Flavour chemistry of methylglyoxal and glyoxal

Yu Wang and Chi-Tang Ho*

Received 30th January 2012
DOI: 10.1039/c2cs35025d

Methylglyoxal (MGO) and glyoxal (GO), known as reactive carbonyl species, can be generated endogenously and exogenously (human body and food system). They are attracting increased attention because of their relationship with diabetes and flavour generation. In this review, their characteristics relating to flavour chemistry are discussed. MGO and GO can be detected in food systems by GC and HPLC after derivatization. MGO and GO formed in the Maillard reaction play important roles as precursors of aroma and colour compounds, especially in Strecker degradation, a major flavour generation reaction. When combined with amino acids they undergo Schiff base formation, decarboxylation and \( \alpha \)-aminoketone condensation leading to heterocyclic aroma compounds such as pyrazines, pyrroles and pyridines. They attack amine groups in amino acids, peptides and proteins to form advanced glycation end products (AGES) and cause carbonyl stress followed by oxidative stress and tissue damage. Therefore, many studies about scavengers of MGO and GO are seen. The influence of these scavengers on flavour generation is also discussed.

1. Introduction

Reactive carbonyl species (RCS) such as glyoxal (GO) and methylglyoxal (MGO), that can be generated endogenously and exogenously, are attracting increased attention because of their relationship with diabetes and flavour generation. In vivo MGO is primarily formed during glycolysis in cells and generated from the metabolism of ketone body degradation of threonine, and by the fragmentation of triosephosphates.\(^1\) In vitro, particularly in the Maillard reaction, RCS can be generated from Schiff’s base and Amadori compounds.

A carbonyl group of RCS can attack an amine group in amino acids, peptides or proteins to form advanced glycation end products (AGES) and cause carbonyl stress, followed by oxidative stress and tissue damage.\(^2\) Increasing evidence in both clinical and pre-clinical studies shows that MGO is associated with hyperglycemia in both Type I and Type II diabetes and diabetes-related complications such as nephropathy, Alzheimer’s disease and cataracts.\(^3\) Therefore, RCS are inducing factors of diabetes or its complications. However, RCS that are formed in Maillard reaction play important roles as precursors of aroma and colour compounds, especially in Strecker degradation, a major flavour generation reaction. MGO and GO, in combination with amino acids, undergo Schiff’s base formation, decarboxylation and \( \alpha \)-aminoketone condensation, leading to heterocyclic aroma compounds such as pyrazines, pyrroles and pyridines. In other words, MGO and GO, that contribute to flavour generation in food, can induce diabetes and diabetes complications. How to evaluate their paradoxical roles becomes very interesting and challenging. Therefore, in this tutorial review, flavour chemistry of MGO and GO is discussed to provide an overview of controlling their formation in food.

2. Chemical properties of methylglyoxal and glyoxal

MGO, a yellow hygroscopic liquid, is known as 2-oxopropanal, pyruvaldehyde, or 2-ketopropionaldehyde. It is present in three rapidly equilibrium forms in aqueous solution; monohydrate is the highest (71%) followed by dihydrate (28%), and the anhydrous form is only about 1%.\(^4\) Depending on temperature and water content, MGO can change from a less reactive noncarbonyl form to more reactive carbonyl and dicarbonyl forms.\(^5\) GO, which is also a yellow coloured liquid, is the smallest dialdehyde.

3. Analytical methods for quantification of MGO and GO

A derivatization process is needed, prior to chromatographic analysis, to quantify MGO or GO. Several derivatization agents listed in Table 1 and their adduct products shown in Fig. 1, including diamino derivatives of benzene and naphthalene, react with MGO to form quinoxalines. Quinoxalines have been analysed.
Table 1 Some examples of derivatization methods for methylglyoxal (MGO) analysis

<table>
<thead>
<tr>
<th>Derivatization reagent</th>
<th>Derivatives products</th>
<th>Detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Hydroxy-2,4,5-triaminopyrimidine</td>
<td>Pteridine</td>
<td>UV</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>2-Acetyltiazolidine</td>
<td>UV</td>
</tr>
<tr>
<td>Meso-stilbenediamine</td>
<td>2,3-Diphenyl-5-methyl-2,3-dihydropyrazine</td>
<td>UV</td>
</tr>
<tr>
<td>GC</td>
<td>O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine-hydrochloride (PFBHA)</td>
<td>Quinoxaline</td>
</tr>
<tr>
<td>1,2-Diaminobenzene</td>
<td>Oxime</td>
<td>MS/SIM, ECD, NPD, FPD</td>
</tr>
</tbody>
</table>

* Source: Ref. 1. Note: ECD, electron-capture detector; NPD, nitrogen phosphorus detector; FPD, flame photometric detector.

4. Generation of MGO and GO in foods

MGO can be exogenously generated from sugar autoxidation, Maillard reaction, as well as degradation of lipid and microbial fermentation. In sugar autoxidation, MGO is formed from fragmentation of sugar by retro-aldol condensation in which oxygen plays an important role. This process mainly occurs in food containing a lot of carbohydrates, especially mono-saccharides, from which the amount of MGO is higher than that from disaccharides. And the amount of MGO produced from glucose is higher than that from fructose. Honey with a high content of glucose and fructose forms MGO through sugar degradation during the heating processes in manufacturing and storage. The concentrations of GO and MGO in honey are in the range of 0.3–1.3 mg kg\(^{-1}\) and 0.8–33 mg kg\(^{-1}\), respectively. The most frequently used sweetener in foods or beverages is high fructose corn syrup which contains 90, 55 or 42% fructose. Levels of GO and MGO in commercial beverages which contain high amount of HFCS were 15.8–104.6 and 23.5–139.5 (µg per 100 ml), respectively. Coffee, as one of the popular drinks, was also studied for its MGO and GO levels. Four types of black coffee (espresso, bold, mild, and a decaffeinated mild roast) were tested. Espresso was shown to contain the highest level of MGO at 230.9 µM, followed by bold coffee. The roasting process influences the content of MGO and GO in coffee beans because of the occurrence of Maillard reaction. When green beans were roasted at 210 °C for 20 min, the glyoxal content increased in the first 6 min, peaked at 13.07 ± 0.39 mg per 100 g, then slowly decreased to 1.93 ± 0.05 mg per 100 g at 20 min. Methylglyoxal showed similar behavior with its peak concentration (21.19 ± 0.42 mg per 100 g) at 10 min and subsequently declined. Accumulation of MGO in lipids is caused by lipid degradation during processing and storage. The amount of MGO formed in fish oils (tuna, cod liver and salmon oils) heated at 60 °C for 7 days ranged from 2.03–0.13 to 2.89–0.11 mg kg\(^{-1}\), whereas among vegetable oils (soybean, olive and corn oil) under these conditions, only olive oil yielded MGO (0.61–0.03 mg kg\(^{-1}\)). During fermentation, microorganisms release MGO into food products most commonly into alcoholic drinks and dairy products. Levels of MGO in brandy, vinegar and wine were 1.9, 35 and 10 ppm, respectively.

5. Relationship between MGO and GO formation and the Maillard reaction

Maillard reaction plays an important role in MGO and GO formation in vitro and in vivo. In fact, scavenging RCS may terminate Maillard reaction, and suppressing Maillard reaction
may reduce the level of RCS. Maillard reaction is the major route for the generation of flavour and colour. Therefore, it is important to study the relationship between flavour generation and RCS formation.

5.1. Chemistry of Maillard reaction

Maillard reaction, which can be generally defined as the chemical interaction involving carbohydrates and amino compounds, is responsible for the generation of roasted, toasted and caramel-like aromas, as well as for the development of brown colour in foods. Maillard reaction also has both nutritional and toxicological effects on processed food.\textsuperscript{13-15} Many of the antinutritional aspects of the Maillard reaction, such as effects on the availability of essential amino acids, on enzyme activity, as well as on the absorption/utilization of minerals, have been extensively studied.\textsuperscript{13,14}

Maillard reaction occurs in three stages.\textsuperscript{13} The initial stage (Fig. 2) is the condensation of the carbonyl group of a reducing sugar with an amino compound to form the Schiff base which then cyclizes to the N-substituted aldosylamine. These Schiff bases can rearrange to form RCS such as 1-deoxyglycosone or 3-deoxyglycosone through amino-deoxyaldose or ketose by Amadori or Heyns rearrangements. The Amadori rearrangement product is not stable, so the intermediate stages (Fig. 2) include enolization, deamination, dehydration, cyclization, retroaldolization, isomerization and fragmentation to carbonyl compounds (small molecular RCS), furan derivatives and other intermediates. The final stage is the reaction of these RCS and furan derivatives to form flavour and colour compounds. In the intermediate stage, an amino group is released from the reaction, this means amino acids play important roles in catalysing sugar activation and fragmentation. On the other hand, sugar can be degraded into carbonyl compounds at high temperature through enolization, dehydration and fragmentation.

5.2. Strecker degradation

Generally, flavour compounds can be categorised into two groups: cyclization/condensation products and fragmentation products.

Strecker degradation, which is considered a significant source of flavour compounds, is associated with the intermediate stage of Maillard reaction. If Maillard reaction can be seen as the degradation of sugar catalysed by amino compounds, from another point of view, Strecker degradation can be taken as the degradation of amino acids initiated by RCS.

In Strecker degradation, dicarbonyl compounds such as MGO and GO react with amino acids to produce carbon dioxide and aldehydes with one less carbon atom and α-aminoketones that are key precursors of heterocyclic flavour compounds such as pyrazines, oxazoles and thiazoles (Fig. 3).\textsuperscript{16}

5.3. Proposed mechanism of methylglyoxal formation

RCS, including glyoxal, methylglyoxal and 3-deoxyglucosone, have been demonstrated to be formed in early glycation in vitro from the degradation of glucose and Schiff base adduct. The reactions were influenced by the concentration of phosphate buffer and availability of trace metal ions.\textsuperscript{17}

Glucosone can be formed from monosaccharide autoxidation.\textsuperscript{18} Glyoxal can be generated in the degradation of glucose by retro-aldol condensation, which is activated by deprotonation of the 2- or 3-hydroxy groups. Hydrogen peroxide, which is formed in autoxidation of glycoaldehyde to glyoxal, and glucose to glucosone, can also stimulate glyoxal formation by hydroxyl radical-mediated acetal proton abstraction from glucopyranose and α-elimination reactions.\textsuperscript{18} Deprotonation of carbon-2 of glucose and re-distribution of the electron density between carbon-1 and carbon-2 or carbon-2 and carbon-3 in glucose lead to dehydration, forming the 1,2-enol or 2,3-enol and thereby 1-deoxyglucosone (1-DG) or 3-deoxyglucosone (3-DG), respectively.\textsuperscript{19} Methylglyoxal may be formed by fragmentation of 3-DG (Fig. 4). The formation of glyoxal, methylglyoxal and 3-DG from glucose is dependent on phosphate buffer and availability of trace metal ions.\textsuperscript{20} This may be due to phosphate dianion HPO\textsubscript{4}\textsuperscript{2-} and metal ions catalysing the deprotonation of glucose and the autoxidation of glycoaldehyde and hydroxyl radical formation implicated in glyoxal formation. Moreover, trace metal ion phosphate complexes may be related to the activation of glucose for 3-DG formation.\textsuperscript{20}

The formation of fructosamine residues is the major pathway of early glycation by glucose, but not the only one. Originally, RCS were considered to be formed from fructosamine only. However, some studies show that 3-DG, MGO and GO were generated throughout the whole reaction and changes in their
a superoxide radical, which is a key in GO generation, can be directly from the Schiff’s base through erythritol, while methylglyoxal is from 3-DG though retroaldolisation. Formation of a superoxide radical, which is a key in GO generation, can be detected in the early stage of glycation. MGO and 3-DG generation, however, does not involve oxidation. The formation of glyoxal, methylglyoxal and 3-DG in glucose-amine reaction is also dependent on phosphate buffer concentration and availability of trace metal ions.

6. Importance of MGO and GO as intermediates in flavour generation

Flavour generated from Maillard reaction can follow three paths, (i) from carbohydrates only, (ii) from a combination of carbohydrates and amino acids, and (iii) from amino acids only, among all of which MGO and GO play important roles as intermediates. Carbohydrates can transform into glycosones at the beginning of Maillard reaction, and then these glycosones either cyclize into flavour compounds or break into α-dicarbonyls such as MGO and GO, then follow a recombination of these intermediates. One of these is 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), which is generally considered the product of cyclization of intact glycosones and/or the recombination of MGO and GO. Aromas formed from carbohydrates and amino acids can be divided into amino acid-specific and amino acid-non-specific pathways. For the amino acid-non-specific pathway, α-dicarbonyl (MGO or GO) reacts with most types of amino acids forming α-amino ketone via Strecker degradation which leads to the formation of alkylpyrazines, oxazoles and oxazolines. In the amino acid-specific pathway, amino acids such as cysteine and proline, α-amino ketone or α-dicarbonyl are involved in the generation of thiazoles, thiazolines, pyrrolines, and pyridines. Although some flavour compounds (e.g., methional, phenylacetaldehyde) are generated from amino acids only, α-dicarbonyl compounds are still involved in their formation pathways, particularly through Strecker degradation. In addition, peptides can also react with α-dicarbonyl compounds to generate some peptide-specific aromas (e.g., pyrazinones).

6.1. Formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) from carbohydrate or MGO

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF, known as furanone), with an intense caramel-like aroma, was originally discovered as a key flavour component of strawberry in 1965. DMHF has been found to be an important odour-active compound in various natural and processed foods, such as pineapple, raspberry, tomato and grape, as well as in roasted coffee, bread crust, roasted almond and soy sauce. DMHF has been found to be an important odour-active compound in various natural and processed foods, such as pineapple, raspberry, tomato and grape, as well as in roasted coffee, bread crust, roasted almond and soy sauce. In fruits, DMHF is usually formed by biosynthesis. Because of its widespread occurrence, DMHF became a major reactant in generating other flavour compounds. At low pH, DMHF has been shown to react with cysteine or hydrogen sulfide in generating meat-like aroma compounds. Some roast aroma compounds such as alkylpyrazines can also be generated through the decomposition of DMHF with phenylalanine.

The formation pathways of DMHF have been studied in model experiments of thermal degradation of 6-deoxysugars, hexoses and pentoses in the presence or absence of amino acids. Generally, DMHF can be formed through 2,3-enolization of 6-deoxysugars, hexoses and pentoses leading to 1-deoxyosones as intermediates. Compared to hexoses and pentoses, 6-deoxysugars, such as rhamnose, are more effective in forming DMHF through 2,3-dioxy-4,5-dihydroxyhexane that is not easily formed from hexoses and cannot be generated from pentoses. Schieberle in 1992, and later Hofmann and Schieberle in 2001 showed that DMHF can be formed from hexose via acetylformoin (2,4-dihydroxy-2,5-dimethyl-3-(H)-furanone) reduction, which may proceed either by disproportionation reaction or a Strecker reaction with amino acids. Only one study has shown the formation of DMHF from pentoses. Blank and Fay indicated that elongation of pentoses by Strecker aldehyde of glycine was an alternative pathway of DMHF formation in which acetylformoin was also proposed as an intermediate.
Hexose or pentose can be cleaved into MGO and 1-hydroxy-2-propanone to generate DMHF. This reaction has been proved to be a major pathway when glucose is reacted with proline in an aqueous solution.32 MGO, depending on the pH, differently affected DMHF generation in the presence or absence of amino acids. The DMHF level increased as pH increased when cysteine reacted with MGO, whereas the trend was reversed in the presence of glycine.24 When MGO was heated alone at 120 °C, the formation of DMHF was observed, and the MGO level was significantly increased as the pH of the reaction increased. MGO may transform into 1-hydroxy-2-propanone and pyruvic acid through the Cannizzaro reaction (Fig. 6), and subsequently lead to DMHF by reacting MGO with 1-hydroxy-2-propanone.32 DMHF formation from MGO was pH-dependent because the Cannizzaro reaction is a base preferential reaction.

### 6.2. Formation of aroma compounds from carbohydrates and amino acids

#### 6.2.1. Formation of alkylpyrazines via Strecker degradation (non-specific amino acids)

Alkylpyrazines, which are nitrogen-containing heterocyclic compounds, occur in a wide range of raw and processed foods with potent and characteristic aroma. The most plausible formation mechanism of pyrazines is the condensation of two α-aminoketones. Dicarbonyls from sugar degradation at the early stage of Maillard reaction react with amino acids via Strecker degradation to form α-aminoketones leading to pyrazine formation through oxidation of dihydro-pyrazines. If the alkyl groups in α-aminoketones are different, the isomers of pyrazines are observed33 (Fig. 7).

#### 6.2.2. Formation of oxazoles and oxazolines via Strecker degradation (non-specific amino acids)

Oxazoles and oxazolines are two closely related heterocyclic flavour compounds containing nitrogen and oxygen atoms. Oxazoles and oxazolines occur in various processed foods, such as roasted and ground coffee, roasted cocoa, heated soy sauce, baked potatoes and roasted peanuts.34,35 Formation pathways of oxazoles and oxazolines were first proposed between a dicarbonyl compound and an amino acid undergoing decarboxylation (Fig. 8).

#### 6.2.3. Formation of 2-acetyltetrahydropyridine and 2-acetylpyrroline via Strecker degradation (specific amino acids)

Flavour generation reactions occur most often at high temperatures especially Strecker degradation in which carbonyl groups react with amine groups through nucleophilic attack. However, some flavour compounds such as popcorn like flavour 2-acetyltetrahydropyridine and roasted aroma 2-acetylpyrroline can be formed from MGO in the presence of a specific amino acid proline, but without forming α-aminoketones. For example, 2-acetyltetrahydropyridine, which is a popcorn like flavour in rice and bread crust, can be formed from Strecker degradation of proline and MGO through decarboxylation and dehydration36 (Fig. 9). The degradation product from the reaction between proline and MGO can continually react with MGO to form 2-acetylpyrroline (Fig. 10). On the other hand, 2-acetyltetrahydropyridine can be biosynthesized through lysine and MGO.37 The gen(1at) encoding the L-lysine α-aminotransferase (LAT) in Streptomyces clavuligerus was cloned and expressed in Escherichia coli. Lysine was found to be transformed to 1-piperideine-6-carboxylic acid. And 2-acetyltetrahydropyridine was characterized from the reaction mixture of 1-piperideine-6-carboxylic acid and MGO (Fig. 11).
6.2.4. Formation of thiazoles and thiazolines via Strecker degradation (cysteine specific). Other aroma compounds formed in the presence of cysteine via Strecker degradation are thiazoles and thiazolines. They are closely related to oxazoles and oxazolines, except a sulfur atom replaces the oxygen atom in position 1 and a nitrogen atom occupies position 3. The first thiazole and thiazoline isolated from foods were 4-methyl-5-vinylthiazole and 2-acetyl-2-thiazoline, respectively.38,39 Later, various thiazoles and thiazolines were identified in many food systems, especially in thermally treated foods such as baked potatoes40 and roasted peanuts.35 Generally, 2-alkylthiazoles possess green, vegetable-like aroma. Increasing substitutions in positions 4 and 5 adds more nutty, roasted and sometimes meaty notes. Because they contain sulfur thiazoles and thiazolines that are formed mostly in the presence of cysteine. The most accepted formation mechanism of these is that \( \alpha \)-dicarbonyls, such as 2,3-butanedione, react with hydrogen sulfide, ammonia and acetaldehyde to form either 3-hydroxy-3-mercapto-2-butanone or 3-mercapto-2-butanone, leading to 2,4,5-trimethylthiazole and 2,4,5-trimethyl-3-thiazoline through condensation, cyclization and condensation with ethylideneamine41 (Fig. 12). MGO and GO should undergo similar reaction.

6.3. Formation of aroma compounds from amino acids via Strecker degradation

Aromas, such as 3-methylbutanal (malty) or phenylacetaldehyde (honey-like), that are derived from leucine and phenylalanine, respectively, in the presence of \( \alpha \)-dicarbonyl, generally are Strecker aldehydes formed in Strecker degradation. Besides aldehydes, some odour-active acids were also identified in the presence of RCS. In Strecker degradation, for example, phenylalanine reacts with MGO or GO, preferentially leading to phenylactic acid generation42 (Fig. 13), whereas reacting with 3-DG favors the formation of phenylacetaldehyde.43

7. Trapping of MGO and GO by phenolic compounds

MGO and GO are extremely reactive and readily modify lysine, arginine, and cysteine residues on proteins in vivo to form advanced glycation end products (AGEs) that are linked to hyperglycemia and diabetes complications. As the first step of AGEs formation, proteins in the tissues are modified by reducing sugars through the reaction between a free amino
group of proteins and a carbonyl group of the sugars, leading to the formation of fructosamines via a Schiff base by Amadori rearrangement. Then, both Schiff base and Amadori product undergo a further series of reactions through dicarbonyl intermediates (e.g., GO and MGO), to form AGEs. The amount of AGEs is dependent on the concentration of dicarbonyl intermediates, reactivity and concentration of amino acid residues, the half-life of protein, and the activity of glyoxalase system.

Accumulation of MGO and GO in cells may cause carbonyl stress that in its first step induces the formation of hydrogen peroxide. The hydrogen peroxide may then increase oxidative stress and, therefore, tissue damage. Moreover, MGO can activate NF-κB and induce the associated gene that is responsible for inflammation and proliferation. Therefore, studies on the prevention of accumulation of dicarbonyl compounds become very urgent. Many synthetic chemicals have been reported to inhibit formation of AGEs. More recently, researchers have documented the scavenging effect of MGO and GO by natural phenolic compounds, such as flavanols, chalcones, stilbenes, isoflavones and phenolic acids.

An inhibition of protein glycation on different stages was studied by using different dietary flavonoids. Luteolin, rutin, epigallocatechin-3-gallate (EGCG), and quercetin demonstrated significant inhibitory effects on MGO mediated AGEs formation by 82.2, 77.7, 69.1 and 65.3%, respectively, while catechin, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), kaempferol, and naringenin showed a lower inhibitory effect (13–54%).

Among all flavonoids, molecules with catechin-like structure show the strongest effect on direct trapping of MGO. In tea polyphenol adduct investigation, the mole ratio of MGO to each specific polyphenol was set at three, and the % reduction of MGO was compared with that of the control sample at 0 °C on an ice/salt bath for 1 h. After 1 h at 37 °C incubation, MGO was very stable, only decreasing by 5.8%. All tea polyphenolic compounds scavenge MGO. Most tea catechins decreased MGO by about 33%, which indicates that one catechin molecule reacts with one MGO molecule, since the initial mole ratio of MGO to a tea polyphenolic compound was 3 to 1. Theaflavins, the main black tea components, were more reactive toward MGO than other polyphenols tested. Theaflavins showed high levels of MGO reduction with respect to the control sample, which suggested that theaflavins would be excellent candidates in the treatment of MGO scavenging in future in vivo studies. The decreased amounts of MGO in TF1 (theaflavin), TF2 (theaflavin-3- and -3’-gallate), and TF3 (theaflavin-3,3’-digallate) were 63.1, 60.1, and 66.7%, respectively. All tested theaflavins decreased MGO by about 66%, which implies that one theaflavin molecule can trap two MGO molecules. In addition, the efficacy of EGCG trapping to GO was much lower than that of MGO, because GO is much easier to polymerise in aqueous solution. Trapping efficacy could be slowed down by the transformation from polymers to free GO. The major adduct between EGCG and MGO has been identified (Fig. 15). It is concluded that the reaction between EGCG and MGO dominantly occurs at the C6- and C8-position in the A ring of EGCG. Slightly alkaline pH facilitates the addition of MGO at these two positions to form mono- and di-MGO adducts. Isomers with R- and S-configuration could also be seen at each position.

Chalcones, represented by phloretin and phloridzin, have been studied for their trapping efficacy of MGO and GO. GO still showed a lower attachment than MGO because of easier polymerization. Phloretin had a much higher trapping rate than did phloridzin suggesting that glycosylation of a hydroxyl group at position 2 significantly slows down the formation of adducts. A similar phenomenon was observed in EGCG. Positions 3 and 5 of the A ring in phloretin and phloridzin were active sites to bind with MGO to form mono- and di-MGO adducts (Fig. 16). Stilbenes were compared for their efficacy of trapping MGO. Among 2,3,5,4’-tetrahydroxy-stilbene-2-O-β-D-glucoside (THSG), pterostilbene and resveratrol, THSG was the most effective trapping agent. Positions 4 and 6 of the A ring in stilbenes were the major active sites for trapping MGO (Fig. 17). Genistein, one of the isoflavones, was reported to trap MGO under neutral and alkaline conditions in vitro. Mono- and di-MGO adducts were identified. Mono-MGO adducted at position 8 on the A ring of genistein, and di-MGO conjugated at both positions 6 and 8 on the A ring of genistein (Fig. 18). Based on all the results obtained here, it is concluded...
that phenolic compounds having the same A ring structure (EGCG, phloretin, chalcone, stilbene and genistein) can efficiently trap MGO or GO to form mono- or di-MGO adducts even with different C rings. The mechanism is that the slightly alkaline pH can increase the nucleophilicity of the unsubstituted carbons at the A ring and facilitate the addition of MGO at these two positions to form mono- or di-MGO adducts. This observation also applies to polymers of flavonoids such as proanthocyanidins. Peng et al. (2008) demonstrated that proanthocyanidin B2 could effectively scavenge MGO, in a similar manner to aminoguanidine. Compounds consisting of a single benzene ring with the addition of at least one hydroxyl group were studied for MGO trapping. Compounds with one hydroxyl group on the benzene ring cannot react with MGO. Benzenetriols showed relatively higher trapping capability. The decreasing rate of MGO trapping for pyrogallol, 1,2,4-trihydroxybenzene, and 1,3,5-trihydroxybenzene were 55%, 49% and 64%, respectively. Steric hindrance and carbon electron charges on the benzene ring were reported as the influential factors. A carbon electron charge of $-0.24$ was the minimum value for high reactivity using a computational chemistry calculation. The carbon electron charges of epicatechin are shown in Fig. 19. The carbon electron charge on position 6 showed the lowest number of $-0.26$, followed by the one on position 8 ($-0.25$). These results explain why positions 6 and 8 were the active sites for flavanols.

8. Effect of polyphenolic compounds on Maillard flavour generations

Some polyphenolic compounds can efficiently trap MGO and GO usually generated from Maillard reaction. Therefore, scavenging RCS may influence Maillard reaction, while suppressing Maillard reaction may reduce the level of RCS. Moreover, Maillard reaction is the major route for the generation of flavour and colour. Thus, it is important to study the effect of polyphenolic compounds on flavour generation by trapping RCS.

Epicatechin was first studied for its influence on flavour generation in an aqueous model with glucose and glycine. Addition of epicatechin (EC) in the reaction decreased the generation of 2,3-butanedione, acetol, pyrazine, methylpyrazine, 2,3,5-trimethylpyrazine and cyclotene, because these are volatile compounds formed from C2/C3 or C3/C3 sugar fragments, and epicatechin can directly react with C2, C3 and C4 sugar fragments. Through NMR analysis, glyoxal, hydroxyacetone, and erythrose were identified as the sugar fragments. Methylglyoxal could also bind with EC, but EC-MGO underwent conformation/constitutional exchange. One of the isomers consisted of a covalent binding between C1 of MGO and either the C6 or C8 position of the EC A ring, in accordance with a previous study. Besides EC, other flavan-3-ols, such as ECG and EGCG, showed the same phenomenon. Generation of phenolic-C2, C3, C4 and C6 fragment adducts statistically fit the reduction of glyoxal, glycolaldehyde, MGO, hydroxyacetone, diacetyl, acetoin, and 3-deoxyglucosone. Under a roast condition, addition of EC can significantly reduce the generation of hydroxyacetone, 2-methylpyrazine, 2,3,5-trimethylpyrazine, furfural, 2-acetylfuran,

![Fig. 16 Adducts of methylglyoxal (MGO) and phloridzin.](image1)

![Fig. 17 Adducts of methylglyoxal (MGO) and stilbenes.](image2)

![Fig. 18 Adducts of methylglyoxal (MGO) and genistein.](image3)

![Fig. 19 Carbon electron charges on epicatechin.](image4)
and phloridzin were also studied for their effect on flavour generation in a model reaction with lysine and glucose. Phloretin and phloridzin were also studied for their effect on the formation of volatile compounds, but phloretin showed amounts of lysine. Both phloretin and phloridzin inhibited the formation of volatile compounds, but phloretin showed a higher activity. Moreover, phloretin and phloridzin were reported to show lower inhibitory effect with a higher content of lysine, suggesting the competition of these phenolic compounds and amino groups for RCS.

9. Conclusion

MGO and GO, which are intermediates in the Maillard reaction, are involved in flavour and colour generation for foods and drinks. Phenolic compounds having the same A ring structure (EGCG, phloretin, chalcone, stilbene and genistein) can efficiently trap MGO or GO to form mono- or di-MGO/GO adducts even with different C rings. Because of the trapping efficacy of phenolic compounds, adding them into Maillard reaction would change the formation of MGO and GO, and change the flavour properties. Therefore, RCS trapping agents provide an alternative way to influence flavour generation and affect the quality of food.

Notes and references