Chapter 6
Advanced Glycation End Products (AGEs)

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6.1 Introduction

Until after the 1940s, when there were some reports about the nutritional loss in milk powder due to the reaction between lactose and milk proteins, the consequences of Maillard reaction were not recognized [1]. Soon after it was understood that this reaction not only takes place during heating of foods but also in vivo, Maillard reaction has gained much more attention. With the identification of a non-enzymatic glycosylated variant of hemoglobin (HbA_{1c}) in the blood of diabetic patients [2], "glycation" term was introduced to the literature. In the 1980s, Monnier and Cerami [3] supposed that the Maillard reaction of proteins could have a causative role in the aging of extracellular matrix proteins and related pathologies, and since then the interest in the field of the Maillard reaction in vivo has increased exponentially.

The chemistry behind the Maillard reaction/glycation is very complicated. Even in simple reaction systems, for example, in glucose and glycine solutions, many tens of reaction products are formed. Therefore, even in such simple systems, the Maillard reaction mechanisms have not been fully elucidated, and all the reaction products have still not been identified.

Maillard reaction/glycation affects many food quality parameters such as color, sensorial properties, textural properties, and protein functionality. However, the so-called advanced glycation end products (AGEs), which are formed at the later

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stages of the Maillard reaction during food processing, might have some undesired properties. The results of the animal and human studies confirm that dietary AGE levels have direct and indirect effects on AGE accumulation in the body and further complications in degenerative diseases. Therefore, inhibition of glycation reactions during food processing is an important issue since this may help to reduce the dietary intake of AGEs.

In this chapter, following brief information about protein glycation, the consequences of glycation and the contribution of dietary AGEs will be discussed. The formation routes of the main glycation products in foods will be explained, and their analysis methods will be summarized. The major mitigation strategies developed so far will be evaluated.

6.1.1 Protein Glycation

Glycation refers to the addition of a sugar moiety to a protein molecule and occurs during the Maillard reaction. In the Maillard reaction, the amine moiety from free amino acids, peptides, or proteins reacts with the carbonyl group of a reducing sugar, oxidized lipids, vitamin C, or quinones. Glycation takes place in three stages as commonly accepted; "early", "intermediate", and "advanced" stages. However, it should be noted that the reactions occur simultaneously depending on conditions [1].

Glycation is initiated with the nucleophilic addition of amino groups of an amino acid-free or within a protein molecule to the carbonyl group of a reducing sugar such as glucose, fructose, lactose, or maltose. The covalent attachment results in the formation of a reversible and unstable Schiff base (Fig. 6.1). After the condensation reaction, the so-called Schiff base undergoes an arrangement to form an Amadori product (or Heyns product, if the reducing sugar is a ketose), which is the first stable

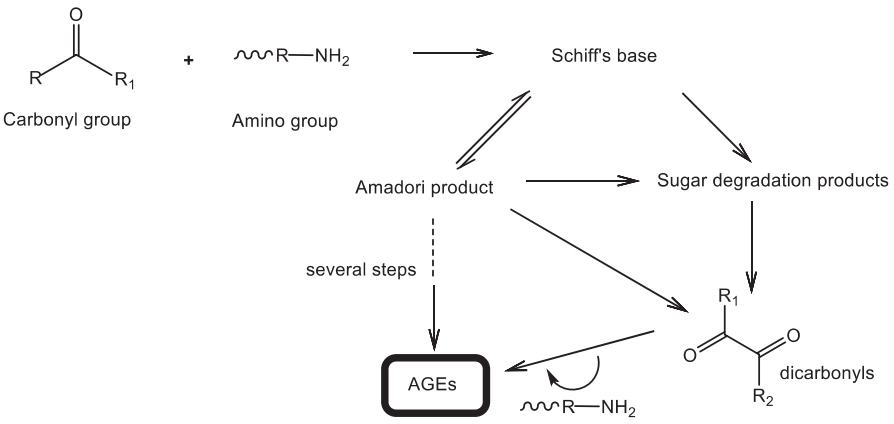

Fig. 6.1 Simplified scheme of advanced glycation in food products

product of the reaction. *N*-ε-fructosyllysine, *N*-ε-maltulosyllysine, or *N*-ε-lactulosyllysine are the major Amadori compounds generated in the early stage of protein glycation during food processing. In the intermediate stage of glycation, by the degradation of Amadori compounds via enolization and elimination reactions, reactive carbonyl species, known as dicarbonyl compounds or oxoaldehydes, are formed. Dicarbonyl compounds might also be formed by caramelization solely during food processing, and this might occur to a larger extent compared to their formation via the degradation of Amadori compounds. The formation of dicarbonyl compounds is discussed in Chap. 2. Some early glycation compounds and dicarbonyl compounds are shown in Fig. 6.2. The dicarbonyl compounds are very reactive, and hence they react immediately with the side chains of peptides and proteins to form advanced glycation end products (AGEs) in the advanced stage. The ε-amino group of lysine, guanidino group of arginine, sulfhydryl group of cysteine residues, and the N-terminal amino group of any amino acids are susceptible for the derivatization by 1,2-dicarbonyl compounds. When oxidation takes place with glycation, the products formed are also called glyco-oxidation products. The great variety of the carbonyl species produced through sugar autoxidation and lipid peroxidation results in a great variety of AGEs in food systems. So far, several glycation products such as *N*-ε-fructoselysine (FL), pyrraline, pentosidine, *N*-ε-carboxymethyllysine (CML), *N*-ε-carboxyethyllysine (CEL), *S*-carboxymethylcysteine, glyoxal lysine dimer (GOLD), methylglyoxal lysine dimer (MOLD), and 3-deoxyglucosone lysine dimer (DOLD) have been identified in processed foods [4–7]. The formation and occurrence of these compounds will be discussed in Sect. 6.2.

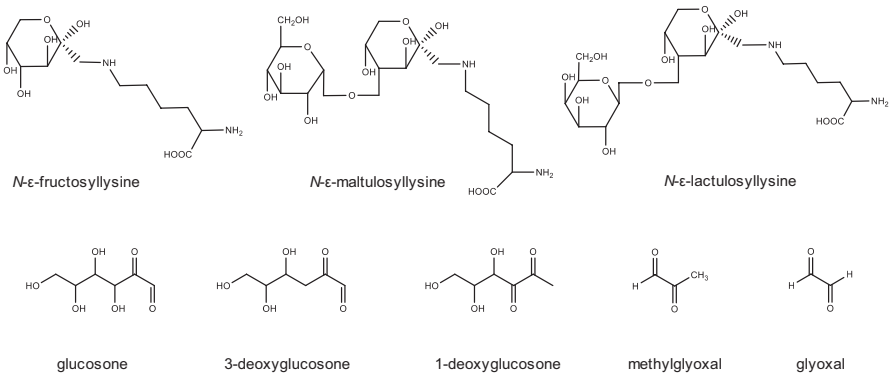

Fig. 6.2 Amadori compounds and 1,2-dicarbonyl compounds formed in the early and intermediate stages of the Maillard reaction

6.1.2 Factors Affecting Glycation

The extent of glycation and the formation of glycation products in food systems depend on several factors such as temperature, reaction time, reaction environment (water content, water activity), reactant species, pH of the reaction medium, the presence of oxygen, and protein conformation.

The extent of glycation is determined by the severity of the heat treatment, either by the increase in temperature or heating time. Glycation is accelerated by the increase in processing temperature. Mild heat treatment results mostly in the formation of Amadori products. However, when the processing temperature or time extends, subsequent degradation of Amadori compounds leads to the formation of dicarbonyl compounds and AGEs. The diversity of the amino acids within a protein molecule involved in the reaction increases by the increase in heating temperature.

Glycation proceeds at a higher rate in dry heating conditions than in aqueous conditions due to the dilution effect of the reactants in the aqueous environment. Since the condensation reaction between the carbonyl and amine group generates water [8], Amadori rearrangement product formation is restricted in the presence of water. The water in the reaction medium also affects the site-specificity of the reaction. In several studies, it was revealed that the lactosylation site of β-lactoglobulin differs when protein was heated in solution or in the dry state. ^{47}Lys [9–11] and ^{100}Lys [9, 12, 13] were found to be preferentially lactosylated during heating in solution, whereas ^{47}Lys and ^{91}Lys were lactosylated during the heating of β-lactoglobulin in the dry state [14, 15].

Due to the fact that water activity (a_w) affects the molecular mobility of the reactants, protein conformation, surface area, dynamics, and accessibility of amino groups besides the dissolved oxygen concentration and pH of the medium, site-specific glycation is affected by a_w [16]. In addition to these, the solvent and its contact with the protein matrix influence the electrostatic and biophysical properties of the protein [16]. Generally, browning is considered to occur at its maximum in a_w values between 0.5 and 0.8 [17], and researchers showed increased glycation of proteins at intermediate a_w values [18, 19]. The reaction rate is decreased at low values of a_w due to the diffusional limitations of the reactants, and at high values of a_w, the decrease is attributed to the dilution effect and inhibition by water. Relative humidity (water activity) affects the formation of dicarbonyl compounds, and the proportions of dicarbonyls formed differ for the samples heated at low, intermediate, and high relative humidities. For instance, it was shown that under high relative humidity values in a model system containing sodium caseinate and lactose, 3-deoxypentosulose and galactosyl 2-pentosulose were produced, whereas galactosyl hexosulose and 1,4-dideoxyhexosulose were produced in higher amounts under low relative humidities [20].

In terms of reactant species, the type of carbonyl source is very important. The reactivity of carbonyl compounds generally increases in the following order [21]:

- Ketoses < aldoses
- Polysaccharides < disaccharides < hexoses < pentoses < tetroses < trioses.
- Oxoacids < saccharides < ketones < aldehydes < α-dicarbonyl compounds.

The carbonyl source has effects on the extent of glycation and site-specificity of the reaction. Aldose sugars are more reactive toward the lysine residues of proteins than ketose sugars, and these sugars prefer different glycation sites. Glycation of β-lactoglobulin was three to four times more efficient with glucose than that of with fructose [22]. ^{13}Lys, ^{16}Lys, $^{93/94}$Lys, ^{98}Lys, ^{108}Lys, ^{114}Lys, and ^{122}Lys residues of α-lactalbumin were glycated by allose and glucose, while ^{13}Lys, $^{98/108}$Lys, and ^{114}Lys were glycated with fructose and psicose [23]. Monosaccharides are generally more reactive than disaccharides; β-lactoglobulin was found to attach more galactose (up to 22 adducts) than lactose (up to 14 adducts) in a study where it was confirmed by LC/MS that the products were mainly the early glycation products [24]. Heating of β-casein with either glucose or glyoxal at 95 °C for 1 h in solution resulted in modification at ^{107}Lys and ^{176}Lys [25]. The Amadori product was formed preferentially on ^{176}Lys rather than ^{107}Lys, while the proportion of *N*-ε-carboxymethyllysine (CML) on both lysine residues was similar. ^{202}Arg was found to be the main modification site of β-casein with glyoxal [25].

Proteins react with carbonyl compounds primarily through the ε-amino group of lysine residues and, to a smaller extent, through the α-amino groups of N-terminal amino acids and other amino acid functional groups, such as the thiol group of cysteine and guanidine group of arginine. The availability of glycation sites within a protein molecule greatly influences the extent of reaction. Given the fact that the accessibility of glycation sites within a protein molecule depends on its conformation, any environmental factor affecting a protein's conformation has an indirect effect on the glycation behavior. pH- or temperature-induced changes (including denaturation, aggregation, or hydrolysis) would have an effect on protein conformation and thus on the glycoforms produced. The composition of the reaction medium (the presence of lipids, minerals, other proteins, and reducing agents) and the molecular weight of the carbonyl attached to the protein influence the conformation of the protein [16]. Glycation of ovalbumin was favored when the tertiary structure was disrupted; after reducing the disulfide bonds, the number of glycated sites was increased from 7 to 12 in a dry state and from 1 to 2 in aqueous conditions [26].

Researchers suggested that the structural accessibility of lysine residues is the most important factor affecting the preferential glycation sites [27, 28]. Hydrogen bonding between the N-H of lysine residues with water and with the C=O with other amino acids in the polypeptide chain may protect lysine residues against glycation [29]. pK_a values, phosphate and bicarbonate ions, and proximate amino acids have effects on the reactivity of lysine residues and play a role especially in the early stages of glycation [45–48]. The reactivity of a lysine residue within a protein

sequence may be explained by their position adjacent to the neighboring basic amino acids in the primary or tertiary protein structure. The Maillard reaction is accelerated when an acidic amino acid is present near the lysine residue in the primary structure or in the 3D conformation. Also, amino acid residues of Ile, Leu, Phe, and Arg increase the lysine reactivity in lysine-containing dipeptides [30]. The presence of histidine or lysine residue near to lysine was shown to promote the glycation tendency of lysine [31–33].

The pH of the reaction medium is another factor affecting protein glycation. The rate of carbonyl-amine addition is related to the pK_a value of the amino compound, which determines the concentration of reactive species at a certain pH. Lysine is the most reactive amino acid in a wide pH range, whereas aliphatic aromatic amino acids valine, leucine, and isoleucine are the least reactive ones. pH is a determining factor whether decomposition takes place by 1,2- or 2,3-enolization. 1,2-Enolization is favored that allows protonization of the Amadori product in acidic media, whereas 2,3-enolization dominates in alkaline solutions and in nonaqueous conditions [21]. Increase in pH led to increased glycation in several studies conducted with milk proteins. The molecular weight of fructosylated and glucosylated β-lactoglobulin was increased with the increase of pH from 5.0 to 8.0 [22]. Thirteen glucose molecules on average were attached to β-lactoglobulin at pH 5.0, while 14 glucose moieties were attached at pH 8.0 [22]. Isoelectric point of a protein is important in terms of glycation rate. Thomsen et al. [18] reported that increasing pH during the preparations of dry reaction media caused an increase in both the rate and the degree of lactosylation. The reaction solution prepared at pH 5.0 was less lactosylated than those prepared at pH 6.0 and 7.0. Since pH 5.0 is close to the pI of β-lactoglobulin, protein-protein interactions might have evolved, thus making it more difficult for free amino groups to react with lactose. The reactivity of amino groups was limited due to the low amount of reactive unprotonated amine groups at low pH. However, when the pH is increased, negative charges on the protein molecule increase, causing repulsion and a decrease in protein-protein interactions. Therefore, the amount of free reactive amino groups increases yielding an increased lactosylation [18].

The presence of oxygen in the medium also affects the glycation of proteins. More glucose attachment is favored in the presence of oxygen. Oxygen level has an important effect in the later stages of glycation; dicarbonyl compounds generated through glycoxidation also participate in the reaction, therefore increasing the glycation rate. It was reported in a study where lysozyme was heated at 50 °C for 14 days that due to the higher reactivity of dicarbonyl compounds (generated in the presence of oxygen) for the guanidine group of arginine residues, the involvement of arginine in glycation favored the glycation rate [34]. The reaction rate for the systems having the same conditions but containing fructose was lower than that of glucose, and this was explained by the fact that glucose was more susceptible to glycoxidation under dry heating conditions. It was stated that fructose mainly reacted with the ε-amino group of lysine residues, whereas glucose reacted with all primary amino groups and guanidine groups of arginine, as well. Due to the lower reactivity of fructose, a narrow distribution of glycoforms was obtained; however, for glucose, a higher glycation rate and a wider range of glycoforms were observed [34].

6.1.3 Consequences of Glycation and Contributions of Dietary AGEs

The glycation of protein is of particular importance for food chemistry since color development (such as the color of bread crust, roasted coffee, French fries, and fried onions) and aroma formation (roasted coffee and bakery products) are typical results of this reaction. On the contrary, undesired color formation as a quality defect (in the production of dried foods, milk powders, as well as fruits and vegetables), formation of off-flavors (such as cooked flavor in UHT milk), reduction of the nutritional value of foods (due to modification in amino acids), and formation of toxic compounds (such as HMF, acrylamide, and furan) are the drawbacks of glycation in food systems.

After the identification of the nonenzymatic glycosylated variant of hemoglobin, HbA_{1c}, in the blood of diabetic patients [2], it was understood that glycation also takes place endogenously; since then, Maillard reaction has attracted attention in the field of biochemistry and medicine. In the human body, AGEs arise not only from glucose but also from the reactive products of glucose metabolism (such as glucose-6 phosphate, triose phosphates, and fructose-3-phosphate) and nonenzymatic degradation. Methylglyoxal, glyoxal, 3-deoxyhexuloses, transformation products of ascorbic acid, or some secondary decomposition products of lipid hydroperoxides react with proteins [21, 35]. AGEs have some undesirable consequences in terms of chronic and especially age-related disorders. They may take part in chronic and degenerative diseases, such as diabetes, renal failure [36], atherosclerosis [37, 38], and Alzheimer's and Parkinson's diseases [39, 40]. Glycation is increased in diabetes mellitus, where glyoxal, methylglyoxal, and 3-deoxyglucose, besides plasma glucose concentration, are increased and in uremia, where many α-oxoaldehydes are increased [41]. The body proteins of diabetic patients were found to be two to three times more glycated than those of healthy humans, due to the increased level of blood sugar [42]. Amadori products are the predominant form of circulating glycated protein in patients with diabetes [43, 44]. Uremic patients accumulate pentosidine or CML in the plasma and tissues [45]. The serum level of pentosidine was found to be 2.5 times greater in patients with diabetes and 23 times greater in patients with diabetes with end-stage renal disease [46]. It was stated that patients with advancing age, diabetes, and end-stage renal disease have a very high incidence of atherosclerotic vascular disease [38, 46]. An excess of blood or tissue AGEs is also associated with rheumatoid arthritis, amyloidosis, and Alzheimer's and other neurodegenerative diseases [47].

AGEs are generally accumulated in long-lived proteins such as collagen and eye lens due to the low turnover of these proteins. Cataract is one of the most common consequences of diabetes. A high correlation was obtained between pentosidine cross-links and the degree of pigmentation in cataractous lenses, indicating that pentosidine formation in human lens leads to brunescent cataracts [48].

There are two major sources contributing to the total pool of AGEs in the body: AGEs that are consumed with foods and endogenous AGEs that are generated by

the nonenzymatic glycation of proteins, lipids, and nucleic acids, especially under hyperglycemic conditions in diabetes [37, 49]. The interrelationship between dietary AGEs and AGEs in the body has been established with several animal and human studies. In a study [50] where laboratory rats were fed with glucose-lysine model food (containing AGEs) for 3 months, dietary dicarbonyl compounds from the diet or dietary CML itself were found to be responsible for CML accumulation in hearts and tendons. Moreover, regular consumption of dietary AGEs in healthy individuals promoted CML accumulation in some organs, such as cardiac tissue and tail tendon [50]. Feeding laboratory mice with high-AGE diet resulted in twofold higher plasma AGE levels than the levels of mice fed with low-AGE diet [51]. Proteinuria increased during feeding with high-AGE diets in remnant kidney models in rats [52, 53]. High-AGE diets were also shown to accelerate the progression of renal fibrosis [52]. In another study [54] where casein-linked lysinoalanine (LAL), *N*-ε-fructoselysine (FL), and *N*-ε-carboxymethyllysine (CML) were administered to rats at different doses for 10 days, it was concluded that kidneys were the predominant sites for accumulation and excretion of LAL, FL, and CML. It was also observed that the endogenous load of compound in either plasma or tissue was increased by its dietary intake [54]. In a mouse model of obesity, targeted reduction of the advanced glycation improved renal function and glycemic control in obesity [55].

Ten percent of consumed dietary AGEs were reported to be absorbed by humans, and this was correlated with the circulating and tissue levels of AGEs [56]. Dietary AGE level of healthy people showed correlation with the circulating AGE levels, such as CML and methylglyoxal, as well as with oxidative stress markers [57]. Furthermore, reduction of AGEs in the diet in diabetes patients [58] and kidney disease patients [59, 60] or healthy individuals [61] also reduced the markers of oxidative stress and inflammation. In a human study where people consumed a standard diet (high amounts of AGE-containing diet) or steamed diet (low in AGEs) for a month, the urinary CML excretion was found to be 40% higher and fasting plasma CML was 7% higher in the standard diet group. This suggested that dietary CML was absorbed in the intestines and rapidly excreted, confirming the results obtained in animals [62].

The results of the animal and human studies confirm that dietary AGE levels have direct and indirect effects on AGE accumulation in the body and further complications in degenerative diseases. Therefore, inhibition of glycation reactions during food processing is an important issue since this may help to reduce the dietary intake of AGEs. The methods useful for the mitigation of glycation will be discussed in Sect. 6.4.

6.2 Occurrence of AGEs in Foods

As stated above, Amadori products are the early products of protein glycation. The condensation reaction between a carbonyl moiety and an amine residue of a protein results in the formation of an unstable Schiff base. After the condensation reaction,

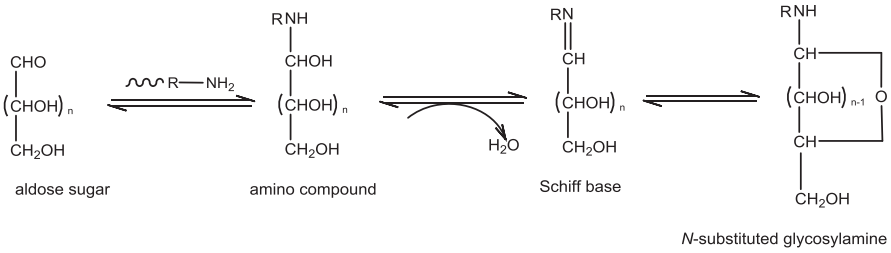

Fig. 6.3 Sugar-amine condensation to form *N*-substituted glycosylamine

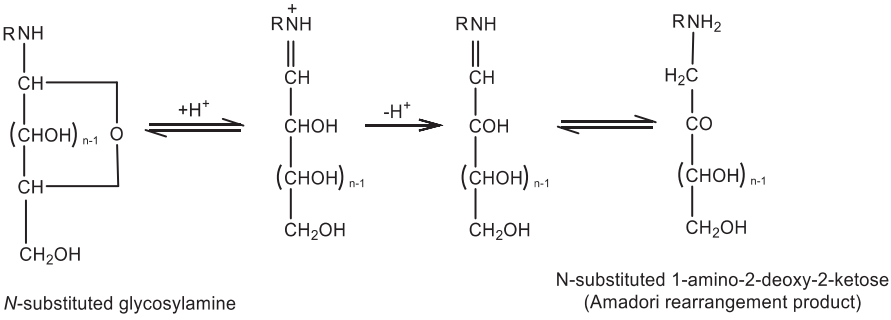

Fig. 6.4 Amadori rearrangement

the so-called Schiff base undergoes an arrangement to form an Amadori product (*N*-substituted 1-amino-1-deoxy-2-ketoses), which is the first stable product of the reaction (Figs. 6.3 and 6.4). In the case of food proteins, the ε-amino group of lysine is the most susceptible target for the attack of carbonyls; so the product formed is mostly lysine derivatives such as *N*-ε-fructosyllysine, *N*-ε-lactulosyllysine, and *N*-ε-maltulosyllysine; however, the *N*-terminal α-amino acids also react to the Amadori compounds. Free amino acids are also significantly modified [63], but it will not be discussed in this chapter.

Amadori products are quantitatively the most prevalent glycation products in many food systems. Depending upon the temperature and time of processing or storage of a food product, up to 70% of lysine might react to the Amadori product [4]. The formation of *N*-ε-fructosyllysine causes the loss of nutritional quality of proteins, since lysine bioavailability is decreased due to lysine modification. Therefore, furosine formation is investigated in many foods for the evaluation of the nutritional quality of heat-treated foods. Furosine content is measured as the quality indicator of milk products, honey, cereals, pasta, and several other food products [63–67]. It is also used for regulatory purposes; in mozzarella cheese, the furosine content indicates the addition of heat-treated cow's milk to the original product made from low temperature-treated buffalo's milk [68].

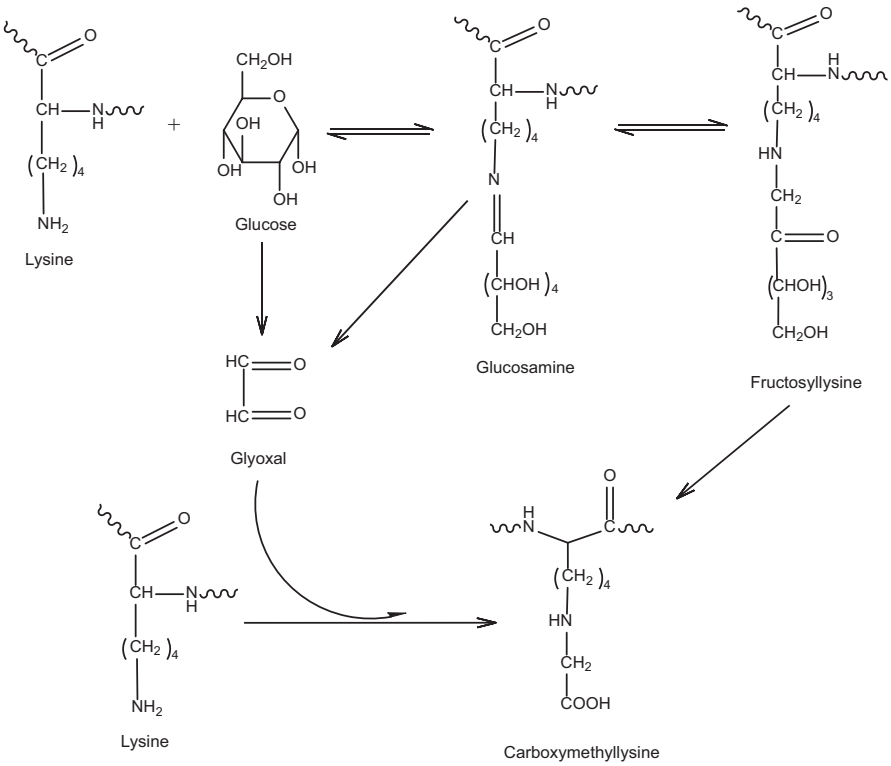

Fig. 6.5 Pathways of CML formation. (Adapted from Han et al. [69])

Furosine concentration in foods does not always correlate well with the severity of heat treatment; it does not increase linearly with heat damage. Amadori compounds may degrade via enolization and elimination reactions in the intermediate stages of glycation, forming dicarbonyl compounds. These dicarbonyl compounds are so reactive that they immediately react with the amine residues of proteins to form the advanced glycation end products. The Amadori compound is also oxidized to form the advanced glycation compounds. CML is the first and the most common amino acid derivative of the advanced glycation that was quantified in foods and a major AGE structure formed in vivo. It can be formed through various pathways as shown in Fig. 6.5 [69]. In the autoxidative pathway, glyoxal is derived from glucose and then reacts with lysine residues to form CML [70]. In the Namiki pathway, CML is formed by the reaction with lysine residues and glyoxal derived from Schiff base [71]. In another pathway, Amadori product is oxidized to form CML [72].

CML is present in a range of heat-treated foods such as dairy products [73–76], cereals and bakery products [7, 77], meat [74, 78, 79], and nuts [80, 81]. Lipid oxidation occurs simultaneously during heating in some food products, and lipid

oxidation products (highly reactive aldehydes and ketones, such as glyoxal) may be involved in the formation of AGEs. In a study [82], vegetable and fish oils were treated under accelerated storage conditions and cooking conditions, and it was found that fish oils with polyunsaturated fatty acids produced more glyoxal than vegetable oils. Glyoxal derived from lipid oxidation participated in food-derived CML formation [82]. Fu et al. [83] also showed that CML was formed in vitro during copper-catalyzed oxidation of PUFA in the presence of protein. Therefore, during thermal processing, CML may be formed through one or more of the mentioned pathways, depending on the food composition (precursors) and process conditions.

N-ε-carboxyethyllysine (CEL) (Fig. 6.6), which is formed by the reaction between methylglyoxal and lysine, is a homolog of CML and is found in several food products. He et al. [7] reported CEL levels ranging between 225 and 820 mg/kg protein in bread crust, between 159 and 452 mg/kg protein in biscuits, and between 146 and 373 mg/kg protein in fried dough sticks. Commercial sterilization of chicken, beef, and pork meat was found to increase protein-bound CML and CEL levels significantly [79]. The amounts of protein-bound CML and CEL in fish muscle increased as the heating (100 °C) time increased [84]. In a study where the effect of irradiation on CML and CEL formation and its relationship with lipid oxidation in meat products during storage was investigated [85], a linear correlation was found between the loss of polyunsaturated fatty acids content and the increase in CML and CEL contents in the irradiated beef samples during 6 weeks of storage. It was indicated that irradiation-induced lipid oxidation promotes CML and CEL formation through oxidation pathway [85].

Another important AGE, pyrraline (Fig. 6.6), which is the product of lysine and 3-deoxyglucosone, is found in high heat treatment-applied foods, such as bread crust (up to 3.7 g/kg protein), cookies (120 mg/kg protein), dried carrot products (up to 378 mg/kg protein), or roasted peanuts (up to 382 mg/kg protein) [80, 86, 87]. Considerable amounts of pyrraline were also reported in beer [88] and peptide-enriched drinks [89].

Pronyllysine results from lysine side chains and acetylformoin (Fig. 6.6) and was quantified up to 62 mg/kg in the crust and 6 mg/kg in the crumb of bread [90–92], whereas 0.43 mg/kg in Pilsner-type pale beer and 0.92 mg/kg in dark beer [90].

Argpyrimidine [*N*-δ-(5-hydroxy-4,6-dimethylpyrimidine-2-yl)-L-ornithine] is an AGE derived from the reaction of methylglyoxal with arginine residues (Fig. 6.6). It was detected as a free amino acid derivative form in beer [88, 93].

Pentosidine is a cross-linker formed by the reaction of pentose with the lysine and arginine residues of proteins (Fig. 6.6). In milk, up to 5 mg pentosidine/kg protein was detected in some samples of sterilized and UHT milk, whereas higher amounts up to 23 mg/kg protein were obtained in alkali-treated bakery products, such as pretzels. The highest amount of pentosidine was found in roasted coffee, ranging from 11 to 40 mg/kg protein [94]. 10–158 μg/100mL of pentosidine was detected in soy sauce, sour-sweet sauce, barbecue sauce, or tomato sauce, and meat treated with sauces also contained high amounts of pentosidine after baking and frying [95].

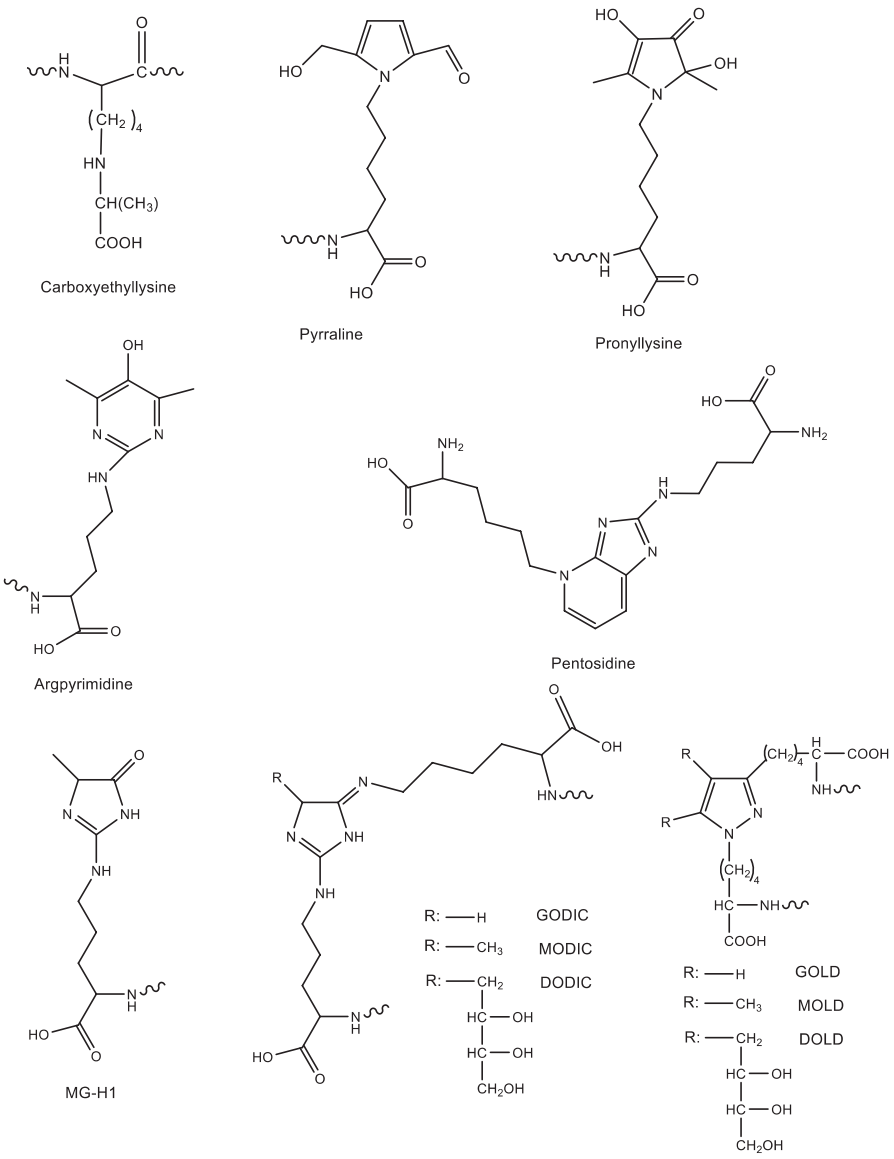

Fig. 6.6 Examples of AGEs found in foods

DOLD, GOLD, and MOLD (Fig. 6.6), the lysine dimers resulting from the reaction between two lysine side chains and two molecules of 3-deoxyglucosone, glyoxal and methylglyoxal, respectively, were found in the enzymatic hydrolysates of bakery products and boiled egg white in the mg/kg level, together with the cross-links between lysine and arginine (DODIC, GODIC, MODIC) [96]. The concentrations of MODIC and GODIC were found to be almost five times higher than those of their

corresponding imidazolium compounds, MOLD and GOLD. 151 mg MODIC/kg protein was found maximum in butter biscuit samples [96]. Soy sauce-based seasonings were also found to contain up to 0.19 mg/L GOLD and up to 0.30 mg/L MOLD in the free form [97].

Imidazolinones are formed by the reactions of the guanidine group of arginine residues with dicarbonyl compounds, such as methylglyoxal and 3-deoxyglucosone (Fig. 6.6). The acid-labile imidazolinone resulting from the reaction between peptide-bound arginine and methylglyoxal was quantified in alkali-treated bakery products [98]. The amounts of imidazolone after complete enzymic digestion ranged between 9 and 13 mg/g protein, indicating that between 20 and 30% of the arginyl residues might react with methylglyoxal during the bakery process [98]. Traces of methylglyoxal-dihydroxyimidazoline were detected at ^{124}Arg of β-lactoglobulin in sterilized and evaporated milk and small amounts of methylglyoxal-imidazolinone were shown to be present at ^{40}Arg and ^{124}Arg in more severely heated products [99]. In different beer types, 35.5–136.6 mg/kg protein MG-H1 was detected [88], whereas free forms of MG-H1 were also determined up to 2.47 mg/L in beer and beer-type liquors [88, 97] and up to 7.75 mg/L in soy sauce-based seasonings [97].

Some AGEs and their concentrations in different food products are given in Table 6.1.

6.3 Analysis Methods

Monitoring of glycation is challenging given the complexity of the reaction. Until today, many techniques have been used to determine the AGEs in food products and the body, including methods that use only simple absorbance measurements or more sophisticated instruments.

Due to the formation of brown-colored products in the Maillard reaction, the absorbance at 420 nm increases by the degree of glycation; hence, absorbance measurements at 420 nm might give an idea about the extent of glycation [104, 105]. Fluorescence measurements at 340–350 nm excitation and 400–440 nm emission have also been carried out to monitor protein glycation [106–109]; however, only AGEs with fluorescent properties such as pentosidine and crossline can be detected by this method, whereas nonfluorescent AGEs such as CML and CEL cannot be detected. Fluorescamine (4-phenylspiro [furan-2 (3H, 1-phtalan]-3–3′-dion) assay, which is based on the reaction between this reagent and the primary amino groups of protein and amino acids [110], also gives an idea about the extent of glycation. The resulting fluorescence decreases in case of glycation due to the decrease in the free amino groups [110].

Immunological detection and quantification of protein glycation based on ELISA [56, 111–114] have been widely used in biomedical and food science investigations. Although the ELISA method is easy and rapid, it is not regarded as reliable at present since the precision and accuracy are not high. The results are expressed in arbi-

Table 6.1 AGE contents in food products

Food category	AGE type	AGE content	Method	References
Dairy products				
Raw milk	Furosine	35–55 mg/kg protein	HPLC	[100]
	CML	Up to 9.3 mg/kg protein	LC-MS/MS	[74, 75]
UHT milk	Furosine	500–1800 mg/kg protein	HPLC	[76, 100]
	CML	Up to 34.1 mg/kg protein	LC-MS/MS	[75, 76]
Pasteurized milk	Furosine	Up to 200 mg/kg protein	HPLC	[76, 100]
	CML	Up to 16.3 mg/kg protein	LC-MS/MS	[74–76]
Sterilized milk	Furosine	5000–12,000 mg/kg protein	HPLC	[100]
	CML	343 mg/kg protein	RP-HPLC	[73]
	Pentosidine	0.1–2.6 mg/kg protein	HPLC-FLD	[94]
Evaporated milk	Furosine	3400–8800 mg/kg protein	HPLC	[100]
	CML	Up to 1015 mg/kg protein	RP-HPLC and UPLC-MS/MS	[73, 74]
	Pentosidine	0.3–0.6 mg/kg protein	HPLC-FLD	[94]
Condensed milk	CML	205 mg/kg protein	LC-MS/MS	[75]
Infant formula (liquid)	Furosine	Up to 12,500 mg/kg protein	HPLC and LC-MS/MS	[76, 100]
	CML	Up to 62.9 mg/kg protein	LC-MS/MS and GC-MS	[76, 77]
Infant formula (powder)	Furosine	Up to 18,900 mg/kg protein	HPLC and LC-MS/MS	[76, 100, 101]
	CML	Up to 148 mg/kg protein	LC-MS/MS and GC-MS	[76, 77, 101]
	CEL	7.1–13.1 mg/kg protein	LC-MS/MS	[101]
Butter	CML	37.1 mg/kg protein	UPLC-MS/MS	[74]
Coffee cream	CML	Up to 618 mg/kg protein	RP-HPLC	[73]
Whey cheese	CML	1691 mg/kg protein	RP-HPLC	[73]
Cheese	Furosine	Up to 290 mg/kg protein	RP-HPLC	[100, 102, 103]
	CML	23.2 mg/kg protein	UPLC-MS/MS	[74]

(continued)

Table 6.1 (continued)

Food category	AGE type	AGE content	Method	References
Bakery products				
Bread crust	CML	58–94 mg/kg protein	LC-MS/MS	[7]
Bread crumb	CML	14–34 mg/kg protein	LC-MS/MS	[7]
Biscuits	CML	50–117 mg/kg protein	LC-MS/MS	[7]
	CEL	462.5 mg/kg protein	LC-MS/MS	[101]
Pasta	Furosine	400–8500 mg/kg protein	HPLC	[100]
Cookies	CML	5–35 mg/kg protein	GC-MS	[77]
Corn flakes	CML	6–8 mg/kg protein	GC-MS	[77]
Meat products				
Raw minced beef	CML	3.9 mg/kg protein	UPLC-MS/MS	[74]
	CML	2.76–4.32 mg/kg	LC-MS/MS	[79]
	CEL	2.32–3.18 mg/kg	LC-MS/MS	[79]
Pasteurized ground beef	CML	3.12–19.96 mg/kg	LC-MS/MS	[79]
	CEL	2.65–11.89 mg/kg	LC-MS/MS	[79]
Boiled minced beef	CML	27.3 mg/kg protein	UPLC-MS/MS	[74]
Fried minced beef	CML	61.1 mg/kg protein	UPLC-MS/MS	[74]
Chicken breast, boiled	CML	17.2 mg/kg protein	UPLC-MS/MS	[78]
Chicken breast, roasted	CML	17.4 mg/kg protein	UPLC-MS/MS	[78]
Chicken breast, fried	CML	23.5 mg/kg protein	UPLC-MS/MS	[78]
Coffee	CML	84.1 mg/kg protein	UPLC-MS/MS	[78]
	Pentosidine	10.8–39.9 mg/kg protein	HPLC-FLD	[94]
Nuts				
Unroasted peanut	Furosine	Up to 24 mg/kg protein	HPLC	[80]
Roasted peanut	Furosine	129–267 mg/kg protein	HPLC	[80]
	CML	5–77 mg/kg protein	GC-MS	[80]
Peanut puffs	Furosine	166–256 mg/kg protein	HPLC	[80]
	CML	61–63 mg/kg protein	GC-MS	[80]
Peanut butter	Furosine	73–91 mg/kg protein	HPLC	[80]
	CML	63–203 mg/kg protein	GC-MS	[80]

(continued)

Table 6.1 (continued)

Food category	AGE type	AGE content	Method	References
Unroasted almond	CML	1.5 mg/kg[a]	LC-MS/MS	[81]
	CEL	1.3 mg/kg[a]		[81]
	Pyrraline	not detected		[81]
Roasted almond	CML	3.7–4.9 mg/kg[a]	LC-MS/MS	[81]
	CEL	5.1–10.1 mg/kg[a]		[81]
	Pyrraline	8.2–42.8 mg/kg[a]		[81]
Beer	Pyrraline	55–400 mg/kg protein	HPLC-MS/MS	[88]
	Pronyllysine	0.43–1.07 mg/kg	HRGC-MS	[90]
	MG-H1	35.5–136.6 mg/kg protein	HPLC-MS/MS	[88]
	MG-H1	0.09–0.23 mg/L	LC-MS/MS	[97]
	Argpyrimidine	0.1–4.1 μg/L[b]	HPLC-MS/MS	[88]
	Argpyrimidine	27 nmol/L[b]	HRGC-MS	[93]

[a]free + bound AGE
[b]free form AGE

trary units rather than actual concentrations. The method requires the use of specific antibodies for each compound, and furthermore, the food matrix affects the specificity of the assay.

Determination and quantification of glycation products might be performed more precisely by HPLC with UV-DAD detectors, LC-MS/MS, and GC-MS. Some AGEs such as furosine and CML are regarded as indicators of glycation, and they have been used as markers for the extent of glycation. Furosine has been used as a reliable indicator of thermal damage in foods since its detection in 1966 [68]. It is one of the first identified early glycation products in foods and is the most common chemical indicator of the Amadori product [68]. Furosine is formed during the acid hydrolysis of the Amadori products, *N*-ε-lactulosyllysine, *N*-ε-fructosyllysine, and *N*-ε-maltulosyllysine and tagatosyllysine [115]. Generally, food products are hydrolyzed by using concentrated acids, such as 6 N or 8 N hydrochloric acid (Fig. 6.7). The yield of furosine from the Amadori compounds during acid hydrolysis is variational between different Amadori compounds but is considered to be constant under controlled conditions. Different yields ranged from 20% to 30% after hydrolysis in 6 N hydrochloric acid, from 29% to 46% after hydrolysis in 7.8 N hydrochloric acid, and from 46% to 51% after hydrolysis with 8 N hydrochloric acid [115, 116]. If the corresponding conversion factors are known, then monitoring of the Amadori product formation in foods may be evaluated. Similarly, other Amadori compounds may be converted into *N*-(2-furoylmethyl) amino acids (FMAAs) by acid hydrolysis and then may be measured by RP-HPLC [67, 86].

CML is frequently used as a marker for AGE formation in food. Chemical analyses of CML concentrations in food products include extraction of the compound from the food and determination of its level by immunochemical assays or instrumental methods [117]. High-performance liquid chromatography (HPLC), gas chromatography coupled with mass spectrometry (GC-MS), and liquid chromatog-

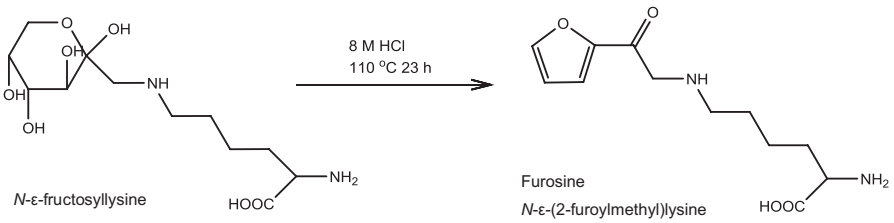

Fig. 6.7 Formation of furosine during acid hydrolysis of Amadori compounds

raphy coupled with tandem mass spectrometry (LC-MS/MS) might be used for the identification and determination of CML. For the determination of protein-bound CML, acid hydrolysis is applied to release CML from the protein. Since CML might be formed from fructosyllysine, sample preparation should be performed with extreme care to avoid any potential undesirable reactions, which might give rise to artifactual CML formation and thus an overestimation of the real content. Therefore, it has been proposed to initially reduce fructosyllysine residues into hexitollysine by sodium borohydride to prevent this process [73, 76]. Delatour et al. [75] proposed that enzymatic digestion might be performed to prevent the artifactual formation of CML mediated by fructosyllysine. However, they concluded that a slight overestimation of CML with enzymatic digestion might be observed. Determination of CML in food products may also be performed by GC analysis [77, 118].

N-ε-carboxyethyllysine (CEL), which is formed by the reaction between methylglyoxal and lysine, is a homolog of CML. Its content may be determined after acid hydrolysis or enzymatic hydrolysis by HPLC and LC-MS/MS methods [7, 81, 84, 101, 119, 120].

Pyrraline, which is the product of lysine and 3-deoxyglucosone, was first identified by amino acid analysis in heated skim-milk powder [121]. Pyrraline amount may be quantified using HPLC techniques either in free form or in protein-bound form after enzymatic hydrolysis, since the pyrrole compound is labile during acid and alkaline hydrolysis [1, 89, 122].

Mass spectrometry is widely used for the analysis of glycation products. Pronyllysine can be determined with HRGC/MS [90–92]; argpyrimidine with LC-MS/MS [81, 88] or high-resolution GC-MS [93]; pentosidine with LC-MS/MS [81] or HPLC with a fluorescence detector [95]; DOLD, GOLD, MOLD, DODIC, GODIC, MODIC, and methylhydroimidazolones with LC-MS [96]; and MG-H1 with HPLC-ESI-MS/MS [88, 99].

The extent of glycation of a protein molecule could be determined by mass spectrometric techniques. ESI-MS and MALDI-MS have been used to evaluate the glycation extent and glycoforms of proteins in different processing conditions [15, 123–125]. In most cases, it was shown that only one or two sugar units were attached to proteins after heating in solution state, whereas multiple glycoforms were obtained in the dry state [15, 123–125]. Mass spectrometry also enables the determination of the glycation sites of the protein molecule. Formation of lactulosyllysine at ^{47}Lys, ^{138}Lys, and ^{141}Lys and also methionine sulfoxide at ^{7}Met, ^{24}Met, and ^{145}Met in β-lactoglobulin was detected by using MALDI-TOF-MS coupled to elec-

trophoretic protein separation and in gel digestion with the endoproteinase AspN [126]. CML formation was shown at different lysine residues of β-lactoglobulin such as ^{47}Lys, ^{60}Lys, ^{91}Lys, ^{135}Lys, and CEL formation at $^{69/70}$Lys and ^{91}Lys by using ultrahigh-performance liquid chromatography tandem mass spectrometry [99]. Traces of methylglyoxal-dihydroxyimidazoline were detected at ^{124}Arg in sterilized and evaporated milk, and small amounts of methylglyoxal-imidazolinone were shown to be present at ^{40}Arg and ^{124}Arg in severely heated products [99].

The use of mass spectrometry also allows the enlightenment of reaction mechanisms for the inhibition of glycation. The ability of phenolic compounds to trap carbonyl compounds and the ability of oxidized forms of catechins to react with the amino groups of proteins were revealed by using different mass spectrometric techniques such as high-resolution ESI-TOF/MS and ESI-ion trap MS [127–130]. The mechanisms of inhibition of glycation will be discussed in the next section.

6.4 Mitigation Strategies

The human organism has a certain protective mechanism to fight against AGE formation. There are chemical and biochemical processes including enzymatic and immune responses. Enzymes such as glyoxalases, aldehyde reductases, aldehyde dehydrogenases, amadoriases, and fructosamine 3-phosphokinases are responsible for the suppression of glycation reactions in the body and the repair of glycated proteins [21]. Nonetheless, in such cases, mainly in the increased level of carbonyl and oxidative stress, these protective mechanisms might be insufficient to struggle with the consequences of glycation. Therefore, AGE inhibitors are used for the treatment of the consequences of glycation.

The medical concept of glycation inhibition includes any mechanism delaying or preventing glycation reactions in vivo. The principle of the inhibition is based on the following strategies [131]:

- Anti-glycation strategies involving scavenging hydroxyl radicals and superoxide radicals to attenuate oxidative stress and reducing the generation of reactive carbonyl compounds.
- Blocking the carbonyl or dicarbonyl attachment to proteins.
- Metal ion chelation since AGE formation is related to the presence of transition metal ions.
- Breaking the cross-linked structures in AGEs.

Pharmaceuticals used as AGE inhibitors (such as aminoguanidine or pimagedine) might cause adverse effects such as gastrointestinal disturbance, anemia, and flu-like symptoms [132, 133]. Therefore, several natural compounds have been investigated for their inhibitory effects on glycation. Food-derived compounds such as spermin and spermidine [134–136], chlorogenic acid [137, 138], and isoflavonoid glycoside puerarin [139] have been shown to exert in vivo anti-glycation effects in human and animal studies.

In a model system composed of bovine serum albumin and glucose/fructose, incubated at 37 °C for 7 days, wild berries were shown to have anti-glycation activity in a concentration-dependent manner, and reduction in the AGE formation was positively correlated with the total phenolic content and related to radical scavenging capacity [140]. In another study [141], vegetable seed extracts were found to exhibit anti-AGE activity in protein-glucose assay (37 °C, 21 days), ranging from 20 to 92% inhibition, while peach and pomegranate extracts exhibited the highest anti-AGE activity in protein-methylglyoxal assay (37 °C, 14 days), ranging from 0 to 79% inhibition [141]. Presence of white grape skin extracts yielded a reduction in the formation of fluorescent AGEs in bovine serum albumin-fructose model system incubated at 37 °C for 3 days [142].

Maillard reaction and glycation have particular importance for the food industry. These reactions affect the organoleptic properties, color development, protein functionality, and nutritional properties of the product. Since glycation reactions are also responsible for the desired flavor and color development, mitigation of glycation in food products is a challenging issue.

The factors affecting glycation was discussed thoroughly in Sect. 6.1.2. Any reaction conditions or environmental factors affecting the rate of glycation such as reactant species, water activity, pH, and oxygen status would affect the progression of glycation; thus, by altering these parameters, glycation could be mitigated. However, addition of functional ingredients able to inhibit glycation is the most frequently used strategy in different food products and food model systems. Table 6.2 summarizes the strategies used for mitigation of glycation in food products and model systems.

Table 6.2 Strategies used for mitigation of glycation

Strategy	Function	Agent	References
Blocking of amines	Covalent attachment to amine residues	Catechins	[127, 128]
		Ferulic acid	[143]
		Green tea infusion	[144]
		Soy isoflavones	[145]
		Chlorogenic acid	[146]
Structural modification	Sterically hindered protein complexes	Epicatechin/calcium	[147]
	Dissociated casein micelles	Tannic acid/calcium	[148]
		–	[149]
Use of polyphenols	Antioxidant	Grape seed extract	[150]
		Ferulic acid	[151, 152]
		Phloretin, naringenin, epicatechin, chlorogenic acid, rosmarinic acid	[153, 154]
Use of polyphenols	Dicarbonyl trapping	Genistein	[155]
		Quercetin	[156]
		Catechins	[157–160]
Use of polyphenols	Scavenging of MR-derived radicals	Catechins	[161]

6.4.1 Use of Polyphenols

Polyphenols are the most widely studied natural ingredients used as anti-glycation agents in food systems. Anti-glycation effect was mostly attributed to their antioxidant activities and their dicarbonyl trapping functions. Antioxidants act as AGE inhibitors, presumably through metal-ion chelation and sequestration of free-radical species, yielding attenuation of oxidative stress [162, 163] and also by trapping carbonyl compounds formed in the intermediate stages of glycation.

Addition of 600 mg and 1000 mg of grape seed extract, which is rich in catechins and proanthocyanidins, to bread (500 g) led to over 30% and 50% reduction, respectively, in the CML content of bread crust [150]. The effect was attributed to strong antioxidant activities of these compounds. Addition of ferulic acid to sponge cake baked at 190 °C for 30 min was found to lower the level of CML and CEL significantly, and the anti-glycation activity was attributed to the free-radical scavenging activity in the intermediate stage of glycation [151]. In the study of Zhang et al. [153], addition of phloretin, naringenin, epicatechin, chlorogenic acid, and rosmarinic acid to the glucose-casein model system showed inhibition on the formation of fluorescent AGEs and CML during heating at 120 °C for 2 hours. Chlorogenic acid, being the most potent inhibitor among the phenolics studied, was found to lower glyoxal and methyl glyoxal formation due to its antioxidant activity. The same phenolics in cookie models had positive correlation between glyoxal formation and antioxidant activity; however, methylglyoxal concentration was found to be unaffected [154]. In a recent study [164], negative correlation was observed between total phenolic compounds and the glyoxal, methylglyoxal, and diacetyl concentrations after baking, indicating the ability of phenolic compounds to trap α-dicarbonyl compounds during baking of cookies made of different cereal species. It was concluded that colored corn flour could be the source of natural dietary anti-glycation agents due to the good abilities of their phenolic compounds to trap C_2, C_3, and C_4 α-dicarbonyl compounds [164].

Genistein was shown to inhibit the cross-links of the glycated β-lactoglobulin and suppress the formation AGEs in a dose-dependent manner by trapping reactive dicarbonyl compounds. By using LC-MS, both mono- and di-methylglyoxal adducts of genistein were detected in the β-lactoglobulin–methylglyoxal assay [155]. Quercetin was also shown to have the ability to trap dicarbonyl compounds in bovine serum albumin-methylglyoxal (or glyoxal) model systems [156]. Catechins were shown as potent dicarbonyl trapping agents in many studies. Maillard reaction model system studies have revealed that catechins sequester reactive dicarbonyl compounds through electrophilic aromatic substitution reactions, primarily on A-ring of flavan-3-ols [157–160]. Catechins have also been reported as trapping agents for the reactive imine intermediates linked to the Maillard reaction [161].

Besides their antioxidant actions and carbonyl trapping functions, polyphenols also may inhibit the glycation through blocking the amine residues of proteins in certain conditions. At alkaline conditions, polyphenols are oxidized to their corresponding quinone forms. Quinone, being a reactive electrophilic intermediate, can

readily undergo attack by nucleophiles such lysine, methionine, cysteine, and tryptophan residues in a protein chain [165, 166].

In a study [143], soy glycinin or bovine serum albumin was incubated at 60 °C for 60 min at pH 12 with ferulic acid, and then fructose was added into the model systems and incubated for further 60 min. Ferulic acid was found to reduce fluorescent AGEs and CML formation by nearly 90% and 85%, respectively [143]. Similar results were reported for the use of soy isoflavone-rich extract (containing daidzein, glycitein, and genistein) at oxidizing conditions (60 °C for 1 or 16 hours at pH 12) in the soy glycinin-fructose model system [145]. It was suggested that the formation of early MR products might be inhibited by the conjugation of isoflavones to the active site of glycation, while AGE formation might be modulated by the trapping of dicarbonyl intermediates and oxygen radical species [145]. Pretreatment of ovalbumin with green tea infusion under oxidized conditions (pH 9.0, 50 °C, 1 h) was shown to be effective in reducing furosine and CML formation in the ovalbumin-glucose model system due to the reduction in the free lysine concentration of ovalbumin [144]. It was explained that the quinone forms of green tea polyphenols might react with the free amino groups of ovalbumin under alkaline conditions (Fig. 6.8). Thereby, the concentration of glycation products occurring during heating of ovalbumin and glucose decreased due to the modified lysine moieties in ovalbumin [144]. A similar explanation was also given for the antiglycoxidative mechanism of chlorogenic acid in a model system composed of bovine serum albumin and methylglyoxal [146]. Evidence of binding between BSA and multiple chlorogenic acids and/or its derivative molecules (isomers and oxidation products) was found. It was also concluded that methylglyoxal and chlorogenic acid competed for free amine groups, which prevents methylglyoxal from binding to BSA, resulting in an effective decrease in AGE formation [146].

By using high-resolution ESI-TOF mass spectrometry and isotope labeling technique, various glycine adducts of catechins were shown for the reaction between glycine and (+)-catechin at 120 °C for 70 min under oxidative conditions [127]. Detailed MS/MS analysis confirmed that amino acids were added to oxidized B-ring of (+)-catechin through the formation of Schiff bases [127]. Similarly, Yin et al.

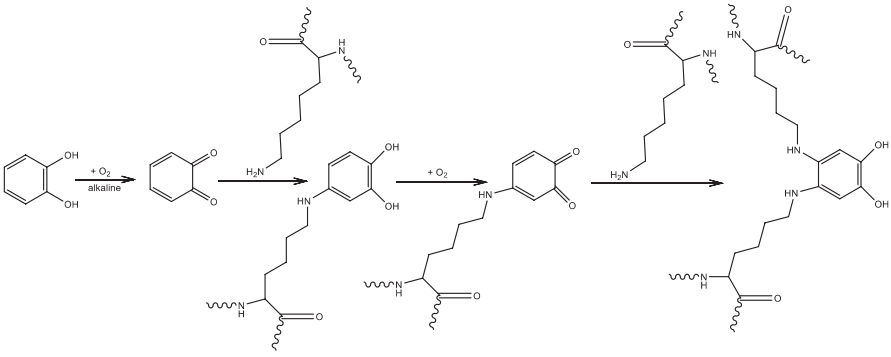

Fig. 6.8 Reaction between green tea polyphenols and proteins at alkaline condition

[128] stated that the inhibitory effect of tea polyphenols on MR might also be correlated with their ability to react with amino acids. It was explained that due to the strong electrophilic nature of quinones, the epicatechin quinone could react with lysine by a Michael-type addition, where lysine is added at the C-5 or C-2 position of the B-ring of epicatechin. It was concluded that tea catechins, epicatechin and epigallocatechin gallate, inhibited the formation of intermediary radicals by the Maillard reaction, by competing with glucose for lysine [128].

6.4.2 Modifications on Physical Structure

The physical structure of proteins affects its glycation tendency. The availability of glycation sites might be changed by the modifications of the protein molecule. A possible anti-glycation mechanism could be due to the physical protection of proteins against glycation by polyphenols. Hydrogen bonding between the phenolic hydroxyl groups and the amine and carboxyl groups of protein is involved in the protein-phenolic interactions. Hydrophobic interaction between the nonpolar regions of the phenolic molecules and the nonpolar domains of the protein may be responsible for weak interactions between the phenolic compounds and proteins [167, 168]. The anti-glycation mechanism involves noncovalent interactions with phenolics and proteins, making the glycation targets on protein molecule inaccessible to react in glycation [169, 170]. Vlassopoulos et al. [169] showed a reduction in fructosamine production in the phenolic preincubated albumin; on the contrary, addition of phenolic acids in the reaction solution throughout the incubation period had no significant effect on fructosamine production compared to glucose alone. It was suggested that physical protection from glycation through protein-phenolic acid interaction is the most likely anti-glycative mechanism especially in oxidative environments. Akıllıoğlu & Gökmen [147] showed that glycation of casein could be reduced by the complexation of casein with epicatechin prior to heating, causing a reduction in the available glycation sites by steric hindrance. Moreover, it was stated that when casein molecule was disintegrated by high-shear treatment before introducing epicatechin, better interaction between epicatechin and casein due to the exposed hydrophobic regions led to a further decrease in the advanced stages of glycation [147]. In the casein-calcium complexes prepared prior to heating in an aqueous solution state, calcium ions acted as cross-linking agents forming bridges between the casein micelles that make it difficult for carbonyl compounds to bind to the glycation sites on protein [147]. In a study where casein glycation was investigated in terms of micellar integrity [149], significantly higher amounts of CML were observed in nonmicellar casein than in the casein micelles after heating for 4 h. The lower amount of CML formation in casein micelle was attributed to the higher amounts of calcium when compared to sodium caseinate suspensions [149].

To evaluate the inhibition of glycation and to determine the mechanism of anti-glycation agents, Akıllıoğlu and Gökmen [148] proposed a kinetic approach, which was similarly derived from the enzyme inhibition kinetic analysis. The kinetic analysis allowed the estimation of the activity of anti-glycation agents comparatively through the calculation of related kinetic constants, in addition to the interpretation of the possible inhibition mechanisms. The effects of tannic acid and calcium ions on the formation of furosine in the ovalbumin-glucose model system in the dry state or aqueous conditions were determined to be noncompetitive [148], which is consistent with the published data about their noncovalent interactions with proteins.

6.5 Concluding Remarks

Since glycation reactions are also responsible for the desired flavor and color development, mitigating glycation in food products is a challenging issue. Thus, particular attention must be paid to the beneficial aspects of the Maillard reaction. Generally, addition of a functional food ingredient is preferred rather than changing the process conditions, to retain the sensorial and textural characteristics of the product. However, the concentration of the inhibitor agent is very important in terms of avoiding any deleterious side effects. It is necessary to know the concentration of the inhibitor agent to be added to food and the kinetics of the reactions taking place in the presence of the inhibitor agent. Unfortunately, most of the studies undertaken until now do not give concentration-dependent inhibition information. Further studies are needed in this regard.

The alternative techniques or agents used for processing yield different products during glycation. For instance, complexation of protein and oxidized phenolic compounds might result in the reduction of the bioavailability of the protein. Due to the fact that lysine is an essential amino acid, there are health concerns about the bioavailability of modified lysine residues in the protein. Therefore, researches with advanced analytical tools should be performed for the identification of neo-formed compounds, and the effects of new techniques should be evaluated in terms of protein digestibility and amino acid bioavailability.

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