-like aromas

12.3.2 Nitrogen-Containing Compounds

12.3.2.1 Pyrazines

These important aroma compounds are believed to contribute to the pleasant and desirable flavour of many different foods. Although tetramethylpyrazine was first isolated from the molasses of sugar beet in 1879 and several alkyl pyrazines were found in coffee in 1928, it was not until the mid-1960s that their occurrence in foods was widely reported, and since then this class of aroma compound has received considerable attention [23]. The alkylpyrazines generally have nutty, roast aromas with some eliciting earthy or potato-like comments [22]. The odour threshold values of the monomethylpyrazines, dimethylpyrazines, trimethylpyrazines and tetramethylpyrazines are all relatively high (above 1 mg/kg), and these pyrazines probably only play minor roles in food aromas. However, replacing one or more of the methyl groups with ethyl can give a marked decrease in the threshold value [24], and some ethyl-substituted pyrazines have sufficiently low threshold values for them to be important in the roast aroma of cooked foods.

Several mechanisms have been proposed for the formation of pyrazines in food flavours [18, 23, 25], but the major route is from α -aminoketones, which are products of the condensation of a dicarbonyl with an amino compound via Strecker degradation (Scheme 12.3). Self-condensation of the aminoketones, or condensation with other aminoketones, affords a dihydropyrazine that is oxidised to the pyrazine.

12.3.2.2 Oxazoles and Oxazolines

Oxazoles have been found in relatively few cooked foods, although over 30 have been reported in coffee and cocoa, and 9 in cooked meat. Oxazolines have been found in cooked meat and roast peanuts, but not to any extent in other foods. 2,4,5-Trimethyl-3-oxazoline has been regularly detected in cooked meat [26], and when it was first identified in boiled beef [27] it was thought that the compound possessed the characteristic meat aroma; however, on synthesis it was shown to have a woody, musty, green flavour with a threshold value of 1 mg/kg [28]. Other 3-oxazolines have nutty, sweet or vegetable-like aromas and the oxazoles also appear to be green and vegetable-like [28]. The contribution of these compounds to the overall aroma of heated foods is probably not as important as the closely related thiazoles and thiazolines.

12.3.2.3

Pyrroles, Pyrrolines and Related Compounds

Pyrroles are found in the volatiles of most heated foods [29], although they have received less attention than some other classes of aroma volatiles. Some pyrroles may contribute desirable aromas, e.g. 2-acetylpyrrole has a caramel-like aroma, and pyrrole-2-carboxaldehyde is sweet and corn-like, but alkylpyrroles and acylpyrroles have been reported to have unfavourable odours [22]. Many more volatile pyrroles have been found in coffee than in other foods [30], and they are common products of amino acid–sugar model systems. Pyrroles are closely related in structure to the furans, and they are probably formed in a related manner from the reaction of a 3-deoxyketose with ammonia or an amino compound followed by dehydration and ring closure (cf. Scheme 12.2).

The characteristic aroma of wheat bread crust has been attributed to 2-acetyl-1-pyrroline, and its formation depends on the presence of bakers' yeast [31]. In model systems it was demonstrated that the acetylpyrroline is formed from the reaction of proline with pyruvaldehyde or dihydroxyacetone. Other compounds with bread-like aromas formed in the reaction of proline with pyruvaldehyde include 1-acetyl-2-pyrroline and 2-acetyltetrahydropyridine (Scheme 12.5). These compounds are unstable, which explains why the characteristic aroma of freshly baked bread disappears quickly during storage.

Since proline already contains a pyrrolidine ring it provides a potential source of nitrogen heterocyclics in the Maillard reaction, and a number of proline-containing model systems have been examined. Tressl et al. [32] identified more than 120 proline-specific compounds in the reaction of proline or hydroxyproline with various sugars. These include pyrrolines, pyrroles, pyridines, indolines, pyrrolizines and azepines, but relatively few of the compounds have been identified among food volatiles.

The roasting of foods such as malt or coffee can result in bitter-tasting compounds; however, until recently little was known about the chemistry of any compounds formed in the Maillard reaction that could be responsible for such tastes. Frank et al. [33] identified a new class of compound, 1-oxo-2,3-dihydro-1*H*-indolizinium-6-oxalates, from reaction mixtures containing xylose, rhamnose and alanine (Fig. 12.1). A number of such compounds have been reported and they appear to have low taste thresholds (below 1×10^{-3} mmol/L).

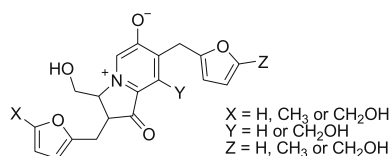


Fig. 12.1 Structures of some bitter tasting 1-oxo-2,3-dihydro-1*H*-indolizinium-6-oxalates found in Maillard reaction systems

12.3.3 Sulphur-Containing Compounds

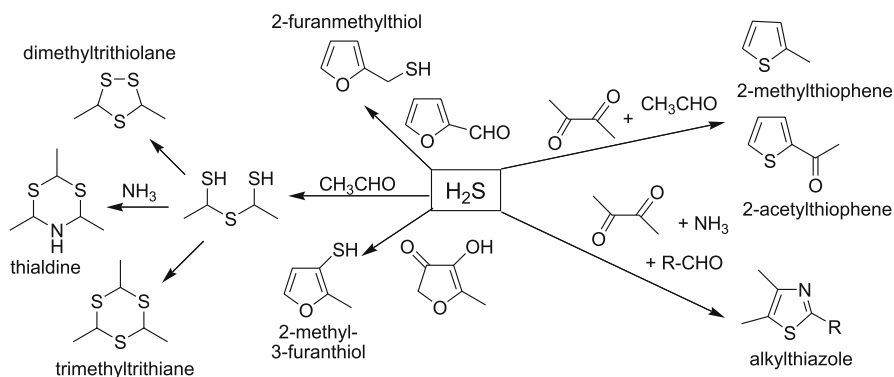
Aliphatic thiols, sulphides and disulphides are found in the volatiles of heated foods; however, the majority of the sulphur compounds produced as a result of thermal treatment of food contain heterocyclic sulphur. These include thio-phenes, thioephones, thiazoles, dithiazines, trithiolanes and trithianes. Over 250 different sulphur-containing volatiles have been reported in heated foods, with the largest numbers in coffee and meat [34]. It is interesting to note that foods from cereals and other plant sources appear to have many more nitrogen-containing than sulphur-containing volatiles, whilst in meat the opposite trend is observed. This may reflect the higher protein content of meat and, therefore, the greater availability of sources of sulphur in the form of the sulphur amino acids.

Hydrogen sulphide is a key intermediate in the formation of many heterocyclic sulphur compounds. It is produced from cysteine by hydrolysis or by Strecker degradation; ammonia, acetaldehyde and mercaptoacetaldehyde are also formed (Scheme 12.4). All of these are reactive compounds, providing an important source of reactants for a wide range of flavour compounds. Scheme 12.6 summarises the reactions between hydrogen sulphide and other simple intermediates formed in other parts of the Maillard reaction.

12.3.3.1

Thiazoles and Thiazolines

Most cooked foods contain thiazoles. Simple alkyl-substituted thiazoles generally have odour threshold values in the range 1–1,000 $\mu\text{g}/\text{kg}$. Odour descriptions include green, vegetable-like, cocoa, nutty, and some are claimed to have meaty characteristics [22]. Although most alkylthiazoles result from thermal



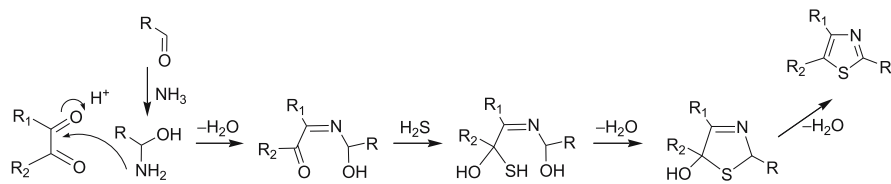
Scheme 12.6 The formation of heterocyclic aroma compounds from the reaction of hydrogen sulphide with intermediates of the Maillard reaction

reactions, some, such as 2-isobutylthiazole, are biosynthesised. This compound makes a very important contribution to the aroma of fresh tomatoes [35]. 2-Acetylthiazole has been reported in a number of cooked foods, including meats, shellfish, coffee, nuts, cereals and some heated vegetables, and it probably makes important contributions to roast, nutty aromas in cooked foods. Mulders [36] proposed a pathway for its formation from the mercaptoiminol intermediate in the Strecker degradation of cysteine and pyruvaldehydhe (Scheme 12.4). The route to alkylthiazoles probably involves the reaction of α -dicarbonyls, such as 2,3-butanedione or 2-oxopropanal (pyruvaldehyde), with ammonia and hydrogen sulphide (Scheme 12.7). This mechanism requires the participation of an aliphatic aldehyde, whose alkyl chain becomes substituted in the 2-position of the thiazole. This aldehyde may be acetaldehyde or a simple Strecker aldehyde, resulting from Strecker degradation of an amino acid. Alternatively, it may be a lipid oxidation product, such as hexanal or nonanal. Several thiazoles with C4–C8 *n*-alkyl substituents have been found in the volatiles of cooked meat [37–39] and, recently, 48 2-alkyl-3-thiazolines were reported in the headspace volatiles of boiled beef from animals in which the meat contained raised levels of poly-unsaturated fatty acids [40]. However, these compounds with long alkyl chains were not found to be potent odorants.

12.3.3.2

Dithiazines

Thialdine (2,4,6-trimethyldihydro-1,3,5-dithiazine) is a six-membered heterocyclic compound containing sulphur and nitrogen in the ring. It was first reported in a food product in 1972 by Brinkman et al. [41], who identified it in heated pork. Subsequently it has been found in other meat species, as well as in peanuts, dry red beans, soybeans, boiled shrimp and several other seafoods [42]. Thialdine was reported to be the major volatile product obtained from a sample of boiled mutton [43]. Thialdine was first reported over 150 years ago by Wöhler and von Liebig [44], who showed that it was formed by the reaction of acetaldehyde, hydrogen sulphide and ammonia. The reaction occurs very readily without heating and, therefore, it is possible that it is formed during flavour-extraction procedures. Nevertheless, there is evidence that dihydrodithiazines do occur in food products and contribute to aroma [45].



Scheme 12.7 Route for the formation of thiazoles

In the 1980s, several other dithiazines were identified in Antarctic krill [46] and later in shrimp [47] and dried squid [48]. They were considered to make important contributions to the aroma of these seafoods. Over 40 different dithiazine derivatives have now been identified in other foods, including beef, pork, chicken, grilled liver, roast peanuts, peanut butter and cocoa [42, 49]. The occurrence and sensory properties of these compounds have been discussed in an excellent review by Werkhoff et al. [42]. They also discuss the formation of these compounds in model systems comprising aldehydes, ammonia and hydrogen sulphide. The odour thresholds are reported to be in the range 5–500 µg/kg and the odour properties of 42 synthesised dithiazines are given in the review. Typical odour descriptors are roasted, onion, garlic-like, meaty, roast peanut, egg-like and sulphury.

12.3.3.3

Furanthiols and Sulphides

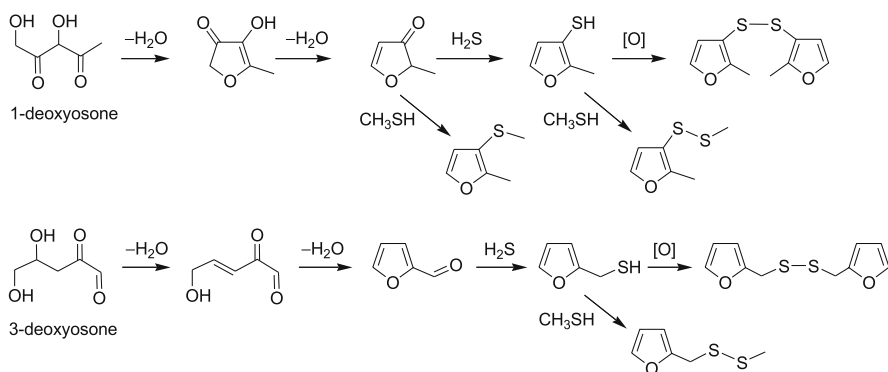
A number of furans with thiol, sulphide or disulphide substitution have been reported as aroma volatiles, and these are particularly important in meat and coffee. In the early 1970s, it was shown that furans and thiophenes with a thiol group in the 3-position possess strong meat-like aromas and exceptionally low odour threshold values [50]; however, it was over 15 years before such compounds were reported in meat itself. In 1986, 2-methyl-3-(methylthio)furan was identified in cooked beef and it was reported to have a low odour threshold value (0.05 µg/kg) and a meaty aroma at levels below 1 µg/kg [51]. Gasser and Grosch [52] identified 2-methyl-3-furanthiol and the corresponding disulphide, bis(2-methyl-3-furyl) disulphide, as major contributors to the meaty aroma of cooked beef. The odour threshold value of this disulphide has been reported as 0.02 ng/kg, one of the lowest known threshold values [53]. Other thiols which may contribute to meaty aromas include mercaptoketones, such as 2-mercaptopentan-3-one. 2-Furylmethanethiol (2-furfurylmercaptan) has also been found in meat, but is more likely to contribute to roasted rather than meaty aromas. Disulphides have also been found, either as symmetrical disulphides derived from two molecules of the same thiol or as mixed disulphides from two different thiols [54].

Disulphides and thiols containing a furan ring have also been found among the volatiles of coffee; however, those containing the 2-furylmethyl moiety are more abundant than compounds with the 2-methyl-3-furyl moiety. 2-Furylmethanethiol was first described as an important constituent of coffee in a patent published in 1926 [55]. Since then its 5-methyl homologue and various other thiols and disulphides have also been found [30]. These thiols have coffee-like characteristics at low concentrations, but are sulphurous and unpleasant at higher concentrations. An interesting bicyclic compound 2-methyl-3-oxa-8-thiabicyclo[3.3.0]-1,4-octadiene (kahweofuran), which is closely related to the 2-methyl-3-furanthio compounds, has also been identified in coffee.

The routes involved in the formation of the various furan sulphides and disulphides involve the interaction of hydrogen sulphide with dicarbonyls, furanones and furfurals. Possible pathways are shown in Scheme 12.8. Furanthiols have been found in heated model systems containing hydrogen sulphide or cysteine with pentoses [56–58]. 2-Methyl-3-furanthiol has also been found as a major product in the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with hydrogen sulphide or cysteine [21, 59]. This furanone is formed in the Maillard reaction of pentoses; alternatively it has been suggested that it may be produced by the dephosphorylation and dehydration of ribose phosphate, and that this may be a route to its formation in cooked meat [21, 60].

12.4 Conclusion

The Maillard reaction is a major source of flavour in cooked foods. The reaction is complex and, because different foods have different profiles of amino acids and sugars, a wide range of flavours are produced when foods are heated. Research over the past 50 years has provided some understanding of the chemical pathways that are involved in the reaction. The identification of a large number of volatile compounds, including many heterocyclic structures, in heated foods has helped flavour scientists understand some of the relationships between the structure of flavour compounds and the perceived flavour. An understanding of the Maillard reaction also provides the potential for improving the sensory quality of heated foods through better control of processing conditions and through the enhancement of the important precursors in the raw materials during the production of both plant and animal foods.



Scheme 12.8 Routes for the formation of furanthiols, sulphides and disulphides in the Maillard reaction

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