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THE MAILLARD REACTION OF REDUCING SUGARS WITH AMINO ACIDS IN BAKING CHEMISTRY

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ABSTRACT

The Maillard reaction often referred to as browning or caramelization occurs when proteins undergo a chemical reaction with reducing sugars upon applied heat. More specifically, proteins, peptides and the free amino groups of amino acids react with free reducing sugars to produce desirable flavors, aroma and crust coloring in many baked goods such as bread, rolls, crackers, cakes, biscuits and any other baked item possible of producing a crust. lactose, maltose, fructose and glucose are the common reducing sugars found in breads. Flour contains most of the free amino acids such as lysine, alanine, cystine and proline. The reducing sugars and amino acids react together in three distinct stages to complete the Maillard reaction. The initial stage is undetectably colorless on crust and enables the sugars as well as the amino acids to condensate. Moving into the intermediate stage which ranges from colorless to light yellow crust, the sugars dehydrate and become fragments, at the meantime the amino acids begin to degrade as well. The final stage is where the most color is produced and exhibited as golden brown on the crust of the baked item. Polymerization of aldehyde-amine compounds and formation of heterocyclic nitrogen compounds occur in the last stage. Once each stage has passed, the Maillard reaction is complete and many aromatic and flavor compounds are produced yielding a high quality baked good that exhibits positive final product attributes such as crust color, crumb grain, volume and mouth feel. The Maillard reaction can occur at room temperature around 20-25°C (68-77°F) in the presence of oxygen. When the temperature is over 30°C (80°F), the reaction rate starts to increase. With every 10°C (50°F) difference, the reaction rate would increase at least 3 times. Over the temperature of 80°C (176°F), the reaction rate will be consistent and unaffected by temperature or oxygen level. Browning, or the Maillard reaction, creates flavor and changes the color of food. Maillard reactions generally only begin to occur above 140°C (285°F). Both caramelization and the Maillard reaction only occur on the right producing the noticeable brown color.

KEYWORDS: Reducing sugars, Amino acids, Maillard reaction, Amadori rearrangement, Ketosamines, Reductones, Melanoidins, Pentosidine, Furfural, Hydroxymethyl furfural, Caramelization, Advanced Glycation end Products, Age-Related Macular Degeneration.

INTRODUCTION

The **Maillard reaction** is a chemical reaction between an amino acid and a reducing sugar, usually requiring the addition of heat. Like caramelization, it is a form of non-enzymatic browning. The reactive carbonyl group of the sugar interacts with the nucleophilic amino group of the amino acid and interesting but poorly characterized odor and flavor molecules result. This process accelerates in an alkaline environment to activate the amino groups. This reaction is the basis of the flavoring industry, since the type of amino acid determines the resulting flavor.[1]

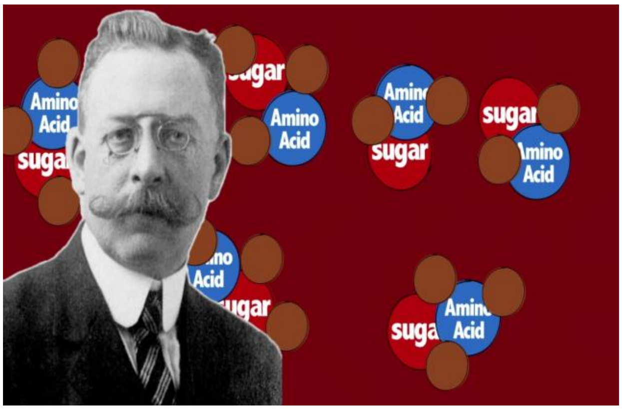


Figure-1: Louis-Camille Maillard

In the process, hundreds of different flavor compounds are created. These compounds in turn break down to form yet more new flavor compounds and so on. Each type of food has a very distinctive set of flavor compounds that are formed during the Maillard reaction. It is these same compounds that flavor scientists have used over the years to create artificial flavors. The Maillard reaction should not be confused with caramelization which occurs with sugars.

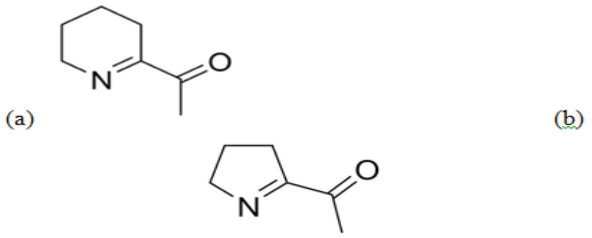


Figure-2: (a) 6-Acetyl-2,3,4,5-tetrahydropyridine (b) 2-acetyl-1-pyrroline

Although used since ancient times, the reaction is named after the chemist **Louis-Camille Maillard** who investigated it in the 1910s. It is named after French chemist Louis-Camille Maillard, who first described it in 1912 while attempting to reproduce biological protein synthesis. The Maillard reaction is named after the French scientist Louis Camille Maillard (1878-1936), who studied the reactions of amino acids and carbohydrates in 1912, as part of his PhD thesis, which was published in 1913.[2]

Products with Maillard reactions:

The Maillard reaction is responsible for many colors and flavors in foodstuffs:

(1) Caramel made from milk and sugar (2) The browning of bread into toast (3) The color of beer, chocolate, coffee and maple syrup (4) Self-tanning products (5) The flavor of roast meat (6) The color of dried or condensed milk.

6-Acetyl-2,3,4,5-tetrahydropyridine is responsible for the biscuit or cracker-like flavor present in baked goods like bread, popcorn and tortilla products. The structurally related compound 2-acetyl-1-pyrroline has a similar smell and occurs also naturally without heating and gives varieties of cooked rice and the herb *pandan* (*Pandanus amaryllifolius*) their typical smells. Both compounds have odor thresholds below 0.06 ng/l.[3]

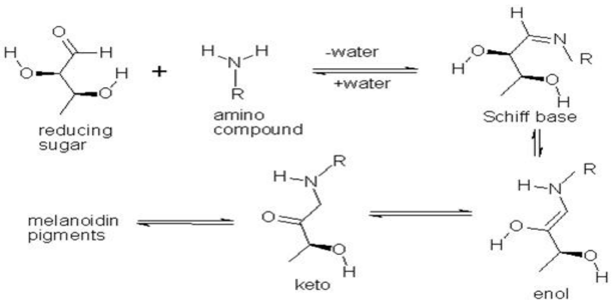


Figure-3: Maillard reaction forming Schiff base by carbohydrate and amino acid turning to Amadori product (melanoidin pigments)

The process

1. The carbonyl group of the sugar reacts with the amino group of the amino acid, producing N-substituted glycosylamine and water.
2. The unstable glycosylamine undergoes Amadori rearrangement, forming ketosamines.
3. There are several ways for the ketosamines to react further:
   - ○ Produce 2 water and reductones.
   - ○ Diacetyl, aspirin, pyruvaldehyde and other short-chain hydrolytic fission products can be formed.
4. Produce brown nitrogenous polymers and melanoidins (Melanoidins are brown, high molecular weight heterogeneous polymers that are formed when sugars and amino acids combine through the Maillard reaction at high temperatures and low water activity. Melanoidins are commonly present in foods that have undergone some form of non-enzymatic browning, such as barley malts (Vienna and Munich), bread crust, bakery products and coffee. They are also present in the wastewater of sugar refineries, necessitating treatment in order to avoid contamination around the outflow of these refineries.)[4]

Key Factors: Pentose sugars react more than hexoses, which react more than disaccharides. Different amino acids produce different amounts of browning. Since the Maillard reaction produces water, having a high water activity environment inhibits the reaction.

Chemistry *in-vitro*: The **Maillard reaction** is a chemical reaction between amino acids and reducing sugars that gives browned food its distinctive flavor. Seared steaks, pan-fried dumplings, cookies and other kinds of biscuits, breads, toasted marshmallows and many other foods undergo this reaction.[5]

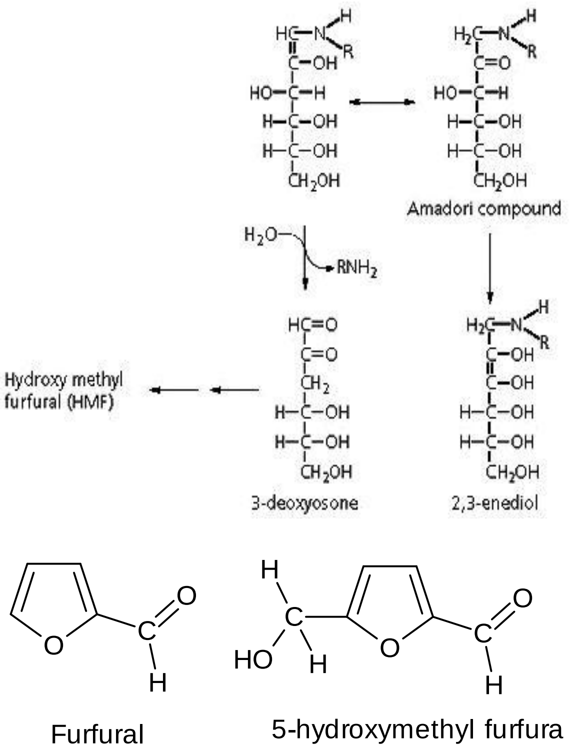


Figure-4: Formation of HMF and Amadori-rearrangement

The reaction is a form of non enzymatic browning which typically proceeds rapidly from around 140-165°C (280-330°F). At higher temperatures, caramelization and subsequently pyrolysis become more pronounced. This process is accelerated in an alkaline environment (*e.g.*, lye applied to darken pretzels), as the amino groups (RNH_3^+) are deprotonated and hence, have an increased nucleophilicity. The type of the amino acid determines the resulting flavor. This reaction is the basis for many of the flavouring industry's recipes. At high temperatures, a potential carcinogen called acrylamide can be formed. In the process, hundreds of different flavor compounds are created. These compounds, in turn, break down to form yet more new flavor compounds and so on. Each type of food has a very distinctive set of flavor compounds that are formed during the Maillard reaction. It is these same compounds that flavor scientists have used over the years to make artificial flavours.[6]

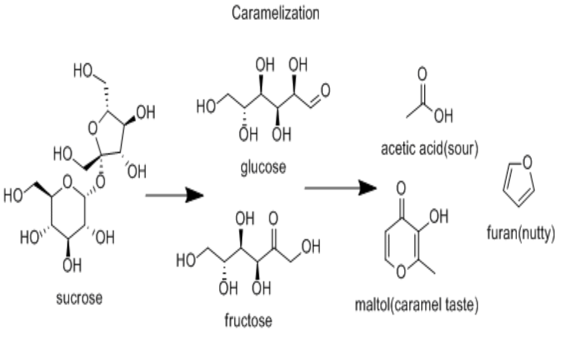


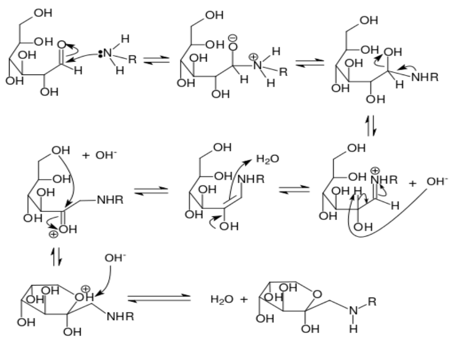
Figure-5: Caramelization

In 1913 Maillard published a paper to explain what happens when amino acids react with sugars at elevated temperatures. However, it was chemist John E Hodge, working at the US Department of Agriculture in Peoria, Illinois, who published a paper in 1953 that established a mechanism for the Maillard reaction.

The Maillard reaction is responsible for many colors and flavors in foods:
(1) The browning of various meats like steak, when seared and grilled (2) The browning and umami taste in fried onions (3) Coffee roasting (4) The darkened crust of baked goods like pretzels, bagels and toast (5) The golden-brown color of French fries and other crips (6) Malted barley, found in malt whiskey or beer (7) Dried or condensed milk (8) *Dulce de leche* (9) Black garlic.

Caramel made from milk and sugar, especially in candies: Milk is high in protein (amino acids) and browning of food involving this complex ingredient would most likely include Maillard reactions: Chocolate and lightly roasted peanuts. French fries: The browning reactions that occur when meat is roasted or seared are complicated and occur mostly by Maillard browning with contributions from other chemical reactions, including the breakdown of the tetrapyrrole rings of the muscle protein myoglobin. Caramelization is an entirely different process from Maillard browning, though the results of the two processes are sometimes similar to the naked eye (and taste buds). Caramelization may sometimes cause browning in the same foods in which the Maillard reaction occurs, but the two processes are distinct. They both are promoted by heating, but the Maillard reaction involves amino acids, as discussed above, whereas caramelization is simply the pyrolysis of certain sugars.[7]

The open-chain Amadori products undergo further dehydration and de-amination to produce dicarbonyls. This is a crucial intermediate. Dicarbonyls react with amine to produce Strecker aldehyde through Strecker degradation. Acrylamide, a possible human carcinogen can be generated as a by-product of Maillard reaction between reducing sugars and amino acids, especially Asparagine both of which are present in most food products.[8]



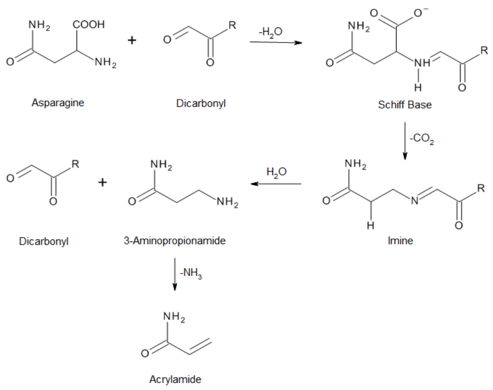
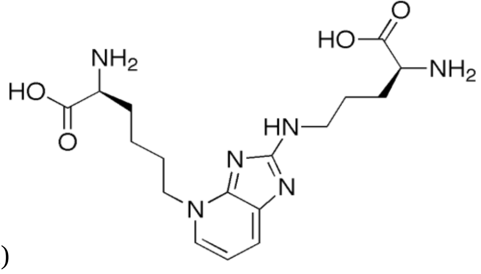


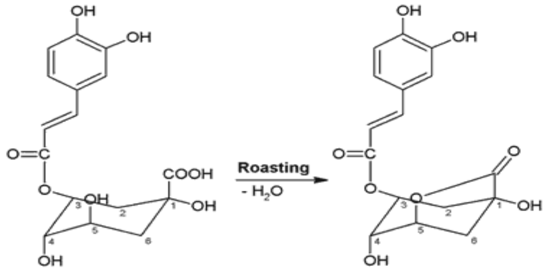
Figure-6: Maillard Reaction Mechanism

Chemistry *in-vivo*: The Maillard reaction also occurs in the human body. It is a step in the formation of advanced glycation end products (AGEs). It is tracked by measuring pentosidine. (Pentosidine is a biomarker for advanced glycation end products, or AGEs. It is a well characterized and easily detected member of this large class of compounds.) Derived from ribose, a pentose, pentosidine forms fluorescent cross-links between the arginine and lysine residues in collagen. It is formed in a reaction of the amino acids with the Maillard reaction products of ribose. Although it is present only in trace concentrations among tissue proteins, it is useful for assessing cumulative damage to proteins—advanced glycation end products—by non-enzymatic browning reactions with carbohydrates. *In-vivo*, AGEs form pentosidine through sugar fragmentation. In patients with diabetes mellitus type 2, pentosidine correlates with the presence and severity of diabetic complications.)[9]

Although the Maillard reaction has been studied most extensively in foods, it has also shown a correlation in numerous different diseases in the human body, in particular degenerative eye diseases. In general, these diseases are due to the accumulation of AGEs (Advanced Glycation end Products) on nucleic acids, proteins and lipids. Though AGEs have numerous origins, they can form from the oxidation and dehydration of Amadori adducts, which themselves are products of non-enzymatic Maillard reactions. Apart from ocular diseases, whose correlation with Maillard chemistry has been more recently studied, the formation of AGEs has also proven to contribute to a wide range of human diseases that include diabetic complications, pulmonary fibrosis and neuro-degradation.



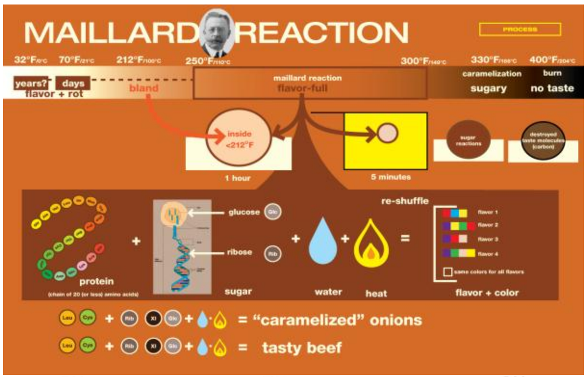
(a)



(b)

Figure-7: (a) Pentosidine (b) Melanoidin

The positron emission tomography imaging agent fluorodeoxyglucose has been shown to undergo the Maillard reaction to form fluorodeoxyglycosamine. Receptor systems in the body have been suggested to have evolved to remove glycation-modified molecules, such as AGEs, to eliminate their effects. The adverse effects of AGE accumulation appear to be mediated by numerous different AGE receptors. Examples include AGE-R1, galectin-3, CD36 and most noted, RAGE, the receptor for AGEs.[10]



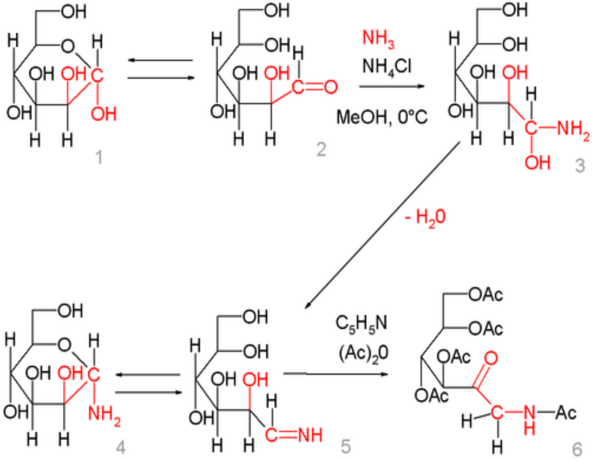


Figure-8: Mailliard reaction and Amadori rearrangement

Advanced glycation in numerous different locations within the eye can prove detrimental. In the cornea, whose endothelial cells have been known to express RAGE and galectin-3, the accumulation of AGEs is associated with thickened corneal stroma, corneal edema and morphological changes within patients with diabetes. Within the lens, Maillard chemistry has been studied extensively in the context of cataract formation.

Advanced glycation is known to alter fiber membrane integrity in the lens and dicarbonyl compounds are known to cause increased aggregate formation within the lens. This effect is exacerbated by both diabetes and aging. Furthermore, it is thought that AGE-inhibiting compounds are effective in preventing cataract formation in diabetics.[11]

Glycation in Maillard reactions may lead to destabilization of the vitreous gel structure within the eye via unnecessary cross-linking between collagen fibrils. Again, this process is more strongly observed within diabetic patients. Within the retina, the accumulation of AGEs in the drusen and Burch’s membrane has been associated with age and has also been observed at a higher level among patients with age-related macular degeneration (AMD). This is manifested by the thickening of the Bruch's membrane. Furthermore, it has been observed that AGE levels increase with age within the lamina cribrosa and the products of the Maillard reaction have been observed there, as well. A wide range of ocular diseases, particularly diabetic retinopathy, may be prevented by the inhibition of the Maillard reaction. This may be achieved in numerous ways: preventing the formation of AGEs, reducing the effectiveness of the AGE signaling pathway and the receptor-ligand interactions, or breaking the AGE cross-links. This latter method has already been achieved to some extent by the breaker alagebrium, though its effectiveness against retinopathy is unknown. Another method is by the use of amadorins, which are able to prevent the reaction of Amadori intermediates, which form into AGEs, by scavenging the reactive carbonyls.[12]

CONCLUSION

The **Maillard reaction** is not a single reaction, but a complex series of reactions between amino acids and reducing sugars, usually at increased temperatures. Like caremelization, it is a form of non-enzymatic browning. In the process, hundreds of different flavour compounds are created. These compounds in turn break down to form yet more new flavour compounds, and so on. Each type of food has a very distinctive set of flavour compounds that are formed during the Maillard reaction. Maillard reactions are important in baking, frying or otherwise heating of nearly all foods. Maillard reactions are (partly) responsible for the flavour of bread, cookies, cakes, meat, beer, chocolate, popcorn, cooked rice. In many cases, such as in coffee, the flavour is a combination of Maillard reactions and caramelization. However, caramelization only takes place above 120-150°C, whereas Maillard reactions already occur at room temperature.

Although studied for nearly one century, the Maillard reactions are so complex that still many reactions and pathways are unknown. Many different factors play a role in the Maillard formation and thus in the final colour and aroma; pH (acidity), types of amino acids and sugars, temperature, time, presence of oxygen, water, water activity (a_w) and other food components all are important. The first step of the Maillard reaction is the reaction of a reducing sugar, such as glucose, with an amino acid. The Amadori compounds easily isomerise into three different structures that can react differently in the following steps. As in food generally over 5 different reactive sugars and 20 reactive amino acids are present, only the first step theoretically already results in over 100 different reaction products. The larger the sugar, the slower it will react with amino acids. The pentose sugars (5 carbon atoms), such as ribose, will react faster as hexose sugars (glucose, fructose) and disaccharides (sugar, lactose). From the amino acids lysine, with two amino groups, reacts the fastest and causes darker colours. Cysteine, with a sulphur group, causes specific flavours, but fewer colours. Sugar alcohols or polyols (sorbitol, xylitol) do not participate in the Maillard reaction. This means that bakery products sweetened with sorbitol will not or hardly change colour during baking. The next steps differ, depending on the isomer of the Amadori compound. Either the amino acid is removed, which results in reactive compounds that are finally degraded to the important flavour components furfural and hydroxymethyl furfural (HMF). The other reaction is the so-called Amadori-rearrangement, which is the starting point of the main browning reactions. Furfural and hydroxymethylfurfural are characteristic flavour compounds of the Maillard reaction. Furfural is the result of a reaction with a pentose sugar (such as ribose); HMF is the result of a reaction with a hexose (glucose, saccharose). After the Amadori-rearrangement three different main pathways can be distinguished:

- Dehydratation reactions,
- Fission, when the short chain hydrolytic products are produced, for example diacetyl and pyruvaldehyde,
- “Strecker degradations” with amino acids or they can be condensated to aldols.

These three main pathways finally result in very complex mixtures, including flavour compounds and brown high molecular weight pigments **melanoidins**. Melanoidins are present in many foods like coffee, bread and beer. However, up to now the knowledge about structural, functional and physiological properties of this group of food components is rather limited. The Maillard reaction products thus change the colour and flavour of food and in most cases these changes are appreciated by people. In addition the melanoidins may have some beneficial anti-oxidant properties.

On the other hand, Maillard reactions may reduce the nutritional value of a product, as amino acids and carbohydrates may be lost. Sometimes the flavour is not appreciated, such as the ‘cooking flavour' in sterilized milk. Some of the Maillard end-products may also be toxic or carcinogenic. One of the Maillard reaction products is acrylamide, a potential toxic compound which is only formed at temperatures above 180°C, especially in baked or fried products (French fries).

When frying below 180°C acrylamide is not formed. In general it can be stated that Maillard products have been present in our foods for many thousands of years and are consumed daily by nearly all people in the world.

Maillard reactions cannot, or hardly, be prevented when heating foods. Only by removing the sugars or the amino acids, or making the product very acid or alkaline, the reactions can be prevented. The **Amadori rearrangement** is an organic reaction describing the acid or base catalyzed isomerization or rearrangement reaction of the N-glycoside of an aldose or the glycosylamine to the corresponding 1-amino-1-deoxy-ketose. The reaction is important in carbohydrate chemistry. The reaction mechanism is demonstrated starting from the reaction of D-mannose in its closed (**1**) and open-form (**2**) with ammonia to produce the 1,1-amino-alcohol (**3**), which is unstable and loses water to the glysosylamine (again the open imine (**5**) and the closed form hemiaminal (**4**), which is the starting point for the actual Amadori rearrangement. By treatment of the glycosylamine with pyridine and acetic anhydride, the imine group rearranges and the intermediate enol, in turn, rearranges to the ketone. In this particular reaction, all the alcohol and amino groups are acylated as well. The reaction is associated with the Mailliard reaction in which the reagents are naturally occurring sugars and amino acids. Recent study is revealed the possibility of Amadori rearrangement during interaction between oxidized dextran and gelatin. An Amadori product is an intermediate in the production of an advanced glycation end-product (AGE) as a result of glycation.

The formation of an advanced glycation end-product involves the following steps:

1. Formation of a Schiff base: for example the aldehyde group of a glucose molecule will combine with the amino group of a lysine molecule (in a protein) to form an imine or Schiff base, which is a double bond between the carbon atom of the glucose and the nitrogen atom of the lysine.
2. Formation of an Amadori product: the Amadori product is a re-arrangement from the Schiff base, wherein the hydrogen atom from the hydroxyl group adjacent to the carbon-nitrogen double bond moves to bond to the nitrogen, leaving a ketone.
3. Formation of an advanced glycation end-product (AGE): the Amadori product is oxidized, most often by transition metal catalysis.

The first two steps in this reaction are both reversible, but the last step is irreversible.

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