Thermal generation or aroma

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8.1 Introduction

Cooking of food is an ancient art that increases the microbiological safety of the food, tenderises and improves the texture, and generates flavour, stimulating the appetite and providing character and variety to our diet. Unprocessed food, particularly grains and meat, are very bland, and the characteristic flavours are only generated during thermal processing. In a domestic kitchen, we use techniques such as boiling, roasting and frying, whereas the food industry uses processes such as pressure cooking (retorting), extrusion cooking, pasteurisation and UHT processing, as well as baking and frying, to name only a few. In all cases, the two major routes to flavour formation are lipid oxidation and the Maillard reaction, whilst other reactions such as caramelisation and degradation of thiamin, vitamin C, carotenoids and ferulic acid are other sources of flavour. These reactions are dealt with briefly in this chapter, but the main focus is the Maillard reaction, looking at the chemistry underlying flavour formation and strategies to control and optimise the reaction. Because sulfur compounds play such a key role in flavour generation, Chapter 9 is dedicated to the role of sulfur chemistry in the generation of aroma; it discusses the interactions between Maillard and lipid pathways. Lipid oxidation, though often associated with rancidity and product deterioration, is also the route to desirable fried notes, which are critical components of foods such as French fries and snacks such as crisps (potato chips). Chapter 12 discusses in detail the mechanism of lipid autoxidation, which is similar whether the end product is a desirable fried note or an objectionable rancid note. The difference is in the relative proportions and concentrations of the volatile lipid-oxidation products and, in the case of desirable fried aromas, the presence of other toasty, roasted notes that signal a fried product rather than a rancid product. The generation of desirable lipid-derived volatile will be discussed briefly in this chapter. Finally, bringing together the three chapters on the Maillard reaction, lipid oxidation and sulfur chemistry, we look at how knowledge of the underlying flavour chemistry has led to the creation and exceptional growth in the manufacture of process flavours, supporting an ever-expanding market for savoury snacks, soups, sauces, gravies and ready-meals.

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8.2 The Maillard reaction

The Maillard reaction is the basis for aroma generation in all thermally processed foods. It involves the reaction between a reducing sugar and an amino compound, initially forming a Schiff base, which breaks down in an array of parallel and sequential reactions to form aroma compounds, colour and antioxidants, and potentially harmful compounds such as acrylamide (Mottram et al., 2002) and heterocyclic aromatic amines (Jaegerstad et al., 1998). It was first reported by French chemist Louis Camille Maillard (1912) and pioneering work by Hodge (1953) published some 50 years later, explained much of the underlying chemistry, which is still the basis for our understanding of the Maillard reaction today. The chemistry has been reported in detail. Among the most comprehensive reviews are those by Ledl and Schleicher (1990) and more recently by Nursten (2005). The aim of this section, therefore, is to summarise the chemistry, highlight recent advances and discuss the implications for the generation of flavour. It will cover recent updates on the breakdown of deoxyhexosuloses, the discovery as oxazolines as novel precursors of Strecker aldehydes in very low-moisture systems and the summary of recent work on pyrazine formation.

8.2.1 Basic chemistry

Before diving into the complexities of the Maillard reaction, it may be useful to revise some basic chemistry. The Maillard reaction may be a complex network of reactions, but the bulk of these reactions can be explained by a few basic chemical reactions that are simplified and reviewed here (see Figure 8.1). Note that for clarity, the arrows are denoting the points of attack rather than the full movement of electrons.

8.2.1.1 Carbonyl-amine condensation

Carbonyl-amine condensation occurs when an amine and a carbonyl react with one another and, if the amine is a primary amine (all amino acids except proline), water is lost. The result is an imine, which, depending on the structure of the rest of the molecule, is often referred to as a Schiff base. The reaction proceeds faster under acidic conditions since protonation of the carbonyl group promotes the reaction. Because water is removed, low-moisture conditions also promote the reaction. Aldehydes are less sterically hindered than ketones and tend to undergo carbonyl-amine condensations more readily, but in sugars, many other factors come into play (see Section 8.2.2.1). The carbonyl-amine condensation is the starting point for the Maillard reaction, but it is also the first step in the Strecker degradation and in the formation of pyrazines and pyrroles, and it is an important reaction in the final stages of colour formation.

8.2.1.2 Keto-enol tautomerisation

Keto-enol tautomerisation is an equilibrium shown in Figure 8.1. The equilibrium normally lies on the left i.e., the keto form predominates unless there are other factors stabilising the enol tautomer. The extent of enolisation is greatly affected by pH,

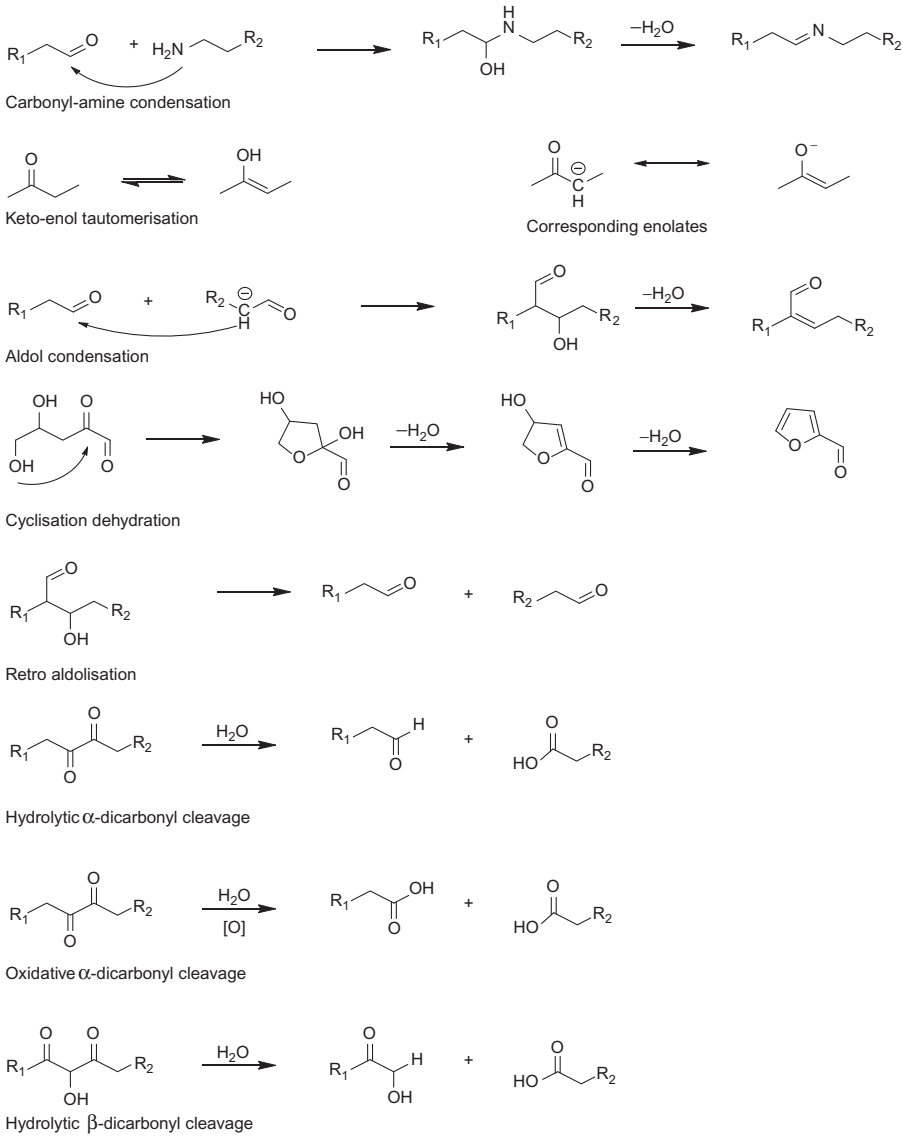

Figure 8.1 Basic chemical reactions involved in the Maillard reaction.

temperature, concentration and, particularly, solvent, which is one reason why Maillard reactions in real food systems (containing lipids, emulsions) may behave differently to aqueous model systems. In the Maillard reaction, keto-enolisation is the basis for the breakdown of the Amadori rearrangement product (ARP) where pH dictates which keto-enol tautomer predominates and, therefore, which aroma

compounds are formed. In alkali conditions, a proton may be lost from either tautomer to produce an enolate ion that is the reactive species in the aldol condensation.

8.2.1.3 Aldol condensation

Aldol condensation is the reaction between two carbonyl groups, one of which must be able to form the enolate species, which is the nucleophile in this reaction. Thus, formaldehyde, glyoxal and benzaldehyde, for example, cannot participate as nucleophiles, although they are susceptible to nucleophilic attack. Figure 8.1 shows the reaction between two aldehydes and the subsequent loss of water. The ability to remove water in the second step to form the conjugated α,β-unsaturated carbonyl also governs which aldehydes are most reactive. 2-Methylbutanal and 2-methylpropanal, for example, do not undergo aldol condensation as the nucleophile, whereas, 3-methylbutanal does. Ketones can also participate, although they are more sterically hindered and are more likely to participate as the nucleophile. Note that Figure 8.1 shows the attack of the R2 molecule on R1 but, depending on the structure of the rest of the molecule, the roles can be reversed to produce a different product. Given the number of carbonyls generated in the Maillard reaction, the number of possible combinations and permutations of the aldol condensation is quite large, especially if we consider that the product is also a reactive carbonyl, so the reaction can continue to make carbonyl compounds of increasing molecular weight. This is the start of polymerisation that contributes to much of the colour formed in the Maillard reaction.

8.2.1.4 Cyclisation and dehydration

Most of the C5 and C6 sugar-related products exist in both cyclic and acyclic forms, as a result of a cyclisation reaction. In Figure 8.1, 3-deoxypentosone is used to illustrate the reaction, where the OH group on C5 is shown to attack the C2 to form a furan ring, but it could equally attack at C1 to form a pyran ring. Indeed, the other OH could also attack at C1; thus, there are often several possibilities for ring closure to form 5- or 6-membered rings. Dehydration often follows a cyclisation reaction and, in the example given, two molecules of water are removed to form 2-furfural.

8.2.1.5 Cleavage reactions

Cleavage reactions are discussed in more detail in Section 8.2.3.1. Figure 8.1 shows a simplified version of four cleavage reactions that may occur during the breakdown of sugar-moieties. The first is the retro-aldol reaction, which is the reverse of the aldol condensation. Hydrolytic α- or β-dicarbonyl cleavage effectively results in addition of water across the cleaved bond: an OH group is added to one part and an H to the other. Hydrolytic cleavage of an α-dicarbonyl (adjacent carbon atoms) results in the formation of one acid and one aldehyde, and either pair could be formed. When the cleavage is oxidative, two acids are formed.

These fairly simple chemical reactions account for a large part of the Maillard reaction.

8.2.2 The early stage of the Maillard reaction

In his 1953 paper, Hodge divided the chemistry of the browning reaction into three stages: the early, the intermediate and the final stage, and these have generally been adopted as the three stages of the Maillard reaction. These stages are also relevant for aroma formation and will be discussed in turn. Hodge defined the early stage of the Maillard reaction, before the formation of any colour or aroma, as the initial sugar-amine condensation and the Amadori rearrangement.

8.2.2.1 The sugar-amine condensation

The first step involves the reaction of a reducing sugar in its acyclic form. Sugars exist in solution in both cyclic and acyclic forms, and it is the ability of the sugar to provide an open chain carbonyl group that determines whether the sugar is a reducing sugar and whether it will, therefore, participate in the Maillard reaction. Glucose, for example, can exist as four different cyclic isomers – either as the α- or β-furanose (5-membered ring) or as the α- or β-pyranose (6-membered ring). In aqueous solution, the pyranose forms predominate (see Figure 8.2), but when the hemiacetal group is cleaved, and the ring opened, there is a carbonyl group available for participation in the Maillard reaction. Some disaccharides can also participate in the Maillard reaction, but only those that are reducing sugars. Maltose for example, because of its $1 \rightarrow 4$ linkage between the two glucose units, has one hemiacetal group that can cleave to give a reactive carbonyl group. However, sucrose, in which the glucose and the fructose units are $1 \rightarrow 2$ linked, cannot ring-open to form a reactive dicarbonyl. Thus, only reducing sugars such as maltose and lactose can participate in the Maillard reaction, and sucrose can only participate if it is first hydrolysed to its component sugars, glucose and fructose, which are both reducing sugars. The relative reactivity of different sugars is often attributed to the relative stability of the acyclic form (Laroque et al., 2008).

The first step in the Maillard reaction is a carbonyl-amine condensation reaction between an amine and a reducing sugar (see the top part of Figure 8.3 for the reaction of a hexose). It is initiated by the nucleophilic attack of the amino group, typically the α-amino group of an amino acid, on the reducing sugar to form a Schiff base, which is in equilibrium with its cyclic form, the *N*-substituted glycosylamine. (All the structures in Figure 8.3 exist in the cyclic form, but for clarity they are not shown.)

8.2.2.2 The Amadori rearrangement

If the sugar is an aldohexose, the Schiff base undergoes acid-catalysed rearrangement via an eneaminol to form the ARP, which in its cyclic furanose form is relatively stable and can be isolated. The aldopentoses are more reactive and isolation of the ARP is trickier. ARPs have been found in many food products, including carrots (Wellner et al., 2011) and dried fruit (Sanz et al., 2001). Protein-bound ARPs are a cause of concern, particularly in milk products, where the ARPs that are formed from the ε-amino group on protein-bound lysine residues cause loss of nutritional quality.

The reaction of ketoses takes a slightly different pathway, which is represented in the bottom part of Figure 8.3 and results in the formation of a Heyns rearrangement

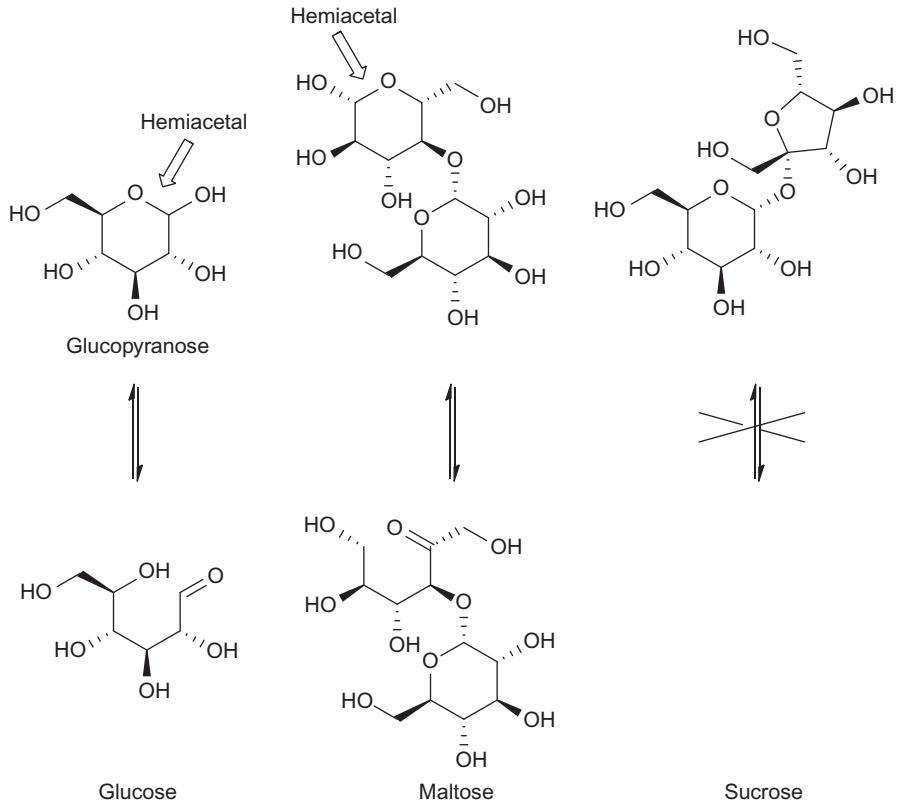

Figure 8.2 Cyclic and acyclic sugar conformations.

product (HRP). However, in the presence of amines, the ARP and the HRP can be interconverted (Teranishi et al., 1998). As an aside, interconversion of aldoses and ketoses without the presence of an amine is called the Lobry de Bruyn-Alberda Van Ekenstein transformation and is important in caramelisation. This takes place under alkaline conditions, or extreme heat conditions, and the lactose–lactulose conversion has been studied in severely heat-treated milk (Berg and Boekel, 1994). In plants, this conversion is catalysed by various enzymes.

8.2.2.3 *Influencing the early stage: the role of amino acid*

The early stage of the Maillard reaction generates the key precursors for subsequent generation of aroma (and colour). The right combination of amino acids and sugars can ensure that the Maillard reaction gets off to a good start. It has been shown (Wedzicha and Leong, 1998) that different amino acids react with sugars at different rates, and some amino acids are more effective in the early stage of the Maillard reaction, whereas others may be more effective during later stages. For the initial condensation of the amino acid with the sugar, lysine and arginine were shown to be amongst the most effective, whereas glutamic acid and asparagine are examples

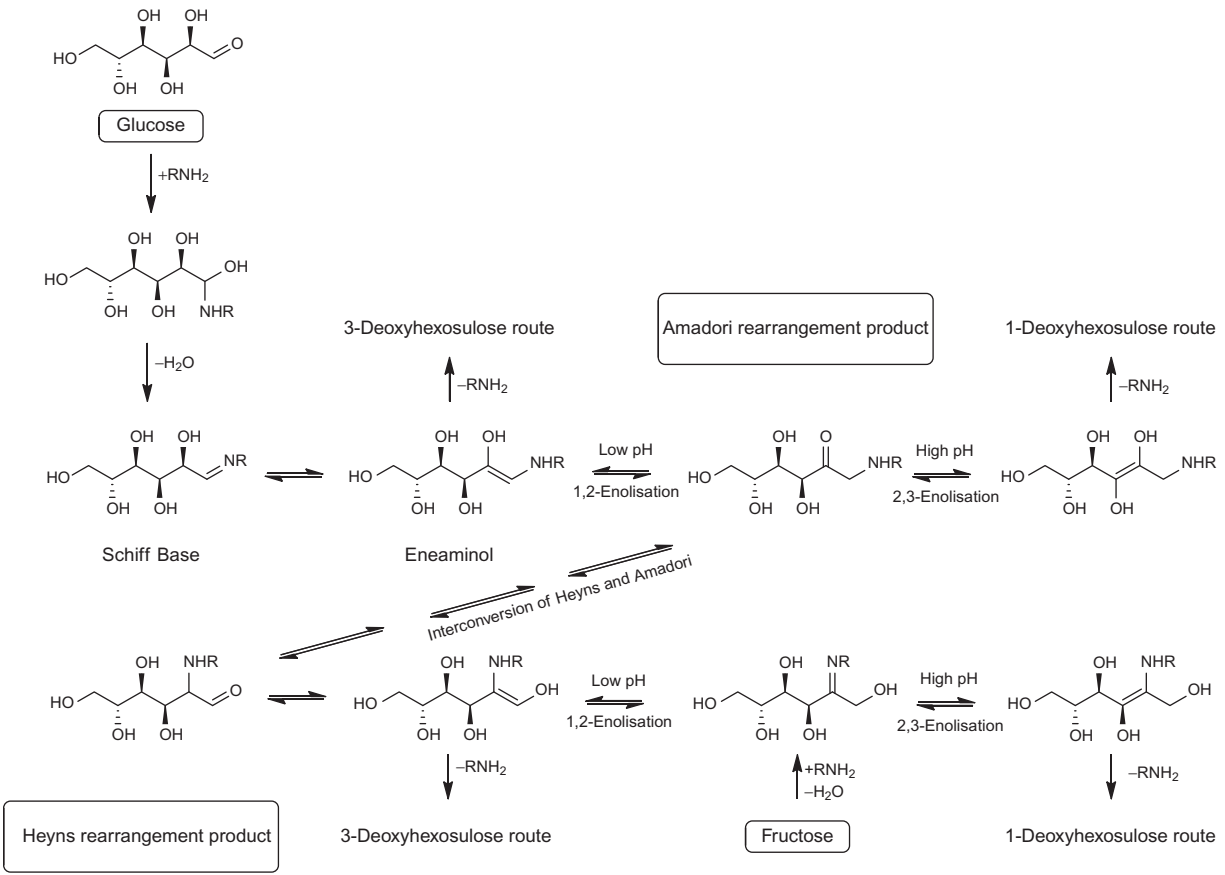

Figure 8.3 Early stage of the Maillard reaction.
Adapted from Teranishi et al. (1998).

of the more unreactive amino acids. This work was directed at colour formation, rather than at flavour formation, but the overall conclusion was that cocktails of amino acids, rather than single amino acids, are more likely to promote browning, and the same is likely to be true of flavour.

8.2.2.4 *Influencing the early stage: the role of sugar*

The first step of the Maillard reaction is also influenced by the type of sugar (Laroque et al., 2008). Pentoses react faster than hexoses because the cyclic forms are less stable. Laroque has also shown that in terms of browning, ribose reacts faster than xylose, which in turn reacts faster than arabinose. These pentose sugars differ only in the relative positions of the OH groups on the ring, and this affects the ring stability and the rate of interconversion between the acyclic and cyclic forms, which in turn affects their ability to participate in the Maillard reaction. It has been estimated that in aqueous solution at 20 °C, ribose does indeed have the highest proportion of molecules in the acyclic form (0.05%). However, this cannot be the full explanation because it does not fit with arabinose being the least reactive, as the figures are 0.03% and 0.02% for arabinose and xylose, respectively (Hayward and Angyal, 1977). There are likely to be several other factors involved as well, such as conformation of the rings, steric hindrance and the gauche effect. Laroque suggested that positioning of the C3 and C4 hydroxyl groups seemed to have the greatest influence on the reaction, and the presence of three adjacent hydroxy groups on the same side of the ring (ribose) created tension in the ring and promoted ring opening.

The comparison of ketoses with aldoses is complex. Sterically, the primary carbonyl of the aldoses might be expected to promote nucleophilic attack faster than the secondary counterpart (ketoses), but often the reverse is found. This is attributed to a greater proportion of the ketose existing in the acyclic form. There are 'diverging' results concerning the relative reactivities of fructose and glucose (Laroque et al., 2008) depending on the conditions employed. It has been shown that the relative reactivities of glucose and fructose vary with respect to the conditions of the reaction and the composition of the reaction mixture (Laroque et al., 2008; Mundt and Wedzicha, 2003). Browning intensity was higher for fructose–glycine systems compared to glucose–glycine systems when the glycine concentration was low, but when the amount of glycine was increased, the results were inverted (Kato et al., 1969). In the case of glucose, the Amadori compound can be isolated, and its formation is one of the rate limiting steps in browning (Wedzicha, 1984). In a casein fructose model system studied by Brands (Brands and van Boekel, 2001, 2002), the Heyns product could not be detected but, in the corresponding glucose system, the Amadori was detected. The relative rates of glucose and fructose need to be assessed on a case-by-case basis.

8.2.3 *The intermediate stage of the Maillard reaction*

Hodge's intermediate stage consists of the breakdown and dehydration of sugar-moieties (starting from the ARPs) and the breakdown of amino acids via the Strecker degradation. At the intermediate stage, colour is just beginning to develop.

8.2.3.1 Sugar breakdown and dehydration

Low pH

ARPs exist in equilibrium with their keto-enol tautomers (see Figure 8.4). At low pH, 1,2-enolisation is favoured, possibly because the electron-rich enolic β-carbon is adjacent to the protonated amine. Enolisation is followed by loss of water, and hydrolysis of the imine releases the amino acid (intact) and the 3-deoxyhexosulose (3DH), which exists predominantly in its bicyclic forms (Weenen, 1998). One of the major breakdown products of 3DH, resulting from dehydration and cyclisation, is 5-hydroxymethylfurfural (HMF) (**1**) which is often used as a marker of the Maillard reaction. If ammonia is in excess then a series of *N*-methylpyrroles is formed. If the original sugar is a pentose, then 2-furfural is the major product (Feather, 1981).

3DH can also undergo fragmentation reactions to form highly reactive short-chain intermediates. Brands and van Boekel (2001) have shown that hydrolytic α-dicarbonyl cleavage produces formic acid (**2**) and a highly reactive C5 moiety, some of which ends up as 2-furfural (**3**). Retroaldolisation has been proposed as the route to methylglyoxal (**4**) and glyceraldehyde (**5**) (Yaylayan and Keyhani, 2000), although it has been suggested that the yield of methylglyoxal (<0.2%) obtained from 3DH under physiological conditions (Thornalley et al., 1999) demonstrates that retroaldolisation cannot be a major breakdown pathway of 3DH (Smuda and Glomb, 2013). However, these conditions are not typical of food processing, and it is likely that different pathways will dominate under different conditions. It is also worth mentioning that aroma compounds are only formed at sub-ppm levels, so even minor pathways can form relevant precursors.

High pH

At high pH, the 1,2-enolisation route is disfavoured as the electron-rich β-carbon of the 1,2-enol is adjacent to an electron-rich amine, and the 2,3-enolisation predominates. The major flavour-forming pathway is via loss of the amino acid to give the 1-deoxyhexosulose (1DH), which fragments and provides a pool of highly reactive dicarbonyl and hydroxycarbonyl compounds. Acetic acid (**6**) can be formed by three different dicarbonyl cleavage reactions: oxidative α-dicarbonyl cleavage, hydrolytic α-dicarbonyl cleavage and β-dicarbonyl cleavage which produce erythronic acid, erythrose or erythrulose, respectively, as the other fragment (Smuda and Glomb, 2013). Hydrolytic β-dicarbonyl cleavage of the isomeric 2,4-dicarbonyl produces hydroxypropanone (acetol) (**7**). Again, retroaldolisation has been proposed as the route for the formation of methylglyoxal (**4**) and glyceraldehyde (**5**) (Weenen, 1998). Voigt has shown that under physiological conditions, the ratio of (**5**):(**4**) is too high to confirm this as a significant pathway, whereas results from our own laboratory performed at 120 °C show the reverse. Clearly, the formation pathway for methylglyoxal is still an area for debate.

Cyclisation and dehydration of 1DH results in the formation of the pyranone (**8**), but the unfavourable conformation of the molecule means that it cannot lose water to form the highly odour-active maltol (**9**).

Rearrangement and dehydration of the 1DH gives diacetylformoin, which is a precursor for the formation of other reactive intermediates such as 2,3-butanedione (**10**), acetaldehyde (**11**) and hydroxypropanone (**12**). Some of these are odour-active in

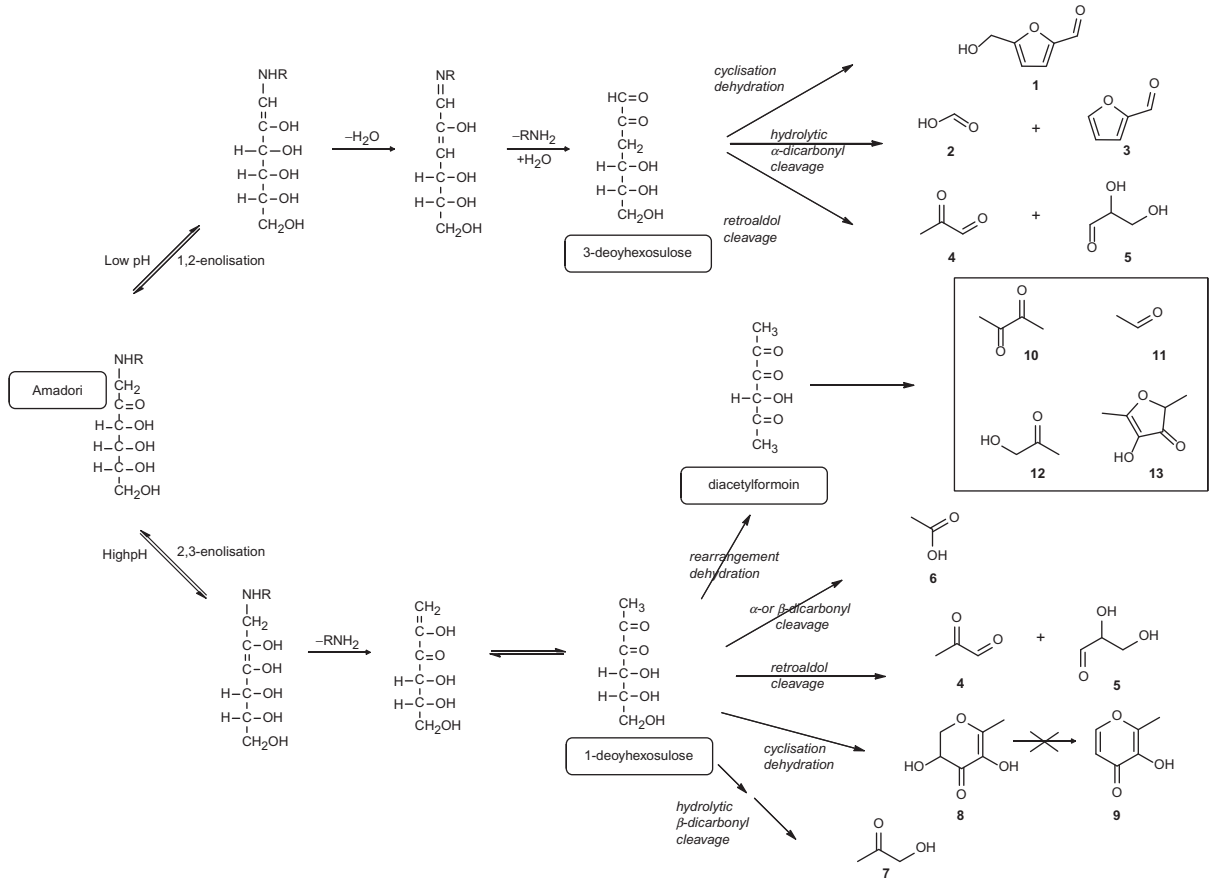

Figure 8.4 Intermediate stage of the Maillard reaction – sugar breakdown.

their own right (for example 2,3-butanedione imparts a characteristic creamy, buttery note) but they all undergo further reactions. Cyclisation and dehydration of diacetylformoin gives the highly odour-active furaneol (**13**). The high pH route is also important in the formation of odour-active furanones and pyranones such as 2-acetyl-3-hydroxyfuran (isomaltol). 4-Hydroxy-5-methyl-3(2H)-furanone (norfuraneol) is formed by the corresponding route from pentoses.

Disaccharides

Kinetic studies of the early stages of the Maillard reaction comparing glucose, maltose and other oligosaccharides revealed that the rate of reaction was independent of the structure of the saccharide but depended only on the concentration of the reducing groups (Wedzicha and Kedward, 1995). Disaccharides such as maltose and lactose undergo very similar reactions to the monosaccharides in the early part of the Maillard reaction, but the second sugar molecule does have an impact on the final products. The initial chemistry is similar, and the corresponding 1DH can be formed with the OH at carbon 4 replaced by an OR, where R is either glucose or galactose, respectively. In monosaccharides, cyclisation of the 1DH and elimination of the OH at C2 results in the formation of pyranone (**8**), which has unremarkable flavour properties. Elimination of a further molecule of water to form maltol does not occur because the stereochemistry of the elimination reaction is unfavourable (Yaylayan and Mandeville, 1994). However, the presence of the OR group directs the elimination of OH to C5, and subsequent elimination of the second sugar results in the formation of maltol (**9**), a powerful odorant with sweet caramel notes. Galactosyl-isomaltol can be formed and is one of the major products found in heated milk. The glucose analogue (from maltose) is not formed to the same extent (Belitz et al., 2004).

One further route exists for the degradation of the 1DHs via the 2,3-enediol, which can eliminate an OH from the 4-position forming the 1,4-dideoxyglucosone without loss of the amino acid. This is particularly relevant for disaccharides where the 4-position contains an OR that is readily eliminated. The breakdown of maltose is considered in detail by Smuda and Glomb (2011).

8.2.3.2 Strecker degradation

The other reaction discussed by Hodge in the intermediate stage of the Maillard reaction is degradation of amino acids by the Strecker degradation. This is one of the most important reactions for generating flavour in which an α-dicarbonyl compounds reacts with an amino acid (carbonyl-amine condensation) to provide the corresponding α-aminoketone and a Strecker aldehyde derived from the parent amino acid, but containing one carbon less (Figure 8.5). The key Strecker aldehydes for aroma generation and their corresponding amino acids are shown in Table 8.1. Many of the Strecker aldehydes have low-odour thresholds and provide a characteristic aroma to the food. For example, the reaction with proline gives a toasted, bread crust aroma, whereas, reaction with leucine or isoleucine gives a malty or cocoa aroma. The Strecker degradation is also a source of further reactive intermediates. Notably, serine and threonine break down under Strecker conditions to provide small carbon fragments,

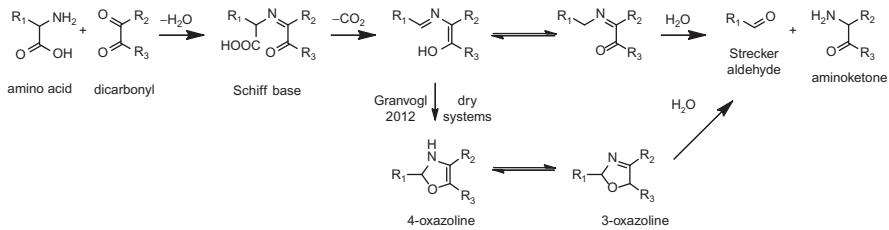

Figure 8.5 Intermediate stage of the Maillard reaction – amino acid breakdown (Strecker degradation).

Table 8.1 The role of amino acids in the Maillard reaction[a]

Amino acid	Strecker aldehyde	Aroma	Odour threshold[a] (µg/kg)
Highly odour-active compounds			
Valine	2-Methylpropanal	Malty	0.5
Leucine	3-Methylbutanal	Malty	0.5
Isoleucine	2-Methylbutanal	Malty	1.5
Phenylalanine	Phenylacetaldehyde	Honey/rose	5.2
Methionine	Methional	Cooked potato	0.4
Reactive intermediates			
Glycine	Formaldehyde		
Alanine	Acetaldehyde		
Cysteine	Acetaldehyde, ammonia and hydrogen sulfide		
Serine	Glycolaldehyde (CH_2OH-CHO)		
Threonine	2-Hydroxypropanal		

[a]Granvogl et al. (2012).

which are involved in the subsequent formation of pyrazines and pyrroles (Yaylayan et al., 2000). The two most important amino acids for the generation of savoury, meat flavour are methionine and cysteine, both of which break down to form reactive sulfur-containing intermediates, and a vast array of potent sulfur compounds are subsequently generated. These are discussed in detail in Chapter 9.

The pathway in aqueous solution, depicted across the top of Figure 8.5, is well understood, and the aminoketone, which is derived from the sugar moiety, reacts further, forming pyrazines (see Section 8.2.4.1). Recently, Granvogl et al. (2012) identified a series of semi-stable intermediates in the form of oxazolines, as precursors of Strecker aldehydes in dry- or low-moisture systems, which release the Strecker

aldehydes upon contact with water or upon mastication. As such, 2-isobutyl-5-methyl-3-oxazoline was identified in dark chocolate.

8.2.4 The final stages

Hodge's classification was based on the browning reaction, so most of the final stages involve polymerisation and colour-forming reactions, particularly carbonyl-amine condensations. However, the early and intermediate stages provide a rich pool of highly reactive precursors of aroma. These undergo an array of reactions that include addition reactions with H_2S and NH_3, aldol condensations, dehydration and cyclisation. Those that are responsible for aroma are discussed below.

8.2.4.1 Formation of pyrazines

Pyrazines are formed from the carbonyl-amine condensation of two amino ketones (generated during the Strecker degradation) to form a dihydropyrazine, which is subsequently oxidised. This is illustrated in Figure 8.6, route 1, showing the formation of 2,3-dimethylpyrazine directly from glyoxal and 2,3-butanedione. However, the more highly substituted pyrazines, which tend to have lower odour thresholds, are more likely to be generated from alternative routes in which one of the substituents comes from an aldehyde. The nature and source of the aldehyde can vary, but pyrazines with substituents derived from Strecker aldehydes are often identified. Methyl substituents can be derived from formaldehyde via the Strecker degradation of glycine, ethyl substituents from acetaldehyde via Strecker degradation of alanine or cysteine (or it can also be lipid-derived), as well as isopropyl, 2-methylbutyl and 3-methylbutyl groups derived from valine, isoleucine and leucine, respectively. In the case of 2,3-dimethyl-5-ethylpyrazine, there are several possible pathways. It may be that the ethyl group is derived from acetaldehyde and the precursors are glyoxal and 2,3-butanedione, in which case, the dihydropyrazine undergoes a condensation reaction with acetaldehyde (illustrated in Figure 8.6, route 2). This is the non-oxidative route that could equally provide one of the methyl groups via formaldehyde condensation. Evidence of these different mechanisms has been found in potato cakes where addition of [2-^{13}C] glycine confirmed that some of the methyl-substituents were derived from glycine (Low et al., 2007). Likewise, use of [$^{13}C_6$] glucose in a meat-based pet food system showed that some of the substituents were not glucose-derived (Parker et al., 2010). These studies also showed that the addition of glycine, which under Strecker degradation forms formaldehyde, resulted in the preferential increase in formation of the more substituted pyrazines. These studies provide a neat example where an understanding of the chemical mechanism has led to targeted manipulation of the pyrazine profile in simplified food systems.

However, Yaylayan has demonstrated by pyrolysis GC–MS that there are other pathways to incorporation of acetaldehyde into an ethylprazine. One is incorporation of the acetaldehyde into the dicarbonyl structure prior to the formation of the pyrazine ring (route 3). This can be achieved through aldol condensation of acetaldehyde with, for example, glyoxal (Figure 8.6, bottom left) to produce 1,2-butanedione. This and

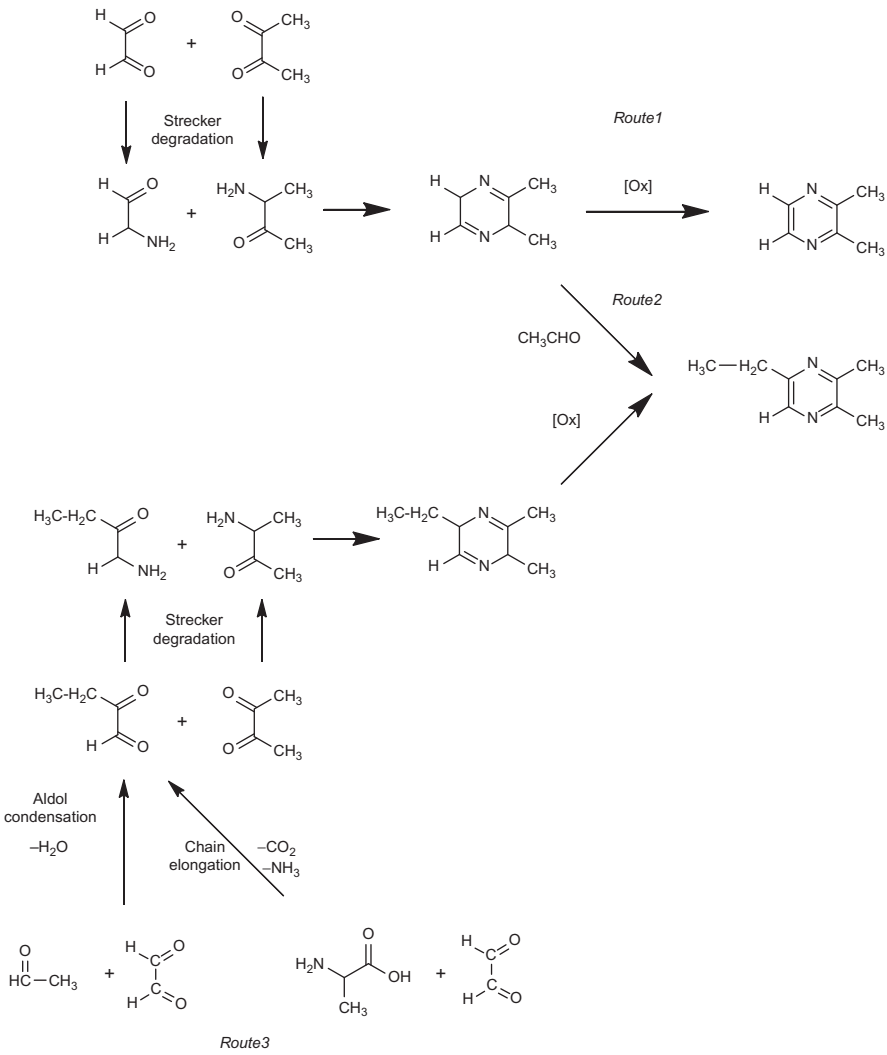

Figure 8.6 Formation of pyrazines.

2,3-butanedione can then proceed via the oxidative pathway to form the same pyrazine. Yaylayan also proposed that condensation of 1,2-butanedione with a second molecule of acetaldehyde could lead to the formation of 3,4-hexanedione and the formation of 2,3-diethylpyrazines. Furthermore, he proposed a mechanism whereby this chain elongation could be achieved directly from the interaction of alanine (or indeed other amino acids such as valine, leucine or isoleucine) with glyoxal (Figure 8.6, bottom right).

Recently, Yaylayan has also shown that under some conditions, part of the glycine carbon skeleton can be found in the pyrazine ring (Guerra and Yaylayan, 2012). In a

dicarbonyl/amino acid soup, there are many permutations and combinations of carbonyl-amine condensations that can lead to the formation of pyrroles and pyrazines. For example, the dicarbonyls, rather than undergoing a Strecker degradation, can form a double Schiff base with glycine that can cyclise leaving two glycine-based carbons in the pyrazine ring. However, these mechanisms have not been identified in food matrices. In two model food systems (Low et al., 2007; Parker et al., 2010), mass spectra of the fragments confirmed that the ring carbons were derived exclusively from glucose. Furthermore, serine has also been shown to be a precursor of pyrazines (Yaylayan et al., 2000). Knowledge of these mechanisms does provide scope for manipulation of the pyrazine profile in real foods.

8.2.4.2 Formation of pyrroles and pyridines

Many nitrogen heterocycles are generated in the Maillard reaction. Those of particular interest are a group of compounds that have low-odour thresholds and impart very characteristic roasted toasted notes. 2-Acetyl-1-pyrroline imparts a characteristic cooked rice or popcorn note, whereas 6-acetyl-1,2,3,4-tetrahydropyridine has a characteristic white bread crust note. Their formation pathways are both well established, and both of these compounds have been shown to arise from 1-pyrroline, which is the Strecker degradation product of both proline and ornithine. 6-Acetyl-1,2,3,4-tetrahydropyridine is formed from the reaction between hydroxypropanone and 1-pyrroline, whereas 2-acetyl-1-pyrroline requires the hydrated form of methylglyoxal (Hofmann and Schieberle, 1998). These mechanisms have been reviewed by Adams and De Kimpe (2006).

8.2.4.3 Formation of sulfur compounds

The formation of sulfur compounds is one of the most complex, and most important of the Maillard reaction pathways, particularly for the formation of meaty aromas. Chapter 9 is dedicated to thermal generation of sulfur compounds.

8.2.4.4 Aldol condensation

The aldol condensation was discussed in some detail in an earlier section. Because of its relevance, it needs to be reiterated as one of the key reactions in the intermediate and final stages of the Maillard reaction. It acts as a sink for excess dicarbonyl compounds, and probably removes many of the short-chain aldehydes generated in the intermediate stages.

In the later stages of the Maillard reaction in food, the opportunities for aldol condensation escalate as there is an abundance of aldehydes that have been generated by both the Strecker degradation and lipid oxidation. Aldol condensation products between simple Strecker aldehydes (formaldehyde, acetaldehyde, 3-methylbutanal, etc.) are often reported in the literature (Elmore et al., 2008) and a series of aldol condensates derived from methional have been reported to have ‘interesting’ aromas that are likely to contribute to the overall aroma profile of potato chips (Buttery, 1973). Aldol condensation can be advantageous, or not, depending on whether your active

aroma compounds are the original aldehydes or are the more complex aldol products. Methylbutanals are typically malty and react with each other and phenylacetaldehyde to produce more chocolaty compounds. Thus, if your aim is a chocolate flavour, then the aldol condensation is desirable.

8.2.5 Generation of taste compounds

One area of Maillard chemistry, which is rapidly expanding, is isolation of taste-active compounds. Much of this work has been carried out by the Hofmann research group. The Maillard-modified nucleotides, which are efficient umami taste enhancers, are discussed in detail in Chapter 15. The most effective was N^2-(1-carboxyethyl)guanosine 5″monophosphate (**14**), which is illustrated, along with other Maillard-derived tastants, in Figure 8.7. In addition, a series of multimodal taste enhancers have been isolated from meat broth that are tasteless in aqueous solution and are only active when tasted in broth. The first of these to be isolated was alapyridaine (**15**), which was isolated from beef bouillon and shown to be vital in recombinants to reproduce the sweet and umami character of the broth (Soldo and Hofmann, 2005). This compound is derived from HMF and alanine. A related series of compounds (**16**) was found to have bitter-suppressing activity, although the longer-chain derivative ($n = 3$) was less active. The reaction between creatine and Maillard-derived dicarbonyls such as glyoxal and methylglyoxal resulted in a series of compounds based on

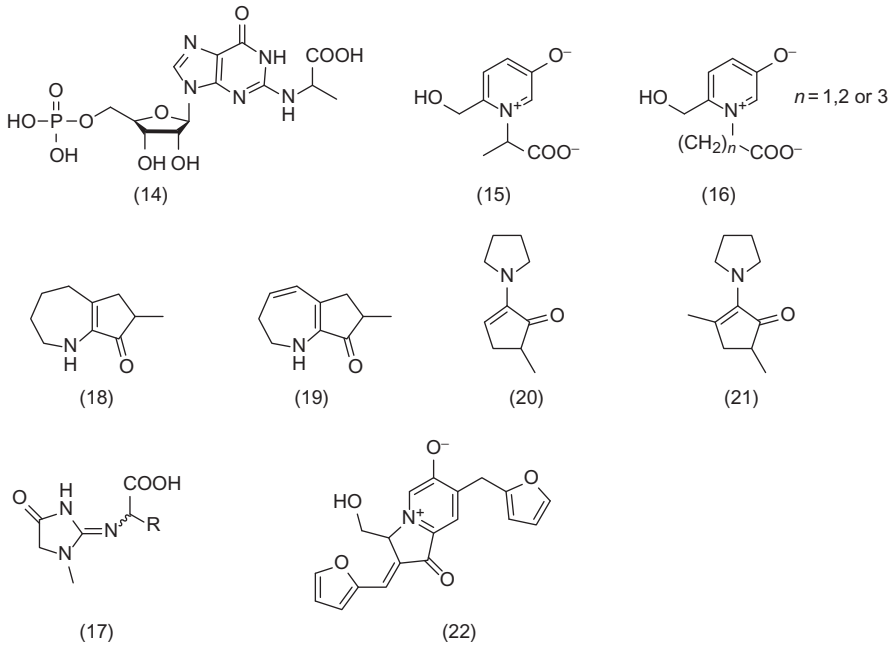

Figure 8.7 Examples of Maillard-derived tastants.

structure (**17**), which gave a thick and sour mouth dryness and mouth fullness to a model broth solution (Kunert et al., 2011).

Two bitter compounds (**18, 19**) and two cooling compounds (**20, 21**) (technically irritants rather than tastants) were isolated from model Maillard systems containing cyclotene and proline (Ottinger and Hofmann, 2002). The different degradation pathways of proline dictated which compounds were formed: thermal decarboxylation of proline produced the cooling compounds, whereas the Strecker degradation of proline gave (**19**). A series of bitter compounds based on the structure of **22** was isolated from xylose alanine mixtures refluxed for 3 h. The parent compound **22** has a bitter threshold of 0.25 μmol/kg (Frank et al., 2003), which was lower when the furan rings were replaced with thiophene rings.

This recent work suggests that generation of tastants needs to be taken into account in Maillard systems, particularly in the process reactions discussed in Section 8.5.

8.2.6 Generation of antioxidants

There is accumulating evidence for the antioxidant properties of Maillard reaction products (MRPs); thus, thermal processing has another potential role in the control of flavour. The antioxidant properties of various simple Maillard model systems applied to emulsions of linoleic acid were reported by Lingnert and Eriksson (1980, 1981). The selection of amino acid (from Arg, Cys, Gla, His, Lys and Val) had the greatest impact with the best results obtained when xylose-lysine or xylose-arginine were refluxed in a pH7 buffer at 100 °C for 5 h. Application of similar mixtures to raw dough used to bake cookies (Lingnert and Hall, 1986) showed that this method worked in real food systems, and that xylose combinations performed better than the corresponding glucose ones. Glucose-casein and lactose-casein were also shown to have antioxidant properties (McGookin and Augustin, 1991). MRPs prepared by reacting sugars and amino acids together at 100 °C for 20 h were applied to ground-pork patties (18–20% fat) prior to cooking (Bedinghaus and Ockerman, 1995). The patties were cooked to an internal temperature 68 °C, stored at 4 °C for 10 days and analysis of the TBA value on days 0, 5 and 10 revealed that xylose MRPs showed higher antioxidant activity than glucose MRPs and that histidine, lysine and tryptophan produced MRPs with good antioxidant activity. Another example in biscuits fortified with lysine (Virag et al., 2013) confirmed glucose rather than other sugars, as a good precursor of antioxidant MRPs.

8.2.7 Generation of potentially harmful compounds

There are several hundred compounds generated by the Maillard reaction, and the discussion above has focussed on those responsible for aroma or taste. Many more are formed as intermediates to colour formation and subsequent polymerisation. However, the Maillard reaction also generates some compounds that are potentially harmful, and the less desirable effects of the Maillard reaction cannot be ignored. Indeed, the greater the understanding of the Maillard reaction, the greater the opportunities to maximise flavour whilst minimising the more harmful reactions.

8.2.7.1 Acrylamide

In 2002, acrylamide was found in potato, wheat and rye products that had been subjected to frying or baking. This caused concern worldwide because acrylamide is classified as a probable human carcinogen by the IARC (1994), and two papers published in *Nature* shortly afterwards indicated that its formation was related very closely to Maillard chemistry (Mottram et al., 2002; Stadler et al., 2002). Since then, much effort has been devoted to identifying key precursors, understanding the formation pathway and developing mitigation strategies (Friedman and Levin, 2008; Stadler, 2005). It is evident that acrylamide is formed during the reaction between asparagine and reducing sugars, and various mechanisms have been proposed based on Maillard chemistry. Two pathways are shown in Figure 8.8. The first is the generic amino acid pathway where the initial reactions are identical to the first step of the Maillard reaction (cf. Figures 8.3 and 8.4), forming reactive dicarbonyls. Asparagine then reacts with these dicarbonyls to undergo the first steps of the Strecker degradation forming the Schiff base (cf. Figure 8.5). However, the breakdown of the asparagine-containing Schiff base is different and acrylamide is formed. The specific amino acid pathway was proposed by Zyzak et al. (2003) and shows acrylamide being formed directly from the glucose-asparagine Schiff base and bypassing both the formation of the ARP and the fragmentation of the sugar (Parker et al., 2012). It is likely that these two routes exist in parallel and their relative contributions are influenced by the other components of the system.

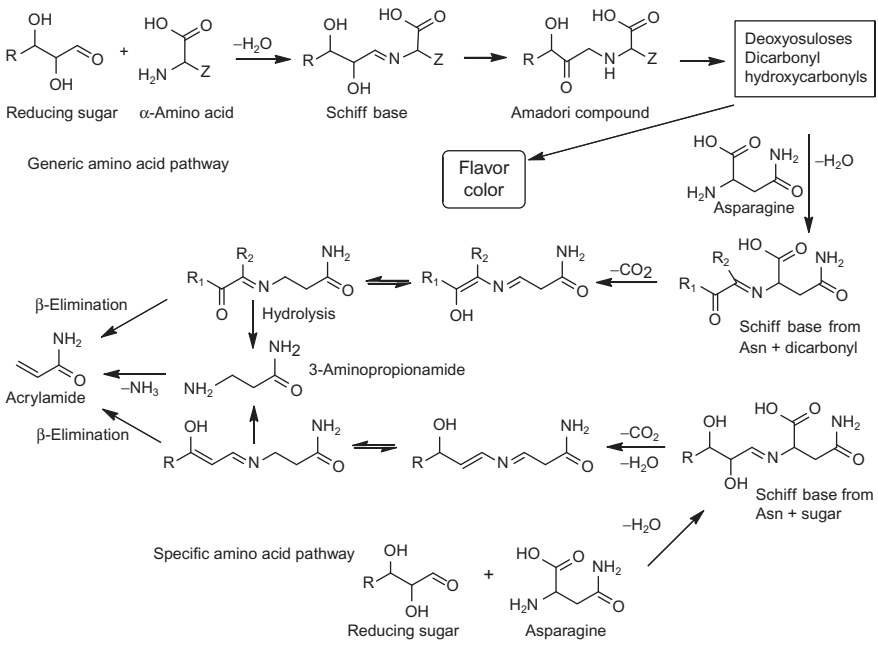

Figure 8.8 Formation of acrylamide.
From Parker et al. (2012).

It is clear that acrylamide formation is inextricably linked with the development of colour and flavour. This poses a challenge for the food industry because many strategies to reduce acrylamide, also reduce flavour formation. Such are the complexities of the Maillard reaction that several authors have turned to mathematical kinetic modelling based on the underlying chemistry to determine the control points in the reaction (Low et al., 2007; Parker et al., 2012) and develop strategies to minimise acrylamide whilst maintaining flavour.

8.2.7.2 Heterocyclic aromatic amines

There is a group of about 20 highly mutagenic heterocyclic aromatic amines (HAAs) that are recognised by their common abbreviations such as IQ, MeIQ, MeIQx and PhiP, and these four in particular have been classified by the IARC as possible or probable human carcinogens. They are formed in mixtures of reducing sugars, creatinine and amino acids and are therefore produced in the Maillard reaction when creatine or creatinine are also present. Their identity has long been established and recent reviews in this area are available (Kizil et al., 2011; Seidel and Pfau, 2012; Alaejos and Afonso, 2011). One major concern is their formation in cooked meat, and marinating has been shown to be effective in reducing their formation. The use of red wine and beer marinades is the subject of a review paper by Viegas et al. (2009) and antioxidant-rich marinades have been shown to be effective in pan-fried beef (Viegas et al., 2012). Some structure activity-based work with polyphenols has recently been used to minimise the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (Salazar et al., 2014), and various other ingredients have been shown to be effective (green tea (Quelhas et al., 2010), grape seed extract (Natale et al., 2014)). Recently, it has been suggested that isothiocyanates are the key ingredient in cabbage that reduce the mutagenicity of cooked meat (Lewandowska et al., 2014). Further discussion on HAAs is beyond the scope of this chapter.

8.2.8 Controlling the Maillard reaction

Given the diversity of compounds formed in the Maillard reaction, it is vitally important to be able to direct the Maillard reaction towards the desirable taste and aroma compounds, preventing the formation of burnt notes and possible carcinogens. Strategies to achieve this are numerous, and some of the more successful approaches are discussed below.

8.2.8.1 Choice of amino acid

Amino acids have two separate roles in the Maillard reaction. The first is to promote the first step in the reaction (a sugar-amino condensation). Any amino acid can participate, but some are far more reactive than others (Wedzicha and Leong, 1998) (see Section 8.2.2.3). The second is the role of specific amino acids to generate specific aromas via the Strecker degradation. These are summarised in Table 8.1. Methionine, for example, giving a characteristic potato note, cysteine being an essential precursors for meaty aroma and leucine providing malty notes. Combinations of Strecker aldehydes via aldol condensation can be used to target other notes.

Chocolaty notes can be produced by combining phenylalanine and leucine – their respective Strecker aldehydes undergo aldol condensation to produce cocoa hexenal (Chapter 1, figure 1, compound 2). However, asparagine, which is the amino acid implicated in acrylamide formation, is the one to avoid.

8.2.8.2 Choice of sugar

The choice of sugar can influence the rate of the Maillard reaction as well as the products formed. The rate of the first step depends on the initial sugar or mix of sugars and is discussed in more detail in Section 8.2.2.3. The main role of sugars is to supply precursors for flavour formation, but swapping sugars can produce subtle differences in flavour and there are instances where the choice of sugar is as important as the choice of amino acid. Disaccharides such as maltose and lactose are required for formation of maltol, and the monosaccharides do not produce this compound. Rhamnose is the best precursor for the formation of furaneol, although there are other pathways from the more common sugars that will produce furaneol in small amounts. Meat flavour requires pentoses (see Chapter 9) as a basis for the reaction with the sulfur-containing amino acids to form the character impact compounds in meat.

8.2.8.3 Concentration of precursors

In many food products, the concentration of precursors is one of the sources of variation that can lead to flavour changes. There is a natural variation in these precursors in living systems, which are further affected by pre- and post-harvest, or pre- and post-slaughter conditions. There are several examples in the literature where a change in the concentration of key precursors in the raw ingredients has been shown to alter the volatile profile of the cooked food. Much of this work, particularly in meat, has been carried out by the Mottram research group at the University of Reading. Postmortem conditioning at 4 °C was shown to alter the natural concentrations of precursors present in beef *M. longissimus lumborum,* particularly ribose and cysteine, which are required for the development of meat aroma (Koutsidis et al., 2008). The addition of ribose to an aqueous beef extract prior to cooking led to an increase in most Maillard volatiles (Balagiannis et al., 2010). The addition of glycine to pet food prior to cooking led an increase in pyrazines (Parker et al., 2009), and small changes in the concentration of ribose, rather than any of the other potential precursors, led to an increase in the meaty character impact compound 2-methyl-3-furanthiol (Aliani and Farmer, 2005). Equally in fish, it was shown that the natural variation of precursors, as well as addition of cysteine, led to significant changes in the aroma profile of cooked salmon (Methven et al., 2007).

The natural variation in plants is just as critical. A deficiency in sulfur during the growth of wheat led to an increase in free amino acids in the raw flour which, when cooked, led to an increase in Strecker aldehydes, aldol condensation products and pyrazines. However, this also led to an increase in acrylamide, which was up to six times higher in the flour from wheat grown in sulfur-deprived soil (Elmore et al., 2008).

Potatoes are particularly susceptible to changes in Maillard precursors, because during storage they are prone to cold-sweetening, a process that produces glucose and fructose thus boosting the supply of Maillard precursors. Elmore showed that storage of potato tubers at 12 °C for 1 month resulted in a doubling of the sugar concentrations compared to the unstored control, but storage at 4 °C led to a much greater increase and there was a concomitant increase in many of the aromagenic amino acids. The result was an increase in the volatile compounds generated in the corresponding baked potatoes. Strecker aldehydes, aldol condensates, pyrazines and sulfides all increased, and there was a sixfold increase in acrylamide, confirming the relationship between acrylamide and flavour and a common formation pathway. Manipulation of glucose and fructose levels in potato strips prior to frying was shown to alter the formation of acrylamide, with exchange of fructose for glucose giving less acrylamide for a given colour in the final French fry (Parker et al., 2012). In one study dedicated to reduction of acrylamide in potato cakes, high levels of glycine were added to potato dough prior to cooking. It was predicted that the addition of glycine would compete with the amino acids for the low levels of highly reactive intermediates in the Strecker degradation thus decreasing the amount of acrylamide formed. This was indeed found to be the case and was accompanied by a change in the volatile profile. There was an increase in pyrazines but only those generated by routes 2 or 3 in Figure 8.6, where one of the substituents is glycine-derived.

Thus, changing levels of precursors in raw ingredients has a significant impact on the volatile profile of the cooked products. Foodstuffs rich in amino acids such as potatoes or meat are likely to be significantly influenced by changes in sugars, rather than by changes in free amino acids, which can be present in excess. Alternatively, in sugar-rich foodstuffs, such as milk powder, the sugar is in excess and the free amino acids limit the production of flavour, which in heated milk is often undesirable anyway.

8.2.8.4 Changes in pH

Most steps in the Maillard reaction are sensitive to pH, and small changes in pH can alter the aroma profile of your product. The first step has an optimum pH that is weakly acidic, maximising protonation of the carbonyl group making it a better electrophile but, at the same time, ensuring that the amine group is not protonated. The pH continues to be important in directing the reaction during the intermediate stage where the pH dictates whether the reaction follows the 1,2- or the 2,3-enolisation pathway (Figure 8.4). The high pH promotes the formation of reactive intermediates, the Strecker degradation and the formation of odour-active compounds such as pyrazines, furaneol and maltol. Low pH tends to favour cyclisation, so the less odour-active compounds such as HMF and 2-furfural are formed. However, low pH also favours the formation of sulfur compounds, and this is discussed in detail in Section 9.2 of Chapter 9. However, these routes are not mutually exclusive so that for example, in meat at pH 5-6, we find plenty of sulfur compounds to impart meaty notes, but there are also sufficient pyrazines and pyrroles present to provide the roasty, toasty character of roast beef. Meat that has been processed at too high a pH lacks flavour.

8.2.8.5 Processing conditions: time, temperature, water activity and pressure

Conditions of time and temperature, not surprisingly, play a role throughout the Maillard reaction. Whilst for stable compounds, such as pyrazines, their generation continues with increased time and temperature, those that are more labile often reach a peak, after which the degradation pathways dominate. Kinetic modelling has been used to predict where this peak might occur under various conditions (Balagiannis et al., 2009) and this is discussed in Chapter 10. Maillard reactivity is also promoted by low-water activity, up to the point where the reactants are no longer solubilised, their mobility is reduced and they do not readily come into contact with each other.

High-pressure processing (of the order of 600 MPa) is being used increasingly for the preservation of fresh fruit, vegetables or seafood where thermal processes such as sterilisation, pasteurisation and blanching cause deterioration in flavour. The laws of physical chemistry mean that high pressure favours reactions where two molecules become one (such as the first step of the Maillard reaction) but not those where molecules fragment. Since much of the Maillard reaction is about the breakdown of sugars and amino acids, not surprisingly, high pressure has been shown to inhibit the formation of Maillard-derived aroma compounds (Hill et al., 1996).

8.2.8.6 Interactions with other components of the food

Interactions with other components of the food adds yet another layer of complexity to the Maillard reaction and is the reason that Maillard reactions in simplified (often aqueous) model systems are difficult to translate into real foods. Certainly, one of the most obvious when moving into a food system is the presence of proteins, which can have a number of effects. Proteins contain additional amine groups that can participate in the initial sugar–amine condensation and can catalyse the Maillard reaction in the absence of free amino acids. This has been studied in casein model systems (Brands and van Boekel, 2001) and is important in meat systems. In the case of participation by the ε-amino groups of lysine, the nutritional quality of the protein is reduced because the lysine can no longer be utilised by the body. In addition, the subsequent breakdown pathways are different, and the formation of protein-bound carboxymethyllysine and lysylpyrraline prevents the formation of aroma compounds. Proteins have also been shown to bind covalently to thiols and disulphides (Adams et al., 2001) thus, removing potent odorants from the system.

Lipids can also alter the Maillard reaction both physically and chemically. Physically, they can alter the solubility and the partitioning of the precursors, and structured lipids have been shown to promote the formation of 2-methyl-3-furanthiol (Vauthey et al., 2000). Chemically, the oxidation of lipids produces a variety of aldehydes that can participate in carbonyl-amine condensation and aldol condensations, potentially competing for reactive intermediates with the Strecker aldehydes. They can also react with H_2S, removing a vital source of sulfur from the system. Lipid–sulfur interactions are discussed in more detail in Section 9.2.2 of Chapter 9.

Furthermore, flavonols (Noda and Peterson, 2007) and hydroxycinnamic acids (Jiang et al., 2009) have been shown to alter the progression of the Maillard reaction. Thus, there is an ever-expanding list of components that can be used to manipulate the

Maillard reaction, and an understanding of the underlying mechanisms helps to direct the Maillard reaction in the desired direction.

8.2.9 Summary of the Maillard reaction

The more we explore the Maillard reaction, the more complex it becomes. From the early days of L.C. Maillard when it was noted for its browning, research has expanded to cover generation of aroma, taste and antioxidant compounds as well as loss of nutritional quality and the generation of potential carcinogens. Nursten (2005) summed up the Maillard reaction thus: 'Overall, the contribution of the Maillard reaction to flavour and off-flavour is striking in its scope and amazing in its intricacies'.

The aim of this discussion is to demonstrate the scope of the Maillard reaction and give insight into the intricacies involved. Unravelling the finer mechanisms involved in the Maillard reaction is likely to keep flavour chemists busy for several decades, but we already have a vast knowledge of the odour-active compounds formed, a good understanding of the major formation pathways and some idea of how the raw ingredients, the processing conditions and other components of food may be used to manipulate the reactions. Armed with this knowledge, the holy grail of the Maillard chemist to control and optimise the Maillard reaction gets closer. The food industry has a good selection of strategies, based on sound chemistry, with which to optimise the flavour characteristics of thermally processed food, whilst minimising less desirable aspects.

8.3 Lipid oxidation

Lipid oxidation is important for flavour formation, particularly in fried foods, where the most odour-active volatile compounds are lipid-derived. The mechanism of lipid oxidation is discussed in detail in Chapter 12, so this section will focus on key volatile compounds formed from autoxidation during thermal processing, using oleic acid as an illustration.

8.3.1 Initiation

During the initiation phase, hydrogen is abstracted from the lipid to leave a lipid radical, which reacts with molecular oxygen to form a lipid hydroperoxide (Figure 8.9). Hydrogen abstraction from unsaturated fatty acids forms more stable radicals due to conjugation of the radical with the double bonds, and unsaturated fatty acids are therefore more prone to oxidation, particularly at low temperatures. Saturated lipids are relatively stable by comparison, and their degradation becomes more important at higher temperatures – i.e., during thermal processing.

8.3.2 Generation of aroma compounds

The second phase involves the breakdown of the lipid hydroperoxides by cleavage of the C—C bond on one side or other of the radical. Because there is no double bond to guide the initial abstraction of hydrogen, saturated fatty acids degrade to form series of alkanes, alkanals and alcohols with a range of chain lengths; however, the major

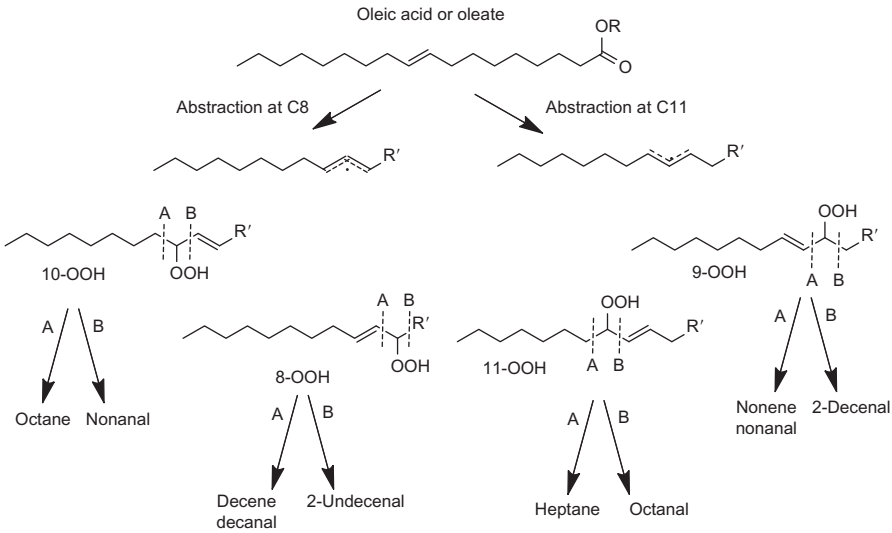

Figure 8.9 Oxidation of oleic acid.
From Parker (in press).

products from triglycerides, such as tristearin, are medium-chain (C6–C10) aldehydes, alcohols, alkanes, carboxylic acids and lactones.

With monounsaturated fatty acids (MUFAs), the degradation is more directed. In oleic acid for example, abstraction of the hydrogen can happen more readily alpha to the double bond at either carbon 8 or 11 (Figure 8.9). At carbon 8, the resultant allylic radical can tautomerise across the conjugated double bond to form the radical on C10. Likewise, both C11 and C9 radicals can be formed from abstraction at C11 and the corresponding hydroperoxides are formed at C8, C9, C10 or C11 in approximately equal amounts (Frankel, 1984). Each of these peroxides can cleave on either side of the hydroperoxide (A or B), resulting in formation of medium-chain aldehydes, 2-alkenals, alkanes and alkenes. The dominant pathways are influenced by the reaction conditions and by the relative stability of the lipid radicals and the compounds that are formed. Model systems (Grosch, 1987) showed that the major degradation product of oleate at ambient temperatures was nonanal (40–60 wt%), whereas at temperatures more relevant to roasting and frying (192 °C), nonanal (22%), 2-decenal (17%) and 2-undecenal (11%) were the major products, imparting green, fatty notes. These three aldehydes are formed from β-cleavage of the 10-OOH, 9-OOH and 8-OOH hydroperoxides, respectively.

The most reactive fatty acids are the polyunsaturated fatty acids (PUFAs), estimated to be about 10 times more reactive than the MUFAs, which, in turn, are 100 times more reactive than the fully saturated fatty acids. For PUFAs such as linoleic acid, there are more possibilities for the abstraction of the hydrogen, but abstraction at C11 is much more favoured because it is alpha to two double bonds. Tautomerism of the resulting radical gives stable radicals at both C9 and C13 so this is the favoured

position for the formation of hydroperoxides. Subsequently, cleavage can occur at A or B resulting in the formation of alkanals, 2-alkenals, 2,4-alkadienals and cyclisation products such as 2-alkylfurans. In a model system at moderate temperatures (Grosch, 1987), hexanal (formed from β-cleavage of the 13-OOH) was quantitatively the major product derived from trilinolein and linoleic acid (50% and 66%, respectively), although organoleptically the most important was the (*E*,*Z*)-2,4-decadienal (6%), which imparts a fatty fried note. However, when the methyl linoleate was irradiated under similar conditions, both (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal were formed in greater amounts (9% and 19%, respectively), formed from β-cleavage of the 9-OOH. At 192 °C, trilinolein produced pentane, hexanal, 2-heptenal and 2,4-decadienal in roughly equal proportions, emphasising the point that under different processing conditions the balance of the lipid degradation pathways changes.

Linolenic and arachidonic acids provide yet more possible pathways, leading to several unsaturated aldehydes. Under moderate conditions, the major oxidation product of linolenic acid (ω-3) was (*E*,*Z*)-2,4-heptadienal (40%) followed by (*Z*)-3-hexenal (11%) and 2,4,7-decatrienal (11%), but in the presence of haem, propanal, (*Z*)-2-hexenal and 3,5-octadien-2-one were the major products. At 250 °C, ethyl linolenate generated 2-ethylfuran and a range of ethyl esters. The degradation products of arachidonic acid (ω-6) were similar to those of linoleic acid (hexanal, (*Z*)-2-heptenal and (*E*,*Z*)-2,4-decadienal). Given the relative reactivity of PUFAs compared to their more saturated counterparts, and the high odour-activity of many of the compounds formed, these are often the compounds that have the greatest impact on the volatile profile. The fatty acid profile of the raw material therefore has a major role in the development of the aroma profile, and this is particularly relevant for the aroma of meat where many factors, particularly species, diet and breed, can influence the fatty acid composition of the animal.

8.4 Other reactions

Although the greatest proportion of aroma is derived from sugars, amino acids and lipids, there are other components of food that generate odour-active compounds. Caramelisation, thermal breakdown of ascorbic acid and thiamine produce reactive intermediates that are common to the Maillard reaction. Ferulic acid, however, is the starting material for generation of vanillin and a series of related methoxyphenols (guaiacols). Carotenoid derivatives (e.g. β-damascenone in boiled sweet potato (Nakamura et al., 2013) and β-ionone in boiled carrots (Buttery and Takeoka, 2013)) as well as terpenes present in herbs and spices also contributes to the aroma of cooked food, particularly of cooked fruit and vegetables, but these are beyond the scope of this chapter.

8.4.1 Caramelisation

Like the Maillard reaction, caramelisation breaks down sugar to produce colour and aroma. In the absence of amino acids to catalyse this reaction, low-moisture conditions and temperatures in excess of 150 °C are required. Sugar breakdown occurs via the Lobry de Bruyn-Alberda Van Ekenstein transformation, with subsequent

dehydration, cyclisation and fragmentation not unlike the intermediates stages of the Maillard reaction. The odour-active compounds are formed by aldol condensation of the sugar fragmentation products such as hydroxpropanone and hydroxybutanone, producing for example cyclotene and sotolone. This reaction is pushed to extremes by the action of base in the formation of Class 1 and Class 2 caramels, or the addition of ammonia in the formation of Class 3 and Class 4 caramels, all of which are used as colourants in the food industry.

8.4.2 Ascorbic acid

The non-enzymic browning of ascorbic acid is well known and is accompanied by the generation of aroma-active compounds due to Maillard type reactions. Degradation of ascorbic acid can provide a pool of intermediates containing reactive dicarbonyls and hydroxycarbonyls. Amongst these are glyoxal, methylglyoxal and 2,3-butanedione (Bradshaw et al., 2011) whilst Vernin et al. (1998) showed a pathway for the degradation of ascorbic acid to 1-deoxyopentosulose. These compounds are typical of those formed during the Maillard reaction and react further to form odour-active compounds. Recently, Yu and Zhang (2010) reported the volatiles generated when ascorbic acid and cysteine were heated in aqueous buffer at 141 °C at different pHs $\geq$5. Many of these were compounds that can contribute to meat flavour such as thiophenes, thiazoles, pyrazines and cyclic sulfur compounds. However, using dynamic headspace extraction rather than SPME, Parker et al. (2013) showed that 2-methyl-3-furanthiol and many related disulfides were formed in buffered model systems containing ascorbic acid and cysteine. They suggested that these are formed directly from ascorbic acid via 1DO, bypassing the early stages of the Maillard reaction and avoiding the possible formation of the semi-stable 2-(1,2,3,4-tetrahydroxybutyl)thiazolidine-4-carboxylic acid, which is readily formed between ribose and cysteine (De Roos, 1992).

8.4.3 Degradation of thiamine

Thiamine is an important precursor of flavour that is exceptionally important in meat aroma, but also can lead to off-flavours in foods where meaty notes are out of place. This is discussed in detail in Chapter 9.

8.4.4 Ferulic acid

Although most of the aroma-active compounds in foods are generated from sugars, amino acids and lipids, one extremely important exception that cannot be overlooked is vanillin. Vanillin and related compounds such as guaiacol (smoky) and *p*-vinylguaiacol (smoky, spicy) are derived from thermal and oxidative breakdown of ferulic acid (Cerny, 2010) (Figure 8.10), which is present in wood lignocelluloses. Rice bran is a rich source of ferulic acid and is used commercially as the starting material for the bioformation of vanillin (see Chapter 11).

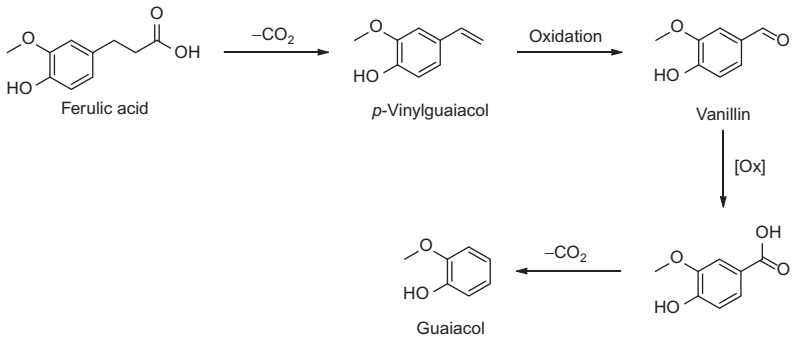

Figure 8.10 Formation of vanillin and guaiacols.
From Cerny (2010).

8.5 Process flavours

Process reactions are designer Maillard reactions performed outside the food environment from highly tailored precursors that are chosen to target specific compounds, groups of compounds and aromas. They are carried out using typical Maillard precursors under carefully controlled conditions of time, temperature and pH, which mimic conventional cooking but have been optimised to maximise the desired flavour. They capitalise on the wealth of flavour chemistry that has been developed over the past 50 years, particularly in understanding the thermal generation of aroma. The more complex examples integrate knowledge of Maillard chemistry, lipid chemistry and vitamin breakdown in a holistic approach to produce a rounded product with aroma, taste (and colour). They are widely used in the food industry, particularly to flavour meat products and meat-flavoured soups and snacks.

8.5.1 The application of the Maillard reaction to produce process flavours

The first commercial use of the Maillard reaction to produce process flavourings took place in the 1960s at the Unilever subsidiary company Food Industries Ltd. (FIL), which later became Quest and eventually Givaudan. Workers at the Unilever Research Centre, Colworth House, in the UK had filed a number of landmark patents (May and Akroyd, 1960; Morton et al., 1960), which protected the reactions of the key precursors of meat flavour. These were applied to the development of the first recognisable meat flavours. Prior to this, savoury character was generated in food products through the use of hydrolysed vegetable proteins (HVPs), spice blends and meat extracts, however, these materials did not possess meat character or were not strong enough. FIL was one of a number of companies manufacturing HVPs through the acid hydrolysis of proteins – a process that resulted in the formation of amino acids and peptides. These products were recognised as useful bases in which the patented Maillard reactions could be undertaken to produce the first commercial meat flavours.

Work on meat flavour started a Colworth House in the mid-1950s and followed two different approaches. The first approach was to isolate and identify the aroma volatiles arising from beef on cooking, and the second approach was to characterise the flavour precursors giving rise to beef flavour. The first approach did not produce any meaningful results for many years because the analytical techniques available at the time were not sensitive enough to detect the very low odour threshold compounds responsible for meat flavour. The second approach, the precursor approach, quickly led to some important discoveries that heralded the start of a new era in flavour technology. The driving force behind this research was the belief at that time that new edible meat-like products derived from texturised proteins would have a major impact on consumer foods of the future. Research at Colworth House was well advanced in developing meat-like products from soy protein using a spinning process, and there was a need to flavour these products like meat.

The study of the precursors of meat flavour at Colworth House led to some important discoveries that laid the foundations for process flavour technology. It was found that when raw beef was extracted with cold water and dialysed, the fraction having a molecular weight of less than 200 Da produced a meaty flavour on heating. The unheated dialysate was analysed by paper chromatography and found to contain around 32 amino acids, small peptides, glucosamine and three sugars – glucose, fructose and ribose. Following heating and further analysis, it was revealed that a number of the compounds in the dialysate were reduced in concentration but two compounds, cysteine and ribose, were completely lost. When these two compounds were heated together in water, a recognisable meaty, beefy flavour was produced. This was one of those truly 'eureka' moments in science, and the discovery was widened to include other pentose sugars such as xylose and arabinose, hexose sugars such as glucose and fructose, other important amino acids such as glutamic acid, alanine and glycine, and peptides such as glutathione and HVPs, which provide more complex amino acid profiles for process flavour reactions.

8.5.2 Examples of process flavours

Examples of some of the early formulations were laid out in the early patents and in a chapter written by May (1995), one of the Unilever inventors. Two examples are shown in Table 8.2. Over the past five decades, many reaction flavour patents have been filed, and process flavourings are manufactured using a range of techniques, such as reflux at atmospheric pressure, pressurised reactions, extrusion, roller and oven drying, vacuum oven drying and surface scraped heat exchange. The extent of the growth of process flavourings over the past 50 years has been remarkable as the savoury food market grows. It is currently undergoing another surge as countries such as China, India, Brazil, Indonesia and others located in Southeast Asia develop consumer food markets.

8.5.3 Legislation

The US Food and Drug Administration (FDA) recognised that flavours produced by thermal processing techniques present a problem in the determination of safety as the end result is not a chemically defined substance, nor is it a simple naturally occurring

Table 8.2 Examples of some early process flavour formulations

Beef flavouring		Chicken flavouring	
Ingredient	**Quantity (kg)**	**Ingredient**	**Quantity (kg)**
L-Cysteine hydrochloride	80	L-Cysteine hydrochloride	100
Protein hydrolysate liquid 40% total solids (from soya, maize or wheat gluten)	1000	Wheat gluten hydrolysate liquid 40% total solids	1000
D-Xylose	100	D-Xylose	60
		Chicken fat	500
		Water	3000
Heated under reflux for 3 h with stirring. Cooled within 30 min. Sieved. Spray dried with carrier (60 parts flavour to 40 parts carrier) to produce a beef flavour powder		Heated under reflux for 2 h with stirring. Cooled to 80–85 °C. Glyceryl monostearate (20 kg) added with vigorous stirring. Carrier added (60 parts flavour to 40 parts carrier). Stirred for 30 min. Homogenised mixture spray dried to produce a chicken powder	

mixture isolated from foods such as orange oil. To address this situation, the Flavour and Extract Manufacturers Association of the United States (FEMA) established a committee to develop information about the practice of making process flavours, and during the 1970s and early 1980s, the FEMA Committee assembled data valuable in the determination of the safe use of process flavours that included the following:

- a review of the practice of the US flavour industry in producing process flavours by a survey of methods, precursors, reaction conditions and volumes produced
- development of a dossier of the manufacture and ingredients used in creating commercial process flavours
- the safety data including toxicological testing undertaken by individual flavour companies

The presentation of the above scientific, manufacturing and useful information by FEMA to the FDA led to the following conclusions (Janiec and Manley, 2005; Newberne et al., 2000).

The FDA considers that process flavours are 'Generally Recognised as Safe' (GRAS) based on the following:

1. The manufacturing process is related to high-temperature cooking.
2. There is a selection of ingredients similar to the preparation of gravy. The belief is that process flavours are produced on the selection of natural ingredients that have been found to create a high flavour note when cooked at a temperature similar to gravy.
3. The use level of process flavours is low, as is true of all flavours; therefore, the consumption rate is low in consumers' diets.

Representatives of the FDA have publicly stated that based on all available information, process flavours meet the requirements of GRAS status (Lin, 1995) but nowhere

in federal law is this statement made in writing. Following the deliberations of the FDA, the International Organisation of the Flavour Industry (IOFI) developed international guidelines for the manufacture of process flavours and these guidelines are adhered to by industry around the world. They were adopted by the Council of Europe based on the conclusions of the Committee of Experts on Flavouring Substances (Gry, 1988).

Thermal process flavourings are legislated in the European Union under Regulation 1334/2008 (European Union, 2008). The definition is:

> *'Thermal Process Flavouring' shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar; the ingredients for the production of thermal process flavourings may be:*
>
> *(i) Food and/or*
> *(ii) Source material other than food.*

There is one other place in the world where there is a legal definition for process flavourings and that is in the Mercosur Technical Regulation No 10/06 (Mercosur, 2006) applying to Argentina, Brazil, Paraguay and Uruguay. The definition is:

> *Reaction/process flavourings are products obtained by heating, in similarity with the cooking of foods, raw materials which are foodstuffs or food ingredients, or a mixture of ingredients which may or may not have flavouring properties on their own, at least one of which needs to contain amino nitrogen and the other is a reducing sugar.*

Reaction/process flavourings are further sub-classified into natural when obtained exclusively from natural raw materials and/or ingredients or synthetic when at least one synthetic raw material is used in preparing them. Under EU Regulation 1334/2008 thermal process flavourings cannot be natural under any circumstances because in Article 16 clause 2 it states;

> *The term 'natural' for the description of a flavouring may only be used if the flavouring component comprises only flavouring preparations and/or natural flavourings substances.*

Article 16 clause 2 does not include thermal process flavourings and therefore by a process of exclusion they cannot be natural in the EU whereas, in the rest of the world where the IOFI Guidelines are implemented, or in the Mercosur countries following Regulation No 10/06, they can be natural. With an increasing demand for natural flavourings across the European Union, this places the flavour industry and food manufacturers under enormous difficulties. No statement has ever been produced by the European Commission that explains the reasoning why thermal process flavourings cannot be natural in the European Union.

8.6 Summary and future work

This chapter has sought to bring together all the thermal generation pathways that lead to the formation of flavour in food during processing. The challenge for the future is to understand how these pathways interact with each other and, more importantly, how they interact with other components of a complex food matrix.

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