ROLES OF REACTVE CARBONYL SPECIES IN HEALTH AND FLAVOR GENERATION

by

YU WANG

A Dissertation submitted to the
Graduate School–New Brunswick
Rutgers, The State University of New Jersey
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
Graduate Program in Food Science
Written under the direction of
Professor Chi-Tang Ho
and approved by

_____

_____

_____

_____

_____

New Brunswick, New Jersey

January, 2009

Abstract of the dissertation

Roles of Reactive Carbonyl Species in Health and Flavor Generation

By YU WANG

Dissertation Director: Professor Chi-Tang Ho

Reactive carbonyl species (RCS) such as deoxyosones, glyoxal (GO) or methylglyoxal (MG), which can be generated endogenously and exogenously (human body and food system), have been attracted more attention because of their relationship with diabetes and flavor generation. In this study, two models i) quantification of RCS in beverages and ii) roles of RCS in furanoid generation were set up to evaluate the amount of RCS from food system and their roles in flavor generation.

In the first model, α-dicarbonyl compounds, namely glyoxal (GO), methylglyoxal (MG) and 3-deoxyglucosone (3-DG), were found and measured in carbonated soft drinks (CSD). By comparing their levels in regular and diet CSDs, it was realized that high fructose corn syrup (HFCS) in regular CSDs was the major source of these α-dicarbonyl compound in beverages.

The second model was conducted in three different experiments, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) generation through MG, amino acid-dependent formation pathways of 2-acetylfuran and DMHF, and sugar-dependent formation of 2-acetylfuran to give an understanding of roles of RCS in furanoid formation in Maillard reaction. MG alone or MG with cysteine could produce increased level of DMHF with pH increased, whereas MG with glycine had contrary trend. For the DMHF, there was

only one major formation pathway in which glucose carbon skeleton kept intact. On the other hand, formation pathways for 2-acetylfuran were more complicated. It could be formed either from glucose or from glucose and glycine. The type of sugars is a major factor regulating the reaction rates and pathways in Maillard reaction. The reactivity of sugars in 2-acetylfuran formation decreased in the order of ribose, fructose, glucose, rhamnose and sucrose. The difference between glucose and ribose in 2-acetylfuran formation was that glucose could form 2-acetylfuran from the direct cyclization of its intact carbon skeleton, whereas ribose has to first undergo degradation into fragments before forming six-carbon unit for 2-acetylfuran.

Acknowledgement

I would like warmly thank my advisor Dr. Chi-Tang Ho for his continuous support and encouragement throughout my PhD studies. His patience, enthusiasm, inspiration, and generous contribution of time and resources have greatly helped me to achieve my goals. I am especially thankful for his trust in my ideas and abilities and for the opportunities he has offered me.

I would like to thank all the members of my PhD committee and particularly Dr. Qingrong Huang, Dr. Xiaogen Yang, and Dr. Hernyk Daun for all the knowledge and support they offered me during my PhD studies at Rutgers University.

Finally, I would like to thank my parents, grandparents for their love and support. Without them this work would never have been accomplished.

.

Table of contents

List of tables

List of figures

Roles of Reactive Carbonyl Species in Health and Flavor Generation

1. Introduction

Reactive carbonyl species (RCS) such as deoxyosones, glyoxal (GO) or methylglyoxal (MG), which can be generated endogenously and exogenously (human body and food system), have been attracted more attention because of their relationship with diabetes and flavor generation. *In vivo*, for example MG is primarily formed during glycolysis in cells and generated from the metabolism of ketone bodies degradation of threonine, and by the fragmentation of triosephosphates (Reichard *et al.*, 1986; Lyles *et al.*, 1992 and Phillips *et al.*, 1993). In *vitro*, particularly in the Maillard reaction, RCS can be generated from *Schiff's* base and Amadori compounds. It seems hardly to bridge diabetes and flavor generation, but in fact when the food is processed and consumed, RCS play such a paradoxical role.

Firstly, the carbonyl group of RCS can attack amine groups in amino acids, peptides or proteins to form the advance glycation end products (AGEs) and cause carbonyl stress followed by oxidative stress and tissue damage (Baynes *et al*., 1999 and Onorato *et al*., 1998). Increasing evidence in both clinical and pre-clinical studies show MG is associated with hyperglycemia in both Type I and Type II diabetes and diabetes-related complications such as nephropathy, Alzheimer's disease and Cataracts (Basciano *et al.*, 2005 and Juergens *et al.*, 2005). Therefore, RCS are inducing factors of diabetes or its complications. Secondly, RCS, which formed in Maillard reaction, play important roles as precursors of aroma and color compounds especially in the *Strecker* degradation, a major flavor generation reaction. RCS particularly dicarbonyl compounds with amino acids undergo *Schiff's* base formation, decarboxylation and α-aminoketone condensation

leading to heterocyclic aroma compounds such as pyrazines, pyrroles and pyridines. In other words, RCS, which contribute to the flavor generation in foods, could induce diabetes and diabetes complications. How to evaluate their contradictory roles in foods becomes very interesting and challenging. Therefore, two models (Chapter I and Π) were set up here to evaluate the amount of RCS absorbed from food system and their roles in flavor generation.

Besides formation of RCS in *vivo*, foods or drinks are the major extrinsic factors that could cause the increase of RCS in normal physiological condition. Foods and drinks contain a lot of sugar especially high fructose corn syrup (HFCS), which can be oxidized to RCS during processing. HFCS containing 90, 55 and 42% of fructose are now widely used in food and beverage industry as a liquid sweetener because of its lower price and ease of handling. The demanding of HFCS can be reflected from the use of HFCS from 0.5 pound per capita in 1970 to 62.4 pounds in 1997 and remained as high as 59.0 pounds in 2005 (Putman et al., 1999 and USDA website). Many nutrition researches had focused on the relation between beverage consumption and obesity, diabetes or related chronic diseases (Basciano et al., 2005; Jüergens et al., 2005; Ebbeling et al., 2006; Wharton et al., 2004 and Bray et al., 2004). Literature data on the detection and quantification of RCS in food or beverage have not been extensive. And also in view of the fact that high consumption of HFCS beverage nowadays, it is meaningful and urgent to screen the possible presence of RCS in commercial beverages. Meanwhile, in our previous study, methylglyoxal can be effectively trapped by polyphenols such as green tea (-)-epigallocatechin gallate (EGCG) (Lo et al., 2006), in the present study, the possible effect

of (-)-epigallocatechin gallate (EGCG) on the levels of RCS in carbonated beverages during storage was also investigated (Chapter I).

As mentioned above, RCS could lead to some important flavor generation in Miallard reaction which has been extensively studied for almost a century, particularly for the development of flavor and color in processed foods. Maillard reaction starts from very simple reactants, a carbonyl group (i.e., reducing sugar) and an amine group (i.e., amino acids), and goes through initial, intermediate and final stages in generation of end products. Formation of those Maillard compounds (i.e., RCS or flavor compounds) depends on the types of sugars and amino acids as well as reaction temperature, time, pH and water content which may alter reaction kinetics and pathways. For example, fructose shows a higher reactivity in 5-hydroxymethyl-2-furaldehyde (HMF) generation compared to glucose and sucrose (Antal et al., 1990; Lee and Nagy, 1990), and the formation pathways of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) vary with different sugars and water content (Hofmann and Schieberle, 1997, 2001).

Furanoids, incorporating a furan ring into its molecular structure such as furfural, 2-acetylfuran, DMHF and 5-hydroxymethy-2-furfural (HMF), are important flavor or flavor intermediates in foods. Most furanoids are formed from RCS in the presence or absence of amino acids during intermediate or final stage of Maillard reaction. HMF or furfural and DMHF are products obtained on the decomposition of 3-deoxyosone and 1-deoxyosone, respectively (Hodge et al., 1972). These furanoid compounds, on one hand, can be formed through cyclization of an intact deoxyosone. On the other hand, the deoxyosone may be cleaved into small fragments of RCS such as MG or 1-hydroxy-2-propanone, which may recombine to form furanoids. Depending upon the reaction

conditions, particularly the type of sugars and amino acids, the formation pathways for furanoid compounds may become more complicated. However, systematic studies of furanoid formation have not been performed yet. Furanoids particularly DMHF and 2-acetylfuran were chosen as the targets, and studied on the relationship between their generation and RCS formation, influence of types of amino acids or sugars (Chapter Π). This part were conducted in three different experiments, DMHF generation through MG, amino acid-dependent formation pathways of 2-acetylfuran and DMHF, and sugar-dependent formation of 2-acetylfuran to give an overview of roles of RCS in furanoids formation in Maillard reaction.

2. Chapter I. Reactive Carbonyl Species in Beverages

2.1. Literature Review

Reactive carbonyl species include a large number of compounds with the carbonyl structure, such as 3-deoxyglucosone (3-DG), 1-deoxyglucosone (1-DG), MG, GO and diacetyl. MG, as one of the most important RCS, has been extensively studied in recent year, so in the literature review, MG was selected as a representative compound for RCS to discuss the occurrence and formation mechanisms.

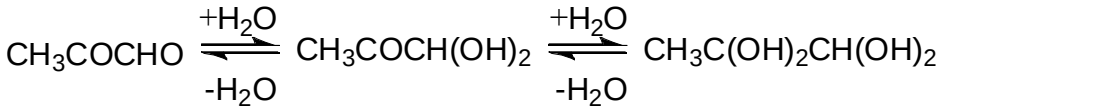
2.1.1. Occurrence of Methylglyoxal

MG, yellow hygroscopic liquid, is known as 2-oxopropanal, pyruvaldehyde, or 2-ketopropionaldehyde (Structure shown in **Figure 1**). It is present in three rapidly equilibrium forms in aqueous solution; monohydrate is the most (71%) followed by dihydrate (28%), and anhydrated form is only about 1% (Creighton *et al*., 1988). Depend on temperature and water content, MG can change from less reactive noncarbonyl form to more reactive carbonyl and dicarbonyl forms (Nemet *et al*., 2004). There are several sources of MG, which can be classified as exogenous and endogenous.

MG can be exogenously generated from sugar autoxidation, Maillard reaction, degradation of lipid and microbial fermentation. In sugar autoxidation, MG is formed from fragmentation of sugar by retro-aldol condensation in which oxygen plays an important role. This process mainly occurs in foods containing a lot of carbohydrate especially monosaccharides from which the amount of MG is higher than that from disaccharides (Hollnagel *et al.,* 1998). And the amount of MG produced from glucose is higher than that from fructose. Honey with a high content of glucose and fructose contains MG through sugar degradation during the heating processes in manufacturing

and storage. The concentrations of MG in honey were in the range of 0.4–5.4 mg/kg (Weigel *et al*, 2004). Mostly used sweetener in foods or beverages is high fructose corn syrup which contains 90, 55 and 42% of fructose. Till now there is no report on the content of methylglyoxal in commercial beverages. Accumulation of MG in lipid is caused by lipid degradation during processing and storage. The amount of MG formed in fish oils (tuna, cod liver and salmon oil) heated at 60 ^{o}C for 7 days ranged from 2.03-0.13 to 2.89-0.11 mg/kg, whereas among vegetable oils (soybean, olive and corn oil) under these conditions, only olive oil yielded MG (0.61-0.03 mg/kg) (Fujioka1 and Shibamoto, 2004). During fermentation, microorganisms release MG into food products mostly alcohol drinks and dairy stuff. Levels of MG in brandy, vinegar and wine were 1.9 ppm, 35 ppm and 10 ppm, respectively (Rodrigues, 1999). Moreover, MG was also quantified in drinking water, rain, even cigarette smoke (Fujioka1 and Shibamoto, 2006; Bao *et al.,* 1998 and Do Rosario *et al.*, 2005).

In vivo, MG can be formed in numerous enzymatic and nonenzymatic reactions, and it widely occurs in biologic matrix such as urine, blood, plasma and organs. The level of MG in whole blood from normal people was 256±92 nM and from diabetic patients was 479±49 nM (McLellan *et al*, 1992). In human plasma, 20.6 ug/dL of MG in diabetic patients was 2-6 folds higher than 4.9 μg/dL of MG in normal people (Odani *et al*., 1999). MG levels were also determined in rat heart, liver, kidney and aorta in which the concentration were 3.4±1.6, 2.5±0.8, 2.0±1.0 and 10.6±1.7 nmole/g wet weight, respectively. Generally, MG level was 10-15 fold higher than that in the erythrocytes of diabetic animals (Nagaraj *et al*., 2002).



Unhydrated Monohydrate Dihydrate

Adapted from Thornalley(1996)

Figure 1. Forms of MG

2.1.2. MG and Maillard Reaction

Thornalley *et al* (1999) have demonstrated that the Maillard reaction plays an important role in MG formation *in vitro* and *in vivo*. In fact, scavenging RCS may terminate Maillard reaction, while suppressing Maillard reaction may reduce the level of MG. However, Maillard reaction is the major route for the generation of flavor and color. Therefore, it is important to study the relationship between flavor generation and MG formation to be able to develop a method to minimize MG without significant adverse effects on flavor.

2.1.2.1. Chemistry of Maillard Reaction

Maillard reaction, which can be generally defined as the chemical interaction involving carbohydrates and amino acids, are responsible for the generation of roasted, toasted and caramel-like aromas, as well as for the development of browned surfaces in foods. The Maillard reaction also has both nutritional and toxicological effects on processed food (Hodge 1953; Hayashi *et al*., 1985 and De Revel *et al.,* 2000). Many of the antinutritional aspects of the Maillard reaction, such as effects on the availability of essential amino acids, effects on enzyme activity, as well as the effects on the absorption/utilization of metals, have been extensively studied. (Hodge 1953; Hayashi *et al.,* 1985) The Maillard reaction is responsible for the formation of potent mutagenic/carcinogenic heterocyclic aromatic amines (HAAs) in heated meat and fish. (Sugimura *et al.,* 2004) Asparagine-mediated Maillard reaction is known to lead to the formation of neurotoxic acrylamide. (Mottram *et al.,* 2002)

Maillard reaction occurs in three stages. The initial stage (**Figure 2**) is the condensation of carbonyl group of a reducing sugar with an amino compound to form the

*Schiff'*s base which then cyclizes to the N- substituted aldosylamine. These *Schiff'*s 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 stage (**Figure 3**) is its 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 flavor and color compounds. In the intermediate stage, amine group is released from reaction, which means amino acids play an important role in catalyzing sugar fragmentation. On the other hand, sugar can be degraded into carbonyl compounds at high temperature through enolization, dehydration and fragmentation (Fagerson, 1969)

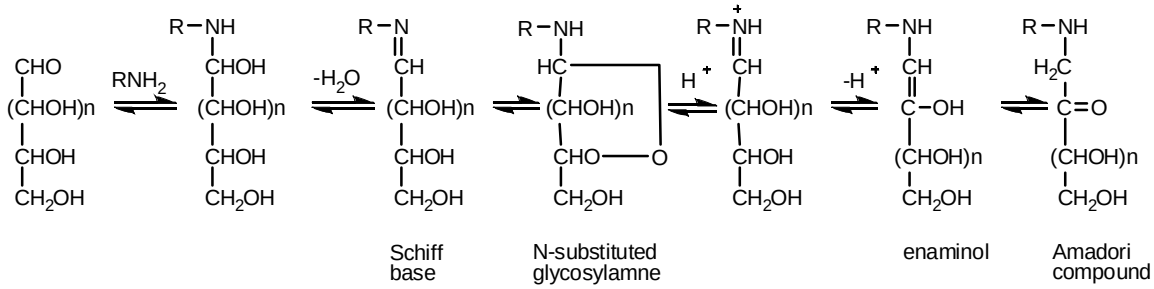


Figure 2. Initial Stage of the Maillard reaction (Hodge, 1953)

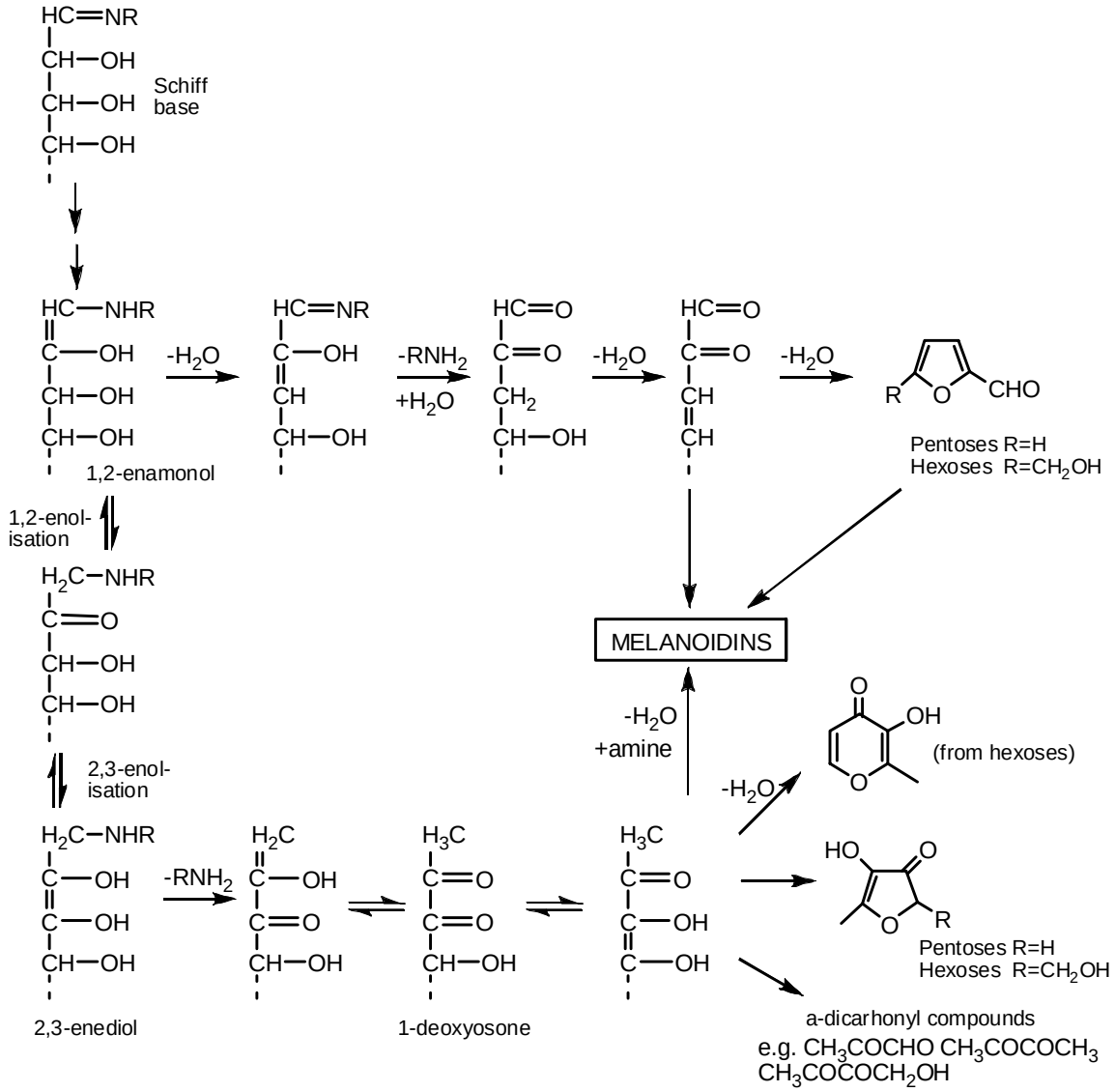


Figure 3. Decomposition of the Amadori compounds in the intermediate stage of the Maillard Reaction (Hodge, 1953)

2.1.2.2 Strecker Degradation

Generally, flavor compounds can be categorized into two groups: cyclization/condensation products and fragmentation products. Strecker degradation, which is considered as a significant source of flavor compounds, is associated with the intermediate stage of Maillard Reaction. If Maillard reaction can be seen as the degradation of sugar catalyzed by amino acids, from another point of view, Strecker degradation can be taken as the degradation of amino acids initiated by RCS.

In Strecker degradation, RCS for example dicarbonyl compounds react with amino acids to produce carbon dioxide and aldehyde with one less carbon atom and α-aminoketones which are key precursors of heterocyclic flavor compounds such as pyrazines, oxazoles and thiazoles (**Figure 4**) (Rizzi, 1972; Vernin and Metzger, 1981).

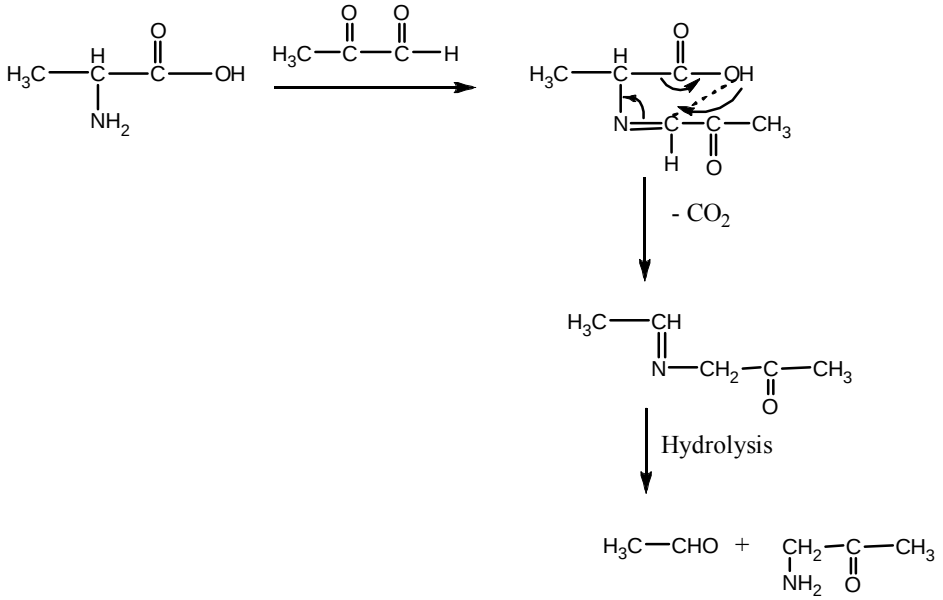


Figure 4. Strecker Degradation (Rizzi, 1972)

2.1.3 Proposed mechanism of Methylglyoxal formation

2.1.3.1 Formation of Methylglyoxal *in Vitro*

The glycation of proteins by glucose has been linked to the development of diabetic complications and other diseases. (Baynes *et al*., 1999 and Onorato *et al.,* 1998) Recent research has shown that physiological glycation processes is also related to the modification of proteins by reactive carbonyl species (RCS) particularly glyoxal, methylglyoxal and 3-deoxyglucosone (3-DG) (Basciano *et al.,* 2005 and Jüergens *et al.,* 2005). Thornalley *et al* (1999) demonstrated the formation of RCS, glyoxal, methylglyoxal and 3-deoxyglucosone in early glycation *in vitro*. All their results suggested that RCS were formed in early glycation from the degradation of glucose and Schiff's base adduct, which were also influenced by the concentration of phosphate buffer and availability of trace metal ions (Thornalley *et al*., 1999).

Glucosone can be formed from monosaccharide autoxidation (Thornalley *et al.,* 1984). And degradation of glucose under physiological conditions also forms GO, MG and 3-DG (Wells-Knecht *et al*., 1995 and Araki *et al*., 1998). 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 β-elimination reactions (Thornalley *et al*., 1984). 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 leads to dehydration forming the 1,2-enol or 2,3-enol and thereby 1-DG or 3-DG, respectively (Thornalley, 1985). Methylglyoxal

may be formed by fragmentation of 3-DG (**Figure 5**). The formation of glyoxal, methylglyoxal and 3-DG from glucose are dependent on phosphate buffer and availability of trace metal ion (Nursten, 2005). This may be due to phosphate dianion HPO_4^{2-} and metal ions catalyzing the deprotonation of glucose and the autoxidation of glycoaldehyde and hydroxyl radical formation implicated in glyoxal formation, respectively (Thornalley *et al*., 1984). Moreover, trace metal ion phosphate complexes may be related to the activation of glucose for 3-DG formation (Nursten, 2005).

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 (Hayashi and Namiki, 1980, Wells-Knecht *et al.,* 1995 and Thornalley *et al*., 1999) showed 3-DG, MG and GO were generated throughout the whole reaction and the changes in concentrations of them did not follow that of fructosamine. In other words, RCS may be formed from other sources such as from glucose degradation and from the *Schiff's* base (Figure 6). The formation mechanism of RCS is similar to glucose degradation except for the presence of aldimine which could be hydrolyzed to MG, GO and 3-DG. And the presence of the aldimine group accelerates the formation of RCS (Nursten, 2005). The formation of 3-DG and fructosamine are parallel reaction pathways in which the deprotonation of carbon-2 of the *Schiff's* base is a critical point. Furthermore, in Namiki pathway, glyoxal can be formed directly from The *Schiff's* base through erythritol, while methylglyoxal is from 3-DG though retro-aldol condensation (Hayashi and Namiki, 1980). Formation of superoxide radical, which is a key in GO generation, can be detected in early stage of glycation (Mullarkey *et al*., 1990). MG and 3-DG generation, however, does not involve oxidation.

The formation of the glyoxal, methylglyoxal and 3-DG in glucose-amine reaction were also dependent on phosphate buffer concentration and availability of trace metal ions (Nursten, 2005).

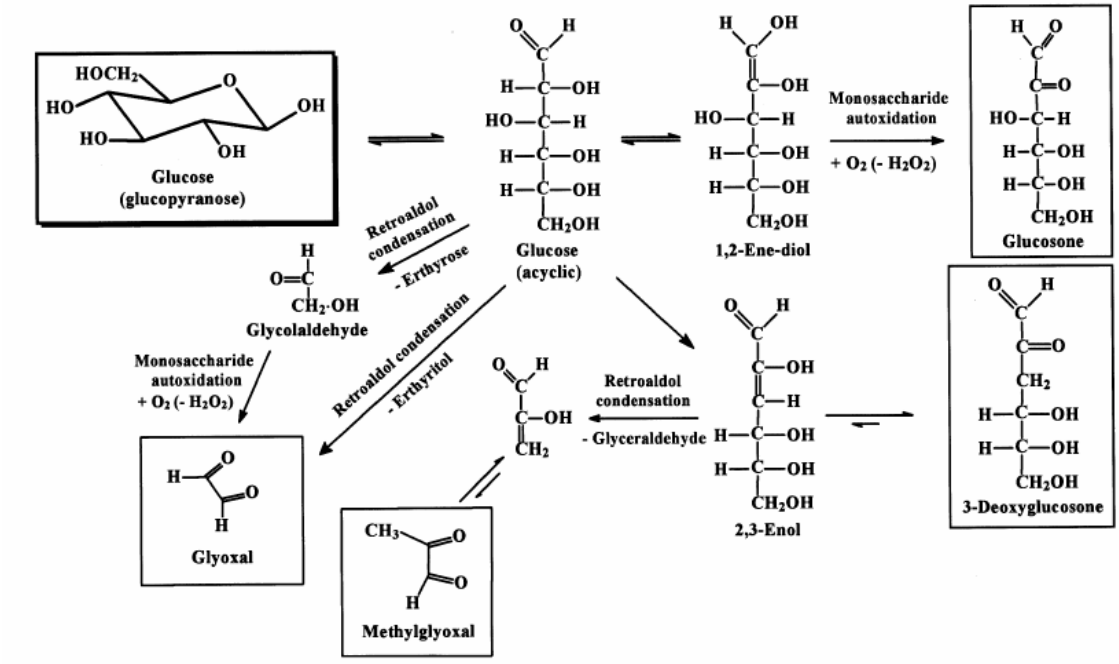


Figure 5. Oxidative Formation of MG from Glucose (Nursten, 2005)

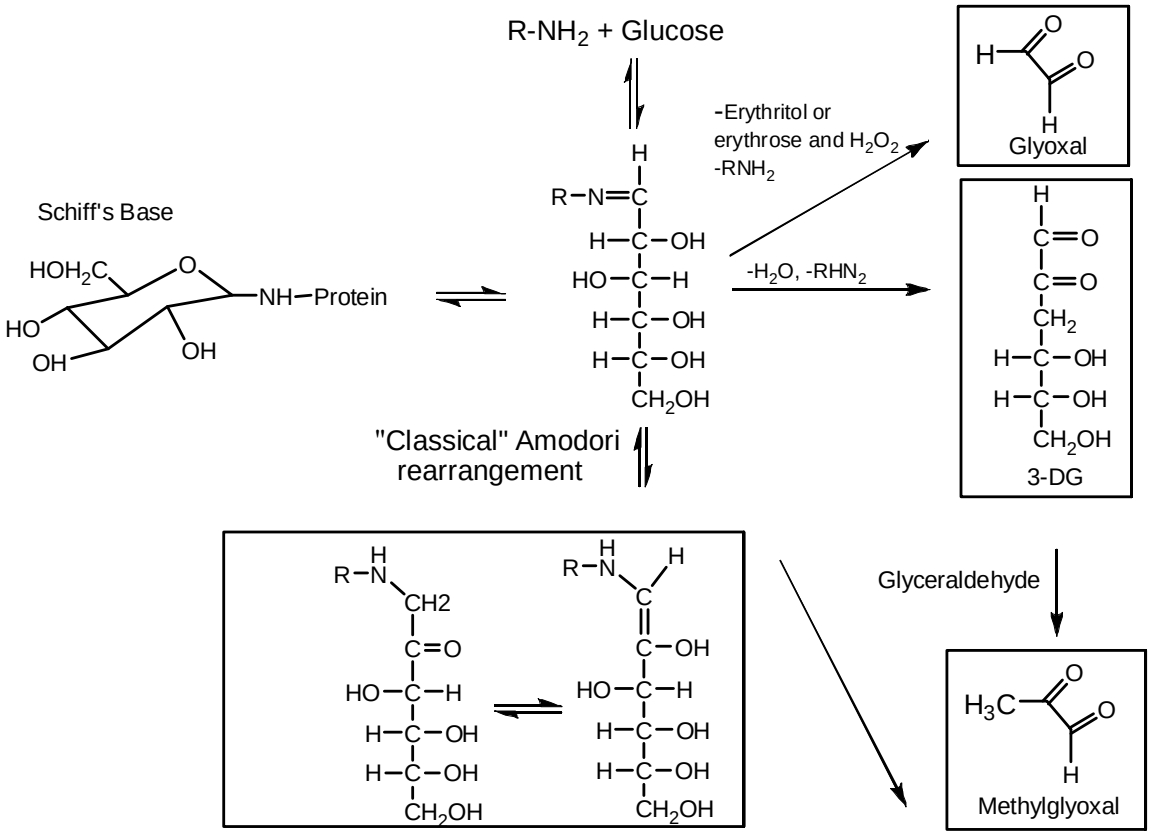


Figure 6. Formation of MG and GO in Maillard reaction (Hayashi and Namiki, 1980)

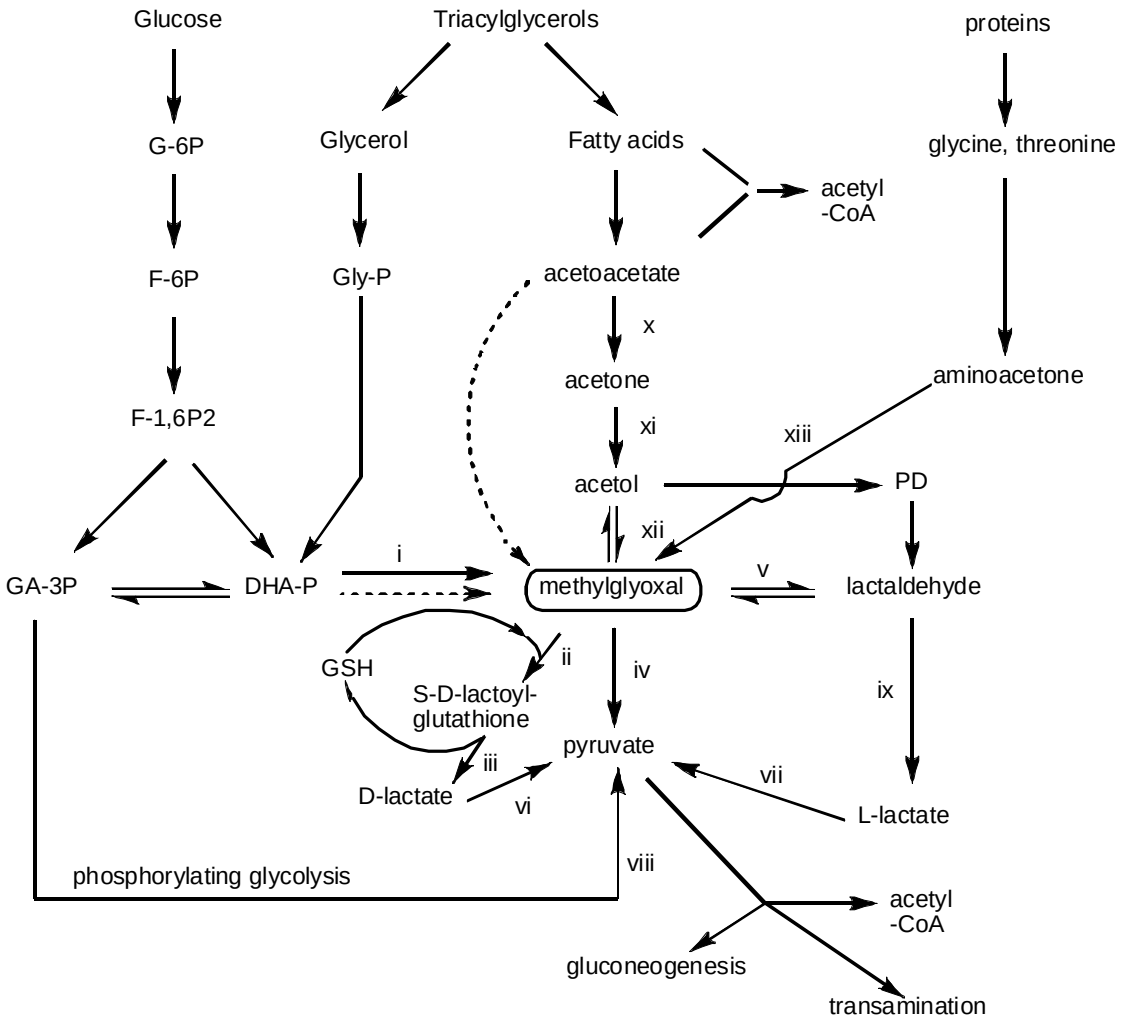
2.1.3.2. Formation of Methylglyoxal in *Vivo*

In *vivo*, MG can be formed in many enzymatic and nonenzymatic pathways. Enzymatic pathways include reactions catalyzed by triosephosphate isomerase, cytochrome P450 2E1, myeloperoxidase, and aminooxidase, whereas nonenzymatic pathways include decomposition of dihydroxyacetone phosphate, the Maillard reaction, oxidation of acetol, and lipid peroxidation (Nemet *et al*., 2006).

One of the most important ways of MG production is the fragmentation and elimination of phosphate from the phospho-ene-diolate form of glyceraldehyde-3-phosphate (GA-3P) and dihydroxyacetonephosphate (DHA-P). This may occur nonenzymatically (Phillips and Thornalley, 1993a) or enzymatically by leakage of phospho-ene-diolate from the active site of triosephosphate isomerase (Pompliano *et al*., 1990) and by methylglyoxal synthase (Ray, 1981). MG is also formed from acetone and aminoacetone by acetone monoxygenase and amino oxidase, respectively. And the aminoacetone is the catabolism of threonine and glycine which are degradated from protein. Although ketone bodies are likely to be an important source of MG, it is also possible that some MG is derived from increased triose phosphate resulting from metabolisms of glucose or the increased production of glycerol caused by accelerated triglyceride breakdown, or from lipoxidation products (Baynes and Thorpe, 2000).

There are four enzyme systems in detoxification of MG, which include the glyoxalase system, aldose reductase (ALR), betaine aldehyde dehydrogenase, and 2-oxoaldehyde dehydrogenase (2-ODH). Glyoxalase system contains two enzymes which are Glyoxalase I and Glyoxalase II. Glyoxalase I catalyses the formation of S-D-

lactoylglutathione from the hemithioacetal formed nonenzymatically from methylglyoxal and reduced glutathione. Glyoxalase II catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, reforming the reduced glutathione consumed in the glyoxalase I-catalyzed reaction (Thornalley, 1996). ALR system catalyzes the NADPH-dependent reduction of MG into lactaldehyde in the presence of GSH and into acetol in the absence of GSH. Lactaldehyde and acetol could be transformed into D-lactate and 1,2-propandiol, respectively. However, when the GSH level decreases, the acetol may increase so as to increase the MG level, because MG can be transformed from acetol. Both betaine aldehyde dehydrogenase and 2-ODH catalyze MG into pyruvate.



Abbreviations used in the figure: G-6P, glucose 6-phosphate; F-6P, fructose 6-phosphate; F-1,6P2, fructose 1,6-bisphosphate; GA-3P, glyceraldehyde 3-phosphate; DHA-P, dihydroxyacetone-phosphate; Gly-P, glycerolphosphate; GSH, glutathione; PD, propanediol. The enzymes involved in the reactions: (i) methylglyoxal synthase; (ii) after a non-enzymatic reaction between methylglyoxal and glutathione glyoxalase I; (iii) glyoxalase II; (iv) a-oxoaldehyde dehydrogenase; (v) e.g. methylglyoxal reductase; (vi) D-lactate dehydrogenase; (vii) L-lactate dehydrogenase; (viii) the enzymes of phosphorylating glycolysis; (ix) e.g. lactaldehyde dehydrogenase; (x) acetoacetate decarboxylase and spontaneously, too; (xi) acetone monooxygenase; (xii) acetol monooxygenase; (xiii) amine oxidase.

Figure 7. Pathways of Methylglyoxal Metabolism (Adapted from Kalapos 1999)

2.1.4. Methylglyoxal in Health

Methylglyoxal, a major α-dicarbonyl compound found in human, is extremely reactive and readily modify lysine, arginine, and cysteine residues on proteins to form advanced glycation end products (AGEs) which is 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 further undergo a series of reactions through dicarbonyl intermediates (e.g., glyoxal and methylglyoxal), to form AGEs. The amount of AGEs is depend on the concentration of MG, reactivity and concentration of amino acid residues, the half-life of protein, and the activity of glyoxalase system.

Accumulation of MG in cell may cause the carbonyl stress which finally induces the formation of hydrogen peroxide (Baynes *et al*., 1999 and Onorato *et al*., 1998). Then the hydrogen peroxide may increase oxidative stress and therefore tissue damage. Moreover, MG could activate NF-κB and induce the associated gene that is responsible for inflammation and proliferation (Wu and Juurlink, 2002).

2.2. Experimental

2.2.1. Materials.

Thirteen different carbonated soft drinks (CSDs) including two diet carbonated beverages were used in this study. Each brand was purchased from three different supermarkets. Honey purchased from a supermarket was a wild flower pure honey product of USA. *o*-Phenylenediamine (o-PDA) was purchased from Sigma (St. Louis, MO, USA). It was the derivative reagent used for dicarbonyl compounds. Quinoxaline (Q) was purchased from Fluka Chemicals (Milwalkee, WI, USA). 2-Methylquinoxaline (2-MQ; 97%), 2-ethylpyrrole (2-EP; technical grade, 90%) and anhydrous sodium sulfate were purchased from Aldrich (St. Louis, MO, USA). EGCG (100% pure) was a gift from Mitsui Norin (Shizuoka, Japan). Glass threaded vials (14.8 mL; 21 mm x 70 mm; od x H), HPLC grade water, acetonitrile, methanol and ethanol were purchased from Fisher Scientific (Springfield, NJ, USA). Deuterated solvents, including D_2O, were purchased from Norell Company (Landisville, NJ, USA). 2-Methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline (97% purity) was purchased from TRC (Toronto Research Chemicals Inc., Toronto, ON, Canada). TLC was performed on 250 μm thickness, 2–25 μm particle size TLC plates (Sigma–Aldrich, St. Louis, MO, USA). Three samples of HFCS were gifts from two beverage companies. Two were claimed to contain 55% fructose and one was claimed to have 42% fructose.

2.2.2. Dicarbonyl Compound Derivatization.

The derivatization reaction was done for GO, MG and 3-DG. Their corresponding quinoxaline analogues were formed by the reaction of dicarbonyl compounds with *o*-phenylenediamine (*o*-PDA). All derivatization processes were the same. After the samples were added with appropriate amount of *o*-PDA in glass vials, glass vials were capped and shaken vigorously by vortexing for 5 s. The reaction was performed in a 60ºC water bath and 50 rpm for 30 min. After centrifuging at 14 x 1000 rpm (16,000g) for 5 min, samples were ready for HPLC analysis.

2.2.3. 2-Methyl-3-(2',3'-dihydroxypropyl)-quinoxaline Preparation and Purification from Honey.

Ten grams of honey were dissolved with 5 mL *o*-PDA (60 mg/mL). After reacting in a water bath at 60 ºC and 50 rpm for 30 min, the solution was applied to a self packed reverse-phase C18 column (Borosilicate glass column; i.d. x Length: 1.5 x 30 cm; Sigma Chemical Co.). 100% water was used as initial mobile phase. After sugars and unreacted *o*-PDA eluted from the column, the mobile phase was changed to 30% methanol. The fractions with major components were checked by TLC. This whole procedure was repeated to collect enough amounts of major components for further purification. The major components obtained from the previous step were further dissolved in a mixture of water (1.5 mL) and ethanol (1 mL) and loaded onto the C18 reverse-phase preparative (XTerra ®MS C18 OBD™, 5 μm) (Waters Corp., Milford, MA, USA) HPLC system. A gradient method was used from 5% acetonitrile-95% water to 20% acetonitrile -80% water in 20 min with a flow rate of 20 mL/min. The fractions were analyzed by LC–ESI-MS. The purified fractions by LC–MS were combined and concentrated or lyophilized to

dryness. The dried compounds were analyzed by MS and NMR and their LC–MS and NMR spectra were the same as the purchased reference.

2.2.4. Screening of GO, MG and 3-DG in Carbonated Beverages.

Thirteen varieties of CSDs (noted as: A, B, C, D, E, F, G, H, I, J, K, AA and BB) used in this study were in aluminum cans. Two of them are diet drinks and they are the same brands with particular brands A and B, respectively (noted as AA and BB). J and K were energy carbonated drinks. For proper sampling of beverages, 13 varieties of CSDs were purchased from three different local supermarkets on different days. The batch sizes depended on the manufacturers' package. There were 6 or 12 cans in each batch. One can for each batch in a particular brand from each supermarket was sampled. Each sampled beverage was measured in triplicates. The can was uncapped before the experiment and enough amount of beverage was poured into glass vials for sonication for 5 min and 0.9 mL beverage was mixed with 0.1 mL (60 mg/mL) *o*-PDA.

2.2.5. GO, MG and 3-DG in High Fructose Corn Syrup (HFCS).

In order to explore the possible source of dicarbonyl compounds in the beverage, the nutrition information from the products were checked. HFCS is the most suspicious source for these compounds. In the investigation of GO, MG and 3-DG in HFCS, 10 g of each HFCS were weighted into 10 mL volumetric flasks and were dissolved to the final concentration as 1 g/mL by o-PDA (60 mg/mL). The dicarbonyl compounds were derivatized under conditions similar to those previously described in the section of dicarbonyl compound derivatization.

2.2.6. Beverage Storage Study.

Brand A CSD was chosen for further accelerated storage study. A 2x2 factorial experiment was designed in this study. The factors were temperature and EGCG concentration. Temperatures were set at 35 or 45ºC and EGCG's final concentrations in beverages were either 0% or 0.1% (w/v). Four systems were used: (i) beverage without EGCG at 35ºC, (ii) beverage with 0.1% EGCG at 35ºC, (iii) beverage without EGCG at 45ºC, and (iv) beverage with 0.1% EGCG at 45ºC. Four aluminum cans (serving size 12 oz) in the same batch were used in this study and one can was studied in each of the four systems. The EGCG solution (0.03 g/mL) was prepared before the experiment. The beverage was poured into a 500 mL beaker and sonicated for 5 min. The beverage (14.5 mL) was transferred to each vial. Afterwards, 0.5 mL HPLC grade water was added to (i) and (iii), and 0.5 mL, of 0.03 g/mL EGCG was added to (ii) and (iv). Under these circumstances, the headspace volume in a vial was minimal. The samples stored in the oven were kept away from light. Time points of 0, 1, 2, 4, 8, 12 and 16 days were set for EGCG and RCS (GO,MG and 3-DG) quantifications.

2.2.7. Sample Preparation/Derivatization for EGCG, GO, MG and 3-DG content analysis in stability study.

For EGCG analysis, samples were centrifuged before HPLC run. For dicarbonyls analyzes, the same procedure was used as described in the section under Dicarbonyl compound derivatization.

2.2.8. HPLC System.

The Dionex UltiMate 3000 LC Modules equipped with a pump (Model: LPG-3400 pump, Sunnyvale, CA), UV–Vis detector (Model: VWD-3400 detector), and an autosampler (Model: WPS-3000 SL) were used. A Luna C18 (Phenomenex, Torrance, CA) column (150x4.6 mm i.d., 3 μm particle size) was used for EGCG and quinoxaline derivatives analysis. The column temperature was maintained at 25°C in column oven (Dionex Model: STH 585). The mobile phase for the HPLC system consisted of HPLC grade water with 0.15% acetic acid (v/v; solvent A) and acetonitrile (solvent B) with a constant flow rate set at 0.8 mL/min. HPLC gradient programs were performed as followings: (i) For EGCG analysis: A curve line number 6 gradient elution was performed as following: Initially, 8% solvent B, and it increased to 40% over 10 min, to 48% over additional 2 min, to 60% over additional 1 min and 5 min for equilibrium. EGCG was detected with a UV wavelength at 280 nm and the injection volume was 15 μL. (ii) For RCS analysis: The same curve gradient program was performed as in EGCG analysis from 0 to 13 min. After 13 min, solvent B increased to 80% for additional 2.5 min and 5 min for equilibrium. Quinoxaline derivatives were detected with a UV wavelength at 313 nm and the injection volumes were varied and dependent on the concentration of quinoxaline derivatives. The external standard quantification method was applied in this study. Every single peak area for the quantification was laid in the linear range of each standard curves.

2.2.9. Extraction Processes for Gas Chromatography (GC) Analysis.

At each specific incubation period at 35 and 45ºC, beverage samples were taken out from oven. The CSD samples were stirred vigorously for 5 s before extraction

process. CSD (3 mL) was transferred into a clean vial. Later, an appropriate amount of internal standard was added to 3 mL CSD. 5-HMF in CSD was extracted twice with 3 mL methylene chloride. For each extraction process, samples were stirred vigorously for 5 s. The methylene chloride and CSD were centrifuged for 5 min. The organic phase was dried over anhydrous sodium sulfate. These extraction samples were concentrated to 0.5 mL final volume under gentle nitrogen gas. The samples were ready for GC injection and quantification.

2.2.10. Capillary GC/FID.

The analysis of standard volatiles samples were performed with an Agilent Gas Chromatograph (Santa Clara, CA). The Agilent Gas Chromatograph (6850 series) equipped with an Agilent autosampler (7683 series Injector) and a flame ionization detector (FID) was used. Samples were analyzed on a Zebron ZB-5 fused silica capillary column, 30 mx0.25 mm i.d., film thickness 1.0 μm (Phenomenex, Torrance, CA). The injector temperature was 250ºC and detector temperature was 300ºC. 1 μL sample was injected for analysis and the injector was in splitless mode. The 1.0 mL/min constant carrier gas (helium) flow rate was set. The GC oven temperature was programmed as followed: the initial oven temperature 40ºC held for 0 min and increased to 164ºC at the rate of 4 ºC/min held for 0 min. Then the temperature was increased to a final temperature of 280ºC at the rate of 120º C/min and held for 3 min. The total run time was 34.97 min.

2.2.11. NMR Instrument.

NMR spectra were recorded on a Varian 300 Spectrometer (Varian Inc., Palo Alto, CA). With TMS serving as internal standard, 1H NMR was recorded at 300 MHz.

2.2.12. Liquid chromatography–electrospray ionization mass spectrometry (LC–MS).

A HPLC–MS system was composed of an autosampler injector, an HP1090 system controller, with a variable UV wavelength (190–500 nm) detector, an ELSD (Evaporative Light Scattering Detector) and an ESI-MS detector from Micromass VG Platform II mass analyzer (Micromass, Beverly, MA). ESI-MS conditions were as follows: Acquisition mode, ESI-positive; mass scan range, 100–800 amu; scan rate, 0.4 s; cone voltage, 25 V; source temperature: 150ºC; probe temperature: 550ºC. Analytical HPLC conditions on HPLC–MS: Column: Chromeabond WR C18 (ES Industries, West Berlin, NJ), 3 μm, 120 Å length and OD: 30x3.2 mm; injection volume: 15 μL; flow rate: 2 mL/min; run time: 3 min. Mobile phase consisted of acetonitrile and H_2O with 0.05% TFA, typical gradient of 10–90% acetonitrile and the gradient varied.

2.2.13. Statistical analysis.

Data were expressed as means ± standard deviation (SD) and represent three independent analyzes. Statistical significance was examined using Student's t-test comparison between the means. A p value of <0.05 was considered statistically significant.

2.3. Results and Discussion

2.3.1. GO, MG and 3-DG in Carbonated Beverages.

Table 1 list the concentrations of GO, MG and 3-DG in 13 brands of carbonated soft drink (CSD) from three different local supermarkets. The HPLC profile of quinoxaline derivatives was shown in **Figure 8**. Their baseline separation can be achieved in a 15 minute gradient run. The peaks for quinoxaline and 2-methylquinoxaline were confirmed with standard compounds at retention times of 11.35 and 12.67 min, respectively. The peak with retention time at 7.81 was first checked with a LC/MS to have a molecular weight at 234 corresponding to the quinoxaline derivative of 3-DG, 2-methyl-3-(2',3'-dihydroxypropyl)-quinoxaline. 2-Methyl-3-(2',3'-dihydroxypropyl)-quinoxaline was purified and identified from a honey derivatized sample as previously reported (Weigel et al., 2004). Its retention time was 7.81 minutes, the same as the peak resulting from CSD. The standard curve for 2-methyl-3-(2',3'-dihydroxypropyl)-quinoxaline was established with a reference compound obtained from TRC Inc.

From the result in **Table 1**, it is obvious that CSD contained significantly high levels GO, MG, especially 3-DG. This is the first report of the presence of reactive dicarbonyl compounds in CSD. AA and BB were both diet drinks and contains aspartame as sweetener, they contain less than 20 μg/100 mL or undetectable amount of 3-DG. The samples of AA and BB drinks from the three supermarkets had low level of GO and MG (2.0 to 9.3 μg/100 mL), except one beverage BB had 31.5 μg/100 mL of MGO from the supermarket C. These amounts of dicarbonyl compounds may contribute from caramel or the contaminated natural and artificial flavors. However, A, B and all other brands contain high amount of HFCS. Their contents of GO, MG and 3-DG range from 15.8-

104.6, 23.5-139.5 and 978.2-3488.9 µg/100 mL, respectively. In Energy drink K, highest amount of MG, above 100 µg/100 mL was quantified.

Table 1. The concentrations (μg/100 mL beverage) of GO, MG and 3-DG in 13 brands carbonated soft drinks from supermarket A, B and C.

Supermarket A			
Brand	**GO**	**MG**	**3-DG**
A	50.8 ± 1.6	88.3 ± 7.2	3488.9 ± 50.6
B	24.8 ± 1.5	92.5 ± 8.4	3338.4 ± 89.9
C	27.3 ± 0.7	54.6 ± 0.6	2647.1 ± 0.7
D	25.2 ± 3.8	48.5 ± 2.1	2813.7 ± 60.3
E	15.8 ± 6.5	41.1 ± 4.8	1779.6 ± 9.4
F	27.8 ± 0.8	42.5 ± 2.0	2229.8± 9.5
G	173.4 ± 2.8	62.2 ± 3.3	2460.6 ± 39.5
H	41.9 ± 2.2	50.1 ± 6.1	2796.6 ± 42.3
I	34.6 ± 0.9	23.5 ± 0.6	1677.4 ± 27.1
J	104.6 ± 14.4	59.7 ± 4.8	1339.5 ± 35.8
K	38.4 ± 1.9	139.5 ± 2.1	1532.5 ± 7.7
AA	2.7 ± 0.7	7.2 ± 1.6	19.2 ± 3.3
BB	2.0 ± 0.2	7.1 ± 1.9	ND
Supermarket B			
Brand	**GO**	**MG**	**3-DG**
A	39.9 ± 8.1	83.6 ± 6.1	3346.7 ± 12.3
B	25.1 ± 0.8	89.2 ± 3.4	2875.4 ± 21.6
C	11.2 ± 0.7	46.5 ± 3.1	2401.8 ± 41.7
D	12.3 ± 1.7	23.6 ± 1.5	1351.6 ± 81.2
E	10.8 ± 2.2	31.6 ± 2.6	1566.4 ± 50.0
F	11.4 ± 1.2	38.5 ± 0.8	2373.6 ± 99.0
G	68.8 ± 2.9	57.8 ± 1.0	2835.1 ± 33.5
H	21.0 ± 1.5	50.2 ± 0.5	2765.6 ± 33.1
I	18.3 ± 0.9	26.2 ± 1.1	1374.5 ± 32.4
J	40.8 ± 8.5	43.2 ± 4.9	1656.2 ± 99.4
K	20.8 ± 4.8	104.2 ± 5.9	978.2 ± 50.8
AA	2.6 ± 0.5	7.6 ± 0.7	10.6 ± 0.7
BB	2.4 ± 0.4	8.2 ± 1.2	ND
Supermarket C			
Brand	**GO**	**MG**	**3-DG**
A	28.9 ± 3.1	78.2 ± 6.7	3197.0 ± 70.2
B	25.5 ± 0.9	87.3 ± 8.8	2636.6 ± 47.1
C	7.2 ± 3.5	60.0 ± 10.7	2638.5 ± 73.1
D	8.5 ± 0.4	41.8 ± 1.3	2733.7 ± 14.5
E	9.5 ± 3.0	31.0 ± 1.6	1549.1 ± 30.9
F	12.9 ± 1.2	29.2 ± 1.2	1647.5 ± 54.5
G	62.9 ± 2.1	62.1 ± 1.5	2788.9 ± 142.8
H	22.0 ± 1.3	27.7 ± 0.2	1286.7 ± 2.8
I	20.5 ± 0.5	26.9 ± 0.8	1334.8 ± 6.5
J	33.9 ± 1.9	33.0 ± 3.2	1697.5 ± 13.5
K	32.7 ± 11.0	111.2 ± 6.0	1016.0 ± 7.4
AA	2.3 ± 0.9	9.3 ± 2.6	13.8 ± 4.1
BB	2.0 ± 0.5	31.5 ± 6.2	ND

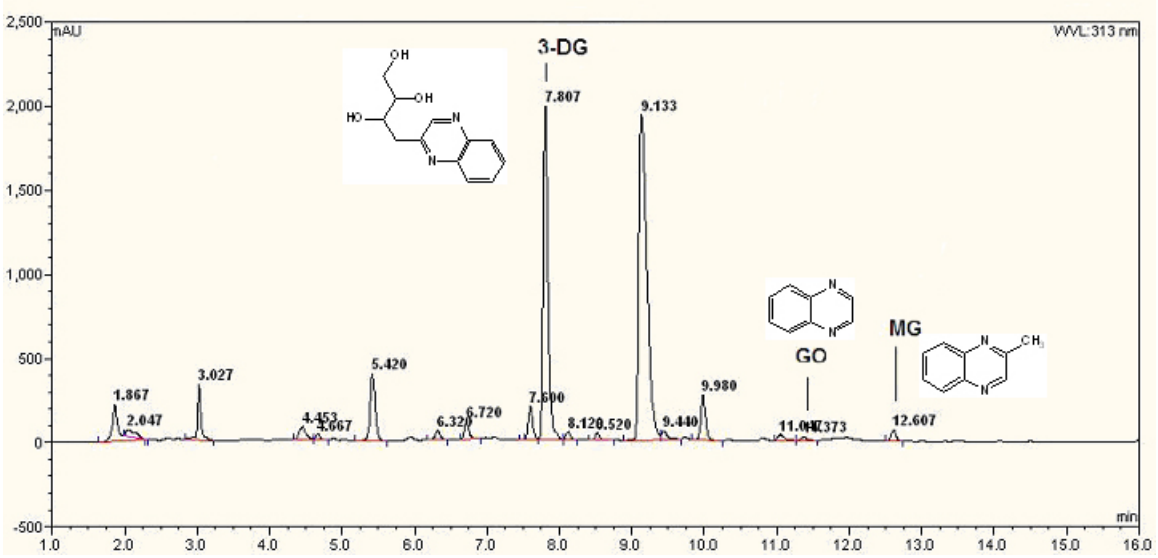


Figure 8. HPLC profile of quinoxaline derivatives from the reaction of carbonated soft drinks containing EGCG and *o*-phenylenediamine (wavelength of 313 nm).

2.3.2. GO, MG and 3-DG in High Fructose Corn Syrup (HFCS).

In order to verify the observations of dicarbonyls in CSD and their possible sources in drink, 3 HFCS samples were obtained from local beverage companies. In **Table 2** high contents of GO, MG and 3-DG were observed. The highest level of 3-DG was in 42% HFCS. More than 7 mg was observed in 100 g of 42% HFCS. It was more than twice higher than 55% HFCS. However, the concentrations of GO and MG vary significantly from different sources of HFCS. Their manufacture processes and storage conditions should play a major role for the variation.

Table 2. The concentrations of GO, MG and 3-DG in HFCS samples. #1 and #2 are 55% HFCS samples and #3 is a 42% HFCS sample.

	GO	MG	3-DG
#1	18.1 ± 0.8	385.1 ± 7.5	2713.1 ± 89.0
#2	50.8 ± 0.2	112.4 ± 0.1	3332.2 ± 34.2
#3	32.7 ± 1.5	366.9 ± 19.5	7449.0 ± 429.5

concentration unit: μg/100ml; values are mean ± standard deviation, *n*=3.

2.3.3. Beverage stability study during storage.

After the discovery of high contents of dicarbonyls in CSD which contained HFCS, the storage study was further investigated. Brand **A** beverage was chosen in this study. A 2 x 2 factorial design was conducted. Temperatures were set at 35 or 45ºC and EGCG's final concentrations in beverages were either 0% or 0.1% (w/v). Besides temperature factor, EGCG is a natural product used in the stability study**. Figure 9** showed the HPLC profile of EGCG. EGCG quantification in EGCG-containing CSDs at the specific time points was monitored from direct HPLC injection of samples without the derivatization of dicarbonyl compounds. Since the UV wavelength at 280 nm has higher sensitivity than 313 nm for EGCG. EGCG was detected with UV wavelength 280 nm and quinoxalines of GO, MG and 3-DG was detected with UV wavelength 313 nm. From the result shown in **Figure 10**, EGCG reacted with reactive components in CSD under acidic conditions at 35 or 45ºC during storage. The rate of reaction at 45 ºC was higher than that of in 35 ºC. The difference of decrease percentage was 3-4% before 2 days. After 4 days, the difference of decrease percentage increased to 12-18%. The highest difference, 18%, occurred on day 8 and the lowest, 12%, occurred on day 4. Only half of EGCG was left on day 16 for 35ºC and one third of EGCG was left on day 16 for 45 ºC under storage.

The changes of GO, MG and 3-DG levels in beverage with and without the addition of EGCG during storage were shown in **Fig 11-13**. In Figure **11**, the original GO levels were among 20 to 35 μg/100 mL. The highest concentration (37 μg/100 mL) occurred at 35 ºC, 16 days. At 45 ºC, GO was in a steady level through 16 days study no

matter EGCG was added or not. Nevertheless, 35 ºC and EGCG model showed the decrease GO level after 16 days storage and the highest relative decrease was at 8 days.

In **Figure 12**, the concentrations of MG among four bottle beverages were between 64-76 μg/100 mL initially. The generation of MG in elevated temperatures was a static kinetics. According to observations, MG concentration for 35 ºC after 16 days storage increased 50% and MG concentration is double after 16 days storage for 45 ºC sample. However, MG levels did not increase when EGCG was added in the samples which were incubated at 35 or 45 ºC. It implied that the same adduct reaction between EGCG and MG we observed previously may occur in acidic condition (Lo et al., 2006). From the constant concentrations of MG in the samples with EGCG through whole storage study, it may indicate that the rates of MG generation and MG trapping should be similar.

From **Figure 13**, the range of 3-DG is among 2968 to 3029 μg/100 mL from sample at 0 day. For 45 ºC storage, 3-DG increased from 2968 to 3214 μg/100 mL. The first stage of increase occurred in the first 4 days incubation and the next stage occurred after 12 day. Its increase could be due to the autoxidation of glucose (Thornalley et al., 1999). For 35 ºC storage, 3-DG increased from 3004 μg/100 mL in 0 day sample to 3108 μg/100 mL in 16 days sample. In contrast with 3-DG amounts for the beverages added with EGCG to the beverages without EGCG, 3-DG decrease in both 35 and 45 ºC when the beverages contained EGCG. This again confirms our previous observation that the dicarbonyl compounds could efficiently react with catechins.

In this study, the levels of RCS in commercial carbonated soft drinks were found to be astonishingly high. Take the methylglyoxal as an example, the range of 23.5-139.5

μg/100 mL was observed, this is significantly higher than the reported level of methylglyoxal, 16-21 μg/100 mL in diabetic patients (Odani et al., 1999; Lapolla et al., 2003). High fructose corn syrup was identified as a source of these reactive carbonyl compounds in CSDs. Furthermore, EGCG and possible other polyphenols may have the potential to reduce the levels of reactive carbonyl compounds in beverages.

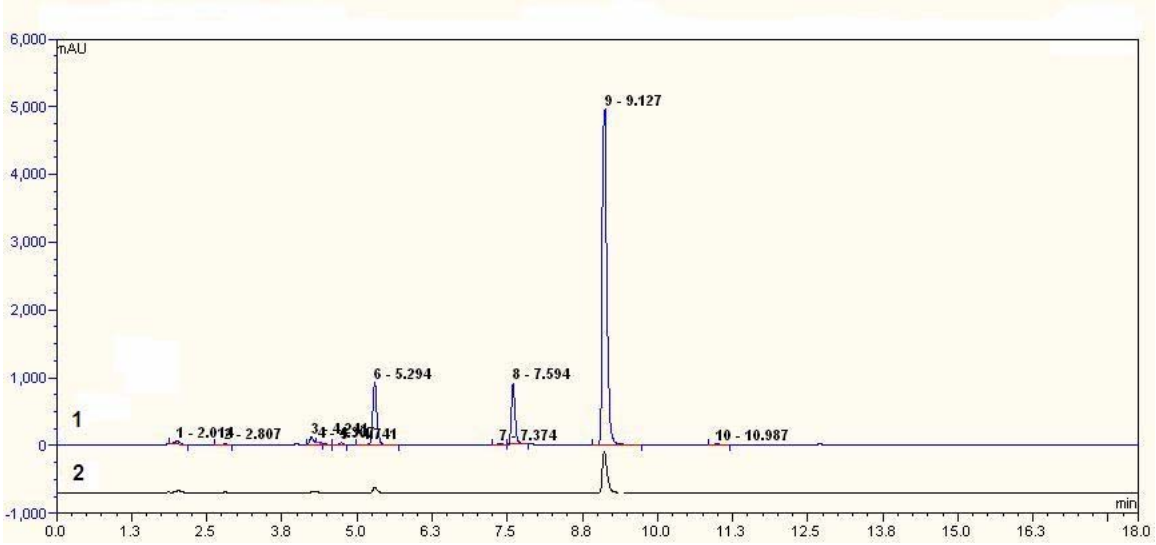


Figure 9. PLC profile for storage test containing 0.1% EGCG (w/v) CSD sample without *o*-PDA treatment. Line 1 represents for UV detection at wavelength 280 nm and line 2 represents for UV detection at wavelength 313 nm. EGCG showed at 9.127 min.

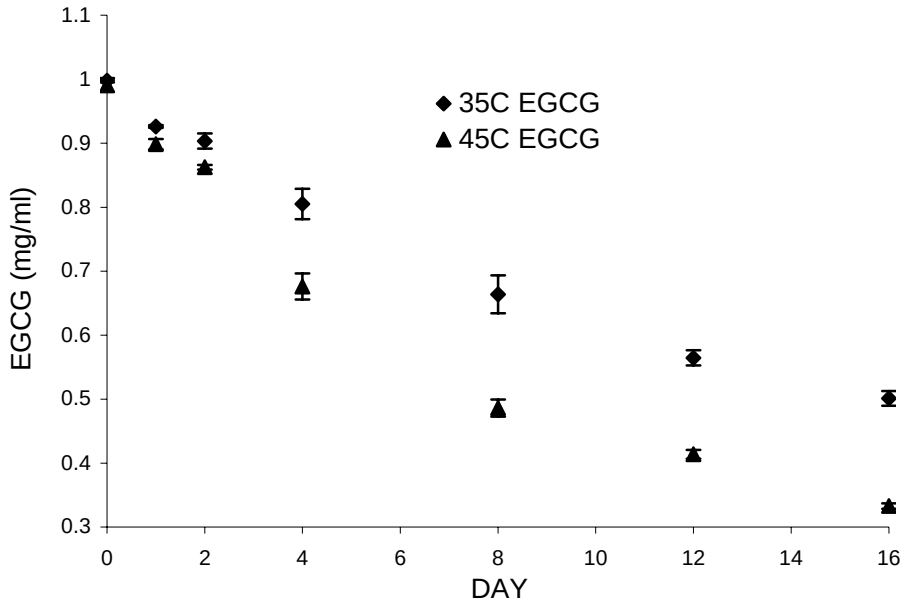


Figure 10. Concentration of EGCG during storage of brand A beverage from 0 to 16 days at 35 ºC (♦) and 45 ºC (▲). *n*=3.

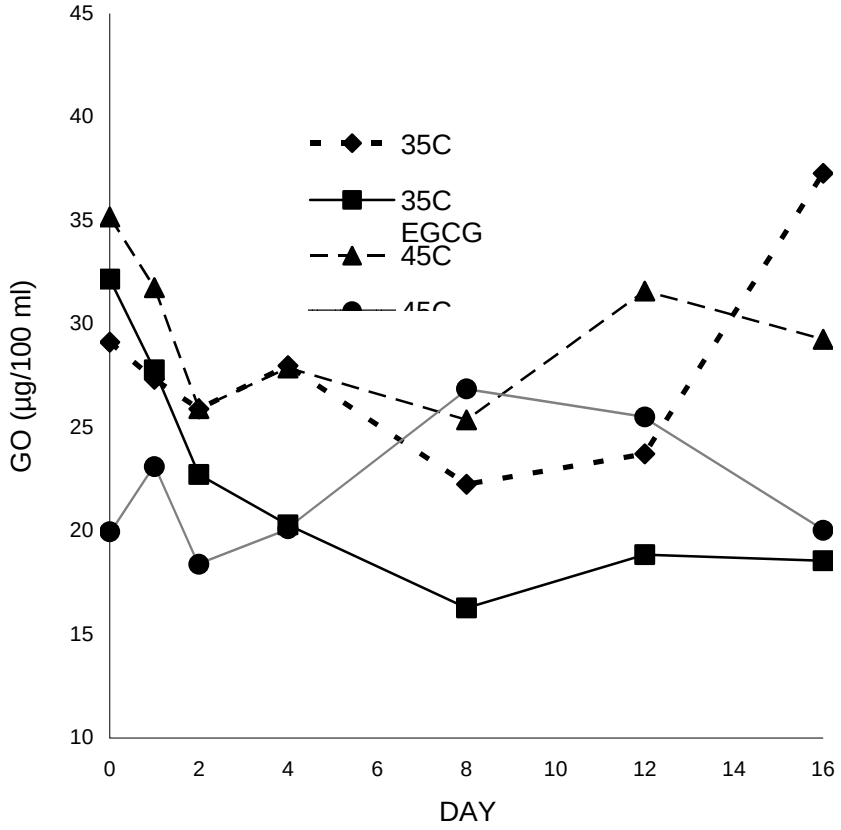


Figure 11. Glyoxal (GO) changes in brand A CSD samples. ♦: 35ºC, without EGCG; ■: 35ºC with 0.1% EGCG; ▲: 45ºC, without EGCG; ●: 45ºC, with 0.1% EGCG. *n*=3.

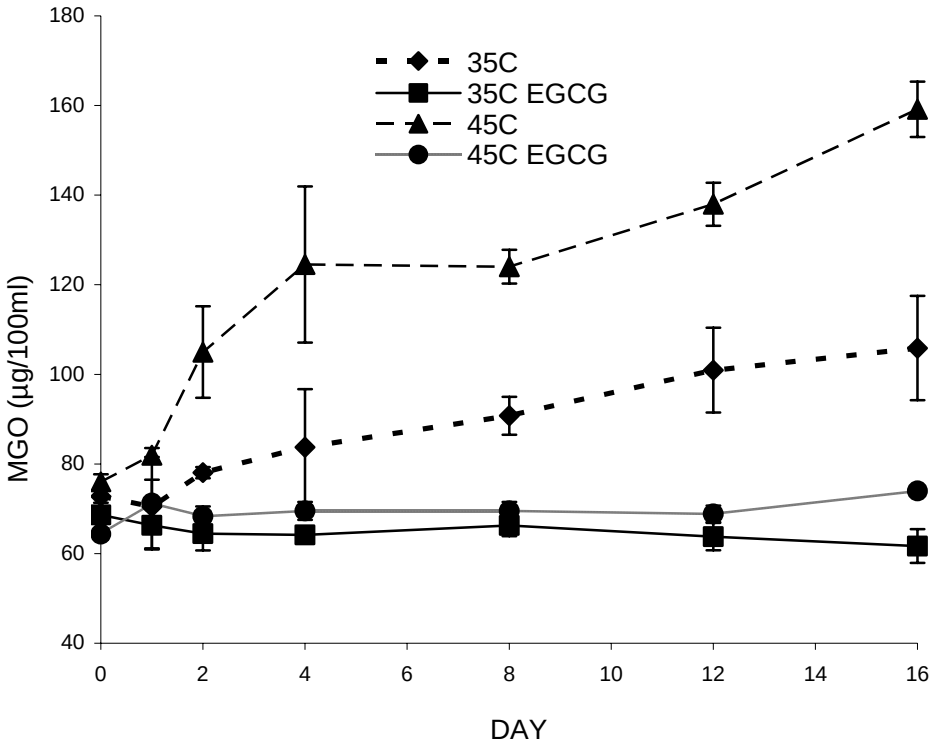


Figure 12. Methylglyoxal (MGO) changes in brand A CSD samples. ♦: 35ºC, without EGCG; ■: 35ºC with 0.1% EGCG; ▲: 45ºC, without EGCG; ●: 45ºC, with 0.1% EGCG. *n*=3.

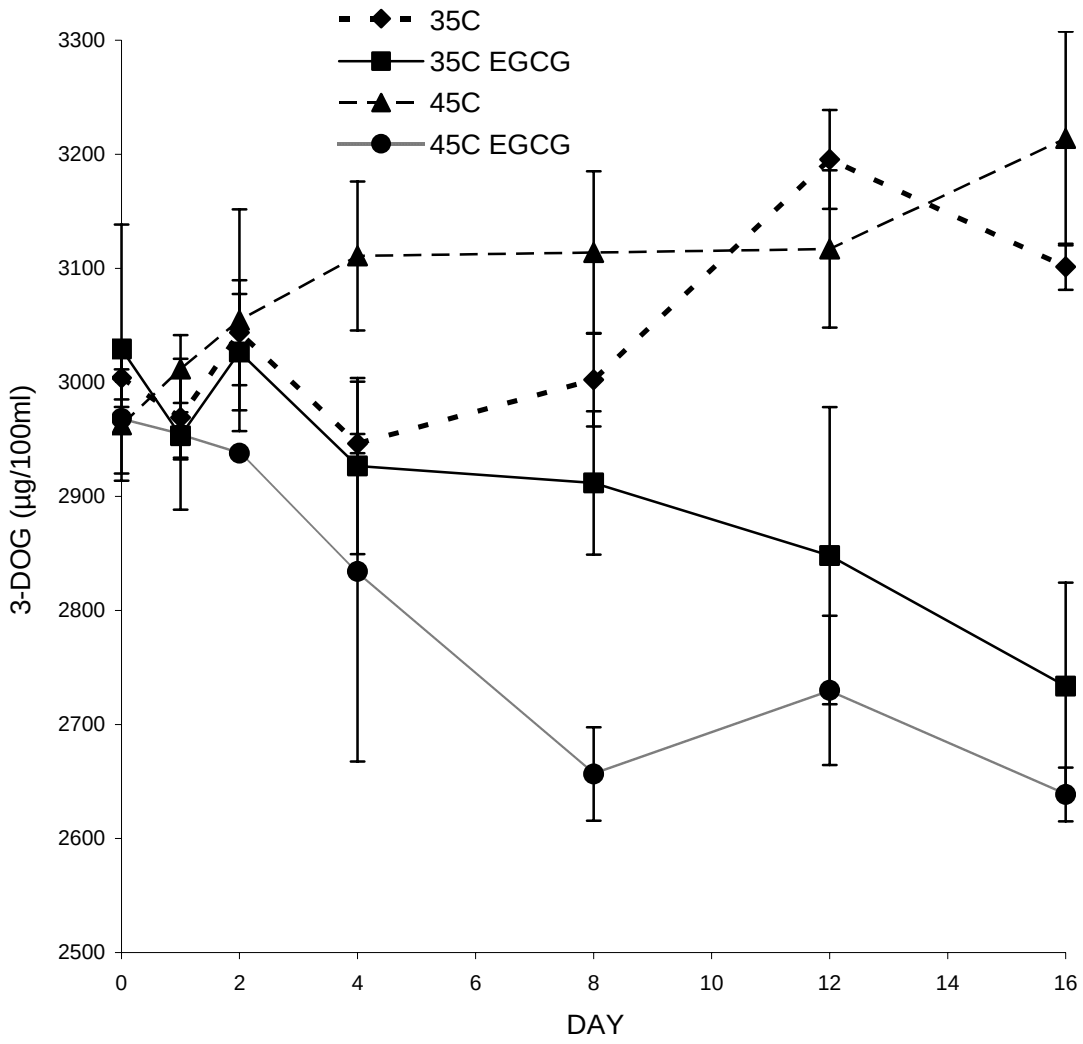


Figure 13. 3-Deoxyglucosone (3-DOG) changes in brand A CSD samples. ♦: 35ºC, without EGCG; ■: 35ºC with 0.1% EGCG; ▲: 45ºC, without EGCG; ●: 45ºC, with 0.1% EGCG. *n*=3.

3. Chapter II. Reactive Carbonyl Species in Flavor Generation

3.1. Literature Review

Flavor generated from Maillard reaction could go through three basic ways, i) from carbohydrates only, ii) from combination of carbohydrates and amino acids, and iii) from amino acids only, among which RCS play important roles. Carbohydrates could transform into glucosones at the beginning of Maillard reaction, and then those RCS either cyclize into flavor compounds or breakdown into small RCS such as diacetyl, methylglyoxal and glyoxal, then followed a recombination of these intermediates. In this part, furanoids discussed as a representative are generally considered from cyclization of intact glucosones (RCS) and/or recombination of small molecular RCS from glucosones. The product formation from carbohydrates and amino acids could be divided into amino acid-specific and non-specific pathways. For the amino acid-non-specifi pathway, α-dicarbonyl reacts with most types of amino acids forming α-aminoketone via Strecker degradation which leads to the formation of alkylpyrazines, oxazoles and oxazolines, whereas for the amino acid-specific pathway, amino acids such as cysteine and proline, α-aminoketone or α-dicarbonyl involve the generation of thiazoles, thiazolines, pyrrolines, and pyridines. Although some flavor compounds (etc. methional, phenylacetaldehyde) are generated from amino acids only, α-dicarbonyl compounds are still involved in their formation pathways particularly through Strecker degradation. Besides involvement of amino acids, carbohydrates or their fragments can react with peptides to generate some specific aromas (etc. pyrazinones).

3.1.1 Formation of aroma compounds from carbohydrates

Furanoids, incorporating a furan ring into its molecular structure such as furfural, 2-acetylfuran, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) and 5-hydroxymethylfurfural (HMF), are important flavor or flavor intermediates in foods. 2-Acetylfuran having a sweet balsamic-cinnamic note, is widely occurred in essential oils, sweet corn products, fruits and flowers (Adams, 1995; Buttery et al., 1995; Nishimura et al., 1989; Yamagushi & Shibamoto, 1979). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (known as Furaneol), with an intense caramel-like aroma, was originally discovered as a key flavor component of strawberry in 1965 (Willhalm et al., 1965). Till now, DMHF has been found as an important odor-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 (Rodin et al., 1965; Buttery et al., 1994; Tressl et al., 1978; Schieberle, 1990; Tei and Yamanaishi, 1974; Steinhaus and Schieberle, 2007). Because of its widespread occurrence, DMHF became a major reactant in generating other flavor compounds. At low pH, DMHF has been shown to react with cysteine or hydrogen sulfide in generating meat-like aroma compounds (Shu and Ho, 1988; Zheng et al., 1997). Some roast aroma compounds such as alkylpyrazines could also be generated through the decomposition of DMHF with phenylalanine (Jutta and Werner, 1990). And furfural is an intermediate of 2-furfurylthiol, a key roast aroma in coffee and the reaction of cysteine and ribose (Cerny & Davidek, 2004).

Most furanoids are formed from deoxyosone in the presence or absence of amino acids during intermediate or final stage of Maillard reaction. HMF or furfural and DMHF are products obtained on the decomposition of 3-deoxyosone and 1-deoxyosone,

respectively (Hodge et al., 1972). The formation pathways of 2-acetylfuran have been barely studied until now. Yaylayan et al. (2003) proposed that 2-acetylfuran was generated from 1-deoxyglucosone-6-phosphate during the thermal degradation of glucose-6-phosphate.

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 (Hofmann and Schieberle, 1997; Schieberle, 1992; Blank and Fay, 1996). Generally, DMHF can be formed through 2,3-enolization of 6-deoxysugars, hexoses and pentoses leading to 1-deoxyosones as intermediates (Hodge et al., 1972). Compared to hexoses and pentoses, 6-deoxysugars such as rhamnose are more effective in forming DMHF through 2,3-dioxo-4,5-dihydroxyhexane which is not easily formed from hexoses and cannot be generated from pentoses (**Figure 14**) (Hofmann and Schieberle, 1997). Schieberle in 1992, and later Hofmann and Schieberle in 2001 showed that DMHF can be formed from hexose via acetylformoin reduction which may proceed either by disproportionation reaction or a Strecker reaction with amino acids (**Figure 15**). In addition, hexose or pentose can be cleaved into methylglyoxal and 1-hydroxy-2-propanone to generate DMHF, and this reaction has been proved to be a major pathway when glucose is reacted with proline in an aqueous solution (**Figure 16**). (Schieberle, 2005). 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 **(Figure 17**).

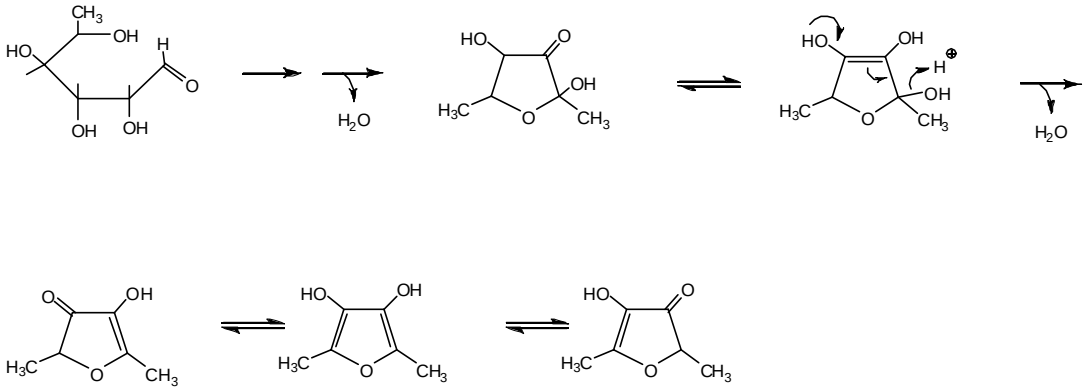


Figure 14. Proposed Pathway of DMHF Generation from Rhamnose (Hofmann and Schieberle, 1997)

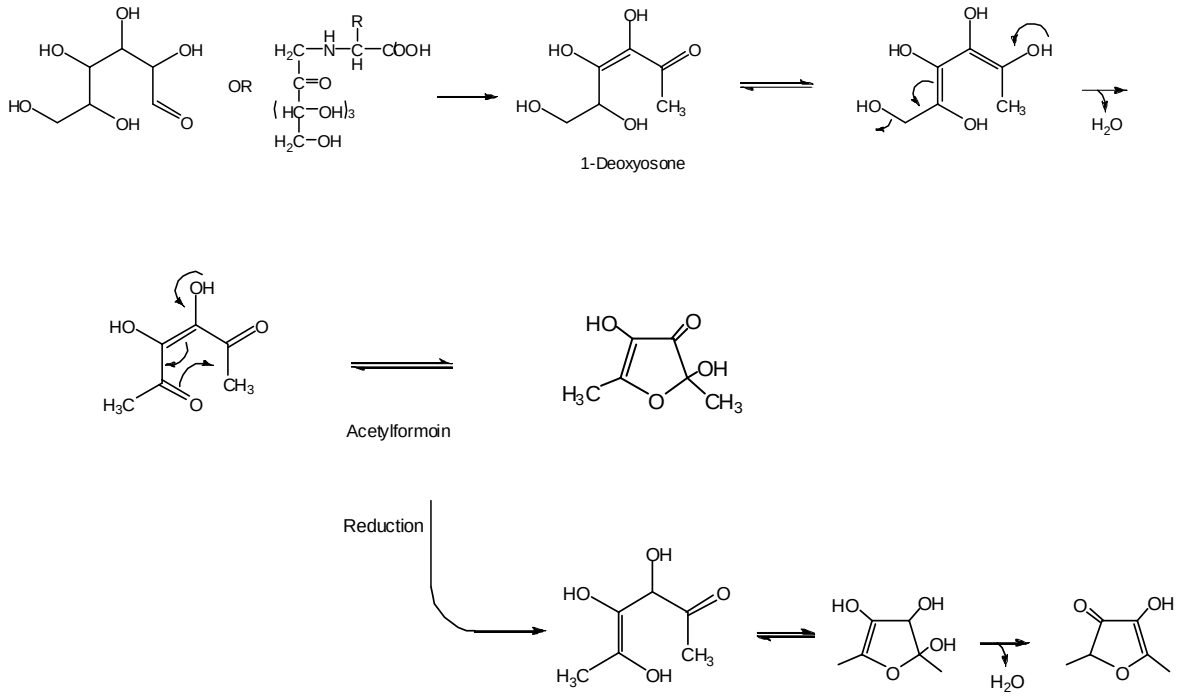


Figure 15. Proposed Pathway of DMHF Generation from Hexose (Hofmann and Schieberle, 2001)

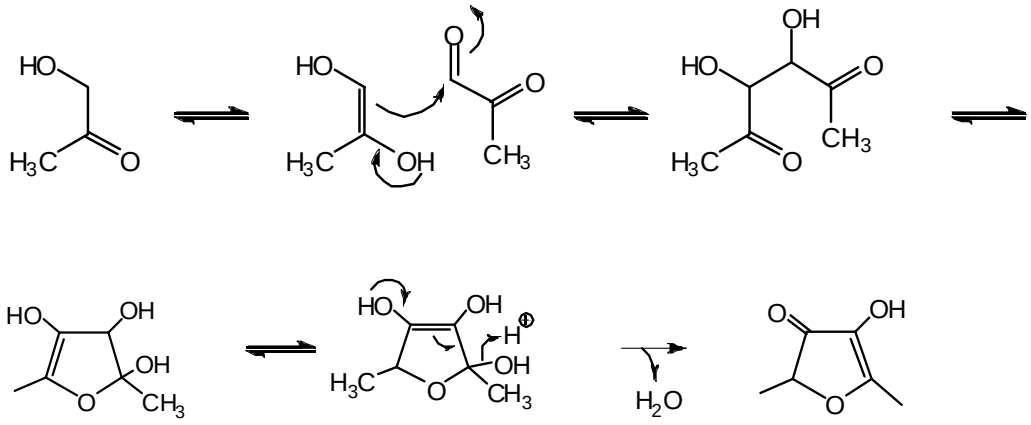


Figure 16. Proposed pathway of DMHF Generation from Glucose Fragments (Schieberle, 2005)

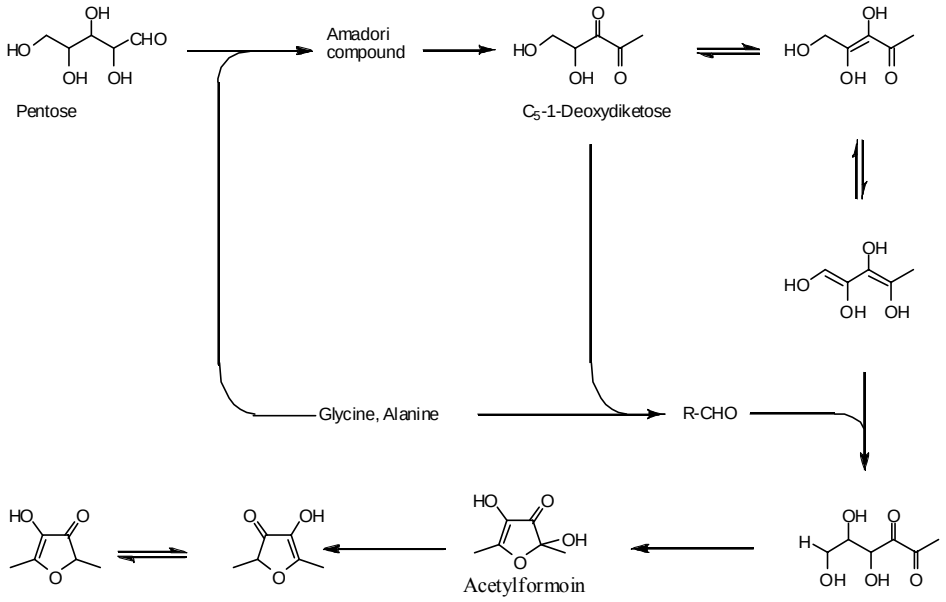


Figure 17. Proposed pathway of DMHF Generation from Pentose (Blank and Fay, 1996)

3.1.2. Formation of aroma compounds from carbohydrates and amino acids

3.1.2.1 Non-specific amino acids

Alkylpyrazines, which are nitrogen-containing heterocylic compounds, occur in a wide range of raw and processed food systems with potent and characteristic aroma (Maga, 1973, 1982). The most plausible formation mechanism of pyrazines is the condensation of two α-aminoketones (Shibamoto and Bernhard, 1977). RCS especially 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 dihydropyrazines. If the alky groups in α-aminoketones are different, the isomers of pyrazines are observed (Vernin, 1982) (**Figure 18**). In addition, dicarbonyl compounds can undergo Strecker degradation with amino acids, while α-hydroxycarbonyls can not undergo Strecker degradation, but will react with ammonia to produce alkylpyrazines (Izzo & Ho., 1992). Ammonia can be derived from amino acids and the major source of that is from deamidation of protein (**Figure 19**) (Wright, 1991). If amide group of glutamine was labeled, amino group and free ammonia could react with dicrabonyl and α-hydroxycarbonyl to form pyrazine with unlabled, one labeled and two labeled nitrogens indicating the deamidation of glutamine involved in pyrazines formation with RCS (**Figure 20**) (Hwang *et al.,* 1993).

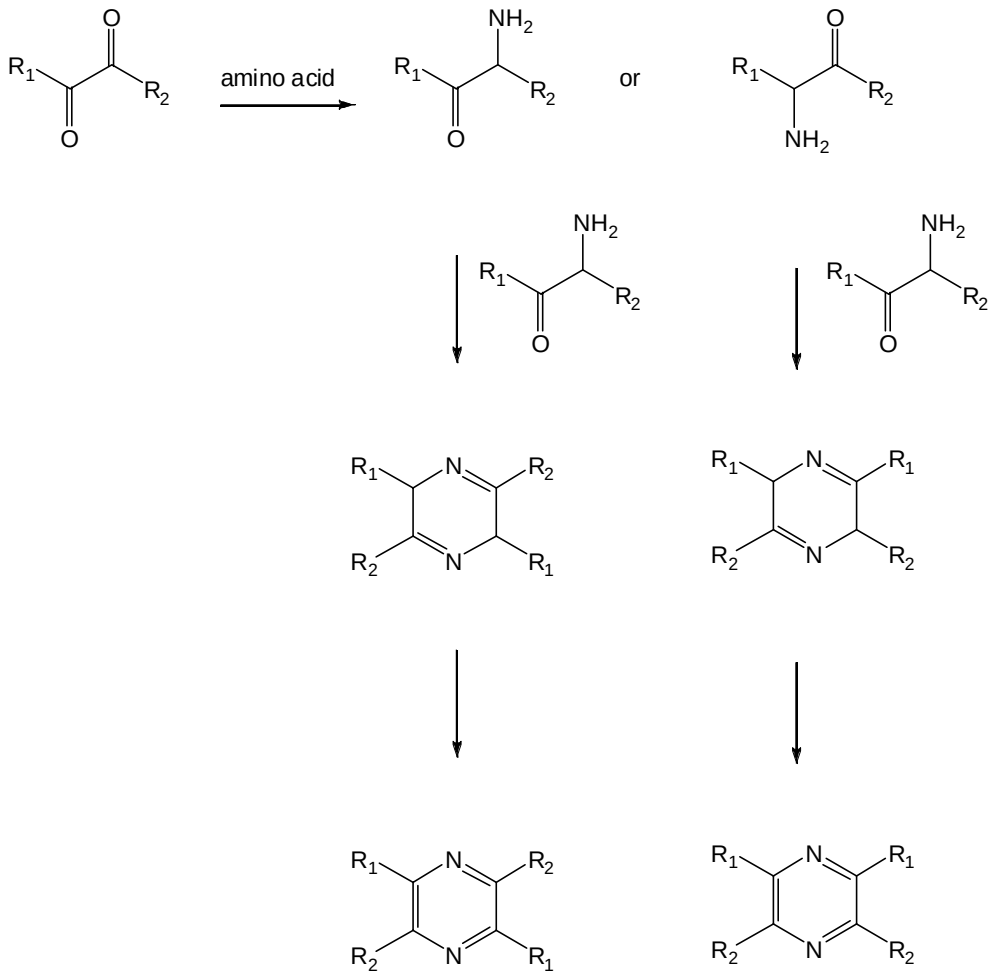


Figure 18. Proposed pathways of Alkylpyrazines Formation (Vernin, 1982)

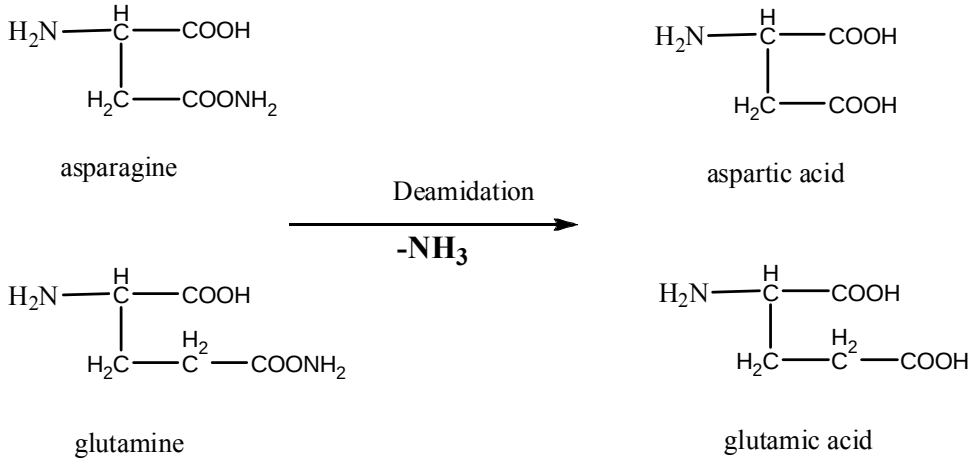


Figure 19. Deamination of Asparagine and Glutamine (Izzo & Ho, 1992)

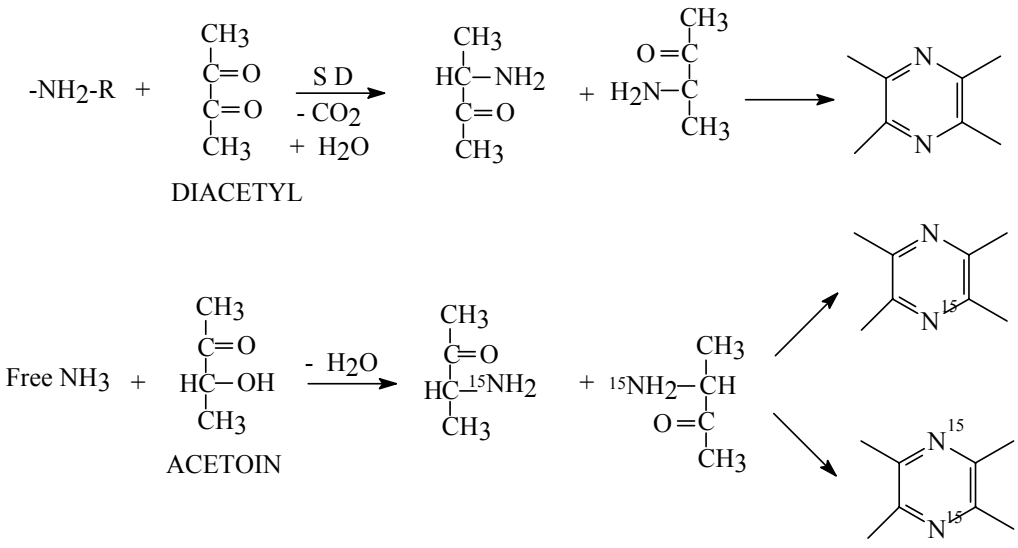


Figure 20. The Reactivity Between α-Amino Group and Free Ammonia towards Dicarbonyl and Hydroxycarbonyl compounds (Hwang *et al.*, 1993)

Oxazoles and oxazolines are two closely related heterocyclic flavor compounds containing nitrogen and oxygen atoms. The occurrences of oxazoles and oxazolines are in various processed foods. Stofflesma et al., (1968) reported the presence of 5-acetyl-methyloxaole in the roasted and ground coffee. Then four alkyloxazoles, 4,5-dimethyloxazole, 2,5-dimethyloxazole, 2,4,5-trimethyloxazole, and 5-methyl-2-propyloxazole, were identified in the roasted cocoa (Vitzthum et al., 1975). Some other thermal treated foods such as heated soy sauce, baked potatoes and roasted peanut also contained alkylyoxaozles (Nunomura et al., 1978; Coleman et al., 1981; Ho et al., 1983a).

Formation pathways of oxazoles and oxazolines were firstly proposed between a dicarbonyl compound and an amino acid undergoing decarboxylation (**Figure 21**). Then another pathway involved α-aminoketones and aldehyde were found to generate oxazoles and oxazolines via condensation and oxidation (**Figure 22**). Mussinan et al. (1976) demonstrated a third pathway which is through the interaction of acetoin, ammonium, and acetaldehyde. Acetoin generated from the reduction of diacetyl reacts with ammonia and acetaldehyde via cyclization and oxidation to form 2,4,5-trimethyloxazole (**Figure 23**).

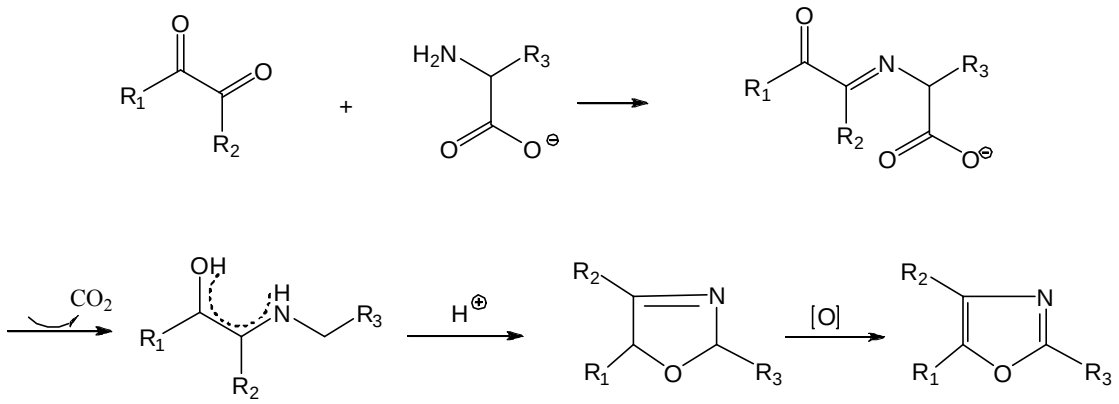


Figure 21. Proposed Pathway of Oxazoles and Oxazolines Formation (Rizzi, 1969).

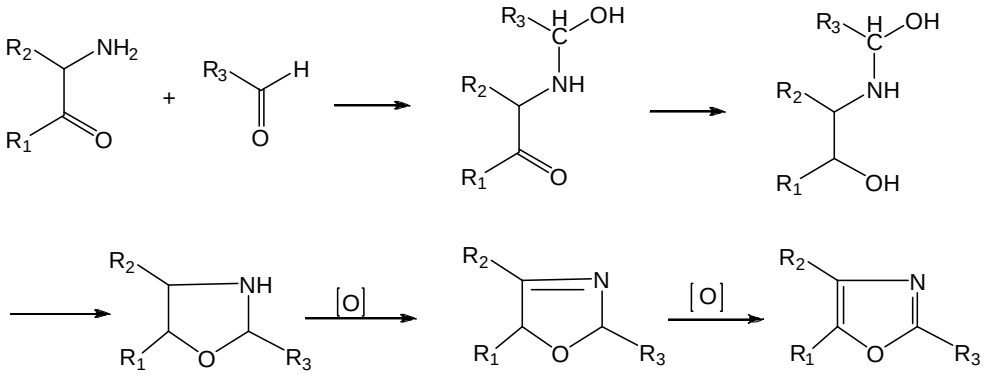


Figure 22. Proposed Pathway of Oxazoles and Oxazolines Formation (Baines and Mlotkiewicz, 1984).

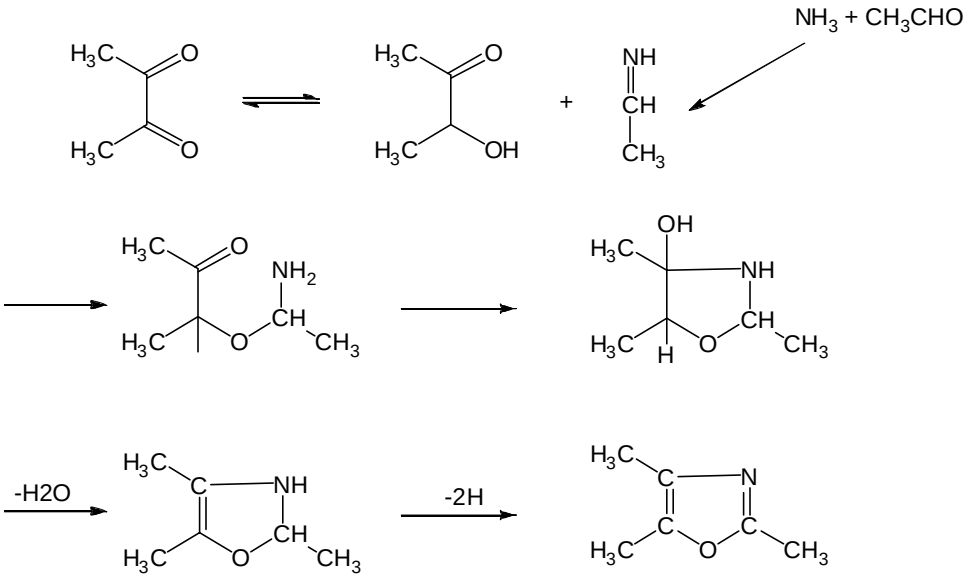


Figure 23. Proposed Pathway of Oxazoles and Oxazolines Formation (Mussinan et al., 1976).

3.1.2.2 Specific amino acids

Flavor generation reactions mostly occur at high temperature especially Strecker degradation in which carbonyl groups react with amine groups through nucleophilic attack. In Strecker degradation, MG can react with amino acids to form α-aminoketones which are key precursors of heterocyclic flavor compounds such as pyrazines, oxazoles and oxazolines (Rizzi, 1972; Vernin and Metzger, 1981). However, some kinds of flavor compounds such as popcorn like flavor 2-acetyltetrahydropyridine and roasted aroma 2-acetylpyrroline can be also formed from MG in the presence of a specific amino acid proline but without forming α-aminoketones. For example, 2-acetyltetrahydropyridine, which is a popcorn like flavor in rice and bread crust, can be formed from Strecker degradation of proline and MG through decarboxylation and dehydration (Tressl *et al.,* 1985) (**Figure 24**). The degradation product from reaction between proline and MG can continue reacting with MG to form 2-acetylpyrroline (**Figure 25**). Moreover, 2-acetyltetrhydropyridine can be also biosynthesized through lysine and MG (Wu *et al*., 2007). The gene(lat) encoding the L-lysine ε-aminotransferase(LAT) in the *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 mixture of 1-piperideine-6-carboxylic acid and methylglyoxal (**Figure 26**).

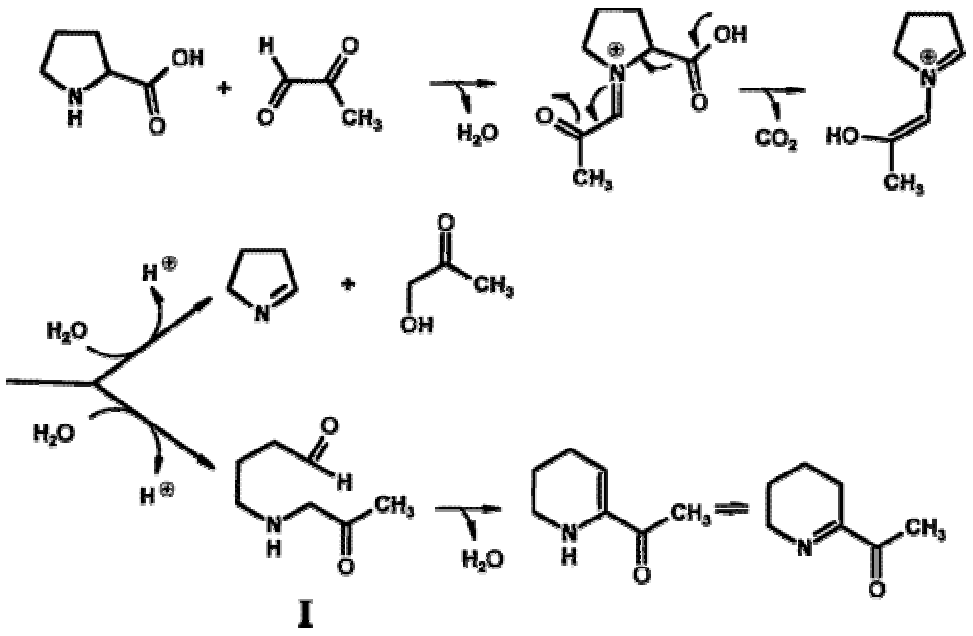


Figure 24. Hypothetical mechanism for the formation of 2-acetyltetrahydropyridine (Tressl *et al.*, 1985)

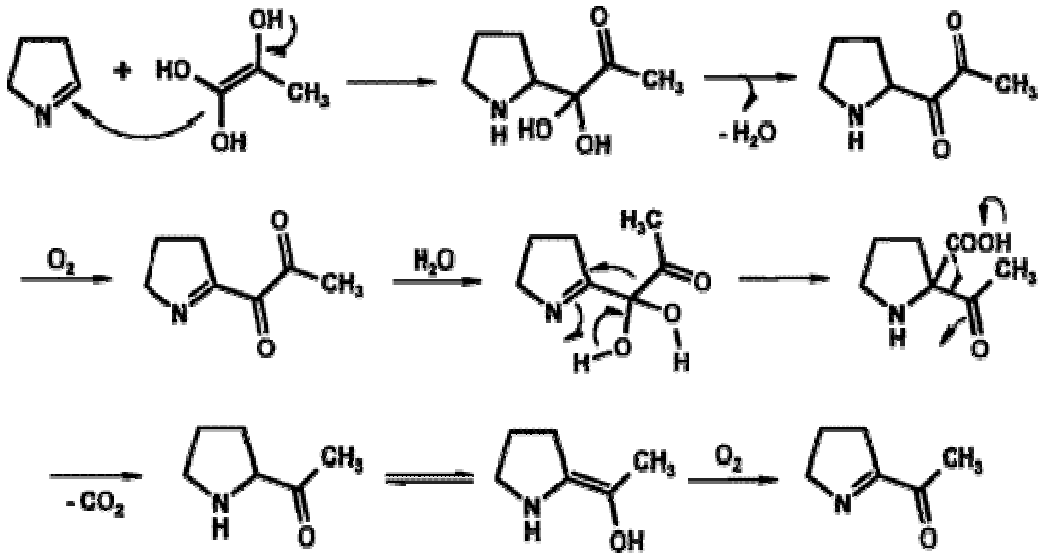


Figure 25. Hypothetical mechanism for the formation of 2-acetylpyrroline (Tressl *et al.,* 1985)

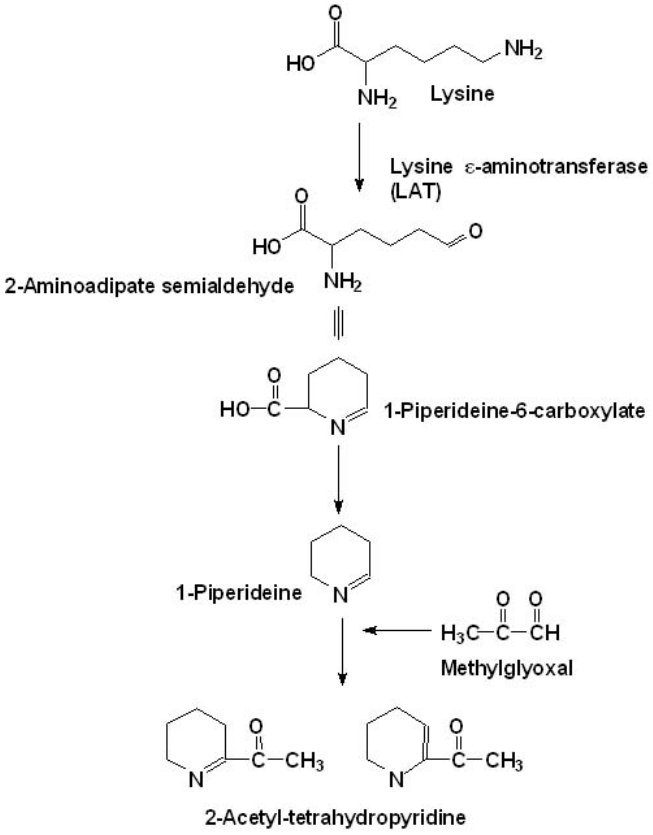


Figure 26. Biosynthesis of 2-acetyltetrahydropyridine (Adapted from Wu et al. 2007)

Other aroma compounds, which are formed in the presence of cysteine, are thiazoles and thiazolines. They are closely related to oxazoles and oxazolines, and the sulfur atom replaces oxygen atom in position 1 and nitrogen occupies position 3. The first thiazole and thiazoline isolated from food systems were 4-methyl-5-vinylthiazole and 2-acetyl-2-thiazoline, respectively (Stoll et al., 1967; Tonsbeek et al., 1971). Later various thiazoles and thiazolines have been identified in many food systems especially in thermally treated foods such as boiled and roasted beef (Wilson et al., 1973; Mussinan and Walradt, 1974.; Mussinan et al., 1976), baked potato (Coleman and Ho, 1981), fried chicken (Tang et al., 1983), and roasted peanut (Ho et al., 1983a). Generally, 2-alkylthiazoles possess green, vegetable-like aroma, with increasing the substitution in position 4 and 5 add more nutty, roasted and sometimes meaty notes. Because of sulfur containing, thiazoles and thiazolines mostly are formed in the presence of cysteine. The most accepted formation mechanism of them was that α-dicarbonyls 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-trimethythiazole and 2,4,5-trimethyl-3-thiazoline through condensation, cyclization and condensation with ethylideneamine (**Figure 27**). In addition, thiamine which contain thiazole moiety, undergoes degradation to form 4,5-dialkyl-substitued thiazoles, and 2-alkylthiazole could also be generated from condensation of aldehyde and cysteine degradations (**Figure 28**).

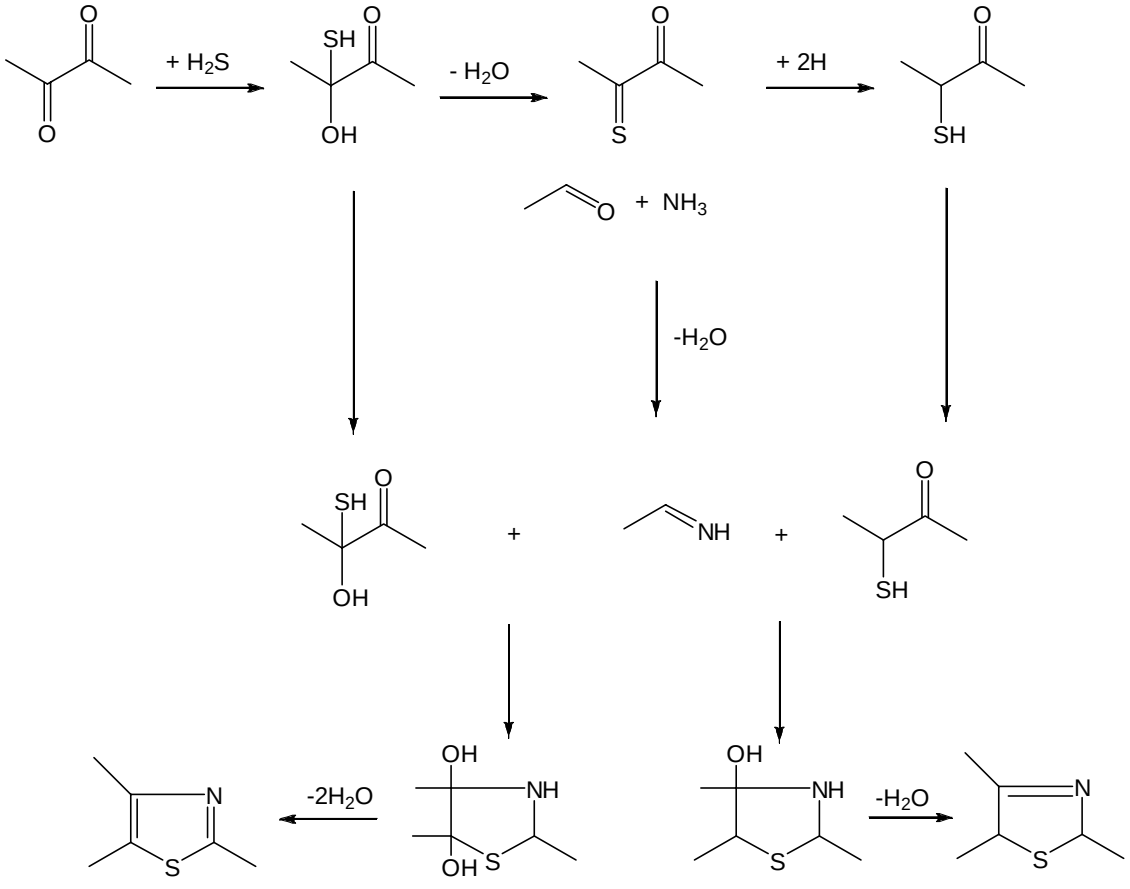


Figure 27. Formation pathways of thiazoles and thiazolines (Takken, 1976)

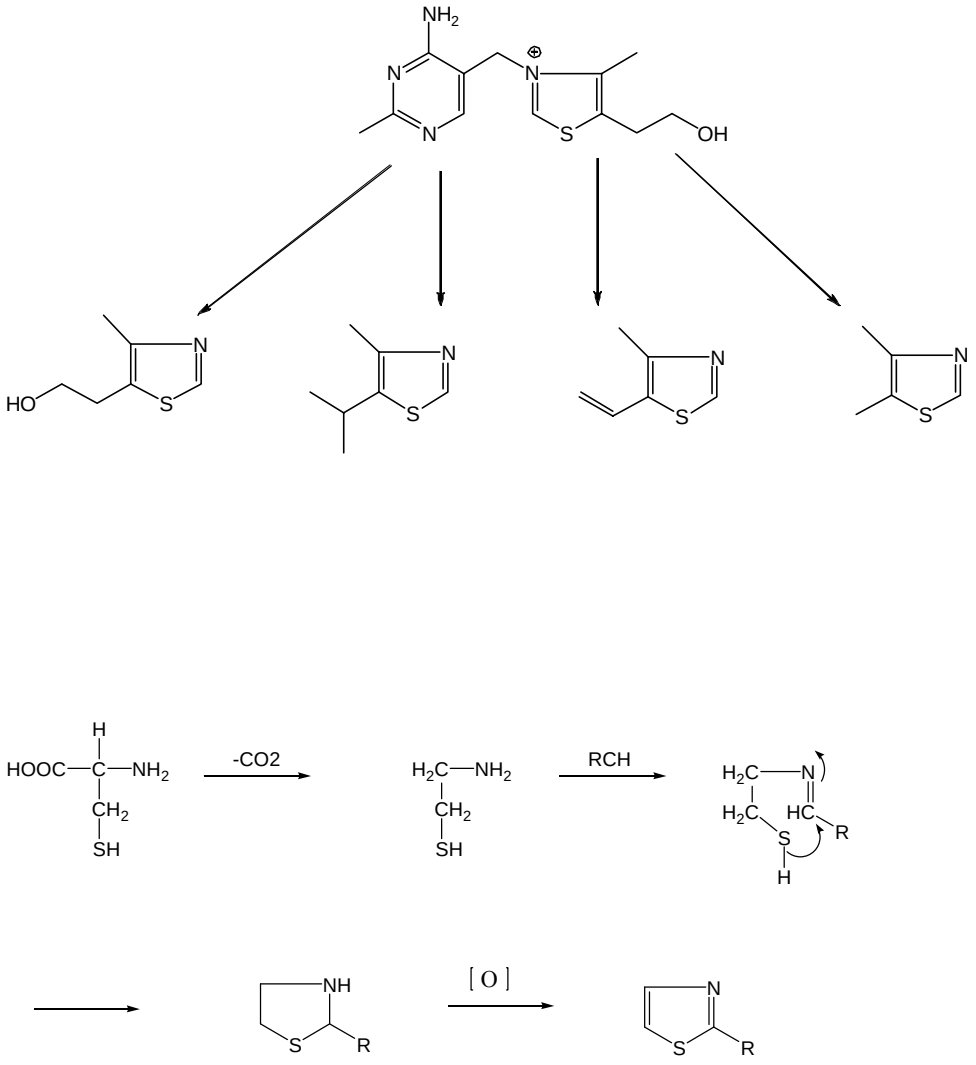


Figure 28. Thiazoles Generated from Thiamine and Cysteine Degradation (Mulder, 1973 and Mottram, 1991)

3.1.3. Formation of aroma compounds from amino acids.

Aromas such as 3-methylbutanal (malty) or phenylacetaldehyde (honey-like) which are derived from leucine and phenylalanine, respectively in the presence of α-dicarbonyl, generally are Strecker aldehydes formed in the Strecker degradation. Besides aldehydes, some odor-active acids were also identified in the presence of RCS. In Strecker degradation, for example, phenylalanine reacts MG or GO, preferentially leading to phenylacetic acids generation (**Figure 29**), whereas reacting with 3-DG favors the formation of phenylacetaldehyde (**Figure 30**).

3.1.4. Formation of aroma compounds from peptides.

Besides different amino acids, peptides are also important precursors of flavor generation with RCS. Lu *et al* (2005) reported the major volatile compounds from Maillard reaction of glycine, diglycine and triglycine with glucose were alkylpyrazines. Triglycine was not stable and degradated to cyclic gly-gly and glycine, whereas diglycine had a higher stability than triglycine toward hydrolytic cleavage of the peptide bond. Pyrazinone, an important toasted aroma is a peptide-specific reaction flavor, and could generate from diglycine, triglycine and tetraglycine with glucose (Oh et al., 1992a). Those compounds are formed from the decarboxylation of 2-(3′-alkyl-2′-oxopyrazin-1′-yl) alkanoic acid at 180 ºC, or the condensation of α-dicarbonyl with alanine amide (Shu and Lawrence, 1995) (**Figure 31**). Model systems of Gly-Leu and Leu-Gly were also studied, as a result, only the amount of pyrazinones slightly changed after heating with glucose at 180 ºC for 2 h (Oh et al., 1992b).

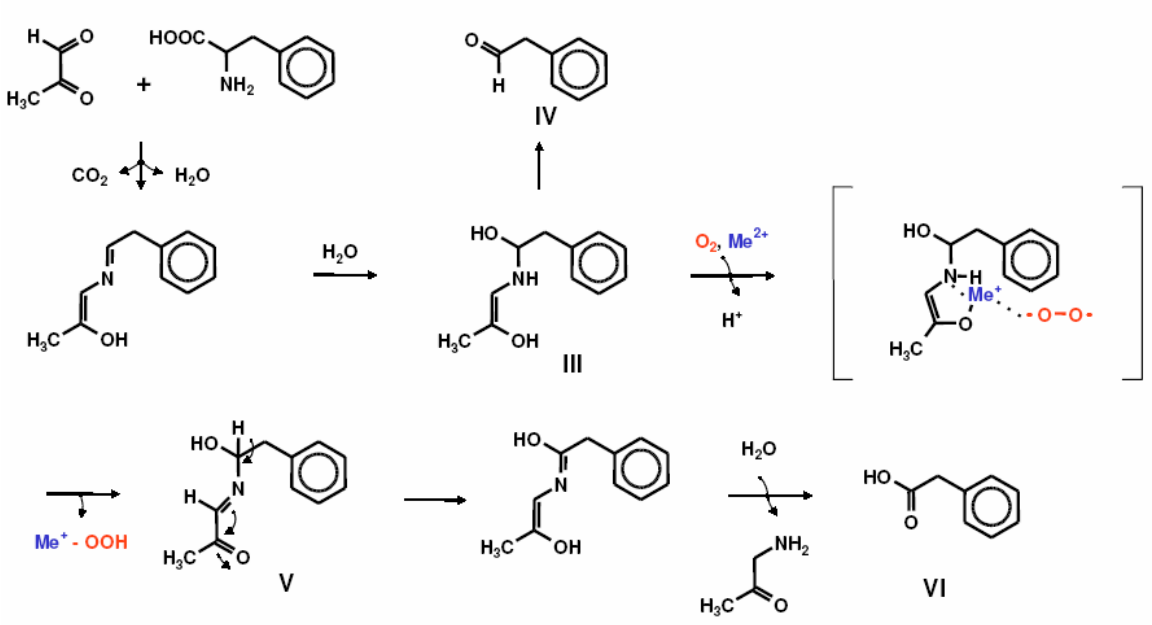


Figure 29. Formation of phenylaldehyde and phenylacetic acid from MG and phenylalanine (Adapted from Hofmann and Scheberle, 2000).

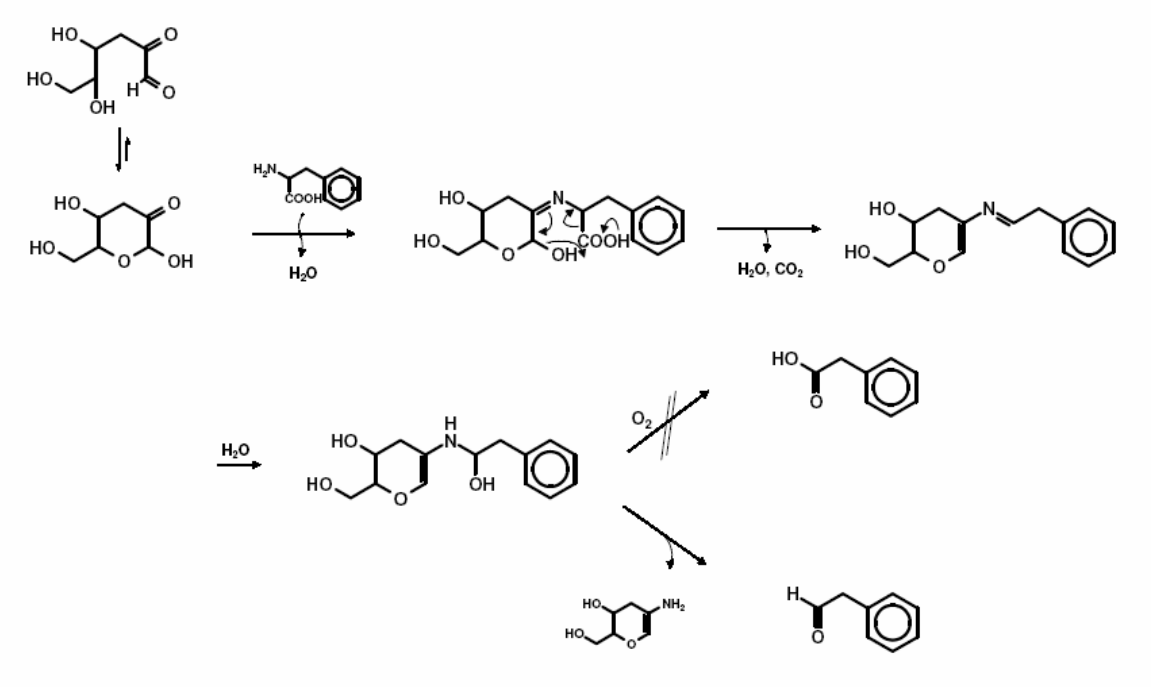


Figure 30. Formation of phenylacetaldehyde from 3-DG and phenylalanine (Adapted from Hofmann et al., 1999).

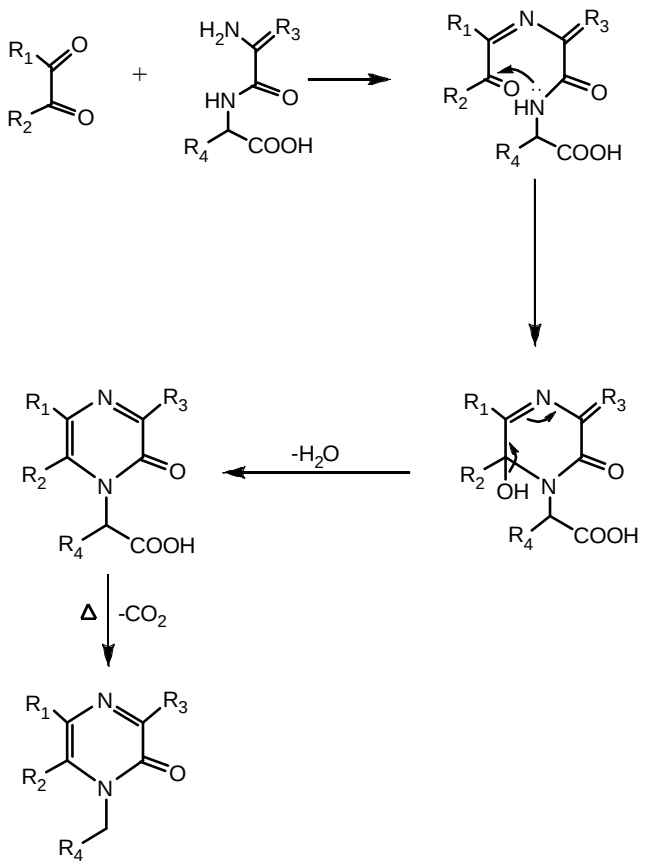


Figure 31. Mechanism of the formation alkylpyrazinone from dipeptide and MG (Shu and Lawrence, 1995)

3.1.5. The Carbon Module Labeling (CAMOLA) Technique

Maillard reaction, as a major flavor generation reaction, contains a lot of complex reaction cascades even if only one carbohydrate and one amino acid evolve. Among those flavor compounds, some are formed only from carbohydrate, some are from the combination of carbohydrate and amino acid, and the rest are only from amino acid. However, even the flavors are generated from carbohydrates, they could either be formed from cyclization of carbohydrate in which the carbon backbone of carbohydrate is kept intact or be formed from degradation of carbohydrate into small fragments, which turn out recombine to form the flavor compounds. In order to evaluate different pathways lead to a certain target compound and their relative importance of each pathway, the carbon module labeling technique was developed. In principle, this technique uses a defined quantification mixture of labeled and unlabeled carbohydrates, and the importance of one pathway will be evaluated through the ratio of isotopomers of target compounds. The mechanism of this approach is shown in **Figure 32** via DMHF formation. During the thermal treatment, a 1:1 mixture of [$^{13}C_6$] and [$^{12}C_6$]D-glucose react with certain amino acids. If the carbohydrate skeleton remains intact during DMHF formation, a 1:1 mixture of [$^{13}C_6$] and [$^{12}C_6$] DMHF should be observed. However, if carbohydrates degrade into small molecular intermediates, five other isotopomers ([$^{13}C_1$] DMHF to [$^{13}C_5$] DMHF) should be obtained.

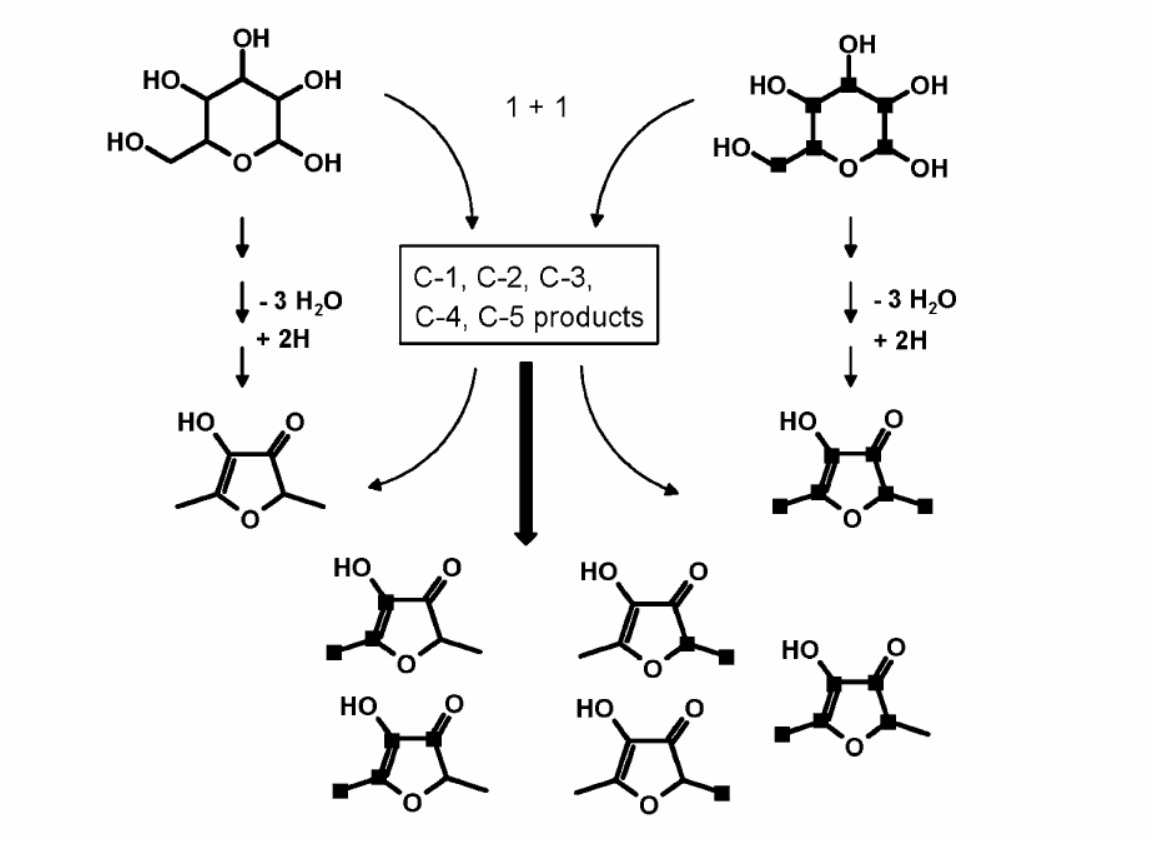


Figure 32. Basis of the carbon (carbohydrate) module labeling technique (CAMOLA) (Adapted from Schieberle, 2005).

3.2. Experimental

3.2.1. Formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) through methylglyoxal (MG) in the Maillard reaction

3.2.1.1 Materials.

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone, methylglyoxal (40 wt % in water), L-cysteine, L-glycine, sodium hydroxide and [$^{13}C_6$] or [$^{12}C_6$]D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC grade water, acetonitrile, dichloromethane and methanol were purchased from Fisher Scientific (Springfield, NJ).

3.2.1.2. Preparation of Model Systems.

MG, glucose, cysteine and glycine were dissolved in the phosphate buffer (0.5 M, pH 3.0, 5.0, and 8.0), separately. The pH was adjusted with 1 N sodium hydroxide. The concentrations were 1.4 M, 1.4 and 1 M for MG, glucose and amino acids, respectively. Two model systems were set up. The first one contained an aliquot (1 mL) of MG and phosphate buffer solution. In the second group, an aliquot of MG was mixed with either glycine or cysteine. All these samples were prepared in sealed glass tubes and heated at 120 °C for 60 min. All reacted samples were cooled by an ice bath and centrifuged at 14 × 1000 rpm (16000*g*) for 5 min before HPLC analysis. The carbon module labeling (CAMOLA) technique (Schieberle, 2005) was used to verify and evaluate the formation pathways of DMHF from MG and glucose. A 1:1 mixture of 1.4 M [$^{13}C_6$]glucose and 1.4 M [$^{12}C_6$]glucose (containing natural abundant ^{13}C) were reacted with 1 M glycine or cysteine at 120 °C and pH 5 for 60 min, and another similar model, a 1:1 mixture of 1.4 M [$^{13}C_6$]glucose and 1.4 M [$^{12}C_3$]methylglyoxal (containing natural abundant ^{13}C) were

reacted with 1 M glycine or cysteine at 120 °C and pH 5 for 60 min. Then, the reaction mixture was extracted three times with 10 mL methylene chloride. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under nitrogen gas for the GC-MS analysis.

3.2.1.3. Analysis of DMHF by HPLC.

The Dionex UltiMate 3000 LC Modules equipped with a pump (model: LPG-3400 pump, Sunnyvale, CA), UV-vis detector (model VWD-3400 detector), and an autosampler (model: WPS-3000 SL) were used. A Luna C18 (Phenomenex, Torrance, CA) column (150 × 4.6 mm i.d., 3 μm particle size) was used for DMHF analysis. The column temperature was maintained at 25 °C in a column oven (Dionex model STH 585). The mobile phase for the HPLC system consisted of HPLC grade water with 0.15% acetic acid (v/v; solvent A), acetonitrile (solvent B) and methanol (solvent C) with a constant flow rate set at 0.5 mL/min. HPLC gradient programs were modified according to the method reported by Haleva-Toledo et al. (1999) for DMHF analysis as follows: 6% solvent B and 6% solvent C, and they increased together to 15% over 16 min, then decreased to 6% over 4 min. The whole program ran for 20 min. DMHF was detected with a UV wavelength at 290 nm and the injection volumes were 30 μL. The external standard quantification method was applied in this study. Every single peak area for the quantification was laid in the linear range of standard curve.

3.2.1.4. GC-MS System.

The analyses of volatiles were performed with a HP6890 Gas Chromatograph. An Agilent Gas Chromatograph (6890 Series) is equipped with an autosampler (7673 Series

Injector) and an Agilent 5973 mass spectrometric detector (EI, 70eV). The column was an HP-1701 (14% (cyanopropyl-phenyl)methylpolysiloxane capillary (60 m × 0.25 mm id, film thickness 0.25 μm). The injector was in 1:1 split mode. The constant carrier gas (helium) flow rate was set at 1.0 mL/min. The GC oven temperature was programmed as follows: the initial oven temperature of 40 °C was set and increased to 280 °C at a rate of 5° C/min and then held at 280 °C for 12 min. The total run time was 60 min. The injector and detector temperatures were both 250 °C.

3.2.1.5. Statistical Analysis.

Data were expressed as means (standard deviation (SD) and represent three independent analyses. Statistical significance was examined using Student's *t* test comparison between the means. A *p* value of >0.05 was considered statistically significant.

3.2.2. Amino Acid-Dependent Formation Pathways of 2-Acetylfuran and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone in the Maillard Reaction

3.2.2.1. Materials.

L-phenylalanine, L-alanine, L-glycine, L-cysteine, L-proline, L-arginine, L-serine, L-lysine, methylglyoxal (40% wt in water), phosphate buffer (pH 7.4, 0.01 M), sodium hydroxide, anhydrous sodium sulfate and [$^{13}C_6$] or [$^{12}C_6$]D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO). [2-^{13}C]-L-glycine was from Cambridge Isotope Laboratories (Andover, MA). HPLC grade dichloromethane were purchased from Fisher Scientific (Springfield, NJ).

3.2.2.2. Preparation of Model Systems.

[$^{13}C_6$] or [$^{12}C_6$] glucose and different amino acids were dissolved in the phosphate buffer (0.01 M, pH 7.4), separately. The pH was adjusted with 1 N sodium hydroxide. The concentrations were 0.6 M and 0.2 M for glucose and amino acids, respectively. The carbon module labeling (CAMOLA) technique (Schieberle, 2005) was used to verify and evaluate the formation pathways of furanoids from glucose. An aliquot (1 mL) of 1:1 mixture of 0.6 M [$^{13}C_6$] glucose and 0.6 M [$^{12}C_6$] glucose was mixed with different amino acids, separately. All these samples were prepared in sealed glass tubes and heated at 145 ºC for 40 min, then cooled with an ice bath. The reaction mixture was extracted three times with 10 mL dichloromethane. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under nitrogen gas for the GC-MS analysis.

3.2.2.3. GC/MS System (Refer to 3.2.1.4. GC-MS System)

3.2.2.4. Calculation of Labeling Percentage.

The percentage of labeling distribution for furanoids was calculated by subtracting the natural abundance of ^{13}C (1.1%). All the percentages below 1% were taken as 0%.

3.2.3. Comparison of 2-Acetylfuran Formation between Ribose and Glucose in the Maillard reaction

3.2.3.1. Materials.

L-phenylalanine, L-glycine, L-cysteine, L-proline, L-lysine, formaldehyde (37% wt in water), phosphate buffer (pH 7.4, 0.01 M), sodium hydroxide, anhydrous sodium sulfate, [$^{13}C_6$] D-glucose, D-glucose, D-ribose, D-fructose, L-rhamnose and sucrose were

purchased from Sigma Chemical Co. (St. Louis, MO). [2-^{13}C]-L-glycine, [^{13}C5] ribose and [5-^{13}C] ribose were obtained from Cambridge Isotope Laboratories (Andover, MA). HPLC grade dichloromethane was purchased from Fisher Scientific (Springfield, NJ).

3.2.3.2. Preparation of Model Systems.

Labeled or unlabeled sugars and different amino acids were dissolved in the phosphate buffer (0.01 M, pH 7.4), separately. The pH was adjusted with 1 N sodium hydroxide. The concentrations were 0.6 M and 0.2 M for sugars and amino acids, respectively. An aliquot (1 mL) of sugar (1:1 mixture of labeled and unlabeled sugars or only labeled sugars) was mixed with different amino acids, separately. All these samples were prepared in sealed glass tubes and heated at 145 ºC for 40 min, then cooled with an ice bath. The reaction mixture was extracted three times with 10 mL dichloromethane. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under nitrogen gas for the GC-MS analysis.

3.2.3.3. GC/MS System (refer to 3.2.1.4. GC-MS System).

3.2.3.4. Calculation of Labeling Percentage.

The percentage of labeling distribution for 2-acetylfuran was calculated by subtracting the natural abundance of ^{13}C (1.1%). All the percentages below 1% were taken as 0%.

3.2.3.5. Calculation of Relative Reactivity.

The relative reactivity was based on the comparison between glucose and other sugars. [^{13}C6] glucose and other unlabeled sugars were used in a ratio of 1:1, and values of the relative reactivity were calculated using the following equations:

RR=PIO/PIG

$PIO=P[^{12}C_n]+(1/n) \times P[^{12}C_1]+ (2/n) \times P[^{12}C_2]+\ldots+ ((n-1)/n) \times P[^{12}C_{n-1}]$

$PIG=P[^{13}C_6]+(1/6) \times P[^{13}C_1]+ (2/6) \times P[^{13}C_2]+\ldots+ (5/6) \times P[^{13}C_5]$

where RR: relative reactivity; PIO: percentage of isotopomers from other sugars, PIG: percentage of isotopomers from glucose, P: percentage of n-number of carbons in certain sugar.

3.3. Results and Discussion

3.3.1. Formation of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) through methylglyoxal (MG) in the Maillard Reaction

3.3.1.1. Quantification of DMHF Formation from MG.

DMHF as an intense caramel-like aroma compound with low odor threshold has been widely used in the flavor industry. So far, the studies on formation pathways of DMHF have been mainly focused on different hexoses, 6-deoxysugars and pentoses. In current study, the generation of DMHF from MG, an important flavor precursor for processed foods was carried out. Generally, MG and 1-hydroxy-2-propanone, the two major degradation products in Maillard Reaction, could react together to form DMHF via 2,5-dioxo-3,4-dihydroxyhexane (Schieberle, 2005). However, when MG was heated alone at 120 °C, the formation of DMHF was observed (**Figure 33**), (Also, provide in Table form!) and the MG level was significantly increased as pH of the reaction increased. MG, one of the dicarbonyl compounds, may transform into 1-hydroxy-2-propanone and pyruvic acid through the Cannizzaro reaction (**Figure 34**), and subsequently lead to DMHF by reacting MG with 1-hydroxy-2-propanone (Schieberle, 2005). DMHF formation from MG was pH-dependent because the Cannizzaro reaction is a base preferential reaction. The aroma compounds generated in Maillard reaction depend on the study models such

as composition of sugars, amino acids as well as the reaction condition. DMHF was produced at different levels either directly from MG or from MG in the presence of glycine or cysteine. At pH 8, Strecker degradation was the major reaction which consumed most of MG in the presence of amino acids. However, cysteine could be degraded into hydrogen sulfide which can be used as a reducing agent to produce 1-hydroxy-2-propanone from MG. At pH 5, Cannizzaro reaction and Strecker degradation became weaker, and reduction activity of cysteine was the main effect on DMHF formation, consequently cysteine reacting with MG generated a high level of DMHF. Cysteine may change its role from a reductant to an inhibitor at pH 3. The inhibitory effect of thiol group in cysteine on DMHF formation has been observed particularly at pH 3 (Friedman and Molnar-Perl, 1990 I П Ш). Haleva-Toledo et al. showed that cysteine and *N*-acetylcysteine inhibited DMHF formation at pH 3 by a nucleophilic attack of thiol group to the open carbonyl form of DMHF (Haleva-Toledo et al., 1999). It was very interesting to observe that the generation of DMHF from MG and glycine increased as pH decreased. Maybe at lower pH, MG can be easily transformed into 1-hydroxy-2-propanone in the presence of glycine.

3.3.1.2. Verification of DMHF Formation through MG and Glucose Pathways.

Maillard reaction involving thermal degradation of carbohydrates and amines could induce a complex reaction cascade in which aroma, taste and color compounds are generated through cyclization and fragmentation of carbohydrates. DMHF, on one hand, can be formed through cyclization of an intact carbohydrate via acetylformoin as an intermediate.

On the other hand, the carbohydrate may be cleaved into fragments such as MG and 1-hydroxy-2-propanone, which may recombine to form DMHF. In order to show DMHF formation mechanisms, the carbon module labeling (CAMOLA) was used. CAMOLA is a powerful technique to elucidate different pathways and evaluate the relative importance of each pathway (Schieberle, 2005). Equal molar of [$^{13}C_6$] labeled and [$^{12}C_6$] unlabeled glucose were mixed in the presence of glycine or cysteine, and the isotopomers of DMHF were analyzed by GC-MS. If the glucose carbon skeleton keeps intact in DMHF formation, equal molar of [$^{13}C_6$] labeled DMHF and [$^{12}C_6$] unlabeled DMHF should be obtained. However, if the fragmentation of glucose occurs before DMHF formation, up to seven isotopomers with different numbers of labeled carbons may be formed. The results demonstrated that five isotopomers [$^{13}C_1$] to [$^{13}C_5$] from glucose fragmentation, were not observed in the presence of glycine and cysteine, and a 1:1 mixture of [$^{13}C_6$]DMHF and [$^{12}C_6$]-DMHF was obtained (**Figure 35**), suggesting no breakdown of glucose during DMHF formation. Generally, fragmentation degree is related to the temperature. When reaction temperature was increased to 165 °C, still no fragmentation of glucose occurred in the DMHF formation. Previous studies suggested that acetylformoin was an important precursor which could be reduced to DMHF (Hofmann and Schieberle, 2001). The chromatograph of DMHF and its precursor acetylformoin was shown in **Figure 36** In the current experiments, molecular ion of *m*/*z* 144 representing the [^{12}C6]acetylformoin was present in an equal intensity to *m*/*z* 150 ([$^{13}C_6$]-acetylformoin) indicating that acetylformoin as one of DMHF intermediates was also generated from intact glucose (**Figure 37**). It is therefore concluded that in the presence of glycine or cysteine, DMHF can only be formed through the intact glucose. As

an important intermediate during thermal degradation of glucose, MG itself could generate DMHF with or without amino acids. If a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_3$]MG reacted with glycine or cysteine at 120 °C, a 4:1 mixture of [$^{13}C_6$]DMHF and [$^{12}C_6$]DMHF was obtained. Because some of the MG involved in the Strecker degradation, only 20% of DMHF was formed from MG, and the rest 80% was formed from glucose (**Figure 38**). However, no [$^{12}C_6$]acetylformoin was observed suggesting that acetylformoin was not a precursor during the DMHF formation from MG (**Figure 39**). In all of DMHF and acetylformoin isotopomers, only [$^{13}C_6$] labeled and [$^{12}C_6$] unlabeled, were observed. Glucose kept carbon skeleton intact during DMHF formation even its fragment MG was present, which indicated that MG and cyclization of intact glucose pathways were parallel since the precursors of these two pathways were different.

In conclusion, the results of this study indicate that MG, depending on the pH differently affected DMHF generation in the presence or absence of amino acids. DMHF level increased as pH increased when cysteine reacted with MG, whereas the trend was reversed in the presence of glycine. When glucose reacted with glycine or cysteine, glucose skeleton kept intact in the formed DMHF as well as its precursor acetylformoin. Acetylformoin was not formed in the reaction between MG and either glycine or cysteine.

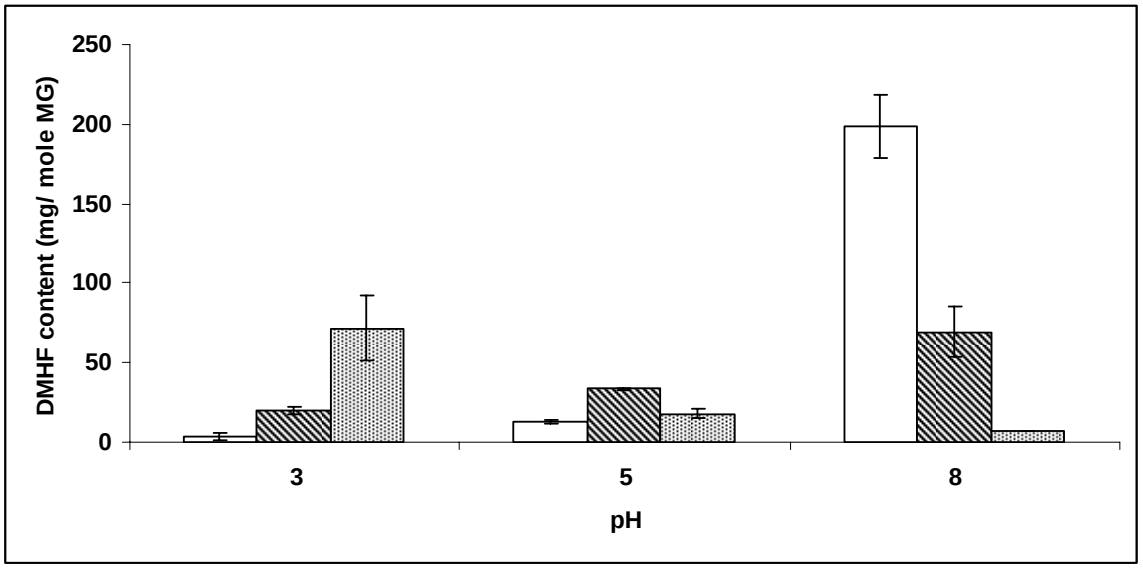


Figure 33. Effect of pH on 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone formation in 1.4 M MG phosphate buffer solution incubated with or without 1.0 M glycine or 1.0 M cysteine: MG only (open bars); MG-cysteine (slash bars); MG-glycine (dotted bars). Values for DMHF are the means ± standard deviation (SD), each analyzed three independent times. Statistical significance was examined using Student's *t*-test comparison between the means. A p value of <0.05 was considered statistically significant.

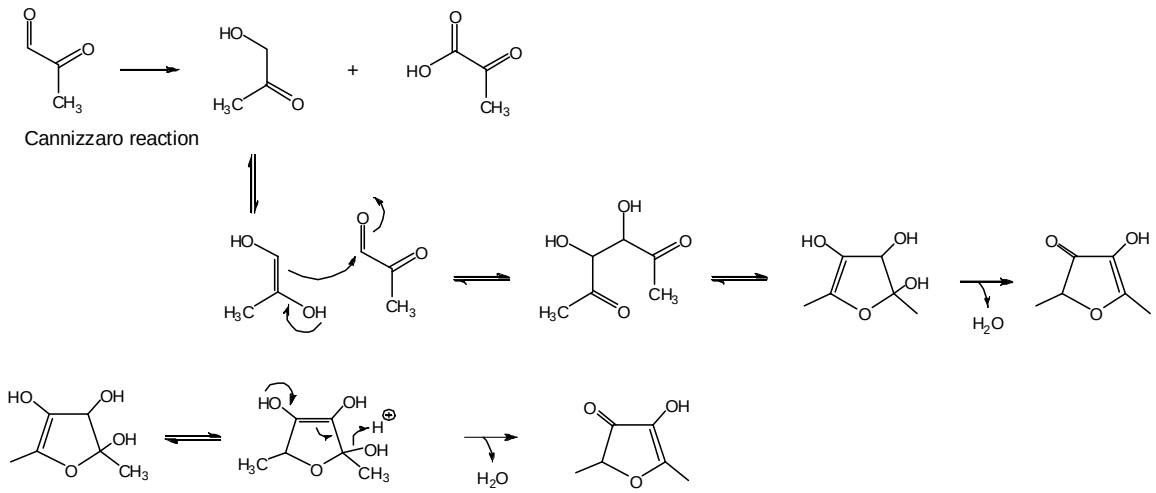


Figure 34. Reaction pathway leading methylglyoxal to 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone via Cannizzaro reaction.

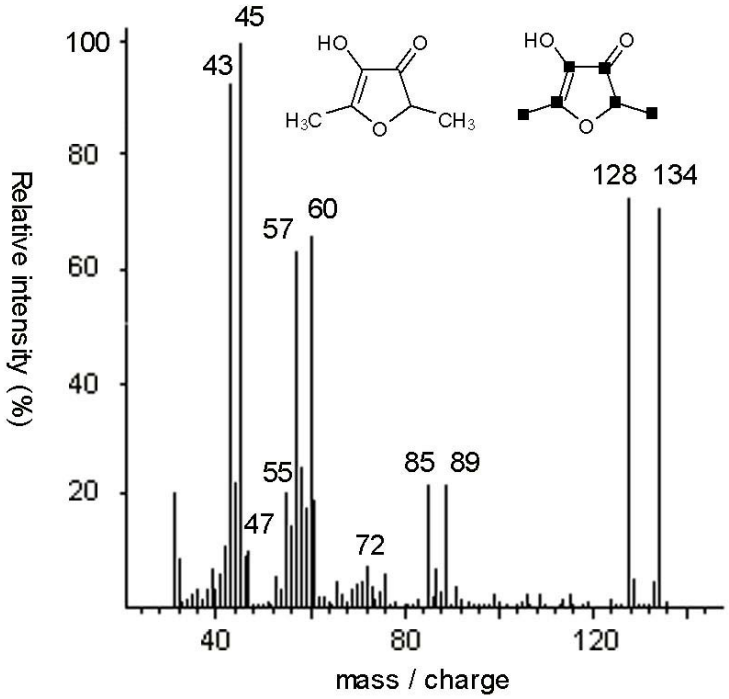


Figure 35. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone from a 1:1 mixture of [^{13}C6]glucose and [^{12}C6]glucose.

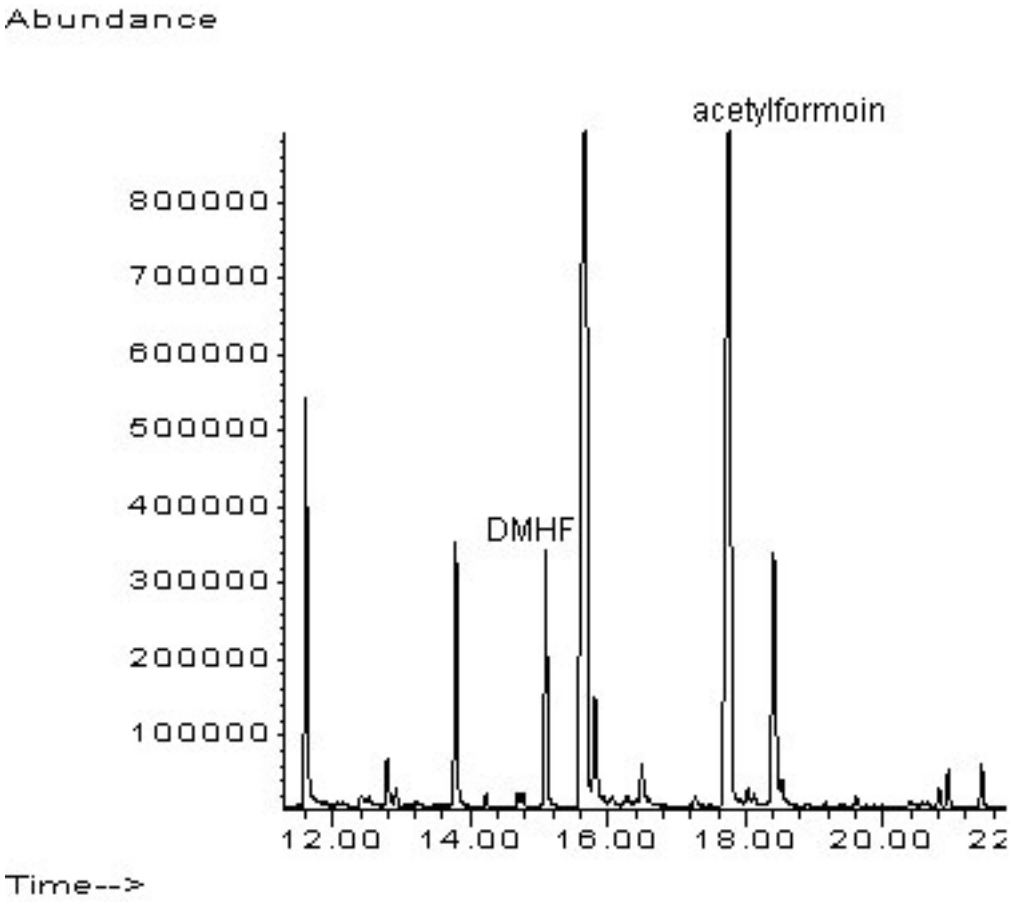


Figure 36. GC Chromatograph of DMHF and acetylformoin.

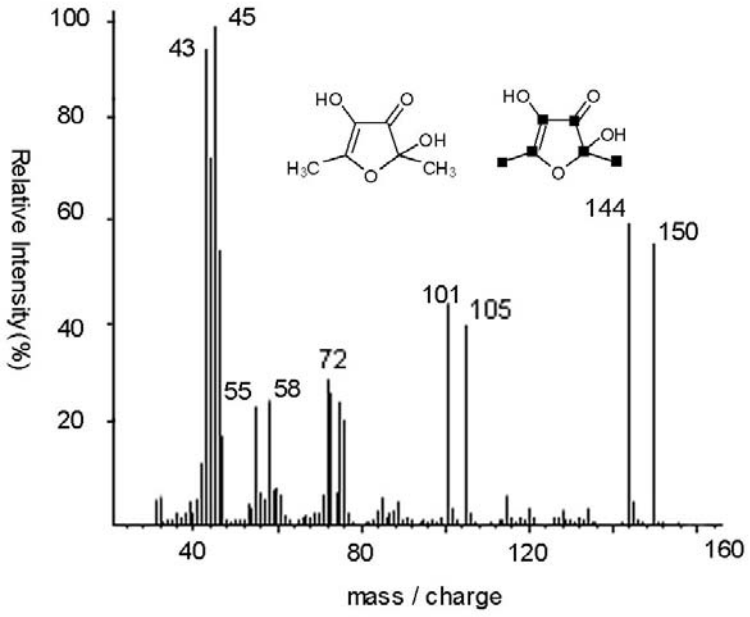


Figure 37. GC-MS spectrum of acetylformoin from a 1:1 mixture of [^{13}C6]glucose and [^{12}C6]glucose.

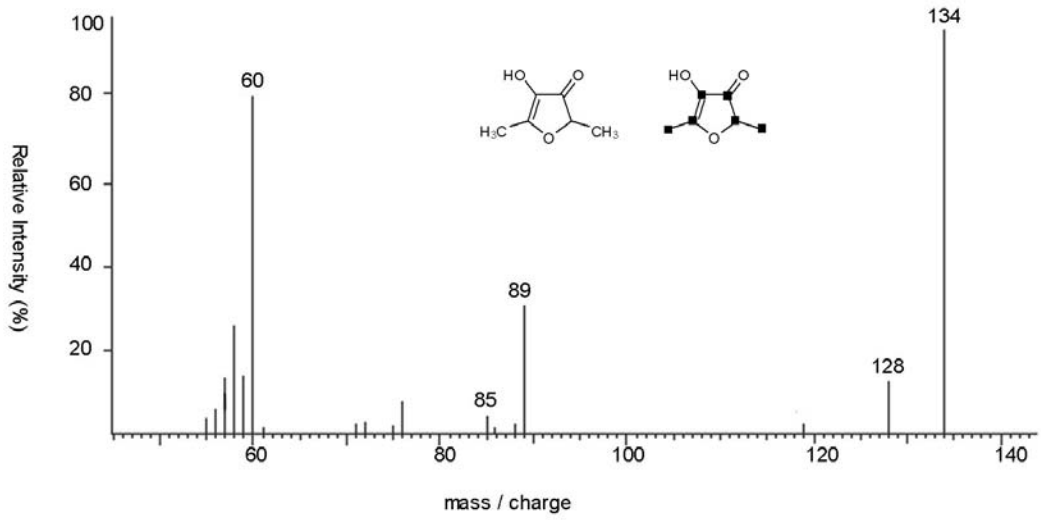


Figure 38. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone from a 1:1 mixture of [^{13}C6]glucose and [^{12}C3]methylglyoxal.

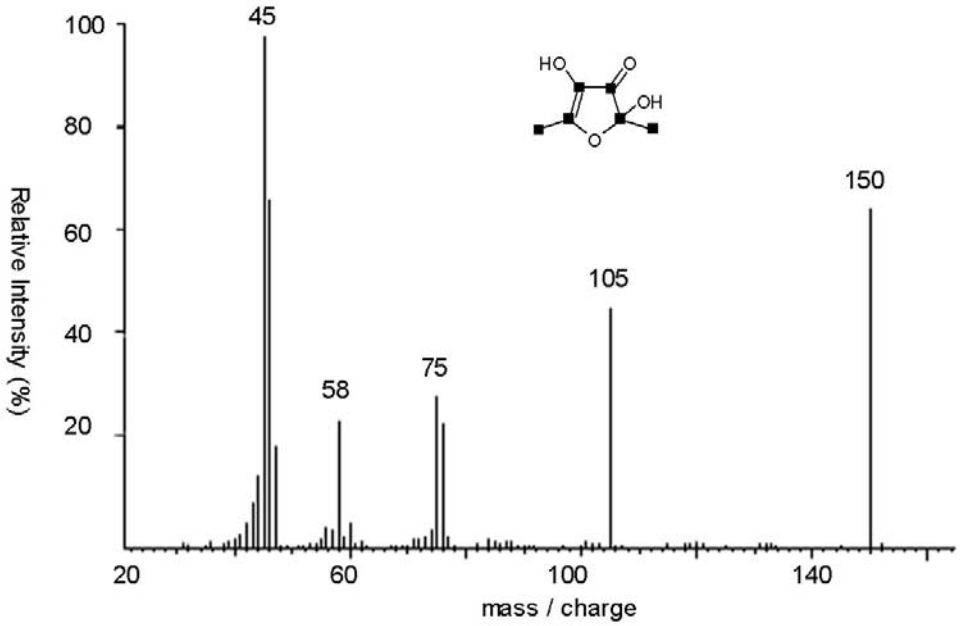


Figure 39. GC-MS spectrum of acetylformoin from a 1:1 mixture of [^{13}C6]glucose and [^{12}C3]methylglyoxal.

3.3.2. Amino Acid-Dependent Formation Pathways of 2-Acetylfuran and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone in the Maillard Reaction

Maillard reaction involving thermal degradation of carbohydrates and amines could induce complex reaction cascades to generate flavor and color compounds. Most volatile furanoid compounds are considered to derive from deoxyosones. They are either from the whole intact carbon skeleton through cyclization or from the cleavage of deoxyosones and recombination of their fragments. In order to understand those formation mechanisms, the carbon module labeling (CAMOLA) was used. Equal molar of [^{13}C6] labeled and [^{12}C6] unlabeled glucose were mixed with different amino acids, and the isotopomers of 2-acetylfuran and DMHF were analyzed by GC-MS. If the glucose carbon skeleton kept intact in their formation, equal molar of [^{13}C6] furanoids and [^{12}C6] furanoids should be obtained. On the other hand, if a fragmentation of glucose occurred before the formation of furanoids, partly labeled isotopomers would be observed.

3.3.2.1. Formation of 2-acetylfuran and DMHF via Glucose and Selected Amino Acids.

Tables 3 and 4 demonstrated the labeling distribution of 2-acetylfuran and DMHF in different amino acid models with equal molar of [^{13}C6] labeled and [^{12}C6] unlabeled glucose (Chromatograph shown in **Figure 40**). For the DMHF formation, except for lysine and arginine, other amino acids with glucose models showed a 1:1 mixture of [^{13}C6] DMHF and [^{12}C6] DMHF in accordance with our previous study of glycine or cysteine with glucose (**Section 3.3.1**). Even there were some fragments involving in the DMHF formation from lysine or arginine models, the percentage of fragmentation (11%-12%) was still a small portion. **Figure 41 and 42** were used to show the mass spectra of

DMHF in the proline and lysine systems. Those results indicated that under our experimental conditions, most glucose carbon skeleton kept intact during DMHF formation despite of type of amino acids used. On the other hand, the formation of 2-acetylfuran showed a different behavior from DMHF. Firstly, a 1:1 mixture of [^{13}C6] 2-acetylfuran and [^{12}C6] 2-acetylfuran was observed in the models of phenylalanine, cysteine and serine suggesting no fragmentation (**Figure 43a**). Secondly, models contained lysine, alanine, proline and arginine showed about 50% of [^{13}C6] 2-acetylfuran and [^{12}C6] 2-acetylfuran, and the rest half of fragmentations. It was calculated that about a quarter of 2-acetylfuran was performed as a [^{13}C3] isotopomer in these models (**Figure 43b**). Thirdly, glycine revealed a different characteristics from other amino acids in 2-acetylfuran formation. [^{12}C6] isotopomer showed a higher level instead of an equal level compared to [^{13}C6] isotopomer. The major fragmentation product was the [^{13}C5] 2-acetylfuran (**Figure 43c**).

Generally, DMHF and 2-acetylfuran can be formed from glucose through 1-deoxyosone and 1,4-dideoxyosone as the intermediates, respectively. DMHF is generated from hexose via acetylformoin reduction which may proceed either by disproportionation reaction or a Strecker reaction with amino acids (Hofmann & Schieberle, 2001). The formation pathway of 2-acetylfuran through 1,4-dideoxyosone from glucose were proposed in **Figure 44A**. 1-amino-4-deoxyosone was formed from glucose and amino acids via 2,3-eneaminol. Then 1-amino-4-deoxyosone was transformed into 1,4-dideoxyosone through Strecker degradation (Cerny & Dvidek, 2003). 2-acetylfuran can be formed via cyclization of 1,4-dideoxyosone by dehydration. In the intermediate stage of Maillard reaction, deoxyosones can be degraded into shorter fragments of carbonyl or

hydroxyl carbonyl compounds which may recombine to form flavor compounds. A 1:1 mixture of [^{12}C6] and [^{13}C6] isotopomers obtained indicating that no shorter chain fragments participated in 2-acetylfuran formation in the model system when phenylalanine, cysteine and serine were used. On the other hand, glycine showed a different mechanism from other amino acids in the formation of 2-acetylfuran. The major fragmentation product was the [^{13}C5] 2-acetylfuran, whereas the percentage of its corresponding isotopomer [^{13}C1] was zero. Moreover, [^{12}C6] isotopomer showed a higher level than [^{13}C6] isotopomer. Therefore, based on the principals of CAMOLA, the glycine may take part in the reaction so as that the distribution of corresponding isotopomers showed a significant difference. In order to confirm this interpretation, [2-^{13}C]-L-glycine was reacted with [^{12}C6] glucose and the result indicated that half of 2-acetylfuran was from recombination of glucose and glycine fragments. Generally, flavor compounds could be generated either from sugars or from sugars and amino acids. In the presence of other amino acids, only glucose contributed to the carbons of 2-acetylfuran, whereas in the presence of glycine, one carbon of 2-acetylfuran was from glycine. Formation pathway of 2-acetylfuran from glucose and glycine fragments was proposed in **Figure 44B**. Glycine can be degraded into formaldehyde via Strecker degradation. Further fragmentation of the 1-deoxyosone may lead to the five carbons aldehyde which could react with formaldehyde via enolization and aldol condensation.

Potential Control of Maillard Reaction. Significant difference was observed in this study between 2-acetylfuran and DMHF formation pathways. For the selected amino acids, high proportions of a 1:1 mixture of [^{12}C6] and [^{13}C6] DMHF demonstrated only one major pathway during DMHF formation in which glucose carbon skeleton was kept

intact. However, in a 1:1 mixture of [^{12}C3] MG and [^{13}C6] glucose model in the presence of glycine and cysteine, [^{12}C6] and [^{13}C6] DMHF were both observed suggesting a pathway for DMHF from MG (Wang & Ho, 2008). MG may transform into 1-hydroxy-2-propanone and pyruvic acid through the Cannizzaro reaction, and subsequently lead to DMHF by reacting MG with 1-hydroxy-2-propanone (Wang & Ho, 2008). In another word, DMHF could be formed from MG out of Maillard system, but in the Maillard reaction system, it can not be formed via the recombination of MG, because Maillard reaction is composed of various sub-reaction cascades which are competitive with each other in the generation of Maillard reaction product. For example, MG could participate in Strecker degradation which is a major flavor generation reaction. Besides Strecker degradation, MG could also involve in other flavor formation pathways. Observation of [^{13}C3] 2-acetylfuran indicated the participation of MG or other [C_3] fragments.

Flavor compound formation in the Maillard reaction depends on various factors such as the type of sugars and amino acids, reaction temperature, time, pH and water content. Generally, sugar and amino acid type may influence the flavor type, yield and formation pathway. In the presence of different amino acids, the yields of DMHF are different (**Section 3.3.1**). Even for the same flavor compound such as 2-acetylfuran, the generation pathways vary with different amino acids. However, this is just one part from a macro perspective to control the Maillard reaction. If all these factors are fixed, for example, in our glucose and glycine model, glucose can be degraded into [C_1-C_5] fragments, and glycine can give rise to formaldehyde. All these moleculars occur in the Maillard reaction, and participate in different reaction cascades competitively. How the reaction goes depends on the competitive ability of each molecules. Therefore, although DMHF and 2-

acetylfuran both contain furan ring, and can be formed from deoxyosone, in the glucose-glycine Maillard reaction, DMHF generated only from cyclization of intact glucose, whereas 2-acetylfuran formed either from intact glucose or from [C_5] fragment and formaldehyde. If Strecker degradation could be attenuated, would much more dicarbonyl compounds involve in DMHF or 2-acetylfuran formation, would fragmentation of glucose be observed during DMHF formation? These discussion and qusetions presented here will be discussed in a following study.

In conclusion, based on selected amino acids, 2-acetylfuran and DMHF demonstrated different formation pathways. For the DMHF, there was only one major formation pathway in which glucose carbon skeleton kept intact. However, 2-acetylfuran could be formed either from glucose or from glucose and glycine. In the presence of glycine, [C-5] unit of glucose combined with formaldehyde from glycine leading to 2-acetylfuran. For other amino acids, either cyclization of intact glucose or recombination of glucose fragments could lead to 2-acetylfuran formation. These results indicated a competitive trend in controlling Maillard reaction. Therefore, besides changing Miallard reaction impact factors (temperature, time, pH etc.), inhibiting or preventing the competitive reaction cascade gave a rise to directing desired pathways of Maillard reaction.

Table 3. Percent Labeling Distribution of 2-acetylfuran generated in different Models.

Sample model	M	M+1	M+2	M+3	M+4	M+5	M+6
Phe+Glc(c12:c13)	50	0	0	0	0	0	50
Lys+Glc(c12:c13)	27	4	4	26	4	4	31
Gly+Glc(c12:c13)	44	0	0	9	0	30	17
Ala+Glc(c12:c13)	38	0	0	27	0	0	35
Cys+Glc(c12:c13)	50	0	0	0	0	0	50
Pro+Glc(c12:c13)	24	9	5	25	6	9	22
Arg+Glc(c12:c13)	27	5	5	31	5	5	22
Ser+Glc(c12:c13)	50	0	0	0	0	0	50
Glu(c12)+glycine(c2-c13)	50	50	0	0	0	0	0

Table 4. Percent Labeling Distribution of DMHF Generated in Different Models

Sample model	M	M+1	M+2	M+3	M+4	M+5	M+6
Phe+Glc(c12:c13)	50	0	0	0	0	0	50
Lys+Glc(c12:c13)	44	4	1	2	1	4	44
Gly+Glc(c12:c13)	50	0	0	0	0	0	50
Ala+Glc(c12:c13)	50	0	0	0	0	0	50
Cys+Glc(c12:c13)	50	0	0	0	0	0	50
Pro+Glc(c12:c13)	50	0	0	0	0	0	50
Arg+Glc(c12:c13)	43	4	1	2	1	3	46
Ser+Glc(c12:c13)	50	0	0	0	0	0	50
Glu(c12)+glycine(c2-c13)	100	0	0	0	0	0	0

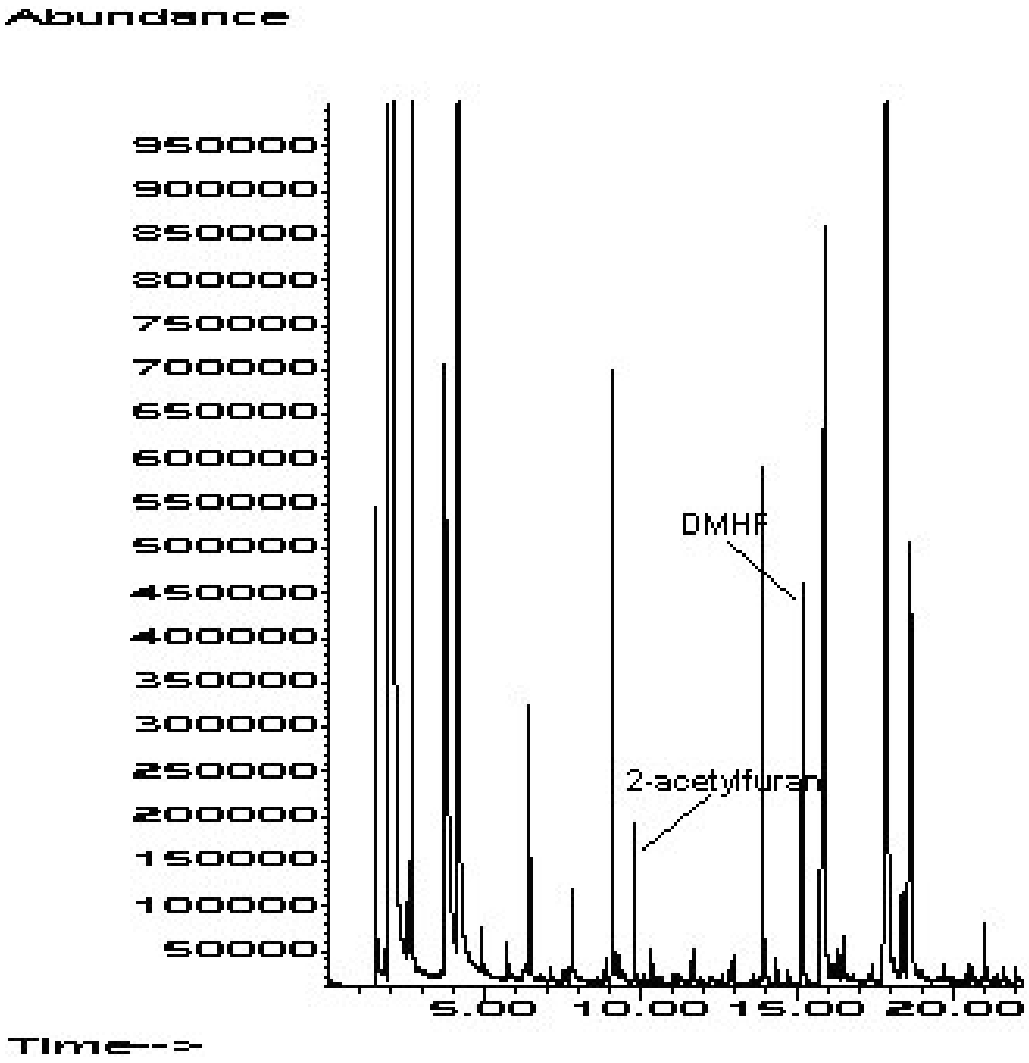


Figure 40. GC Chromatograph of 2-acetylfuran and DMHF.

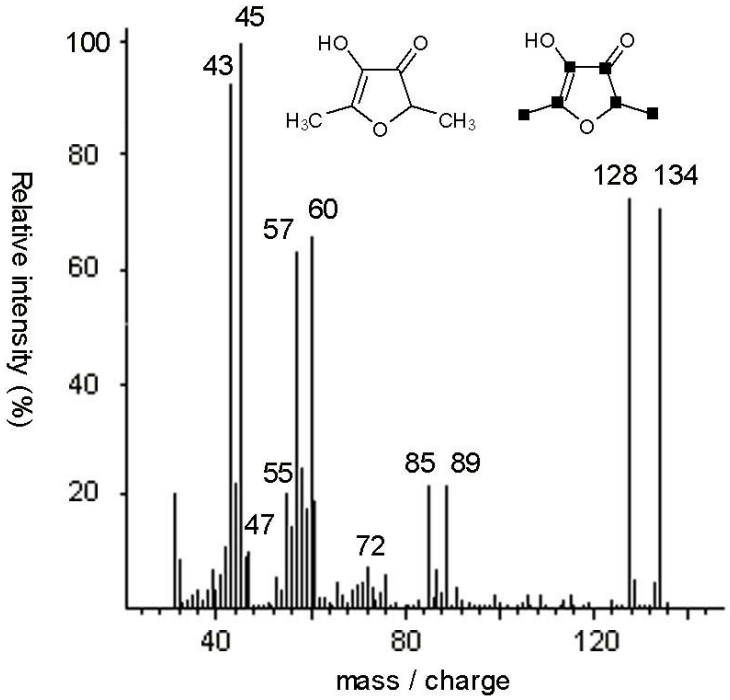


Figure 41. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone from a 1:1 mixture of [^{13}C6]glucose and [^{12}C6]glucose in the presence of proline.

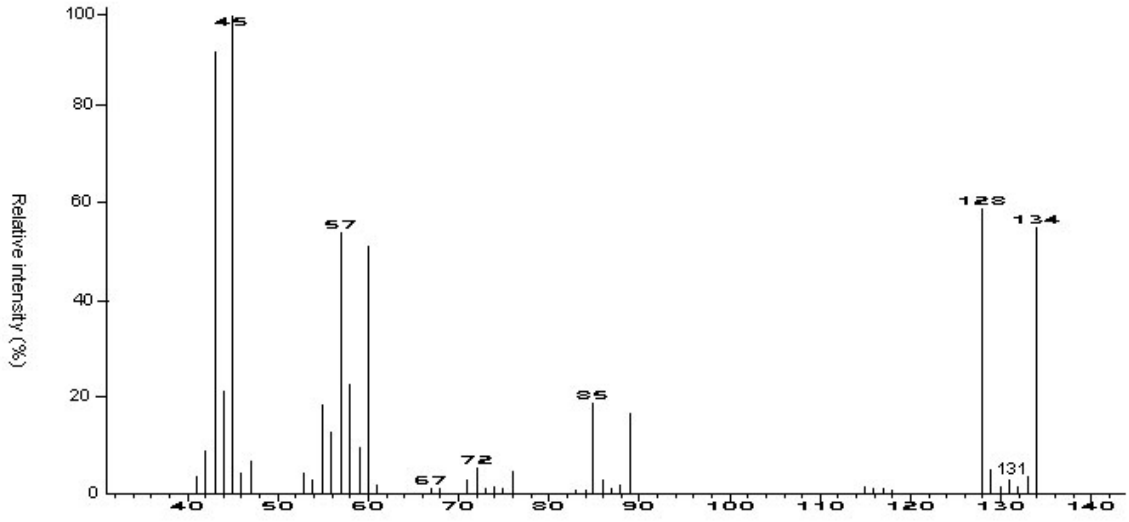


Figure 42. GC-MS spectrum of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone from a 1:1 mixture of [^{13}C6]glucose and [^{12}C6]glucose in the presence of lysine.

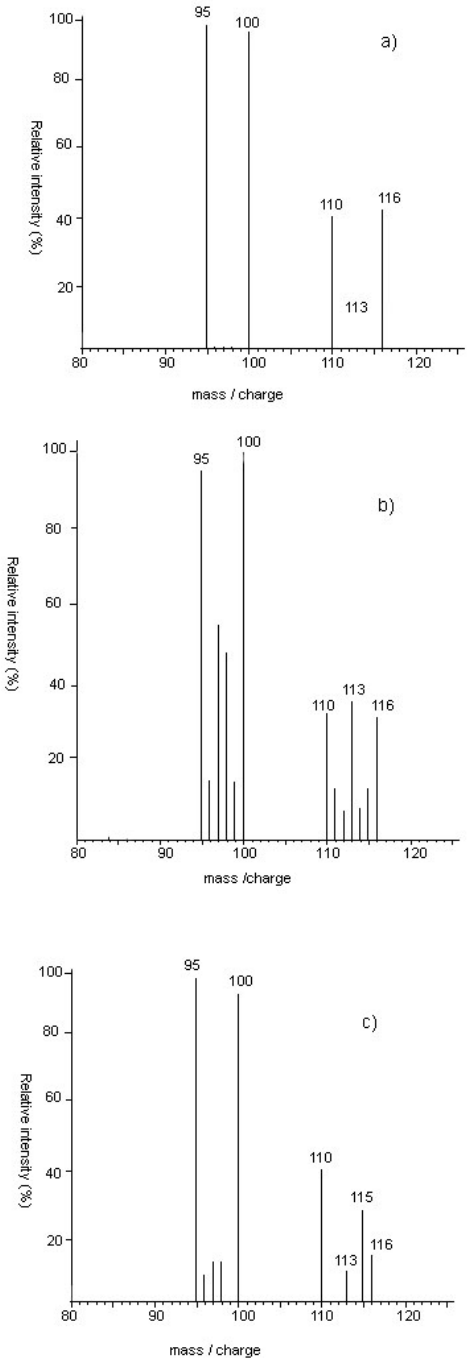


Figure 43. GC-MS spectra of 2-acetylfuran from a 1:1 mixture of [^{13}C6] glucose and [^{12}C6] glucose in the presence of (a). phenylalnine, cysteine or serine (b). proline (c). glycine

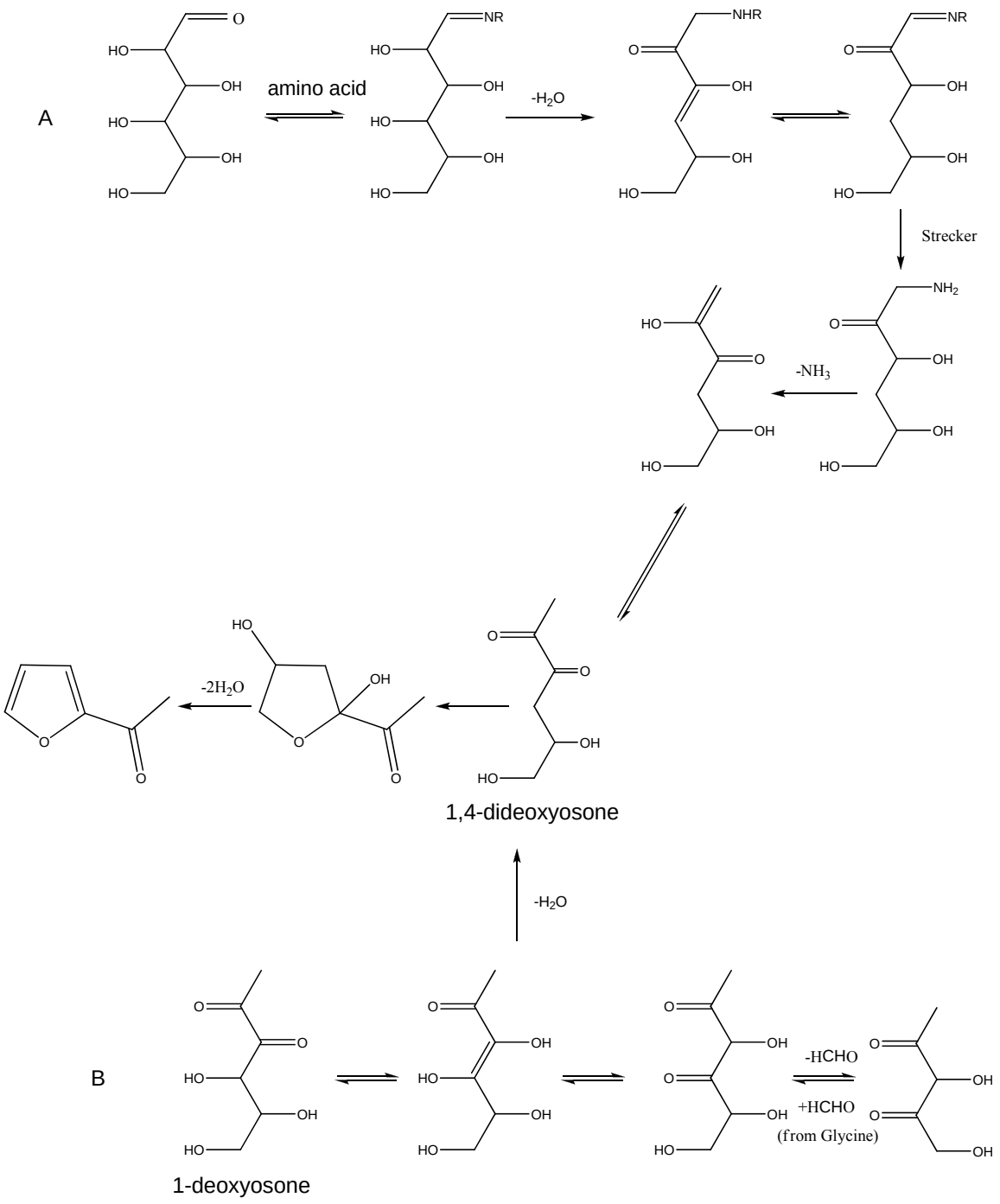


Figure 44**.** Proposed formation of 2-acetylfuran from glucose and glycine (A) or glucose (B) via 1,4-dideoxyosone.

3.3.3. Comparison of 2-Acetylfuran Formation between Ribose and Glucose in the Maillard reaction

3.3.3.1. Measuring Relative Reactivity of Sugars on 2-Acetylfuran Formation.

Maillard reaction is a complicated reaction consisting of many parallel and consecutive reactions. Many factors such as types of reactants and reaction conditions (temperature ranges, pH and water activity) play key roles in controlling the reaction rates and mechanisms. Generally, reactants mainly regulate the type of Maillard products and formation pathways, and reaction conditions may influence the kinetics (Van Boekel et al., 2006). However, for some specific compounds, type of sugars or amino acids could also affect the reaction rate. For example, fructose browned faster than glucose and sucrose (Reyes et al., 1982). Therefore, in the current study different sugars were used to study their relative reactivity in 2-acetylfuran formation. A novel method was developed to obtain the relative reactivity by reacting a 1:1 mixture of [^{13}C6] glucose and another unlabeled sugar and then comparing the ratio of labeled and unlabeled isotopomers. **Figure 45** showed relative reactivity of glucose and other sugars in 2-acetylfuran formation in the presence of proline. At 145ºC and pH of 7.4, the reactivity of ribose was 6.5-fold higher than glucose, followed by fructose of 3.3-fold, and sucrose was only 0.3-fold of glucose. Therefore the reactivity of sugars in 2-acetylfuran formation declined in the order of ribose, fructose, glucose, rhamnose and sucrose. It is generally accepted that the higher concentration of open chain form of the sugar, the higher reactivity it has (Van Boekel., 2001). In other words, compared to glucose, ribose and fructose are easier to keep the open chain form which may influence the relative reactivity in 2-acetylfuan formation.

The uniqueness of this newly developed method for measuring relative reactivity of sugars in Maillard compound formation was giving a direct visual comparison from the mass spectrum of the compound. A higher activity of ribose compared to glucose in 2-acetylfuran generation was clearly indicated in **Figure 46**. Take another example, 6-deoxysugars such as rhamnose are more effective in forming 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone (DMHF) compared to hexoses (Hofmann and Schieberle, 1997) in accordance with the spectrum showed in **Figure 47a** when a 1:1 mixture of labeled glucose and unlabeled rhamnose was used. In the presence of proline, the intensity of *m*/z 128 ([^{12}C6] DMHF) which was generated from [^{12}C6] rhamnose was significantly higher than that of *m*/z 134 ([^{13}C6] DMHF) which was formed from cyclization of [^{13}C6] glucose. In addition, this method could also be used to study the formation mechanism of a Maillard compound. It could differentiate precursors of one compound from different sugars. For instance, acetylformoin is considered as a precursor of DMHF from the degradation of hexoses (Hofmann and Schieberle, 2001). In the same model of a 1:1 mixture of labeled glucose and unlabeled rhamnose, the [M+6] isotopomer of acetylformion from glucose could be observed, whereas the [M] isotopomer from rhamnose was not identified. DMHF, thus, is formed from rhamnose through a different precursor (**Figure 47b**). The same method was also used in the discussion of DMHF formation pathway from methylglyoxal.

3.3.3.2. Formation of 2-Acetylfuran via Ribose.

It is a challenge to study 2-acetylfuran formation pathways from ribose, because pentose must degrade into different fragments, and form six carbons 2-acetylfuran via complicated recombination reaction. Glucose has been reported to have three pathways

in forming 2-acetylfuran based on the variety of amino acids. In the presence of glycine, [C-5] unit of glucose combines with formaldehyde from glycine leading to 2-acetylfuran. For other amino acids, either cyclization of intact glucose or recombination of glucose fragments can lead to 2-acetylfuran formation (**Section 3.3.2**).

In the presence of cysteine, a 1:1 mixture of [^{13}C6] 2-acetylfuran and [^{12}C6] 2-acetylfuran is observed from a 1:1 mixture of [^{13}C6] glucose and [^{12}C6] glucose suggesting no fragmentation of glucose during 2-acetylfuran formation (**Part 3.3.2**). If a 1:1 mixture of the [^{13}C6] glucose and [^{12}C5] ribose was reacted with cysteine, and the reactivity of ribose was higher than glucose, besides [^{13}C6] isotopomer, all other isotopomers formed in this reaction system could attribute to ribose. However, it was very interesting to observe that no other isotopomers formed except [^{13}C6] isotopomer (**Table 5**) indicating the inhibition of fragmentation of ribose by cysteine. In order to prove the inhibition ability of cysteine, a 1:1 mixture of [5-^{13}C] ribose and unlabeled ribose was reacted with cysteine, and there was no 2-acetylfuran detected. The difference between glucose and ribose in 2-acetylfuran formation was that glucose could undergo the cyclization via its intact carbon skeleton to form 2-acetylfuran, whereas ribose must degrade into fragments before it can form 2-acetylfuran with 6-carbon unit. Thus, 2-acetylfuan could be hardly formed from ribose in the presence of a fragmentation inhibitor.

When glucose reacts with glycine, 2-acetylfuran is formed from [C-5] unit of glucose and formaldehyde of glycine degradation. Glycine can be degraded into formaldehyde via Strecker degradation. Further fragmentation of the 1-deoxyglucosone may lead to the five-carbon hydroxyl-carbonyl intermediate which could react with

formaldehyde via enolization, aldol condensation and cyclization and lead to 2-acetylfuran (**Part 3.3.2**). Two models containing a 1:1 mixture of [^{13}C6] glucose and [^{12}C5] ribose with glycine or [2-^{13}C] glycine were set up to study the formation of 2-acetylfuran from ribose and glycine and their similarity to glucose. The percentage of [^{12}C6] isotopomer was higher than that of [^{13}C6] isotopomer suggesting that in the presence of glycine, ribose still had a higher reactivity. When [2-^{13}C] glycine was used instead of unlabeled glycine, the percentage of [^{13}C6] isotopomer increased 9%, while the percentage of [^{13}C5] decreased 9% (**Table 5**). This result indicated the involvement of one carbon atom from glycine in the 2-acetylfuran formation from glucose in accordance with our previous data (**Part 3.3.2**). The same result was observed in the ribose. The percentage of [^{12}C6] isotopomer decreased 26%, while [^{12}C5] isotopomer increased 24%. In order to elucidate formaldehyde reacting with ribose to form 2-acetylfuran, a [^{13}C5] ribose was reacted with formaldehyde, the [M+5] isotopomer of 2-acetylfuran was obtained suggesting one possible formation pathway of 2-acetylfuran from ribose and formaldehyde. 1-Deoxypentosone, after dehydration, could react with formaldehyde from glycine to form 2-acetylfuran via enolization and aldol condensation which were similar to the proposed pathway for the formation of 2-acetylfuran from glucose (**Figure 48a**).

The third type of 2-acetylfuran formation from ribose was the fragmentation and recombination. A 1:1 mixture of [5-^{13}C] ribose and unlabeled ribose was reacted with proline. A 1:1 mixture of [M] and [M+1] isotopomers was observed, and the percentage of [M+2] isotopomer was only 14% (**Table 5**). Those data were not enough to speculate the formation pathways. Firstly, a 1:1 mixture of [5-^{13}C] ribose and unlabeled ribose was reacted with either phenylalanine or lysine, the percentages of isotopomers distribution

were the same as reaction with proline suggesting except cysteine and glycine, and other amino acids may not have a great influence on the fragmentation of ribose. Therefore, proline was used as a representative. Secondly, only [5-^{13}C] ribose was mixed with proline. A 1:1 mixture of [M+1] and [M+2] isotopomers was showed (**Figure 49b**), and compared with the model of 1:1 mixture of [5-^{13}C] ribose and unlabeled ribose (**Figure 49a**) indicating the possibility of breakages of ribose from C1-C2 or C2-C3 was equal to that from C3-C4 or C4-C5, respectively. In other words, a symmetric structure occurred from ribose during 2-acetylfuran formation. One possible pathway was proposed in **Figure 48b.** Ribose was rearranged into 3-pentulose through keto-enol tautomerism. By retro-aldol condensation, either carbon at position 1 or position 5 might be cleaved into formaldehyde which could react with ribose to form 2-acetylfuran.

In conclusion, the results of this study showed the difference between ribose and glucose in 2-acetylfuran formation from reaction reactivity and mechanism pathways. The reactivity of ribose in 2-acetylfuran formation was higher than that of glucose. In the presence of cysteine, 2-acetylfuran could not be generated from ribose. When ribose was reacted with glycine, formaldehyde generated from glycine would involve in the formation of 2-acetylfuran. For other amino acids, symmetric structure of ribose was formed.

Table 5. Percent labeling distribution of 2-acetylfuran generated in different models.

[$^{13}C_6$] Glc+Rib+Cys	0	0	0	0	0	0	100
[5-C^{13}] Rib+Rib+Cys	0	0	0	0	0	0	0
[$^{13}C_6$] Glc+Rib+Gly	71	7	4	6	0	9	4
[$^{13}C_6$] Glc+Rib+Gly'	45	31	4	7	0	0	13
[5-C^{13}] Rib+Rib+Pro	41	45	14	0	0	0	0
[5-C^{13}] Rib+Pro	6	47	44	3	0	0	0

Figure 45. Relative reactivity of glucose and other sugars in 2-acetylfuran formation in the presence of proline: Black line at 1 represents glucose reactivity. All models were heated at 145ºC for 40min.

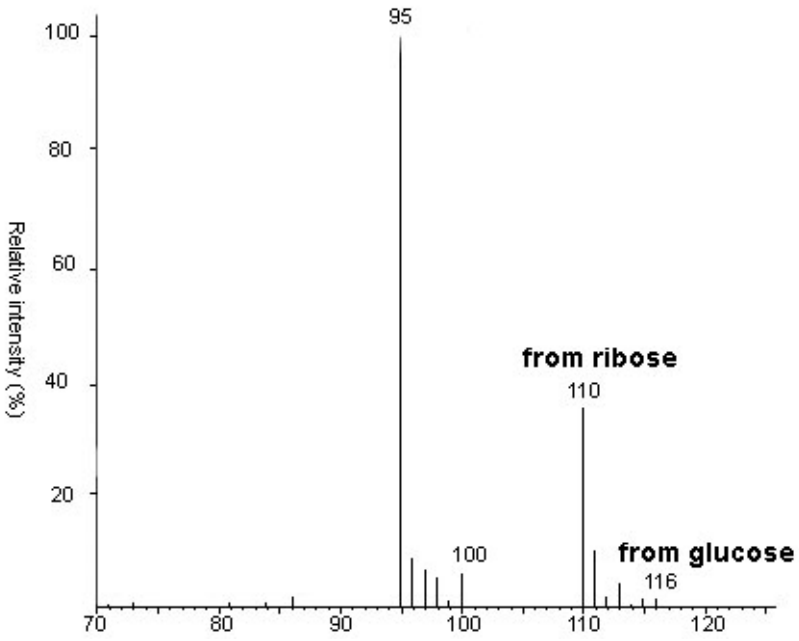


Figure 46. Mass spectrum showed the relative activity of ribose and glucose in 2-acetylfuran formation

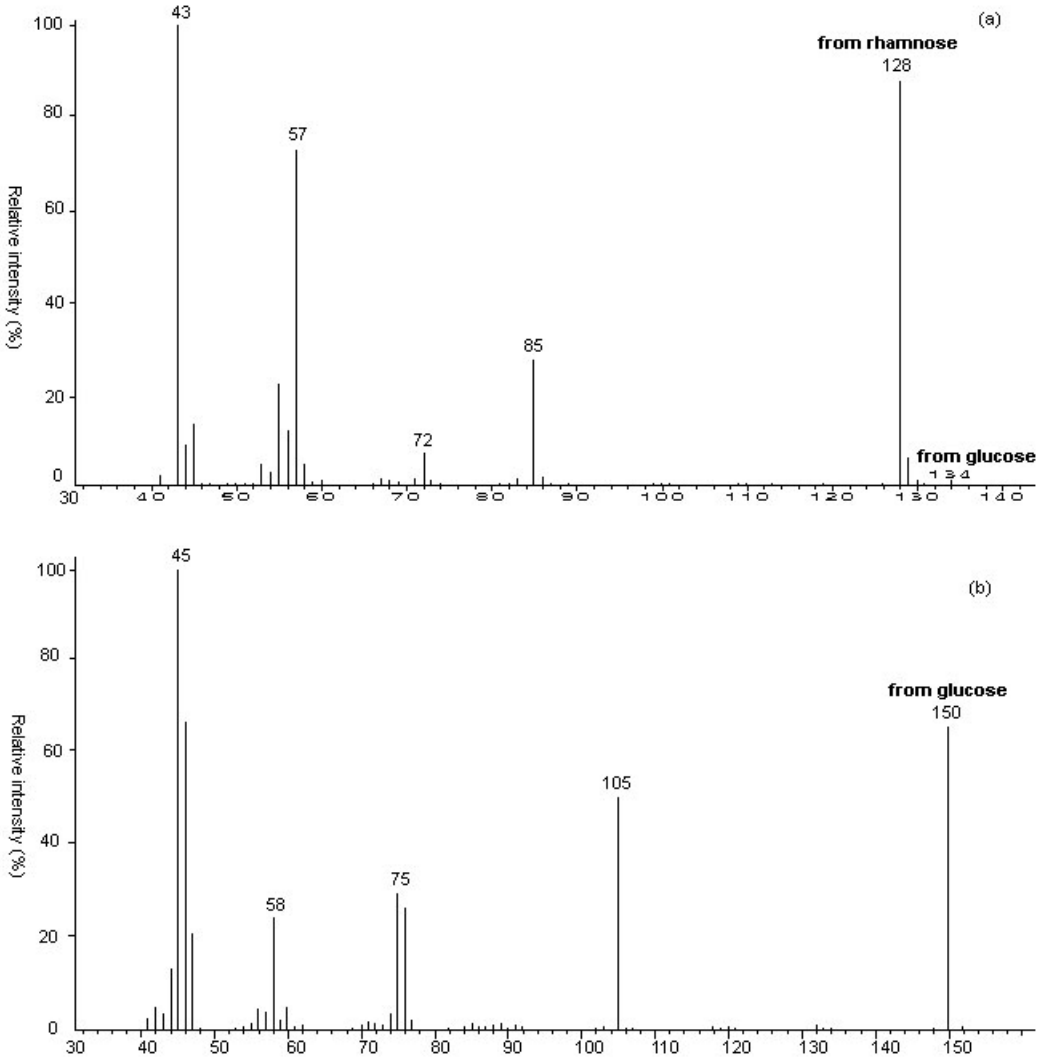


Figure 47. GC-MS spectra of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone (DMHF) (a) and acetylformoin (b) from a 1:1 mixture of [^{13}C6] glucose and [^{12}C6] rhamnose in the presence of proline.

Figure 48. Proposed formation of 2-acetylfuran from ribose and formaldehyde (A) and formation of formaldehyde from a symmetric structure of ribose (B).

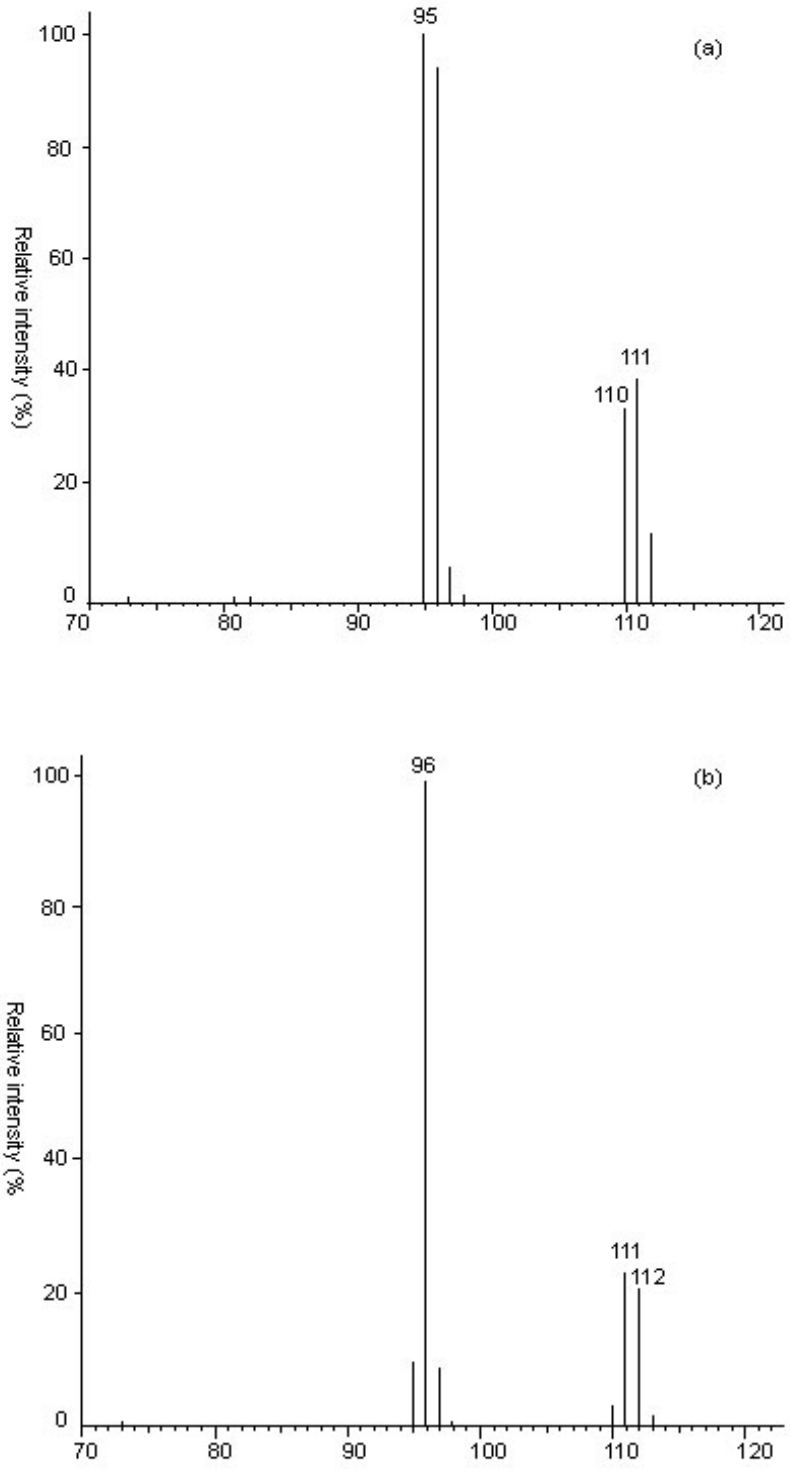


Figure 49. GC-MS spectrum of 2-acetylfuran from a 1:1 mixture of [5-^{13}C] ribose and [^{12}C5] ribose (a) and [5-^{13}C] ribose (b) in the presence of proline.

4. Conclusion

1. The levels of RCS in commercial carbonated soft drinks were found to be astonishingly high. High fructose corn syrup was identified as a source of these reactive carbonyl compounds in CSDs. Furthermore, EGCG and possible other polyphenols may have the potential to reduce the levels of reactive carbonyl compounds in beverages or foods.

2. MG, depending on the pH differently affected DMHF generation in the presence or absence of amino acids. When glucose reacted with glycine or cysteine, glucose skeleton kept intact in the formed DMHF as well as its precursor acetylformoin. Acetylformoin was not formed in the reaction between MG and either glycine or cysteine.

3. For the DMHF, there was only one major formation pathway in which glucose carbon skeleton kept intact. On the other hand, formation pathways for 2-acetylfuran were more complicated. It could be formed either from glucose or from glucose and glycine. These results indicated a competitive trend in controlling Maillard reaction. Therefore, besides changing Miallard reaction impact factors (temperature, time, pH etc.), inhibiting or preventing the competitive reaction cascade may direct desired pathways of Maillard reaction.

4. Big differences occur between ribose and glucose in 2-acetylfuran formation from reaction reactivity and mechanism pathways. The reactivity of ribose in 2-acetylfuran formation was higher than that of glucose. In the presence of cysteine, 2-acetylfuran could not be generated from ribose. When ribose was reacted with glycine, formaldehyde generated from glycine would involve in the formation of 2-acetylfuran. For other amino acids, symmetric structure of ribose was formed.

In summary, RCS particularly these dicarbonyl compounds play a paradoxical role in food for the consumers. On one hand, high levels in foods or beverages may cause serious health problem; On the other hand, they are key precursors for flavor generation in foods. How to find a way to decrease the RCS levels in foods but meanwhile pleasure flavors are still kept becomes a big challenge for recent food science research. This study provides several possible solutions, one is to use trapping agent either in or after food processing, and another one is controlling flavor generation reaction especially Maillard reaction to a desired direction by changing parameters.

References

Adams, RP. Identification of essential oil components by gas chromatography/mass spectrometry. **1995**. Allured Publishing Corporation, Carol Stream, IL.

Antal, MJ.; Mok, W. S. L.; Richards, G. N. Mechanism of formation of 5-(hydroxymethy)-2-furaldehyde from D-fructose and sucrose. *Carbohydr. Res*. **1990**, 199, 91–109.

Araki, A., Nourooz-Zadeh, J., Glomb, MA., Monnier, VM. and Wolff, SP. in *The Maillard Reaction in Foods and Medicine* (O'Brien, J., Nursten, H. E., Crabbe,M. J. C. and Ames, J. M., eds.), **1998**, p. 401, RSC Pub., Cambridge, U.K.

Baines, DA., Mlotkiewicz, JA. The chemistry of meat flavours. In *recent advances in the chemistry of meat.* Bailey, AJ., Eds. Royal Soc. Chem. Publication, London, 1984,119-164.

Bao, M., Pantani, F., Griffini, O., Burrini, D., Santiani, D., Barbieri, K., Determination of carbonyl compounds in water by derivatization-solid-phase microextraction and gas chromatographic analysis. *J. Chromatogr.* A **1998**, 809, 75-87.

Basciano, H.; Federico, L.; Adeli, K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr. Metab*. **2005**, *2*, doi: 10.1186/1743-7075-2-5.

Baynes, JW., Thorpe, SR., Glycoxidation and lipoxidation in atherogenesis. *Free Radic. Biol. Med.* **2000**, 28, 1708-1716.

Baynes, JW.; Thorpe, SR.; Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* **1999**, 48, 1-9.

Blank, I.; Fay, LB. Formation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone through Maillard reaction based on pentose sugars. *J. Agric. Food Chem.* **1996**, 44, 531–536.

Boelens, M., van der Linde, LM. , de Valois, PJ. , van Dort, HM. , Takken, HJ. , Organic sulfur compounds from fatty aldehydes, hydrogen sulfide, thios and ammonia as flavor constituents, *J. Argic. Food Chem*. **1974**, 22, 1071-1076.

Bray, GA.; Nielsem, SJ.; Popkin, BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am. J. Clin. Nutr*. **2004**, *79*, 537-543.

Buttery, RG., Stern, DJ., & Ling, LC. Studies on flavor volatiles of some sweet corn products. *J. Agric. Food Chem.* **1994**, 42, 791-795.

Buttery, RG., Takeoka, GR., Krammer, GE., Ling, LC. Identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) and 5-methyl-4-hydroxy-3(2*H*)-furanone in fresh and processed tomato. *Lebensm. Wiss. Technol.* **1994**, 27, 592–594.

Cerny, C., Davidek, T. α-Mercaptoketone formation during the Maillard reaction of cysteine and [1-13C] ribose. *J. Agric. Food Chem*. **2004**, *52*, 958-961.

Coleman, EC., Ho, CT., Chang, SS. Isolation and identification of 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-thithiolane in the volatile flavor compounds from backed potatoes. *J. Agric. Food Chem.* **1981**, *29*, 42-48.

Creighton, DJ., Migliorini, M., Pourmotabbed, T., Guha, MK., Optimization of efficiency in the glyoxalase pathway. *Biochemistry* **1988**, 27, 7376–7384.

De Revel, G., Pripis-Nicolau, L., Barbe, J.-C., Bertrand, A., The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivatives. *J. Sci. Food*

Agric. **2000**, 80, 102-108.

Do Rosario, PM. ., Cordeiro, CAA., Freire, AP., Nogueira, JMF. Analysis of methylglyoxal in water and biological matrices by capillary zone electrophoresis with diode array detection. *Electrophoresis* **2005**, 26, 1760 –1767.

Ebbeling, CB., Feldman, HA., Osganian, SK., Chomitz, VR., Ellenbogen, SJ., Ludwig, D S. Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. *Pediatrics*. **2006**, 117, 673-680.

Fagerson, I. S. Thermal degradation of carbohydrate. *J. Agric. Food Chem*. 1969, 17, 747-750.

Friedman, M., Molnar-Perl, I. Inhibition of browning by sulfur amino acids. I. Heated amino acid-glucose systems. *J. Agric. Food Chem*. **1990,** 38, 1642-1647.

Fujioka, K., Shibamoto, T. Determination of toxic carbonyl compounds in cigarette smoke. *Environ. Toxicol.* **2006**, 21, 47-54.

Fujioka, K., Shibamoto, T. Formation of genotoxic dicarbonyl compounds in dietary oils upon oxidation. *Lipids*. **2004**, 39, 481–486.

Haleva-Toledo, E., Naim, M., Zehavi, U., Rouseff, RL. Effects of *L*-cysteine and *N*-acetyl-*L*-cysteine on 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol), 5-(hydroxymethyl)furfural, and 5-methylfurfural formation and browning in buffer solutions containing either rhamnose or glucose and arginine. *J. Agric. Food Chem*. **1999,** 47, 4140-4145.

Hayase, F., Shibuya, T., Sato, J., Yamamoto, M. Effects of oxygen and transition metals on the advanced Maillard reaction of proteins with glucose. *Biosci. Biotech. Biochem.* **1996**, 60, 1820-1825.

Hayashi, T., Namiki, M. Formation of two-carbon sugar fragments at an early stage of the browning reaction of sugar and amine. *Agric. Biol. Chem.* **1980***, 44*, 2575-2580

Hayashi, T., Shibamoto, T., J. Analysis of methylglyoxal in foods and beverages. *Agric. Food Chem.* **1985**, 33, 1090-1093.

Ho, CT., Jin, QZ., Lee, MH., Chang, SS. Positive identification of new alkyloxazoles, alkylthiazoles, and piperidine in roasted peanut flavor. *J. Agric. Food Chem.* **1983a,** 31, 1384-1386.

Hodge, JE. Dehydrated foods: Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, 1, 928–943.

Hodge, JE., Mills, FD., Fisher, BE. Compounds of browned flavor derived from sugar-amine reactions. *Cereal Sci. Today* 1972. 17, 34–40.

Hofmann, T., Munch, P., Schieberle, P. Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker reaction. *J. Agric. Food Chem*, **1999**, 48, 434-440.

Hofmann, T., Schieberle, P. Acetylformoin—an important progenitor of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-acetyltetrahydropyridine during thermal food processing. *In Flavour 2000, Perception Release Evaluation Formation Acceptance Nutrition/Health.* Proceedings of the 6th Wartburg Aroma Symposium. Eisenach, M. Rothe, Ed. **2001,** 311–322. Eisenach, Germany.

Hofmann, T., Schieberle, P. Acetylformoinsan important progenitor of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 2-acetyltetrahydropyridine during thermal food processing. In *Flavour 2000 Perception Release EValuation Formation Acceptance*

Nutrition/ Health; Proceedings of the 6th Wartburg Aroma Symposium; Eisenach, M., Rothe, Eds.; **2001**, 311-322.

Hofmann, T., Schieberle, P. Formation of aroma active strecker aldehydes by a direct oxidation degradation of Amadori compounds. *J. Agric. Food Chem*, **2000**, 48, 4301-4305.

Hofmann, T., Schieberle, P. Identification of potent aroma compounds in thermally treated mixtures of glucose/cysteine and rhamnose/cysteine using aroma extract dilution techniques. *J. Agric. Food Chem*. **1997,** 45, 898-906.

Hollnagel, A., Kroh, L. W. Formation of α-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Z. Lebensm. Unters. Forsch. A*. **1998**, 207, 50–54.

Homoki-Farkas, P., Orsi, F., Kroh, L. W. Methylglyoxal determination from different carbohydrates during heat processing. *Food Chem.* **1997**, 59, 157–163.

http://www.ers.usda.gov/Data/FoodConsumption/FoodAvailQueriable.aspx#midForm

Hwang, HI., Hartman, TG., Rosen, RT., Ho. CT. Formation of pyrazines from the Maillard reaction of glucose and glutamine-amide-15N, J. *Argic. Food Chem*. **1993**, 41, 2112-2115.

Izzo, HV., Ho, CT. Ammonia affects Maillard chemistry of an extruded autolyzed yeast extract: pyrazine aroma generation and brown color formation. *J. Food Sci*. **1992**, 57, 657−659 & 674.

Jüergens, H., Haass, W., Castañeda, TR., Schüermann, A., Koebnick, C., Dombrowski, F., Otto, B., Nawrocki, AR., Scherer, PE., Spranger, J., Ristow, M., Joost, H., Havel, PJ., Tschoep, M. H. Consuming fructose-sweetened beverage increases body adiposity in

mice. *Obesity Res.* **2005**, 13, 1146-1156.

Jutta, KK., Werner, B. Model reaction on roast aroma formation. VIII. Volatile reaction products from the reaction of phenylalanine with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) by cooking in a laboratory autoclave. *Z. Lebensm. Unters. Forsch.* **1990**, 190, 14–16.

Lapolla, A,, Flamini, R., Dalla Vedova, A., Senesi, A., Reitano, R., Fedele, D., Basso, E., Seraglia, R., Traldi, P. Glyoxal and methylglyoxal levels in diabetic patients: quantitative determination by a new GC MS method. *Clin. Chem. Lab. Med*. **2003**, 41, 1166-1173.

Lee, H. S., Nagy, S. Relative reactivities of sugars in the formation of 5-hydroxymethyl furfural in sugar-catalyst model systems. *J. Food Process. Preserv*. **1990**, 14, 171–178.

Lo, CY., Li, S., Tan, D., Sang, S., Pan, M. H., Ho, CT. Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Mol. Nutr. Food Res.* **2006**, 50, 1118-1128.

Lyles, GA., Chalmers, J. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. *Biochem. Pharmacol*. **1992**, 43, 1409–1414.

Maga, JA., Pyrazines in foods: an update. CRC *Crit. Rev Food Sci. Nutri*. **1982**, 16,1-96.

Maga, JA; Sizer, CE. Pyrazines in Foods. CRC *Crit. Rev. Food Technol*. **1973**, 4 ,39-115.

McLellan, AC., Phillips, SA., and Thornalley, PJ. The assay of methylglyoxal in biological systems by derivatization with 1,2-diamino-4,5-dimethoxybenzene. *Anal. Biochem*. **1992**, 206, 17-23

Molnar-Perl, I., Friedman, M. Inhibition of browning by sulfur amino acids. II. Fruit juices and protein-containing foods. *J. Agric. Food Chem*. **1990,** *38*, 1648-1651.

Molnar-Perl, I., Friedman, M. Inhibition of browning by sulfur amino acids. III. Apples and potatoes. *J. Agric. Food Chem*. **1990,** 38, 1652-1656.

Mottram, DS. Meat. In Volatile compounds in foods and beverages. Maarse H., Eds.; Marcel Dekker, Inc., New York, NY., 1991, 107-177.

Mottram, D. S., Wedzicha, B. I., & Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature*. **2002**, 419, 448–449.

Mulders, EJ. Volatile compounds from the non-enzymatic browning reaction of cysteine/cysteine-ribose system. *Z.Lebensm Unters-Forsch*, 1973, 152, 193-201.

Mullarkey, CJ., Edelstein, D. and Brownlee, M., Free radical generation by early in diabetes 1990 *Biochem. Biophys. Res. Commun.* **1990**, 173, 932-939.

Mussinan, CJ., Walradt, JP. Volatile constituents of pressure cooked pork liver. *J. Agric. Food Chem*, **1974**, 22, 827-831.

Mussinan, CJ., Wilson, RA., Kazt, I., Hruza, A., Vock, MH. Identification and flavor properties of some 3-oxazolines and thiazolines isolated from cooked beef. *In Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors.* Charalambous, G.; Katz, I.; Eds..; ACS Symp. Ser.26, American Chemical Society: Washington, DC, **1976**, 133-145.

Nagaraj, RH., Sarkar, P., Mally, A., Biemel, KM., Lederer, MO., Padayatti, PS. Effect of pyridoxamine on chemical modification of proteins by carbonyls in diabetic rats: characterization of a major product from the reaction of pyridoxamine and methylglyoxal. *Arch. Biochem. Biophys*. **2002**, 402, 110–119.

Nemet, I., Vikic-Topic, D., Varga-Defterdarovic, L. Substrate product equilibrium on a reversible enzyme, triosephosphate isomerase *Bioorg. Chem.* **2004**, 32, 560–570.

Nursten, H. The Maillard Reaction: Chemistry, Biochemistry and Implications. The Royal Society of Chemistry, Cambridge, UK, 2005

Nishimura, O., Yamaguchi, K., Mihara, S., & Shibamoto, T. Volatile constituents of guava fruits (*Psidium guajava* L.) and canned puree. *J. Agric. Food Chem*. **1989**. 37, 139-142.

Nunomura, N., Sasaki, M., Asao, Y., Yodotsuka, T. Shoyu (soy sauce) volatile flavor components: basic fraction. *Agri. Biol. Chem.* **1978**, 42, 2123-2128.

Odani, H., Shinzato, T., Matsumoto, Y., Usami, J., Maeda, K. Increase in three μ, β-dicarbonyl compound level in human uremic plasma: Specific *in vivo* determination of intermediates in advanced maillard reaction. *Biochem. Biophys. Res. Commu*. **1999**, 256, 89-93.

Oh, YC., Hartman, TG., Ho, CT. Volatile compounds generated from the Maillard reaction of Pro-Gly, Gly-Pro, and a mixture of glycine and proline with glucose. *J. Agric. Food Chem.* **1992a**, 40, 1878-1880.

Oh, YC., Shu, CK., Ho, CT. Formation of novel 2(1H)-pryazinones as peptide-specific Maillard reaction products, *J. Argic. Food Chem.* **1992b,** 40 118-121.

Onorato, JW., Thorpe, SR., Baynes, JW. Immunohistochemical and ELISA assays for biomarkers of oxidative stress in aging and disease. Ann. NY *Acad. Sci*. **1998**, 854, 277-290.

Pabst, A., Barron, D., Etie´vant, P., Schreier, P. Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *J. Agric. Food Chem.* **1991**, 39, 173–175.

Phillips, SA., Thornalley, PJ. The formation of methylglyoxal from triose phosphate. *Eur. J. Biochem*. **1993**, 212, 101-105.

Pompliano, DL., Peyman, A., Knowles, JR. Stabilization of a reaction intermediate as a catalytic device: definition of the functional role of the flexible loop in triosephosphate isomerase. *Biochemistry*, **1990**, 29, 3186–3194.

Putnam, JJ., Allshouse, JE. *Food consumption, prices and expenditures*, 1970-97. US Department of Agriculture Economic Research Service statistical bulletin no. 965, April 1999. Washington, DC: US Government Printing Office, **1999**.

Rapp, A., Knipser, W., Engel, L., Ullemeyer, H., Heimann, W. Off-flavor compounds in the berry and wine aroma of grapevine hybrids. I. The strawberry-like aroma. *Vitis* **1980**, 19, 13–23.

Ray, S., Ray, M**.** Isolation of methylglyoxal synthase from goat liver. *J. Biol. Chem*. **1981**, 256, 6230-6233.

Reichard, GA., Skutches, CL., Hoeldtke, RD., Owen, OE. Acetone metabolism in humans during diabetic ketoacidosis. *Diabetes* **1986**, 35, 668–674.

Reyes, FGR., Poocharoen, B., Wrolstad, R. Maillard browning reaction of sugar-glycine model systems: Changes in sugar concentration, color and appearance. *Journal of Food Science* **1982**, 47, 31376–31377.

Rizzi, GP. Mechanistic study of alkylpyrazine formation. *J. Agric. Food Chem.* **1972**, 20, 1081-1085

Rodin, JO., Himel, CM., Silverstein, RM., Leeper, RW., Gortner, WA. Volatile flavor and aroma components of pineapple. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. *J. Food Sci.* **1965**, 30, 280–285.

Rodrigues, JA., Barros, AA., Rodrigues, PG. Differential pulse polarographic determination of α-dicarbonyl compounds in foodstuffs after derivatization with *o*-phenylenediamine. *J. Agric. Food Chem.* **1999**, 47, 3219-3222

Rahbar, S., Yerneni, KK., Scott, S., Gonzales, N., Lalezari, I. Novel inhibitors of advanced glycation endproducts (part II), *Mol Cell Biol. Res. Commun.* **2000**, *3,* 360–366.

Schieberle, P. Studies on bakers yeast as source of Maillard type bread flavour compounds. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R., Eds.; Birkha¨user; Basel, Switzerland, **1990**; pp 187-196.

Schieberle, P. Studies on the formation of furaneol in heat processed foods. In *Fla*V*or Precursors: Thermal and Enzymatic Con*V*ersions*; Teranishi, R., Takeoka, G. R., Gu¨ntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 164-175.

Schieberle, P. The carbon module labeling (CAMOLA) technique. A useful tool for identifying transient intermediates in the formation of Maillard-type target molecules. *Ann. N.Y. Acad. Sci.* **2005**, 1043, 236–248.

Fujioka, K., Shibamoto, T. Formation of genotoxic dicarbonyl compounds in dietary oils upon oxidation. *Lipids*. **2004**, 39, 481-486.

Fujioka K, Shibamoto T. Determination of toxic carbonyl compounds in cigarette smoke. *Environ. Toxycol*. 2006, 21, 47–54.

Shibamoto, T., Bernhard, RA. Investigation of pyrazines formation pathways in glucose-ammonia model systems. *Agri. Biol.Chem*, **1977**, 41,143-153.

Shu, CK., Ho, CT. Effect of pH on the volatile formation from the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2*H*)- furanone. *J. Agric. Food Chem.* **1988**, 36, 801–803.

Shu, CK., Lawrence, BM. 3-Methyl-2(1H0-pyrazinones, the asparagines-specific Maillard products formed from asparagines and monosaccharides. *J. Agric. Food Chem.* **1995**, 43, 779-781.

Steinhaus, P., Schieberle, P. Characterization of the key aroma compounds in soy sauce using approaches of molecular sensory science. *J. Agric. Food Chem.* **2007**, 55, 6262-6269.

Stofflesma, J., Sipma, G., Kettenes, DK., Pypker, J. New Volatile compounds of roasted coffee. *J. Agric. Food Chem.* **1968**, 16, 1000-1004.

Stoll, M., Dietrich, P., Sundte, E., Winter, M. Compounds associated with the aroma of cocoa. Helv. Chim.Acta. **1967**, 50, 2065.

Takken, HJ., van der Linde, LM., de Valois, PJ., van Dort, HM., Boelens, M. Reaction products of α-dicarbonyl compounds, aldehydes, hydrogen sulfides, and ammonia. In Phenolic, sulfur, and nitrogen compounds in food favors. Charalambous, G., Katz, I. Eds. ACS Symp. Ser. 26, American Chemical Society: Washington, DC, 1976, 114-121.

Tang, J., Jin, QZ., Shen, GH., Ho, CT., Chang, SS. Isolation and identification of volatile

compounds from fried chicken. *J. Agric. Food Chem*, **1983**, 31, 1287-1292.

Tei, Y., Yamanaishi, T. Flavor components of roasted almond. *Agric. Biol. Chem.* **1974**, *38* (12), 2329–2336.

Thornalley, PJ., Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. *Chem. Biol. Interact.* **1998**, 111–112, 137 –151.

Thornalley, P. J., Monosaccharide autoxidation in health and disease. *Environ. Health Prospect.* **1985**, 64, 297-307.

Thornalley, PJ., Wolff, SP., Crabbe, J., Stern, A. The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalysed by buffer ions. *Biochim. Biophys. Acta*. **1984**,797, 276-287

Thornalley, PJ., Langborg, A., Harjit, S. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J*. **1999**, 344, 109-116.

Thornalley, PJ. Pharmacology of methylglyoxal: Formation, modification of proteins and nucleic acids, and enzymatic detoxification-A role in pathogenesis and antiproliferative chemotherapy, *General Pharmocology* **1996**, 27, 565-573.

Tonsbeek, CH., Copier, H., Placken, AJ. Compounds contributing to beef flavor. Isolation 2-acetyl-2-thiazoline from beef broth. *J. Agric. Food Chem.* **1971**, 19, 1014-1016.

Tressl, R., Grunewald K. G., Helkk, B., Formation of flavor components from proline and hydroxyproline with glucose and maltose and their importance to food flavour, in Flavour ’81 (ed. P. Schreier), Walter de Gruyter, Berlin, **1981**, pp. 397-416.

Tressl, R., Bahri, D., Ko¨ppler, H., Jensen, A. Diphenols and caramel compounds in roasted coffees of different varieties. II. *Z. Lebensm. Unters. Forsch.* **1978**, 167, 111–114.

Tressl, R., Rewicki, D., Helak, B., Kamperschroer, H. Formation of pyrrolidines and piperidines on heating L-proline with reducing sugars. *J. Agric. Food Chem.* **1985**, 33, 5, 924-928.

Van Boekel, MAJS. Formation of flavour compounds in the Maillard reaction. *Biotechnology Adavnces*. **2006**, 24, 230-233.

Van Boekel, MAJS. Kinetic aspects of the Maillard reaction: a critical review. *Nahrung/Food*. **2001**, 45, 150-159.

Vernin, G., Metzger, J. La chimie des aromes: les heterocycles. *Bull. Soc. Chim. Belg*. **1981**, 90, 553-587.

Vernin, G. The chemistry of heterocyclic flavoring and aroma compounds. Ellis Horwood, Chichester, UK, **1982**.

Vitzthum, OG., Werkhoff, P., Hubert, P. Volatile compounds of roasted cocoa: basic fraction. *J. Food. Sci.* **1975**, 40, 911-916.

Weigel, K. U., Opitz, T., Henle, T., Studies on the occurrence and formation of 1,2-dicarbonyls in honey. *Eur. Food Res. Technol.* **2004**, 218, 147–151.

Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, Baynes JW. Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. *Biochemistry* **1995**, 34, 3702-3709.

Wells-Knecht, KJ., Zyzak, DV., Litchfield, JE., Thorpe, SR., Baynes, JW., Wharton, CM.,

Hampl, JS. Beverage consumption and risk of obesity among Native Americans in Arizona. *Nutr. Rev*. **2004**, 62, 153-159.

Wharton, CM., Hampl JS. Beverage consumption and risk of obesity among Native Americans in Arizona. *Nutr. Rev*. **2004**, 62, 153-159.

Willhalm, B., Stoll, M., Thomas, AF. 2,5-Dimethyl-4-hydroxy- 2,3-dihydrofuran-3-one. *Chem. Ind. (London)* **1965**, 38, 1629–1630.

Wilson, RA., Mussinan, CJ., Katz, I., Sanderson, A. Isolation and identification of some sulfur chemicals present in pressure-cooked beef. *J. Agric. Food Chem.* **1973**, 21, 873-876.

Wu, L., Juurlink, BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* **2002**, 39, 809–814.

Wu, ML., Chen, JH., Ho, CT., Huang, TC. Synthesis of 1-Piperideine-6-carboxylic Acid Produced by L-lysine-ε-aminotransferase from the Streptomyces clavuligerus Gene Expressed in *Escherichia coli. J. Agric. Food Chem*. **2007**, 55, 1767-1772.

Yamaguchi, K., Shibamoto, T. Volatile constituents of Castanopsis flower. *J. Agric. Food Chem.* **1979**, 27, 847-850.

Yaylayan, VA., Machiels, D., Istasse, L. Thermal decomposition of specifically phosphorylated D-glucose and their role in the control of the Maillard Reaction. *J. Agric. Food Chem.* **2003**, 51, 3358-3366.

Zheng, Y., Brown, S., Leding, W. O., Mussinan, C., Ho, CT. Formation of sulphur-containing flavour compounds from reactions of furaneol and cysteine, glutathione, hydrogen sulphide, and alanine/hydrogen sulfide. *J. Agric. Food Chem.* **1997**, 45, 894-897.

Curriculum Vita

Yu Wang

Education

2005-2009 PhD in Food Science, Rutgers, the State University of New Jersey, New Brunswick, NJ

2004-2005 MS in Food Science, Louisiana State University, Baton Rouge, LA

1999-2003 BS in Food Science and Engineering, Hefei University of Technology, Hefei, Anhui, P.R. China

Selected Publications

Wang, Y., Juliani, HR., Simon, JE., Ho, CT. Amino acid-dependent formation pathways of 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3(2H)-furanone in the Maillard reaction. *Food Chemistry*, 2008, submitted.

Wang, Y., Ho, CT. Comparison of 2-acetylfuran formation between ribose and glucose in the Maillard reaction. *Journal of Agricultural and Food Chemistry*, 2008, submitted.

Wang, Y; Ho, CT. (2008): Metabolism of dietary plant phenolic acids. *Critical Reviews in Food Science and Nutrition*, 2008, submitted

Wang, Y., King, JM., Xu, Z., Losso, J., Prudente A. Lutein from Ozone-treated corn retains antimutagenic properties. *Journal of Agricultural and Food Chemistry*, 2008, 56(17), 7942-7949.

Wang, Y., Ho, CT. Formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone through methylglyoxal - A Maillard reaction intermediate. *Journal of Agricultural and Food Chemistry*, 2008, 56, 7405-7409.

.

Tan, D., **Wang, Y**., Lo, CY., Sang, S., Ho, CT. Methylglyoxal: Its presence in beverages and potential scavengers. In The Maillard Reaction: Recent Advances in Food and Biomedical Sciences, Edited by Erwin Schleicher; Veronika Somoza; Peter Schieberle; *Annals of the New York Academy of Sciences*, 2008, 1126, 72-75.

Lo, CY., Li, S., **Wang, Y**., Tan, D., Pan, MH., Sang, S., Ho, CT. Reactive dicarbonyl compounds and 5-(hydroxymethyl)-2-furfural in carbonated beverages containing high fructose corn syrup. *Food Chemistry*, 2008, 107, 1099-1105.

Wang, Y., Raghavan, S., Ho, CT. Process flavors of Allium vegetables. In Fruit and Vegetable Flavour: Recent Advances and Future Prospects, Edited by B. Brückner and S. G. Wyllie, Woodhead Publishing Ltd., Cambridge, U.K., 2008, pp. 200-226.