CHAPTER 14

The Maillard Reaction

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CONTENTS

14.1 INTRODUCTION

Non-enzymatic catalyzed browning reactions in food and drink are classified into several distinct reaction types including lipid oxidation, caramelization, ascorbic acid oxidation, and the Maillard reaction. The last set of reactions, Maillard, are non-enzyme catalyzed reactions whose product is formed as the result of heat generated during the processing, preparation, and storage of meat, vegetables, fruit, and liquids. These reactions produce both desirable and unwanted results for food preparation. Tea, beer, bread, and syrup are all enhanced by the rich flavor profiles of the Maillard reaction while the organoleptic properties reduce the qualities and appearance of other flavorants and volatiles in foods such as milk. Thus, a complete understanding of the Maillard reaction and its implications in food preparation is very important (Hellwig and Henle, 2014; Bertrand et al., 2018). The Maillard reaction, a condensation of a reducing sugar with amino groups of amino acids, peptides, or protein, is a complex reaction with multiple phases and possible pathways providing a multitude of possible colored and flavored compounds (Figure 14.1). The initial reaction was first observed by Louis-Camille Maillard as he investigated peptide synthesis while heating amino acids in the presence of glycerol and then glucose (Hellwig and Henle, 2014). The key product was a brown precipitate with the concomitant liberation of carbon dioxide. This work led to further investigations into understanding the condensation reactions between amino acids and the carbonyls of reducing sugars (Maillard, 1911, 1916). The first mechanism describing Maillard browning was proposed by Hodge and Rist (1953) with additional modifications suggested by others (Eskin and Shahidi, 2013). While there continues to be an active focus on biomedical applications, where the Maillard reaction is known as glycation producing advanced glycation end products (AGE) with a number of associated pathologies, much of the work in the food industry is concerned with the impact of the reaction on monitor processing,

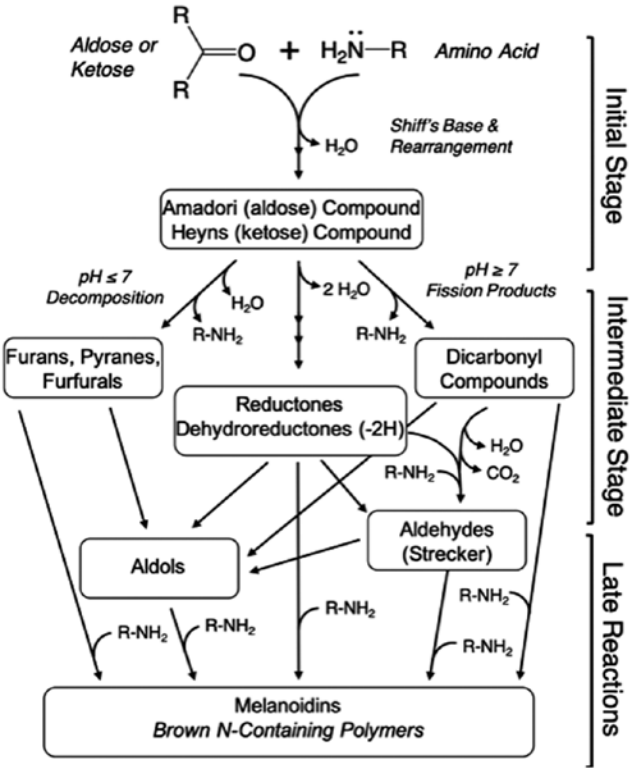

FIGURE 14.1 The Maillard reaction.

flavor where Maillard products are both advantageous and to be avoided and toxic products including acrylamide. We will highlight this complex reaction and describe where AGE products are formed, the addition of caramelization intermediates as substrates of the reaction, the role lipids and other biomolecules have on the reaction, as well as the factors including pH and water activity that affect the final products.

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14.2 MAILLARD CHEMISTRY

14.2.1 Initial Stage

As initially described by Hodges, the Malliard reaction is a complex set of pathways consisting of three phases. The initial stage is a condensation between the amino group and the carbonyl of an oxidized reducing sugar coupled with an Amadori or Heyns rearrangement. This stage is followed by an advanced or intermediate phase where sugar products fragment or dehydrate and amino acids degrade (the Strecker degradation). The reaction is completed with the final stage of aldol condensation and the formation of brown melanoid polymers and co-polymers. While the entire set of Maillard reactions have been described as a "non-classical" chemical reaction format because of the pool of complex reactions taking place after the initial formation of Heyns and Amadori products (Yaylayan, 1997), they are not linear and vary greatly depending on the conditions and competing reactants; the initial step remains a simple condensation, dehydration,

and rearrangement reaction pathway. This initial reaction begins as a nucleophilic substitution by the nitrogen of an amine onto the partially positive carbonyl carbon of an oxidized reducing sugar. This reaction is followed by a dehydration forming an intermediate imine which will cyclize forming an unstable Schiff base, which in turn is transformed into an N-substituted glycosylamine. The source of electron-rich nitrogen is commonly described as the amino acid side group for lysine (-amino) and the guanidinium group of arginine. All free amino acids have the potential for the reaction via the free amino group. Heating ribose with amino acids without nitrogen-containing side-chains results in the browning reaction. Interestingly, besides lysine, glycine, tryptophan, and tyrosine all react strongly with reducing sugars, providing a significant Maillard product. Protease protection and the nutritional availability of the internal peptide bonds of proteins have been suggested as another source of the reaction as the imine of a peptide bond condensing with a reducing sugar (Horn et al., 1968). The length of the peptide/protein may impact the rate of the reaction (Kim and Lee, 2009). However, this isn't as straight forward as chain length because dipeptide glycine reacted at a greater rate than free amino acid alone or the tripeptide (Lu et al., 2005). The likely explanation was that the peptide bond hydrolysis and greater stability of the tripeptide decreased the rate of the reaction (Kim and Lee, 2009). However, lysine remains the key amino acid thought to be involved in browning reactions.

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AU: 'All free amino acids have the potential for the reaction via the free amino group.' This is unclear, please rephrase.

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Both ketone and aldehyde reducing sugars can participate in the reaction. Aldoses in an oxidized state will react with forming aldosylamines after the Schiff rearrangement ultimately giving rise to a 1-amino-1-deoxy-ketose Amadori compound. On the other hand, ketose sugars will produce a ketosylamine and rearrange into a 2-amino-2-deoxy-aldose Heyns compound (Figure 14.2). In general, pentoses react with a faster rate than hexoses, and monosaccharides are significantly more reactive than disaccharides (Eskin and Shahidi, 2013). The availability of the oxidized anomeric carbon of a reducing sugar is critical for the nucleophilic amine to attack. Thus, the reaction is enhanced in alkali conditions when the equilibria of a closed-ring hemi-acetal to an open chain of reducing simple carbohydrates shifts into the open form of the reducing sugar. A simple study comparing the rate of the browning of foods dipped in water (pH 6.90) vs. a dilute solution of baking soda (pH 9.0) showed that carrot, potato, onion, and chicken all browned two or even four times faster in basic conditions than neutral (Provost, 1987).

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In general, lysine with both a side-chain amine and an -carbon amino group, is highly reactive with most sugars. However, glycine shows the highest rate of reaction when heated with fructose, ribose, or lactose (Ashoor and Zent, 1984). When heated in a 1:1 ratio at pH 9.0, both arginines produced only 30% of the product of either lysine or glycine with the sugars tested. Surprisingly, several free amino acids reacted ~20% as effectively as lysine, presumably through the -carbon amino group (tryptophan, tyrosine, proline, leucine, isoleucine, and alanine). Therefore, a protein hydrolysate or foods with a high concentration of free amino acids (such as shrimp with significant levels of glycine supporting osmotic pressures) are able to brown easily.

Likewise, the type of sugar has an important impact on the browning reaction. Monosaccharides react with greater rates than disaccharides, and five-carbon sugars are more reactive than six-carbon sugars (Pastoria et al., 2018). Generally, sugars react in the following order, pentoses > hexoses and disaccharides. However, this depends on the model system used. One report showed that ribose will react with lysine at the greatest rate with xylulose while moderately reacting with arabinose. Both glucose and fructose produced an almost undetectable product with protein hydrolysate (Eskin and Shahidi, 2013), and when tested against each amino acid both ribose and lactose showed nearly

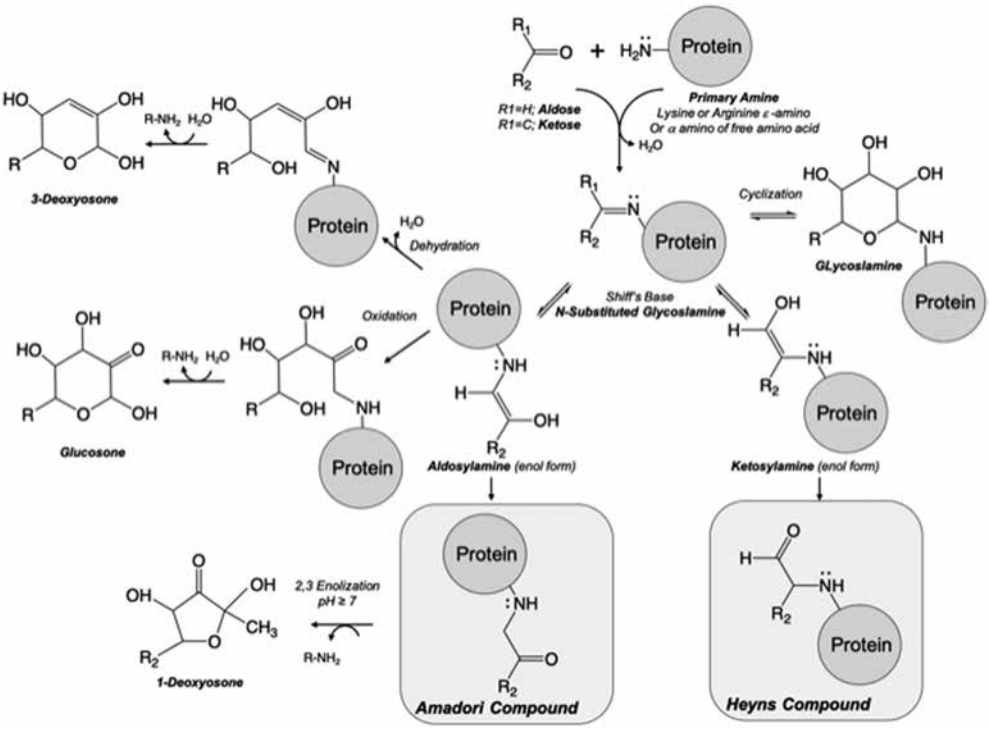

FIGURE 14.2 Formation of Amadori and Heyns compounds in an early Maillard reaction.

identical rates of browning with most amino acids (lactose slightly > ribose > fructose > glucose) (Ashoor and Zent, 1984). A conservative estimate of possible reaction combinations of the first step of the Maillard reaction demonstrates the possibility of diverse aroma, flavor, and color products. Considering each of the amino acids with a free amino group plus the side groups of lysine and arginine and limiting the reducing sugars to eight of the more common simple carbohydrates, there are over 576 Amadori or Heyns potential products at this point alone. The path each intermediate may take through the rest of the reaction pathway, as well as possibilities for the intermediates of the condensation product to react with other substrates, highlights the diversity of this reaction. Following the condensation reaction and loss of water forming an intermediate imine, there are two potential paths: a reversible cyclization generating a glycosylamine or the formation of a Schiff base (Figure 14.2). The reaction generating the Schiff base is reversible, especially so in acidic conditions, where the initial products are easily regenerated. However, the N-glycosylamine rearrangement into the Amadori or Heyns compound is irreversible, as the precursor Schiff base can be converted into a cyclic hemiaminal which easily mutarotates, whereas the formation of a furanose (Amadori) or ketose (Heyns) hemiacetal has a similar mutarotation to carbohydrates where the equilibria lies in the cyclic form. The Schiff base will form an enaminol (enol form) and, depending on the initial sugar and pH, will have one of three possibilities. At a higher pH, a loss of amine generates a deoxydicarboyl compound. At a lower pH, a 1–2 enolization results in the generation of the keto Amadori product, while oxidation and hydrolytic loss of the amine results in a

glucosone cyclic product. Under heat, each of these intermediates can form into flavor/aroma products via the next set of advanced Maillard reactions.

14.2.2 Intermediate Stage

Depending on the initial condensation and the various pathways already presented, there are several different fates for the Amadori, Heyns, and other intermediates formed thus far, in what is classically called the intermediate stage (Figure 14.1). An example of aroma and flavor compound generation through deoxyosone dehydration includes the generation of furanones, isomaltol, and maltol in meats (Eskin and Shahidi, 2013). Although this is perhaps more accurately described as a pool of possible reactants, intermediates, and products, which we will discuss later, Amadori and Heyns products will degrade into one of several possible pathways depending on pH and temperature. Further decomposition and dehydration of the three waters of Amadori products in acidic conditions will lead to the formation of a furan ring containing hydroxymethlfurfural and furfural. Glycine has been reported to increase the formation of this set of products, presumably as this increases the reaction from the Amadori product over an alternative degradation of glucose without utilizing the Maillard reaction (Pastoriza et al., 2018). Also, under acidic conditions, sugar dehydration results in the production of deoxyosone osloses 1-, 2-, 3, or 4-deoxysones. These can then cyclize into the maltols, and furanones described earlier, and can in subsequent reactions with ammonia and hydrogen sulfide produce a number of meaty and other savory flavors. An additional pathway of both Heyns and Amadori compounds involves dehydration under more neutral or basic conditions resulting in the loss of two water molecules generating the antioxidant reduced-enediol, the reductones. The acid-catalyzed fragmentation and loss of amine generates the dicarbonyl deoxyosones. Further dehydration creates dehydroreductones. Both forms of reductones serve as a fork in the degradation pathways, where in basic conditions they undergo a retro-aldolization into acetone compounds, diacetyle, glyoxal, pyruvaldehyde, glycolaldehyde, and glyceraldehyde, each with their own potential as a flavorant/odorant, or they can further react with food compounds on their own or in reactions with amino acids (Strecker reaction). The presence of the carbonyl group stabilizes the enediol form as an a-oxo-enediol with a strong acidic tendency leading to a strongly reducing potential for the compounds. As such, both the reductones and the dehydroreductones play a key part in browning in subsequent reactions. A third pathway involves the fission or fragmentation of Amadori/Heyns degradation compounds (Figure 14.1). The reaction is initiated by an oxidative fission or reversal of the aldol condensation reaction. Through rearrangement, reduction, and saccharinic rearrangement (migration of alcohol transforming from a dihydroxyl enol to a carboxyl compound), the cleavage reaction forms small decarbonylated aldehyde, alcohol, and acetic compounds, including acetic acid, formaldehyde, diacetyl, glyoxal, acetal and acetaldehyde and 2,3 butanedione (Weenen and Apeldoorn, 1996; Nursten, 2005). An important pathway that involves the loss of free amino acids and for the most part not peptides or proteins, as observed in the initial Maillard condensation reaction, is the oxidative degradation of amino acids in the presence of -dicarbonyls formed by the Amadori/Heyns compounds (reductones and fission products). Deoxyosones, diacetyl, pyruvaldehyde, hydroxyacetone, glyoxyal, and other decomposition products are potential Strecker reactants. The diversity of potential degradation dicarbonyl compounds and free amino acids combine to make this reaction a major contributor to the flavor profile of food and beverages. Because this reaction

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proceeds at a greater rate with free amino acids, the pathway is more prevalent in foods high in free amino acids or protein hydrolysates. The reaction involving cysteines and ribose is responsible for many of the aroma and flavor of cooked meats. Interestingly, the reactive -carbonyl of glyoxal can react with the lesser reactive arginine side chain (in the absence of competing reactant cysteine) preferred over lysine. While the Strecker degradation reaction imparts a key effect on the flavor profiles of roasted foods including meats and cocoa and coffee beans, both the higher and lower molecular weight products of the reaction play a contributory role. It is the further reactions (with a second amino acid or condensation of various intermediates) that produce the compounds involved in the flavor of heated foods. These aroma and flavor products from the Strecker degradation (Figure 14.3) are often organized by the heterocyclic products: pyrroles, oxazoles and oxazolines, and thiazole derivatives. Heterocyclic nitrogen-containing compounds such as pyrazine have been reported to be important in the flavor of many heated and toasted foods, from meats, broths, and vegetables, most with a low odor threshold. Pyrroles were first identified in roasted coffee beans and are a key component of the heterocyclic compounds formed during non-enzymatic browning. Ribose and b-hydroxy amino acids are precursors for these compounds, which are further processed into furans and fururyl-substituted pyrroles. When heated in the presence of serine, threonine, and various monosaccharides, the important flavors of heat-treated cereal and popcorn were identified. Some of the compounds bring about a caramel-like aroma of cooked meats. The Strecker degradation production of several oxazoles further provides complex green and vegetable-like notes in cooked meats. Cysteine and methylglyoxyl Strecker degradation is an example of the thiazole heterocyclic compounds. The nucleophilic attack of the sulfur of cysteine at the carbon of an imine intermediate formed by the reaction between ammonia and an aldehyde creates a range of similar cyclic molecules. These low odor threshold volatiles give sulfurous, onion-like aromas to meats, potato chips, and roasted peanuts.

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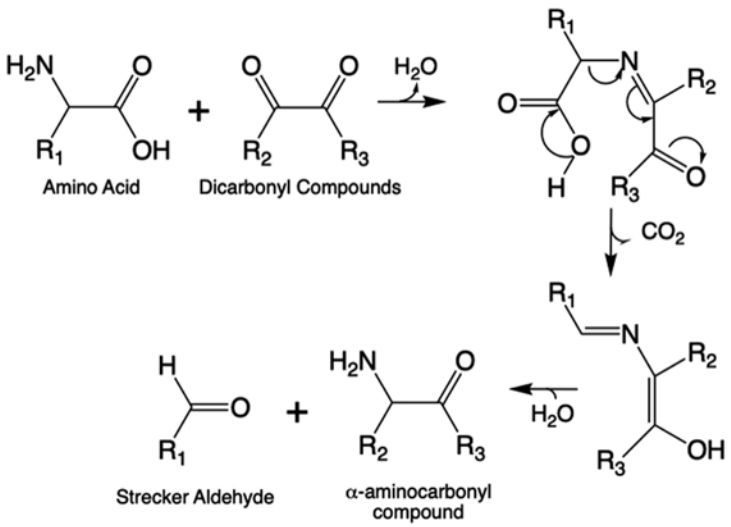

FIGURE 14.3 The Strecker reaction.

14.2.3 Late Reactions

While many intermediates provide both volatile and soluble components of flavor and aroma, the reactions generate brown-colored compounds as well as flavors. This final step is a polymerization of many of the substances into both high and low molecular weight melanoids. Much of these are condensation reactions potentially between all of the intermediates presented thus far. Polymerization of aminated products including pyrrols, furaldehyes, and others create a brown poorly defined anionic compound. If the backbone of the browning pigment is built from a carbohydrate backbone, such as those found in dark liquids, they are known as melanosaccharides. The browning reactions found on crusts of bread and baked goods often have protein at the core and are then called melanoproteins. The constitution of melanoids, in general, is dependent on the amount of aldehyde and amines involved in the polymerization. Often the condensation products are polymers of hetercyclic compounds. Proteins, via linkage with arginine or lysine side groups, act as a scaffold for carbohydrate-involved melanoidins. Thermal processing of meats shows high levels of carbohydrate-induced browning in this fashion. Despite a growing role in health as both an antioxidant and other biological effects, very little has been learned about the structure of these compounds. Most of our understanding comes from using model systems, taking a reductionist approach mixing various intermediates, and investigating the final products.

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14.3 POOL THEORY

The difficulty in determining the intermediates and the final melanoid polymers stems from the diversity, condition (pH, water availability, temperature), and concentration of potential reactants in the processed foods. Thus, a particularly interesting and descriptive theory first described by Yaylayan in 1997 is that a cascade of reactions does not follow the paths described (Figure 14.4). Rather the products are propagated by chemical pools generated from various precursors. The pools result from three defined pools of precursor parents: sugars, amino acids, and Amadori/Heyns products. In addition to the interaction between pool constituents, each parental compound can fragment as they interact in the "pool." Depending on the condition, the initial pool will direct the final product into polymers, dimers, heterocycles ore "other compounds." As the reactions propagate, each pool can easily interact and within or between pools, generate new Maillard reaction products. While making it difficult to predict these products, it is a more applicable description vs. the pathway model.

14.4 ADVANCED GLYCATION END PRODUCTS

For some time, there have been concerns about the loss of nutritional value of foods or even potential toxicity due to the formation of Maillard products. The loss of lysine as a nutritional source in processed foods and a decrease in protein function, solubility, and digestibility after the reaction with Maillard products are examples of the role of these reactants in food processing. In addition to changes in the nutritional state of foods due to the Maillard reaction, there are biological health concerns with some of the side reactions between Maillard intermediates and proteins or lipids. Advanced glycation end products (AGEs) are the result of side reactions from the Amadori product and -dicarbonyl

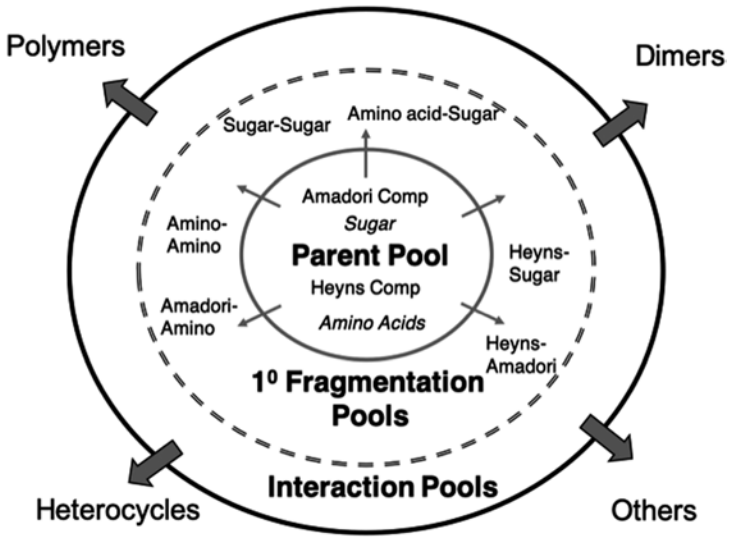

FIGURE 14.4 Pool theory of the Maillard reaction.

compound reaction with proteins or lipids. In general, these products are formed by the reactions of reducing sugars or the degradation of biological macromolecules (carbohydrates, lipids, proteins) or ascorbic acid. The results are that proteins or lipids that have become modified (glycated) with carbohydrates in a non-ATP dependent process. Foods especially high in AGE products include those sterilized, subjected to ultra-high-temperature processing for pasteurization or roasting. Once absorbed, these compounds have been implicated as factors in a number of degenerative and aging-related diseases.

Focusing on protein–AGE formation, the end products can result in either a protein–AGE adduct (mono or polysubstituted) or proteins crosslinked with AGE (protein–AGE–protein). In addition to late/advanced stage reactants, Amadori products also degrade into reactive carbonyls, which in turn react with amino groups forming AGE compounds. Most of the detected AGE products are modifications of primarily lysine or arginine side groups with a limited set of reactions known involving cysteine side groups (Lund and Ray, 2017). Production of AGE products via the Hodge pathway involving the degradation of Amadori or Hayns intermediates can happen in a single step producing a carboxylated methyllysine or other AGE products depending on the reducing sugar (Figure 14.5). Formation of reactive dicarbonyls and their condensation with amino groups of proteins via the Namiki pathway results in another suite of AGE products from glyoxal, methyl-glyoxal, and 2-deoxy-glucose intermediates.

AGE products are based on a multitude of reactants with highly diverse and heterogeneous structures (Figure 14.6). One of the earliest AGE crosslinked proteins and highly prevalent in tissues and cooking is carboxymethl-lysine (CML) formed by the degradation of Amadori products or the addition of a reactive glyoxal to a protein's lysine residue. Reactions initiated with ribose will result in pentosidine, a crosslinked AGE formed between an arginine and a lysine. The reaction is a ribose-Amadori product involving ascorbic acid via the Hodges pathway. The reactive dicarbonyl also can generate a number of AGE products. Glyoxal-lysine dimer (GOLD) is an imidazolium ion formed from the cyclic dimerization of two lysine side groups and glyoxal. A similar AGE product is produced when the crosslinked proteins start instead with methyglyoxyl and two lysine

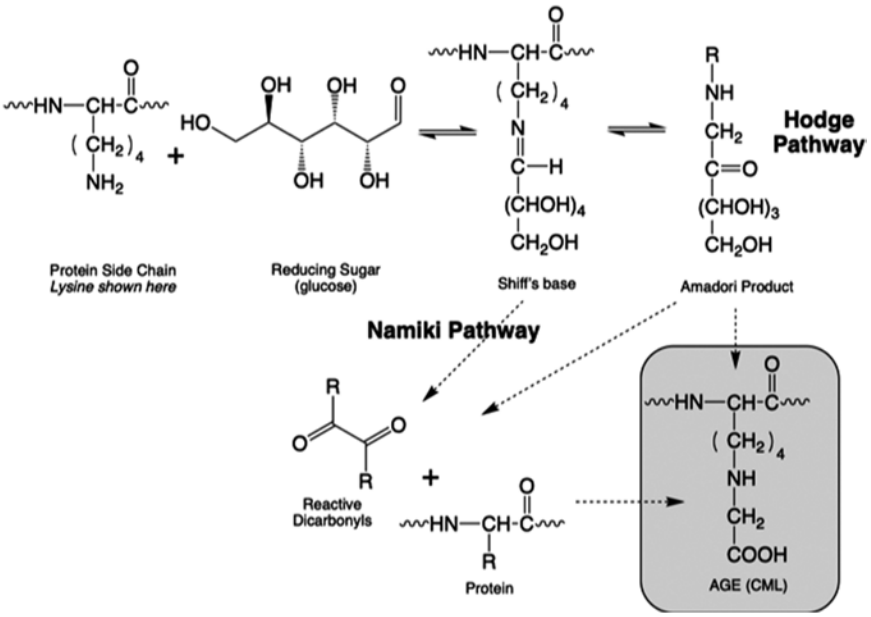

FIGURE 14.5 Maillard production of AGE compounds.

residues (MOLD). Similarly, dimers of lysine and arginine are formed with either glyoxal or methyglyoxal. The reaction between arginine and cysteine with glucose results in 8-hydroxy-5-methyldihydrothiazolo (3,2 alpha) pyridinium-3-carboxylate, also named Maillard reaction product X (MRX). MRX is found in both prepared food and in vivo on long-lived proteins and may be an important participant in the progression of diabetes. This compound is likely formed from glucose with proteins and has been identified after cysteine and arginine mixtures were incubated with glucose.

AU: 'This compound is likely formed from glucose with proteins and has been identified after cysteine and arginine mixtures were incubated with glucose.'

Foods high in fat and protein show the highest levels of AGE products after processing or cooking. Foods cooked in high fat and protein content including those prepared with mayonnaise, olive oils, or almonds all show significant AGE products although the temperature and process highly impact the final AGE levels (Nguyen, 2006). Common mono- and disaccharide glucose displays the slowest rate while fructose, ribose, and glucose-6-phosphate generate AGE crosslinked proteins at the fastest rate (Pasupulati et al., 2016). Foods high in AGE products at first were thought to be poorly absorbed, and the biomedical impact of AGE compounds focused on endogenously produced glycans. However, measuring the health impact of a diet high in AGE products increased tissue AGE content, and ingesting foods high in AGE products increased the risk of heart, kidney, and other diseases including diabetes in mice (Gkogkolou and Bohm, 2012). Interestingly in diabetic animals, clearance of AGE products was reduced, indicating a longer transit time concomitant with increased risk of renal-vascular injury (Nguyun, 2006). Fortunately, a number of approaches have been formulated to create effective inhibitors of AGE compounds. Trapping the reactive alpha-dicarbonyls using epicatechin from green tea reduced off-flavors and AGE products in processed milk. A number of other phenolic flavonoids are under investigation for their trapping and radical scavenging ability in addition to other approaches limiting high heat processing times (Lund and Ray, 2017).

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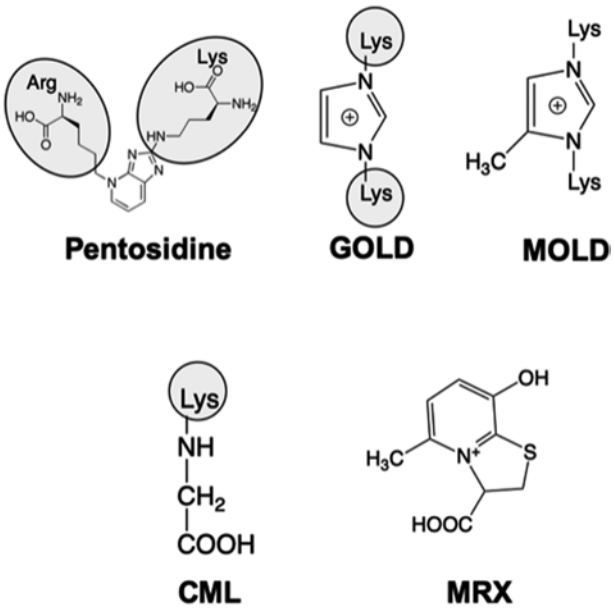

FIGURE 14.6 Select AGE reactants and crosslinkers+.

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