Investigation of the role of glycine/sugar ratio, sodium and hydrochloride salts of glycine, glucose or fructose on the profile of the Maillard reaction products

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Abstract

Maillard reaction refers to the non-enzymatic interaction of reducing sugars with amino acids, and plays a significant role in determining the flavour sensation of many food products. Since flavour sensation is dictated by a profile of hundreds of compounds, modification of this profile can be an effective method to control the over-all flavour sensation of foods. Three important factors affecting the Maillard reaction have been investigated using a simple model system: sugar type, amino acid salts and their ratios. Labelled or unlabeled pure reactants - sugars and amino acid salts- were homogenized and pyrolyzed at 250℃ for 20 seconds, and reaction products were separated by Gas Chromatography and then identified by Mass Spectrometry. Results indicated that the sugar type, the amino acid form (free or salt), and their ratios had a significant influence on the total abundance, total number of components, formation of specific peaks as well as on the intensity changes of the common peaks in the various model systems. The data generated may provide useful information on the modification and optimization conditions of the Maillard reaction profiles using practical solutions.

Résume

Réaction de Maillard est basée sur l'interaction non- enzymatique des sucres réducteurs avec des acides aminés, et joue un rôle important dans la détermination des saveurs de nombreux produits alimentaires. La saveur est dictée par le profil d'une centaine de composés, et la modification de ce profil peut être une méthode efficace pour contrôler la saveur des aliments dans son ensemble. Trois facteurs importants qui affectent la réaction de Maillard ont été étudiés en utilisant un système de modèle simple: le type de sucre, les sels d'acides aminés et de leurs rapports. Réactifs purs ou non marqués étiquetés - sucres et les acides aminés - ont été homogénéisés et pyrolysés à 250 ℃ pendant 20 secondes ; et les produits de réaction ont été séparés par chromatographie en phase gazeuse et ensuite identifiés par spectrométrie de masse. Les résultats indiquent que le type de sucre, sous forme d'acides aminés (libre ou de sel), et leurs rapports ont une influence significative sur l'abondance totale, le nombre total de composants, la formation de pics spécifiques, ainsi que sur les changements d'intensité des pics communs aux divers systèmes de modèles. Les données générées peuvent fournir des informations utiles sur les conditions appropriées de modification pour l'optimisation des profils de réaction de Maillard en utilisant des solutions pratiques.

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General Introduction

Maillard reaction is widely recognized as a non-enzymatic reaction that occurs between reducing sugars, such as D-glucose, and amino compounds, such as amino acids or proteins under thermal conditions. Since food matrix contains various and abundant reducing sugars, such as glucose, fructose, ribose and so forth, and amino acids, Maillard reaction becomes a prominent reaction during the thermal processing of foods. Flavour is well accepted as the most important but challenging aspect of food products and Maillard reaction plays a crucial role in determining the flavour sensation including aroma, taste and colour of processed food especially during roasting, baking and toasting. Flavour sensation is not determined by one single chemical compound but with a profile which may contain hundreds of compounds. For example, more than 875 compounds have been reported in roasted coffee aroma. If we can elucidate all the products formed and exercise control over the Maillard reaction successfully, optimization of the aroma and taste of food products can be realized.

There are many factors that affect the Maillard reaction profile such as sugar and amino acid type and their ratios, pH, temperature, water content, time etc. According to the literature, the rate of the Maillard reaction is governed by the concentration of the reactants; there is only limited information on the effect of amino acid/sugar ratios on

the intermediates or end product formation of Maillard reaction. In aqueous systems, pH exerts a crucial effect on the rate of formation of the Amadori compound and the Schift-base (Nursten, 2005). It also influences the chemical activity of the sugars and amino acids (Van Boekel, 2001). Finally, the type of sugars has an effect on both the reaction rate and the structure of the end products formed. However, there are no studies on the effect of amino acid salts such sodium or hydrochloride on the Maillard reaction. In addition, there are no systematic studies published on the effect of sugar type and the ratios of amino acids to sugars on the distribution of Maillard reaction end products. These research gaps lead us to systematically investigate the effect of sugar type, amino acid salts and their ratios on the modification of Maillard reaction profile.

Chapter 1

Literature Review

1.1 Introduction

Mechanism of browning in food can be separated into two main types, enzymatic and non-enzymatic depending on whether the reaction is mediated by enzymes or not (Nursten, 2005). Under heating, it is supposed that enzymes are inactivated and thus only non-enzymatic browning takes place. Non-enzymatic browning can be roughly divided into three reactions: Maillard reaction, which generally takes place between reducing sugars and amino acids; caramelization, which needs only sugars; and ascorbic acid oxidation. Most academic efforts are put into Maillard reaction, because not only people regard caramelization and ascorbic acid oxidation as special cases of Maillard reaction, but also it is of great commercial, nutritional and physiological significance (Nursten, 2005).

Maillard reaction is widely recognized as a reaction that occurs between reducing sugars, such as D-glucose, and amino compounds, such as amino acids or proteins under thermal conditions (Hodge, 1953). Maillard reaction plays an important role in determining the aroma, taste and colour (Arnoldi, et al., 1997;Frank and Hofmann,

2000;Gerrard, et al., 2002;Hofmann, 1998;Rizzi, 1997;Yaylayan and Haffenden, 2003) of many roasted, baked and toasted foods (Ledl and Schleicher, 1990a). Flavour is well recognized as the most important quality aspect of food products and Maillard reaction plays a key role in their flavour development. Understanding its mechanism and control over its reaction pathways will highly improve the appearance and taste of the food products and thus have an effect on the commercial success of these food products.

The Maillard reaction can have both beneficial effect by forming antioxidant compounds during processing or it can have detrimental effect by decreasing digestibility and by possible formation of toxic and mutagenic compounds and decreasing the nutritional value of foods in three ways (Chevalier, et al., 2001;Erbersdobler and Dummer, 1971;Martins, et al., 2001): first, it may damage the protein quality by the formation of lysine-bound Amadori compounds and thus destroying essential amino acids; second, the premelanoidins are able to react with and damage some vitamins; finally, they also influence trace element metabolism. The reduction of protein quality caused by Maillard reaction attracts most attention from nutritionists specially in infant foods (Hurrell, 1990). Thus, it is important to study the factors which affect the Maillard reaction to be able to

modify or change the processing parameters to minimize the browning phenomenon brought by interaction between reducing sugar and amino compounds.

Furthermore, the Maillard reaction can also occur in the human body at physiological conditions through glycation of various proteins and formation of AGEs (Advanced Glycation End products) such as pentosidine (Grandhee and Monnier, 1991). Although the Maillard reaction has been studied most extensively in food, however, it has been shown the existence of a relation between Maillard reaction and numerous diseases in the human body, in particular degenerative eye diseases. In general, these diseases are attributed to the accumulation of AGEs on nucleic acids, proteins, and lipids. Though AGEs have numerous origins, they can be formed from the oxidation and dehydration of Amadori adducts, which themselves are formed during Maillard reaction. Apart from ocular diseases, a wide range of human diseases are believed to be due to the formation of AGEs, that include diabetic complications, pulmonary fibrosis, and neurodegeneration (Thorpe and Baynes, 1996). To effectively prevent these diseases, inhabitation of Maillard reaction need be performed and thus the mechanism of Maillard reaction should be clarified.

The Maillard reaction is named after the person who first proposed it, French chemist Louis Camille Maillard. In 1912, he addressed a very simple but still-significant observation: the formation of a yellow-brown colour by slightly heating sugars and amino acids in water (Maillard, 1912). This paper didn't gain much attention from academia until 1941. In 1948 Maillard reaction was eventually considered as the cause of browning and nutrition loss of many heated foods (Finot, 2005). Since then, an increasing number of scholars have been involved in the research of this impressive reaction, and thousands of references have been published in many disciplines, including organic chemistry, food science, nutrition, biology, medicine and so forth (Fayle and Gerrard, 2007). Even with the recent advances in analytical separation methodologies and the growing attention to the Maillard reaction, the complexity of this reaction remains a major challenge. In the first place, the large number of products formed proves to be too difficult to handle (Chu, 2009). In a single roasted coffee, there are more than 875 compounds reported. Additionally, the reactive intermediates produced in Maillard reaction can further react with other food components and thus increase the diversity of the products(Adams, et al., 2005;Hidalgo, et al., 1999;Weenen, 1998). Furthermore, Maillard reaction is affected by various environmental factors such as presence or absence of oxygen, temperature and heating time, type of reactants,

amino acid/sugar ratios, pH of the system, water activity, and so forth(Bemis-Young, et al., 1993;Chu and Yaylayan, 2008a;b;Fayle, et al., 2001;Tehrani, et al., 2002). All these factors mentioned above may influence the specific course of Maillard reaction and the final physical and chemical properties of the food product. As a consequence, the Maillard reaction can be recognized as a complex network of interactions among different precursors and undergo through a complex series of stages influenced by various factors. In this section, the literature regarding the influence of three main factors on Maillard reaction will be reviewed; amino acid/sugar ratios, pH, and sugar type in addition to the techniques involved in their analysis.

1.2 Basic mechanism of the Maillard reaction

The Maillard reaction consists of a highly complex network of reactions. The very first publication of Maillard (1911) initiated interest and research on the chemical aspects of the reaction. In 1953, Hodge proposed a general scheme of the reaction pathways in his well-known review integrating all published data (Hodge, 1953). This paper is regarded as a breakthrough in the understanding of the Maillard reaction mechanism and remains a crucial reference document for all Maillard researchers. Hodge (Hodge, 1953) pointed out the significant role of the Amadori compound with its various

pathways of degradation and an important step has been the possibility of obtaining the Amadori compound of the amino acids in order to study their properties. Hodge (Hodge, 1953) described the first coherent scheme of Maillard reaction shown in Figure 1.

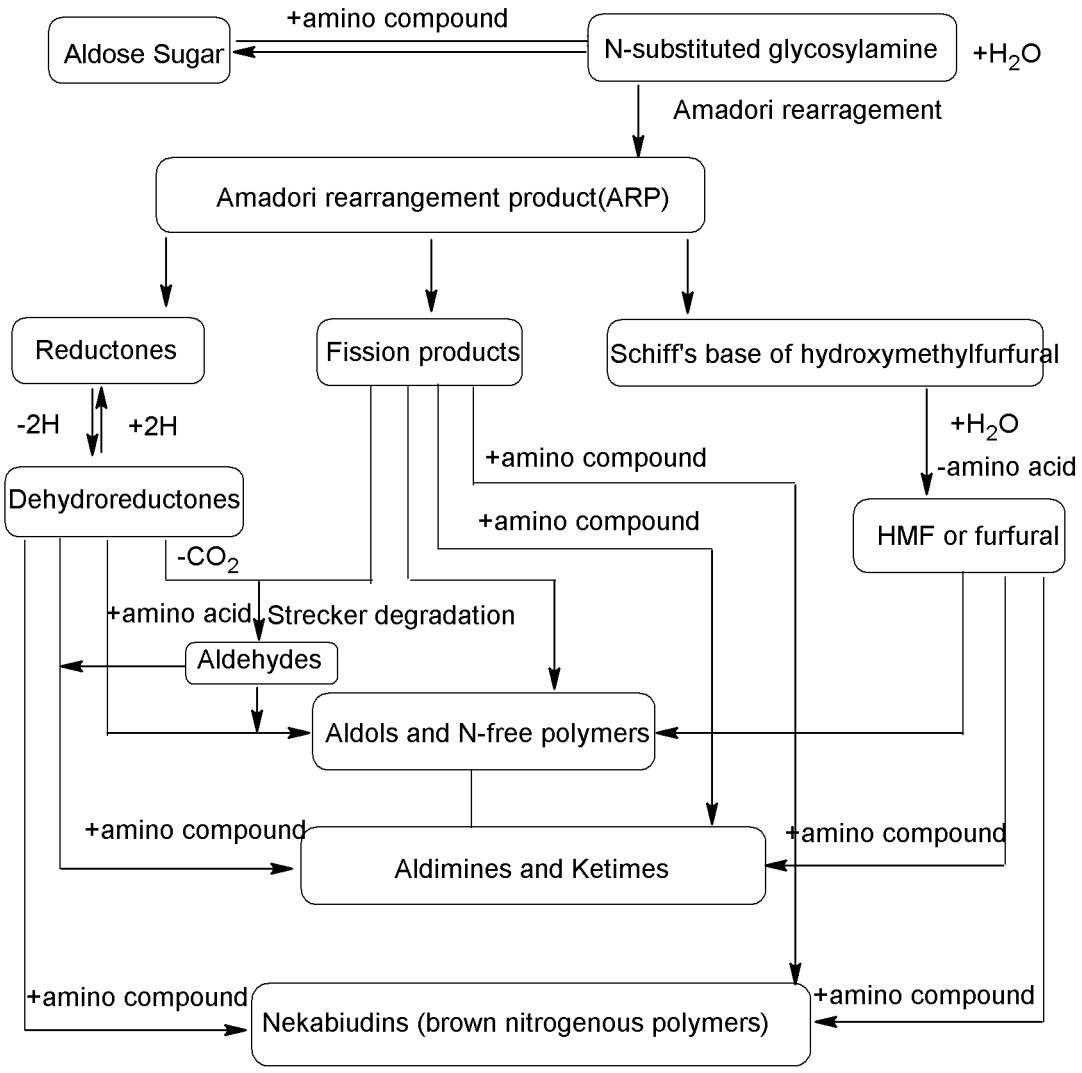


Figure 1.1 Maillard reaction scheme adapted from Hodge (Hodge, 1953;Martins, et al., 2001)

Hodge also subdivided Maillard reaction into three stages as follows:

I. Initial stage: products colourless, without absorption in the ultraviolet (about 280 nm).

Reaction A: Sugar–amine condensation

Reaction B: Amadori rearrangement

II Intermediate stage: products colourless or yellow, with strong absorption in the ultraviolet.

Reaction C: Sugar dehydration

Reaction D: Sugar fragmentation

Reaction E: Amino acid degradation (Strecker degradation)

III. Final stage: products highly coloured.

Reaction F: Aldol condensation

Reaction G: Aldehyde–amine condensation and formation of heterocyclic nitrogen compounds

There is no strict limit among these three stages, since all reactions take place interactively. In an early stage, a reducing sugar reacts with a compound having a free amino group to produce a condensation product N-substituted glycosylamine, which rearranges to give the Amadori rearrangement product (ARP). If the sugar involved is a

ketose, the reaction product is known as Heyns rearrangement product. The degradation of the Amadori product depends on the pH of the system. At pH 7 or below, 1,2-enolisation takes the priority resulting in the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH >7, 2,3-enolisation is predominant, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3- one and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed. All these compounds are very reactive and participate in later stage reactions. Carbonyl groups can condense with free amino groups, incorporating nitrogen into the reaction products. Dicarbonyl compounds can react with amino acids, which leads to formation of aldehydes and a-aminoketones. This reaction is known as the Strecker degradation. Subsequently, in an advanced stage, a set of reactions takes place, including cyclization, dehydrations, retro-aldolisations, rearrangements, isomerization and further condensations, which eventually, in a final stage, results in brown nitrogenous polymers and co-polymers, called melanoidins (Martins, et al., 2001).

The initial phase of the Maillard reaction is a well-known process, which yields glycosylamine or the Schiff base. However, the subsequent reactions after the Schiff

base formation need further investigation. Due to its complexity, the detailed reaction mechanism is still being investigated.

1.3 The effect of amino acid/sugar ratios, pH and sugar type on the mechanism of Maillard reaction

1.3.1 The effect of amino acid/sugar ratios on the mechanism of Maillard reaction

In aqueous systems, the rate of the Maillard reaction is governed by the concentration of each reactant. Higher concentrations results in higher reaction rates. For instance, different concentrations of glucose and glycine were stored at 37℃ in a 0.1M phosphate buffer at pH 7.0, the rate of browning and fluorescence formation was elevated when either reactant concentration was increased while the other remaining constant up to a certain level (Baisir and Labuza, 1992). Additionally, the molar ratios of amino acid and sugar have an effect on the reaction rate in aqueous systems. For example, with a water activity of 0.52 and temperature 45℃, the rate of pigment formation increased linearly as the starting molar ratio of glucose to available lysine from one-half to three (Warmbier, et al., 1976). At 100℃ and pH 9 and 10 in aqueous system, samples with excess glycine had higher mean color values than that of sample prepared using glucose/glycine ratios of 1:1 or 2:1 (Renn and Sathe, 1997).

However, the effect of amino acid/sugar ratios on the intermediates or end products of Maillard reaction has not been studied in detail. Kroh and his colleagues demonstrated that, under constant reaction conditions, the fundamental composition of model melanoidins was slightly affected by the molar ratio of the reactants in solvent-free reaction systems (Cammerer and Kroh, 1995). Some research reported that amino acid/sugar ratio indeed has an effect on the Maillard reaction products. For example, products requiring two moles of amino acid per mole of sugar can be enhanced when excess amino acids are used (Keyhani and Yaylayan, 1996;1997).

1.3.2 The effect of pH on the mechanism of the Maillard reaction

In aqueous systems, the pH interferes with Maillard reaction by influencing the formation of important intermediates and affecting the chemical reactivity of the precursors (reducing sugars and amino acids). Since the various steps in the reaction are acid-base catalyzed, understandably, pH becomes a vital parameter (Martins and Van Boekel, 2005). Furthermore, the open chain concentration of the sugars depends on pH, and the active form of the amino acid whether protonated or un-protonated is also dependent on pH (Van Boekel, 2001).

The pH exerts a significant effect on the Maillard reaction after the formation of the Amadori compound. At that point it determines the degree at which the degradation of

Amadori compounds proceed by either 1,2- (low pH) or 2,3- (higher pH) enolisations (Nursten, 2005). In addition, the major degradation pathways of the Amadori compound, namely enolisation and retro-aldolisation, have been reported to be strongly pH-dependent (Huyghues-Despoints and Yaylayan, 1996;Ledl and Schleicher, 1990b;Smith and Thornalley, 1992).

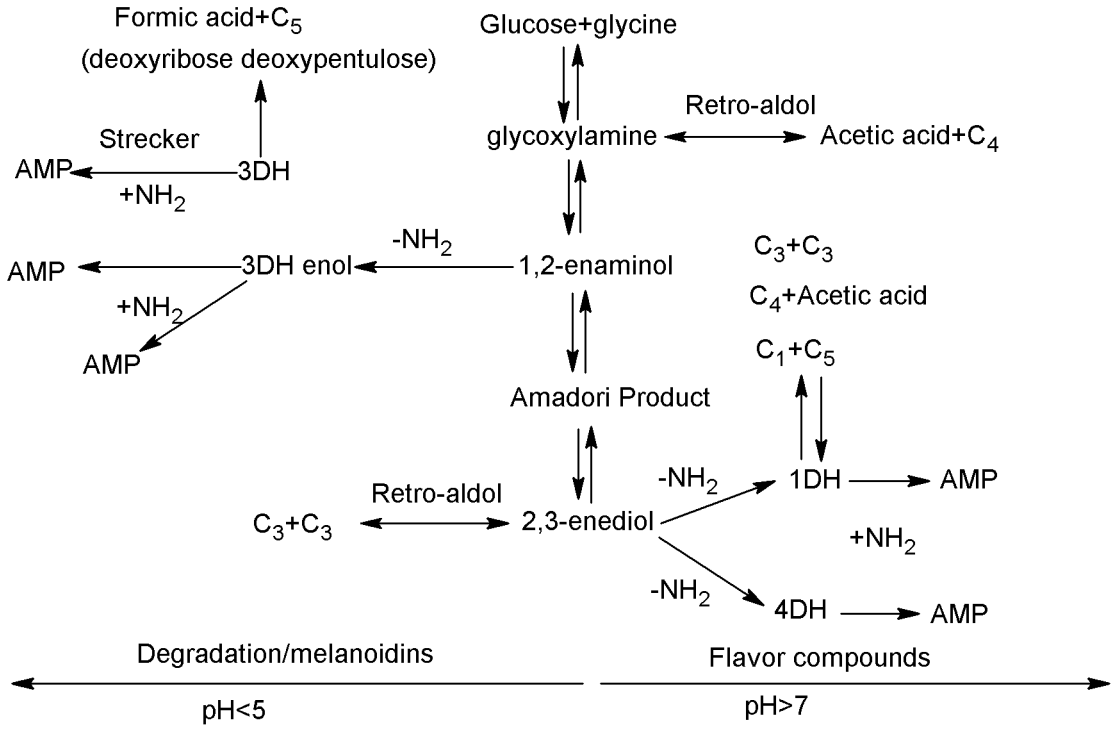


Figure 1.2 Scheme glucose/glycine Maillard reaction adapted from Tressl. et al (1995).(Tressl, et al., 1995)

Additionally, the pH-dependence of the Maillard reaction can also be described by its effect on the protonation of amino acids, and the amount of un-protonated amino group,

which is considered to be the reactive species, obviously this form increases with increasing the pH (Martins and Van Boekel, 2005). That means, at higher pH values, more reactive species are present in the mixture.

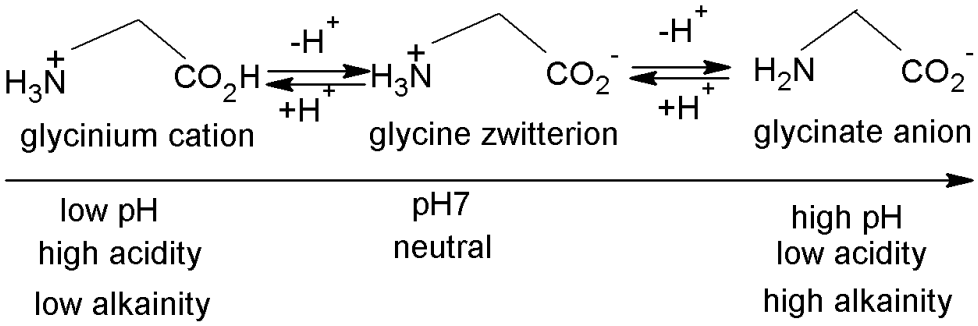


Figure 1.3 Glycine protonation status under different pH

Furthermore, pH has also an effect on the reactant sugar. First, the open chain form of the sugar is recognized to be the reactive species, and the amount of open chain increases with the value of pH (Yaylayan, et al., 1993). Second, the formation of the enediol anion is considered as a key reaction in both reversible and irreversible sugar reactions (De Bruijn, 1986). Reversible sugar reactions are ionization, mutarotation, enolization and isomerisation, and these reactions imply that the sugar carbon skeleton remains intact. Irreversible sugar reactions imply that the sugar moiety is degraded into different fragments. Since ionization is a rate-determining reaction (De Bruijn, 1986), pH obviously has an influence on sugar reactions and hence on the Maillard reaction.

1.3.3 The effect of the sugar type on the mechanism of the Maillard reaction

The sugars that can participate in the Maillard reaction are limited to reducing types. Reducing sugar refers to the sugar which has an open-chain form with a carbonyl group or a free hemiacetal group. Monosaccharides which include an aldehyde group are called aldoses, and those with a ketone group are known as ketoses. Sugars with ketone groups in their open chain form are able to isomerize through a set of tautomeric shifts to form an aldehyde group in solution. Therefore, ketone-bearing sugars like fructose are considered reducing sugars as well. Reducing monosaccharides include glucose, glyceraldehyde and galactose. Many disaccharides, like lactose and maltose, also have a reducing form, as one of the two units may have an open-chain form with an aldehyde group. (Campbell and Farrell, 2012)

The structure of reducing sugar affects both the rate and the end products of Maillard reaction. Reducing sugars with a smaller number of carbon atoms are considered more reactive. Pentoses (e.g. ribose) are more reactive than hexoses (e.g. glucose) which, in turn, react more rapidly than disaccharides (e.g. lactose) (Ames, 1990;Spark, 1969). For example, at 55℃ and pH 6.5 with a shrimp hydrolysate, results demonstrated the prevailing propensity of pentoses over hexoses to react in the Maillard reaction, with a discernable behaviour for the ribose-hydrolysed system (Laroque, et al., 2008). Under

the same solvent-free reaction condition, more than 4 mol ribose were incorporated into the melanoidin per mole of amino acid, while, merely half the number of moles for hexoses were involved (Cammerer and Kroh, 1995). In addition, among the same type of sugar, the location of the carbonyl influences its chemical reactivity. Individual sugars within each of these groups exhibit different reaction rates: in particular, aldoses, such as glucose behaves differently than ketoses, such as fructose (Ames, 1990). Suarez compared the nonenzymatic glycation by glucose with that of fructose, and results indicated that the rate and extent of protein-bound fluorescence generation upon fructation was about 10 times more relative to glycation (Suarez, et al., 1989). Another example is that the reactivities of glucose and fructose with glycine at 1:1 molar ratio, at 60℃ and at pH of 3.5 over long time exposure (280 hours) were compared; The results indicated that fructose underwent browning at a faster rate at the beginning, but it was overtaken by glucose after 80 hours (Reyes, et al., 1982).

There are conflicting reports in the literature regarding the issue of reactivity of sugars, several studies (Kato, et al., 1969;Mauron, 1981;Suarez, et al., 1995;Walton, et al., 1989) support that fructose is more reactive, while other researches (Baxter, 1995;Naranjo, et al., 1998;Spark, 1969) claim that glucose is more reactive. It has also been reported that fructose is more effective than glucose in causing protein

crosslinking and in generating protein-bound Maillard fluorescence (Sakai, et al., 1990;Suarez, et al., 1991;Walton, et al., 1989). The discrepancies in the literature may be associated with the differences in the experimental conditions (Baynes, et al., 1989;Wu, et al., 1990) under which the Maillard reaction were conducted and the methods used to monitor the reaction (Yeboah, et al., 1999). The type of sugar also has an effect on the intermediates and end products formed during the reaction. At the initial stage, glucose produces Amadori compound while fructose results in Heyns compound (Figure 1.4).

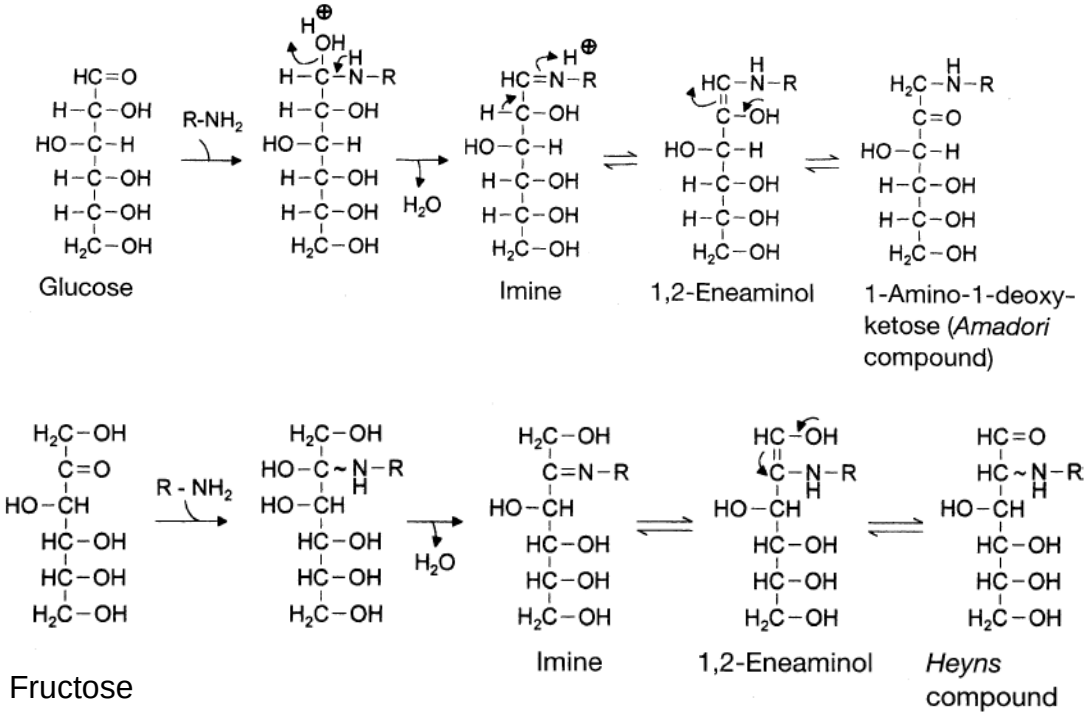


Figure 1.4 Glucose forms Amadori product and Fructose forms Heyns product due to different structures (Fennema, 1996)

After enolization and isomerization, reducing sugars undergo the following fragmentations: retro-aldol cleavage, hydrolytic α-cleavage, oxidative α-cleavage, hydrolytic β-cleavage, and amine-induced β-cleavage (Figure 1.5) and different number of carbon atoms will results in different fragments which will lead to different end products in further Maillard reaction phases (Weenen, 1998).

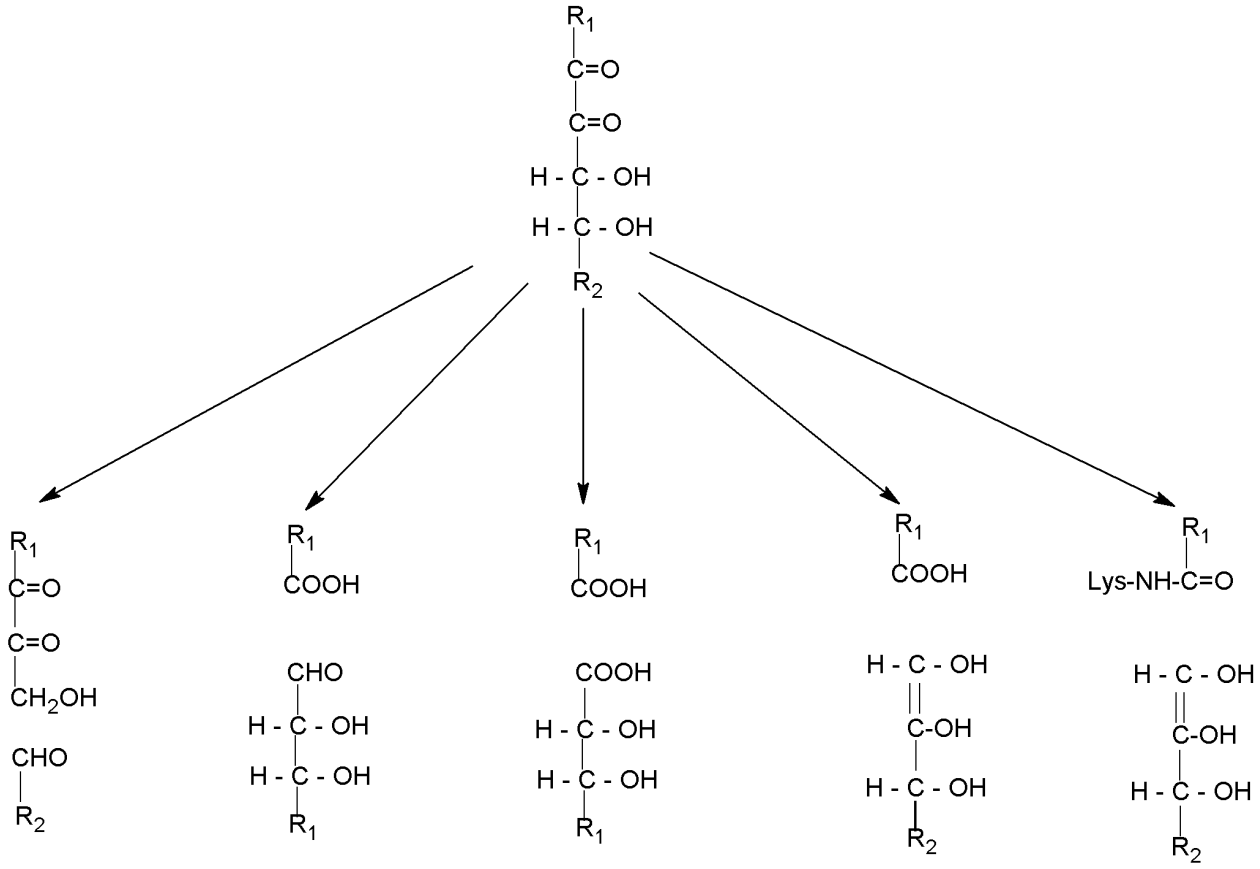


Figure 1.5 Fragmentation pathways of α-dicarbonyl compounds reported in the Maillard literature (Smuda and Glomb, 2013)

When different types of sugars react with the same amino acid, the products are not completely different; they may lead to some common end products. Take furan for instance (Figure 1.6). Hexoses, pentoses and tetroses all can produce the characteristic Maillard reaction product furan.

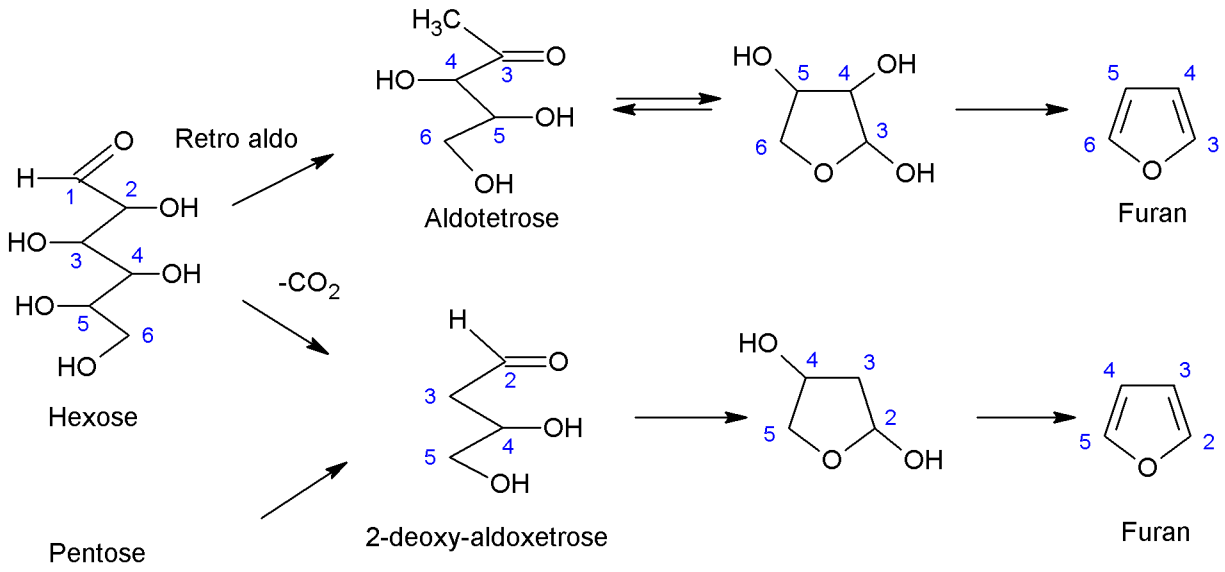


Figure 1.6 Mechanistic pathways of formation of the parent furan from hexoses, pentoses, and tetroses based on labelling studies (Locas and Yaylayan, 2004)

1.4 Experimental approaches to study the Maillard reaction

1.4.1 Model system approach

Model systems refer to the use of pure chemicals to mimic reactions occurring in complex matrices, such as food. Food is a complex reaction matrix, it contains hundreds of components such as various carbohydrates, proteins, lipids, vitamins,

minerals and so forth, this can lead to different and complicated chemical interactions happening at the same time, such as caramelization, lipid oxidation, Maillard reaction and so forth. It is difficult to observe and analyze a specific reaction mechanism under such an intricate background. Compared to the quantity of compounds produced in heated foods, the use of model systems leads to reducing the complexity and number of volatiles, in this way increasing the chances of successful identification (Parliment, 1989). Thus in this study model systems are employed, in which selected chemicals will be used, instead of real food. By using the same precursors and mimicking similar reaction conditions that exists in food systems, we can induce the mechanism of food reactions under laboratory experimental conditions. The current studies of the mechanism of the Maillard reaction are mainly based on the analysis of model systems using various reducing sugars and amino acids as simple models to represent a complex food matrix. Thus a single amino/sugar model system is widely applied in the research of the reaction mechanism since it establishes a direct correlation between the products and the specific amino acid and specific reducing sugar (Keyhani and Yaylayan, 1996).

1.4.2 Py-GC/MS (Pyrolysis/ Gas chromatography/ mass spectrometry)

Pyrolysis can be considered as a thermal extraction method for the analysis of volatiles. The thermal environment not only provides energy for the chemical reactions but also assists in the structural identification of compounds by decomposing large complex molecules into smaller fragments with more analytical utility (Irwin, 1982). It enables samples to undergo derivatization and reactions using minimum amount of reactants, with flexible reaction parameters, and to perform isotope labelling studies (Chu, 2009). Pyrolysis has been employed for rapid determination of the volatiles from whole foods including roasted coffee, chocolate, biscuits, tea and milk powder (Halket and Schulten, 1988) and beef and soy protein (Raghavan, et al., 1986). Pyrolysis has been applied on a variety of amino acid and sugar model systems in characterization reaction products and mechanisms in the Maillard reaction (Huyhgues-Despointes, et al., 1994;Keyhani and Yaylayan, 1996).

The modern Py/GC/MS has become a sophisticated method for polymer characterization (Tsuge, et al., 2012). Py-GC/MS has been proven to be a fast and convenient method for the analysis of Maillard reaction products, especially those from isotopically enriched compounds for mechanistic studies (Keyhani and Yaylayan, 1996).

Py/GC/MS can be used to identify primary and secondary pyrolysis products of the proline Amadori compound with minimum sample preparation and analysis time. Pyrolysis of the proline Amadori compound in the quartz tube at 250°C for 20s is equal to the effect of autoclaving of proline/glucose mixture at 150 °C for 1.5 h in water (Keyhani and Yaylayan, 1996). Quartz tube Py/GC/MS is intentionally designed to perform small scale reactions without isolation or extraction of the reaction mixture, which may result in the loss of valuable isotopically labeled products.

Aqueous incubation model systems, conducted under aerobic or anaerobic conditions, is another modern approache to study the Maillard reaction mechanism, which provides information about mechanistically related compounds and identification of precursor structures (Gobert and Glomb, 2009;Smuda and Glomb, 2011). Microwave technique have also been applied in aqueous system to synthesize and extract selected Maillard reaction products (Yaylayan and Keyhani, 1998).

1.4.3 Isotope labelling technique

Isotope labelling is a technique applied to track the passage of an isotope, or an atom, through a reaction or a metabolic pathway. The reactant is 'labelled' by replacing

specific atoms by their isotopes. Because the labelled atom has the same number of protons as the unlabeled counterpart, it reacts in exactly the same manner and will not change the nature of the reaction. Stable isotope labelling technique can be used to study chemical and biochemical reactions. By using isotope labeled reactants, we can trace the formation pathway of certain characteristic products. For example, from figure 1.7, we can see the formation of pyruvaldehyde, glyceraldehyde and hydroxyacetone from 1-^{13}C-Glucose. The most common stable isotopes used in Maillard reaction studies are ^{13}C, and ^{15}N which allows the unambiguous detection of the number of labelled atoms incorporated in an analyte with MS (Schieberle, 2005). MS instruments separate a particular isotopomer distribution by its molecular weight. All isotopomers of a particular compound are collected in one peak signal. Because all isotopomers contribute to exactly one chromatographic peak, the percentage values can be calculated for each peak from the observed masses. Hence, from the mass spectrum, we can judge for each compound if it contains labeled isotope, how many atoms it has and its possible location.

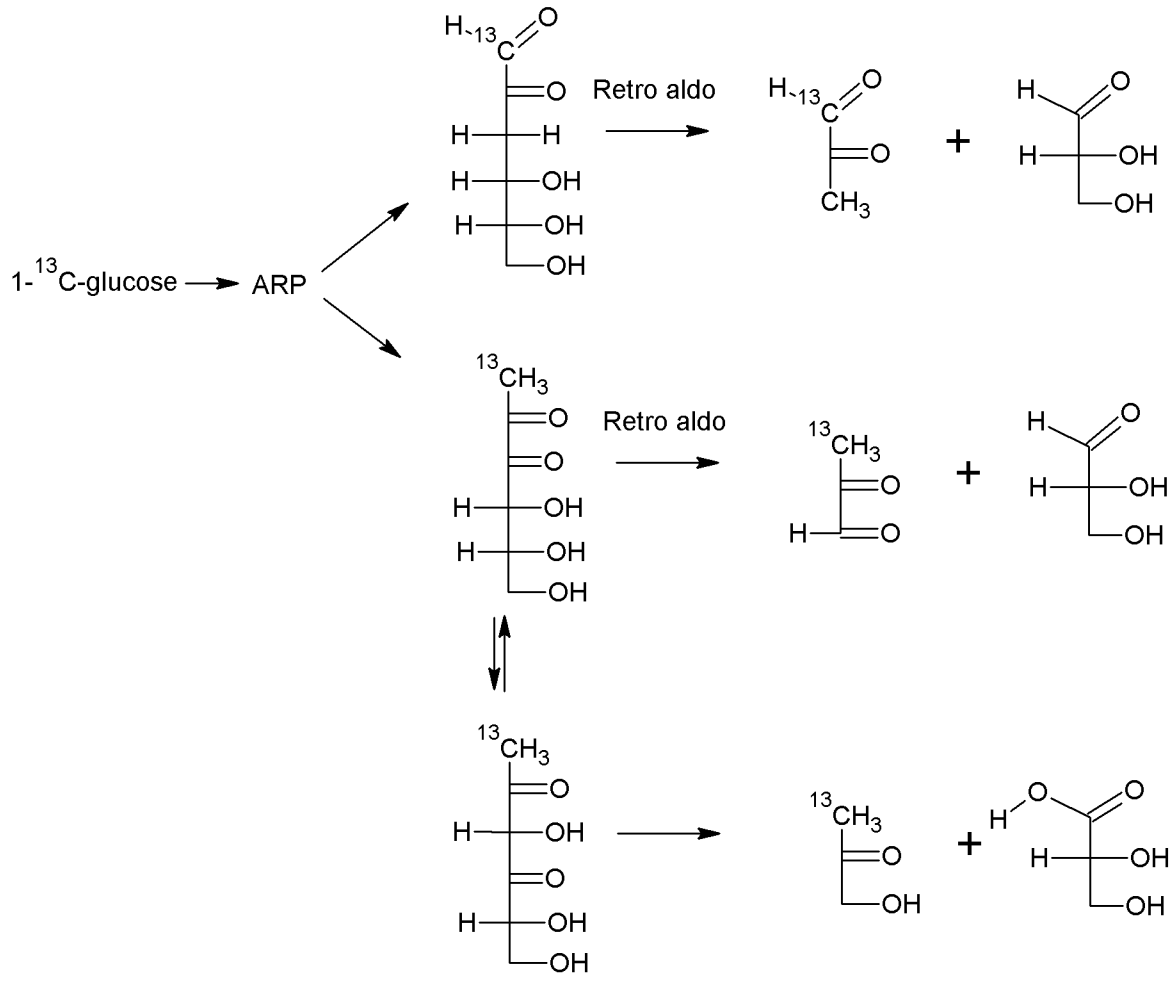


Figure 1.7 Formation of pyruvaldehyde, glyceraldehyde and hydroxyacetone from 1-^{13}C-Glucose (Weenen, 1998)

The application of stable isotopes in the research on the mechanism of Maillard reaction started in the early 1990s. For example, ^{13}C-1 labeled glucose, arabinose and fructose reacted with 4-aminobutyric acid were employed to clarify the intermediates and formation pathways of a set of pyrroles and 2-pyrrolidones (Tressl, et al., 1993). Also, a

sugar and two amino acid model system, where glycine was kept constant while the other amino acid was changed, is used to elucidate the formation of flavour compounds using ^{15}N glycine. In this study the precise contribution that the glycine made to the resulting end products was determined (Hwang, et al., 1995a;b). Labelling studies have also been reported using lysine, which has two amino groups, to differentiate the behaviour of the alpha and epsilon groups (Hwang, et al., 1994). The existence of multiple formation pathways for the carcinogenic compound furan has been elucidated with labelling studies, including a mechanism where the sugar skeleton remained intact and another in which sugar C2 and/or C3 fragments merged together (Limacher, et al., 2008). A particular method is called CAMOLA or Carbon Module Labelling which was designed to rationalize the existence of multiple pathways (Limacher, et al., 2008). In another study isotope labelling method has been used to demonstrate the existence of five different mechanisms resulting in the formation of 2,3-pentanedione from glucose/alanine models (Yaylayan and Keyhani, 1999).

Chapter 2

Hypothesis and Objectives

Maillard reaction refers to the non-enzymatic interaction of reducing sugars with amino acids. According to current literatures, there are many environmental factors that can affect this reaction however, there are no studies on the effect of amino acid salts on the Maillard reaction; and also there is no systematic study on the effect of sugar type and amino acid/sugar ratio on the profile of the Maillard reaction. Thus, the research objective is to systematically investigate the effect of glucose vs fructose, amino acid salts and their ratios on the modification of the profile of the Maillard reaction. Glycine, glucose and fructose were chosen as representative amino acid and sugars. Glycine being the smallest amino acid reduces the complexity of reaction products profile and requires less labeled reactants (C-1, C-2 and N-labelled only). Glucose and fructose are the most common reducing sugars in foods. Pyrolysis can be considered to mimic heating foods under high temperature conditions. The advantage over other methods is that pyrolysis required small amounts of expensive labelled starting materials and can complete the reaction within much shorter period of time (20 seconds). Through linking pyrolysis to GC/MS the reaction products can be collected, separated and identified

under experiment conditions, and the use of isotope labelling technique can enable the characterization of the formation pathways.

This general objective is divided into four specific ones:

1. Effect of glucose and fructose, glycine salts and their ratios on the total abundance and total number of components of the Maillard reaction.

2. Effect of amino acid/sugar ratios on the changes in the profile of the Maillard reaction.

3. Effect of amino acid salts on the on the changes in the profile of the Maillard reaction.

4. Effect of glucose and fructose on the changes in the profile of the Maillard reaction.

(Changes in the profile mentioned above refer to new or specific peak formation and intensity changes of common peaks.)

Chapter 3

Materials and Methods

3.1 Reagents and chemicals

D-glucose (99%) was purchased from BDH (Toronto, Ontario). D-fructose (>99%), glycine (98%), glycine hydrochloride (>99%), sodium glycinate (98%), Potassium hydroxide Kaliumhydroxid (KOH) (>85%), Oxalic acid (>99%) and [^{15}N] glycine were purchased from Sigma-Aldrich (St. Louis, MO). D- [U-^{13}C6] glucose (99%), [1-^{13}C] glycine (99%), and [2-^{13}C] glycine (99%) were purchased from Cambridge Isotope Laboratories (Andover, MA). D- [U-^{13}C6] fructose was purchased from Omicron Biochemicals (South bend, IN). All chemicals were used without further purification.

3.2 Experimental procedures

Py-GC/MS was used as an integrated reaction, separation and identification system. Amino acid (or their salts) and reducing sugars were mixed and homogenized according to different molar ratios shown in Table 3.1. Sample mixtures (0.6 mg) were introduced into a quartz tube plugged with quartz wool on both sides. The tubes were inserted into the pyroprobe, pyrolyzed at 250℃ and analyzed by GC/MS.

Table 3.1 Model systems analyzed by Py-GC/MS[a]

Model system	Amino acid/sugar ratio
Glucose alone	-
Fructose alone	-
Glycine+ D-glucose	1:1, 1:2, 2:1
Glycine ǂHCl + D-glucose	1:1, 1:2, 2:1
Glycine ǂNa + D-glucose	1:1, 1:2, 2:1
Glycine ǂNa + Glycine ǂHCl+ D-glucose	1:1, 1:2, 2:1
Glycine+ D- [U-^{13}C6]glucose	1:1, 1:2
[1-^{13}C]Glycine+ D-glucose	1:1, 1:2
[2-^{13}C]Glycine+ D-glucose	1:1, 1:2
[^{15}N] Glycine+ D-glucose	1:1, 1:2
Glycine+ D-[U-^{13}C6]glucose+ Oxalic acid	1:1:1
[1-^{13}C]Glycine+ D-glucose+ Oxalic acid	1:1:1
[2-^{13}C]Glycine+ D-glucose+ Oxalic acid	1:1:1
[^{15}N]Glycine+ D-glucose+ Oxalic acid	1:1:1
Glycine+ D- [U-^{13}C6]glucose+ KOH	1:1:1
[1-^{13}C]Glycine+ D-glucose+ KOH	1:1:1
[2-^{13}C]Glycine+ D-glucose+ KOH	1:1:1
[^{15}N] Glycine+ D-glucose+ KOH	1:1:1

Glycine + D-Fructose	1:1, 1:2, 2:1
Glycine·HCl + D-Fructose	1:1, 1:2, 2:1
Glycine·Na + D-Fructose	1:1, 1:2, 2:1
Glycine+ D-[U-^{13}C6]Fructose	1:1
[1-^{13}C]Glycine + D-Fructose	1:1
[2-^{13}C]Glycine + D-Fructose	1:1
[^{15}N]Glycine + D-Fructose	1:1

[a] Each model system had two replicates and results were reported as an average value

3.3 Pyrolysis/ Gas chromatography/ Mass spectrometry

Experiments were performed on a Varian CP-3800 GC coupled with a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA). The pyrolysis unit which included a CDS Pyroprobe 2000 and a CDS 1500 valved interface (CDS Analytical, Oxford, PA) is installed onto the GC injection port. The quartz tube with 0.6 mg sample was inserted inside the coil probe, and pyrolyzed for 20 s at 250 °C. The sample separation was carried out on a DB-5MS (5% diphenyl, 95% dimethyl polysiloxane) capillary column with dimensions of 50 m length by 0.2 mm internal diameter and 0.33 μm film thickness (J&W Scientific, ON, Canada), using helium as the carrier gas. The GC column flow rate was regulated by an electronic flow controller (EFC) and set at a delayed (30s) pressure

pulse of 70 psi for the first 4 min and later maintained with a constant flow of 1.5 mL/min for the remainder of the run. The GC oven temperature was set at −5 °C for 5 min using CO_2 as the cryogenic cooling source. The temperature was increased to 50 °C at a rate of 50 °C/min and then to 270 °C at a rate of 8 °C/min, and kept at 270 °C for 5 min. The samples were detected by using an ion trap mass spectrometer with a scan range of m/z 20–650. The MS transfer line temperature was set at 250 °C, the manifold temperature was set at 50 °C, and the ion trap temperature was set at 175 °C. The ionization voltage of 70 eV was used, and EMV was set at 1500 V. (Gueera and Yaylayan, 2013)

3.4 Data analysis

The tentative structures of various products were confirmed either by comparison of their MS data to those published in the literature or by searching the AMDIS (v. 2.65) and NIST Standard Reference Databases (data v. NIST-5 and software). The purity, total abundance and the number of the chromatographic peaks were determined using NIST AMDIS version 2.1 software. The reported percent label incorporation values (corrected for natural abundance and for percent enrichment) are the average of duplicate analyses and are rounded off to the nearest multiple of 5%. Abundance values and total components were reported as average of two measurements.

Chapter 4

Effect of glycine/sugar ratios, amino acid salts and sugar type on the general profile of the Maillard reaction

4.1 Introduction

Based on the objective of this research which attempts to investigate the factors affecting the changes in the Maillard reaction profile, this chapter will first discuss how these factors (ratio, salts and sugar type) will affect the general profile (total abundance and total number of components of GC chromatograms) of the different model systems.

4.2 Materials and Methods

As described in chapter 3.

4.3 Results and discussion

4.3.1 Comparison of total abundances of glycine/glucose model systems

The table 4.1 displays the total abundance values of nine model systems studied with different glycine/glucose ratios and amino acid salts. The values are reported relative to the model system containing only glucose which generated an abundance value of 46774, which was converted into 1.4×10^{10} abundance value per molar of glucose and then normalized as 1. The total abundances of these models varied from a three-fold increase to ten-fold increase relative to glucose alone, with glycine

hydrochloride/glucose model (ratio 1:1 and 2:1) yielding the highest intensities among all the models studied.

Table 4.1 Effect of glycine/glucose ratios and amino acid salts on the relative increase in total abundance [a] per mole of glucose

	1:1	1:2	2:1
Free Glycine	5x	7x	4x
Glycine·HCl	10x	9x	10x
Na·Glycine	3x	5x	3x

[a] Relative to the abundance of model system containing only glucose (1x)

In case of free glycine, ratio 1:2 generated the most abundance in its category; for glycine hydrochloride, ratio 1:2 produced slightly less than other two ratios; for sodium glycinate, again the ratio 1:2 yielded more abundance than other two models. Thus, for free glycine, glycine hydrochloride or sodium glycinate, ratio 1:2 is the best parameter to result in most abundance. Comparing the efficiencies of different forms of the amino acid in generating Maillard reaction products, it seemed that glycine hydrochloride resulted in most abundance in all the three ratios studied indicating that glycine-glucose reaction is more sensitive to acid environment.

4.3.2 Comparison of total number of components of glycine/glucose model systems

The table 4.2 displays the total number of components in nine model systems studied with different glycine/glucose ratios and amino acid salts. The values are reported relative to the model system containing only glucose which generated a total of 187 peaks, which was normalized as 1. The total number of peaks of these models varied from no change when compared to glucose alone to seven-fold increase when sodium glycinate/glucose model (ratio 1:2) was used.

Table 4.2 Effect of glycine/glucose ratios and amino acid salts on the relative increase in total number of peaks [a]

	1:1	1:2	2:1
Free Glycine	3x	3x	5x
Glycine·HCl	1x	2x	1x
Glycine·Na	1x	7x	1x

[a] Relative to the total peaks of model system containing only glucose (1x)

The efficiency of three glycine/sugar ratios to produce the number of Maillard reaction products was compared. In case of free glycine models, ratio 2:1 produced higher number of components than other two models; glycine hydrochloride and sodium

glycinate, ratio 1:2 generated more components than the other two ratios. The amino acid salts have an effect on the total number of components. Ratio 1:1 and 2:1, free glycine/sugar yielded much more components than glycine hydrochloride/glucose or sodium glycinate/glucose; while, at ratios 2:1 sodium glycinate produced 7 times more components as the other two forms of glycine.

4.3.3 Comparison of total abundances of glycine/fructose model systems

The table 4.3 displays the total abundance values of nine model systems studied with different glycine/fructose ratios and amino acid salts. The values are reported relative to the model system containing only glucose which generated an abundance value of 46774, which was converted in to 1.4×10^{10} intensity units per molar of glucose and then normalized to 1. The total abundances of these models varied from a two fold increase to twenty fold increase relative to glucose alone, with glycine hydrochloride/fructose model (ratio 2:1) yielding the highest intensity among all the models studied.

Table 4.3 Effect of glycine/fructose ratios and amino acid salts on the relative increase in total abundance [a] per mole of glucose

	1:1	1:2	2:1
Free Glycine	8x	8x	8x
Glycine·HCl	12x	7x	**20x**
Glycine·Na	4x	6x	2x
Fructose	6x		

[a] Relative to the abundance of model system containing only glucose (1x)

Glycine/fructose ratios had no effect on the total abundance of free glycine/fructose models, but influenced glycine hydrochloride/fructose and sodium glycinate models; glycine hydrochloride, ratio 2:1 yielded more abundance than other two ratios; and sodium glycinate, ratio 1:2 generated most intensity.

The efficiency of three forms of glycine to generate more intense Maillard reaction products was compared. Ratio 1:1, glycine hydrochloride produced much more abundance than free glycine and sodium glycinate; ratio 1:2 of free glycine yielded slightly more intensity than other two models; ratio 2:1 of glycine hydrochloride generated more than twice the abundance of the other two models. Thus, generally

speaking, glycine hydrochloride resulted in the highest intensity of products while sodium glycinate the least.

4.3.4 Comparison of total peaks of glycine/fructose model systems

The table 4.4 displays the total number of peaks in nine model systems studied with different glycine/fructose ratios and amino acid salts. The values are reported relative to the model system containing only glucose which generated a total of 187 peaks which was normalized as 1. The total number of peaks of these models varied from two-fold increase to fifteen-fold increase relative to glucose alone, with sodium glycinate/fructose model (ratio 1:2) yielding predominantly the highest number of peaks among all the models studied.

Table 4.4 Effect of glycine/glucose ratios and amino acid salts on the relative increase in total peaks [a]

	1:1	1:2	2:1
Free Glycine	6x	5x	4x
Glycine·HCl	4x	3x	4x
glycine·Na	2x	15x	2x
Fructose	2x		

[a] Relative to the total peaks of model system containing only glucose (1x)

The glycine/sugar ratios have an effect on the total components of Maillard reaction products. The three ratios of free glycine model system generated similar number of components with ratio 1:1 produced slightly; glycine hydrochloride, ratio 1:2 generated slightly less than the other two models; sodium glycinate, ratio 1:2 produce more than 7 times than other two ratios.

The efficiency of three forms of glycine to yield components of Maillard reaction products was compared. Ratio 1:1 of free glycine yielded much higher number of peaks than glycine hydrochloride and sodium glycinate; ratio 1:2 of sodium glycinate produced predominantly more components than the others; ratio 2:1 of free glycine and glycine hydrochloride generated twice as many components as that of sodium glycinate.

4.3.5 Comparison between glycine/glucose and glycine/fructose model systems

Because of the difference in the locations of carbonyl functionality, glucose and fructose didn't act exactly in the same manner in generating the Maillard reaction products. From tables 4.1 and 4.3, we observed that the abundance of fructose was 6 times as much as that of glucose, and in most model systems, fructose generated more abundance values than glucose, and especially glycine hydrochloride at ratio 2:1, fructose yielded twice as much abundance as that of glucose. From tables 4.2 and 4.4, it could be seen

that fructose produced twice as many components as glucose, and in most model systems, fructose generated more components than glucose. From table 4.1 and table 4.2, we can conclude that glycine/glucose model systems generated more abundance values and number of components than glucose alone. However, glycine/fructose model systems didn't necessarily yield more abundance or components than fructose alone.

4.4 Conclusion

Total abundance and total number of components generated by GC chromatograms were selected as parameters to describe the general profile of Maillard reaction, and results indicated that there was no necessary connection between these two properties. Both sugars with glycine hydrochloride caused the generation of high abundance values. For glucose, glycine hydrochloride at ratio 1:2 yielded most abundance among all models; and for fructose the ratio 2:1 generated the most. For both sugars, sodium glycinate at ratio 1:2 generated highest number of components. Generally, fructose produced more abundance values and higher number of components than glucose.

Chapter 5

Investigation of the role of amino acid/sugar ratios on the generation of specific Maillard reaction products

5.1 Introduction

This chapter mainly focuses on the effect of amino acid/sugar ratio on the profile of Maillard reaction products. Three amino acid/sugar ratios; 1:1, 1:2 and 2:1, will be analyzed and compared. Two terms will be used to describe the changes in the profile of the products. One is “specific peak”, meaning that these peaks can only be found in one specific amino acid/sugar ratio and cannot be formed in other two ratios under the same reaction conditions. Another is significant intensity changes of common peaks. The same peak may appear in all these three ratios, but in different intensities in each ratio. By monitoring these two properties, we can describe how changes in amino acid/sugar ratio can affect the reaction profile.

5.2 Methods and Materials

As described in chapter 3.

5.3 Results and discussion

5.3.1 The effect of glycine/ glucose ratios on the formation of specific peaks

Specific peaks are peaks that are observed only at one ratio of sugar amino acid mixtures and whose intensity exceeds 10% of total abundance of the model system. Peaks that are specifically formed at one ratio with abundances less than 10% are greyed out in Table 5.1 below.

Table 5.1 General chromatographic properties of specific peaks formed under different glycine/sugar ratios

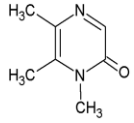
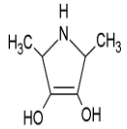
Ratio	Free Glycine			Glycine·HCl			Glycine·Na		
	RT(min)	Intensity	%[a]	RT(min)	Intensity	%[a]	RT(min)	Intensity	%[a]
1:1	16.605	5,419	3%	No specific peaks were detected					
	17.684	1,141	1%						
	25.211	1,853	1%						
1:2	**20.931**	**33,895**	**12%**				14.550	5515	3%
							15.431	7232	4%
							18.578	3528	2%
2:1	17.258	1,208	1%				**16.658**	**71572**	**56%**
	19.304	1,518	1%						
	21.829	3,163	2%						
	25.318	**42,653**	**26%**						
	25.436	8,579	5%						
	26.464	10,523	6%						
	31.572	9,072	6%						
	31.615	4,802	3%						

[a] Percentage of specific peak intensity upon the whole chromatogram abundance

The table 5.1 above lists the retention time, intensity and relative intensity of each specific peak. Free glycine, ratio 1:1 yielded three minor specific peaks in the middle of the chromatogram, ratio 1:2 generated one major specific peak around 21min, and ratio 2:1 produced eight new peaks in the later part of the chromatogram. No specific peaks were detected using glycine hydrochloride /glucose model. Sodium glycinate, ratio 1:1 didn't yield any new peaks, ratio 1:2 produced three minor specific peaks in the middle of the chromatogram, and ratio 2:1 generated one major specific peak with high relative intensity (56%) around 16 min. Comparing the number of specific peaks under three models we can observe that free glycine/glucose model generated the highest number of specific peaks, and sodium glycinate/glucose model yielded less, and glycine hydrochloride /glucose didn't produce any specific peaks.

5.3.2 Structural information on the specific peaks

Table 5.2 Proposed structures and isotope incorporation patterns of specific peaks observed under different glycine/glucose ratios

Model	RT(min)	structure	U6[a]	C-1[b]	C-2[c]	N[d]
Free Glycine 1:2	20.931	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0
Free Glycine 2:1	25.318	 1,5,6-trimethylpyrazin-2(1*H*)-one	+3	+1	+3	+2
Glycine·Na 2:1	16.658	 2,5-dimethyl-2,5-dihydro-1*H*-pyrrole-3,4-diol	+6	0	0	+1

[a] All glucose carbons labelled, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

To rationalize why some compounds were mainly generated under specific amino acid/sugar ratio, a few significant peaks were selected and their structures were proposed based on mass spectral library data search or published literature. Obtained structures were verified by the isotope labelling information.

Regarding free glycine/glucose model, ratio 1:2 generated one significant specific peak, 5-(hydroxymethyl) furan-2-carbaldehyde (HMF). HMF is a well-known sugar

degradation product and its formation pathway is shown in Figure 5.1 based on (Putten, et al., 2013). Due to excess glucose in glycine/glucose model system (1:2) it was not surprising to find high intensity peak of HMF. Model systems with ratios 1:1 or 2:1 contained less glucose, the small amount of generated HMF degraded or reacted with other amino compounds thus generating less intense peaks.

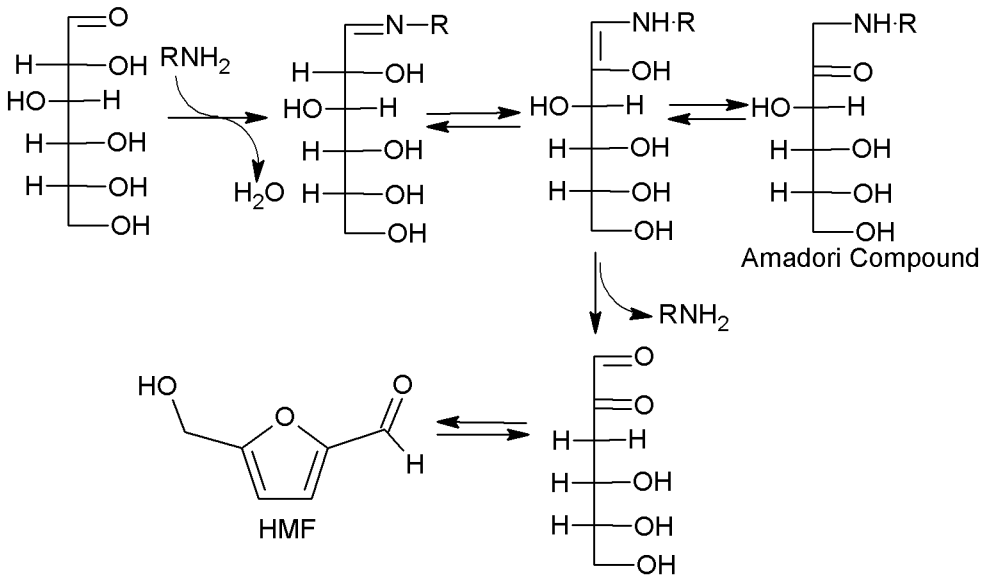


Figure 5.1 Amadori rearrangements and the subsequent formation of HMF (Putten, et al., 2013)

Glycine/glucose 2:1 yielded one major specific peak characterized as 1,5,6-trimethylpyrazin-2 (1H)-one, which didn't appear in the chromatograms of either ratio

1:1 or 1:2. Figure 5.2 shows the proposed formation pathway based on literature data (Keyhani and Yaylayan, 1996).

According to the mechanism proposed in the literature (Keyhani and Yaylayan, 1996), the formation of this product requires excess glycine as shown in Figure 5.2. Thus, 1,5,6-trimethylpyrazin-2 (1H)-one only appeared in glycine/glucose 2:1 model system where glycine is twice as much as glucose.

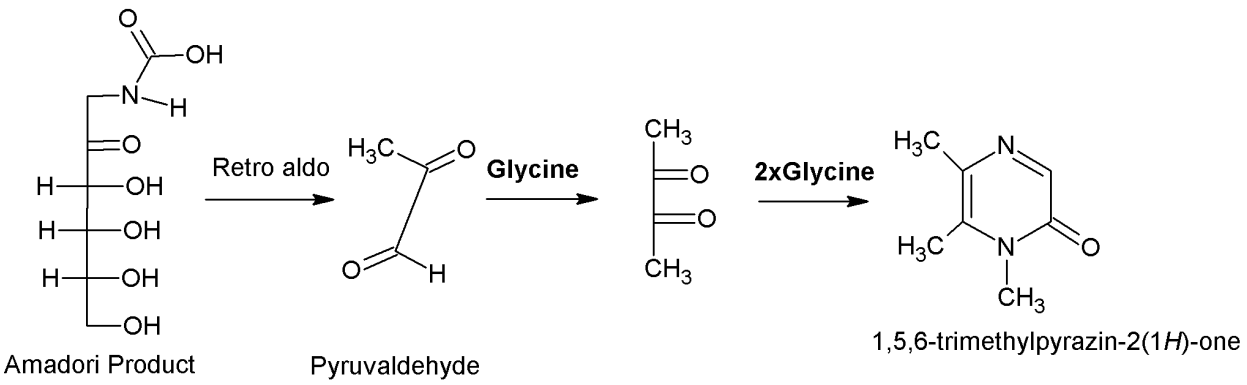


Figure 5.2 Formation of 1,5,6-trimethylpyrazin-2(1H)-one from Amadori product (Keyhani and Yaylayan, 1996)

Sodium glycinate/glucose 2:1 model generated a significant peak with high intensity at retention time of 16.65 min that was tentatively assigned to 2,5-dimethyl-2,5-dihydro-

1H-pyrrole-3,4-diol structure based and the molecular weight and on the isotope incorporation data which indicated the presence of six carbon atoms all originating from glucose and one nitrogen atom from glycine. Figure 5.3 shows the proposed formation pathway of this compound starting from a well-known intermediate 1-deoxy-glucoson which undergoes Strecker reaction after a dehydration step and followed by cyclization to generate a six carbon structure with a single nitrogen atom.

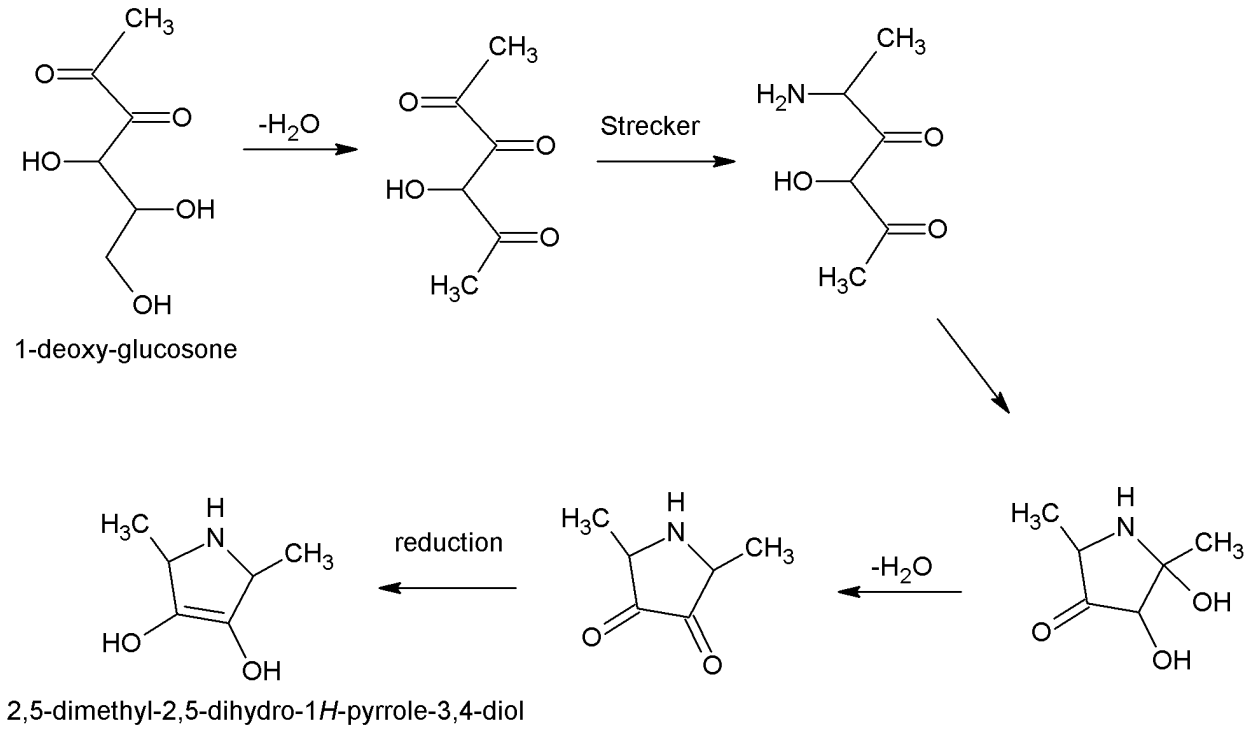


Figure 5.3 Proposed formation pathway of 2,5-dimethyl-2,5-dihydro-1H-pyrrole-3,4-diol

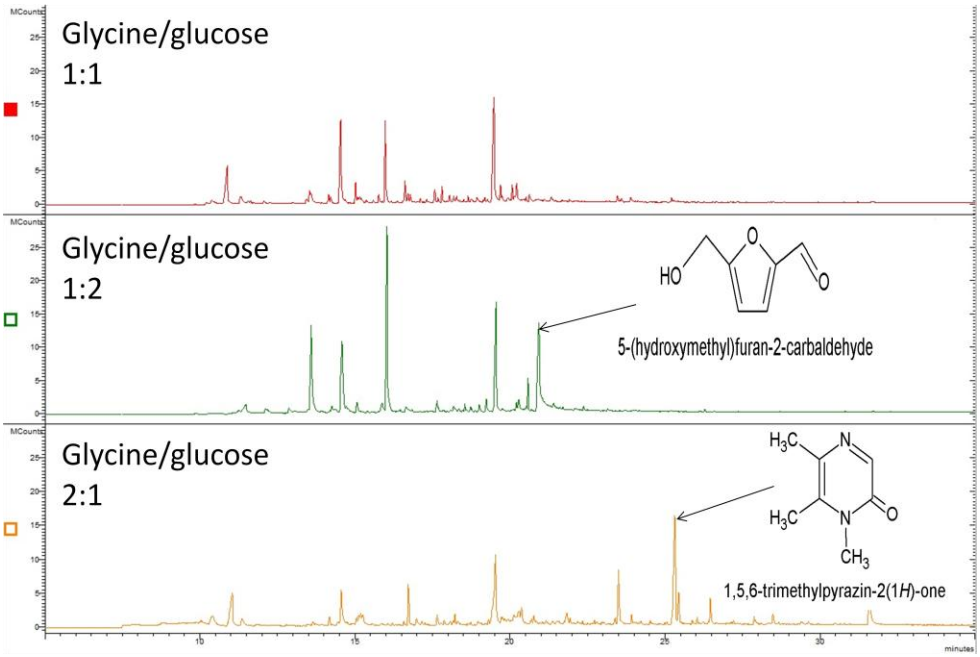


Figure 5.4 Chromatograms with proposed structures of selected specific peaks at glycine/glucose ratios 1:1, 1:2, and 2:1

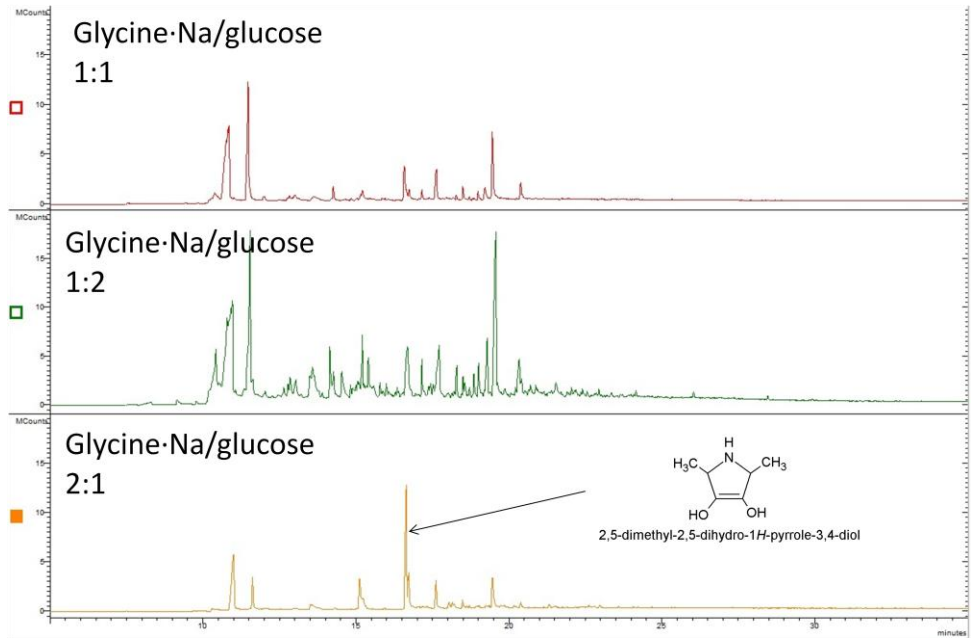


Figure 5.5 Chromatograms with proposed structures of selected specific peaks at glycine·Na/glucose ratios 1:1, 1:2, and 2:1

5.3.3 The effect of amino acid/sugar ratios on the changes in the intensity of the common peaks

Common peaks that underwent significant changes in intensity are peaks that were observed in all the three ratios of sugar amino acid mixtures but with intensity changes exceeding 10%. Common peaks that underwent changes in less than 10% are greyed out in Table 5.3 below.

From table 5.3, we can easily observe the extent to which the intensity of each compound has changed. The first peak of the first column for example, was produced by all the three ratios of glycine/glucose (1:1, 1:2 and 2:1), but the intensity changed from 2% to 19% to 0% respectively. In general, free glycine/glucose models produced most peaks with intensity changes compared to models with amino acid salts.

Table 5.3 Common peaks that underwent significant changes in intensity under different glycine/sugar ratios

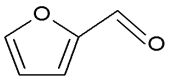
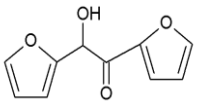
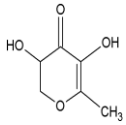
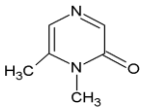
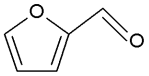
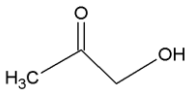
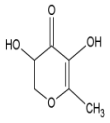
Ratio		Free Glycine				Glycine . HCl				Glycine . Na		
		RT(min)	Intensity	%[a]		RT(min)	Intensity	%[a]		RT(min)	Intensity	%[a]
1:1	1	13.541	3,785	2%	1	13.546	11,727	4%	1	11.484	61,798	50%
	2	14.537	36,422	23%	2	19.56	1,127	0%	2	15.155	1,507	1%
	3	15.018	8,454	5%	3	19.983	6,935	2%	3	16.581	11,324	9%
	4	15.975	32,321	21%	4	21.565	2,601	1%	4	17.164	1,516	1%
	5	16.707	3,148	2%	5	26.301	2,276	1%	5	19.477	19,397	16%
	6	17.805	5,835	4%								
	7	19.49	83,450	53%								
	8	23.45	1,630	1%								
1:2	1	13.581	54,319	19%	1	13.556	44,192	13%	1	11.536	73,715	41%
	2	14.575	28,523	10%	2	19.52	2,509	1%	2	0	0	0
	3	15.059	4,096	1%	3	19.968	13,701	4%	3	16.688	14,815	8%
	4	16.035	106,549	38%	4	21.525	4,333	1%	4	17.174	7,147	4%
	5	0	0	0	5	26.268	1,309	0%	5	19.573	97,996	55%
	6	0	0	0								
	7	19.555	98,169	35%								
	8	0	0	0								
2:1	1	0	0	0	1	13.556	5,239	2%	1	11.631	13,620	11%
	2	14.555	9,931	6%	3	0	0	0	2	15.131	9,333	7%
	3	15.053	3,271	2%	4	0	0	0	3	0	0	0
	4	0	0	0	5	0	0	0	4	0	0	0
	5	16.721	17,549	11%	6	0	0	0	5	19.465	5,719	5%
	6	17.875	1,767	1%		0	0	0				
	7	19.525	46,486	29%								
	8	23.509	18,342	11%								

[a] Percent of total intensity

5.3.4 Structural information on common peaks that underwent significant changes in intensity

Table 5.4 shows proposed NIST library structures of common peaks that underwent significant intensity changes under different glycine/glucose ratios and these structures are also consistent with the labelling information. Figures 5.6, 5.7 and 5.8 show the appropriate chromatograms.

Table 5.4 Proposed structures and isotope incorporation patterns of common compounds undergoing significant changes in intensity under different glycine/glucose ratios

Amino acid (or salts)	Percentage			structure	Labelling information			
	1:1	1:2	2:1		U6[a]	C-1[b]	C-2[c]	N[d]
Free Glycine	2%	19%	0%	 furan-2-carbaldehyde	+5	0	0	0
Free Glycine	23%	10%	6%	 1,2-di(furan-2-yl)-2-hydroxyethanone	+10	0	0	0
Free Glycine	21%	38%	0%	 (5-methylfuran-2-yl)methanol	+6	0	0	0
Free Glycine	53%	35%	29%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0
Free Glycine	1%	0%	11%	 1,6-dimethylpyrazin-2(1*H*)-one	+2	+1	+2	+2
Glycine . HCl	4%	13%	2%	 furan-2-carbaldehyde	+5	0	0	0
Glycine . Na	50%	41%	11%	 1-hydroxypropan-2-one	+3	0	0	0
Glycine . Na	16%	55%	5%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0

[a] All glucose carbons labelled, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

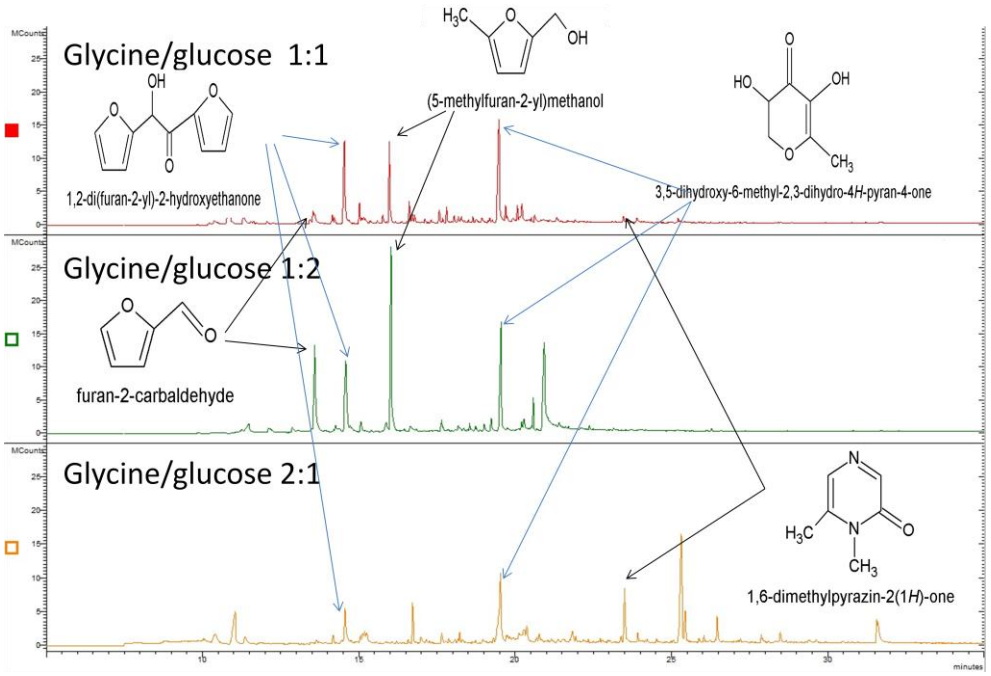


Figure 5.6 Chromatograms with proposed structures of selected peaks of significant intensity alternation at glycine/glucose ratios 1:1, 1:2, and 2:1

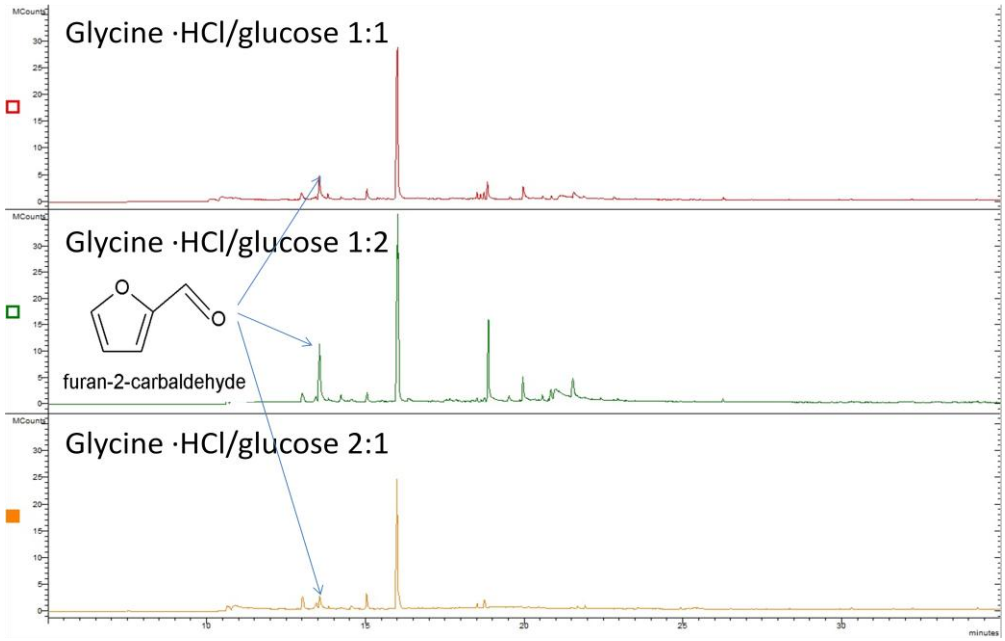


Figure 5.7 Chromatograms with proposed structures of selected peaks of significant intensity alternation at glycine·HCl/glucose ratios 1:1, 1:2, and 2:1

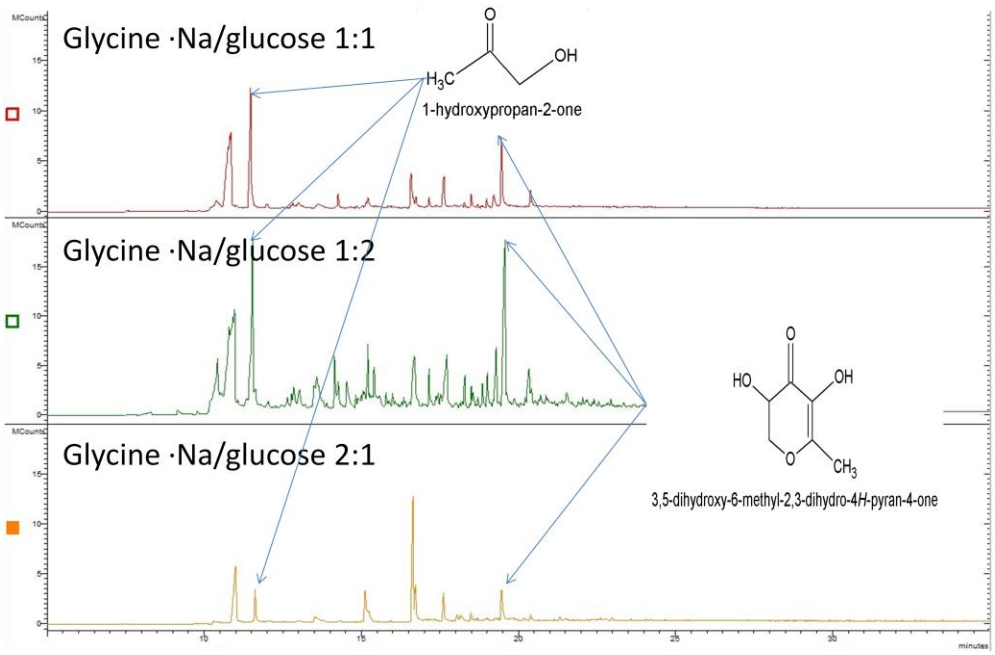


Figure 5.8 Chromatograms with proposed structures of selected peaks that underwent significant intensity changes in glycine·Na/glucose model systems

5.4 Conclusion

Amino acid/sugar ratios indeed have an impact on the generation of specific peaks and on the changes in intensities of common peaks. However, the degree of this influence varies based on the different glycine/glucose models. In general, ratio had most powerful effect on free glycine/glucose models, and less impact on models containing amino acid salts.

Chapter 6

Investigation of the role of sodium and chloride salts of amino acids on the Maillard reaction products

6.1 Introduction

Use of the amino acid salts could be considered another significant factor affecting Maillard reaction profile which has not been investigated yet. Under high temperatures such as 250℃, the hydrochloride salt of amino acids may release HCl gas and provide gas phase acidity in the interface. And the sodium salt of amino acids may melt to give basic environment to the carbonyl-amine reaction. Thus, comparing the products of free glycine to glycine hydrochloride or sodium glycinate may provide information on the changes in the reactivity of glycine and glucose under these altered environmental conditions and changes in the profile of the end products.

6.2 Methods and materials

As described in chapter 3.

6.3 Results and discussion

6.3.1 The effect of sodium and chloride salts of amino acids on the generation of specific peaks

Specific peaks are peaks that are observed only at one ratio of sugar amino acid mixtures and whose intensity exceeds 10% of total abundance of the model system. Peaks that are specifically formed at one ratio with abundances less than 10% are greyed out in Table 6.1 below. Comparing free glycine, glycine hydrochloride and sodium glycinate, results indicated that amino salts change the profile of Maillard reaction end products completely. At ratio 1:1 and 2:1, free glycine/glucose yielded much more specific peaks than other two forms of glycine while at ratio 1:2, sodium glycinate/glucose generated more specific peaks than the other two.

Table 6.1 General chromatographic properties of specific peaks formed under different glycine/sugar ratios using glycine salts

Amino acid form	1:1			1:2			2:1		
	RT (min)	Intensity	%[a]	RT (min)	Intensity	%[a]	RT (min)	Intensity	%[a]
Free Glycine	14.152	2,865	2%	**14.575**	**28,523**	**10%**	11.361	2,268	1%
	14.537	**36,422**	**23%**	20.58	8,220	3%	14.176	3,520	2%
	16.605	5,419	3%				14.554	9,931	6%
	16.795	2,557	2%				17.258	1,208	1%
	17.684	1,141	1%				17.875	1,767	1%
	17.805	5,835	4%				19.304	1,518	1%
	19.691	4,584	3%				21.829	3,163	2%
	20.072	3,541	2%				**23.509**	**18,342**	**11%**
	20.153	1,155	1%				23.924	2,886	2%
	20.218	5,022	3%				**25.317**	**42,653**	**26%**
							25.438	8,579	5%
							26.467	10,523	6%
							27.879	1,934	1%
							31.572	9,072	6%
							31.615	4,802	3%
Glycine . HCl	18.843	6,701	2%	21.532	4,333	1%	**16.003**	**87,784**	**37%**
	19.983	6,935	2%						
Glycine . Na	**11.481**	**61,798**	**50%**	**11.536**	**73,715**	**41%**	**11.631**	**13,620**	**11%**
	14.257	9,717	8%	13.565	12,189	7%	**16.658**	**71,572**	**56%**
	16.581	11,324	9%	14.166	13,634	8%	17.626	5,049	4%
	20.389	9,226	7%	15.233	12,129	7%			

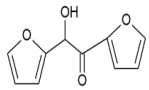
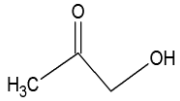
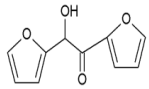
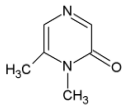
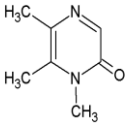
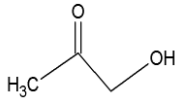
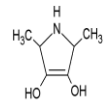
		15.431	7,232	4%	
		16.684	14,815	8%	
		17.174	7,147	4%	
		18.309	6,768	4%	
		18.581	3,528	2%	
		18.886	2,641	1%	
		19.029	7,799	4%	

[a] Percentage of total intensity

6.3.2 Structural information on the specific peaks

Table 6.2 shows all proposed structures of the specific peaks based on the NIST library search and confirmed by isotope labelling information. The following figures 6.1, 6.2 and 6.3 show their corresponding chromatograms.

Table 6.2 Proposed structures and isotope incorporation patterns of specific peaks observed under different amino acid salts

Amino acid salts	RT(min)	structure	U6[a]	C-1[b]	C-2[c]	N[d]
Free Glycine 1:1	14.537	 1,2-di(furan-2-yl)-2-hydroxyethanone	+10	0	0	0
Glycine．Na 1:1	11.481	 1-hydroxypropan-2-one	+3	0	0	0
Glycine．Na 1:2	11.536					
Free Glycine 1:2	14.575	 1,2-di(furan-2-yl)-2-hydroxyethanone	+10	0	0	0
Free Glycine 2:1	23.509	 1,6-dimethylpyrazin-2(1*H*)-one	+2	+1	+3	+2
Free Glycine 2:1	25.317	 1,5,6-trimethylpyrazin-2(1*H*)-one	+3	+1	+3	+2
Glycine．HCl 2:1	16.003	 (5-methylfuran-2-yl)methanol	+6	0	0	0
Glycine．Na 2:1	11.631	 1-hydroxypropan-2-one	+3	0	0	0
Glycine．Na 2:1	16.658	 2,5-dimethyl-2,5-dihydro-1*H*-pyrrole-3,4-diol	+6	0	0	+1

[a] All glucose carbons labelled, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

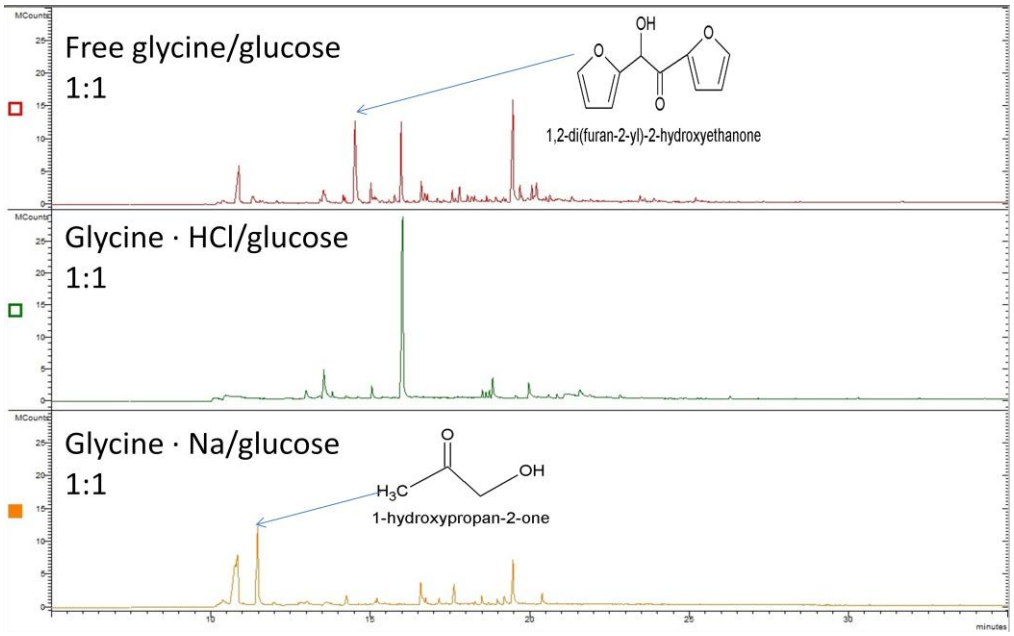


Figure 6.1 Chromatograms with proposed structures of selected specific peaks at amino acid/sugar ratios 1:1

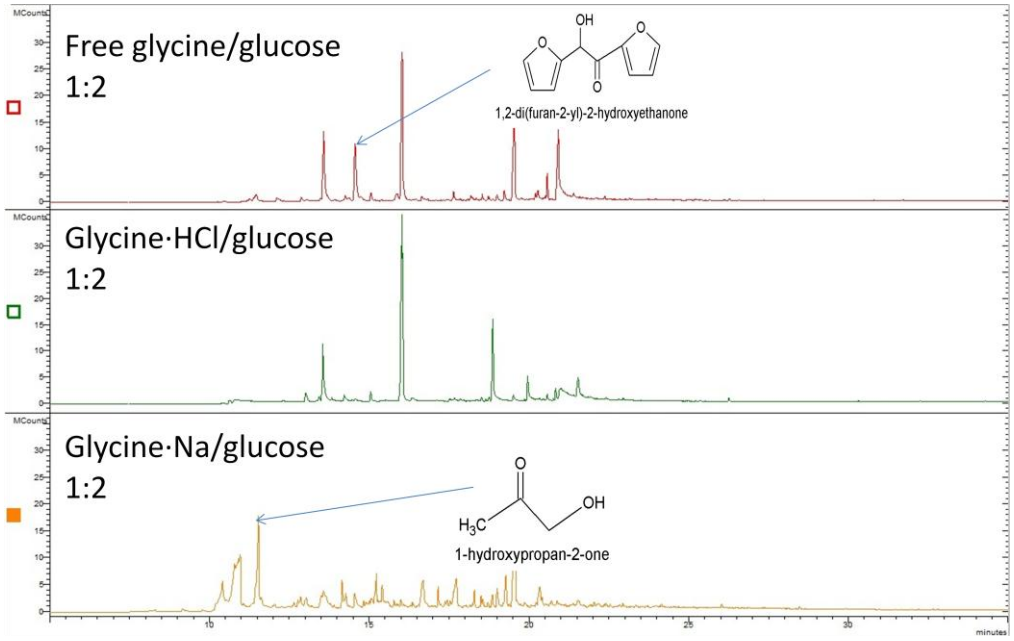


Figure 6.2 Chromatograms with proposed structures of selected specific peaks at amino acid/sugar ratio of 1:2

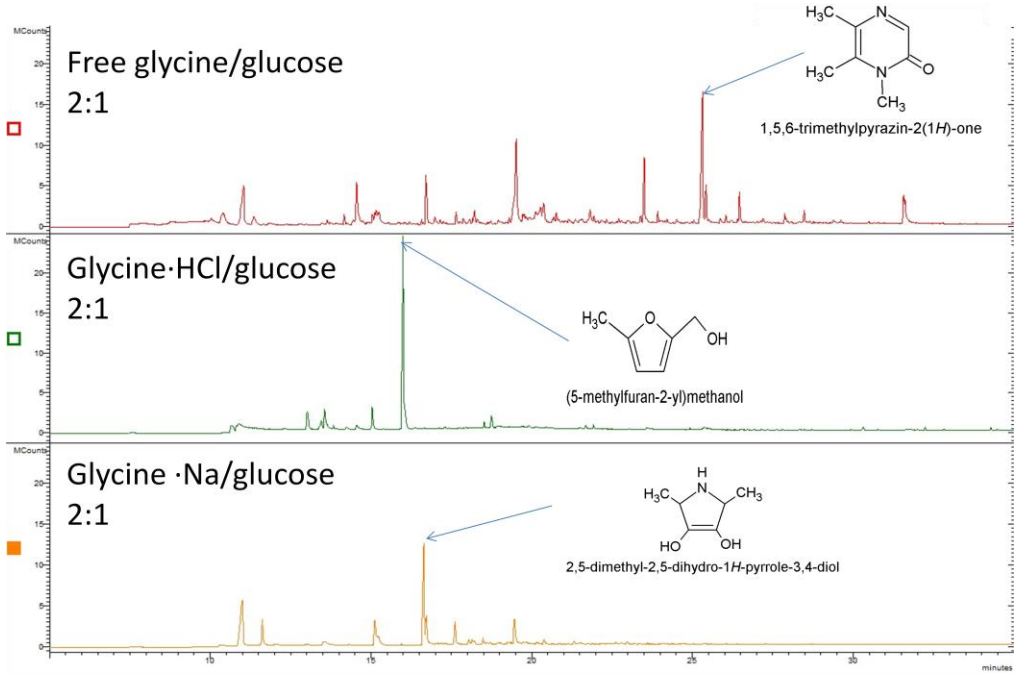


Figure 6.3 Chromatograms with proposed structures of selected specific peaks at amino acid/sugar ratio of 2:1

6.3.3 The effect of sodium and chloride salts of amino acids on the changes in the intensity of the common peaks

Common peaks that underwent significant changes in intensity are peaks that are observed in all three ratios of sugar amino acid mixtures but with intensity changes exceeding 10%. Common peaks that underwent changes in intensity in less than 10% are greyed out in Table 6.3 below. Comparing free glycine, glycine hydrochloride and sodium glycinate, results indicated that amino salts changed the profile of the Maillard reaction end products completely. At ratio 1:2, there were more peaks with intensity changes than at ratio 1:1, and the ratio 1:1 more than in ratio 2:1.

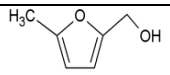
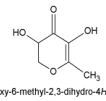
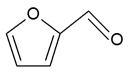
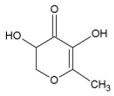
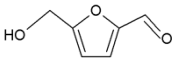
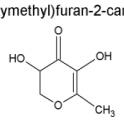
Table 6.3 General chromatographic properties of the common peaks that underwent significant changes in intensity with different amino acid (salts)

Ratio		1:1				1:2				2:1		
		RT(min)	Intensity	%		RT(min)	Intensity	%		RT(min)	Intensity	%
Free Glycine	1	13.541	3,785	2%	**1**	**13.581**	**54,319**	**19%**	1	15.053	3,271	2%
	2	15.018	8,454	5%	2	14.575	28,523	10%	2	15.121	3,386	2%
	3	**15.975**	**32,321**	**21%**	**3**	**16.035**	**106,549**	**38%**	3	16.721	17,549	11%
	4	17.572	3,476	2%	4	17.646	3,610	1%	**4**	**19.529**	**43,878**	**27%**
	5	**19.487**	**83,450**	**53%**	5	19.232	4,807	2%	5	0	0	0
					6	**19.555**	**98,169**	**35%**				
					7	**20.931**	**33,895**	**12%**				
Glycine • HCl	1	13.544	11,727	4%	**1**	**13.556**	**44,192**	**13%**	1	15.052	9,401	4%
	2	15.046	5,665	2%	2	0	0		2	0	0	0
	3	**16.015**	**105,756**	**37%**	**3**	**16.037**	**118,734**	**34%**	3	0	0	0
	4	0	0	0	4	0	0		**4**	**0**	**0**	**0**
	5	**19.56**	**1,127**	**0%**	5	0	0		5	0	0	0
					6	**19.52**	**2,509**	**1%**				
					7	**20.979**	**4,073**	**1%**				
Glycine • Na	1	13.578	1,170	1%	**1**	**0**	**0**	**0**	1	0	0	0
	2	0	0	0	2	14.552	5,515	3%	2	15.131	9,333	7%
	3	**0**	**0**	**0**	**3**	**16.006**	**3,951**	**2%**	3	16.727	8,855	7%
	4	17.632	5,485	4%	4	17.738	14,451	8%	**4**	**19.465**	**5,719**	**5%**
	5	**19.474**	**19,397**	**16%**	5	19.294	13,616	8%				
					6	**19.573**	**97,996**	**55%**				
					7	**0**	**0**	**0**				

6.3.4 Structural information on the common peaks that underwent significant changes in intensity

Table 6.4 shows all proposed structures of the common peaks that underwent significant intensity changes. The structures were based on NIST library searches and were consistent with the isotope labelling information. The following figures 6.4, 6.5 and 6.6 indicated their corresponding chromatograms.

Table 6.4 Proposed structures and isotope incorporation patterns of common compounds undergoing significant changes in intensity under different glycine salts

Ratio	% Relative intensity			structure	Labeling Information			
	Free Glycine	Glycine. HCl	Glycine. Na		U6[a]	C-1[b]	C-2[c]	N[d]
1:1	21%	37%	0%	 (5-methylfuran-2-yl)methanol	+6	0	0	0
1:1	53%	0%	16%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0
1:2	19%	13%	0%	 furan-2-carbaldehyde	+5	0	0	0
1:2	38%	34%	2%	 5-methylfuran-2-carbaldehyde	+6	0	0	0
1:2	35%	1%	55%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0
1:2	12%	1%	0%	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0
2:1	27%	0%	5%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0

[a] All carbons labelled glucose, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

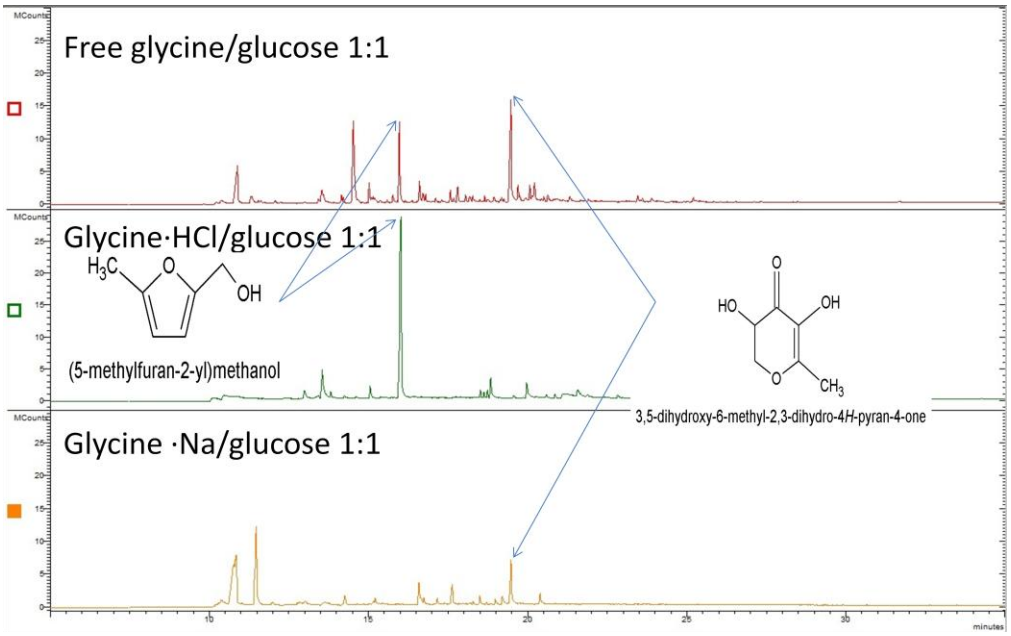


Figure 6.4 Chromatograms with proposed structures of significant intensity alternation of common peaks at amino acid/sugar ratio of 1:1

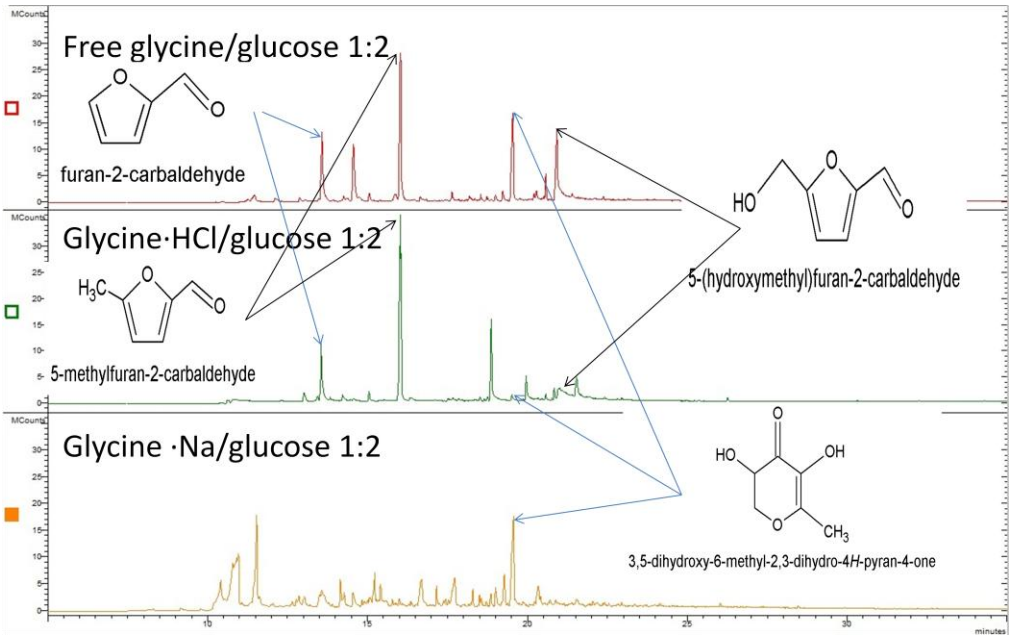


Figure 6.5 Chromatograms with proposed structures of significant intensity alternation of common peaks at amino acid/sugar ratio of 1:2

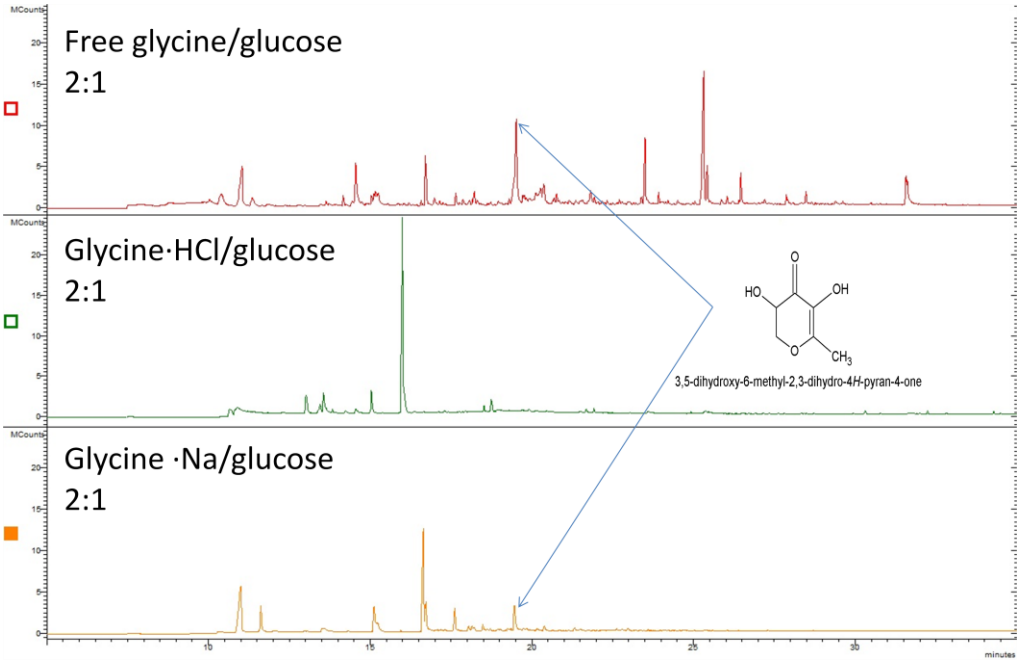


Figure 6.6 Chromatograms with proposed structures of significant intensity alternation of common peaks at amino acid/sugar ratio of 2:1

6.4 Conclusion

Amino acid salts have a noticeable influence on the generation of specific peaks and on changes in the intensity of common peaks.

Chapter 7

Investigation of the role of glucose and fructose on the profile of the Maillard reaction

7.1 Introduction

Glucose and fructose are common reducing sugars that are found extensively in food, and are considered important source of carbonyls for the Maillard reaction. At the initial stage of the Maillard reaction, glucose reacts with amino acid and form Amadori compound while fructose generates Heyns product. In this chapter, the reaction profiles of both sugars will be compared under similar reaction conditions.

7.2 Methods and materials

As described in chapter 3.

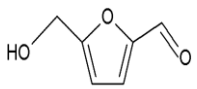
7.3 Results and discussion

7.3.1 Comparison between glucose and fructose based on the formation of specific peaks

In table 7.1, the profiles of Maillard reaction products from glycine/glucose and glycine/fructose were compared in 9 model systems: free glycine/sugar 1:1, 1:2 and 2:1, glycine hydrochloride /sugar 1:1, 1:2 and 2:1, and sodium glycinate/sugar 1:1, 1:2 and

2:1. Glucose and fructose generated quiet similar chromatograms and they produced limited number of specific peaks. No specific peaks were detected in sodium glycinate/sugar model systems, when comparing glucose and fructose. In free glycine/sugar model, ratios 1:1, fructose merely yielded one specific peak-5-(hydroxymethyl) furan-2-carbaldehyde (HMF). In glycine hydrochloride /sugar model, fructose generated several specific peaks. Relative to hundreds of peaks per chromatogram, one or two specific peaks do not constitute a significant change. As indicated above, HMF is considered as one of the important characteristic compounds that allows us to distinguish glucose from fructose under certain conditions such as free glycine/sugar 1:1, glycine hydrochloride/sugar 1:1 and 2:1, in these models only fructose generated HMF but not glucose. This is may be due to the fact that fructose is reported to exhibit much higher efficiency to yield HMF than glucose (Locas and Yaylayan, 2008).

Table 7.1 Proposed structures and isotope incorporation patterns of specific peaks formed from glycine/glucose and glycine/fructose model systems

	Sugar Type	RT (min)	Intensity	%	Structure	U6[a]	C-1[b]	C-2[c]	N[d]
Free Glycine 1:1	Fructose	21.012	57,892	21%	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0
Glycine．HCl 1:1	Fructose	21.048	80,344	25%	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0
Glycine．HCl 2:1	Fructose	19.984	113,922	27%	 5-(chloromethyl)furan-2-carbaldehyde	+6	0	0	0
	Fructose	21.016	59,291	14%	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0

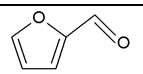
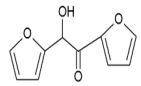
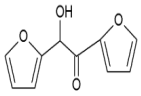
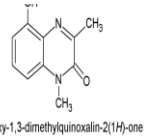
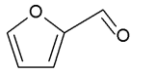
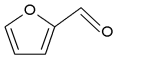
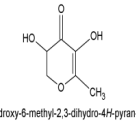
[a] All sugar carbons labelled, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

7.3.2 Comparison between glucose and fructose based on common peaks that underwent significant changes in intensity

In table 7.2, the profiles of Maillard reaction products of glycine/glucose and glycine/fructose models were compared based on common peaks that underwent significant changes in intensity. Glucose and fructose caused least intensity changes of common peaks under sodium glycinate/sugar model systems having only one peak with different intensities at ratio 1:1. Regarding glycine hydrochloride/sugar model systems,

furan-2-carbaldehyde had different intensities at ratio 1:2 and 2:1. For free glycine/sugar model systems, there were more peaks with changes in intensity as listed in table 7.2.

Table 7.2 Proposed structures and isotope incorporation patterns of common compounds undergoing significant changes in intensity from glycine/glucose and glycine/fructose model systems

Amino acid (salts)	Glucose %	Fructose %	Structure	U6[a]	C-1[b]	C-2[c]	N[d]
Free Glycine 1:1	2%	23%	 furan-2-carbaldehyde	+5	0	0	0
	21%	2%	 1,2-di(furan-2-yl)-2-hydroxyethanone	+10	0	0	0
Free Glycine 1:2	10%	2%	 1,2-di(furan-2-yl)-2-hydroxyethanone	+10	0	0	0
	38%	18%	 (5-methylfuran-2-yl)methanol	+6	0	0	0
	12%	34%	 5-(hydroxymethyl)furan-2-carbaldehyde	+6	0	0	0
Free Glycine 2:1	6%	40%	 5-hydroxy-1,3-dimethylquinoxalin-2(1*H*)-one	+6	+1	+3	+2
Glycine．HCl 1:2	13%	32%	 furan-2-carbaldehyde	+5	0	0	0
Glycine．HCl 2:1	2%	29%	 furan-2-carbaldehyde	+5	0	0	0
Glycine．Na 1:1	12%	4%	 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	+6	0	0	0

[a] All sugar carbons labelled, [b] C-1 labelled glycine, [c] C-2 labelled glycine, [d] Nitrogen labelled glycine

7.4 Conclusion

The common reducing sugars found in food, glucose and fructose have different chemical structures, surprisingly, however, the results indicate that they yield quiet similar Maillard reaction profiles under different glycine/sugar ratios and amino acid salts. This may be due to the fact that glucose and fructose or their Amadori or Heyns rearrangement products can be easily interchanged and transformed to one another and yield the same reactive intermediates such as 3-deoxy- and 1-deoxy glucosones, through common isomerization, enolization, or tautomerization reactions.

Chapter 8

General conclusions

Sugar type (glucose and fructose), amino acid salts (glycine hydrochloride and sodium glycinate) and their ratios (1:1, 1:2, 2:1) have been investigated to study their effect on the modification of the Maillard reaction profiles. For both sugars, glycine hydrochloride generated the highest abundance values and sodium glycinate models (ratio 1:2) generated the highest number of peaks. Formation of specific peaks and significant intensity changes of the common peaks were used to describe the differences in the Maillard reaction profiles. Firstly, amino acid/sugar ratios indeed had an influence on the changes in the profile of Maillard reaction, and more specifically, it had most powerful influence on free glycine models compared to models containing amino acid salts. Secondly, amino acid salts indeed had a noticeable influence on the generation of specific peaks and on the intensity changes of the common peaks, under all amino acid ratios. Surprisingly, glucose and fructose exhibited highly similar profiles. The data generated may provide useful information on the modification and optimization conditions of the Maillard reaction profiles using practical solutions.

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