

The role of sulfur chemistry in thermal generation of aroma

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9.1 Introduction

Sulfur volatiles frequently play a key role in the aroma of processed foods and tend to have low-odour thresholds, very often orders of magnitudes below those of corresponding alcohols (Table 9.1). For example, the roasted, coffee-like smelling compound 2-furanmethanethiol, a key aroma compound in coffee, has an odour threshold of 0.005–0.12 µg/kg, which is 10,000–100,000-times lower than the corresponding 2-furanmethanol. The thiols shown in Table 9.1 have average odour thresholds that are 1000 to 100 million times lower than the related alcohols.

A review of the contribution of sulfur compounds to heated foods (Mottram and Mottram, 2002) identifies meat (chicken, beef, mutton, pork), coffee, cocoa, tea, rice, bread and peanut as foods rich in sulfur aroma compounds and cysteine, methionine and thiamine as particularly important aroma precursors. Table 9.2 shows examples of aroma-active sulfur compounds together with examples of their occurrence in thermally processed foods. The roasted smelling 2-acetylthiazole forms in various meats (beef, chicken, pork), salmon and asparagus during cooking, as well as in roasted sesame seeds. The typically boiled beef-like smelling 2-methyl-3-furanthiol is key for the aroma of all boiled meats but also contributes to coffee and sesame aroma. Sometimes, reported sulfur odourants actually do not occur in the analysed food but have been formed as artefact during analysis. Siegmund et al. (1997) proved that thialdine (1), which was previously reported in various types of meat, actually forms during simultaneous distillation-extraction of meat from the acetaldehyde, hydrogen sulfide and ammonia that were present in the meat aroma (Figure 9.1).

The following sections in this chapter discuss the principle chemical reactions leading to the formation of sulfur aroma compounds in heated foods. They include the Maillard reaction (i.e. the reaction of carbohydrates with amino compounds), Strecker degradation and the decomposition of thiamine. Review articles from Baines and Mlotkiewicz (1984) on meat flavour and from Tressl et al. (1993) on Maillard-derived aroma compounds serve as a good base for further reading on the subject.

Table 9.1 Odour thresholds of thiols and their corresponding alcohols (van Gemert, 2003)

Thiol	Odour threshold (µg/kg)	Alcohol	Odour threshold (µg/kg)
Ethanethiol	2	Ethanol	3500–190,000,000
2-Furanmethanethiol (furfural mercaptan)	0.005–0.12	2-Furanmethanol	1900–2000
3-Mercapto-2-butanone	3	3-Hydroxy-2-butanone	14–10,000
Methanethiol	0.02–4	Methanol	14–10,000
2-Phenylethanethiol	0.05 ^a	2-Phenylethanol	0.015–75,000
3-Methyl-2-buten-1-thiol (prenyl thiol)	0.0002–0.005	3-Methyl-2-buten-1-ol (prenyl alcohol)	250–7800
4-Mercapto-4-methyl-2-pentanone	0.0001–0.005	4-Hydroxy-4-methyl-2-pentanone	44,000–100,000
1- <i>p</i> -Menthen-8-thiol	0.0001 ^b	α-Terpineol	4.6–15,000

^aSchieberle (1996).

^bBuettner and Schieberle (2001).

9.2 The Maillard reaction

The Maillard reaction is one of the key pathways for aroma generation during thermal food processing (see [Chapter 8](#)). It plays a central role for sulfur aroma compound formation during cooking, for example, in meat or vegetables like tomato, asparagus and potato. [Table 9.3](#) lists some important sulfur odourants together with their most relevant precursors in food. Obviously, cysteine is a vital precursor for multiple odourants in the table. In fact, it is the most important precursor for Maillard reaction-derived sulfur volatiles. Other amino acids that are precursors are methionine and *S*-methylmethionine, a non-proteinogenic amino acid.

9.2.1 The Maillard reaction of cysteine

Model reactions are a common approach to study the formation pathways in the Maillard reaction. Hence, a large number of studies also looked at the formation of sulfur odourants in the Maillard reaction. [Mulders \(1973\)](#) identified thiazoles, pyrazines and thiophenes in a ribose/*L*-cysteine/cystine browning system. The proposed formation pathway for the heterocyclic odourant 2-acetylthiazole (**6**) is shown in [Figure 9.2](#). The carbon skeleton of methylglyoxal (**3**), a well-known Maillard intermediate, stems from the degradation of reducing sugars. **3** condenses with *L*-cysteine (**2**) to form the Schiff base, which undergoes decarboxylation and, upon cyclisation,

Table 9.2 Aroma-active sulfur compounds in thermally processed foods (examples)

Compound	Odour	Occurrence (examples)
2-Acetylthiazole	Roasted	Asparagus ^a , beef ^b , chicken ^c , pork ^d , salmon ^e , sesame ^f
Dimethyl sulfide	Sulfury, tomato-like	Asparagus ^a , potato ^g , tomato ^h
Dimethyl trisulfide	Onion-like	Beef ^b , coffee ⁱ , cocoa ^j , peanut ^k , pork ^d , potato ^g , sesame ^f , shallots ^l
2-Furanmethanethiol	Coffee-like	Beef ^b , chicken ^c , coffee ⁱ , peanut ^k , pork ^d , salmon ^e , sesame ^f
Methanethiol	Cabbage-like	Beef ^b , chicken ^c , peanut ^k , potato ^g , salmon ^e
Methional	Potato-like	Asparagus ^a , beef ^b , chicken ^c , coffee ⁱ , pork ^d , hazelnut ^m , peanut ^k , salmon ^e , sesame ^f , tomato ^h , potato ^g
2-Methyl-3-furanthiol	Meaty	Beef ^b , chicken ^c , coffee ⁱ , pork ^d , sesame ^f
2-Methyl-3-(methylthio)furan	Meaty	Beef ^b , cocoa ^j
Methyl 1-propenyl sulfide	Garlic-like	Shallots ^l
4-Methyl-3-thiazoline	Earthy, burnt	Sesame ^f
3-Mercapto-2-methyl-1-pentanol	Meaty, onion-like	Onion ⁿ

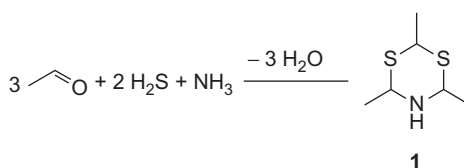
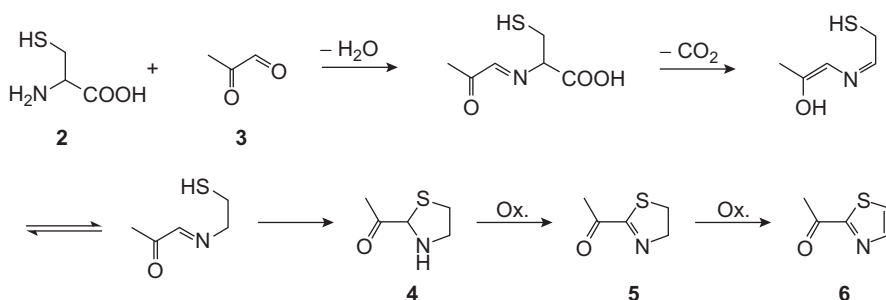
^aUlrich et al. (2001).^bGasser and Grosch (1988), Kerscher and Grosch (1997).^cGasser and Grosch (1990).^dGasser and Grosch (1991).^eMethven et al. (2007).^fTamura et al. (2010).^gMutti and Grosch (1999).^hButtery et al. (1990).ⁱGrosch et al. (1996).^jFrauentorfer and Schieberle (2006).^kChetschik et al. (2010).^lKrammer et al. (2006).^mBurdack-Freitag and Schieberle (2010).ⁿGranvogl et al. (2004).**Figure 9.1** Artefact formation of thialdine (**1**) during simultaneous distillation–extraction (Siegmund et al., 1997).

Table 9.3 Aroma-active sulfur compounds from Maillard reaction and their precursors in food

Compound	Precursor(s)
2-Acetylthiazole	Cysteine/monosaccharides
2-Acetyl-2-thiazoline	Cysteine/monosaccharides
Dimethyl sulfide	S-Methylmethionine
2-Furanmethanethiol	Cysteine/ribose
Methanethiol	Methionine/ monosaccharides
3-Mercapto-2-butanone	Cysteine/monosaccharides
2-Mercapto-3-pentanone	Cysteine/ribose, thiamine
3-Mercapto-2-pentanone	Cysteine/ribose
Methional	Methionine/ monosaccharides
2-Methyl-3-furanthiol	Thiamine, cysteine/ribose

**Figure 9.2** Formation of 2-acetylthiazole (6) from L-cysteine (2) and methylglyoxal (3) (Mulders, 1973).

gives 2-acetylthiazolidine (4). Oxidation of 4 ultimately produces 2-acetyl-2-thiazoline (5) and 2-acetylthiazole (6). Both 5 and 6 possess a roasted, popcorn-like aroma, and both occur in meat. The level of 2-acetyl-2-thiazoline was reported to be 14 $\mu\text{g/kg}$ in pressure-cooked chicken (Kerler and Grosch, 1997) and 28 $\mu\text{g/kg}$ in fried beef (Cerny and Grosch, 1993). Its formation can also be postulated by the reaction of cysteamine, the decarboxylation product of L-cysteine, with methylglyoxal and subsequent oxidation. A thermally reacted model system consisting of cysteamine and glucose actually produced both 2-acetyl-2-thiazoline and 2-acetylthiazole (Sakaguchi and Shibamoto, 1978) supporting this hypothesis.

A similar compound, 5-acetyl-3,6-dihydro-2H-1,4-thiazine, was identified in a heated solution of L-cysteine and ribose, together with 5 and 6. (Hofmann and Schieberle, 1995). It imparts a similar roasted, popcorn-like note. Figure 9.3 suggests its formation from L-cysteine (2) and 2,3-butanedione (7) as precursors. Analogous to the formation of 6, the first step is a condensation of L-cysteine and 7. The resulting Schiff base loses carbon dioxide, and subsequent ring-closure of the enamine leads to

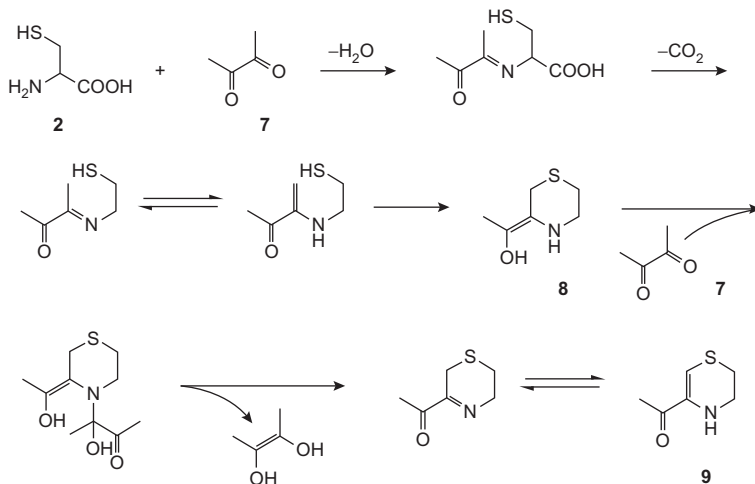


Figure 9.3 Formation of 5-acetyl-3,6-dihydro-2H-1,4-thiazine (**9**) from L-cysteine (**2**) and 2,3-butanedione (**7**) (Hofmann and Schieberle, 1995).

the heterocyclic compound **8**. Oxidation of **8** (for example by 2,3-butanedione) gives 5-acetyl-3,6-dihydro-2H-1,4-thiazine, which can tautomerise to **9**. The proposed pathway represents only a side reaction. The main reaction product from L-cysteine and 2,3-butanedione is by far 2-methyl-2-acetylthiazolidine (Huang et al., 1998). Compound **9** has not yet been identified in food (unlike **5** and **6**), but it occurs in commercial flavourings.

Both L-cysteine and ribose are constituents of raw meat and supposedly key precursors for cooked meat aroma. Consequently, numerous model reactions with these precursors are described in literature with the aim to elucidate aroma formation during cooking. The aqueous reaction of just these two precursors creates a complex mixture of a large number of volatiles. By narrowing down the analytical identification to odour-active compounds by techniques like gas chromatography/olfactometry and aroma extract dilution analysis (AEDA), Hofmann and Schieberle (1995) identified the following aroma-relevant sulfur molecules in the volatile fraction of a heated L-cysteine/ribose solution: 2-furanmethanethiol, 3-mercapto-2-pentanone, 2-methyl-3-furanthiol, 5-acetyl-3,4-dihydro-2H-1,4-thiazine, 3-mercapto-2-butanone, bis(2-methyl-3-furyl) disulfide, 2-formylthiophene, 2-acetyl-2-thiazoline, 1-mercapto-2-propanone, 2-methyltetrahydrothiophen-3-one and 3-methyl-3-thiophenethiol.

Experiments with isotopically labelled ribose provided evidence that, under aqueous conditions and at pH 5, the carbon skeleton of ribose remains intact in 2-methyl-3-furanthiol, 2-furanmethanethiol and 3-mercapto-2-pentanone, which are key odourants (Cerny and Davidek, 2003). In 3-mercapto-2-butanone, either the C1 or C5 moiety is split off from ribose (Cerny and Davidek, 2004). The formation of 1,4-dideoxypentose (**14**), the alleged key intermediate for 2-methyl-3-furanthiol and 2-furanmethanethiol from ribose and L-cysteine is hypothesised in Figure 9.4. The reaction of ribose (**10**) with L-cysteine produces the Schiff base **11** and, subsequently,

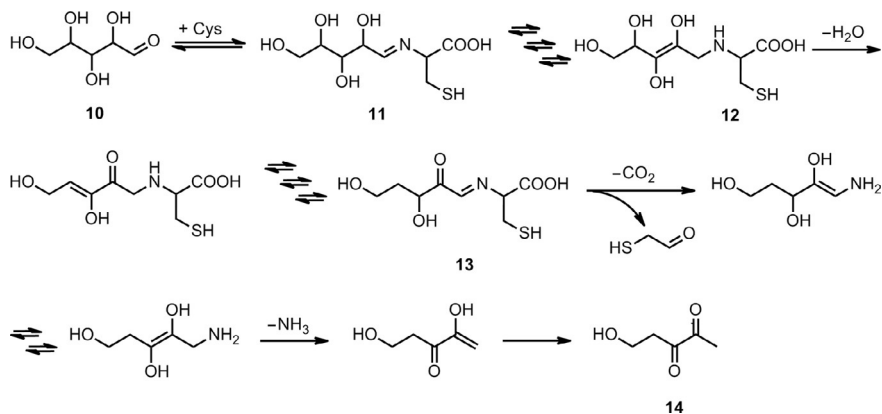


Figure 9.4 Formation of 1,4-dideoxypentose (14) from ribose (10) (Cerny and Davidek, 2003).

after rearrangements, the Amadori compound **12** in its enol form. Strecker degradation of the α -ketoimine **13** and further reactions finally lead to **14**. Compound **14** can add hydrogen sulfide (originating from L-cysteine) and lose water to give 5-hydroxy-3-mercapto-3-penten-2-one (**15**) as depicted in Figure 9.5. Cyclisation and dehydration result in 2-methyl-3-furanthiol (**16**). On the other hand, the Strecker reaction of **15** and subsequent reactions give rise to 3-mercapto-2-pentanone (**17**). It is noteworthy that the hypothetical pathway in Figure 9.5 explains the formation of **17** without the necessary concomitant formation of its isomer, 2-mercapto-3-pentanone. Hence, this pathway agrees with the experimental absence of 2-mercapto-3-pentanone in the

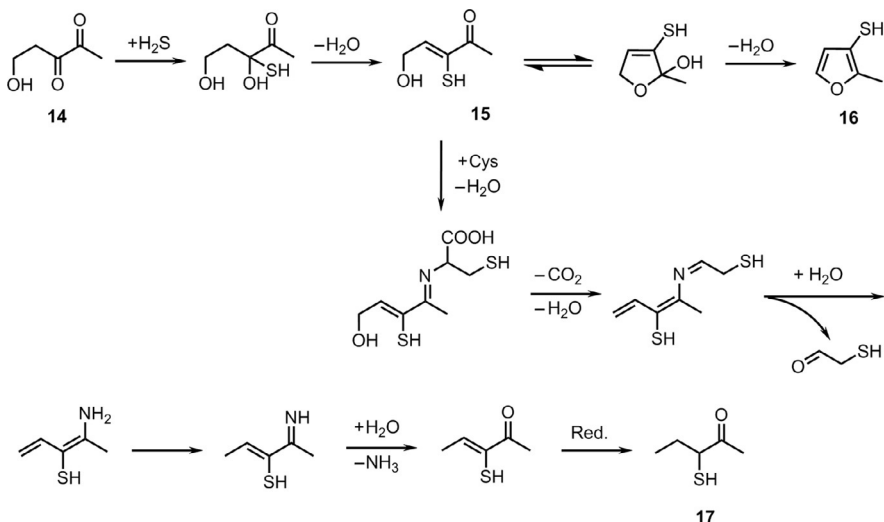


Figure 9.5 Formation of 2-methyl-3-furanthiol (**16**) and 3-mercapto-2-pentanone (**17**) from 1,4-dideoxypentose (**14**) (Cerny and Davidek, 2003).

reaction of ribose with L-cysteine at 95 °C, and pH 5 for 4 h (Cerny and Davidek, 2003).

An alternative formation pathway to α -mercaptoketones passes through dicarbonyl compounds, which are known intermediates in the Maillard reaction. These can add hydrogen sulfide (originating from L-cysteine decomposition) and then undergo reduction. Actually, 3-mercapto-2-butanone was found in the model reaction of hydrogen sulfide with 2,3-butanedione, and 3-mercapto-2-pentanone and 2-mercapto-3-pentanone in the reaction with 2,3-pentanedione (Mottram et al., 1995). Additionally, corresponding disulfides were formed. Apparently, reaction conditions determine the prevailing formation pathway. In the Maillard reaction of L-cysteine with pentoses, the reaction pathway controls whether 2-mercapto-3-pentanone is formed in addition to 3-mercapto-2-pentanone. For example, in the reaction of L-cysteine with molar excess of ribose (molar ratio 1:3), either at 95 °C for 4 h (Cerny and Davidek, 2003) or at 145 °C for 20 min (Hofmann and Schieberle, 1995), only 3-mercapto-2-pentanone is produced and the formation corresponds to the pathway in Figure 9.5. In contrast, in the reaction of equimolar amounts of L-cysteine and ribose (at 140 °C for 30 min), both isomers are formed (Mottram and Nobrega, 2002) and the reaction pathway via 2,3-pentanedione appears dominant.

Contrarily to aqueous conditions, dry roasting (145 °C) of L-cysteine/ribose afforded 2-acetyl-2-thiazoline (**5**) and 2-furanmethanethiol as odourants with the highest aroma impact and not 2-methyl-3-furanthiol (Hofmann and Schieberle, 1998a).

Most of the ribose in meat occurs in bound form, i.e., inosine 5'-monophosphate (IMP). When ribose in the Maillard reaction with L-cysteine was replaced by IMP, the same sulfur compounds (including 2-methyl-3-furanthiol, 2-furanmethanethiol and α -mercaptoketo compounds) dominated the volatiles (Mottram and Nobrega, 2002). However, the IMP system produced much lower quantities. IMP is the *N*-glycoside of ribose 5-phosphate with inosine. Consequently, it requires hydrolysis first to release ribose and become active towards Maillard reaction. However, IMP is fairly stable in aqueous solution and, hence, less efficient in producing meat aroma. In contrast, ribose 5-phosphate produces larger quantities of volatiles with L-cysteine than ribose.

Furfural is thought to be the key intermediate for 2-furanmethanethiol. Furfural is nearly always detected in reactions of L-cysteine with pentoses. Therefore, it is no surprise that 2-furanmethanethiol was identified in the reaction product of furfural with hydrogen sulfide and ammonia (Shibamoto, 1977). Most likely, the thiol contributes to its sweet, roast beef-like odour. When furfural was replaced by glucose, furfural and 2-furanmethanethiol were still detected, suggesting that glucose can likewise serve as a precursor for furfural and 2-furanmethanethiol, even though glucose is less effective as a precursor than pentoses (ribose, xylose), as shown by Hofmann and Schieberle (1998b).

In fact, 2-furanmethanethiol, together with 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine, was among the most important aroma compounds identified in a thermally treated solution of L-cysteine and glucose and played a more important role than 2-methyl-3-furanthiol, which is key in aqueous L-cysteine/pentose systems (Hofmann and

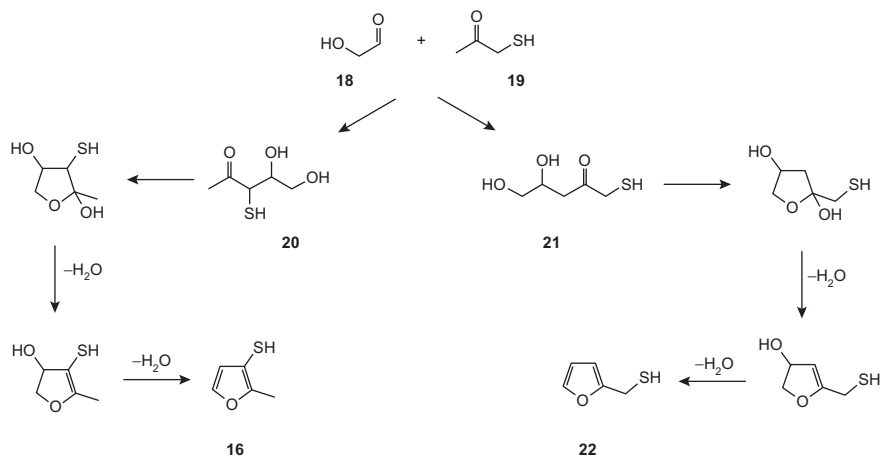


Figure 9.6 Formation of 2-methyl-3-furanthiol (**16**) and 2-furanmethanethiol (**22**) from glycolaldehyde (**18**) and mercaptopropanone (Hofmann and Schieberle, 1998) (**19**).

Schieberle, 1997). Although glucose is a less efficient precursor (compared to pentoses) for the sulfur aroma compound 2-methyl-3-furanthiol (**16**), it still gives significant yields (Hofmann and Schieberle, 1998b). The authors propose formation pathways (Figure 9.6) for both 2-methyl-3-furanthiol (**16**) and 2-furanmethanethiol (**22**) via hydroxyacetaldehyde (**18**) and mercaptopropanone (**19**), two intermediates that are known to occur in heated L-cysteine/glucose systems. The formation of 2-methyl-3-furanthiol (**16**) starts with aldol reaction of hydroxyacetaldehyde (**18**) and mercaptopropanone (**19**) to give 4,5-dihydroxy-3-mercapto-2-pentanone (**20**). The intermediate **20** cyclises and dehydrates to yield 2-methyl-3-furanthiol (**16**). The alternative aldol reaction of **18** and **19** produces 4,5-dihydroxy-1-mercapto-2-pentanone (**21**) and, after few more steps, 2-furanmethanethiol (**22**). Other important molecules for the aroma of the heated aqueous L-cysteine/glucose solution are 3-mercapto-2-pentanone (**17**), 3-mercapto-2-butanone, mercaptopropanone (**19**), 2-acetylthiazole (**6**), 2-(1-mercaptoethyl)thiophene, 2-formylthiophene, 2-thienylmercaptan, 2-propionylthiazole, 2-acetyl-2-thiazoline (**5**), 2-acetylthiophene, 5-methyl-2-formylthiophene, 2-mercaptopropionic acid and various disulfides.

Dry heating of L-cysteine/glucose instead of an aqueous reaction promotes 2-acetyl-2-thiazoline, 2-propionyl-2-thiazoline and 2-furanmethanethiol, which become key for the aroma (Hofmann and Schieberle, 1998a). Dry heating of L-cysteine systems seems to favour the formation of thiazolines. Umamo et al. (1995) observed 2-methylthiazolidine as the major volatile in a dry-heated L-cysteine/glucose mixture, although this most probably has only a minor impact on the flavour. Possibly it is formed via a reaction of the intermediate acetaldehyde with cysteamine originating from L-cysteine decarboxylation.

Natural beef broth contains 4-hydroxy-5-methyl-3(2*H*)-furanone (Tonsbeek et al., 1968). Reacting it with hydrogen sulfide generates a complex mixture with roasted meat-like odour (van den Ouweland and Peer, 1975). Numerous mercapto-substituted

furan and thiophene derivatives are formed, including 2-methyl-3-mercapto-4,5-dihydrofuran and 2-methyl-3-thiophenethiol (the thiophene analogue of 2-methyl-3-furanthiol). 4-Hydroxy-5-methyl-3(2*H*)-furanone was reported as an important aroma intermediate in the Maillard reaction, in particular in reactions with pentoses as educts. Reactions with 4-hydroxy-5-methyl-3(2*H*)-furanone (Whitfield and Mottram, 1999, 2001), carried out at pH 4.5 or 6.5 in the presence of either hydrogen sulfide or L-cysteine, gave caramel-like, roasted-smelling products with complex volatile profiles. Still, complex but different volatile profiles were obtained depending on the pH conditions. At pH 4.5, sulfur compounds dominated the volatile fraction, whereas with pH 6.5 nitrogen compounds prevailed. Among the sulfur compounds were 3-mercapto-2-pentanone and 2-mercapto-3-pentanone, demonstrating 4-hydroxy-5-methyl-3(2*H*)-furanone as a suitable precursor for both isomers. The authors propose a formation pathway involving 2,3-pentanedione.

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone is a homologue of 4-hydroxy-5-methyl-3(2*H*)-furanone and has a caramel-like tonality. It is a key odourant in cooked meat and many other thermally treated foods. It can also act as an intermediate and react further, for example, with L-cysteine. The main volatiles from the reaction of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone with L-cysteine at pH 5.1 are 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophene and 3,5-dimethyl-1,2,4-trithiolane (Shu and Ho, 1988). The formation of the latter can be explained by the reaction of acetaldehyde with hydrogen sulfide originating from L-cysteine. In the same reaction at pH 7.1, heterocyclic compounds (thiazoles, thiazolines, pyrazines and others) dominate. When L-cysteine is thermally reacted alone at pH 2.3, 1,2,3-trithia-5-cycloheptene and 2-thiophenethiol are major compounds. At pH 5.1, several 3,5-dimethyl-1,2,4-trithiolane isomers are formed (Shu et al., 1985). These findings suggest that both acetaldehyde and hydrogen sulfide can originate from L-cysteine.

The pH value plays a crucial role among various other factors for the volatile profile of Maillard reaction products. The pH value of matured meat is generally around 5.6. Modifying this pH will change the flavour after cooking, as shown by Madruga and Mottram (1995). Minced meat that was previously adjusted to pH 4.0–5.6 prior to cooking produced a higher number of volatiles at lower pH. The meat cooked at pH 5.6 had a natural, boiled meat, fatty aroma, but a more kidney and liver-like odour was obtained with pH 4.0. At low pH, levels of 2-methyl-3-furanthiol and 2-furanmethanethiol are, in general, higher. Consistent with these findings, model reactions between L-cysteine and ribose at pH 4.5–6.5 showed drastically reduced levels at pH 6.5 for the sulfur odourants 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol as well as for their corresponding disulfides (Farmer and Mottram, 1990). Higher pH produced more thiazoles (e.g. 2-acetylthiazole). The aforementioned thiols are constituents of the volatile fraction of boiled beef (Farmer and Patterson, 1991; Gasser and Grosch, 1988). The reduction in thiol formation at high pH is in agreement with the common observation that so-called dark-cutting meat, which has a high pH (caused by pre-slaughter stress of the animal and consequently reduced formation of lactic acid post-slaughtering), is low in meat aroma. The increased formation of thiols at low pH was also observed by Cerny and Briffod (2007) who found that formation of 2-furanmethanethiol, 3-mercapto-2-pentanone

and 4,5-dihydro-2-methyl-3-furanthiol was favoured in heated aqueous model systems of L-cysteine, xylose and thiamine with decreasing pH from 7 to 4. Additional experiments with isotopically labelled xylose showed that the 2-furanmethanethiol carbons stemmed exclusively from xylose, and 4,5-dihydro-2-methyl-3-furanthiol and 3-mercapto-2-pentanone primarily from thiamine.

9.2.2 Interaction of the Maillard reaction and lipid oxidation

Both the Maillard reaction and lipid oxidation take part in aroma generation in most heated foods that contain precursors for both reactions, amino acids/reducing sugars and lipids (such as unsaturated fatty acids and phospholipids). Both reactions influence each other by either suppressing or enhancing effects or even yielding new compounds that are formed neither by lipid oxidation nor the Maillard reaction alone. Farmer et al. (1989) found that a solution of L-cysteine and ribose changed its odour from sulfury, rubbery to more cooked beef-like when egg yolk phospholipid was present during heating. Sulfur compounds such as 2-methyl-3-furanthiol, 3-mercapto-2-pentanone and 2-furanmethanethiol predominated in the absence of phospholipid. Additional phospholipids resulted in the formation of lipid degradation products as well as compounds formed by the interaction of intermediates from the Maillard reaction and lipid oxidation. Examples are various alkyl thiophene derivatives, alkanethiols (e.g. 1-heptanethiol, 1-octanethiol) and 2-pentylpyridine.

The level of disulfides with a 2-methyl-3-furylthio moiety decreased in a heated L-cysteine/ribose solution in the presence of egg phospholipids compared to the lipid-free system (Farmer and Mottram, 1990). These disulfides exhibit, in general, meaty, roasted aromas and their formation is influenced by phospholipids. Other heterocyclic compounds are quenched, too, by the presence of lecithin in the Maillard reaction (Whitfield et al., 1988). Also, unsaturated fatty acids or fatty acid esters impeded the formation of many volatiles including 2-methyl-3-furanthiol and 2-furanmethanethiol in a heated aqueous L-cysteine/ribose system (Elmore et al., 2002). Possibly, hydrogen sulfide reacts with lipid degradation products and is, therefore, unavailable for further reaction with Maillard reaction intermediates, resulting in lower levels of typical Maillard sulfur volatiles. The authors also observed a modified pattern of lipid oxidation products. Alcohols (e.g. pentanol) and alkylfurans (e.g. 2-pentylfuran) were preferably formed in Maillard/lipid systems at the expense of saturated and unsaturated aldehydes.

Elmore and Mottram (2000) observed the formation of 2-alkylthiophenes (alkyl = C₂–C₇) and alkylthiapyrans (alkyl = C₁–C₅) in cooked lamb. They proposed the formation pathway shown in Figure 9.7, starting with the addition of hydrogen sulfide to the C-4 carbon of 2,4-alkadienal **23**, followed by the nucleophilic attack of the sulfur on the aldehyde group to form the intermediate **24** and loss of water to yield the alkylthiophene **25**. The attack to the C-4 carbon is unusual because it does not follow the general Michael reaction mechanism. On the other hand, the analogical formation of alkylthiapyrans (**26**) starts with the Michael addition of hydrogen sulfide to the C-5 carbon of **23** and subsequent reaction to **26**. The first reaction step

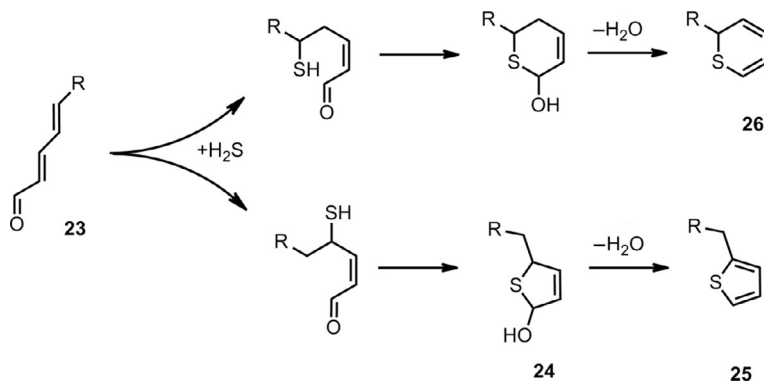


Figure 9.7 Formation of 2-alkylthiophenes (**25**) and 2-alkylthiapyrans (**26**) from 2,4-alkadienals (**23**) and hydrogen sulfide (Motttram and Elmore, 2002).

implicates the isomerisation of the $\text{C2}=\text{C3}$ double bond of **23**, possibly via keto-enol tautomerism.

Various C_3 – C_9 alkyl-substituted thiazoles and 3-thiazolines are the results of the Maillard reaction interacting with lipid oxidation (Elmore et al., 1997). These molecules have been previously identified in cooked beef. The authors explain their formation by the reaction of aldehydes that are intermediates from lipid oxidation with ammonia, hydrogen sulfide and α -dicarbonyl compounds coming from the Maillard reaction. This hypothesis is based on results from corresponding model reactions, which produced the expected thiazoles and 3-thiazolines. Figure 9.8 illustrates the proposed formation pathway. The reaction of hydrogen sulfide with a dicarbonyl (**27**) and reduction produces α -mercaptoketone **28**. The imine **29**, formed from an aldehyde and ammonia, reacts then with **28** resulting in a thioaminal. Ring closure and loss of water lead to the 3-thiazoline **30** and the corresponding thiazole **31** after

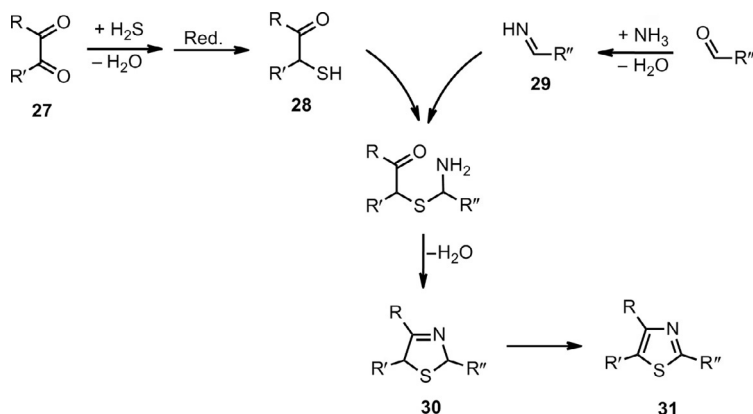


Figure 9.8 Formation of 3-thiazolines (**30**) and thiazole derivatives (**31**) from α -dicarbonyls (**27**), ammonium sulfide and lipid-derived aldehydes (Elmore and Motttram, 1997).

oxidation. It is unclear if 4-methyl-3-thiazoline, which was identified by Schieberle (1996), forms by a similar mechanism.

Unlike in aqueous systems, the addition of phosphatidylcholine has little effect on the volatile profile of a dry-reacted or a low-moisture-reacted mixture of L-cysteine and ribose (Mottram and Whitfield, 1995). Only small amounts of lipid degradation products were formed under these conditions.

9.2.3 Stability of sulfur compounds

The stability of thiols and other sulfur aroma compounds in the respective food after thermal treatment is of paramount importance for the shelf life of food and for whether we still can perceive them when we actually consume the food. Odour-active thiols such as 3-mercapto-2-butanone, 2-methyl-3-furanthiol and 2-furanmethanethiol are slowly oxidised to the corresponding disulfides when dissolved in organic solvents (Hofmann et al., 1996). For example, 2-methyl-3-furanthiol in diethyl ether is reduced by 30% after one day at 6 °C with concomitant formation of bis(2-methyl-3-furyl) disulfide. The 2-methyl-3-furanthiol that is part of a process flavour, decreases by 59% within one day at 50 °C, followed by 2-furanmethanethiol with 28% reduction (van Seeventer et al., 2001). The authors propose a polymerisation mechanism to explain the degradation of 2-methyl-3-furanthiol caused by the electrophilic nature of 2-methyl-3-furanthiol that is protonated in 2-position and then can readily react with a nucleophile. L-Cysteine had a stabilising effect on the thiols.

Thiols are particularly instable in coffee beverages. This explains why brewed coffee aroma rapidly changes in the fresh brew and loses its attractiveness. This is largely due to the quick degradation of 2-furanmethanethiol (75% after 60 min in a thermos flask) and other thiols (Hofmann and Schieberle, 2002). The coffee-like smelling 2-furanmethanethiol (**22**) is irreversibly covalently bound to pyrazinium radical cations (**32**) in the coffee brew. **32**-like compounds are part of the coffee melanoidins and are formed by the Maillard reaction of protein-bound lysine. The binding mechanism is illustrated in Figure 9.9.

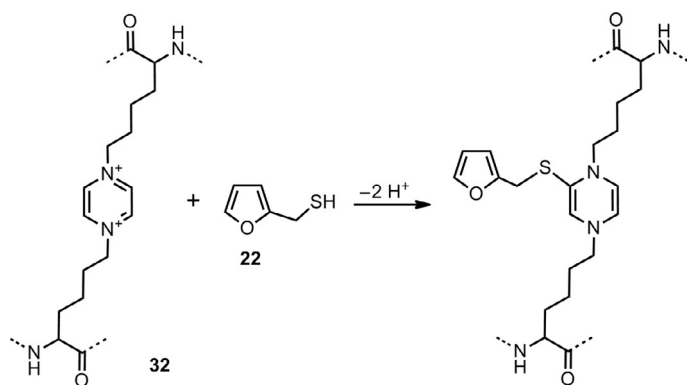


Figure 9.9 Irreversible binding of 2-furanmethanethiol (**22**) to pyrazinium Maillard intermediates (**32**) from coffee melanoidins (Hofmann and Schieberle, 2002).

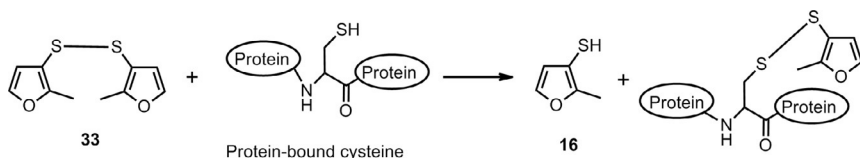


Figure 9.10 Formation of 3-methyl-3-furanthiol (**16**) from its disulfide (**33**) and egg albumin (Mottram et al., 1996).

Like thiols, disulfides show reduced stability in food. The corresponding thiols are formed when disulfides are heated in water, supposedly by hydrolysis (Guth et al., 1995). In food, disulfides can additionally interact with the food protein. When disulfides were heated in solution with egg albumin, conversion into the corresponding thiols 2-methyl-3-furanthiol, 2-furanmethanethiol occurred, triggered by the redox reaction with protein-bound L-cysteine (Mottram et al., 1996). The authors propose an interchange of disulfide and the thiol groups of the protein as depicted in Figure 9.10. An example is bis(2-methyl-3-furyl) disulfide (**33**), which releases 2-methyl-3-furanthiol (**16**).

9.2.4 Cysteine S-conjugates from the Maillard reaction

Cysteine S-conjugates occur in a variety of fruit and vegetables (passion fruit, bell pepper, onion and others) and are known to release the corresponding thiols during consumption of the food (Starkenmann et al., 2008). The release is enzymatic and triggered by the oral microflora. The L-cysteine S-conjugates of 2-methyl-3-furanthiol (**38**) and of 2-furanmethanethiol (**37**) have recently been discovered in the non-volatile fraction of a thermally reacted L-cysteine/xylose solution (Cerny and Guntz-Dubini, 2013). Both conjugates undergo enzymatic cleavage by oral microflora when aqueous solutions are tasted and release the corresponding odourant thiols, which cause meaty and roasted odour impressions. The formation of **37** and **38** in the Maillard reaction from xylose (**34**) and L-cysteine is hypothesised in Figure 9.11.

Furanmethanol (**35**), a Maillard intermediate from xylose, undergoes a nucleophilic attack from L-cysteine and produces S-(2-furfuryl)-L-cysteine (**37**).

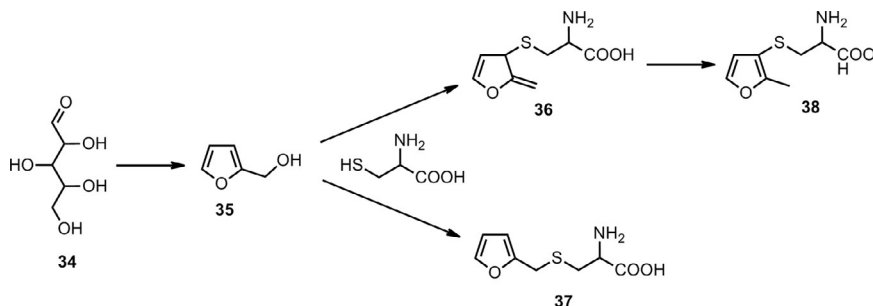


Figure 9.11 Formation of cysteine-S-conjugates **37** and **38** from xylose (**34**) and L-cysteine (Cerny and Guntz-Dubini, 2013).

Nucleophilic attack at the 3-position of **35** results in the intermediate **36** and via rearrangement in *S*-(2-methyl-3-furyl)-L-cysteine (**36**).

9.3 The Strecker degradation

The Strecker degradation occurs during the thermal treatment of food and is important for the creation of several aroma impact odourants. It is named after Adolph Strecker, who observed the formation of purple colour, carbon dioxide and an aldehyde when reacting the triketo compound alloxan (2,4,5,6-pyrimidinetetrone) with alanine or leucine (Strecker, 1862). The resulting aldehydes are acetaldehyde and 3-methylbutanal, respectively. The Strecker degradation in food is often associated with the Maillard reaction because the latter provides dicarbonyl compounds (e.g. deoxyosones, 2,3-butanedione, 2,3-pentanedione) for the reaction with amino acids. L-Cysteine and methionine are the two only sulfur-containing protein-derived amino acids. The Strecker degradation with methionine (**39**) is illustrated in Figure 9.12. After formation of the Schiff base **40** and decarboxylation, an intermediate is formed that exists in two tautomeric forms, **41** and **42** (Figure 9.12a). Hydrolysis of the keto form (**42**) yields the Strecker aldehyde methional (**44**) and the α -aminoketone **43** (Figure 9.12b). Methional can undergo subsequent degradation to acrolein (**45**) and methanethiol (**46**). Methanethiol easily oxidises to dimethyl disulfide (**47**). Recently,

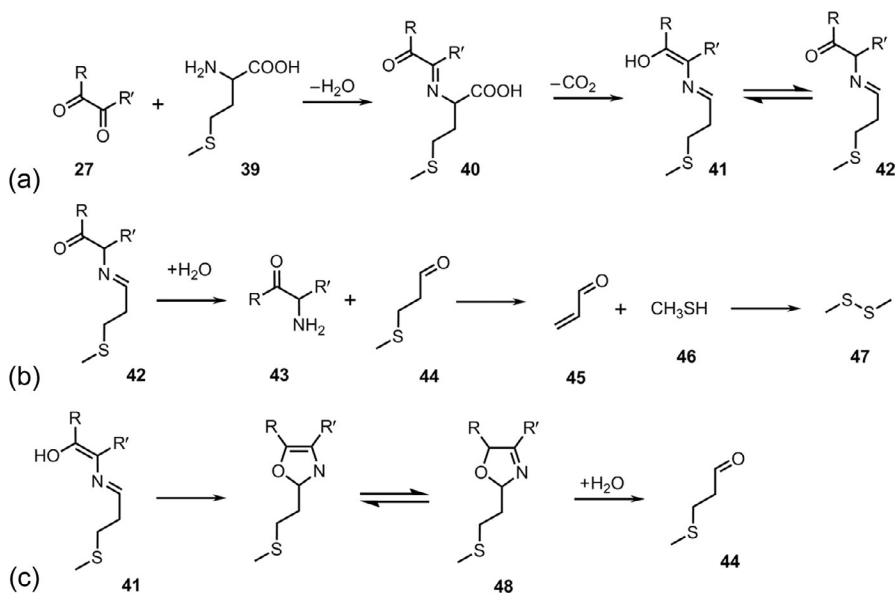


Figure 9.12 Strecker reaction of methionine: (a) Formation of the decarboxylated Schiff base intermediate **42**; (b) Formation of methional (**44**) under aqueous conditions and subsequent decomposition to methanethiol (**46**) and dimethyl disulfide (**47**); (c) Formation of 3-oxazoline derivative **48** under dry conditions and release of methional (**44**) in the presence of water (analogous to Granvogl et al., 2012).

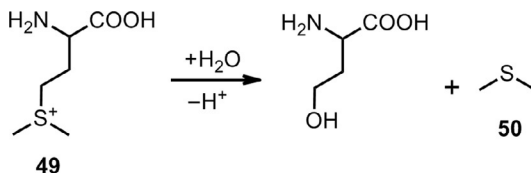


Figure 9.13 Formation of dimethyl sulfide (**50**) from *S*-methylmethionine (**49**) (Scherb et al., 2009).

Buhr et al. (2010) discovered that foods that had undergone a dry heating process (e.g. roasting of malt, cocoa) release aldehydes when exposed to water, as it is the case during mastication. For example, the concentration of methional increased 100 times when malt was watered prior to solvent extraction and analysis. Recent studies identified 3-oxazolines (**48**) as effective in-mouth precursors for the respective aldehyde (Granvogl et al., 2012). They are formed during the Strecker degradation under dry conditions by ring closure of the enol Strecker intermediate **41** (Figure 9.12c). An alternative formation pathway for 3-oxazoline derivatives encompasses the oxidative decarboxylation of Amadori compounds (Granvogl et al., 2012). The Amadori compound of methionine and glucose after oxidation and decarboxylation (**41**; $\text{R}' = \text{H}$ and $\text{R} = \text{erythrosyl}$) results in the corresponding 3-oxazoline **48**. The erythrosyl (1,2,3,4-tetrahydroxybutyl) radical R' corresponds to the C3–C6 fragment of glucose. The Amadori compound can be efficiently generated by a mild reaction from methionine and glucose (40 °C and 15 days), preferably in the presence of high sucrose levels (Cerny et al., 2011).

The *S*-methylated form of methionine, *S*-methylmethionine (**49**) is a non-proteinogenic amino acid that occurs in various vegetables, but also in malt. Its content is 2.8 mg/kg in tomatoes, 6 mg/kg in malt, 81 mg/kg in cabbage, 176 mg/kg in celery and 53–252 mg/kg in asparagus (Scherb et al., 2009). Its thermal degradation (Figure 9.13) produces dimethyl sulfide (**50**). With its sulfurous odour **50** contributes strongly to the aroma of the foods mentioned above. Excessive levels in malt cause an off-note.

9.4 Thiamine degradation

The furan derivative 2-methyl-3-furanthiol (**16**), as well as its disulfide, belongs to the aroma impact compounds of boiled beef and other meats conferring a meaty odour note to it. The previous section highlighted the Maillard reaction of *L*-cysteine and ribose as an efficient mechanism to generate this odourant. However, comparative studies with low precursor concentrations approximating those in raw meat spotted thiamine (**51**) as the more relevant precursor in meat (Grosch et al., 1993). Boiling an aqueous solution of *L*-cysteine (0.65 mM) and ribose (1.34 mM) produced only 0.2 µg/L 2-methyl-3-furanthiol. However, when ribose was replaced with thiamine at a very low concentration (0.06 mM), the yield increased 1500 times to 300 µg/L. Consequently, the most important precursor for **16** in meat aroma appears to be thiamine, in combination with *L*-cysteine, which serves as a hydrogen sulfide source.

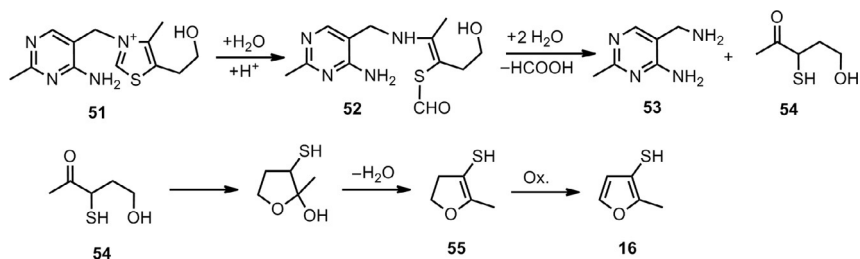


Figure 9.14 Formation of 2-methyl-3-furanthiol (**16**) from thiamine (**51**) via 5-hydroxy-3-mercapto-2-pentanone (**54**) (Van der Linde et al., 1978).

Under acidic conditions, as in meat, the thiazole ring of thiamine (**51**) hydrolyses. The intermediate **52** loses formic acid and splits into the primary degradation products **53** and 5-hydroxy-3-mercapto-2-pentanone (**54**), as illustrated in the upper part of Figure 9.14. Van der Linde et al. (1978) postulated **54** as key intermediate for 4,5-dihydro-2-methyl-3-furanthiol (**55**) and 2-methyl-3-furanthiol. Ring closure of **54** followed by dehydration results in **55** and by subsequent oxidation, 2-methyl-3-furanthiol. Guentert et al. (1993, 1994) emphasised the key role of 5-hydroxy-3-mercapto-2-pentanone as an aroma intermediate as well, for numerous other identified sulfur volatiles (mercaptoketones, thianes, dithianes, thiolanes, dithiolanes and others). Interestingly, **54** was only recently identified and characterised by mass spectrometry and NMR after its isolation from a thiamine model reaction (Cerny and Guntz-Dubini, 2008).

In alkaline conditions, a hydroxide ion attacks thiamine at the carbon atom linking both heterocycles (Figure 9.15). The pyrimidine derivative **56** and 5-(2-hydroxyethyl)-4-methylthiazole (sulfurol) (**57**) are formed as primary degradation products (Van der Linde et al., 1978). Sulfurol was repeatedly claimed as an important odourant from thiamine degradation and has therefore found use as an ingredient in the flavour industry. However, freshly prepared sulfurol has only weak odour (Stoll et al., 1967). Rather, impurities like 4-methyl-5-vinylthiazole (**58**), a known degradation product from thiamine, account for the reported nutty odour often misattributed to sulfurol itself. Sulfurol qualities with reported meat-like odour possibly contain impurities like 4,5-dihydro-2-methyl-3-furanthiol (**55**), a by-product from certain sulfurol syntheses

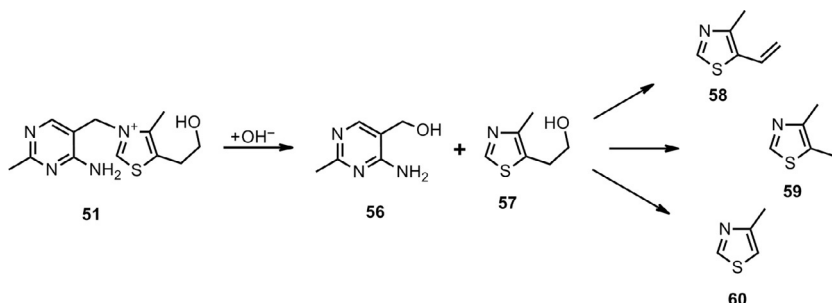


Figure 9.15 Formation of thiazoles (**58–60**) from thiamine (**51**) (Guentert et al., 1990).

with the sulfury, meat-like smell. At pH 8, thiamine degradation still produces 2-methyl-3-furanthiol (**16**) and 4,5-dihydro-2-methyl-3-furanthiol (**55**), according to the mechanism shown in Figure 9.14. However, thiazole derivatives like 4-methyl-5-vinylthiazole (**58**), 4,5-dimethylthiazole (**59**) and 4-methylthiazole (**60**), which are derived from sulfurol, become more important (Guentert et al., 1990).

9.5 *Allium* species

The aroma of bulbs from the *Allium* species, like onion (*A. cepa* L.) and garlic (*A. sativum* L.), is largely dominated by sulfur odourants. The main precursors are *S*-alk(en)yl-L-cysteine sulfoxides (Figure 9.16). *S*-Allyl-L-cysteine sulfoxide (**61**) is the main *S*-alkenyl-L-cysteine sulfoxide in garlic (up to 2.4 g/kg), while *S*-1-propenyl-L-cysteine sulfoxide (**62**) (≈ 2 g/kg) dominates in onion (Whitaker, 1976). *S*-Methyl-L-cysteine sulfoxide (**63**) and *S*-propyl-L-cysteine (**64**) belong to both species. *S*-Methyl-L-cysteine sulfoxide occurs also in the *Brassicaceae* species, like cabbage, cauliflower and broccoli. Apart from L-cysteine sulfoxide derivatives, *S*-alkyl-L-cysteines and *S*-alkenyl-L-cysteines occur in onion and garlic, for example, *S*-allyl-L-cysteine in garlic.

After crushing or cutting an onion or garlic bulb, the non-thermal aroma generation of sulfur volatiles starts as soon as the enzyme alliinase comes in contact with *S*-alk(en)yl-L-cysteine sulfoxides generating sulfenic acids and, subsequently, alkylthiosulfonates (Belitz et al., 2009; Block, 1992). Figure 9.17 shows the reaction cascade with *S*-1-propenyl-L-cysteine sulfoxide (**62**) as an example. Alliinase catalyses the loss of ammonia and pyruvate from **62** to build the unstable 1-propenyl sulfinic acid (**65**). Isomerisation of **65** produces propanthial *S*-oxide (**66**), the lachrymogenic factor in onion. On the other hand, disproportionation of **65** gives 1-propenyl sulfinic (**67**) and 1-propenylthiol (**68**), which combine to form 1-propenyl 1-propenethiosulfinate (**69**) and, through subsequent oxidation, to 1-propenyl 1-propenethiosulfonate (**70**), which plays a role in raw onion aroma (Belitz et al., 2009). Thiosulfinates and thiosulfonates are rather unstable molecules and change during cooking causing, together with other chemical reactions, a complex mixture of volatiles. In cooked onion, propyl and 1-propenyldisulfide (**71**) are formed by oxidation of thiols, e.g., 1-propenylthiol (**68**) and are important for the aroma. In garlic, allyl disulfide dominates.

Another thiol, 3-mercapto-2-methylpentan-1-ol (**72**), was recently identified as a very potent aroma compound in onion. Its concentration in raw onions (8–32 $\mu\text{g/kg}$), which is already high above its odour threshold (0.15 $\mu\text{g/L}$), increases during cooking to 34–246 $\mu\text{g/kg}$ (Granvogl et al., 2004). A plausible formation of the molecule via

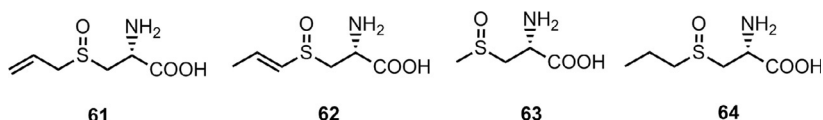


Figure 9.16 *S*-Alk(en)yl-L-cysteine sulfoxides in onion and garlic.

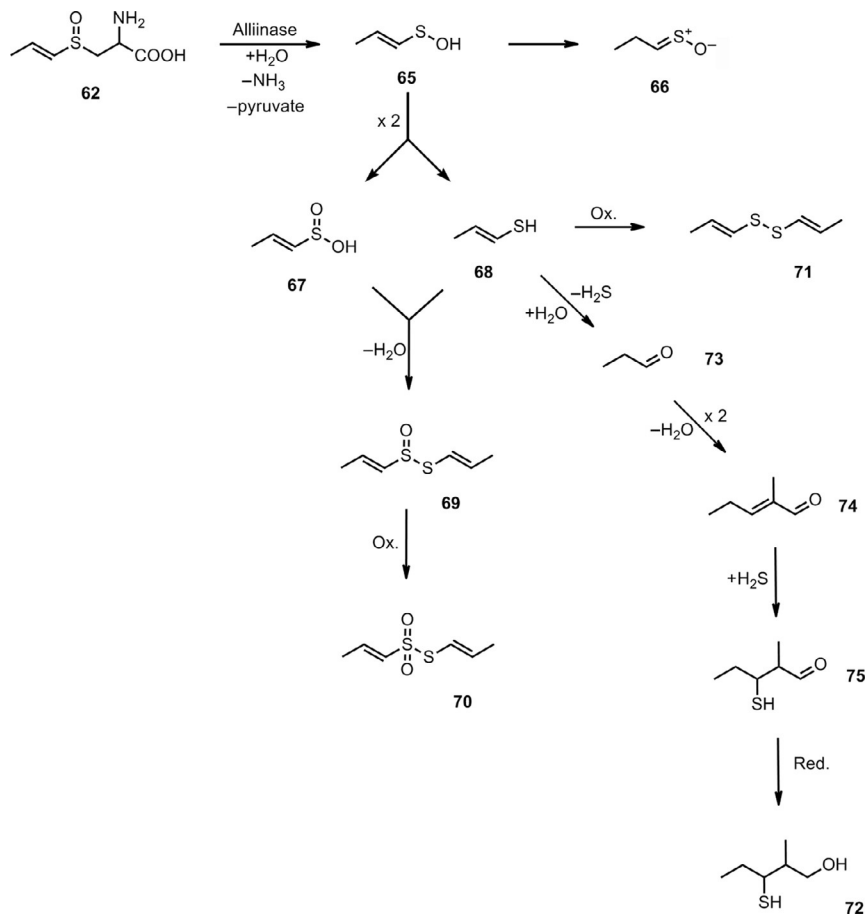


Figure 9.17 Formation of aroma compounds from S-(1-propenyl)-L-cysteine sulfoxide.

aldol condensation of propanal (**73**) and addition of hydrogen sulfide to the resulting intermediate (**74**), followed by reduction of the resulting 3-mercapto-2-methylpentanal (**75**), is shown in Figure 9.17.

It was mentioned before that S-alk(en)yl-L-cysteine derivatives occur in the *Allium* species alongside L-cysteine sulfoxides. S-Allyl-L-cysteine in garlic (Lawson and Gardner, 2005) possibly contributes to aroma formation during cooking. The thermal reaction of S-allyl-L-cysteine with glucose produces a garlic-like and green aroma with diallyl sulfide and diallyl disulfide as main volatiles (Kimura et al., 1990). Dipropyl disulfide was mainly formed when S-allyl-L-cysteine was replaced by S-propyl-L-cysteine.

The relevant sulfur odourants of fried shallots (*A. ascalonicum*) were studied by Krammer et al. (2006). AEDA showed methyl propenyl sulfide, methyl propenyl disulfide, methyl propenyl trisulfide, dimethyl trisulfide, propenyl propyl disulfide and methyl propyl trisulfide to be aroma-relevant. Adding garlic to shallots during frying triggered the additional formation of 2-vinyl-(4*H*)-1,3-dithiine (green, pungent, garlic-like) and diallyl trisulfide (leek-like, garlic-like) by degradation of

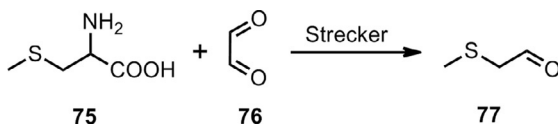


Figure 9.18 Formation of 2-methylthioacetaldehyde from *S*-methylcysteine.

allylthiosulfonate (allicin). Allicin itself is made responsible for the lingering, sulfury, onion-like aftertaste (Krammer et al., 2006).

Possibly, the Strecker degradation of *S*-alkyl-L-cysteines and sulfoxides plays a role in aroma formation during cooking of alliaceous and other vegetables (Roessner et al., 2000). The reaction of *S*-methyl-L-cysteine (**75**) with glyoxal (**76**) produced the Strecker aldehyde 2-methylthioacetaldehyde (**77**), an odourant with a burnt, roasted, pungent smell (Figure 9.18). **77** also occurs in cooked broccoli (0.5 mg/kg). Likewise, the Strecker degradation of *S*-allyl-L-cysteine produced 2-allylthioacetaldehyde, which occurs in cooked garlic (1 mg/kg).

9.6 Roasted sesame seeds

The roasted seeds of sesame (*Sesamum indicum* L.) effuse a pleasant roasted, burnt, sulfury aroma. AEDA and quantification experiments evidenced that it is largely characterised by the sulfur molecules 2-furanmethanethiol (**22**), 2-phenylethanethiol (**78**) and by 2-acetyl-1-pyrroline and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Schieberle, 1996). Remarkably, 2-furanmethanethiol stems from a water-insoluble precursor of unknown structure and not from the Maillard reaction system L-cysteine/ribose. More recent research on the aroma-relevant compounds in pan-roasted sesame seeds showed the compounds depicted in Figure 9.19 to have high FD factors

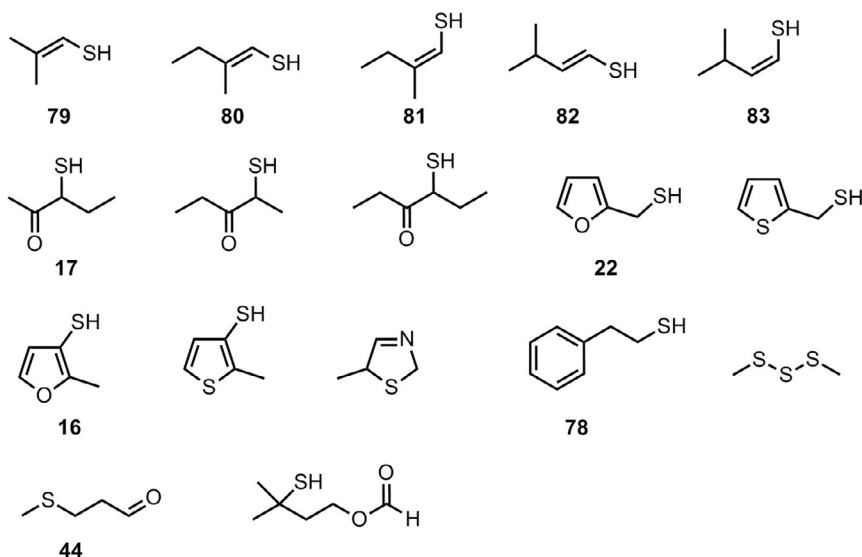


Figure 9.19 Aroma-active thiols in roasted sesame seeds (Tamura et al., 2010, 2011).

(≥ 32) in AEDA (Tamura et al., 2010) or high OAV (Tamura et al., 2011). It is known that the aroma of freshly roasted sesame seeds, though very pleasant, is labile. The sulfury, meat-like odourants 2-methyl-1-propene-1-thiol (**79**), 2-methyl-1(*E*)-butene-1-thiol (**80**), 2-methyl-1(*Z*)-butene-1-thiol (**81**), 3-methyl-1(*E*)-butene-1-thiol (**82**) and 3-methyl-1(*Z*)-butene-1-thiol (**83**) occur at levels of 20–800 $\mu\text{g/kg}$ in roasted sesame seeds and reach OAVs of 27–2400 (Tamura et al., 2011). However, they are fairly volatile. Due to their ene-thiol structure, they can hydrolyse with water into the corresponding aldehyde and hydrogen sulfide, similar to the decomposition of 1-propenylthiol (**68**) to propanal (**73**) (cf. Figure 9.17). The precursors and formation pathways of 1-alkenethiols are unknown and remain a challenge for future research.

9.7 Conclusion

Sulfur chemistry plays a particularly important role for aroma generation during cooking. Some of the resulting sulfur volatiles, despite low concentrations, are key odourants because of their extremely low odour thresholds. Relevant sulfur precursors include cysteine, methionine and thiamine. Important chemical reactions comprise Maillard and Strecker reactions, thiamine degradation as well as the thermal generation of thiols and disulfides from *Allium* species and sesame seeds.

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