Shuo Wang *Editor*

Chemical Hazards in Thermally-Processed Foods

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Chapter 1
Brief Introduction of Food Processing Methods and Chemical Hazards Formed during Thermal Processing

Shujun Wang, Hanbin Xu, Huiyu Luan, and Jingjing Cai

1.1 Introduction

Food processing methods are used to convert varieties of raw agricultural materials or ingredients into safe, nutritious, and convenient food products. In the development of food processing, the key issues of improving food quality, safety, and nutrition remain to be addressed. According to the heat input during processing, food processing methods are generally divided into two categories: thermal processing and non-thermal processing. Thermal processing normally requires high temperature generated by heating, whereas non-thermal processing is often conducted by high-pressure, sonication, pulsed electric field, microwave, infrared, and cool plasma.

Thermal processing, which is one of the most important processes in the food industry, usually includes operation processes such as canning, baking, roasting, frying, pasteurization, boiling, and steaming of various food items. Initially, thermal processing is mostly used to inactivate enzymes or kill pathogens and microorganism in raw food materials and products to enhance the safety and preservation of foods. Nowadays, thermal processing techniques are used widely to improve the palatability, flavor, nutrition quality, and shelf-life of finished food products.

Non-thermal processing is an emerging or novel technology for the improvement or replacement of conventional processing technologies, with the aim of delivering higher quality or better consumption-oriented food products. The physical phenomena used in non-thermal processing of foods include pressure waves, sonication, high hydrostatic pressure, and electric/electromagnetic fields [1]. In addition to processing foods, non-thermal processing technologies have been widely used to modify the properties of food components (such as starch and protein) and widen their application.

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During thermal processing, non-enzymatic browning often occurs. The non-enzymatic browning contains three types of reactions: the Maillard reaction, caramelization, and ascorbic acid oxidation. Of these, much attention has been given to the Maillard reaction, since caramelization and ascorbic acid oxidation often occur at special conditions. The Maillard reaction is incredibly complex and important in the generation of reaction flavors and colors in food processing. Nevertheless, many chemical hazards can also be formed in this reaction. In this chapter, food processing methods and chemical hazards formed during thermal processing will be discussed.

1.2 Thermal Processing Methods

Thermal processing, which is referred to as heating foods at a certain temperature for a length of time, exists in many processes of food engineering universally. The increasing demand for enhancing food safety and quality leads to the development of novel thermal processing technologies. According to the heating forms, thermal processing can be divided into two categories: dry-heat process and moist-heat process. The dry-heat process refers to any technique where the heat is transferred to the food items without any moisture. However, the moist-heat method includes any technique that involves heating with moisture.

1.2.1 Dry-Heating Methods

Foods are subjected to dry-heating either using a flame directly or by surrounding foods with hot air or oil indirectly. Both these operations are conducted at higher temperature (up to 300 °C), which leads to a series of organoleptic changes differing from cooking using moist heat [2]. Particularly, surface browning and flavor development due to Maillard reactions, as well as crust variation resulting from denaturation of surface proteins, are important features, especially in roast meat products and baked cereal-based products. There are at least seven common dry-heating methods, such as broiling, grilling, roasting, baking, sautéing, deep-fat frying, and pan-frying [2].

1.2.1.1 Broiling

Radiant heat, which is from an overhead source, is utilized to cook foods in this process. Foods are put on a heated metal grate to produce crosshatch marks, and the radiant heat cooks foods from above. Foods may be placed on a preheated platter on condition that crosshatch marks are not desirable.

1.2.1.2 Grilling

Similar to broiling, this method uses a heat source located either above or beneath the cooking surface. Grills may be electric, gas, or wood- or charcoal-fired to produce a smoky flavor in the food [2].

1.2.1.3 Roasting and Baking

Foods are heated through hot air in a closed environment. The term "roasting" is usually applied to meats and poultry, whereas "baking" is used for fish, fruits, vegetables, breads, or pastries [2]. Heat is transferred by the form of radiation and convection to the food surface and then penetrates the food through conduction. The surface dehydrates and the food browns because of Maillard reactions and caramelization.

1.2.1.4 Sautéing

The method uses the conduction of heat from a hot pan to the food using a small amount of oil heated to its smoke point. Heat penetrates the food by conduction. High temperatures are used to sautéing foods which are usually cut into small pieces to promote uniform cooking. Stir-frying is a variation of sautéing in which a wok is used instead of a sauté pan; the curved sides and rounded bottom of the wok diffuse heat and facilitate tossing and stirring of the food [2].

1.2.1.5 Deep-Fat Frying

The heat is transferred to the food immersed or free-floating in the hot oil by convection at a temperature range of 160–190 °C and cooks the interior of the food by conduction. Foods are characterized by a golden brown color. As with pan-frying, deep-fried foods are often coated in batter or breading to keep moisture into the food and prevent the food from absorbing excessive quantities of oil [2].

1.2.1.6 Pan-Frying

This method is similar to both sautéing and deep-fat frying where heat is transferred from the pan to the food by conduction using a small amount of oil at a lower temperature than that used in sautéing. Pan-fried foods usually contain breadcrumbs, which keep the food moist and prevent hot oil from penetrating into the food to cause the food greasy [2].

1.2.2 Moist-Heating Methods

Moist heating is performed by placing food materials or food products in hot liquid, which can be water, wine, stock, or steam. Compared to dry heating, moist heating employs lower temperatures, ranging from 60 to 100 °C. The commonly used moist-heating methods are poaching, simmering, boiling, steaming, braising, and stewing. Boiling and steaming are used in processing of meat and oriental cereal-based products. Steaming, braising, and stewing are usually applied to process poultry products.

1.2.2.1 Poaching

Poaching involves processing by submerging food in hot water, wine, stock, or milk, and it is different from other moist-heating processing methods, such as simmering and boiling. Poaching usually operates at a relatively low temperature (60–80 °C), which makes it particularly suitable for foods with delicate flavors, such as eggs, fish, poultry, and fruit. Poaching is usually regarded as a healthy way of cooking because it does not make use of fat to cook or flavor the food. According to the amount of liquid used, poaching is also classified into two categories: shallow poaching and deep poaching. Shallow poaching uses a sautoir or other shallow cooking utensils, in which heat is rapidly transferred by conduction from the pan to the liquid and then to the food. Shallow poaching is best suited for single serving size, naturally tender, boneless, diced, or sliced pieces of poultry, meat, or fish. Deep poaching is similar to shallow poaching; but the food is fully submerged in liquid.

1.2.2.2 Simmering

Simmering uses higher temperatures from 80 to 95 °C, at which steam bubbles are visible, but the cooking liquid does not boil. The food is sorrounded by this liquid at a fixed temperature, which results in even cooking. It is a standard method to prepare stocks and soups, and to cook starchy foods such as potatoes or pastas. However, water-soluble vitamins and minerals leached out from the food into the cooking liquid are lost if the liquid is not consumed [2].

1.2.2.3 Boiling

Boiling at 100 °C is utilized less frequently because some foods may be damaged by the violent agitation owing to steam bubbles. There are many foods suitable for boiling, such as vegetables; starchy foods including rice, noodles, and potatoes; stocks; sauces; eggs; soups; and meats. It is simple and fit for large-scale cookery as a cooking method. It takes a long and slow cooking for tough meats or poultry to

produce a nutritious stock. There is loss of water-soluble vitamins and minerals during boiling. Polythene sachets are sometimes used to pack commercially prepared foodstuffs, which are sold as “boil-in-the-bag” products.

1.2.2.4 Steaming

Steaming is defined by boiling water continuously to vaporize into steam. Upon steaming, foods are separated from boiling water but in direct contact with steam to give it a moist texture. Steaming is suitable for cooking vegetables, seafood, Chinese bread, and other foods having delicate textures or flavors. It can be cooked quickly, with some losses of water-soluble nutrients owing to leaching. Low-pressure steam cooking is a rapid and efficient process of heating and mixing high-protein liquid products, such as cheese, milk, sauce, or soup. The product can not be burnt as there is no direct contact with the heated surface. High-pressure steam at 110–120 °C is used to produce meat casserole products to reduce the time required to soften the meat, usually from a few hours to a few minutes [2].

1.2.2.5 Pasteurization and Sterilization

Pasteurization and sterilization, to inactivate microorganisms existing in foods, are widely used to ensure food safety and extend the shelf-life of foods in the food industry. Pasteurization is a relatively mild treatment in which liquid foods are mostly heated to below 100 °C. The pasteurization and sterilization techniques are initially used in liquid foods such as milk and fruit juices, and they are also applied to particulate food products recently [3]. Heat sterilization is a unit operation where food is heated at a temperature high enough for a long time to destroy vegetative microbial cells and spores and inactivate enzymes. Therefore, the packaged sterile foods have a shelf-life of more than six months at room temperature.

1.2.2.6 Braising and Stewing

Both methods are a combination of dry-heat and damp-heat cooking. The food is first roasted or sautéed and then partially covered with liquid and simmered at a relatively low temperature in a closed container. This may require the use of a pan heated from the bottom, but an oven, a bratt pan, or a jacketed kettle is better in that the food is heated more evenly from all sides. Braising and stewing, which will dissolve the connective tissues and soften the meat by prolonged heating, are important methods to cook harder pieces of meat. Moisture is absorbed in muscle fibers from the cooking liquid, which can increase juiciness of the meat, and flavors from stock vegetables and any herbs or seasonings included are absorbed as well. Braising and stewing are also utilized to cook vegetables such as cabbage, carrots, and aubergine.

1.2.3 Application of Thermal Processing

Thermal processing methods are generally used for food production and manufacturing in food industry. Heat treatment is still one of the most important methods used in food processing in terms of food safety, nutrition, and organoleptic properties. Thermal processing can not only affect desirably the quality of food consumed (many foods consumed in cooked forms and processed such as baked and grilled flavors cannot be produced by other means), but also have a preservative effect on foods by the destruction of enzymes, microorganisms, insects, and parasites.

1.2.3.1 Meat Products

Since the concept of heat treatment to preserve food proposed by Nicolas Apppert, thermal processing of meat and its products has been greatly developed, which can be used alone or in combination with other novel food processing techniques [4]. The main purpose of heat treatment is to improve food safety and extend the shelf-life of products, and it also provides an opportunity to develop the sensory attributes required for varieties of meat and its products. The thermal processing technology used for meat preservation and development of new products can be classified as dry, wet, novel thermal (microwave or infrared), or a combination of technology with minimal changes in meat quality. Thermal processing of meat can be broadly divided into batch type that heating, holding, and cooling phases are provided using a cooker or steamer, or continuous type with the above operations taking place in series [5, 6].

Although new technologies such as irradiation, high-intensity electric fields, ultra-high pressure, and high-intensity light have been widely used in the food industry, thermal processing is still the preferred method to ensure microbiological safety of poultry products. Heat treatment will continue to be the primary method of imparting safety, flavor, and value to poultry products. The use of hot water or steam pasteurization is an effective method to reduce the pathogenic bacteria content on the surface of poultry meat.

1.2.3.2 Fish Products

Pre-cooking is a severe heat treatment before sterilization. Fishes are usually pre-cooked with steam in a steamer at 100–102 °C, and the pre-cooking time depends on the type, size, quality, and temperature of the fish. Pasteurization is a mild or moderate treatment that typically pasteurizes fishery products placed in hermetically sealed containers to extend the refrigerated shelf-life of different seafood products. The heat treatment of canned fish is to eliminate the pathogenic microorganisms and other microorganisms that cause deterioration during storage.

1.2.3.3 Milk Products

The application heat treatment in milk or its products depends on a trade-off between safety/shelf-life and quality. Thermal processing is used to inactivate the microbial organisms to keep safety and prolong the shelf-life of mild products while ensuring the quality of the product. However, the degree of microbial inactivation required to ensure safety and extend the shelf-life by an acceptable factor and changes in quality of the product is always inversely correlated. Milk is an extremely complex raw material for processing, which has a range of constituents with their nature, stability, and properties affected by the types of heat treatments. Therefore, thermal processing can result in changes of sensory, nutritional, and physicochemical properties of milk. Ultra-high temperature (UHT) is a common heating method in the milk production process, which can inactivate enzymes and kill pathogens and harmful microorganisms, thus prolonging the shelf-life of milk.

1.2.3.4 Canned Foods

Thermal processing of canned foods mainly includes in-flow sterilization and in-container sterilization. The former refers to aseptic processing and aseptic packaging, whereas the latter generally involves the canning process in which the prepared food is filled into a package before sealing and sterilization. There is an increasing interest in the canning industry on using new process modeling and process calculation method for food safety and for optimizing the product quality and process efficiency [7, 8].

1.2.3.5 Ready Meals

Ready meals are multicomponent foods, typically comprising of a meat or vegetable component and a rice, pasta, or potato component. Thermal sterilization is applied to ready meals that are stored at ambient temperature, whereas the pasteurization is used for chilled or frozen ready meals. The latter has advantages in maintaining product quality because of the milder process.

1.2.3.6 Vegetable Products

Vegetable products are usually subjected to rigorous thermal treatment during processing to eliminate pathogenic microorganisms, which is termed as canning. Quality of thermally processed vegetables involves organoleptic properties (appearance, texture, flavor), nutrition value, chemical compositions, mechanical properties, functional characteristics, and deficiencies. The quality of thermally processed vegetables has aroused wide public concern. The main focus on thermal processing

of vegetables is to maintain the nutritional and sensory quality through optimizing processing design while reducing the microorganisms to a safe level.

1.2.3.7 Concentrated Juices

Concentrated juices are popular and widely consumed around the world. The fruit products only require mild heat treatment (pasteurization) for long-term conservation because most fruits have a relatively low pH (less than 4.5). The main targets of heat treatment include the elimination of microorganisms and enzyme inactivation. The effects of thermal treatment can be evaluated by the microorganism destruction, the enzyme inactivation, the quality loss, etc. These food component modifications are generally modeled with the reactions kinetic concept [9].

1.3 Non-thermal Processing Methods

Traditional thermal sterilization techniques are widely applied to improve food safety and stability, but it can also cause extensive chemical changes in foods. Comparatively, non-thermal food processing, as a novel technology, is in line with the trend of food safety. The food industry has been improving traditional technologies and developing new technologies to meet the consumer demand for the safety and quality of high nutritional value foods. The non-thermal processing methods, generally used for sterilization and inactivating enzymes, include high-pressure processing (HHP), pulsed electric fields (PEF), high-pressure carbon dioxide (HPCD), high-pressure homogenization (HPH), ionizing radiation (IR), and pulsed magnetic field (PMF). HHP and PEF are most widely investigated in these non-thermal inactivation technologies [10]. HHP, which can improve the microbiological safety of foods while retaining its nutritional and sensory properties, is considered as a promising processing method [11].

1.3.1 High-Pressure Processing (HPP)

In HPP, the pressure between 100 and 1000 MPa is applied to foods for several seconds to minutes. The temperature during high pressure treatment can be set from below 0 °C to above 100 °C. The method can inactivate microorganisms and enzymes without impacting their sensory properties and nutritional value. Moreover, the functional properties of some foods can be improved by this method, producing new value-added products. In addition, there are some other advantages including low energy consumption, short processing times, and no effluents. The main defects of this method include the relatively high capital costs and the inability to

process dry foods or foods that contain entrapped air, such as strawberries, which would be crushed by the high pressure.

1.3.2 Pulsed Electric Fields (PEF)

In PEF processing, foods placed between two electrodes are subjected to pulses of high voltage (typically 20–80 kV/cm). The PEF treatment is performed at sub-ambient, ambient or slightly above ambient temperature for less than 1 second to minimally reduce energy loss. The key points in the application of PEF technology involve the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods, and the electrodes that minimize the effect of electrolysis. The high electric field intensities are achieved by storing a large amount of energy in a capacitor bank from a direct current power supply, which is then discharged in the form of a high voltage pulse [12, 13]. PEF has been mainly used for maintaining the quality of foods, such as improving the shelf-life of bread, milk, orange juice, liquid eggs, and apple juice and the fermentation properties of brewer's yeast.

1.3.3 High-Pressure Carbon Dioxide (HPCD)

HPCD is a kind of non-thermal sterilization technology which combines pressure with carbon dioxide. As a kind of natural antimicrobial agents, carbon dioxide can inhibit the aerobic microorganism rather than kill it. Only combining with high pressure, carbon dioxide can meet the demand of production standard. It has been proposed that HPCD can be used as an alternative cold pasteurization technique for foods. There are some fundamental advantages of HPCD technology, for example, it allowed processing at a much lower temperature than thermal pasteurization. The HPCD preservation technology has not been widely used on the large-scale production in food industry, although it has been researched extensively and deeply over the past few years.

1.3.4 High-Pressure Homogenization (HPH)

The definition of mechanical homogenization is the ability to generate a distribution of particles of a homogeneous size, in a liquid, by forcing the liquid under high pressure through a disruption valve [14]. High-pressure homogenization (HPH), also termed dynamic high-pressure homogenization, utilizes pressure in the range from 100 to 400 MPa, and the range of 300–400 MPa is generally referred to as ultra-high pressure homogenization (UHPH). HPH has confirmed its potential for low-temperature pasteurization of food matrices, and the disruption of vegetative

microorganisms was suggested to result from a combination of temperature, cavitation, shear, turbulence, impingement, and high pressure [15–18]. However, previous work has shown that bacterial spores are resistant to low homogenization pressure and/or low-temperature treatments, thus limits the application of pasteurization [19, 20].

1.3.5 Ionizing Radiation (IR)

Ionizing radiation has frequencies greater than 10^{18} Hz and carries sufficient energy to eject electrons from molecules it encounters. In practice, three different types are used.

1. High-energy electrons in the form of β particles produced by radioactive decay or machine-generated electrons. Strictly speaking, they are particles rather than electromagnetic radiation, although they do exhibit the properties of waves in some behaviors. Because of their mass and charge, electrons tend to be less penetrating than ionizing radiation; for example, 5 MeV β particles will normally penetrate food materials to a depth of about 2.5 cm.
2. X-rays generated by impinging high-energy electrons on a suitable target.
3. Gamma (γ) rays produced by the decay of radioactive isotopes. The most commonly used isotope cobalt 60, 60Co, is produced by bombarding non-radioactive cobalt, 59Co, with neutrons in a nuclear reactor. It emits high-energy γ rays (1.1 MeV) which can penetrate food up to a depth of 20 cm (cf. β particles). An isotope of cesium, 137Cs, which is extracted from spent nuclear fuel rods, has also been used but is less favored for a number of reasons.

Ionizing radiation can affect microorganisms directly by interacting with key molecules within the microbial cell or indirectly through the inhibitory effects of free radicals produced by the radiolysis of water.

1.4 Recently Developed Food Processing Methods

1.4.1 Ohmic Heating

Ohmic heating, also known as “electroconductive heating,” “resistance heating,” or “Joule heating,” allows alternating current to pass through the food and generate heat due to the electrical resistance of the food. Since food is the electrical component of the heater, its electrical characteristics are required to match the capacity of the heater. The way to use electrical resistance for heating purposes is not new, and it developed into a commercial process during the 1980s–1990s. This process is

mainly used for pasteurization or UHT sterilization of foods, especially those foods containing larger particles that are difficult to handle by other methods.

Ohmic heating has some merits, such as a deeper heat penetration and a higher energy conversion efficiency (90% of the energy is converted into heat in the food), compared to dielectric heating (such as a microwave). The electrode in Ohmic heating is required to contact well with foods, whereas this is not required in dielectric heating. The food should have sufficient fluidity to be able to pump it through the heater (i.e., food containing up to about 60% solids) due to the special requirement of Ohmic heating. Ohmic heating has several advantages compared with conventional heating. Firstly, the rate of Ohmic heating is very high, which will result in a uniform heating of solids and liquids when their electrical resistance is the same. Secondly, there are no hot surfaces for Ohmic heating, so there is no risk of food burning or damaging by equipment surfaces or localized overheating. Thirdly, particles in liquids are not subject to shearing forces during Ohmic heating, thus this method is suitable for viscous liquids, such as applesauce or carbonara sauce. Lastly, Ohmic heating has a low capital cost, and it is suitable for continuous processing with the instant switch on and shut down.

Ohmic heating has been commercialized and is mainly used as an aseptic processing method for high value-added ready-to-eat foods stored at ambient or refrigerated temperatures. It also serves as a pasteurization method for granular foods, including pasta in tomato or basil sauce, ratatouille, beef bourguignon, lamb curry, vegetable stew, and minestrone soup concentrate, and as a preheating method for canned product [20]. It is also used as a pasteurization method instead of heat treatment and can be applied to milk, liquid eggs, and juices to produce high-quality whole fruit required for yogurt.

Despite the widespread application, this technology is limited by several factors. For example, the electroconductibility of the solid and liquid components is different and varies with increasing temperature, which causes abnormal and intricate heating patterns and difficulties in forecasting the heating features. In addition, there is a shortage of data on the key factors that impact the heating rate and precise temperature-monitoring techniques to describe heat distribution, which results in dangers under processing and the resulting survival of pathogenic spores in low-acid foods.

1.4.2 Microwave Heating

In classical thermal processing, energy from the surfaces of the material is transferred to the material by transmission, conduction, and irradiation of heat. On the contrary, microwave energy is transferred directly to materials through molecular interactions with the electromagnetic field. In traditional methods, energy is transferred on account of thermal gradients, but microwave heating is the conversion from electromagnetic energy to thermal energy by direct mutual effect of the incident irradiation with the molecules of the target stuff. The diversity in the method

energy can lead to many potential advantages to employing microwaves for processing of materials [21].

1.4.3 Irradiation

Irradiation takes advantages of ionizing radiation (γ rays from isotopes or, commercially to a fewer extent, from electrons and X-rays) to store foods. It is employed to destroy pathogens or spoilage bacteria, or to prolong the shelf-life of fresh produce by disinfestation or slowing the rate of generation ripening, or sprouting. Sensory and trophic properties are not greatly altered because the treatment does not involve heating foods in any significant extent. The process is mostly used as a sanitary and phytosanitary treatment to help meet the recommendations of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) of the World Trade Organization (WTO, 2016). Its purpose is to ensure the quality and safety of foods and to fill quarantine requirements in the commerce of products, including the disinfestation of fresh fruits and disinfection of dried vegetable seasonings and spices.

1.5 Chemical Hazards Formed during Thermal Processing

In food industry, the Maillard reaction plays an irreplaceable role in the protection of food nutrition, the flavor of food, and the colors. Meanwhile, some hazardous compounds generated during the Maillard reaction are considered to be related with diabetes, Alzheimer's disease, and atherosclerosis [22]. Due to the effects of these hazards on the health concerns, there have been many studies on this topic. The mostly reported chemical hazards contain α-dicarbonyl compounds, furan, trans-fatty acids (TFAs), heterocyclic amines (HAs), and acrylamide (AA).

1.5.1 α-Dicarbonyl Compounds

α-Dicarbonyl compounds (α-DCs), for instance, methylglyoxal (MGO) and glyoxal (GO), are chemically and biologically reactive components which are generated endogenously and exogenously [23]. In vivo, as endogenous physiological metabolites, they can be generated by many types of intestinal bacteria by human beings [24]. In vitro, α-DCs are produced during thermal processing (barbecue, baking, and frying) and prolonged storage, especially in sugar-containing foodstuffs and beverages. There are four main pathways to result in the formation of α-DCs, including sugar autoxidation, Maillard reaction, degradation of lipid and microbial fermentation. Generally, α-DCs are easily generated in sugar-rich foods or lipid-rich foods like honey, cookies, coffee, wine, beverages, vinegar, and other fried or

baked foods [25]. They also occur in atmospheric aerosols, fume smoke, and medical products (peritoneal dialysis fluids) [26].

There are about 18 kinds of α-DCs which have been found in foods until now, and the most-studied are methylglyoxal (MGO), glyoxal (GO), 2,3-butanedione (2,3-BD), 3-deoxyglucosone (3-DG), and 3-deoxypentosulos. These chemicals are important intermediates of the Maillard reaction and significant precursors of flavoring and coloring media. Strecker degradation is the intermediate stage of Maillard reaction. In this stage, α-DCs, including aldehydes with at least one atom and α-aminoketones, such as MGO and GO, can react with amino acids to generate carbon dioxide. These are pivotal forerunners of heterocyclic compounds which are known to enhance cooked flavors like oxazoles, pyrazines, and thiazoles [27]. DMHF (known as furaneol), which is generally formed by cyclization of complete glycosides and/or the reorganization of MGO and GO, has been detected to be a key odor-active compound in many raw and processed foods, such as pineapple grape, as well as in soy sauce, roasted coffee, roasted almond, and bread crust [28]. The accumulation of α-DCs in human body is harmful to health, which has been paid increasing attention recently. The formation of α-DCs can destroy the nutrients in the food. α-DCs can react further with peptides, amino acids and proteins, and phospholipids to produce advanced glycation end products (AGEs) [29]. AGEs may lead to many potentially mediates chronic diseases, such as hyperglycemia, nephropathy, cataracts, and Alzheimer's disease. α-DCs are not only responsible for the formation of many advanced glycation end-products (AGEs) but also of some flavors, fragrance, and colors associated with the Maillard reaction. MGO is a physiological α-dicarbonyl compound that is developed from glycolytic intermediates and generated during the Maillard reaction. It leads to diabetes-associated complications and the aging of proteins. In comparison with non-diabetics, higher levels of MGO are existed in diabetic patient plasma. Due to its correlation with diabetes and involvement with flavor generation, methylglyoxal is arousing increased interest [30]. During extended storage for one year, 3-DG was found to be formed in honey examples at diverse temperatures (10, 20, and 40 °C) [31].

1.5.2 Furan

As a cyclic ether, Furan is a colorless liquid which volatility is high and boiling point is low (32 °C) [32]. Furan has been found in some thermally treated foods, such as coffee, canned meat, baked bread and cooked chicken, etc. In canned and jarred foods, furan will not be lost by evaporation when sealed in vessels that receives a considerable thermal load, resulting in its accumulation on the contrary. The concentration of Furan in foods vary greatly from <1.0 ng/g to hundreds of ng/g. For example, the content of furan was detected from 0.77 ng/g in red ginseng drink and 193.95 ng/g in Korean seasoned beef [33]. The furan unit is not only as a versatile precursor for the synthesis of many cyclic and acyclic compounds, but also as the pivotal structural units which impart the required performances in functional materials [34, 35].

1.5.3 Trans-Fatty Acids (TFAs)

Trans-fatty acids (TFAs) which present at a high level in diet are generated from partial hydrogenation of fats and vegetable or fish oils [34]. Due to microbial hydrogenation in ruminant animals, not only processed foods but also some natural foods such as tallow, butter, and milk also contain small amounts of trans-fatty acids [36]. Compared with cis-fatty acids, hydrogenated fats and oils have higher melting points and stability and prevent rancidity, so they are used in foods to improve texture and stability and extend shelf-life [37, 38]. What has increased our attention is TFAs' unfavorable effects TFAs can increase serum LDL-cholesterol and decrease HDL cholesterol concentrations and is concerned with cardiovascular disease [39].

Various studies have pointed out that the total daily intake of TFAs at baseline is positively associated with the daily intake of energy, saturated, total fat and unsaturated fat, and cholesterol, and inversely correlated with the daily intake of protein, alcohol, carbohydrates, and the use of vitamin supplements [40]. In western Europe, the range of TFAs intake is between 0.5% and 2.1% of energy intake and about 2% in the US food and drink [41, 42]. And the label to report the TFA content of a food as "0" means less than 0.5 or 0.2 g per serving (on the basis of the US and Canadian regulations, separately), which requires the knowlege of the minimum amount that can be exactly measured [43, 44].

1.5.4 Heterocyclic Amines (HAs)

Heterocyclic amines (HAs) are composed of three fused aromatic rings having at least one nitrogen atom in the ring structure, one extra cyclic amino group, and up to four methyl groups as substituents. It is a group of structurally like compounds [45]. Heterocyclic amines are detected mainly in fried, grilled, broiled, roasted, and barbecued meat samples and are considered as highly potential mutagens and carcinogens [46]. Until now, there are in excess of 25 types of HAs which have been isolated and determined in cooked foods [47]. The most studied HAs are as follows: IQ (2-amino-3-methylimidazo[4,5-f]-quinoline), MeIQ (2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline), MeIQx (2-amino-3,8-dimethlimidazo[4,5-f]-quinoxaline), DiMeiQx (2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f] quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) [48]. According to the formation process, HAs can be divided into two types: thermic HAs and pyrolytic HAs. Pyrolytic HAs are generated when proteinaceous foods are cooked above 300 °C, while thermic HAs are generated under 300 °C [49]. Excessive consumption of HAs in meat goods can lead to breast and prostate cancer, colon cancer, and lung cancer [50]. These amines, also called thermic HAs, are the produce of a response between amino acids creatine, hexose, and creatinine during common cooking (150–300 °C) [51]. Amino carbolines, also called non-IQ type or pyrolytic

HAs, are generated when protein and amino acids are pyrolyzed at temperatures above 300 °C [52].

1.5.5 Acrylamide (AA)

Acrylamide (AA) occurs in many commonly consumed processed foods, and it is colorless and odorless but potentially hazardous to humans and animals. Because of the Maillard reaction between amino compounds and reducing sugars, acrylamide is formed from food ingredient during heat treatment under low-moisture conditions [53]. The Maillard reaction is the reason for the tasty flavor and golden color of heat-treated foods, and AA is an adverse by-product of Maillard reaction [54, 55].

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Chapter 2
Alpha-Dicarbonyl Compounds

Jie Zheng, Juanying Ou, and Shiyi Ou

2.1 α-Dicarbonyl Compounds Existing in Foods

α-Dicarbonyl compounds (α-DCs) are intermediate compounds widely present in thermally treated foods. They are produced from reactions of caramelization, the Maillard reaction, and lipid peroxidation during food processing. They are also formed in fermented foods and beverages via microorganism metabolism and in plants through the formation of defense products against environmental stresses, such as salinity, drought, and cold [1].

α-DCs are receiving more and more attention because of their beneficial roles in the formation of color and aroma in foods, as well as the possible adverse effects in vivo [2–4]. They have been paid first attention because of the correlation between their existence in glucose-containing peritoneal dialysis solutions and the possible adverse effects in vivo [4]. Later, they were also reported to be responsible for the adverse effects in vivo through the glycation reactions in the appearance of proteins to form advanced glycation end products (AGEs). Nevertheless, α-DCs also show beneficial contributions to the appearance and sensory properties of food, since some important Maillard reaction products such as flavors, aromas, and colors are generated through the interactions between them and various amino acids [5, 6].

Until now, 21 α-DCs (Fig. 2.1) have been qualitatively and quantitatively detected in a wide range of foods, among them, glyoxal (GO), methylglyoxal (MGO), 2,3-butanedione (2,3-BD), and 3-deoxyglucosone (3-DG) represent the most important ones [7–9].

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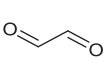
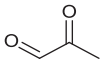
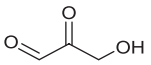


Glyoxal

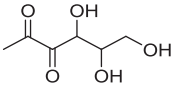


Methylglyoxal

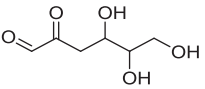


Hydroxypyruvaldehyde

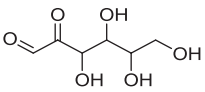
2,3-Butanedione



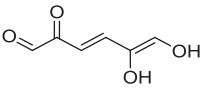
1-Deoxyglycosone



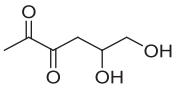
3-Deoxyglycosone



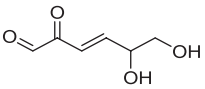
Glucosone



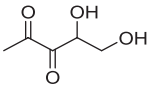
3,4-Dideoxyglucosone-3,5-diene



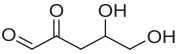
1,4-Deoxyglycosone



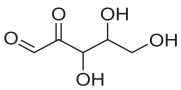
3,4-Dideoxyglycosone-3-ene



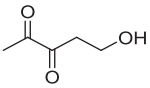
1-Deoxypentosulose



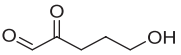
3-Deoxypentosulose



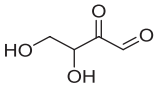
Ribosone



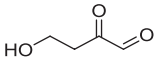
1,4-Dideoxypentosulose



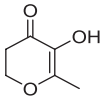
3,4-Dideoxypentosulose



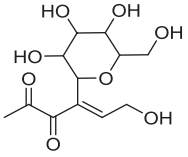
Erythrosone



3-Deoxyerythrosone



2,3-Dihydro-5-hydroxy-
6-methyl-4*H*-pyran-4-one



1,5-Dideoxy-4-glucopyranosyl-
2,3-hexodiulos-4-ene

Fig. 2.1 Structures of α-dicarbonyl compounds [7, 10–12]

2.2 Formation of α-Dicarbonyl Compounds in Thermally Processed Foods

α-Dicarbonyl compounds could be derived from reactions of caramelization, Maillard reaction, and lipid peroxidation during food processing. The pathway through which α-DCs are formed largely depends on the composition of food materials and processing parameters. For example, caramelization requires temperatures over 120 °C or pH in the range of 3–9, whereas the Maillard reaction proceeds effectively at temperatures over 50 °C and pH of 4–7 [11]. Among these reactions, the Maillard reaction and the degradation of sugars were the most investigated. However, the fates of the reactions were still far from being discovered. New products and intermediates of the reactions are being reported continuously.

2.2.1 Formation Through the Maillard Reaction

The Maillard reaction involves a series of reactions between proteins and carbohydrates and is responsible for the generation of roasted, toasted, and caramel-like aromas in foods, as well as the brown color of the food products [10]. The processes are also collectively called the browning reactions or nonenzymatic glycation, in which proteins are subjected to spontaneous posttranslational modifications by reducing sugars. As virtually all foods contain both proteins and carbohydrates, α-DCs as intermediate products of the Maillard reaction are present in the daily diet in considerable amounts. These reactions occur during storage of food at room temperature, as well as during thermal processing of food materials. An increase in temperature always associated with the acceleration of the reaction rate.

The Maillard reaction includes three stages, namely, the initial stage, intermediate stage, and the final stage. During the initial stage, the Maillard reaction starts with the condensation of the aldehyde group of a reducing sugar, in most case glucose, with the amino group of proteins to form an unstable Schiff base. The unstable Schiff base, mainly existing in the cyclic glycosylamine form, can undergo Amadori or Heyns rearrangement under acidic conditions to the more stable 1-amino-l-deoxy-2-ketose, known as Amadori product (Fig. 2.2). In the case of glucose as the precursor, a fructosamine, N_{ε}-(1-deoxy-d-fructose-1-yl) amino acid residue, was formed through Amadori rearrangement [13–15]. The Amadori rearrangement products may further undergo reactions of enolization, deamination, dehydration, cyclization, retroaldolization, isomerization, and fragmentation (Fig. 2.2) under severe heating or prolonged storage and lead to the formation of carbonyl compounds, furan derivatives, and other intermediates [10]. Among the α-DCs formed during the intermediate stages, 3-DG, MGO, and GO represent the most important and thoroughly investigated compounds. When the Maillard reaction continues as the higher temperatures are applied for longer times, advanced brown-pigmented MRPs, termed “melanoidins,” are formed in the final stage [5].

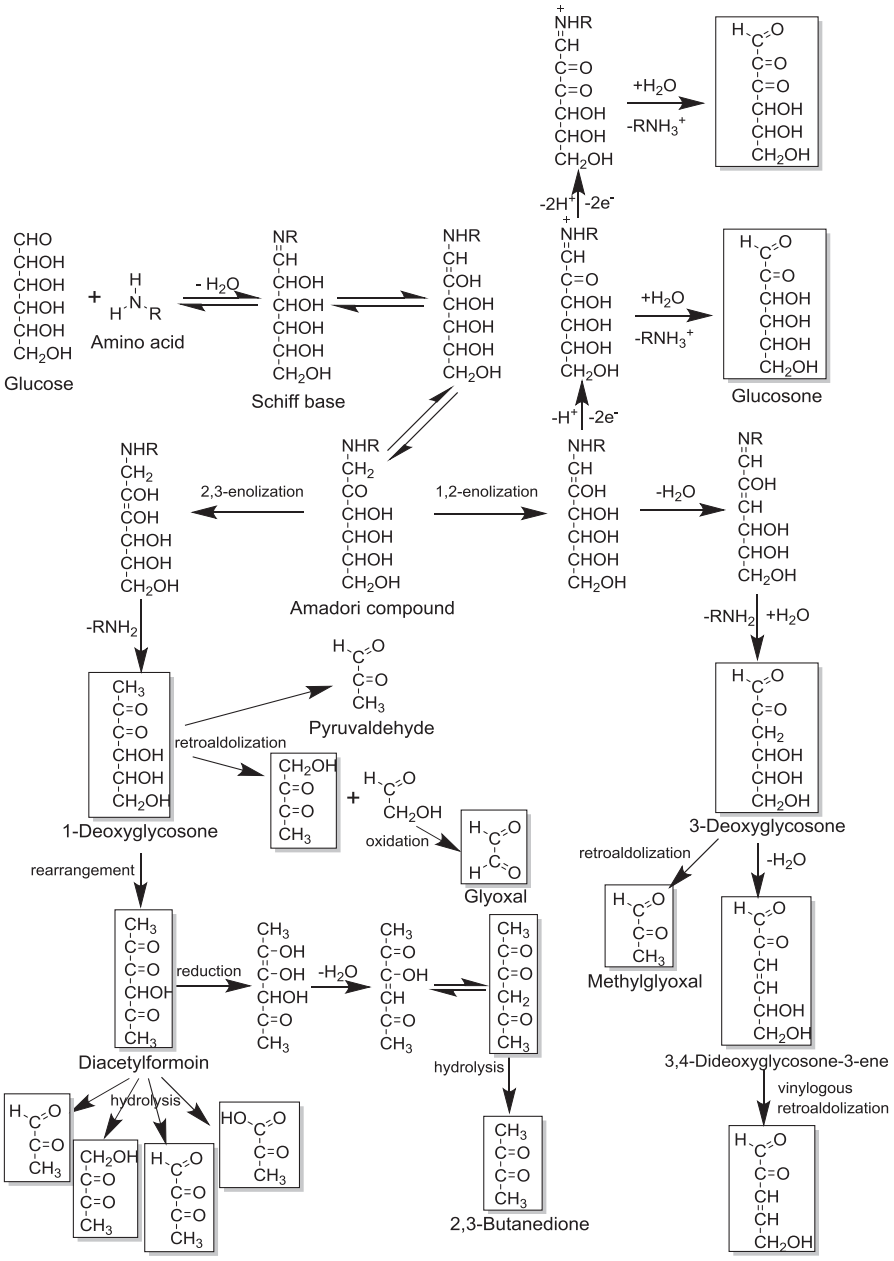


Fig. 2.2 Formation of dicarbonyl compounds from glucose and amino acid via the Maillard reaction [10, 13, 16, 17]

Other reducing sugars can also act as the precursors for the production of α-DCs. 3-Deoxypentosone (3-DPS) resulting mainly from the degradation of 1,4-glycosidically linked di- and oligosaccharides has been detected in heated glucose-lysine solutions [18, 19]. It has been seldom reported in food samples because the analysis of 3-DPS is difficult in complex food samples due to the coeluting matrix peaks (e.g., vanillin), which cause deviations in UV absorbance pattern. However, it could be identified and quantified using mass spectrometry (MS) [6]. Degen et al. [6] found that 3-DPS was only detected in alkali-treated pretzels (up to 2.3 mg/kg), possibly because the fragmentation of carbohydrates is favored under alkaline conditions [20].

3-Deoxypentosulose was the predominant α-DC formed from maltose and maltotriose during the Maillard reaction as well as caramelization. However, 3-deoxypentosulose is formed in a much lower concentration when an amino compound is absent from the substrates. The fact that this α-dicarbonyl compound is formed mainly from the α-1 → 4 glucans such as maltose and maltotriose instead of from D-glucose indicates that the glycosidic bond determines the type of degradation reactions. Hollnagel et al. [18] proposed a feasible pathway of the formation of 3-deoxypentosulose from oligo- and polysaccharides, including the formation of 1-amino-1,4-dideoxyhexosulose by vinylogous β-elimination from the 2,3-ene-diol structure after the Amadori rearrangement. Such reaction is favored by planar alignment of the bonds between C1 and C4 in the oligo- and polysaccharides. Subsequent rearrangement of 1-amino-1,4-dideoxyhexosulose through keto-enol tautomerization leads to a 1-imino-3-keto structure, which could be attacked by a hydroxyl anion to cause a splitting off at the C1 position. This reaction can be characterized as a retro-Claisen ester condensation, producing a formic acid or formamide and a pentose derivative as products. Finally, the subsequent oxidation of the pentose intermediate can lead to the production of 3-deoxypentosulose (Fig. 2.3).

Other than the commonly known α-DCs, Marceau et al. [7] discovered an unknown α-dicarbonyl compound in honey samples and tentatively identified it as an oxidation product of 3,4-dideoxyglucosone-3-ene (3,4-DGE), which was termed as 3,4-dideoxyglucosone-3,5-diene (3,4-DGD). The possible synthetic pathway of 3,4-DGD from 3-DG was described in Fig. 2.4.

2.2.2 Formation via Autoxidation and Degradation of Sugars

Other than the Maillard Reaction, α-dicarbonyl compounds can also be formed by fragmentation of the sugar moiety during retro-aldol condensation and autoxidation without the presence of proteins. For instance, the formation of MGO, the most common α-dicarbonyl compound, was observed during the heat treatment of glucose, fructose, maltose, and maltulose, where the amount of MGO obtained from monosaccharides was remarkably higher than that from disaccharides, and so was its amount obtained from glucose in comparison to fructose. This formation of

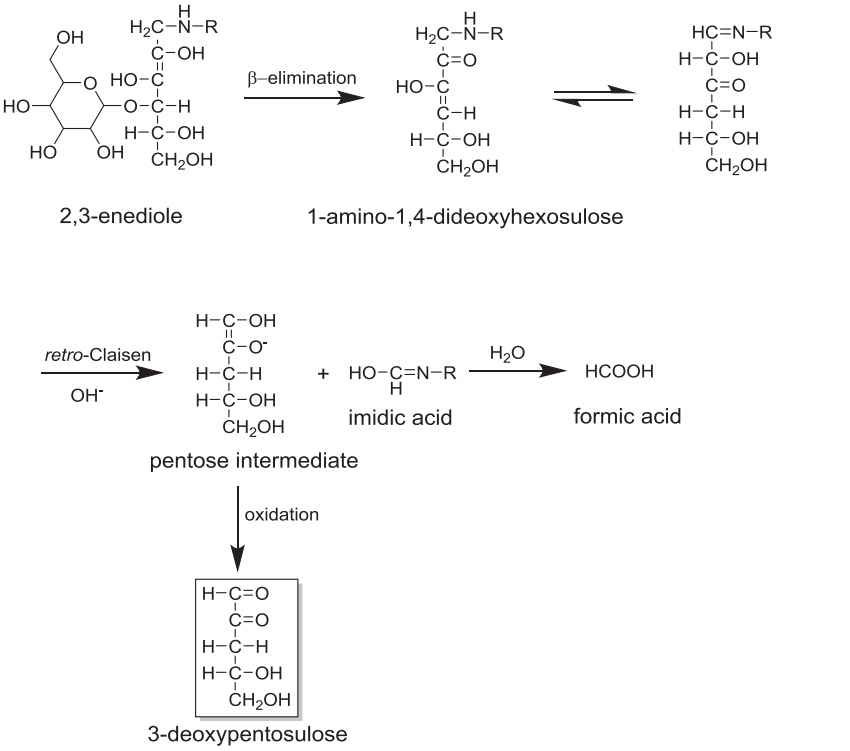


Fig. 2.3 Formation pathway of 3-deoxypentosulose from maltose [18]

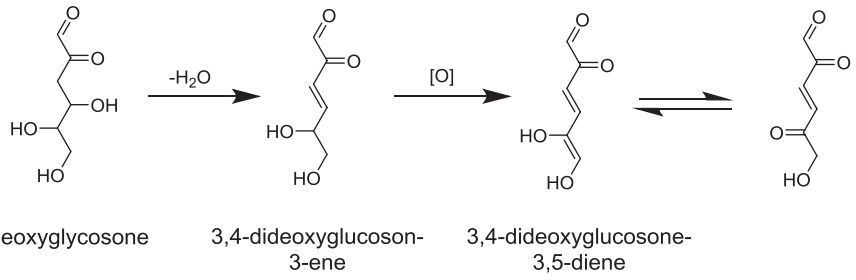


Fig. 2.4 Proposed pathway of formation of 3,4-dideoxyglucosone-3,5-diene from 3-DG and its transformations [7]

α-dicarbonyl compounds through the degradation of sugars could take place under all conditions that are relevant for food processing, although it is highly favored by alkaline conditions.

The degradation of reducing sugars is a fundamental process responsible for the formation of aroma, taste, and visual color compounds during food processing. The breakdown of reducing sugars, e.g., aldoses and ketoses, generally involves myriad

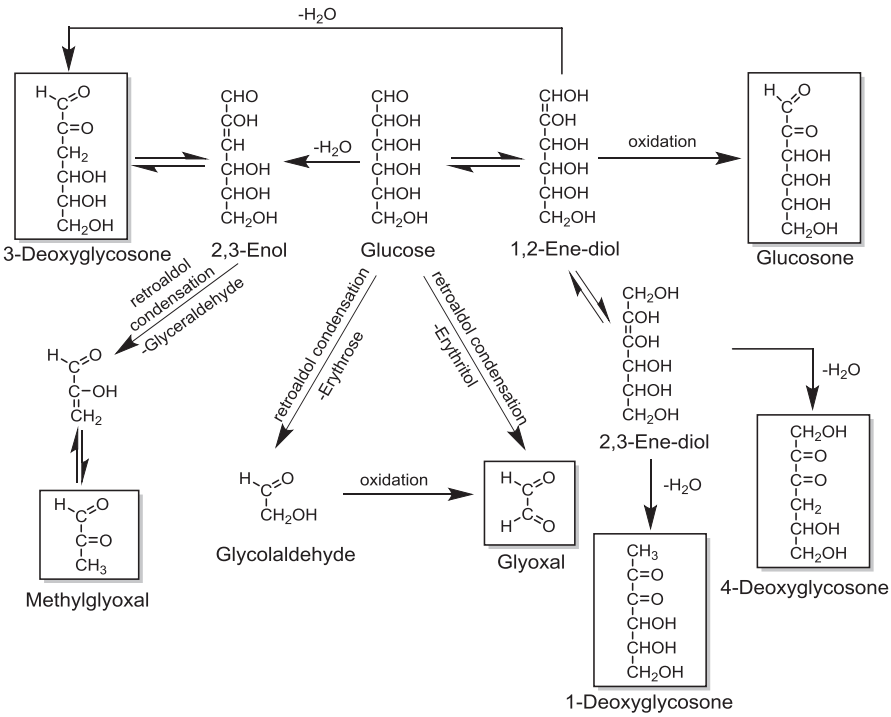


Fig. 2.5 Formation of α-dicarbonyl compounds through degradation and oxidation of glucose [10, 11, 16]

isomerization, dehydration, or oxidation reactions, leading to relatively more reactive α-dicarbonyl intermediates [16]. Many of these reactions are catalyzed by acid/base reactions or by bidentate polyatomic anions like phosphate. A key step involved in the formation of α-dicarbonyl compounds is the enolization of reducing sugar to form enediols, which is known as the Lobry de Bruyn–van Ekenstein rearrangement (Fig. 2.5, [11]). It includes deprotonation of carbon-2 of glucose, and redistribution of the electron density between C1 and C2 or C2 and C3 in glucose leads to dehydration, forming the 1,2-enol or 2,3-enol, respectively. (Fig. 2.5, [10]). The consequent dehydration of enediols leads directly to the well-established Maillard reaction intermediates 3-deoxy-1,2-osones and 1-deoxy-2,3-osones, as well as 4-deoxy-2,3-osones. Oxidation of enediols can also form carbohydrate osones (Fig. 2.5). Despite of the well-known α-dicarbonyl compounds with the structures of an "intact" C-6 backbone, such as 3-deoxyglucosone and glucosone, other breakdown products like methylglyoxal and glyoxal are also produced following the cleavage via retro-aldol reactions in which oxygen plays an important role [6, 10]. The formation of MGO through retro-aldol condensation mainly occurs in food containing a lot of carbohydrates, especially monosaccharides. The amount of MGO produced from monosaccharides was reported to be higher than that from disaccharides. Moreover, the production of MGO from glucose is higher than that from fructose.

As a result, honey with a high content of glucose and fructose forms MGO through sugar degradation during the heating processes [10]. The generation of Glyoxal through the degradation of glucose by retro-aldol condensation could be activated by deprotonation of the 2- or 3-hydroxy groups (Fig. 2.5, [10]).

Despite glucose, which is considered as the predominant precursor of α-DCs in food, D-galactose and some oligosaccharides may also produce α-DCs, such as 3-deoxygalactosone (3-DGal), galactosone, and 3-deoxypentosone (3-DPS) [8, 9, 18]. Two pathways of 3-DGal formation were hypothesized by Bravo et al. [9] and Hellwig et al. [8] as described in Fig. 2.6. As shown in Fig. 2.6, an important source of 3-DGal must obviously be galactose, e.g., in lactose-hydrolyzed milk products, and glucose contributes as another source through the epimerization of 3-DG via 3,4-dideoxyglucoson-3-ene (3,4-DGE) [8]. 3,4-DGE will only react to HMF when it is present in the *Z*-form. However, (*Z*)-3,4-DGE is sterically not favored, and therefore, (*E*)-3,4-DGE might accumulate to some extent instead. Rehydration of (*E*)-3,4-DGE should be favored at the C4 and lead to both 3-DG and 3-DGal [8]. Degen et al. [6] proposed that the dehydration of intermediate 3,4-DGE should be hindered in aqueous systems like juices or beer, so that any thermal impact during food processing should rather induce 3-DGal rather than HMF formation. In the case of fortified wines and liqueurs, the higher concentration of HMF was detected compared to beers. This might be explained by that the 3,4-DGE tended to react to HMF rather than to 3-DGal when water became limited. When foods are heated and/or water becomes limited, the intermediate 3,4-DGE dehydrates and forms the thermodynamically stable end product HMF [6].

2.2.3 Formation via Degradation of Lipids

Decomposition of different lipids, caused by storage and processing, could also contribute to the accumulation of MGO in foods. Depending on oil origin (tuna, salmon, cod liver, soybean, olive, and corn oils) under accelerated storage conditions (60 °C for 3 and 7 days) or cooking (200 °C for 1 h), a broad range in MGO levels was obtained [21].

The formation mechanisms of these compounds are rather complex. It is well known that numerous carbonyl compounds are produced from oxidation of lipids, especially of which containing high proportion of long-chain polyunsaturated fatty acids (PUFA) [22, 23] since the rate of oxidation always correlates positively with the numbers of double bonds in the fatty acids of a triacylglycerol [24]. Niyatishirkhodaee et al. [25] reported the formation of glyoxal and methylglyoxal from low-molecular-weight carbonyl compounds, including acetaldehyde, acrolein, propanal, and acetone on 6 h of UV irradiation. Therefore, the formation of glyoxal and methylglyoxal was hypothesized to be a result of lipid oxidation (Fig. 2.7). The oils containing higher levels of long-chain PUFA, such as fish oils, produced more genotoxic dicarbonyl compounds.

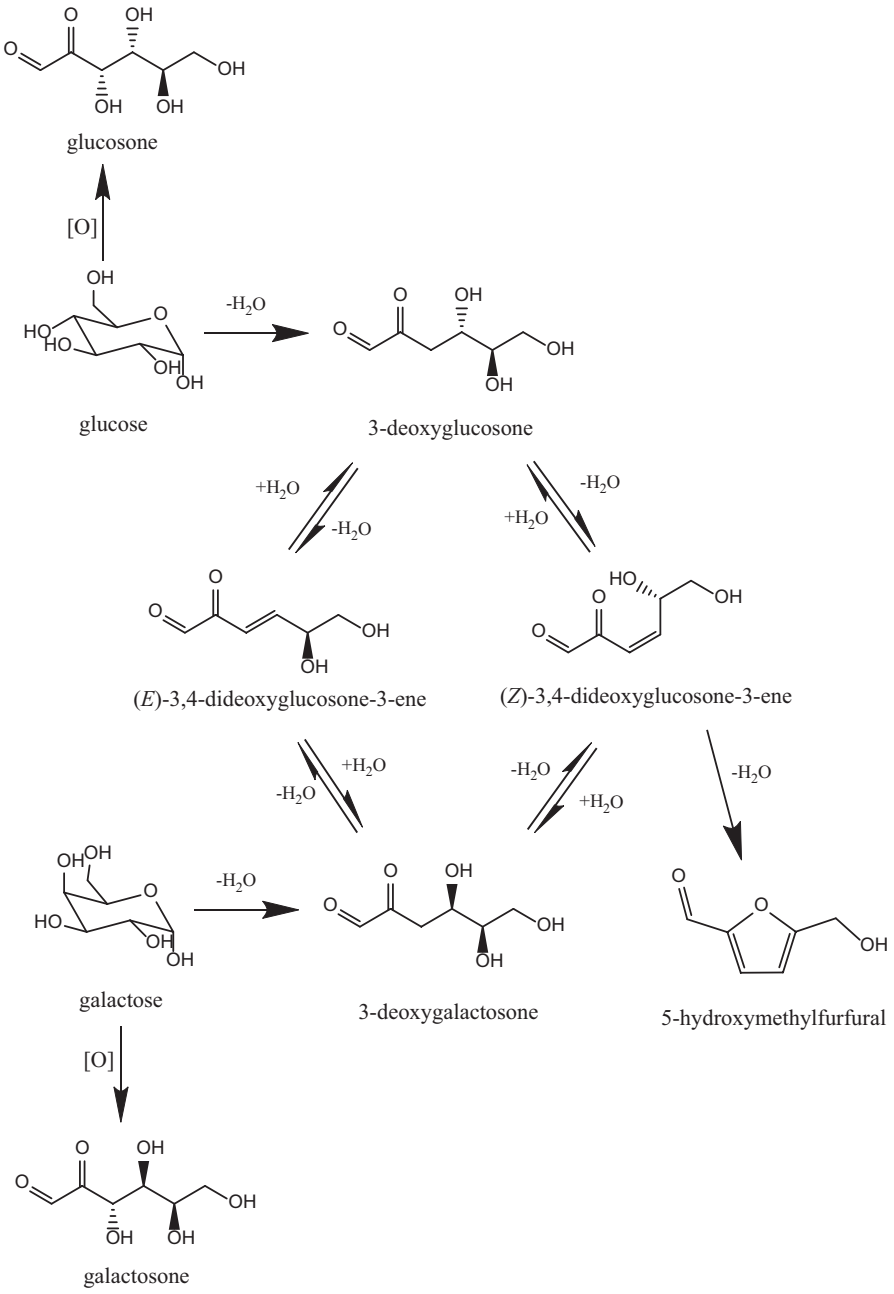


Fig. 2.6 Proposed pathways of generation of 3-deoxygalactosone (3-DGal) and galactosone during caramelization [8]

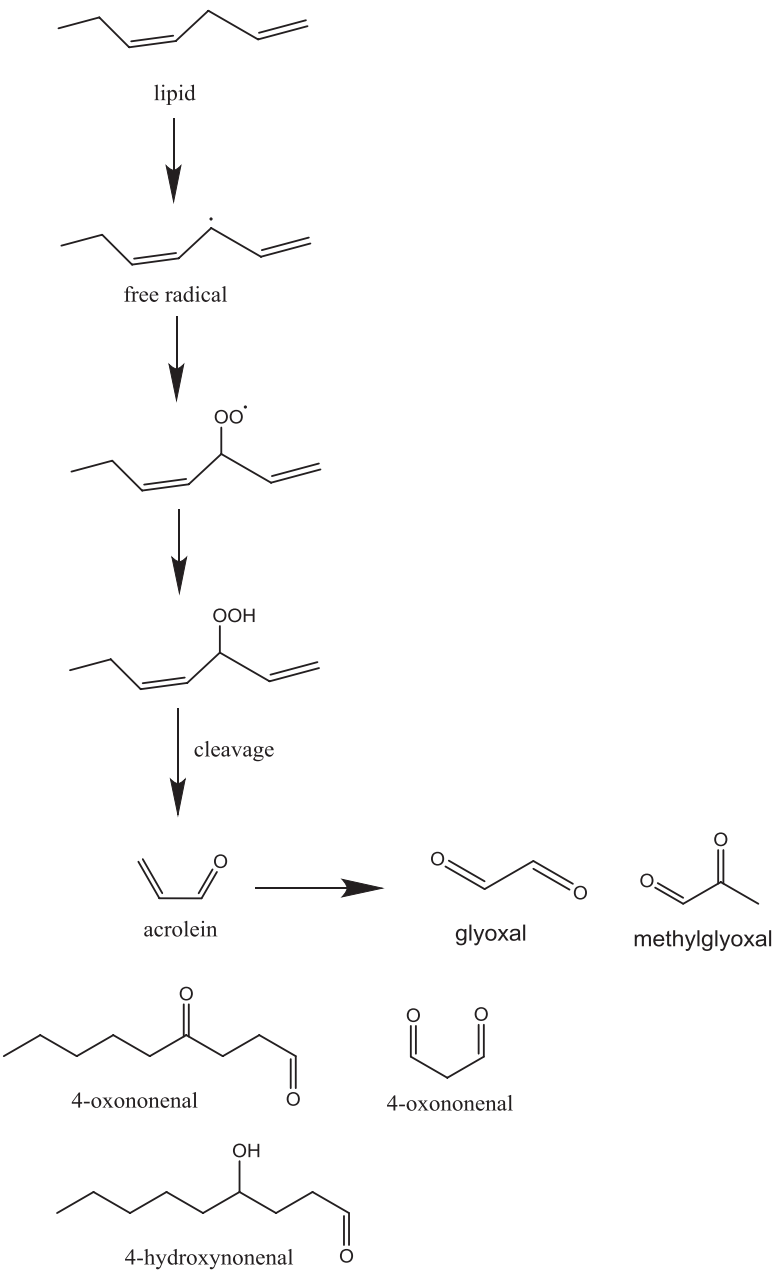


Fig. 2.7 Formation of carbonyl compounds from lipid autoxidation

2.2.4 *Formation via Degradation of Vitamin C*

L-Ascorbic acid, being relatively unstable under common storage and processing conditions such as heat and oxygen, may contribute as another source of α-dicarbonyl compounds. Thermal treatment of L-ascorbic acid could lead to the formation of various types of α-dicarbonyl compounds as important degradation intermediates through both the oxidative and non-oxidative pathways [26]. Mass spectrometric investigation of the degradation products of Vitamin C yielded the following α-dicarbonyl compounds: glyoxal, methylglyoxal, diacetyl, L-threosone, 3-deoxy-L-threosone (4-hydroxy-2-oxo-butanal), and 3-deoxy-L-pentosone. Among these, 3-deoxy-L-pentosone was exclusively formed from non-oxidative reaction of L-ascorbic acid. The oxidative pathway involves the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid as an initial step in the formation of α-DCs. Figure 2.8 describes the possible

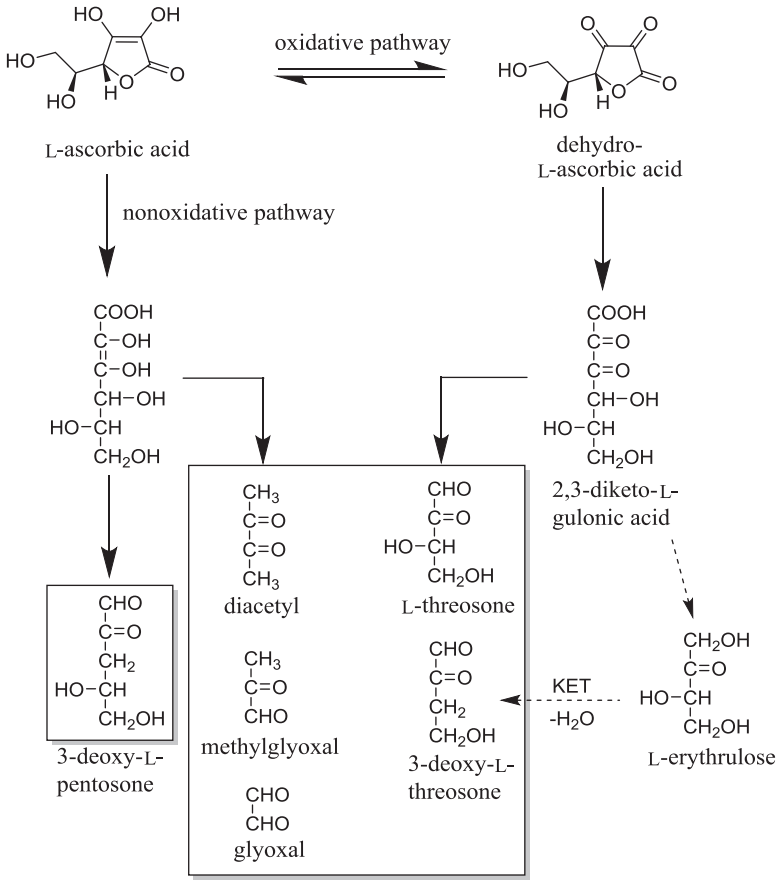


Fig. 2.8 Postulated formation pathway of α-dicarbonyl compounds from L-ascorbic acid. KET, keto-enol tautomerization [26]

oxidative and non-oxidative degradation pathways of L-ascorbic acid and the formation of α-dicarbonyl compounds. Investigations of isotopic-labeled ascorbic acid indicated that diacetyl, methylglyoxal, 3-deoxy-L-pentosone, L-threosone, and 3-deoxy-L-threosone were formed via a decarboxylation step with the loss of CO_2, irrespective of the oxidative or non-oxidative pathway. In contrast, glyoxal was suggested to be at least partially formed by the cleavage of the C2-C3 bond of L-ascorbic acid. The ^{13}C label found exclusively in oxalic acid in the oxidative degradation pathway of L-ascorbic acid corroborated the formation of the L-erythrulose by cleavage of the C2-C3 bond, which leads to the formation of 3-deoxy-L-threosone by further keto-enol tautomerization and dehydration (Fig. 2.8, [26]).

2.3 Analysis of α-Dicarbonyl Compounds in Food

Due to their contributions to the sensory properties of food as well as the potential adverse effects on human health, considerable effort was undertaken not only to elucidate the synthetic pathways and nature of the α-dicarbonyl compounds but also to develop valid methods for their determination in food [27]. Since α-dicarbonyl compounds are highly reactive, and often undergo reversible binding to matrix components [28], they are difficult to be quantified per se in complex systems. Efficient analytical techniques become a challenged requirement for the quantitative analysis of these compounds in complex food matrices.

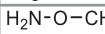
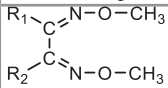
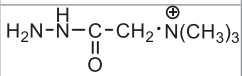
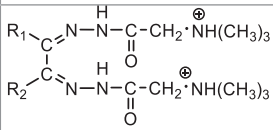
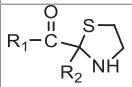
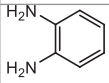
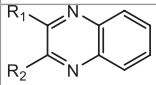
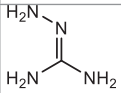
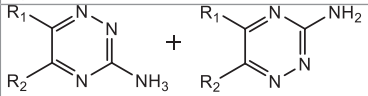
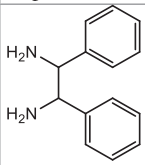
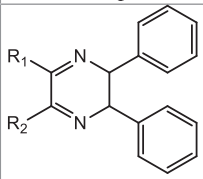
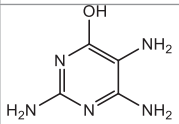
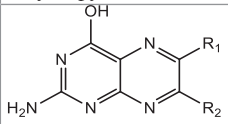
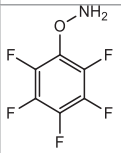
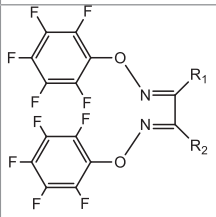
During the past years, various methods for quantification of α-dicarbonyl compounds have been developed. Among them, the most extensively published methods are those with the application of High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). For all the methods, a derivatization process, prior to the chromatographic analysis, is required to transform the α-dicarbonyl compounds to more stable chromophores and/or fluorophores which can be quantified by HPLC or GC coupled with different detectors. Earlier, Thornalley et al. [14] used 1,2-diamino-4,5-dimethoxybenzene dihydrochloride (DDB) as derivatization agent to determine the concentrations of glyoxal, methylglyoxal, and 3-DG. After derivatization, the compounds were analyzed with HPLC coupled with fluorescence detection ($\lambda_{excitation}$ 352 nm, $\lambda_{emission}$ 385 nm) and internal standard because of the low recoveries (52–70%) of derivatized compounds. Nowadays, *o*-phenylenediamine was the most accepted derivatization agent to derivatize the 1,2-dicarbonyl compounds to the stable, UV-active quinoxalines [6]. Degen et al. [6] performed a comprehensive HPLC-UV analysis of the contents of 3-DG, 3-DGal, and MGO, after derivatization with *o*-phenylenediamine, in 173 food items like bakery products, pasta, nonalcoholic and alcoholic beverages, sweet spreads, and condiments. The usage of other derivatization agents, also called trapping reagents, including *O*-aryl or *O*-alkyl hydroxylamines, hydrazines, cysteamine, *meso*-stilbenediamine, aminoguanidine, 6-hydroxy-2,4,5-triaminopyrimidine, and *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine, was also reported. Other than the commonly used UV and fluorescent detection, mass spectrometric (MS) detection of the consequent

derivatized products also became possible by use of diamino derivatives of benzene and naphthalene, 6-hydroxy-2,4,5-triaminopyrimidine, cysteamine, and *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine. The structures of representative agents and their corresponding derivatized products are listed in Table 2.1 [10, 15, 27].

Before quantitative analysis of the α-dicarbonyl compounds, food samples are normally undergoing different preparation procedure depending on the texture and composition of the sample. Liquid samples were mostly analyzed without further preparation, except for carbonated drinks and beer, which were freed from the gas by use of an ultrasonic bath [6]. In contrast, extraction procedures are required for semisolid and solid samples. Water is normally added as extracting solvent. For sugar-rich semisolid materials like honey, preparation by dissolving the sample in water is enough. However, for fibered semisolid samples like jam and solid samples like candy, a homogenization step by Ultra-Turrax mixing after dissolution is generally required [6].

The analytical procedure for the detection of α-dicarbonyl compounds normally includes three basic steps: deproteinization, incubation with derivatization agent, and chromatographic analysis with or without preliminary extraction of the formed products (Fig. 2.9, [15]). Since the insolubility of proteins may cause difficulties in the consequent chromatographic analysis, deproteinization is an important step to get rid of the insoluble protein, as well as liberate the reversibly bound α-dicarbonyl compounds from the proteins. Perchloric acid is a widely used agent for deproteinization. The use of perchloric acid possessed another benefit as to keep the samples in low pH, which prevents degradation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate to MGO during the analysis [10]. However, for foodstuffs that are protein free or have a low protein content, the deproteinization step could be neglected, making the methods easier to perform [15]. However, the second step, derivatization of α-dicarbonyl compounds, cannot be omitted because of the impossibility of direct α-DCs' measurement. Diamino derivatives of benzene or naphthalene were the most used derivatization agents in α-DC quantification [15]. Their adductive products, quinoxalines, could be analyzed with HPLC and be monitored by a UV detector at 300–360 nm, by the fluorescent detector with excitation wavelengths at 300–360 nm and emission wavelengths at 380–450 nm (depending on the diamine used), or by an MS detector. During derivatization, the samples are incubated with the excess of derivatization agents. The incubation period depends mostly on both the derivative and the temperature applied, which varied between 45 min at 60 °C to 18–20 h at 4 °C. However, the degradation of compounds for derivatization provoked by incubation conditions occasionally posed a great problem. Therefore, the incubations had to be performed in the dark and even in the atmosphere of nitrogen. Another challenge for the determination of dicarbonyl compounds was that some derivatization agents suitable for α-DC quantification are not commercially available and have to be synthesized and characterized before analysis [10, 15]. After the derivatization step, the chromatographic analysis is the final step in the determination of α-DC. HPLC analysis was usually performed on the reverse-phase HPLC columns by elution with water solutions of acids or buffers in combinations with methanol or ACN under the gradient or isocratic conditions,

Table 2.1 Derivatization reagents for the determination of α-dicarbonyl compounds

Derivatization reagent	Representative reagent	Derivatized products
O-aryl or *O*-alkyl hydroxylamines	 *O*-Methylhydroxylamine	 Oximes
Hydrazines	 Girard T reagent	 Osazones
Cysteamine		 Thiazolidines
o-diaminobenzene derivatives	 *o*-Phenylenediamine	 Quinoxaline derivatives
Aminoguanidine		 3-Amino-1,2,4-triazines
Derivatization reagent	Representative reagent	Derivatized products
Meso-stilbenediamine		 2,3-Diphenyl-5-methyl-2,3-dihydropyrazine
6-Hydroxy-2,4,5-triaminopyrimidine		 Pteridine
o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine		 Oxime

Note: α-dicarbonyl compound is represented by the structure of  [10, 15, 27]

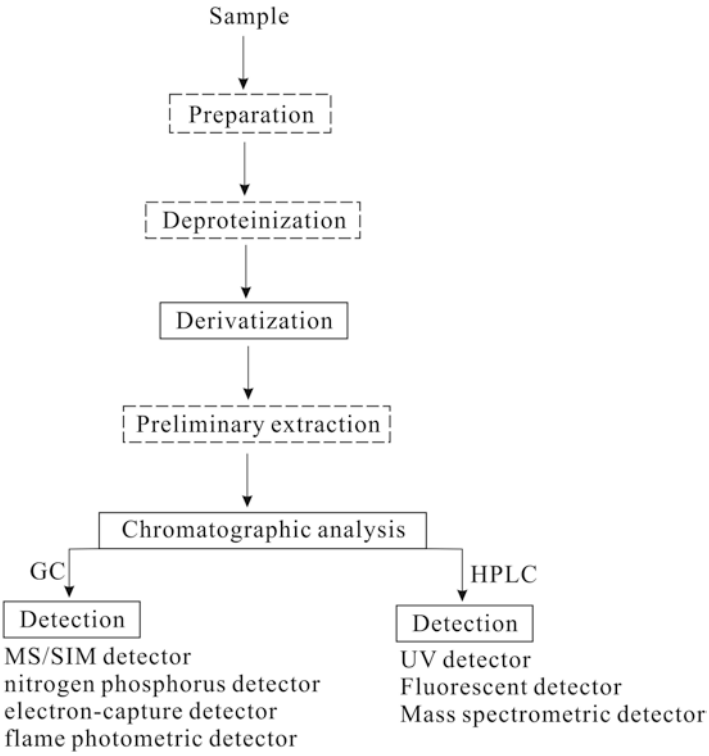


Fig. 2.9 Analytical procedure for the detection of α-dicarbonyl compounds. Procedure in the dashed border is negligible depending on the characteristics of the sample

whereas GC analysis was performed on fused-silica capillary columns with He as a carrier gas. Preliminary concentration of the sample is required if the amount of α-DC is very low in the sample, which can be performed by liquid-liquid extraction or SPE with the additional evaporation of the organic solvent or by freeze-drying of samples deproteinized by TFA [15]. Although chromatographic purification step is commonly required for the detection of the derivatized α-DCs, Schulz et al. [26] introduced a non-chromatographic mass spectrometric method which allows the direct analysis of α-dicarbonyl intermediates generated from the degradation of L-ascorbic acid in Vitamin C containing esculents. Such method was proved to be suitable at least for Baby-C-juice and vitamin C-enriched fruit tea.

During the analysis, caution should be also paid for samples containing constituents that may coelute with derivatized quinoxalines under UV detection. As a resolution, for a novel food sample, an additional measurement without *o*-phenylenediamine derivatization should be applied with HPLC-UV analysis in order to recognize possible coeluting substances originating from the matrix and avoid possible false-positive results. For such food like mustard that could not be measured using UV detection because of the coeluting components, MS detection should be performed instead. Moreover, the derivatization agent should also be

tested for its UV chromatograms at wavelengths for detection to rule out possible interfering compounds present in the solution [6].

Other than quinoxalines that could be analyzed with HPLC, other agents such as 6-hydroxy-2,4,5-triaminopyrimidine and cysteamine that forms pteridine derivative and 2-acetylthiazolidine, respectively, have also been analyzed by HPLC. In the GC method, oxime derivatives of MGO derivatized by *o*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) can be detected by a flame ionization detector, an MS/SIM detector, an electron-capture detector, or a flame photometric detector. Moreover, 1,2-diaminobenzene derivatives of MGO can be analyzed by GC using a flame ionization detector, an MS/SIM, or a specific nitrogen/phosphorus detector [10].

Until now, various foods have been investigated for the occurrence and concentration of α-dicarbonyl compounds (Table 2.2). According to Table 2.2, 3-DG was proved to be the predominant α-dicarbonyl compound in all kinds of food. This could be explained by that during heating or storage, 3-DG as a kinetically stable intermediate can accumulate, due to the delayed generation of thermodynamically stable HMF [6]. 3-DGal was found to be of relevance in many foods even in the absence of galactose. MGO was only of minor quantitative importance in all foods studied, except for manuka honey and coffee bean [6]. The concentration of α-DCs varied a lot between different condiment groups (Table 2.2). Pepper sauce, ketchup, or oyster sauce showed significantly lower α-DC contents than vinegar and soy sauces, probably because of the lack of the relevant manufacturing processes like fermentation, ripening, or long, intense heating during the condiment production [6]. In accordance, plum butter and pear butter, which are thickened by prolonged cooking, and sugar beet syrup made of molasses from industrial sugar production are exposed to much higher thermal impact than jams, resulting in higher concentrations of 3-DG and 3-DGal in the products. The crust, which was exposed to more intensive heat impact during the baking process, contained a significantly higher amount of 3-DG than the crumb (22 mg/kg versus 3.3 mg/kg, $p < 0.05$; [6]).

Degen et al. [6] compared the occurrence of 3-DG in nonalcoholic beverages and found 3-DG content was significantly higher in fruit juices than in soft drinks. They attributed such differences to the variety of sugar existing in the original food materials. Monosaccharides, mostly glucose and fructose, are the predominant sugars in the fruits used for juice processing. On the contrary, sucrose predominates in soft drinks as an exogenous sweetener. Compared to glucose and fructose, sucrose is less susceptible to degradation due to its full acetal structure. This was proved by the fact that highest amounts of 3-DG in soft drinks were detected when using glucose-fructose syrup as an additional sweetener [6, 29]. Another evidence may support this hypothesis is that among all the sweet spreads and sweeteners tested by Degen et al. [6], the lowest 3-DG levels were detected in two maple syrups (1.7–4.7 mg/kg), where sucrose is the major sugar in maple syrup and only small amounts of monosaccharides are present. However, although cookies contain high amounts of sugars and are exposed to intensive heat treatment during baking, smaller amounts of 3-DG were detected in comparison to candies and jams. This can be due to the

Table 2.2 α-Dicarbonyl compounds in various food materials

Food	GO	MGO	2,3-BD	3-DG	1-DG	3-DGal	3-DP	Glucosone	References
Carbonated soft drinks	0.02–1.73	0.07–1.40		nd-34.89					[29]
Soft drinks		nd		nd-28		nd-3.1			[6]
Juices		nd-2.2		nd-410		nd-60			[6]
UHT milk, 1.5% fat	nd-3.2	nd-tr		nd-18		nd-11	nd		[8]
Infant milk	nd	0.6		17		nd	nd		[8]
Infant formula		0.4–17.3		3.9–827.1	nd-50.6			nd-4.8	[30]
Infant fruit puree		nd		26.7–92.3	nd-0.7			0.8–7.6	[30]
Infant vegetable puree		nd-1.4		9.9–49.3	nd			0.2–0.9	[30]
Infant pudding		nd		1.9–98.1	nd-0.7			nd-3.2	[30]
Evaporated milk	0.6–0.8	nd		2.0–2.2		0.9–1.0	1.2–1.5		[8]
Cream, 30% fat	nd-1.0	nd		tr-4.6		nd-5.3	nd		[8]
Coffee cream, 10% fat	nd-tr	nd		tr-16		nd-19	nd-tr		[8]
Yogurt, 3.5% fat	nd-tr	tr-0.7		nd-tr		nd-0.7	nd-tr		[8]
Fruit yogurt	nd-tr	tr-2.3		3.8–11		tr	nd		[8]
Probiotic drink	1.4	1.6		8.6		nd	nd		[8]
Whey drink	nd	1.1		13		nd	nd		[8]
Coffee drinks		nd-16.6		nd		nd			[6, 31]
Coffee bean (raw to different degrees of roast)	10.0–130.7	10.0–211.9							[32]
Beer		nd-1.0		18–136		4.8–33			[6]
Wine		nd-4.5		2.2–95		nd-49			[6]
Vinegars	nd-4.04	nd-53	0.32−13.99	4.6–2622		1.1–162			[6, 33]
Soy sauces		nd-12		32–832		12–71			[6]
Liquid condiments/seasonings		nd-3.9		nd-212		nd-22			[6]
Jams, jellies, sweeteners		nd-13		1.7–1061		nd-124			[6]

(continued)

Table 2.2 (continued)

Food	GO	MGO	2,3-BD	3-DG	1-DG	3-DGal	3-DP	Glucosone	References
Honey	0.2–7.0	nd-761	0–4.3	79–1641 143–1099[a]		14–46			[6, 7, 34, 35]
Candies		nd-1.1		141–1011		nd-36			[6]
Bread		nd-28		13–619		nd-47			[6]
Cheese	nd	nd		nd-tr		nd-tr	nd		[8]
Alkali-treated pretzel		2.5–16		4.5–34		tr-6.4			[6]
Cookies	4.8–26.0	1.8–81.4		8.5–385		tr-88			[6, 36–38]
Pasta (cooked)		nd		nd-8.8		nd			[6]
Potatoes (cooked/fried)		nd-tr		nd-18		nd			[6]
Oils (raw)	< 1.2	nd-0.8							[21]
Oils (cooked)	0.8–4.0	0.2–1.3							[21]

Note: Data are given as ranges in mg/L or mg/kg, respectively; *GO* glyoxal, *MGO* methylglyoxal, *2,3-BD* 2,3-butanedione, *3-DG* 3-deoxyglucosone, *1-DG* 1-deoxyglucosone, *3-DGal* 3-deoxygalactosone, *3-DP* 3-deoxypentosone, *nd* not detectable, *tr* traces. [a]Calculated with glyoxal response factor

possibility of Maillard reactions occurring in the baking process. In the Maillard reactions, the presence of amines not only promotes the degradation of sugars but is also involved, as reactants, in the modification of dicarbonyl compounds. For instance, 3-DG and, probably, 3-DGal as well react with lysine side chains of proteins to form the advanced glycation end product pyrraline, which is comparatively stable [6].

Therefore, some conclusions could be drawn about the conditions in food matrices which exert a major impact on the occurrence of α-dicarbonyl compounds, particularly of 3-DG: (1) The type of sugar (monosaccharide versus disaccharide and reducing versus non-reducing sugar) present in food samples; (2) The extent of heat impact during manufacturing accompanied by a reduction of the water content; (3) Processes like ripening, fermentation, and storage promote the formation of α-DCs; (4) The presence of potential reactants like amino acid side chains of proteins [6].

According to the levels of α-DCs detected in 173 food items, Degen et al. [6] made a rough estimation of the daily intake of α-DCs from common diets. Different types of diets were assumed to cover all population, including the high 3-DG-containing diet rich in sugar-rich products like sugar beet syrup together with the consumption of fruit juices and beer and the low 3-DG-containing diet mainly based on fresh fruits, vegetables, and milk products. As a result, the dietary intake of 3-DG and MGO was estimated to range between 20 and 160 mg/day, and 5 and 20 mg/day, respectively. The ingested amounts of 3-DG exceed those of the other α-DCs, such as MGO and 3-DGal, and HMF as well about ten-fold [6].

2.4 Mitigation Strategies of α-Dicarbonyl Compounds

Since α-dicarbonyl compounds widely occur in a number of foods and beverages (Table 2.2), their effects on human health are of great importance to be investigated and understood. α-DCs were reported to induce in vitro cellular damages by reactions with DNA and proteins [2, 3]. Wu et al. [39] reported that the accumulation of MGO and GO in cells may cause oxidative stress and finally tissue damage by inducing the formation of hydrogen peroxide. MGO was also reported to activate NF-κB and induce the associated gene responsible for inflammation and proliferation. However, Amoroso et al. [1] pointed that the cytotoxicity of α-DCs against human cells was transient and detectable only at very high doses that were not easily reachable with diet, however, with the exception of the HCT116 cell line. Tomo et al. [40] reported the synergistic cytotoxicity of 3,4-dideoxyglucosone-3-ene (3,4-DGE) and acidity under the existence of lactate on human peritoneal mesothelial cells (HPMC), since the 3,4-DGE and acidity alone, in absence of lactate, did not reduce the cell viability of HPMC.

Although the effects of α-DCs on human health still require further investigations, the adverse effects of their products, advanced glycation end products (AGEs), via reactions with proteins/amino acids, were extensively claimed. α-DCs are highly reactive alkylating agents, which can react with amino and sulfhydryl groups of side

chains of proteins to form AGEs during prolonged heating or storage of food. The consequent irreversibly linkage of AGEs to proteins will cause damage of proteins and change their physicochemical and biochemical properties as well as their stability [15] and hence induce a negative impact on the digestibility and nutritional value of proteins [41], as well as a linkage to hyperglycemia and diabetes complication [10]. Moreover, glycation of proteins has been linked to various diseases, such as diabetic retinopathy, neuropathy, and nephropathy [42–44], nondiabetic nephropathy [45], macrovascular disease [46, 47], Alzheimer's disease [48], cataract [49–53], uremia [54], and aging [55]. Other than the endogenous formation of AGEs, the exogenous AGEs from the diet were also considered as risk factors for healthy subjects, from which Peppa et al. [56, 57] recommended diets with a low amount of AGEs for populations.

Since the concentration of dicarbonyl intermediates correlated closely to the number of AGEs formed, the studies on the prevention of accumulation of dicarbonyl compounds are highly focused. The mitigation strategies of α-dicarbonyl compounds in food were required by food manufacturers and widely investigated to process the products with minimal amounts of AGEs. Among all the strategies, the reduction of α-dicarbonyl compounds in food processing by use of naturally derived compounded, especially polyphenols, has been deeply investigated.

2.4.1 Trapping of Dicarbonyl Compounds by Polyphenols

Natural phenolic compounds, such as flavanols, chalcones, stilbenes, isoflavones, and phenolic acids, showed the good capacity of inhibiting the formation of AGEs by trapping the dicarbonyl intermediates, MGO and GO, formed in the Maillard reaction. Luteolin, rutin, epigallocatechin-3-gallate (EGCG), and quercetin showed significant inhibitory effects on MGO-mediated AGEs formation by 82.2, 77.7, 69.1, and 65.3%, respectively, while catechin, epicatechin, epicatechin gallate, epigallocatechin, kaempferol, and naringenin showed a lower inhibitory effect (13–54%) [58]. Polyphenols were found to react with MGO to form adducts at different efficiency and exhibit a different inhibitory efficiency of AGEs formation depending on their structures. Different adducts of MGO and phenolic compounds were identified and listed in Fig. 2.10. Lo et al. reported the reduction of MGO by theaflavins (60.1–66.7%) was much higher than that by catechins (17.1–45.7%). They suggested theaflavins to be the excellent candidate for the treatment of MGO scavenging in vivo in the future. Other phenolic compounds like chalcone [59], stilbenes [60], quercetin [61], and isoflavones [62] were also well studied for their trapping efficacy of MGO and GO. Based on these results, phenolic compounds having the same A ring structure showed efficient trapping capacity of MGO or GO to form mono- or di-MGO adducts even with different C rings. The mechanism is that the slightly alkaline pH can increase the nucleophilicity of the unsubstituted carbons at the A ring and facilitate the addition of MGO at these two positions to form mono- or di-MGO adducts. Such observation also applies to polymers of flavonoids such

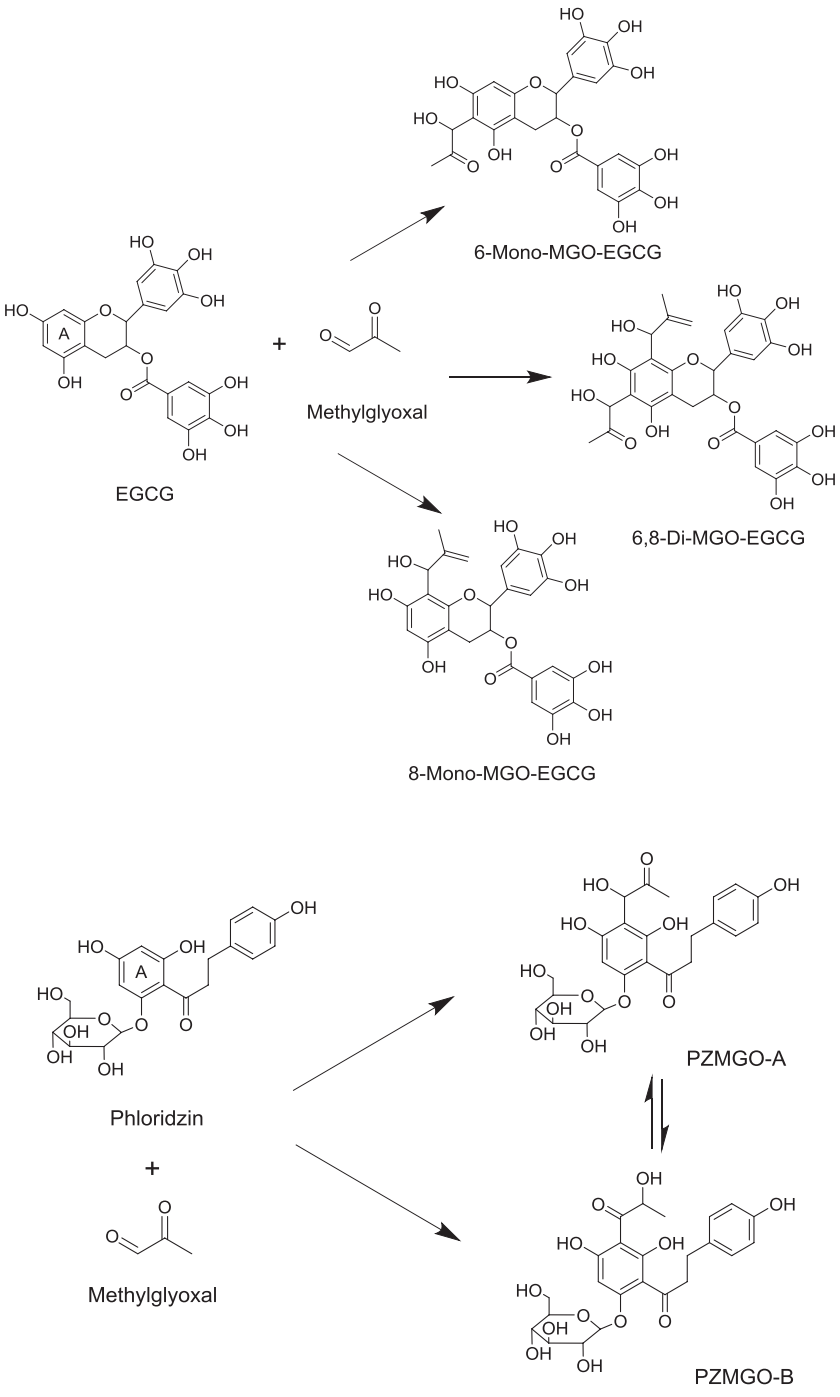


Fig. 2.10 Adducts of methylglyoxal (MGO) and various phenolic compounds [59–62, 67]

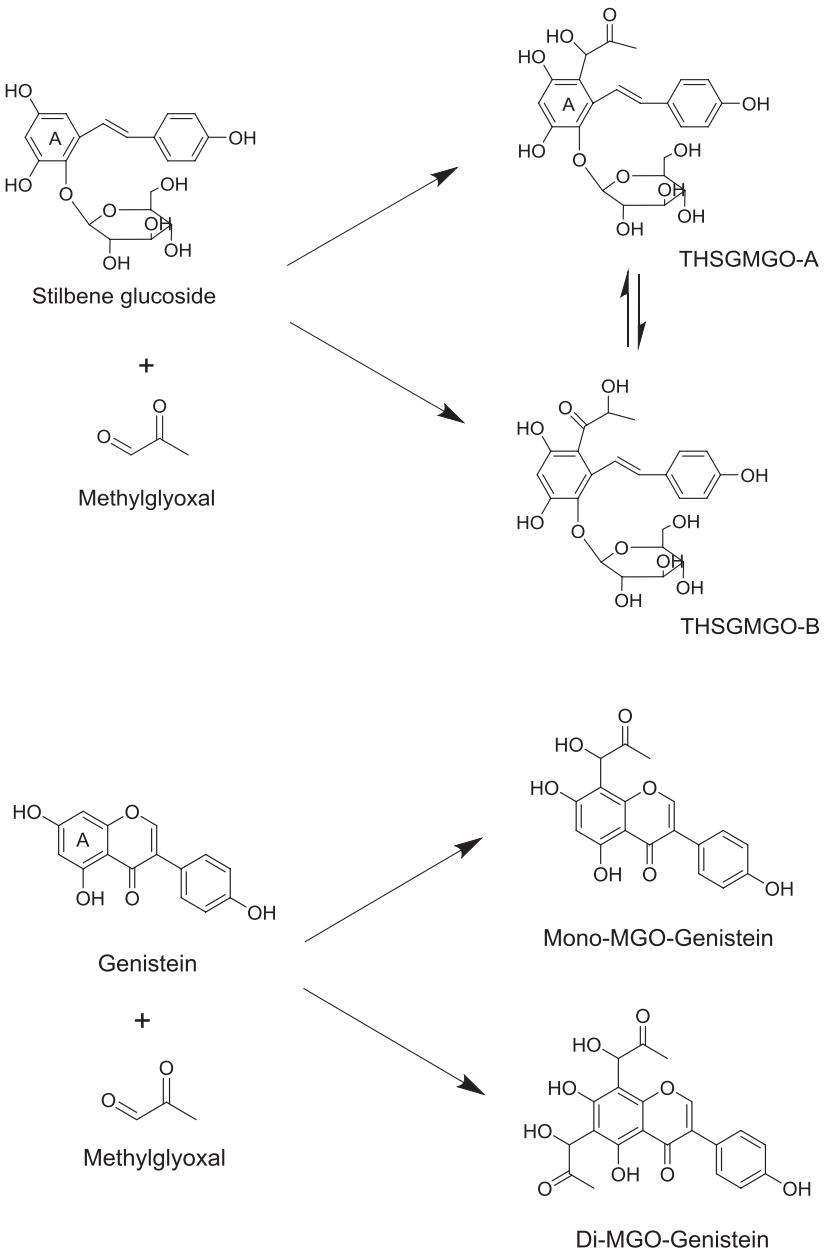


Fig. 2.10 (continued)

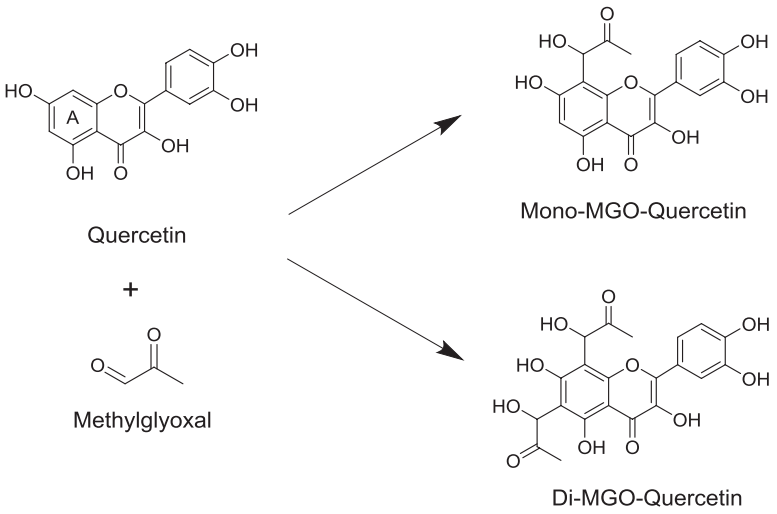


Fig. 2.10 (continued)

as proanthocyanidins [63]. The trapping efficacy and the reactive positions of the phenolic compound depend on the steric hindrance and carbon electron charges on the benzene ring. Compounds with one hydroxyl group on the benzene ring cannot react with MGO, whereas benzenetriols showed relatively higher trapping capability. Lo et al. [64] reported the electron charge of the carbons was required to be less than −0.24 for the high potent target sites for MGO trapping.

The observation by Ou et al. [65] that the addition of rosmarinic acid (RA) and carnosic acid (CA) decreased the fluorescence intensity of AGEs in both BSA/GO and BSA/MGO model may also be explained by the trapping of GO and MGO by RA and CA, resulting in the prevention of the reaction between both GO and MGO with the protein. In accordance, Navarro et al. [66] reported that the olive mill wastewater extracts reduced the formation of fluorescent AGE by trapping reactive dicarbonyls, such as MGO and GO, which was mostly contributed to the compounds of hydroxytyrosol and verbascoside through the partial fractionation study.

2.4.2 Inhibition of α-Dicarbonyl Compound Formation by Polyphenols

In addition to the trapping capacity of RA and CA detected on GO and MGO, Ou et al. [65] also revealed the inhibitory effect of RA and CA on the formation of MGO. However, in the case of GO, only CA presented an inhibitory effect on its formation, while RA showed, in contrary, a modest but significant promoting effect. Zhang et al. [68] studied the effects of chlorogenic acid (CGA) on the formation of 5-hydroxymethylfurfural (HMF) and found that the application of CGA caused a

significant increase in the accumulation of 3-DG ($p < 0.05$), while no impact on MGO ($p > 0.05$), and, as a result, increased the HMF formation. The effects of hydroxytyrosol, quercetin, and gallic acid on the formation of MGO, GO, and 3-DG in real cookies were investigated by Navarro et al. [38], who found that only hydroxytyrosol exerted inhibitory effect on 3-DG formation in biscuits. In contrast, the addition of gallic acid increased significantly the amount of 3-DG in biscuits. Unlike 3-DG, the accumulations of MGO and GO were hardly influenced by the application of all the phenolic compounds tested [38]. In another study using real cookies as objects, all of the five dietary polyphenols tested (naringenin, quercetin, epicatechin, chlorogenic acid, and rosmarinic acid) exhibited an effective inhibitory effect on the formation of GO but not on that of MGO [37]. Zhang et al. [37] also detected a positive correlation between the polyphenols' antioxidant activity and their inhibitory activity on the GO formation ($R^2 = 0.975$), leading to a hypothesis that the inhibitory activity of these polyphenols against GO formation during cookie baking might be attributable to the phenolics' free radical scavenging capability. However, their results are in contrast to those reported by Ou et al. [65] and Navarro et al. [38].

In most studies as mentioned above, the quantification of α-DCs in food, biological systems, and model systems typically involves derivatization processes utilizing *o*-phenylenediamine (*o*-PD) to generate stable quinoxaline moieties for detection. It was hypothesized that the phenolic-dicarbonyl adducts in samples mentioned in the Sect. 3.4.1 would be susceptible to the derivatizing agent and liberate the corresponding α-DC moieties [69]. Therefore, the quantitative analysis of α-dicarbonyl compounds included not only the free α-DCs but also the bound α-DCs, which represent the total formation of α-dicarbonyl compounds in the investigated samples. Therefore, Kokkinidou et al. [69] introduced a solid-phase extraction (SPE) preparation step to the quantification method to evaluate only the free α-dicarbonyl compounds. In their study on UHT milk, pretreatment of a combination of three phenolic compounds, catechin, daidzein, and genistein, showed a clear inhibitory effect on the accumulations of free GO, MGO, and 3-DG. By investigating the inhibitory effects by phenolic mixtures of different concentration ratios, linear effects of phenolic compounds applied were revealed on the concentration of GO in UHT processed milk, showing no significant interactions between different phenolic compounds. However, catechin and daidzein were the most influential factors followed by genistein considering their influence on GO concentration. In contrast, these compounds showed linear, cross product, and quadratic effects on MGO concentration, which indicate complicated interactions between phenolic compounds themselves and methylglyoxal. In the case of 3-DG, both linear and quadratic effects were reported [69].

The contradictory results of the role of phenolic compounds on the formation of α-dicarbonyl compounds indicated the complex mechanisms behind inhibitory/promoting properties. The contrary observations of these investigations might be due to the structural differences between the compounds studied. Until now, no solid conclusion could be made on the effects, and corresponding mechanisms, of phenolic compounds on the formation of α-dicarbonyl compounds. Further researches are highly demanded.

2.4.3 Inhibition of α-Dicarbonyl Compound Formation by Alkaloid Glycosides

In an in vitro study based on bovine serum albumin (BSA)/fructose model system [70], 95% ethanolic extracts of black nightshade were found to inhibit the generation of AGEs in a dose-dependent manner, probably by suppressing the formation of fructosamine and α-dicarbonyl compounds. Solasonine and solamargine were demonstrated to be the active compounds responsible for such suppressing effects. However, solasonine showed stronger antiglycative activities for attenuating AGEs, fructosamine, and α-dicarbonyl compounds generation than solamargine did. These results suggest that black nightshade, with solasonine as a major ingredient, might serve as a novel source of functional ingredients that exert antiglycation activities.

2.4.4 Temperature

The Maillard reaction plays an important role in the formation of reactive carbonyl species (RCS), such as MGO and GO, in vitro and in vivo. Scavenging reactive carbonyl species may terminate the Maillard reaction, and suppressing the Maillard reaction may reduce the level of RCS [10]. The investigations of the degradation pathway of model Amadori compound (N-(1-deoxy-D-fructose-1-yl)-glycine (DFG)) conducted by Martins et al. [71, 72] showed that pH had almost no effect on MGO formation. In contrast, an increase in the temperature from 100 to 120 °C induced an increase by double in the formation of MGO. In accordance, Nedvidek et al. [73] reported a very low increase in the MGO amount as pH changed from 5 to 7 in a glycine/β-alanine reaction mixture heated in phosphate buffer for 12 h. Therefore, temperature contributes as a key factor that influences the formation and concentration of dicarbonyl compounds through the Maillard reactions occurred in the thermal processing of food products.

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Chapter 3
Acrylamide

Yuan Yuan and Fang Chen

3.1 Occurrence, Exposure Assessments, and Toxicity

3.1.1 Occurrence in Foods

Acrylamide (AA), $CH_2CHCONH_2$ (Fig. 3.1), is a low-molecular-weight vinylic compound with a molecular weight of 71.09 g/mol. AA is a chemical monomer that is widely used in polyacrylamide synthesis and a variety of other chemicals in industry. AA has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer on the basis of sufficient evidence for carcinogenicity in experimental animals and mechanistic considerations [1].

The Swedish National Food Administration (SNFA) and the University of Stockholm reported that AA was present in wide varieties of consumed foods for the first time in 2002 [2]. After that, several countries had analyzed the AA contents in foods and verified the results found by Swedish scientists. Since 2003, data on the occurrence of AA in food commodities had been submitted to the Joint Research Center (JCR) of the European Commission by member states both from authorities and food industries [3]. The Food and Drug Administration (FDA) published data about AA concentrations in foods and confirmed that AA concentration in foods was in the range of 0–2510 μg/kg in 2004 [4]. Food categories that contained higher levels of AA were listed as follows: potato products (French fries, oven-baked chips, potato crisps), different cereal-based foods such as breakfast cereals, cookies, bis-

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cuits, bread (especially toasted bread), coffee, and other coffee substitutes, chocolate, baby rusks, and various other baby foods. Table 3.1 showed the mean levels of AA in different food categories from 2010 to 2015 [5].

Fig. 3.1 The structure of AA

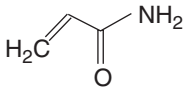


Table 3.1 Distribution of AA in different food categories from 2010 to 2015 by EFSA

Food categories	Mean (μg/kg)
1 Potato fried products (except for potato crisps and snacks)	
French fries and potato fried, fresh or precooked	
1.1 / sold as ready-to-eat	308
1.2 /sold as fresh or precooked, analyzed as sold	367
1.3 /sold as fresh or precooked, prepared as consumed	244.5
1.4 /sold as fresh or precooked, preparation unspecified	368
1.5 Other potato fried products	606
2 Potato crisps and snacks	
2.1 Potato crisps made from fresh potatoes	521
2.2 Potato crisps made from potato dough	327
2.3 Potato crisps unspecified	519
2.4 Potato snack other than potato crisp	283
3 Soft bread	
3.1 Wheat soft bread	38
3.2 Other soft bread	113.5
3.3 Soft bread unspecified	40
4 Breakfast cereals	
4.1 Maize, oat, spelt, barley, and rice-based products	122.5
4.2 Wheat- and rye-based products	160
4.3 Bran products and whole grains cereals	165
4.4 Breakfast cereals, unspecified	124
5 Biscuits, crackers, crisp bread, and similar	
5.1 Crackers	231
5.2 Crisp bread	279
5.3 Biscuits and wafers	201
5.4 Gingerbread	407
6a Coffee (dry)	
6a.1 Roasted coffee (dry)	510
6a.2 Instant coffee (dry)	695
6b Coffee substitutes (dry)	
6b.3 Substitute (dry), based on cereals	510
6b.4 Substitute (dry), based on chicory	2942
6b.5 Substitute (dry), unspecified	415

(continued)

Table 3.1 (continued)

Food categories	Mean (μg/kg)
7 Baby foods, other than processed cereal based	
7.1 Not containing prunes	18
7.2 Containing prunes	107
7.3 Plum content unspecified	33
8 Processed cereal-based baby foods	
8.1 Biscuits and rusks	106
8.2 Other processed cereal-based foods	53.5
8.3 Processed cereal-based foods unspecified	19.5
9 Other products based on potatoes, cereals, and cocoa	
9.1 Porridge	19
9.2 Cake and pastry	66
9.3 Savory snacks other than potato	171
9.4 Other products based on cereals	68
9.5 Other (non-fried) products based on potatoes	108
9.6 Other products based on cocoa	104
10 Other products not based on potatoes, cereals, coffee, and cocoa	
10.1 Roasted nuts and seeds	93
10.2 Black olives in brine	454
10.3 Prunes and dates	89
10.4 Vegetable chips	1846
10.5 Paprika powder	379
10.6 Other	68

3.1.2 Exposure Assessments

JECFA had assessed the AA dietary exposure and the risk of human cancer from the consumption of AA-contaminated foods [6]. The most recent analysis of human food consumption and an AA dietary exposure assessment for eight countries were evaluated at a JECFA meeting held in early 2010. For the general adults, the mean dietary exposures at the national level ranged from 0.2 to 1.0 μg/kg b.w. per day. For adult consumers at the high (95th–97.5th) percentile, the estimates of dietary exposure ranged from 0.6 to 1.8 μg/kg b.w. per day. Based on the few data available for children, it was noted that the dietary exposures of AA were about twice of those for adults when expressed on a body weight basis. The major foods contributing to the total mean dietary exposures for most countries were fried potatoes (in the United States, French fries) (10–60%), potato crisps (in the United States, potato chips) (10–22%), bread and rolls/toast (13–34%), and pastry and sweet biscuits (in the United States, cookies) (10–15%). Generally, other food items contributed less than 10% of the total dietary exposure. Table 3.2 showed the levels of AA exposure in diet (mean) from different regions in recent years.

Table 3.2 Exposure of AA (mean) in diet from different regions and countries

Origin of data	Dietary exposure	Group	References
Poland	0.16 μg/kg b.w./day	Women	[7]
China	0.319 μg/kg b.w./day	Population	[8]
Lebanon (from potato/corn chips)	0.204 μg/kg b.w./day	Population	[9]
Spain (from potato chips)	0.035 μg/kg b.w./day	Population	[10]
Poland	0.09 μg/kg b.w./day 0.13 μg/kg b.w./day	Girls Boys	[11]
Hong Kong	0.213 μg/kg b.w./day	Adults	[12]
Croatia	0.122 μg/kg b.w./day	Female	[13]
Turkey	1.43 μg/kg b.w./day	Toddlers	[14]
Canada	0.29 μg/kg b.w./day	Adolescents	[15]
Western diet	0.441 μg/kg b.w./day	Teenagers	[16]

3.1.3 Toxicity of AA

The metabolic kinetics of AA on human, mouse, and rat had been widely reported in recent years. After digestion and absorption, AA can rapidly distribute throughout the body, which can be detected in most organs, such as thymus, liver, heart, kidney, brain, and even in placenta and breast milk [17–19]. This indicated that AA could pass through the placental barrier, from pregnant mother to the fetus, posing a potential threat to the development. The two main metabolic pathways of AA in the body were shown in Fig. 3.2. Firstly, AA is oxidized to glycidamide (GA) by cytochrome P450 CYP2E1 after the absorption by the body. Secondly, AA can be combined with glutathione and form a copolymerized co-dicarboxylic acid salt. Toxicology experiments showed that 60% of the absorbed AA was excreted from urine, 86% of AA were excreted in the form of thiol and urate, and the unconverted AA accounted for only 4.4% [20]. N-Acetyl-S-(2-ethyl ester)-L-cysteine sulfoxide (AAMA-sulfoxide) was a specific metabolite of the human body and had not been detected in animals. It had been proved to be nephrotoxic and bladder toxic [21]. The study on rodents showed that as a result of the metabolic reactions, AA was transformed to GA, which then induced the formation of DNA adducts, resulting in mutagenesis and the development of cancers [22–24]. Most studies had pointed out that AA was neurotoxic to animals and humans, and AA was also known for its reproductive toxicity, genotoxicity, teratogenicity, mutagenicity, and carcinogenicity in animals.

3.1.3.1 Neurotoxicity

Neurotoxicity was firstly found in highly exposed occupational workers. The main mode of infection was through inhalation of the nose, mouth, and dermal absorption. The main manifestations of infection were ataxia, neuralgia, and numbness of limbs [25]. Early studies reported that peripheral nerve atrophy induced by AA was the

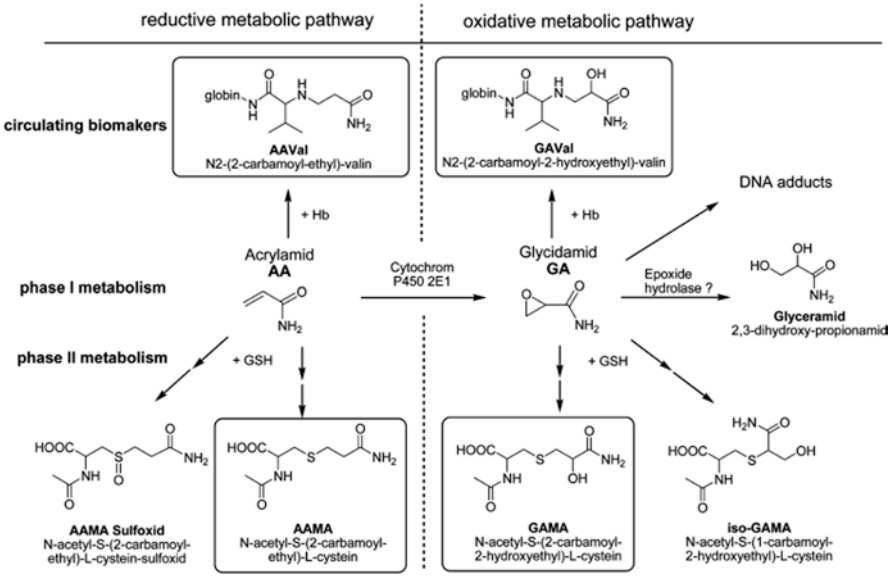


Fig. 3.2 Proposed metabolism of AA

major cause of neurotoxicity. Morphological examination of peripheral nerves revealed that this phenomenon occurred only at low doses of AA [26, 27]. Subsequently, there was a study confirming that AA toxicity also caused peripheral nerve endings by silver staining [28–30]. The combination of morphological, electrophysiological, morphological, and chemical results illustrated that toxicity of AA would damage the nerve transmission. As nerve endings were the primary targets of AA, it would result in damages to the central and peripheral nervous system synapses in a series [31]. With the development of mass spectrometry and nuclear magnetic resonance technology, AA was regarded as an electrophilic reagent, and it was easy to react with DNA, protein, and other macromolecules to form adducts. For example, toxicity induced by AA can attack the residue Cys342, thereby inhibiting the Na^+-dependent presynaptic dopamine transporter function [32]; tandem mass spectrometry results showed that AA at low doses could react with the active site of GADPH and Cys152, while AA at high doses would add a site to Cys156 and Cys247 to inhibit the normal physiological role [33]. As a typical 2-olefin structure, AA can easily perform soft ionization and attack sulfhydryl groups in proteins and DNA. The attack of AA on some proteins can cause transmission disorders, which resulted in significant damages of ending synaptosome [32]. The nerve ending without transmission and translation ability would not have self-protection function. Therefore, it needed other cells to help to achieve its own regulation, and the injured part was difficult to be repaired. This caused insufficient signal supply and led to neurotoxicity [34–36]. Although the maximum dose of AA to cause neurotoxicity was found to be between 0.2 and 10 mg/kg b.w. in the rat model, there was no significant neurotoxicity observed below this range of doses [6]. However, some researchers had pointed

out that neurotoxicity induced by AA might have chronic accumulation effect. Although the average daily exposure amount was far below the maximum dose which had no effects, the risk of accumulation of AA due to long-term dietary exposure to high doses of it still should not be ignored [25]. 2-Olefins at low doses may contribute to the development of stroke, atherosclerosis, Alzheimer's disease, diabetes mellitus, and spinal cord injuries because of the intrinsic oxidative stress [37–41]. Further studies had shown that oxidative stress would lead to highly active 2-olefin derivatives, such as acrolein and HNE (Hydroxynonenal), which might be an important pathological progression of Alzheimer's disease [42–44]. And in Alzheimer's disease, the initial manifestation of pathophysiology was the damage of nerve endings, which occured prior to neurodegeneration and was similar to what AA was induced in animal models [45–47]. Based on a common structure and a similar toxic phenotype, oxidative damage caused by AA may be a potential cause of neurotoxicity and should be paid attention to.

3.1.3.2 Hepatotoxicity

The liver lesion can lead to metabolic detoxification, which causes systemic reactions, and is one of the major target organs that AA attacked after entering the body [48]. As the primary metabolic organ, metabolic activity in the liver was active after exposing to AA. Zhang L et al. reported that AA at 50 mg/kg b.w. could induce liver damage in mice by inducing excessive production of ROS (Reactive oxidative species), consuming GSH and breaking the redox balance [49]. Similarly, Sen et al. also pointed out that 2.5 mM AA on liver cell model for 24 h significantly increased the expression of GST and cytochrome P450-related genes such as deethylase, demethylase, and hydroxylase as well as the protein levels [50]. And a higher dose of AA could cause the death of liver cells [50]. Zhao M et al. reported that 50 mg/kg b.w. AA could cause DNA damage in liver cells of mice and lead to excessive ROS production. The activities of antioxidant reductase GST, GPx (glutathione peroxidase), and SOD (superoxide dismutase) were also changed, leading to break liver cellular redox balance and liver injuries [51]. AA also produced an electron leak by altering the enzyme activity in mitochondrial complexes, stimulating excessive amounts of ROS, and causing damage to liver mitochondrial function [52]. Liver injuries induced by AA were mainly caused by excessive ROS production, resulting in redox imbalance, but the pathway and mechanism on liver toxicity between ROS and AA still needed further investigation.

3.1.3.3 Carcinogenicity

AA had been shown to cause carcinogenicity in several organs in animals and caused tumor formation in the lungs, uterus, dermis, breast, and brain tissues [53]. Studies were carried out on F344 rats and B6C3F1 mice to study the carcinogenicity induced by AA. The results suggested that AA might cause breast fibroadenoma or malig-

nancy in female rats and lead to the formation of the scrotum sheath tumor and the thyroid adenoma in male rats. Conversely, no tumor was found in the target organs of the AA, including the livers, kidneys, lungs, and lacrimal glands [54–56]. Most studies had suggested that the carcinogenicity of AA was caused by the metabolic product GA in vivo. In vivo and in vitro studies illustrated that GA was more mutagenic and genotoxic than AA. Adducts formed by DNA and AA, GA were considered to be the key factor to induce carcinogenesis [57, 58]. Studies had suggested that the combination of AA and GSH led to the depletion of glutathione in vivo, breaking the original redox balance as well as resulting in oxidative stress and thereby affecting gene expression [59]. It was also reported that AA/GA inhibited mitotic or meiotic regulating proteins, and the disruption induced by this may be the cause of genotoxicity and carcinogenicity [60, 61]. In addition, the abnormal hormone levels induced by AA are also a possible reason for gonadal cancer and thyroid cancer [6]. Many references linked the carcinogenicity of AA with its genetic damage and genotoxicity. However, the exact relationship between genotoxicity and carcinogenicity induced by AA had not been established. The United States National Cancer Research Center (NCTR) treated newborn mice and rats with drinking water containing AA/GA for a period of 2 years. The results showed that in the carcinogenic mechanism induced by AA, GA contributed to the distortion in DNA directly [56]. At the same time, AA could cause tumors in a variety of different sites, and there was specificity related to species in mice and rats. Moreover, researchers had found tumors in neonatal mice, which might be related to the expression of cytochrome P450 2E1, a key enzyme in AA transformation to GA, in early embryonic development. Therefore, the harm of AA to infants was worrisome [56].

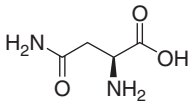
3.2 Formation Mechanisms

In 2002, two articles had been published in *Nature* about AA formation mechanisms. The Maillard reaction between asparagine (Asn) and reducing sugar, represented by glucose (Glc) and fructose (Fru), is the main route for AA formation [62, 63]. In addition, the triacylglycerol oxidation pathway of acrolein can also form small amounts of AA.

3.2.1 Asparagine Pathway

The Maillard reaction is considered as the main AA formation pathway. Stadler and Mottram [62, 63] heated individual amino acids at a certain time above 100 °C, and only a small number of amino acids (such as Asn, glutamic acid, tryptophan, and methionine) produced a small amount of AA. When Asn and reducing sugar were mixed, the amount of AA formed was 500 times higher than that of amino acids heated alone. Zyzak et al. [64] proved that nitrogen atom and carbon atom of AA

Fig. 3.3 Asn structure



from Asn by isotope tracing method (^{15}N and ^{13}C). Asn can be directly converted to AA by the removal of carboxyl and amino group under heating conditions without the addition for reducing sugars from the structure of Asn (Fig. 3.3). However, the results obtained in actual experiments showed that the pyrolysis products of Asn were mainly maleimide, and the reasons were owing to the rapid intramolecular cyclization of Asn molecules under heating conditions, thus inhibiting the formation of AA [65]. While in the presence of reducing sugars, after the formation of a reaction of N-glycosylated intermediates by Asn and reducing sugar, the energy of the decarboxylation pathway was reduced, thereby bypassing intramolecular cyclization and promoting the generation of AA [65].

The beginning of the asparagine pathway was the initial stage of the Maillard reaction (Fig. 3.4). When the Schiff base intermediate was formed in equilibrium with the N-amino acids, two different reaction routes led to the formation of AA.

The first pathway for AA formation was the N-glycosidic pathway (Fig. 3.5). Under the neutral condition, Schiff base intermediates formed by Asn and Glc generate oxazolidinone intermediate by intramolecular cyclization. This intermediate can decarboxylate to Azomethine ylide even at room temperature [66, 67]. It could also form the decarboxylation of Amadori products, and then the C-N bonds were cleavaged to generate AA. The limited step of this process was the formation of AA between the C-N bonds through the elimination reaction [65]. Under the alkaline conditions, Schiff base can also be directly decarboxylated to decarboxylation Schiff base. The decarboxylation Schiff base and the Amadori product directly generated AA or by 3-amino amide pathway. In the N-glycoside pathway, the 3-amino amide was a key intermediate for the formation of AA [64, 68]. Since this decarboxylate reaction was a mild reaction, it could occur at room temperature, and more AA could be produced as compared to the Strecker pathway [69].

The second pathway for AA formation was the Strecker pathway (Fig. 3.6). Schiff base generated by Asn and Glc would produce Amadori products and then dehydrate ammoniagenesis. Asn could be degraded to AA by Strecker degradation in the presence of carbonyl molecule [62]. This pathway suggested that two carbonyl compounds played a crucial role in the formation of AA.

3.2.2 Acrolein Pathway

In addition to the asparagine pathway, AA can also be produced by acrolein or acrylic acid in the high-fat systems (Fig. 3.7) [70]. Yasuhara [71] found that Asn and three oleic acid glycerin could generate more AA after mixing and heating in the model systems. Ammonia and acrolein were heated at 180 °C to produce a consider-

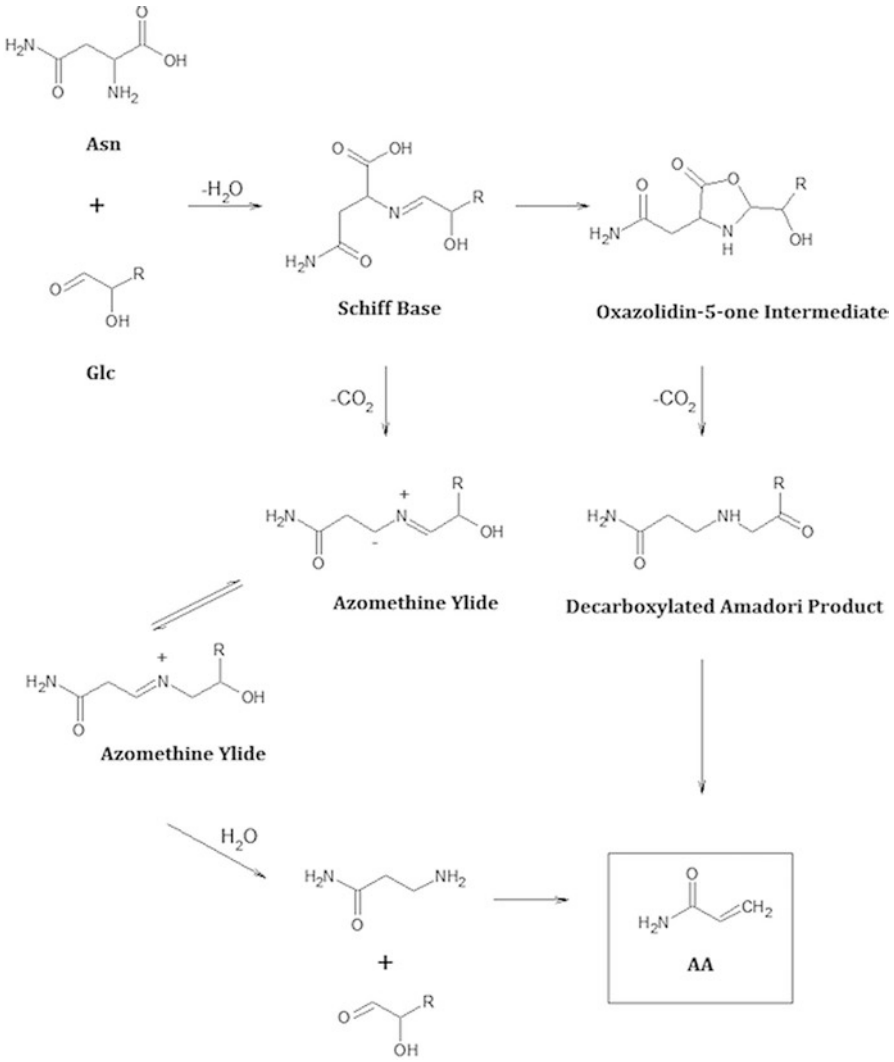


Fig. 3.4 Formation mechanism of AA via the asparagine pathway

able amount of AA. When ammonia and acrylic acid react under the same conditions, more AA would be produced. The study also found that ammonia and acrolein could react at various temperatures, and even at room temperature. The findings suggested that some low-temperature processed foods also had the potential to be contaminated by AA.

Acrolein came from a wide range of sources, and three major nutrients could produce acrolein during food processing. The thermal degradation of monosaccharide which occurred in the heating process of high-temperature protein and carbohydrate decomposition reaction would produce a large number of small molecular

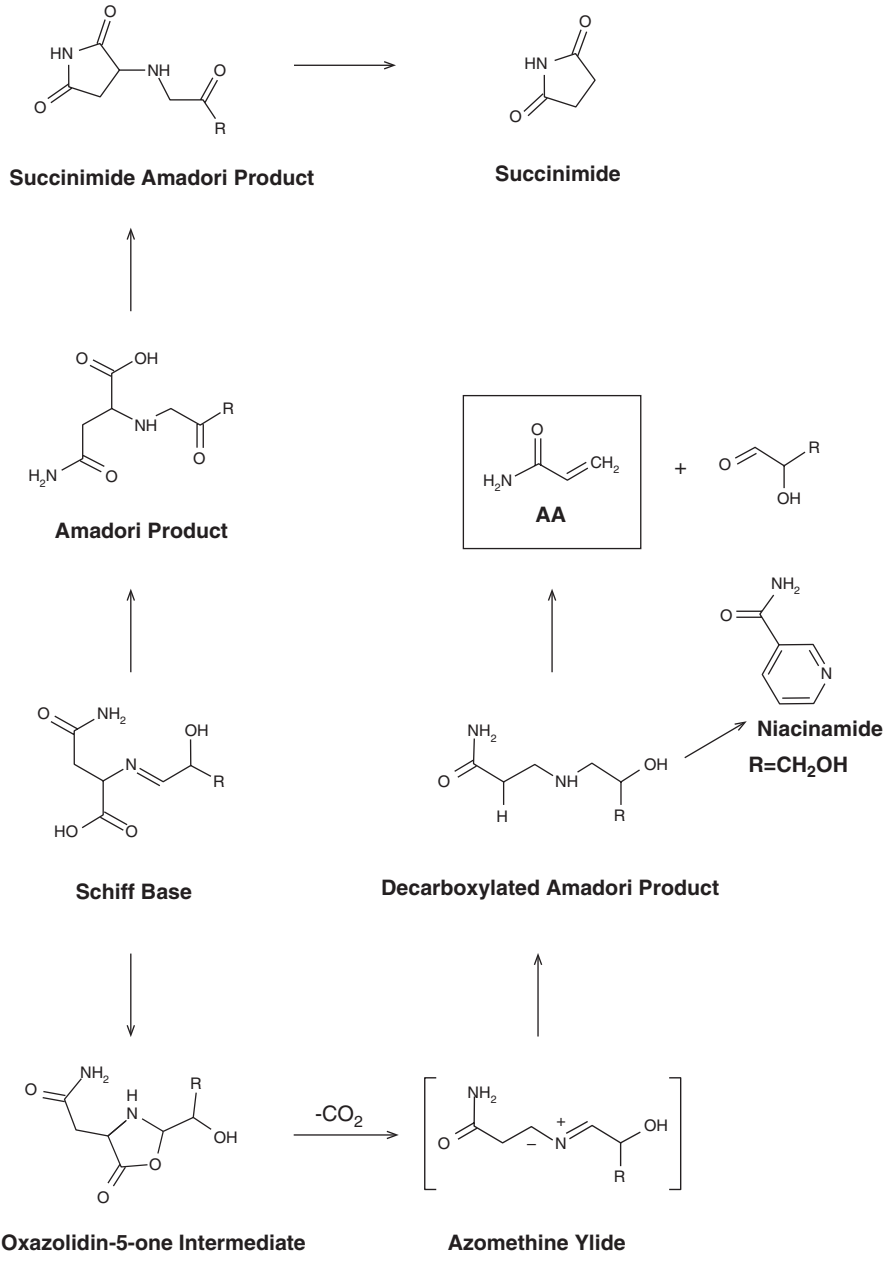


Fig. 3.5 AA formation by N-glycoside pathway

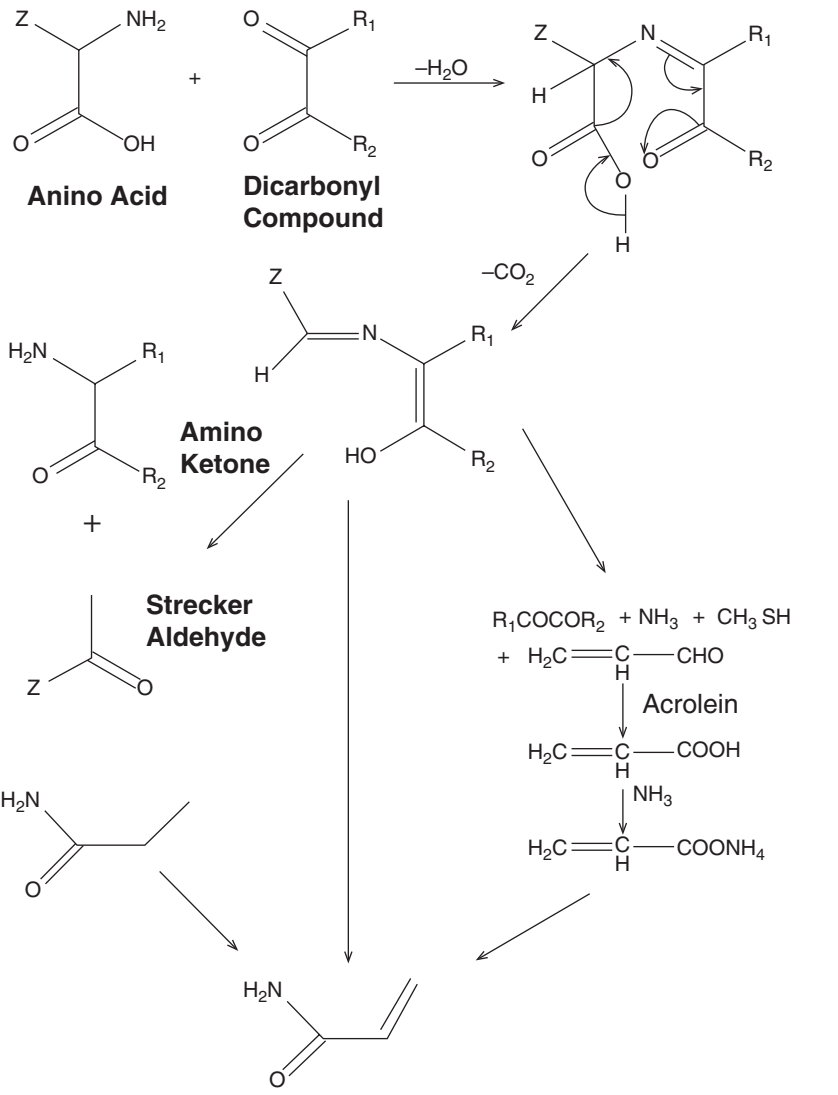


Fig. 3.6 AA formation by Strecker pathway

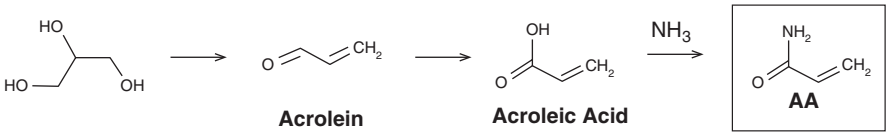


Fig. 3.7 Formation of AA from acrolein

aldehydes (such as formaldehyde, acetaldehyde, and so on). These aldehydes can form acrolein under suitable reaction conditions and then produce AA by the acrolein pathway [72]. The release of oil at high temperature in the heating process of glycerol three vinegar can be decomposed into glycerol and fatty acids, then further dehydration of acrolein produced glycerol [73], and fatty acids and glycerol were generated by oxidation of acrolein and acrylic acid, which provided precursors for the formation of AA. Protein and amino acids in foods, such as aspartic (Asp), can be degraded into acrylic acid. Alanine and arginine heated at high temperature can be converted into acrylic acid. Serine and cysteine generated acrylic acid by pyruvic acid and lactic acid under the condition of heating [69]. In meat products, carnosine can release β-alanine by hydrolysis, after deaminate to produce acrylic acid [74]. Although acrolein was widely used in food processing, the amount of AA produced by acrolein via the acrolein pathway was much smaller than that of the asparagine pathway [75] because of the presence of free ammonia in the acrolein pathway.

3.3 Analysis Methods

3.3.1 Pretreatments for AA Analysis

3.3.1.1 Extraction Technology

The initial focus in the analysis of AA was to obtain a representative homogenous sample. The whole portion or serving of food should be homogenized thoroughly before the extraction and analysis steps. The greatest differences between the analytical methods were the extraction and cleanup step [76]. In recent years, some new extraction technologies were used in the extraction of AA for further analysis. Abd and Albishri presented the simultaneous online preconcentration and separation technology for AA, Asn, and Glc using analyte focusing on ionic liquid micelle collapse capillary electrophoresis (AFILMC) [77]. The coupling of AFILMC with IL-based ultrasonic-assisted extraction (ILUAE) was successfully applied to the efficient extraction and determination of AA, Asn, and Glc in bread samples. Furthermore, an eco-friendly and costless coupling of the HPLC-UV with ionic liquid-based ultrasonic-assisted extraction (ILUAE) was developed to determine the AA content in food samples. ILUAE was proposed for the efficient extraction of AA from bread and potato chips samples [78]. Sun et al. demonstrated a facile MS-free method to determine AA content in microwaved and conventional heated popcorn and rice, using activated carbon-packed extraction column coupling with simple GC analysis [79]. Gökmen et al. studied the effects of single- and multiple-stage extraction procedures on the extraction yield of AA for various cereal- and potato-based thermally processed foods [76]. The extractability was an exponential function which could be used to optimize the multiple extraction conditions during the analysis of foods for AA. In general, the aqueous extraction using 10 mM formic acid was found to be more effective than the methanol extraction.

3.3.1.2 SPE and SPME Methods

For the cleanup of AA from complex samples, accelerated solvent extraction (ASE), liquid-liquid extraction (LLE), and solid-phase extraction (SPE) were used alone or in combination with other purification steps to extract AA. ASE and LLE with conventional organic solvent were time-consuming and labor-intensive methods for AA extraction and cleanup. Furthermore, these methods can easily lead to loss of AA in the scavenging process and require large amounts of organic solvents. The SPE method is simple and stable, has easy automation methods, and has been developed and applied in the cleanup of AA. Can and Arli developed an HPLC method and indicated AA levels in some traditional and non-traditional foods consumed in Turkey [80]. AA content of samples was determined using HPLC coupled to photodiode array detection. Samples were prepared by utilizing Oasis HLB (Waters) cartridges for cleanup and analyzed with no further preconcentration steps. Zhang W et al. researched a rapid and effective HPLC method, using tetraazacalix arene triazine-modified silica gel (NCSi) as SPE sorbent, which was developed for the purification and determination of trace AA in starchy foodstuffs [81]. The improved method was simple, rapid, accurate, and promising for the determination of trace AA in starchy foods with a complex matrix. Zhao H et al. synthesized a functionalized material by immobilizing an ionic liquid onto an activated silica gel surface [82]. Using this material as a sorbent, a method of SPE coupled with HPLC (SPE-HPLC) for analysis of AA in foods was developed. And this method was successfully applied to quantitative detection of AA in bread crust and cracker samples. Bortolomeazzi et al. developed a rapid and reliable purification method based on a single mixed SPE column, for the determination of AA in roasted coffee by LC-MS [83]. The results showed that the SPE method could be used in the pretreatment of AA in food. For SPE, the most important effect on the enrichment efficiency was the adsorption performance of the sorbent. Thus, a functionalized material with high adsorption ability was highly desirable.

The analysis of AA in food always required several pretreatments and cleanup steps. Conventional extraction techniques used SPE to purify crude sample extracts prior to the analysis. However, due to the multiple SPE steps, these methods were often time-consuming and achieving unsatisfactory limits of detection (LOD) [84]. Over the past few decades, solid-phase microextraction (SPME) had become a popular extraction technique in food analysis, due to its exceptional simplicity and ease of use. SPME was an equilibrium-based extraction/preconcentration technique that enabled the consolidation of sample preparation, cleanup, and sampling into one simple step. Compared to SPE, SPME was a non-exhaustive extraction technique that used considerably smaller sample volumes and was not susceptible to analyte breakthrough [85]. Qu et al. developed an SPME fiber coated with SWC-NTs/Ppy coupled to GC with electron capture detection (ECD) method for the detection of AA in different food samples [86]. Ghiasvand and Hajipour found a rapid, easy, and low-cost HS-SPME-GC-FID method to determinate the AA in foodstuffs (chips and French fries), using GC-flame ionization detection (GC-FID) system after its direct trapping in the upper atmosphere of samples by headspace SPME (HS-SPME) [87]. Chen L et al. established a new approach for the determination of AA in foods by SPME-GC

after derivatization [88]. The 2-bromoacrylamide (2-BAM), transformed from AA, was extracted by a commercial SPME fiber, 75 μm Car/PDMS fiber, for GC detection. The presented method was applied to the determination of AA in fried foods.

3.3.1.3 MSPD Methods

MSPD was a sample extract technology based on the traditional SPE method. In recent years, a number of applications of MSPD in food analysis had been reported [89–92]. Zhao H et al. synthesized and characterized the chitosan-grafted multiwalled carbon nanotubes using Fourier transform infrared spectroscopy, transmission electron microscopy, X-ray diffraction, thermogravimetric analysis, and static and kinetic adsorption experiments [93]. And it could be used as a sorbent for development of MSPD extraction coupled with HPLC for the determination of trace AA in foods. Xu X et al. found a quantitative method for the simultaneous determination of 3-MCPD and AA in food by GC-MS/MS. The analytes were purified and extracted by MSPD technique with Extrelut NT [94]. Soares and Fernandes described the development of an optimized MSPD procedure for the analysis of AA in a variety of food matrices (cereal products, chocolates, and baby foods) [92].

3.3.1.4 Derivatization Methods

Bromination was often used to determine the AA content in water or foods. Although bromination was a well-known and widely used derivatization technique, it needed a large number of reagents. In addition to this bromination method, some derivatization reagents such as 2-mercaptobenzoic acid [95, 96], dansulfinic acid [97, 98], and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) [99] had been applied for the determination of AA.

Cengiz and Boyaci Gündüz developed a method involving defatting with n-hexane, extraction with water, precipitation of proteins, bromination, extraction into ethyl acetate, and injection into a GC-MS system to detect AA of 62 different cereal-based baby foods [100]. The method was more eco-friendly and less expensive because it consumed very little solvent relative to other methods using bromine solutions and ethyl acetate. Surma et al. reported the development of a rapid and simple sample preparation method for AA determination in cocoa powder through extract purification by dispersive SPE and derivatization with BSTFA [101]. The developed method had been successfully applied to the determination of AA in 9 cocoa powder and 30 chocolate (dark, milk, and white) samples purchased at a Polish supermarket. Molina-Garcia et al. developed an AA derivatization technique using xanthydrol and analyzed by GC-MS in selected ion monitoring (SIM) mode in order to analyze AA in fried potatoes [102]. This method was applied to diverse foodstuffs and water, and it was claimed to be more environmentally friendly. A series of drawbacks that occurred with bromination derivatization were avoided when xanthydrol was used.

3.3.1.5 QuEChERS Methods

QuEChERS (quick, easy, cheap, effective, rugged, safe) was the latest rapid sample pretreatment technology developed by the US Department of Agriculture in 2003. QuEChERS had the advantages of high recovery rate, good accuracy, a wide range of analytical pesticides, fast analysis speed, and easy operation. In 2006, the QuEChERS method was developed and optimized by Mastovska and Lehotay as a high-throughput extraction method for AA analysis in various food matrices using LC-MS/MS or GC-MS [103]. The method involved the extraction with acetonitrile, liquid-liquid partition by salting out with sodium chloride and magnesium sulfate, and the cleanup step with the dispersive solid-phase extraction (dSPE) such as a primary secondary amine (PSA) [104].

The usefulness and effectiveness of QuEChERS method combination followed by AA silylation had been proven. Modification of the QuEChERS sample preparation method for the determination of AA in coffee and coffee substitutes via silylation had been reported. A sample of instant coffee was used to develop an analytical method in the optimization experiment, which involved the selection of the most suitable sorbents for dSPE cleanup. The AA level after conversion to N,O-bis-(trimethylsilyl)-AA (BTMSA) was determined with GC-MS in a single ion monitoring mode (GC-SIM-MS). The usefulness of the method had been verified based on the recovery ratio of AA (fortified samples analysis). The chosen method was successfully applied for the AA determination in 17 roasted, 10 instant, and 8 coffee substitute samples [105]. And the method for AA extraction and determination in dried fruits (dried prunes and raisins) and edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, and walnuts) using more efficient QuEChERS approach was set up. The study described a quick and easy method for AA determination by QuEChERS approach coupled to LC-ESI-MS-triple quadrupole technique with a “one-pot” sample preparation. Linearity, sensitivity, accuracy, and precision of the method were satisfactory [106]. A sample preparation method based on modified QuEChERS with aluminum oxide (Al_2O_3) as dSPE material and HPLC LTQ-Orbitrap MS was established. The performance of two analytical columns namely Kinetex C_{18} and Rezex ROA-organic acid was compared for AA separation. The proposed Al_2O_3 dSPE method was successfully applied to the analysis of AA in real food samples. And the Kinetex C_{18} column can be used for routine analysis of AA in a huge number of food samples, and ROA-organic acid column can be used for analysis of samples containing low contents of AA [107]. The use of the QuEChERS method, being nowadays one of the most widespread techniques in food analysis, can provide the ability to analyze various food contaminants at the same time.

3.3.2 Instrument Analysis

Hitherto, LC and GC coupled to MS appear to be acknowledged as the most useful and authoritative methods for the determination of trace AA.

3.3.2.1 HPLC Technology

HPLC with liquid mobile phase separated the different polarity of a single solvent or different proportions of mixed solvents. Xu L et al. reported a sensitive method of HPLC based on SPE using a reversed phase C_{18} column, which was characterized by adsorption experiments with good adsorption ability and rapid adsorption dynamic toward AA [108]. And the developed method was applied to the extraction and determination of AA in deep-heated food samples with a good accuracy. Jiang et al. successfully synthesized a novel AA molecularly imprinted material based on silica microparticles [109]. The molecularly imprinted polymers (MIP) on the surfaces of silica microparticles were characterized using infrared spectroscopy and scanning electron microscopy, and the amount of AA adsorbed on the MIP was measured using HPLC and equilibrium binding experiments. HPLC as one of the most widely used technologies, coupled with MS detection, was the most preferred.

The use of HPLC for the analysis of AA was another way to avoid derivatization steps, which were often laborious and resulted in variable yields. HPLC analyses of AA that employed UV-detection suffered from high detection limits (3–10 μg/L) [110]. Wang H et al. studied on a simple and cost-effective method using HPLC-UV technology to quantify the content of AA in baked and deep-fried Chinese foods [111]. HPLC-UV methodology was equipped with a vacuum degasser, a binary pump, a diode array detector, and a temperature-controlled column oven. And the AA contents were found to be 86.3–151 μg/kg. A fast and cost-effective method using HPLC-UV had been developed for the determination of AA in deep-fried flour-based leaven dough foods in Hong Kong [112]. AA was detected at UV wavelengths of 210 and 225 nm. The amounts of AA in eight food samples (stuffed glutinous rice ball, "oxtongue" fritter, sesame ball, deep-fried sesame cookies, glutinous rice sesame ball, fried sweet dumpling, fried egg pastry, and sweet potato crisps) were 27–198 μg/kg when 1 g samples were analyzed. Paleologos and Kontominas developed a method using normal phase with UV detection for the analysis of AA and methacrylamide [113]. This method was applied for the determination of AA and methacrylamide in spiked food samples without native AA yielding recoveries between 95% and 103%.

More recently, HPLC-MS methodology is used due to its high selectivity and sensitivity. Solid-phase cleanup coupled to LC-MS detection was nowadays the gold standard procedure for AA quantitation owing to the high reproducibility, good recovery, and low relative standard deviation. Backe et al. developed a simple and sensitive analytical method to quantify levels of AA in environmental and drinking waters by HPLC-MS [110]. The method detection limit and reporting levels were 2.4 and 17 ng/L, respectively. Troise et al. researched an LC method for AA determination using HRMS detection. It was proved to be solid and robust with LOD of 2.65 ppb and LOQ of 5 ppb. The method was tested on four AA-containing foods: cookies, French fries, ground coffee, and brewed coffee [114].

LC-MS/MS showed high sensitivity, strong anti-interference ability, and high qualitative and quantitative accuracy in the multi-residue trace detection. Recently,

HPLC-MS/MS and UHPLC-MS/MS had been developed to simultaneously analyze AA, Asn, and sugars in the Maillard reaction. Lim and Shin developed an LC-MS/MS method to determine the amount of AA in foods after derivatization with D-cysteine [115]. The method was successfully applied to determine the amount of AA in potato chips, French fries, and coffee. Zhang Y et al. developed an automated microwave digestion labstation (MDL) combined with UHPLC-MS/MS method which was successfully validated and applied to the determination of AA and its precursors and intermediates during Maillard reactions and kinetic elucidation [116]. Zhang C et al. employed UPLC-MS/MS and QuEChERS dispersion to establish the detection method for four AA compounds (AA, methacrylamide, N-methylolacrylamide, N-(Methoxymethyl)methacrylamide) in food-contact paper products and food simulants, which could provide reference for the detection and specific migration study of related compounds in foods [117].

3.3.2.2 GC Technology

GC methods have generally measured the derivatization of AA with potassium bromate/potassium bromide to improve the GC properties. A recent GC method employing a high-resolution time-of-flight mass analyzer was developed for direct analysis (no derivatization) of AA in various heat-processed foodstuffs [118]. Another GC method was based on derivatization of the target analytes with bromination and detection by an electron capture detector (ECD) [119]. Qu et al. developed an SPME coupled to GC with ECD method for the detection of AA in food samples, which possessed good extraction efficiency of AA [86]. The linearity range between the signal intensity and the AA concentration was found to be in the range 0.001–1 μg/mL, and the coefficient of determination was 0.9985. Notardonato et al. proposed a new method for quantitative analysis of AA in cereal-based foods and potato chips [120]. This method used reaction with trifluoroacetic anhydride and analyzed the resulting derivative by GC-ECD. Under the optimum conditions, good retention and peak response were achieved for the AA derivative. The analytical method was fully validated by assessment of LODs and LOQs.

A European interlaboratory study was conducted to validate two analytical procedures for the determination of AA in bakery ware (crisp bread, biscuits) and potato products (chips), within a concentration range from about 20 μg/kg to about 9000 μg/kg. The two methods were based on GC-MS of the derivatized analyte and on HPLC-MS/MS of native AA [121]. A sample preparation technique based on MSPD was applied to the determination of AA in potato chips. After bromination of the extract, the samples were analyzed by GC-MS in selected ion monitoring mode. The method presented good recoveries, and the LOD and LOQ were 12.8 and 38.8 μg/kg, respectively [122].

A GC-MS/MS method was used to measure AA in aqueous matrices extracted from French fries and potato crisps using direct immersion SPME without derivatization. The concentrations of AA detected in French fries and potato crisps were 1.2 and 2.2 μg/g, respectively [123]. Another GC-MS/MS method was developed for separa-

tion, detection, identification, and quantification of AA in bread, biscuits, and similar products, which showed good precision with values lower than 6%. A good sensitivity was achieved for bread with 2.41 (LOD) and 7.23 μg/kg (LOQ), respectively, while for biscuits, LOD and LOQ were 4.63 and 13.89 μg/kg, respectively [124].

In addition, GC-nitrogen phosphorus detector (GC-NPD), GC-MSD, and other technologies could be used in the analysis and detection of AA. Kim et al. developed an improved analytical method for the quantification of AA using a GC-NPD, which showed reasonable recovery, sensitivity, and accuracy in monitoring AA levels [125]. A new protocol combining GC-NPD and LC-tandem mass spectrometry (MS/MS) could be applied to analyze AA accurately and precisely, contributing to the development of a prescreening tool to analyze AA in the food industry. A direct GC-MS method for quantitative determination of AA in several foods was described. The use of diatomaceous earth as sorbent was in combination with ethyl acetate as desorbing solvent. This allowed us to make use of a mass selective detector (MSD), which was cheaper, and available in more laboratories than tandem mass spectrometers [126].

3.3.2.3 Capillary Electrophoresis (CE) Technology

CE had the merits of high efficiency, fast analysis, and low reagent consumption and became an effective alternative for the analysis of a great variety of target substances, but its stability is poor [108]. A non-aqueous CE method was developed for the quantitative determination of AA in the processed food like potato chips and French fries. The AA amount of these samples was found to be 2.95 ± 0.11 mg/kg. A field-amplified sample stacking technique with the non-aqueous capillary electrophoresis method was introduced for the online concentration of AA to increase the poor sensitivity of UV detection at 210 nm by diode array detection [127]. The application of the sample stacking was performed on potato chips and almond extracts. The AA content in almond extract and some potato chips was found to be 95.5 ± 7.6 and 4.3 ± 1.5 μg/kg, respectively [127]. Chen Q et al. [128] detected AA levels in potato crisps by CE with quantum dot-mediated LIF detection. The method had been successfully applied in the determination of sub-parts per million levels of AA or 2-propenamide in a complex sample such as potato crisps.

3.3.3 Rapid Detection Technologies

Since the industries and factories are trying to control the levels of AA in food, there is a need for fast and routine quality control methods for the detection of AA. The current techniques used for AA detection had the following steps: aqueous extraction of AA from food, the concentration of the sample, and analysis by either GC/LC-MS or HPLC. The whole process took a considerable amount of time and required both sophisticated equipment and trained personnel. For this reason, there was a need for a simple, robust, and fast detection method for AA [129]. LC-MS/

MS and GC-MS methods as standard detection methods showed high sensitivity, selectivity, stability, and repeatability. However, these methods required expensive instruments, skilled technicians in laboratories, industries and factories, and high testing costs, and could not meet the needs for real-time and online detection of AA in foods. Therefore, rapid detection methods had developed based on the similar sensitivity and selectivity, but required less time and cost in comparison with standard methods for AA detection.

3.3.3.1 Elisa Methods

Immunochemical methods such as enzyme-linked immunosorbent assays (ELISA) had become a popular and useful screening tool for deleterious compound residues because the methods are of low cost, high selectivity, and specificity. Moreover, ELISAs were able to simultaneously analyze a large number of samples with a simple extraction process. Zhu Y et al. coupled the AA derivative (4-mercaptobenzoic acid) to a carrier protein such as bovine serum albumin and ovalbumin [130]. The conjugates were used as the immunogen and coating antigen. A rapid and sensitive indirect competitive enzyme-linked immunosorbent assay (icELISA) against AA-4-mercaptobenzoic acid was obtained by optimizing the experimental parameters. The monoclonal antibody had no specificity for AA or 4-MBA, but had a high affinity for AA-4-MBA, with an IC_{50} of 32 ng/mL and LOD of 8.87 ng/mL. The quantitative working range was 8.87–112.92 ng/mL (IC_{20} to IC_{80}). Cross-reactivity with other analogs was lower than 10%. These results indicated that the developed icELISA was a fast and efficient method for the detection of AA in food [131].

An enhanced chemiluminescence ELISA had been developed to quantitate AA in food products, such as potato chips, instant noodles, cookies, and cakes [132]. As AA was easily reacted with 4-MPA at high derivation yield, a competitive indirect enzyme-linked immunosorbent assay (ciELISA) for AA via a pre-analysis derivatization was developed. The derivatization and ELISA conditions were fully optimized for AA assay, and the IC_{50} of the method was 2.86 μg/kg, LOD was 0.036 μg/kg, and linear range was 0.25–24.15 μg/kg. This method was thus deemed suitable for routine AA screening in food samples at low cost [133]. Singh et al. [134] raised the polyclonal antibodies against a hapten derived from AA and 3-mercaptobenzoic acid. An indirect competitive ELISA was developed to rapidly quantify AA in complex food matrices and water. Good recoveries for AA were observed in all matrices tested, and the results using this method were comparable to those obtained from mass spectrometry methods.

3.3.3.2 Sensor Methods

Gezer et al. tested a biodegradable zein-based sensor with surface-enhanced Raman spectroscopy (SERS) platform as a potential tool to detect AA for the first time [129]. This was a proof-of-concept study to investigate the potential of a SERS sensor, and it was shown that it could be potentially used with a careful design of AA

extraction from food samples. Liu X et al. successfully constructed a sensitive molecularly imprinted electrochemical sensor [135]. It was applied to the detection of AA in potato chips. HPLC analysis was also conducted to detect AA in the same samples to demonstrate the applicability of the electrochemical molecularly imprinted polymer sensor. The electrochemical sensor had received increasing attention in many fields due to their low cost, small size, fast response time, the possibility of achieving low detection limits, and strong operability [136, 137].

3.3.3.3 Nondestructive Methods

Conventional methods of AA identification in food items were time-consuming, expensive, and might need specialized manpower. It was necessary to propose some nondestructive methods for identifying the presence of AA in food.

Dutta et al. proposed a computer vision work-based nondestructive method to identify the presence of AA in potato chips [138]. The proposed method was based on analysis and classification of the discriminatory features of the image in the spatial domain. The potato chips were automatically segmented from the image followed by statistical and texture features extraction from the segmented image in the spatial domain. These statistical features were then analyzed for identification of AA content using support vector machine (SVM) classifier. The experimental results had shown accuracy over 94% and sensitivity of 96% indicating that this method could be explored for viable commercial use.

While, in 2016, Dutta et al. presented an efficient image processing-based nondestructive method for identification of the presence of AA from potato chips based on statistical analysis and classification of the image features in the wavelet domain [139]. This proposed work involved segmentation of the area of the potato chip from the background of the image followed by feature extraction and strategical analysis in the wavelet domain. Wavelet features had discriminatory properties for identification of the presence of AA in food items. The experimental results of 97% accuracy and 100% sensitivity could be explored for commercial use.

Adedipe et al. investigated the ability of near-infrared spectroscopy (NIRS) to predict AA content in French fried potato [140]. Reflectance spectra (400–2500 nm) of each freeze-dried sample were captured on a Foss XDS Rapid Content Analyzer-NIR spectrometer. NIRS could accurately detect AA content as low as 50 μg/kg in the model potato matrix. The findings indicated that NIRS could be used as a screening tool in potato breeding and potato processing research.

3.4 Inhibition Methods of AA Formation in Foods

As AA widely existed in different food categories, the control and prevention of AA intake along human had become a hot topic within the food safety field in recent years. Because of the obvious matrix effects on the food system and various AA

formation mechanisms, several factors were needed to be considered for reducing the content of AA in foods. According to the mechanisms of AA formation, the widely used methods of inhibiting AA were mainly in the following three ways: the first one was to reduce or eliminate the AA formation precursors to decrease the content of AA formed in the end-products. The second was to optimize the processing technology, such as adjust the processing mode, processing temperature, processing time, pH values, moisture content, and other factors. The third was to add exogenous compounds to inhibit the formation of AA.

3.4.1 Reducing the AA Formation Precursors in Food Systems

According to the formation mechanisms of AA, Asn and reducing sugar (mainly Glc and Fru) were the major precursors for AA formation. Reducing the substrate content of the reactions was found to be an effective way to reduce the AA levels in the food products. Screening the crop varieties and cultivation conditions can control the content of AA precursors [141] from the first beginning. Postles et al. found that Asn was the main amino acid in potato tubers, and Asn concentrations were significantly different among different varieties [142]. Asn concentrations in the three tested samples were between 1621 and 3512 mg/kg, while Glc was between 985 and 1781 mg/kg; the low sugar level tubers had the lowest AA yield which was only 58% of the high sugar level variety. The limited factors in the formation of AA in potato tubers were mainly relevant to sufficient content of Asn and the low concentration of Glc [143], so the use of low-sugar varieties can effectively reduce the AA content in the food products. For cereal products, Asn was a limiting factor for the formation of AA, and low Asn content crop varieties can reduce the AA content of cereal products effectively [142]. These results showed that an AA mitigation strategy focused on developing potato cultivars with low Asn and reducing sugars was likely to be an effective and sufficient strategy. Gene silence technology was used to minimize the AA contents by Zhu et al. [144]. They generated a large number of silenced lines of potato cultivar *Russet Burbank* by targeting the vacuolar invertase gene *VInv* and the asparagine synthetase genes *StAS1* and *StAS2* with a single RNA interference construct. The transcription levels of these three genes were correlated with reducing sugar and Asn content in tubers. Fried potato products from the best VInv/StAS1/StAS2-triple silence lines contained only one-fifteenth of the AA content compared to the control groups.

The cultivation conditions had important effects on the content of amino acids and reducing sugar in the crops, and the AA content of the final products could be reduced by controlling the cultivation conditions of the crops. The use of sulfur fertilizer in the process of potato cultivation can significantly affect the ratio between free amino acid and sugar, as well as free Asn and total amino acids in potato tubers. By reducing the amount of sulfur fertilizer, the ratio of free Asn occupied in total amino acids could be also lowered [145, 146], which resulted in a decrease in the amount of AA produced during subsequent thermal processing. The amount of

nitrogen applied was closely related to the Asn content in rye. Excessive fertilization will lead to a significant increase in the amount of Asn, thereby increasing the AA content in rye [142].

The storage conditions of the crop also affected the free Asn and reducing sugar content. In the low-temperature storage conditions, the potato tubers will undergo a phenomenon called "low-temperature saccharification," which led to the metabolism of carbohydrates in the direction of reducing sugars. A large amount of reducing sugar was beneficial to the formation of AA in the final products. Reducing sugar content in potato tubers can be effectively reduced by selecting the appropriate storage temperature and storage conditions. The AA in the final product could be reduced by up to 16 mg/kg [147, 148] when the potato tubers were stored at 8 °C compared to the low-temperature stored potato tubers.

3.4.2 Optimizing the Processing Technologies

The Maillard reaction is the main way to form AA in food. By adjusting the heating temperature, heating time, pH value, water content of food systems, and other factors during processing, the degree of Maillard reaction and AA formation could be effectively controlled. In addition, during the heating process, we can modify or add certain processing techniques to reduce the production of AA, such as using vacuum baking instead of conventional baking methods [149], pretreatment, altering the oil used for frying [150], blanching [151], fermentation [152], and other processes.

3.4.2.1 Controlling the Processing Temperature and Time

The Maillard reaction is a typical reaction of temperature and time. It was beneficial to reduce the accumulation of AA in the product by reducing the heating temperature and reducing the heating time [153]. In the general food systems, AA could be formed when the heating temperature was above 120 °C, and the amount of AA would increase along the heating temperature and time. But when AA reached a certain amount range, AA contents began to decrease as the reaction time prolonged [62]. Truong found that the frying time significantly affected the AA content in the fried sweet potato fries, and the AA yields were 124.9, 255.5, and 452.0 ng/g (fresh weight) when frying 2, 3, and 5 min, respectively [154]. Tuta et al. pretreated frozen fries by microwave and fried at 190 °C for 2.5 min [155]. AA content in these fries reduced by 64% compared to the traditional fried whose frying time was 3.5 min.

3.4.2.2 Adjusting the pH Values

The Maillard reaction is a pH-depended reaction. Under different pH values, the Maillard reaction route will change accordingly. The potato powder model system (containing 41% potato powder, 38% water, and 21% oil) with different initial pH

values was heated at 170 °C for 6 min, and the largest AA content was found when the pH value was between 7 and 7.5 [156]. For AA, the most proper pH value range for AA formation was between 7 and 8 [153]. Reducing the pH value of the system can limit the nucleophilic addition of Asn and reducing sugars, thereby preventing the formation of Schiff base [153, 157, 158]. Thus, the accumulation of AA in the final product can be limited by the addition of an acidifying agent in the food processing process [159]. After lowering the pH of the dough, the AA content of the flour was significantly reduced, but the level of 3-chloro-1,2-propanediol, another hazardous substance, was significantly increased [159]. Therefore, in the actual food processing, it was necessary to take into account not only the qualities of the final products but the formation of other harmful substances.

3.4.2.3 Adopting New Processing Methods

In recent years, new processing technologies had been widely used in food processing. Banchero et al. used a supercritical fluid extraction process to extract AA from coffee, which would reduce the AA contents by 79% without affecting the caffeine content in the coffee [152]. With the vacuum frying method, the fried temperature can be reduced from 180 to 165 °C, and the AA content in the end product can be reduced by 51% [160]. Wei invented a novel microwave-infrared combined baking process, which could reduce the baking time by 27% and the energy consumption by 85% compared to the traditional baking method [161]. The AA content in the cookies produced by this method was reduced by 66.7%, and the sensory quality of the cookies was not significantly different from that of the conventional baking method [161].

3.4.2.4 Using of the Asparaginase

AA was considered to form from the reaction of Asn and reducing sugars contained in foods, so the free asparagine was a key limiting factor for AA formation. The content of AA in the final product can be reduced by treating with asparaginase. Asparaginase can decompose Asn in raw materials to inhibit AA production effectively [162–164]. Onishi et al. treated sliced potatoes with *Bacillus subtilis* L-asparaginase II (BAsnase; 4 U/g potato), and approximately 40% of L-Asn in the sliced potatoes was converted into L-aspartic acid (L-Asp), resulting in the hydrolysis of approximately 90% of L-Asn to L-Asp [165]. The AA content of BAsnase-treated fried potato chips decreased to below 20% compared to BAsnase-untreated fried potato chips.

Some new types of L-asparaginase showed even better performance in inhibiting AA than the commercial enzymes. *Escherichia coli* type L-asparaginase (EcAII) was a commercial enzyme trademarked Elspar (R) that was widely used for medical applications. Dias et al. produced and purified an L-asparaginase from native *Aspergillus oryzae* CCT 3940 with high potential for AA mitigation [166]. The

results indicated the AA concentration of the fried potato treated with the L-asparaginase from *A. oryzae* CCT 3940 and treated with commercial enzyme reduced 72% and 92%, respectively, compared to control sample [122]. Moreover, the L-asparaginase from *A. oryzae* CCT3940 showed no L-glutaminase activity, while commercial enzyme promoted a decrease in the L-glutamine content (25%). Sun et al. reported the identification of a novel bacterial type II L-asparaginase, abASNase2, from *Aquabacterium* sp. A7-Y [167]. Compared to the untreated potato strips (AA content: 0.823 +/− 0.0457 mg/kg), 88.2% AA was removed in the abASNase2-treated group (AA content: 0.097 +/− 0.0157 mg/kg). Bhagat et al. purified an extracellular asparaginase from an endophytic bacteria *Pseudomonas oryzihabitans* isolated from *Hibiscus rosa-sinensis* [168]. Addition of purified asparaginase (2.8 U/g dried potato), 90% AA was reduced in fried potatoes. Shi R et al. cloned and expressed a novel L-asparaginase gene (PbAsnase) from *Paenibaeillus barengoltzii* CAU904 in *Escherichia coli* [169]. They found that pre-treatment by PbAsnase significantly decreased the AA contents in potato chips and mooncakes by 86% and 52%, respectively [169]. All these results indicated that the novel L-asparaginase could be a potential candidate for applications in the food processing industry.

3.4.2.5 Blanching

Blanching was another important strategy to control the formation of AA in food systems. Potato chips or fries could be blanched to reduce the sugar and Asn on the surface. After the potato chips were soaked in distilled water for 90 min and then fried at 150 °C, 170 °C, and 190 °C, the amount of AA was reduced by 27%, 38%, and 20%, respectively. After the blanching treatment, Glc and Asn contents in the material were significantly reduced [170].

Zuo et al. developed an extremely thermostable L-asparaginase which could be used in the reduction of AA level through blanching [171]. The purified enzyme displayed the maximum activity at pH 8.5 at 90 °C, and the temperature was the highest ever reported. When potato samples were treated with 10 U/mL of L-asparaginase at 80 °C for only 4 min, the AA content in the final French fries was reduced by 80.5% compared with the untreated group. This result showed that a combination of different inhibition methods could be used in the further processing industry.

3.4.2.6 Fermentation

Fermentation treatments were an effective way to reduce AA formation in fried potatoes without influencing product quality. Using lactic acid bacteria in rye dough, the AA in rye bread was reduced by 40–59.4% [172], and the reason was related to the activity of starch decomposition reduction caused by lactic acid bacteria, accompanied by pH reduction in the food system.

Potato chips can also reduce the amount of AA by yeast fermentation. Zhou et al. found that the optimum conditions for decreasing reducing sugar contents were a 1:4 solid-liquid ratio and a fermentation temperature of 37 °C with 0.5% yeast [173]. Potato strips treated under optimum conditions had 70% less AA than the control group chips.

The effect of the fermentation process by four lactic acid bacteria and yeast on an industrial scale was studied on AA reduction in bread [174]. AA content in the control fermented by yeast contained the highest amount of reducing saccharides (239.12 mg/kg). After being treated with four lactic acid bacteria, fermented bread with *Lactobacillus paracasei* showed the lowest amount of AA (131.06 mg/kg) due to its lower pH of sourdough (3.51) and glucose content (5.44 mg/g).

Bartkiene studied AA reduction technology in wheat flour biscuits supplemented with lupine and flaxseed treated by solid state (SSF) and submerged (SMF) fermentations by *Lactobacillus sakei*, *Pediococcus pentosaceus,* and *Pediococcus acidilactici* strains, respectively [175]. The most effective AA reduction in biscuits (78% and 85%, respectively) was reached by using *P. acidilactici* for flaxseed (SMF) and lupine (SSF). Using *L. sakei* for SSF of flaxseed and SMF of lupine, the average AA reduction rate was 83.4%.

3.4.3 Adding Exogenous Compounds

The changes of the raw materials of the food ingredients and processing conditions will affect the food quality, which is difficult to be accepted by manufacturers and consumers. Nowadays, some additives are used to inhibit the formation of AA in food systems and show good effects.

3.4.3.1 Adding Metal Ions

Studies showed that the addition of the metal ions could effectively inhibit the formation of AA. The addition of sodium chloride (NaCl) in food reduced AA formation significantly [176–178]. However, the inhibitory effect of NaCl on AA was not consistent with the concentration. Studies had shown that AA formation was reduced by 32%, 36%, and 40%, respectively, after adding 1%, 5%, and 10% NaCl in equimolar Asn/Glc model systems [179, 180]. In industrial production, the addition of 1% NaCl had a large application prospect, since 1% NaCl was the salt content actually required in many food products [181]. Friedman and Levin proposed the addition of Na^+ could alter the ionic strength of the reaction environment, thus affecting the combination of amino groups of amino acids and AA [182]. In addition, using DSC and infrared spectroscopy technologies, Kolek et al. found that NaCl could catalyze the polymerization of AA to polyacrylamide [176, 179, 180]. The study also demonstrated that the addition of potassium ferrocyanide ($K_4[Fe(CN)_6]$) and potassium iodate (KIO_3) had better performance in AA polymerization than using

NaCl. However, as a commonly used leavening agent in baking, ammonium bicarbonate (NH_4HCO_3) could significantly promote the formation of AA, and it was because of the promotion of NH_4HCO_3 on the formation of α-dicarbonyl compounds and the reaction with NH_3 to form glycosylamine [182]. When sodium bicarbonate ($NaHCO_3$) replaced NH_4HCO_3, the AA in the final product reduced by 70% [183]. Other metal ions, such as K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, and VO^{2+}, had also reported the abilities to inhibit the formation of AA [156, 184–187]. The current studies had shown that metal ions inhibited the formation of AA by affecting the formation of Schiff base [185, 186, 188] and the pH values of the solutions [156, 187, 189, 190].

3.4.3.2 Adding Vitamins

Zeng et al. found that water-soluble vitamins could inhibit the formation of AA in the Maillard model systems and the food system effectively, while the inhibitory effect of fat-soluble vitamins was weak [191]. The inhibitory effect of water-soluble vitamins such as biotin, pyridoxine, pyridoxamine, and vitamin C on the Asn/Glc model systems was up to 50% (170 °C, heating for 30 min). Except for VB_5 and pyridoxal, the total inhibitory rate of all the water-soluble vitamins in the food system was lower than that of the model system. The phenomenon might be due to the final functional groups (hydroxyl groups, amino groups, and aldehydes) of the side chains of the compounds, which played a reductive role in the formation of AA. In the low-moisture system, the addition of pyridoxamine could effectively inhibit the formation of AA, but had no influence on the degradation of AA precursors Glc and Asn, which indicated the inhibitory effect of pyridine on AA formation did not occur at the beginning of the Maillard reaction. It could be concluded as pyridoxamine could capture the dicarbonyl cyclides and sugar fragments in the Maillard reaction through the phenolic groups and aminomethyl groups on the pyridine ring to achieve the effect of inhibiting AA formation [192].

Kamkar et al. studied the efficiency of vitamins B_3, B_6, and the autolyzed yeast on the decrease of AA formation in potato chips [193]. Potatoes were soaked in different concentrations (0.1%, 0.5%, and 1%) of vitamin B_3, B_6, and the autolyzed yeast treatments. Results indicated a 58%, 50%, and 33% of the reduction, respectively, in AA formation in final products without affecting the flavor of the chips when the addition levels were 1 g/100 g. Although the inhibitory effects of on AA have been reported many times, there is no uniformity on its inhibition mechanism, and further study is still needed.

3.4.3.3 Adding Antioxidants and Plant Extracts

In recent years, some plant extracts were found to be able to affect the amount of AA in food and model systems. Cheng et al. found that apple extracts demonstrated potent inhibition on AA formation [194], whereas blueberry, mangosteen, and lon-

gan extracts did not have a significant impact on AA reduction. Column chromatography technology showed that the proanthocyanidin-rich subfraction played a key role in mediating the inhibitory activity. The inhibitory activity can be corroborated in fried potato crisps. Zhu et al. provided a possible use of crude aqueous plant extracts to mitigate AA formation in the Maillard reaction [195]. Effects of 35 crude aqueous extracts from dietary plants (spices, fruits, tea, beans, and herbs) and 11 phenolic compounds on the mitigation of AA in an Asn/Glc model system were investigated. Extracts of mint, cumin seeds, and star anise caused the greatest reduction in AA formation of 75%, 73%, and 69% respectively, but Huanggang Ku-Jing-Cha extract facilitated AA formation with increases of 18%. Of the phenolic compounds, *p*-coumaric acid caused the most significant reduction (53%), whereas hesperetin increased AA levels by 9%. Zhang et al. optimized the method and the frying processing parameters of the addition antioxidant from bamboo leaves to fried chicken wings and also compared the relationship between the contents of total flavonoids in three extracts (EBL971, EBL972, and antioxidant from bamboo leaves) [196]. The results showed that nearly 57.8% and 59.0% of AA in fried chicken wings were reduced when the antioxidant from bamboo leaves addition ratios were 0.1% and 0.5% (w/w), respectively. The maximum inhibitory rate was achieved when antioxidant from bamboo leaves was chosen as the additive with a total flavonoid content of 32% compared with other two extracts, and antioxidant from bamboo leaves mixed with flour was selected as the method of addition.

Using plant polyphenols in inhibiting the formation of AA also became a focus of food safety field. The polyphenol was a potent antioxidant widely contained in fruits and vegetables [197, 198]. The results showed that polyphenols had a good inhibitory effect on the formation of AA in food and model systems, and the uses of some typical polyphenols on AA inhibitions were shown in Table 3.3. But the effects of plant polyphenols on AA were complicated; some polyphenols such as t-butylhydroxyanisole and dibutylhydroxytoluene even promoted the formation of AA [199]. The results indicated that the inhibitory effect of polyphenols on AA was not depended on its antioxidant properties [70, 72, 200, 201], but the detailed inhibition mechanisms of polyphenols were still not clear.

3.4.3.4 Adding Amino Acids and Proteins

As the components of food, amino acids and proteins can also inhibit the formation of AA in the model systems and food systems [153, 208, 209]. Previous studies had shown that glycine (Gly) was effective in inhibiting the formation of AA [210, 211]. The α-amino group on Gly can directly link to the double bond of AA as Michael addition, or Gly can be oxidized and react with AA to eliminate [210]. The kinetic data showed that there was a relative competition inhibition mechanism between the reactors, and Gly and AA elimination reaction was the main mechanism of inhibition of AA formation [131]. The addition of amino acids could significant reduce AA formation while the color and flavor of the final product would change as well [156, 190, 212]. At the same time, some non-protein amino acids, such as alliin and

Table 3.3 Summary of studies and findings on the reduction of AA formation by antioxidant

Matrix	The type of antioxidant	Inhibitory effect	References
Cookie	Eugenol and cinnamaldehyde	The maximal inhibitory rates of eugenol and cinnamaldehyde were 42.3% and 34.0%	[202]
	Tert-butylhydroquinone	The inhibition rate was 54.1%	[203]
Emulsification system	Protocatechuic acid and gallic acid	The maximum inhibitory rates of protocatechuic acid and gallic acid were 70.0% and 50.0%.	[204]
Potato model system	Epicatechin (EC), epigallocatechin gallate (EGC), epicatechin gallate (ECG), apigenin, celeryin, vitexin, isovitexin, luteolin, lute 7-O-glucoside, luteolin-4′-O-glucoside, orientin, alfalfin, kaempferol, kaolin-3-O-glucoside, quercetin, quercetin-3-glucose Glycosides, rutin, myricetin, daidzin, daidzin, genistein, genistin	All the antioxidants can inhibit the produce of AA, the inhibition differs from different concentrations, the best AA inhibition rates of various substances were between 25.3% and 71.6%.	[205]
model systems	Epigallocatechin gallate (EGCG)	The maximal inhibition rate is 91.9%	[205, 206]
	Ferulic acid	The maximum inhibition rate is 47.1%	[207]
	Iso-orientin	The maximum inhibition rate of 61.8%	[205]

taurine, were also found to have the ability to inhibit AA formation [212–214]. In fact, in addition to amino acids, protein could also achieve the purpose of inhibiting the formation of AA in food. Amaranth protein could inhibit the formation of AA in the model and food systems. The inhibition rate in the model system was 35%–40%, in the cookie system was 89%, and in the fried corn flakes was 51%. When frying potato chips were wrapped with Chickpea batter, it was found that AA content reduced by about 30% compared to the unpackaged chips [72]. The addition of soy protein hydrolyzate to fries could also reduce AA formation [215]. Not only the plant but the animal proteins could inhibit the formation of AA in food, and it was deduced as the protein nucleophilic group (-SH or -NH_2) could react with AA and finally eliminate the formation of AA [153].

3.4.3.5 Other Additives

Sansano et al. found that the addition of chitosan might be an excellent alternative for reducing the formation of AA because of their richness in amino groups [216]. The results showed that chitosan was capable of inhibiting the formation of AA in

model systems and in fried batters. In model systems, the reduction in AA levels ranging from 49% to 85% was achieved for only 1% of chitosan. In the fried batter, AA was mitigated by 59 ± 6% with a chitosan concentration of 0.27% in batter formulations.

3.5 Conclusion and Outlook

In recent years, there have been significant improvements in the AA field including the detection methods, the formation mechanisms, and mitigation strategies. Several researchers have investigated many rapid and sensitive AA analysis methods. The developments of some pretreatments technologies gave us more choices for different food matrix. But the complexities of food matrixes also gave us more challenges for AA detections. Rapid detection methods played important roles in the detection of AA, including computer vision, ELISA, electrochemical biosensors, and fluorescent methods with the advantages of being low cost, simple, easy to handle, and portable for detecting AA in thermally processed foods compared with standard methods such as LC-MS/MS and GC-MS. These rapid detection methods have the ability to satisfy the need for food industries, regulatory agents, and customers. However, they still need further improvements to make themselves more accurate, sensitive, repeatable, reproducible, and/or portable to achieve online and real-time detection of trace AA. Besides, simplified pretreatment is essential in the detection of AA using a rapid (except for computer vision method) method.

Various AA mitigation methods have been successfully tested in the lab, but seldom applied in food industries at a commercial scale, partially because those current practical measures to reduce AA in foodstuffs are taken voluntarily [108]. Further researches in this area should focus on the following aspects: development of standardized quantitative methods to facilitate the proper and accurate evaluation of AA levels in foods, building up a corresponding AA database worldwide, and the development of new technologies to successfully reduce AA in food at a commercial scale.

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Chapter 4
Furan

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4.1 Introduction

Furan (C_4H_4O) (CAS No. 110-00-9) is a heterocyclic organic compound, consisting of a five-membered aromatic ring with four carbon atoms and one oxygen (Fig. 4.1). It is an intermediate in the production process of tetrahydrofuran, pyrrole, and thiophene, in the manufacturing of lacquers and resins [1], and for the production of pharmaceuticals, agricultural chemicals (insecticides), and stabilizers [2]. It is a colorless, volatile liquid having a relatively low boiling point of 31.3 °C.

The existence of furan and furan-substituted compounds in foods has been known for quite some time to contribute to the sensory properties of food. It is one of the volatile aroma compounds formed in a number of heated foods through thermal degradation of natural food constituents. On the other hand, the presence of furan in diet is considered as a concern as it is an animal carcinogen and classified as "possibly carcinogen to humans" (Group 2B) by the International Agency for Research on Cancer (IARC) [1, 3, 4]. Due to its toxicity, scientific community and food regulatory agencies have intensely focused on the occurrence, source, formation, and mitigation of furan in foods, and considerable research has been devoted to investigating this volatile food-borne contaminant.

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Fig. 4.1 Chemical structure of furan

4.2 Formation Mechanisms of Furan

Since its discovery, monitoring the formation and level of furan in foods became an essential issue for FDA, which has taken an action first by publishing an analysis method in foods and starting to build a broad database [5]. The database includes different varieties of foods, such as coffee, baby foods (porridge, juice), canned or jarred vegetables, infant formula, meat products, fruit juices, fish, milk products, cereal products, soups, and sauces. The furan levels in these foods range from not detected to 174 μg/kg [5]. The European Food Safety Authority (EFSA) has also started to collect data in EU and published scientific reports in 2009, 2010, and 2011 on the results of the monitoring of furan levels [6–8].

According to the latest report including the data submitted up to the end of 2010 from 20 countries, highest furan levels were found in coffee with the mean values of 45 μg/kg for brewed coffee, 394 μg/kg for instant coffee powder, 1936 μg/kg for roasted ground coffee, 2016 μg/kg for nonspecified coffee, and 3660 for roasted coffee beans [6]. The same report indicated the maximum level of 11,000 μg/kg in roasted coffee bean as the highest among all the food groups.

Elucidating the mechanism of furan formation is an important issue to develop mitigation strategies. Several model studies have been carried out to identify potential precursors and enlighten the formation pathways [9–11]. EFSA report has indicated that foods having high levels of carbohydrates were most likely to form furan [7]. Amino acids and reducing sugars forming Maillard reaction products and lipid oxidation of polyunsaturated fatty acids (PUFAs) or triglycerides, carotenoids, and ascorbic acid are responsible for furan formation. Among them, ascorbic acid and polyunsaturated lipids (such as linoleic and linolenic acid) were reported as the most effective precursors [12]. The US FDA has reported that a variety of carbohydrate/amino acid mixtures or protein model systems (e.g., alanine, cysteine, casein) and vitamins (ascorbic acid, dehydroascorbic acid, thiamin) have been used to produce, isolate, and identify furans in food [5]. The potential routes of furan formation from different components present in food are summarized in Fig. 4.2.

Maga first reported that the primary source of furans in foods is thermal degradation and the rearrangement of carbohydrates such as glucose, lactose, and fructose [3]. Many researchers have further shown that pyrolysis of carbohydrates at extreme temperatures of up to 300 °C formed furan, 2-methylfuran (MF), and further alkylated derivatives [14, 15]. Heyns has revealed that the pyrolysis of several carbohydrates, D-erythrose, D-xylose, D-ribose, D-arabinose, L-sorbose, D-fructose, D-glucurono-6,3-lactone, cellobiose, maltose, lactose, sucrose, raffinose, amylose, amylopectin, and cellulose, at 300–500 °C for a short period of time resulted in the same volatile products including furan.

Formation of furan in model systems has been extensively studied by Perez Locas and Yaylayan [10] using pyrolysis-GC-MS analysis and ^{13}C-labeled sugars, amino acids, and ascorbic acid. The study has revealed that furan could be formed

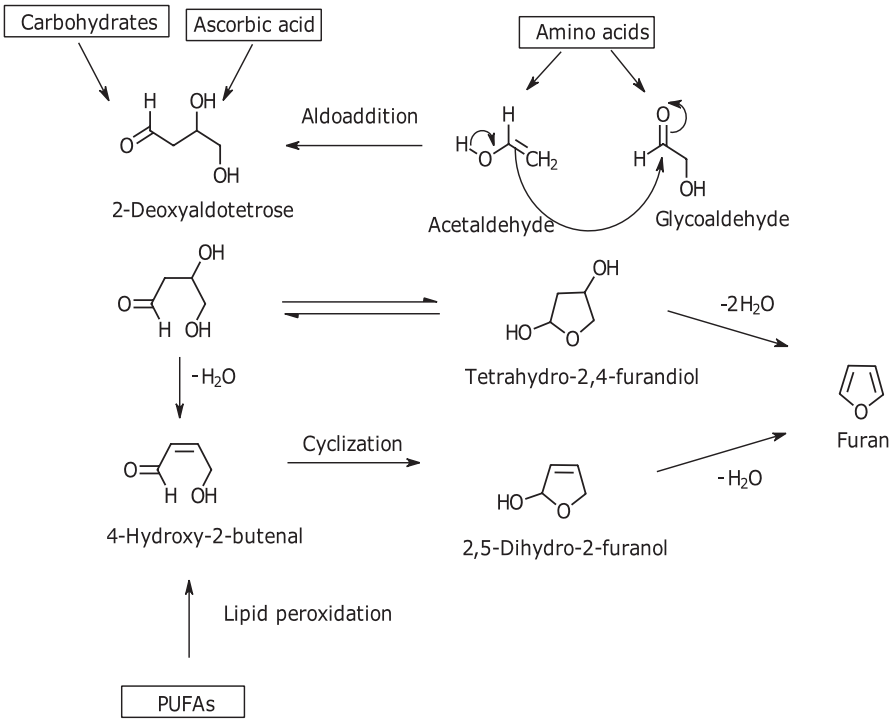

Fig. 4.2 Summary of possible routes for furan formation [2, 10]. (Reused with permission by [13])

from the thermal degradation of certain amino acids such as serine and cysteine, resulting in the formation of two key aldehyde intermediates, acetaldehyde and glycolaldehyde. They could undergo aldol addition forming 2-deoxyaldotetrose, which further reacts to form furan [10]. Hexoses were found to mainly degrade into aldotetrose derivatives to produce the parent furan. Thermal degradation of hexoses leads to the formation of 2-deoxy-3-ketoaldotetrose and 3-deoxyosone, which further react to form furan. Maillard reaction is also responsible for furan formation. The same study has further demonstrated that certain amino acids such as aspartic acid, threonine, and α-alanine require the presence of a sugar to form furan. Another study conducted by Cho and Lee [16] has also demonstrated that when the ribose/serine model system was heated at 90, 121, and 150 °C, it contained higher amounts of furan compared to other Maillard systems tested. The molar ratio of reactants also affected the furan formation from the Maillard model systems. The glucose/serine and glucose/alanine model systems have formed the highest furan at a molar ratio of 0.5:0.5.

Owczarek-Fendor et al. [17] have revealed that the addition of whey proteins into starch-based model food systems enhanced the generation of furan considerably at pH 4 and pH 6 when glucose, fructose, or lactose is present. The opposite trend was observed for sucrose.

Ascorbic acid is one of the important precursors of furan. Pyrolysis of ascorbic acid could generate furan via the formation of the 2-deoxyaldotetrose moiety, a direct precursor of the parent furan. Oxidative degradation of PUFAs forms lipid peroxidation products such as 4-hydroxy-2-butenal, which then undergo cyclization reaction and form furan [10].

Becalski and Seaman [18] have proved furan formation from the decomposition of ascorbic acid and from the oxidation of polyunsaturated fatty acids at elevated temperatures. Kinetic studies have revealed that ascorbic acid oxidation is the critical step in furan formation in tomato paste and pulp during heating [19].

Limacher, Kerler, Conde-Petit, and Blank [9] have investigated the formation of furan from ascorbic acid and related precursors in model systems simulating food preparation conditions such as roasting and pressure cooking. The results have confirmed that ascorbic acid is the major precursor of furan under roasting conditions despite low yield (<1 mol%). Pressure cooking conditions has led much lower furan formation than roasting conditions. However, pH has been found to play an important role in furan formation from ascorbic acid model systems treated with pressure cooking such that furan formation is much higher at pH 4 (57.5 μmol/mol) than pH 7 (3.69 μmol/mol). Finally, the researchers have suggested ascorbic acid as the potential precursor but depending on the pH.

2-Furaldehyde and 2-furoic acid, the degradation products of ascorbic acid and dehydroascorbic acid, have been reported to form furan [9, 20, 21]. 2-Furaldehyde is found to be a furan precursor in both dry and aqueous model systems, where 2-furoic acid is effective only under roasting conditions [9].

It is well known that thermal process is responsible for furan formation. According to the furan survey of FDA, fruit juices contain a certain amount of furan as a result of its thermal treatment as they are rich in ascorbic acid and carbohydrates, the precursors of furan. Not only thermal process but also ionizing radiation as an alternative nonthermal process has been used in fruit juices to ensure inactivated food-borne pathogen. According to the study conducted by Fan [22], both ionizing radiation and thermal treatments induce furan formation in apple and orange juice. Increased radiation dose from 0 to 5 kGy has resulted in increased furan formation. Another study has observed that irradiation induced low ng/g levels of furan only in grape and pineapple among several fresh fruits tested [23]. On the other hand, radiation to 4.5 kGy at 5 °C or to 10 kGy in the frozen state does not significantly induce furan formation in ready-to-eat meat and poultry products [24].

4.3 Occurrence of Furan in Foods

Due to its low boiling point, furan, formed during thermal processing, easily vaporizes. However, this gives rise to concern in canned or jarred foods as furan accumulates in the headspace. Furan levels have been monitored in a broad range of products (roasted coffee, bakery products, baby foods, etc.) and found to be from none detectable to 11,000 μg/kg [7, 25–33] (Table 4.1).

Table 4.1 Furan contents in certain food products

Product category	Furan content (μg/kg or L)		References
	Mean	Maximum	
Coffee, instant	394	2200	[6]
	91.37	145.2	[34]
Coffee, roasted bean	3660	11,000	[6]
Coffee, roasted ground	1936	6900	[6]
	<100	587	[35]
	89.3	352	[36]
Coffee, roasted ground, decaffeinated	53.1	121	[36]
Coffee, not specified	2016	6588	[6]
Coffee brew	42–45	360	[6]
	51.34	74.3	[34]
Baby foods, cereal	23–25	96	[6]
	5.15	8.6	[37]
	4.41	7.2	[34]
	10.9	53.5	[38]
Baby foods, fruits, and vegetables	10–12	66	[6]
	38.8	38.8	[37]
	7.71	13.36	[34]
Baby foods, fruits only	2.5–5.3	58	[6]
	1.6	2.7	[39]
	5.79	12.2	[37]
	13.6	92.6	[38]
Baby foods, meat, and vegetables	40	169	[6]
	35	64	[39]
	34.6	52.7	[37]
	72.3	224.4	[38]
Baby foods, vegetables only	48–49	233	[6]
	10	29	[39]
	38.83	81.9	[37]
Baby foods, fish-based	49	84	[39]
Baby foods, nonclassified	29–30	215	[6]
	40.7	52.5	[37]
Infant formula	0.2–3.2	2.2–10	[6]
Baked beans	22–24	80	[6]
Beer	3.3–5.2	28	[6]
Cereal product	15–18	168	[6]
Canned fish or meat	17	172	[6]
	26	70	[40]
	25.42	48.5	[34]
Fruit juice	2.2–4.6	90	[6]
Fruit drink	3.69	5.64	[34]

(continued)

Table 4.1 (continued)

Product category	Furan content (μg/kg or L)		References
	Mean	Maximum	
Orange juice	1.18–9.12	27.39	[41]
Fruits	2–6.4	36	[6]
Meat products	13–17	160	[6]
Milk products	5–5.6	80	[6]
Sauces	8.3–11	175	[6]
	1.27–26.07	69.13	[34]
Soups	23–24	225	[6]
Soy sauce	27	78	[6]
	44.32	69.13	[34]
	31.60	215.33	[42]
Vinegar	48.15	68.24	[34]
Vegetable juice	2.9–9	60	[6]
Vegetable	6.9–9.6	74	[6]
Cocoa	9–10	40	[6]
Snacks and crisps	9.6–10	47	[6]
	14.92–79.32	95.64	[34]
Soft drinks	0.8–1.2	4.5	[6, 41]
Soya products	6.7	28	[6]
Sweets	5–6	34	[6]
Tea	1–1.7	3.7	[6]
	68.28	71.58	[34]
Vegetable fats	1.5–1.7	10	[6]
Wine and liquors	1.3	6.5	[6]
	40.44–92.26	112.5	[34]

Among the foods containing furan, coffee has been reported to contain the highest amount of furan, and high consumption of coffee increases the exposure of carcinogenic furan. Baby foods (jarred/in closed containers) also take special attention due to its sensitive consumer group. Therefore, these two food groups are particularly discussed in this chapter.

4.3.1 Coffee

Roasted coffee is the group with the highest furan levels up to 11,000 μg/kg. Coffee preparation involves different steps starting from roasting the green beans, following grinding and brewing. These steps affect the final concentration of furan in coffee brew.

Roasting occurs at higher temperatures than most other thermal processes, which causes the formation of furan in addition to other certain thermal process contaminants [43]. Roasting degree plays an important role in the final furan content of

coffee bean. Guenther, Hoenicke, Biesterveld, Gerhard-Rieben, and Lantz [25] have reported that the formation of furan during roasting is dependent on roasting conditions and is, therefore, directly linked to achieving targeted flavor profiles. They have also found that furan formation has not significantly varied for different green coffee types, which has been previously reported by other researchers [35]. But, in another study, higher concentrations in *Coffea canephora* (robusta) than *Coffea arabica* species have been reported [44].

Although roasted coffee bean contains certain amount of furan, the steps prior to coffee consumption cause furan loss due to its high volatility and low solubility in water. Only approximately 10% of the initially generated furan during roasting is estimated to get into the cup of coffee for consumption [25].

As a first step of coffee preparation, grinding causes significant loss of volatile aroma compounds as well as furan due to the opening of the cell structures. Smaller mean particle size has been related to increased furan losses, such that around 40% of furan loss occurs at the typical European drip filter grind sizes (350–500 mm) [25]. Degassing process, which is applied to vacuum-packed roasted or ground coffee to remove CO_2 formed during roasting, also decreased the furan content by approximately 20%. In packed coffee, furan loss does not occur during the first 3 months of shelf life [25]. Zoller, Sager, and Reinhard [27] have assumed that more than 50% of the initial amount of furan is preserved until use, if the coffee beans and the soluble coffee are stored more or less airtight or aroma-tight. Different researchers have found out that the initial furan content of coffee powder and the type of brewing greatly affect the furan content of coffee brew [25, 27, 45]. In the brewing step, the type of preparation and brew recipe play an important role on the final amount of furan in the cup. Furan losses in various brewing techniques, such as drip filtering, fully automated machines, machines using coffee pads or capsules, are different. Among these brewing techniques, espresso-type coffee contains the highest concentration, while filter coffee contains the lowest.

Filtered coffee continues losing furan during the time the pot was held warm [27]. A study conducted by Zoller, Sager, and Reinhard [27] has shown that most of the furan (29%) is extracted with the first 65 mL when espresso is brewed with a semiautomatic machine and further 65 mL extract increases the furan extraction only to 32% with a high variation. Goldmann et al. [46] have reported that furan concentration steadily decreases due to the exposure of brew to the atmosphere. Another study has confirmed the furan loss as 50% after 1 h of brewing [27]. Soluble coffee brews have lower concentration of furan (0.91 mg/kg), while brew produced with automatic coffee machines has the highest [45, 47]. Closed systems, e.g. automatic machines, could keep furan from moving away, which leads to higher furan content in those systems. In addition, a high ratio of coffee powder to water could obviously transfer higher amounts of furan to brew.

Mesías and Morales [48] have investigated the amount of furan removed from the coffee beverage by volatilization during consumption time. Among different scenarios tested, maximum furan loss (97%) occurred when the brew is kept in a sealed thermo for 8 hours. Furthermore, placing the brew on one side at room temperature for 5 min alone or after 30 s of stirring (simulating sugar-added brew) is the

most common behavior, which causes 74% and 64% furan loss, respectively. Becalski, Halldorson, Hayward, and Roscoe [36] found 50% loss of furan in coffee brew at 30 min that was stored in the pot followed by storing in the cup.

Overall, these studies highlight the need for a careful evaluation of the dietary exposure to furan by consuming coffee. Despite the fact that furan loss is inevitable upon drinking the brew, Arisseto, Vicente, Ueno, Tfouni, and Toledo [44] have claimed that the remaining levels of furan in the beverages should be carefully evaluated and it might be still relevant to furan exposure due to the high consumption of coffee.

4.3.2 Baby Foods and Foods in Closed Containers

It is a fact that furan is volatile and could easily move away from certain food systems. However, foods processed in closed containers could contain volatilized furan in the headspace [33, 46]. Among these foods, baby foods have received considerable critical attention due to high sensitivity of babies to carcinogens and the larger amount of the foods consumed by this consumer group (relative to body weight) [49].

According to FDA report, infant formula products (N = 31) contain furan about 8–10 μg/kg, while EFSA report has indicated that baby food and infant formulae (N = 1628) contain furan (median) in the range of 0–38 μg/kg [5, 7].

A very recent survey has been conducted in the Spanish market to determine the furan concentration in commercial baby foods [39]. Researchers have detected the lowest furan concentration in infant formula (up to 0.33 μg/L) and cereal-based baby food (0.15–2.1 μg/kg), while baby food containing meat (7.9–61 μg/kg) and fish (19–84 μg/kg) showed the highest concentrations. Another study performed with a total of 35 infant food samples from Chinese market has not reported detectable amount of furan in some baby rice and infant formula samples, but muddy flesh samples contained 86.9 μg/kg as the highest [34].

As indicated in EFSA report, researchers have found that baby foods containing vegetables (48.0–49.0 μg/kg) or vegetable and meat mixtures (40 μg/kg) have higher furan levels than baby foods containing fruit (2.5–5.3 μg/kg) only, meat only, or meat and starches [6, 50]. Similar results were published by other researchers in a more recent study; vegetables and/or meat-based baby foods contain mean furan concentration of 72.3 μg/kg, while lesser amount has been reported in fruit-based (13.6 μg/kg) and cereal-based (10.0 μg/kg) baby foods [38]. Unlike commercially jarred baby foods, freshly home-prepared baby foods contained furan concentration below the limit of detection (0.20 μg/kg) [51]. The reason could be attributed to the different thermal processes, such that home-cooking occurs in open containers causing furan to be removed [50]. Another study, testing the furan levels in reheated retail canned or jarred foods including baby foods, has demonstrated an increase in furan when the food is closed during heating due to there being no loss by evaporation, leading to accumulation; however, heating the samples in open vials has caused very little furan accumulation [33]. Similarly, Roberts et al. [52] have determined various domestic

cooking regimes, such as cooking in saucepan or in a microwave oven, which affects the final concentration of furan in complete ready-to-eat meals (convenience foods) packed in cans or plastic trays and soups and sauces packed in cans, cartons, or jars. In general, heating leads to a decrease in most canned and jarred foods, while decrease is much when heated in a saucepan rather than microwave. Simulated domestic cooking conditions have indicated that the levels of furan also decrease slightly when foods are left to stand on plates due to their volatility.

4.3.3 Other Foods

Other foods, e.g., potato chips (16 μg/kg), crackers and crisp breads (12 μg/kg), and toasted breads (18 μg/kg) have also been reported to contain furan although they have not been processed in closed jars [25, 27, 53]. Lower amount of furan was also detected in cocoa (9–10 μg/kg), soya products (6.7 μg/kg), sweets (5–6 μg/kg), tea (1.0–1.7 μg/kg), vegetable fats (1.5–1.7 μg/kg), and wine and liquors (1.3 μg/kg) [6]. Roasted hazelnut has also been declared to contain certain amounts of furan due to the thermal process it undergoes [54]. In fact, increasing roasting temperatures from 100 to 150 °C has led to an increasing furan content of hazelnuts from 4 μg/kg to about 450 μg/kg.

4.3.4 Analysis Methods

FDA has published the first quantitative method for furan in foods [55]. This method defines the basic steps of the analysis, sample preparation using headspace (HS) sampling, addition of d_4-furan in sealed HS vials, incubation, and gas chromatography-mass spectrometry analysis. Prior to incubation, sample preparation was advised to be under cold conditions in order to eliminate the loss of furan.

The low boiling point of furan presents a challenge in handling it for analysis. Especially, sample preparation should be performed carefully to attain accurate results. Solid samples may need to be carefully homogenized before putting an appropriate amount to the headspace vial, while liquid samples can be directly transformed. Refrigerated or cryogenic conditions are necessary throughout the homogenization, such that a chilled blender is used to homogenize the prechilled sample placed in an ice bath [56]. Becalski et al. [57] have reported an increased furan loss with prolonged blending time. However, approximately 10% loss in spiked furan occurs when moderate-speed homogenization is used for 1 min.

Generally, headspace sampling is a sample preparation method enabling analysis of volatile analytes that are in the gaseous or vapor phase, either injected directly (HS) or interacted, and mostly equilibrated with a polymeric material [58]. Headspace sampling techniques, e.g., directly from the headspace in gaseous phase or solid phase microextraction (SPME), are well-established techniques for furan analysis.

Both HS and SPME methods should ensure that the present furan is removed from the food matrix. For this purpose, incubation is carried out before analysis. Different factors, such as time, temperature, and the nature of the sample, affect the efficiency of incubation [53]. A homogenized sample is mixed with water, if necessary, to ensure that the analyte is mobile [57]. Due to the decreased solubility of furan in a salt-saturated aqueous phase, sodium chloride or sodium sulfate may be added to the vial [46, 57].

According to the study investigating the effect of heating temperature on incubation efficiency, increasing the incubation temperature in aqueous solutions from 30 to 50 °C increased detected furan by 50% [57]. On the other hand, extra formation of furan at higher temperatures during incubation could cause overestimation [53, 59]. Analyses of first results from FDA were carried out at an incubation temperature of 80 °C [55]. FDA then decreases the incubation temperature to 60 °C to prevent low, ng/g, levels of furan formation that can occur during the analysis of a few relatively high-fat foods [60]. Interestingly, Şenyuva and Gökmen [59] have found continuous furan formation during headspace equilibration at 40 and 70 °C in unprocessed food samples, such as green coffee, freshly squeezed tomato, and orange juice. Therefore, matrix-matched calibration for each particular food matrix is highly recommended.

Several studies have reported methods comprising SPME for furan extraction from different food matrices and model systems [9, 29, 46, 50, 58, 61–64]. In principle, furan desorbed from the food matrix during incubation is adsorbed onto the fiber of SPME. It provides concentration of volatile analyte onto the fiber. Then, the analyte is desorbed thermally (1–5 min; 90–300 °C) from the fiber into the injection port of GC-MS. Goldmann, Perisset, Scanlan, and Stadler [46] have tested a number of different fiber types, including polyacrylate; Carbowax®/divinylbenzene; polydimethylsiloxane/divinylbenzene; and Carboxen®/polydimethylsiloxane (CAR/PDMS). Their results have revealed that CAR/PDMS shows decisive advantages in terms of sensitivity. However, the desorption temperature of SPME should be carefully selected as researchers have found out that some volatile furan precursors, such as 2-butenal and furfural, adsorbed on CAR-PDMS SPME fiber could form furan during high-temperature desorption leading to overestimation of the furan content of the sample (Fig. 4.3) [65].

Stir bar sorptive extraction (SBSE) technique has been tested as an alternative to static headspace analysis for coffee and jarred baby food samples [66]. In general, results from the SBSE technique using d_4-labeled furan as an internal standard gave results comparable to that of the static headspace (LOD: 2 μg/kg). One advantage of this extraction method has reported that extra furan formation is eliminated during extraction as it is performed at ambient temperature.

HS and SPME are extensively used for furan analysis, and both analysis principles give satisfactory results, if applied correctly according to the proficiency test conducted in EU [67]. On the other hand, SPME method has some other advantages over HS, such as allowing sample concentration and having lower detection limit than HS.

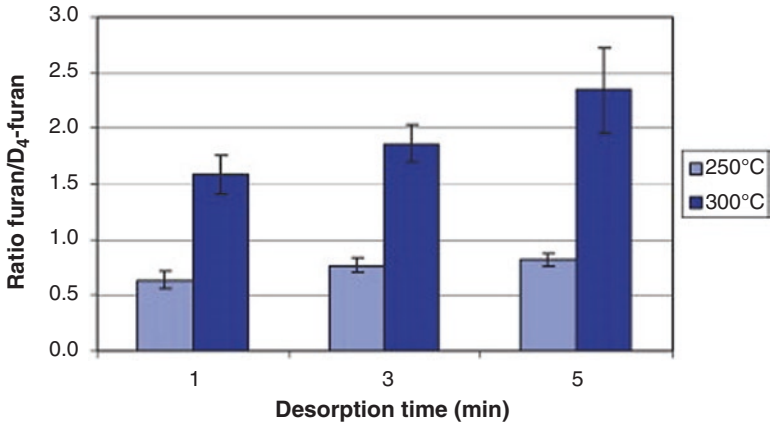

Fig. 4.3 Ratio of furan/d_4-furan (ion 68/ion 72) after SPME-GC–MS analysis of 0.25 mmol of 2-butenal in 1 ml water, spiked with d_4-furan (0.87 μg), as a function of desorption time and temperature. Reused with permission [65]

Quantitative analysis is challenging in headspace sampling when the analyte needs to be emitted from the food matrix. The physical form of the matrix including furan, the standardization and/or normalization of the accumulating polymer(s), and the quantitation approach used have been reported to be important in terms of reliable quantitation [58]. Standard Addition and Stable Isotope Dilution Assay are commonly used as quantifying methods for furan. In 2004, FDA used a static headspace-gas chromatography-mass spectrometry method for the quantification of furan by using the standard addition approach [5, 55]. In 2005, Becalski, Forsyth, Casey, Lau, Pepper, and Seaman [57] have validated a headspace method by using d_4-furan as an internal standard together with an external calibration curve as quantitation approach and attained higher sensitivities and 1 μg/kg limit of quantification. In a previous study, Multiple Headspace Extraction method has been newly proposed for furan quantification and compared with Standard Addition and Stable Isotope Dilution Assay [58]. According to the comparison results, these three techniques have been found to be reliable for furan analysis in combination with HS-SPME-GC-MS.

After FDA has published the first quantitative GC-MS method, researchers started to use this method, but thereafter several papers modifying this method have been published [18, 50, 54, 55, 63, 64]. In these papers, a PLOT (porous layer open tubular), PLOTQ (based on bonded polystyrene-divinylbenzene phase), or an equivalent column for the separation of furan was commonly used. MS was operated in Selected Ion Monitoring (SIM) mode by monitoring major ions at m/z 68, 72 and confirming by monitoring of the ions at m/z 39, 42 for furan and d_4-furan, respectively. The identification of furan is performed by the ratio of m/z 68 compared to its fragment ion at m/z 39.

4.3.5 Toxicity

Furan can pass through biological membranes, and it is rapidly absorbed from the lung or intestine and probably also through the skin due to its low polarity [68]. Rats and mice extensively metabolize after ingestion, whereas human body can be exposed to furan by both ingestion and inhalation while preparing food. Studies with F344 rats using isotopically labeled furan (2,5-^{14}C) have shown that radioactivity has remained in the liver tissues (13%) 24 h post dosing followed by the kidney and gastrointestinal tract (together 1%) [69]. The same study has also demonstrated that elimination of the isotopically labeled furan (80%) occurs mainly by air (40%), followed by feces (22%) and urine (20%) after 24 h of oral administration [69]. Burka, Washburn, and Irwin [69] found out that ingested furan is metabolized to different species in the liver, and these metabolites, including furan itself, react with protein rather than binding to DNA. However, latter studies have revealed that furan is metabolized by cytochrome P-450 to the dialdehyde, *cis*-2-butene-1,4-dial, which can interact directly with DNA [70, 71].

NOAELs based on a 2-year bioassay have been identified for cytotoxicity and hepatocarcinogenicity of 0.5 and 2 mg/kg bw, respectively [72]. It was reported that the margin of exposure for furan indicated a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite [73]. Based on the presently available data, it appears that both genotoxicity and chronic cytotoxicity may contribute to furan-induced tumor formation [2].

4.3.6 Mitigation Strategies

Several attempts have been made to develop mitigation strategies of furan in foods (Fig. 4.4). Due to its carcinogenicity, the ALARA ("as low as reasonably achievable") concept should be applied to furan levels in food. As furan is a consequence of thermal process, one might think to decrease the thermal load applied to food. However, this could not be practical especially for the sealed containers as they undergo pasteurization and sterilization for microbiological safety.

As reviewed by Anese and Suman [74], mitigation of furan formation could be achieved by two conceptually different technological approaches: (a) preventive interventions and (b) removal interventions. Preventive interventions aim to limit the reaction by creating less favorable reaction conditions, while the latter approach aims to decrease the furan content, which is already formed in the food during processing. To prevent furan formation, limiting the precursors in processed foods is not always a viable approach, as furan has not only one precursor.

Ascorbic acid has the highest potential to form furan, followed by polyunsaturated fatty acids, which are both desired food components due to their positive effect on health [53]. However, addition of ascorbic acid to a heat-processed food creates concern with regard to furan formation. In a previous model study, ascorbic acid

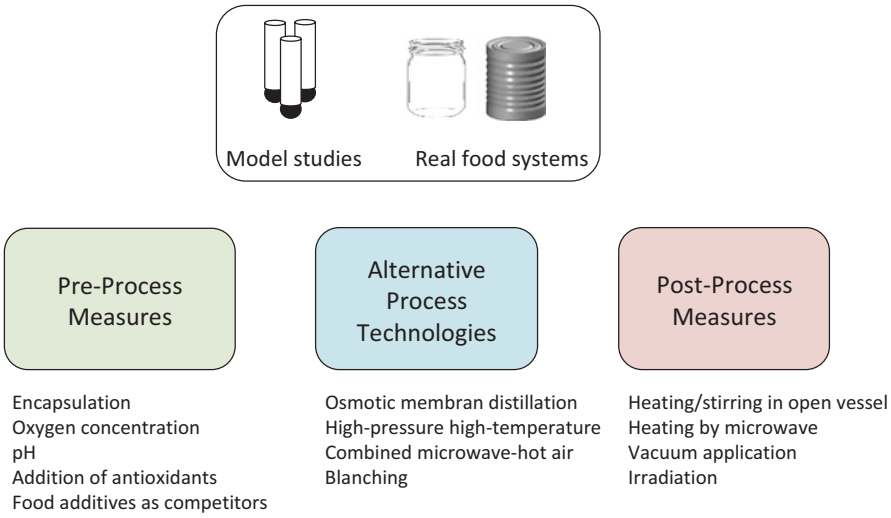

Fig. 4.4 Scheme summarizing the mitigation strategies for furan

was heated in the presence of iron to simulate dry foods containing these ingredients for their nutritional properties [75]. In these model systems, the furan formation rate constant from ascorbic acid increased by 369-fold due to the presence of iron creating oxidizing conditions. At this point, encapsulation may come into prominence as a mitigation strategy. Encapsulation not only preserves the function of ingredient, but also prevents it to react and eventually form furan. Further study has reported that the encapsulation of ascorbic acid by arabic gum and maltodextrin has decreased furan formation up to 57% in model systems containing iron heated at 120 °C ($p < 0.05$) [76].

Reaction conditions, including oxygen concentration, pH, presence of antioxidants, or other certain additives, have been reported to affect the furan formation in model or model food systems. Therefore, researchers have proposed to modify these factors to eliminate furan formation. Palmers et al. [77] have suggested lowering the oxygen concentration or pH prior to thermal processing for furan mitigation in thermally treated plant-based foods.

Becalski and Seaman [18] have shown that the presence of tocopherol acetate and butyl hydroxyanisole (BHA) reduces furan formation from PUFA as much as 70% under pressure cooking conditions. In a very recent study, chlorogenic acid has been found to be the most efficient antioxidant to reduce furan formation in ascorbic acid model systems, while butylated hydroxytoluene (BHT) has shown 92% and 80% reduction of furan in linoleic and linolenic acid model systems, respectively [78]. In soy sauce model systems, BHT and BHA reduced furan by 84% and 56%, respectively [79]. Another study has demonstrated that furan can be reduced in a canned-coffee model system by addition of epicatechin (by 65.3%), epigallocatechin gallate (by 60.0%), catechins (by 44.7%), chlorogenic acid (by 67.0%), ferulic acid (by 57.6%), Trolox (by 50.1%), and caffeic acid (by 48.2%) [80]. Shen et al.

[81] have proposed adding furan formation suppressors such as glutamic acid and/or avoiding from furan forming promoter such as ferric to decrease furan formation from PUFA.

The formation of furan in soy sauce model system was investigated by Kim, Her, Kim, and Lee [79]. The furan content of soy sauce model system has increased by 211% during fermentation up to 30 days after sterilization when compared to the model system without sterilization. The addition of magnesium sulfate and calcium sulfate to soy sauce model system reduced the furan concentration by 36–90% and 27–91%, respectively.

Studies performed on model systems have revealed that furan formation is negatively affected by the presence of additional molecules, which may increase the fragmentation rate of precursors or change the redox status of the reaction system [9]. However, this concept could not be easily extrapolated to food products from simple model systems. Food systems are complex mixtures, and various reactions occur at the same time during heating. These competitive reaction paths lead to significantly lower furan concentration than that obtained in dedicated model systems [9].

Different alternative technologies could be applied to specific food groups to prevent furan formation during processing. For example, tomato paste concentrated using osmotic and membrane distillation systems contains lower amount of furan than that concentrated using conventional thermal evaporation [82]. In the case of vegetable purées, high-pressure high-temperature (HPHT) processing has been suggested as an alternative and successfully applied to reduce furan formation to 1–2 μg/kg purée while ensuring a sterile product [83]. HPHT process has also been proved in sardine in olive oil to achieve reduced furan content such that the furan decreased from 57.9 μg/kg to 16.6 μg/kg by HPHT process when used as an alternative to retort [84]. This process has also been scaled up from lab-scale to the pilot scale [85]. The scale-up has also ensured reduction of furan between 41 and 98% to retorting depending on the food system.

Another technology, combined microwave-hot air for malt roasting was used to mitigate thermal process contaminants, including furan [86]. Combined microwave-hot air reduced furan content in black malt by 23% when compared to conventional process.

Blanching as a pretreatment for potato slices leached out ascorbic acid and reducing sugars, leading to decreased furan concentration by 91% in potato chips; therefore, it is proposed as an alternative unit operation to produce healthier potato chips [87].

Taking the advantage of its volatility, furan could be removed from the food matrix. Based on the evidences from previous researches, EFSA concluded in their report that volatilizing furan by heating and stirring in an open saucepan before consumption has reduced furan levels in canned/jarred foods [6, 27, 33, 52, 88]. However, this technique would be technically difficult for certain foods, for example, for coffee while retaining all the flavor and aroma substances that the consumer demands [53].

Composition of food matrix has an effect on the efficiency of furan volatilization. Van Lancker, Adams, Owczarek, De Meulenaer, and De Kimpe [26] have reported that furan is mainly retained by the lipophilic fraction but not by starch. However, in the case of coffee, defatted coffee and coffee grounds also show the ability to retain furan. Anese et al. [89] have investigated possible vacuum application to remove furan from the food matrix and found that it can effectively be removed from meat sauce, having a high moisture content.

A very recent study has reported that complete elimination of furan via evaporation from baby food samples is not possible even after a long-standing time [39]. Heating promoted diffusion of furan from the food matrix such that if the baby food is heated using a microwave oven or by means of hot water bath, furan content could be decreased by 26% and 42%, respectively. The authors further suggest standing the baby food for 5 min after heating as the furan loss reaches about 50%.

As mentioned in the "Formation" section, irradiation of certain foods could induce furan formation. However, a number of studies indicate that irradiation might be a suitable tool to reduce the furan formation. For example, researchers have revealed that irradiation at doses of 2.5–3.5 kGy that can inactivate 5 log of most common pathogens has significantly reduced the furan levels by 25–40% in all foods studied, e.g., frankfurters, sausages, and infant sweet potatoes [90]. It was confirmed that irradiation reduced furan formation in frankfurters that contained relatively high levels of furan due to previous thermal processing [24]. The composition, particularly the water content, has been declared to be important for the degree of furan reduction in particular foods by irradiation. Although irradiation reduces furan formation in certain foods, it is thought to be unlikely used as the only tool due to its limited effectiveness in most foods, caused nutrient loss, formation of off-odor compounds, and economical aspects [56].

4.3.7 Concluding Remarks

The volatility of furan raises difficulties in handling for analysis. Starting from sampling prior to analysis, the samples should be kept in cold conditions and/or sealed in vials. Headspace sampling, either by direct injection or by using SPME, is a well-established technique for furan analysis, and they are validated and commonly used by researchers. However, using these techniques need additional attention as misapplication could lead to forming extra furan and overestimation.

There are several studies published on the elimination of furan formation in different foods. Different food matrices imply different reaction mediums with numerous variables, such as composition (presence of precursors, i.e., sugars, amino acids, ascorbic acid, PUFA), pH, oxygen concentration, and water content, and might need specific approach. On the other hand, different food products require and undergo relevant thermal process, which ultimately determines the final furan content of food. Eliminating precursors from the food formulation or modifying formulation

or thermal processes may not always be viable approaches, as desired sensory property and/or microbiological safety of the food should be attained at the same time. For example, although coffee contains the highest amount of furan, there is no applicable strategy to be applied without affecting the sensory properties. Or, decreasing the thermal process temperature of canned/jarred foods, particularly baby foods, would not ensure the inactivation of pathogens. Incorporation of certain additives (furan inhibitors) to food products could also yield change in the organoleptic properties. As complex systems, foods may possess more than one potential precursor leading to furan formation through different mechanisms during processing. Mitigation of furan in foods is not an easy task. As reviewed in this chapter, there is no sole method to be applied for controlling furan formation in various kinds of foods. Future studies on developing efficient and applicable strategies are therefore recommended.

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Chapter 5
Heterocyclic Amines in Foods: Analytical Methods, Formation Mechanism, and Mitigation Strategies

Qi Wang, Yuge Bi, Feng Chen, and Ka Wing Cheng

5.1 Introduction

During heating of meat-based food products, a group of structurally close compounds collectively known as heterocyclic amines (HAs) can be formed. Drastic heating conditions such as grilling and roasting could lead to significantly higher HA contents. Precursors for this group of polycyclic aromatic compounds are fundamental food components including sugars, amino acids, and creatine, which undergo the Maillard reaction to form HAs [1]. More than two dozen HAs have been identified in thermally processed foods.

HAs are highly mutagenic in bacteria mutagenicity tests [2], and some of them are classified as potential human carcinogens by the IARC. HAs indeed represent a significant health risk considering the fact that they are widely present in some of our most important dietary components. In this chapter, we will give an overview of key analytical methods for the qualitative and quantitative analysis of HAs, their formation mechanisms, and major strategies for mitigation of HA-associated health risk.

5.2 Analytical Methods for HAs

HAs often occur in the ppb range in foods. Furthermore, they are often present in complex food matrices, which have numerous concurring compounds. Some of these compounds might significantly interfere with the analysis of HAs. Therefore,

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the determination of HAs in food samples requires selective and sensitive methods, which include efficient and robust sample preparation and analytical techniques. One of the most widely used approaches is solid phase extraction (SPE), which in essence incorporates the power of selective types of chromatographic materials packed in one or more solid supports. Chromatography remains the most important analytical technique for HAs. Although some studies also use gas chromatography (GC) for the analysis of HAs, the fact that most HAs are nonvolatile entails a derivatization step prior to quantitative and/or qualitative analysis. Hence, high-performance liquid chromatography (HPLC) coupled to photodiode array detection (LC-PDA) or mass spectrometry (LC-MS) has been the most popular instrument for HA analysis and will be reviewed herein.

5.2.1 Sample Preparation by SPE

SPE is a powerful sample preparation method owing to its capability to accomplish extraction and concentration of target analytes in a single run. This can then be translated into enhanced sensitivity to facilitate subsequent qualitative and quantitative analysis [3, 4]. Depending on the instrumental setup, the SPE process might be completely separated from or coupled to the chromatographic process [5]. Furthermore, the complexity of the sample matrix is also an important determinant of the optimal type of solid adsorbent. For simple matrices, such as those from chemical model reactions using pure precursor compounds of HAs, a combination of a diatomaceous earth cleanup step, followed by a cation exchange and a C-18 reverse phase concentration step, is sufficient to obtain samples amenable to subsequent chromatographic analysis [6, 7]. More recent studies have employed various forms of polymers or composites aiming to enhance the performance of the cleaning and concentration process. For instance, Zhang and co-workers synthesized a derivative of graphene with acrylamide, which exhibited good stability and permeability in both aqueous and organic phases [5]. This modified SPE adsorbent possessed a significantly improved efficiency of extraction of HAs from the sample matrix to allow online coupling to LC analysis.

5.2.2 Determination of HAs by LC-UV and LC-Fluorescence Detection

The presence of a heterocyclic aromatic system in HAs renders them amenable to detection by a UV detector. Although HAs often exhibit very similar chromatographic behavior, especially with most binary mobile phase system, the availability of authentic standards enables unambiguous identification in most cases. Of note, since HAs of the same subclass (e.g., aminoimidazole-azaarenes) only show subtle

difference in their UV absorption spectra, it would be of high importance to have concentrated and clean samples, which faciliate resolution and minimize interference from the sample matrix. Some HAs including the nonpolar ones and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) have an extensive conjugated pi-electron moiety and thus can be detected with a fluorescence detector. The sensitivity was found to be 100–400 times more sensitive than with a UV detector [8, 9]. Some studies have also used a combination of both UV and fluorescence detectors for the analysis of a large number of HAs. For instance, Gibis and Weiss successfully identified and quantified 15 polar and nonpolar HAs in fried beef patties by HPLC coupled to UV and fluorescence detection [9].

Electrochemical detection (ECD) has been a relatively less popular option for the analysis of HAs although it also attains high selectivity and sensitivity, which are key parameters in the method selection for handling samples with complex matrices. An isocratic mobile phase is required for analysis in the high sensitivity range. This criterion, however, made simultaneous analysis of a large number of HAs in one run a daunting task [10]. Improvement in the mobile phase system with the use of ionic liquids such as 1-butyl-3-methylimidazolium tetrafluoroborate, 1-hexyl-3- methylimidazolium tetrafluoroborate, and 1-methyl-3-octylimidazolium tetrafluoroborate (especially the first one) could improve the chromatographic separation of HAs [11].

5.2.3 Determination of HAs by LC-MS

LC-MS is one of the most powerful and versatile hyphenated analytical techniques as it elegantly integrates the separation capacity of column chromatography and the sensitivity and selectivity of MS. This feature makes it a particularly suitable tool for the analysis of HAs. The development of tandem MS has further elevated the resolution power of this already highly efficient analytical tool and enabled the use of much simplified sample preparation procedures [12–14]. As HAs are stable during the ionization process, the protonated molecular ion peaks may thus serve as markers in mass selective detection of HAs [15]. TriMeIQx has been widely used as an internal standard for quantitative analyses [12, 16]. Some studies also used isotopically labeled HAs as internal standards, typically at a hydrogen or carbon atom, and less commonly at nitrogen [12, 15, 17, 18]. The similar chemical properties and mass of these internal standards offer improved accuracy for evaluation of HAs compared to the use of other compounds such as aromatic acids [19].

Various ionization interfaces have been used to couple the liquid chromatograph and mass spectrometer. Major types include thermospray ionization (TSI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI). TSI was used in early studies to detect a single or few HAs [20]. This ionization technique, however, is prone to result in relatively more fragmentation and generate doubly charged ions [21], which would compromise accurate determination of multiple HAs, especially in samples from complex food products. Therefore, ESI and APCI have largely dominated in LC-MS analysis of HAs over the past two decades

due to the soft ionization process. These two interfaces used to be considered complementary to each other since the former is more suitable for analyzing polar HAs while APCI is suitable for less polar ones [22, 23]. Nonetheless, the utilization of ESI and tandem MS has allowed the determination of different categories of HAs from a wider range of food matrices in a single operation. With this configuration, it has become commonplace to simultaneously measure >10 HAs with satisfactory accuracy [14, 15, 24]. More recent studies employing ultra-performance LC tandem MS (UPLC-MS/MS) and principal component analysis have enabled qualitative and quantitative analysis of even larger numbers of different groups of HAs.

Triple quadrupole mass spectrometer has been the most popular mass analyzer for HAs due to its accurate quantitation capability. Operation is almost exclusively in the positive ionization mode given the high proton affinity of HAs, especially with the use of an ammonium salt (e.g., ammonium formate or acetate) in the mobile phase. Data acquisition in multiple reaction monitoring (MRM) mode has accomplished great selectivity and sensitivity (<3.1 ppm) for complex matrices, especially when combined with the use of isotopically labeled reference standards [14]. Further improvement in mass accuracy and sensitivity could be achieved with the use of quadruple time-of-flight (Q-TOF) [25]. The availability of these robust methods makes it feasible to quantitatively monitor total dietary exposure to HAs, and the data generated could be applied to assess long-term health risk.

5.3 Mechanisms of HA Formation

HAs are hazardous by-products of heat treatment in animal products. Their formation is affected by both intrinsic and extrinsic factors. The former mainly include contents and types of amino acids and sugars and characteristic of the food matrix (which could affect interaction among the purported precursors). The latter include heating conditions (e.g., temperature, use of a naked flame, etc.) and sample preparation methods prior to heating (e.g., type of marinade, additives, etc.). Therefore, the same foodstuff might have completely different HA content and profile after different heat treatments and vice versa [26].

According to their chemical structures, HAs can be broadly divided into two categories, namely amino-carbolines and aminoimidazole-azaarenes [27]. Amino-carbolines are generally formed at much higher temperature (>300 °C) via pyrolysis of amino acids or proteins. The formation of aminoimidazole-azaarenes requires lower temperatures. HAs can also be classified based on their polarity. Thus far, important HAs which have demonstrated carcinogenic activity in rodent studies and/or have been associated with increased health risk in epideomiological studies belong to the polar group [28–31]. Structures of polar HAs which have been most extensively studied are shown in Fig. 5.1.

Since amino-carbolines are pyrolytic products and creatine is not required for their formation, they could be present even in foods of plant origins although animal products are the major sources [32]. Thus far, heat-induced generation of free radi-

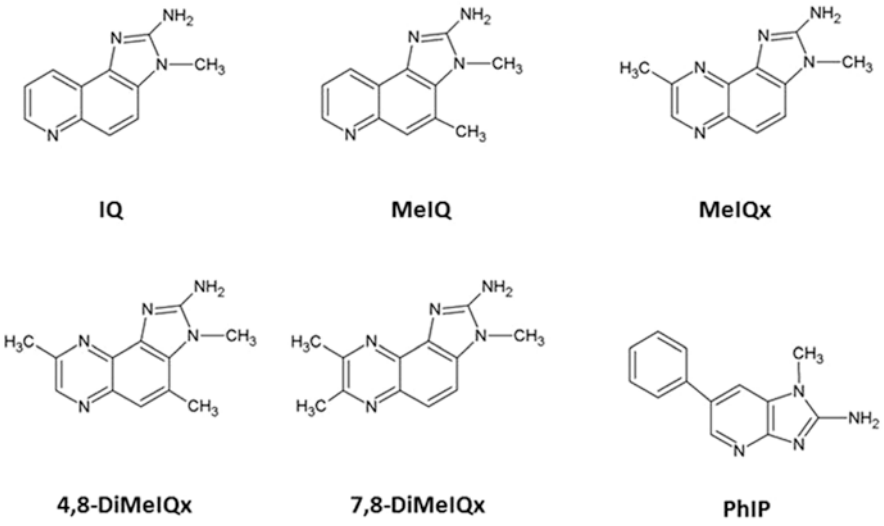

Fig. 5.1 Chemical structures of major polar heterocyclic amines

cals and the subsequent generation of reactive fragments has been proposed to be a major mechanism involved in amino-carboline formation [1]. Condensation of these reactive fragments and rearrangement give rise to various new structures.

In contrast to amino-carbolines, creatin(in)e has been identified to be an important component in the formation pathways of aminoimidazole-azaarenes. In essence, it is creatinine, but not creatine, that contributes to the formation of aminoimidazole-azaarenes including IQ, IQx, and PhIP. The amount of creatinine, a natural breakdown product of creatine phosphate, in muscle is very low. Interestingly, addition of extra creatinine to the surface of meat prior to frying increased the yield of HAs [33]. During heating, creatine can be converted to creatinine via cyclization and water elimination. Meanwhile, intermediary compounds such as pyridines and pyrazines and reactive carbonyls can be formed from the Maillard reaction between amino acids and reducing sugars and Strecker degradation reactions. Creatinine forms the amino-imidazo part which likely joins with the above reactive fragments via aldol condensation, giving rise to different aminoimidazole-azaarenes [32]. Relatively lower heating temperatures (< 300 °C) are sufficient to drive the Maillard reaction and Strecker degradation to generate reactive fragments from the precursors (sugars, amino acids, and their derivatives) [1]. In general, the contents of HAs in foodstuffs and chemical models increase with heating temperature and time [32, 34]. It has also been observed that the content of HAs correlates with the surface color of meat – the darker the color, the higher the HA concentration [35]. Some studies have also reported that radicals, especially pyridine and pyrazine radicals, might also be involved in the formation of aminoimidazole-azaarenes [36]. Nonetheless, findings from more recent studies suggest that free radical-mediated pathway is not likely the principal mechanism of aminoimidazole-azaarene formation [37, 38].

5.4 Mitigation Strategies to Reduce HA-Associated Health Risk

Given the genotoxic potential of HAs and the fact that humans might be exposed to HAs through the consumption of ordinary household dishes on a daily basis, there has been extensive research aiming to develop strategies to reduce HA-associated health risk, which mainly include the following two approaches. The first approach aims to prevent or reduce the content of HAs in our dietary components. This is considered a more desirable approach since it prevents our body tissues and organs from exposing to HAs in the first place. As mentioned above, HA content in foodstuffs is a result of a number of factors, some of which are amenable to manipulation. In other words, it is highly possible to adjust one or more of these parameters to control or interrupt the HA formation process. The second approach targets HAs which have entered the human body through the consumption of foods containing HAs. This approach aims to attenuate the potential pathological consequences of HAs by one or more of the following mechanisms: (1) reducing their bioavailability, (2) suppressing their metabolic activation enzyme systems and/or enhancing their elimination from the body, and (3) modulating metabolic activation at genetic levels [39–41]. The focus of this section will be on the first approach which has been the most widely accepted approach and holds great promise for incorporation into our daily cuisine.

It has almost become a rule of thumb that lowering heating temperature and/or shortening heating time can effectively reduce the content of HAs in foods. Paradoxically, heat-driven Maillard reaction plays a crucial role in the generation of desirable flavor and aroma compounds. This apparently most straightforward approach might negatively impact sensory quality of food products. Therefore, a general consensus in this area is that complete avoidance of dietary exposure to HAs is infeasible and ideal strategies to mitigate HA-associated health risk should meet the following criteria: (1) significant inhibition of HA formation or reduction of HA content, (2) do not give rise to new genotoxicants or promote the formation of other concurrent hazardous compounds, and (3) do not have negative impact on sensory quality (desirably could enhance it).

This section will give an overview of major inhibitory strategies which hold great promise for application in the food industry or household cooking.

5.4.1 Careful Choice of Sugars in Marinades

It is known that sugars, especially reducing sugars, are principal precursors of HAs. Therefore, change in the content and type of sugars may significantly affect the formation of HAs in foods, and the effect may be completely different at different

heating temperatures. In the lower temperature range (~100 °C), increasing the level of reducing sugars was reported to favor HA formation [42]. In contrast, it was found that addition of reducing sugars beyond a certain concentration range decreased HA formation in ground beef and beef patties heated between 150 and 200 °C [43, 44]. In practice, honey is sometimes used as a substitute for sugar to add sweetness to marinades. A recent study [45] which evaluated the effect of different types of sugar on the formation of HAs in grilled chicken found that marination with honey led to lower HA contents in chicken breast samples than those marinated with brown sugar or table sugar. However, the authors did not further verify whether the observed inhibitory effect was caused by the sugar in honey or was it an integrated effect from sugar and other components.

5.4.2 Addition of Synthetic Antioxidants

Knowledge about the formation mechanism of HAs has greatly facilitated the development of more targeted inhibition methods. Free radicals and other reactive intermediary compounds especially carbonyls have been demonstrated to be among the key participants in HA formation. In accordance with this mechanistic basis, antioxidants have been the most important group of candidate inhibitors. In early proof-of-concept experiments, researchers tested the effect of some common synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ). Interestingly, their effect turned out to be highly dependent on the experimental system employed. In food systems, these antioxidants were able to inhibit HA formation even at low levels of addition [46, 47]. In chemical models containing pure HA precursors, BHT and TBHQ were found to promote HA formation. The effect was particularly strong with TBHQ which increased MeIQx formation by >200% [48–50]. Despite the inconsistent findings on the activity of synthetic antioxidants against HA formation, these proof-of-concept studies at least added to the strength of the evidence that antioxidation is likely to be an important inhibitory mechanism under certain circumstances.

5.4.3 Addition of Natural Antioxidants

Natural agents are generally more preferred to synthetic ones as culinary ingredients or additives. A wide range of natural antioxidants including vitamins, phenolics, and carotenoids have been evaluated for their effect on the formation of HAs. Similar to the aforementioned synthetic antioxidants, vitamins like vitamin C and α-tocopherol failed to demonstrate consistent effects on HA formation [43],

although they possess the capability to interrupt free radical-mediated reaction pathways [51]. As a matter of fact, natural polyphenols are a large group of phytochemicals with a wide range of antioxidant or free radical scavenging capacities. Thus far, reports on the relative effectiveness of natural polyphenols as HA formation inhibitors have been more consistent compared to those on synthetic antioxidants and vitamins. They can be added in the form of pure phytochemicals isolated from plants or in the form of polyphenol-rich extracts. Most of them originated from fruits, vegetables, or spices which could be easily incorporated into daily cuisine.

Considering the sometimes inconsistent data from chemical model and real food experiments, more recent studies have used both systems or verified the findings from chemical model screening assay in at least one food system. In an attempt to identify potent inhibitors of HA formation, we evaluated 12 food-derived phenolic antioxidants in simple chemical model systems and in fried beef patties [52]. The results did not support a significant positive correlation between antioxidant and HA formation inhibitory activity. Of note, naringenin, a rather weak antioxidant, turned out to have comparable inhibitory capacity to theaflavin-3,3′-digallate and epigallocatechin gallate, which are well-known potent natural antioxidants. At 0.1% (w/w) level of addition, they were able to reduce the content of total HAs by >50% relative to control, and the inhibition was consistent in both chemical model and beef patties. Curcumin, a principal bioactive polyphenol in turmeric, was also found to dose-dependently inhibit mutagenic HA formation in several model systems [53, 54], although it is not a potent free radical scavenger. The discordance between radical scavenging/antioxidant activity and inhibitory activity of HA formation suggests that alternative (if not dominant) mechanism likely contributes to the inhibitory effect of many natural polyphenols.

As mentioned in Sect. 5.3, certain Maillard intermediates, especially reactive carbonyl species (RCS) generated from thermal and/or Strecker degradation reactions, are likely key intermediates in the formation of HAs [32, 49, 55, 56]. Our group were among the first to report the scavenging of reactive carbonyls as a key mechanism responsible for the inhibitory effect of certain natural polyphenols [57]. By doing so, the added polyphenolic inhibitors combine with the reactive HA intermediates to form adducts. PhIP has been the most well-studied HA with regard to this postulated action mechanism, and phenylacetaldehyde is a major reactive carbonyl intermediate involved. A more recent study has further confirmed the importance of this mechanism for the inhibitory activity of polyphenols against HA formation [58]. The authors demonstrated a significant positive correlation between the phenylacetaldehyde-scavenging capacity and PhIP formation inhibitory activity of a group of polyphenols with a diverse range of antioxidant capacities. An example of this recently discovered and yet amply demonstrated inhibitory mechanism is presented in Fig. 5.2. Structures of some potent natural HA formation inhibitors are presented in Fig. 5.3.

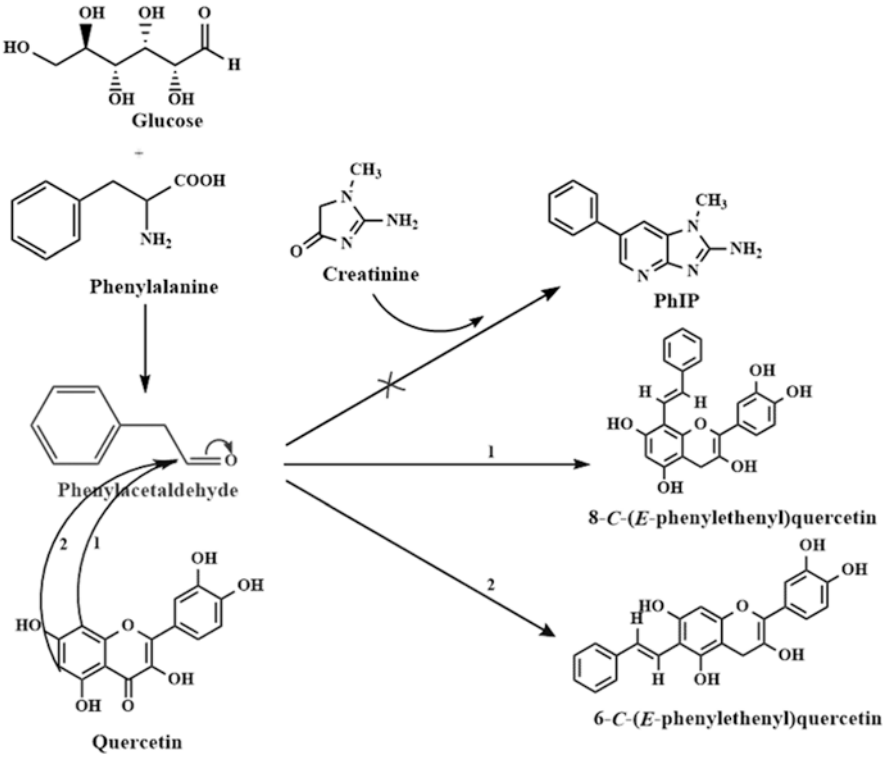

Fig. 5.2 Postulated pathway for the inhibitory activity of quercetin in PhIP formation

5.4.4 Addition of Phytochemical Extracts and Herbs

Fruits, vegetables, and certain seasoning herbs or spices are known to be valuable natural sources of a wide range of phytochemicals, especially polyphenols. Therefore, it is not surprising that more and more studies have reported the inhibitory effect of plant extracts or herbs on the formation of HAs. As some of the most common seasoning ingredients in Western countries, rosemary, thyme, sage, and garlic were reported to significantly reduce HA content in meat by applying them to the surface prior to heating [59]. Other promising spices include turmeric, torch ginger, pepper, onion, lemongrass, and curry leaves [60, 61]. Fruit and vegetable extracts have also been evaluated for their potential to inhibit HA formation. Our group and others have identified a small number of extracts through systematic comparison using both chemical models and real food systems [61, 62]. One common feature is that they are all rich in polyphenols and could effectively reduce the content of HAs even at low levels of addition. For example, marinating minced beef

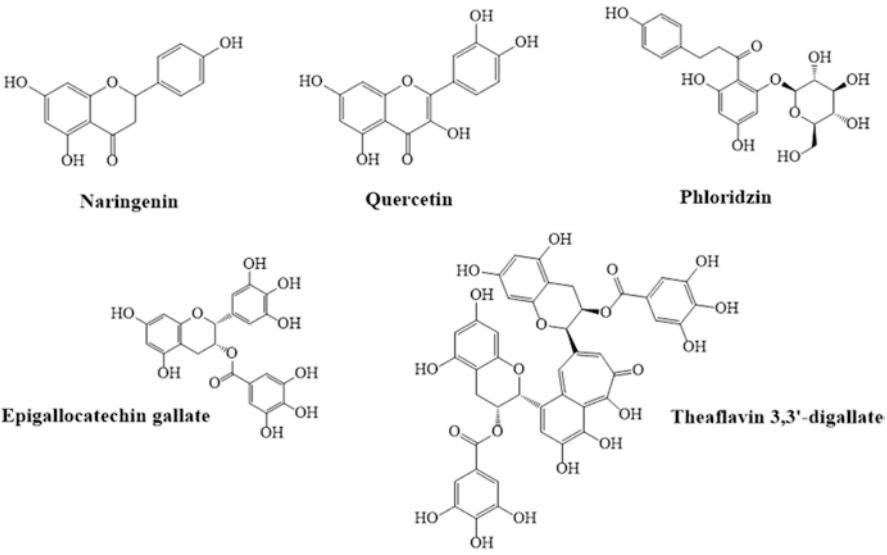

Fig. 5.3 Structures of potent natural HA formation inhibitors

with 0.1% (w/w) apple or grape seed extracts prior to heat treatment decreased individual HAs (MeIQx, 4,8-DiMeIQx and PhIP) and total HA content by >70% in the fried beef patties [62]. Nevertheless, very few studies have performed activity-guided investigation to identify the principal HA formation inhibitors in these extracts, nor has there been sufficient research on the potential synergic effect among some of the purported inhibitors. This warrants further evaluation. It is conceivable that knowledge about the principal inhibitors and/or synergism (if any) among them in these crude extracts would facilitate the preparation of extracts which are selectively concentrated with the inhibitors identified.

5.5 Concluding Remarks

Heating is an important process to ensure microbiological safety, eliminate antinutritional factors, and enable the development of desirable profiles of color and flavor attributes of meat and fish. However, this process also gives rise to HAs and other potentially hazardous by-products which may be a significant health risk for humans in the long term. Robust analytical tools, especially liquid chromatography coupled to diode array detector and mass spectrometer are crucial for the accurate determination of the content of HAs in foods to thus monitor dietary intake and assess the associated health risk. Given the genotoxic potential of HAs and their occurrence in a wide range of major dietary components, development of strategies to inhibit their formation during heat processing or reduce their content in foodstuffs would remain

a key direction in the field of food-borne genotoxicants. Further research should continue to explore how the application of inhibitory strategies could be optimized by integrating with physical conditions such as temperature and duration of cooking, as well as the potential interactions among purported inhibitors of HA formation with various culinary ingredients.

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Chapter 6
Advanced Glycation End Products (AGEs)

Halise Gül Akıllıoğlu and Vural Gökmen

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

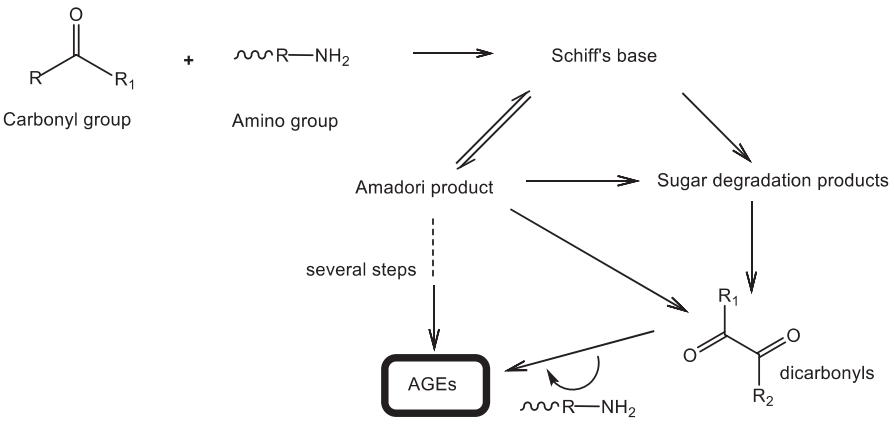

Fig. 6.1 Simplified scheme of advanced glycation in food products

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.

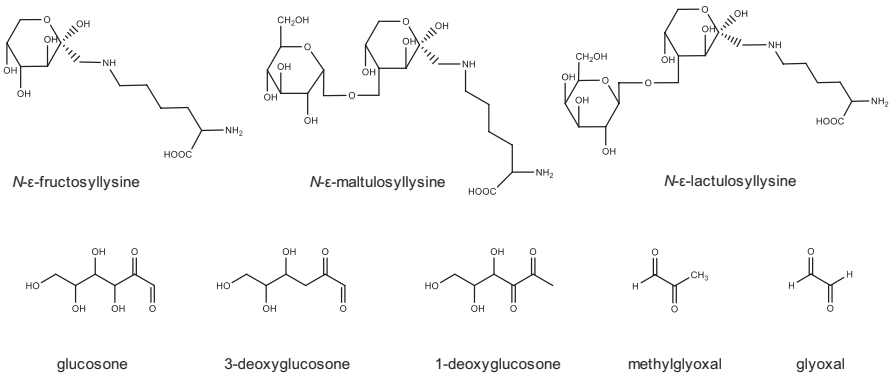

Fig. 6.2 Amadori compounds and 1,2-dicarbonyl compounds formed in the early and intermediate stages of the Maillard reaction

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,

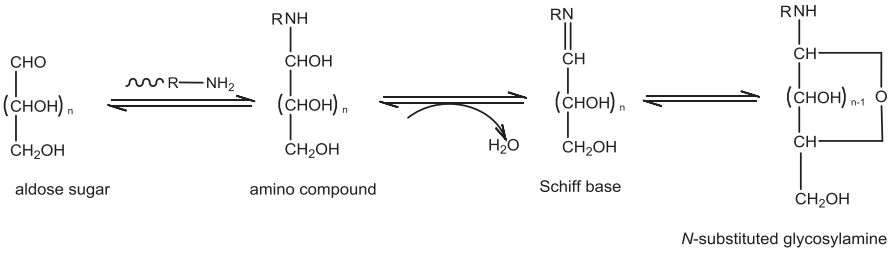

Fig. 6.3 Sugar-amine condensation to form *N*-substituted glycosylamine

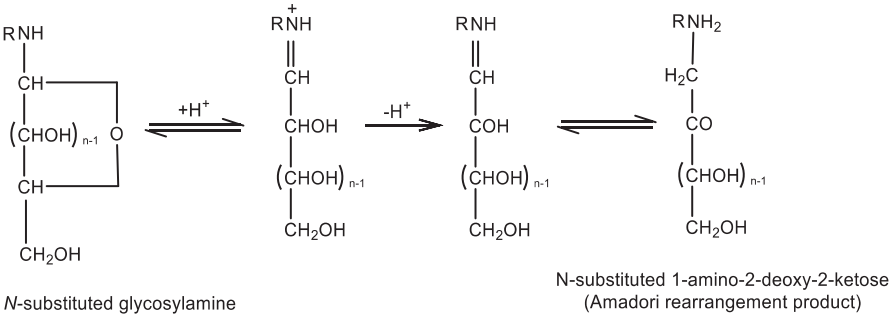

Fig. 6.4 Amadori rearrangement

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].

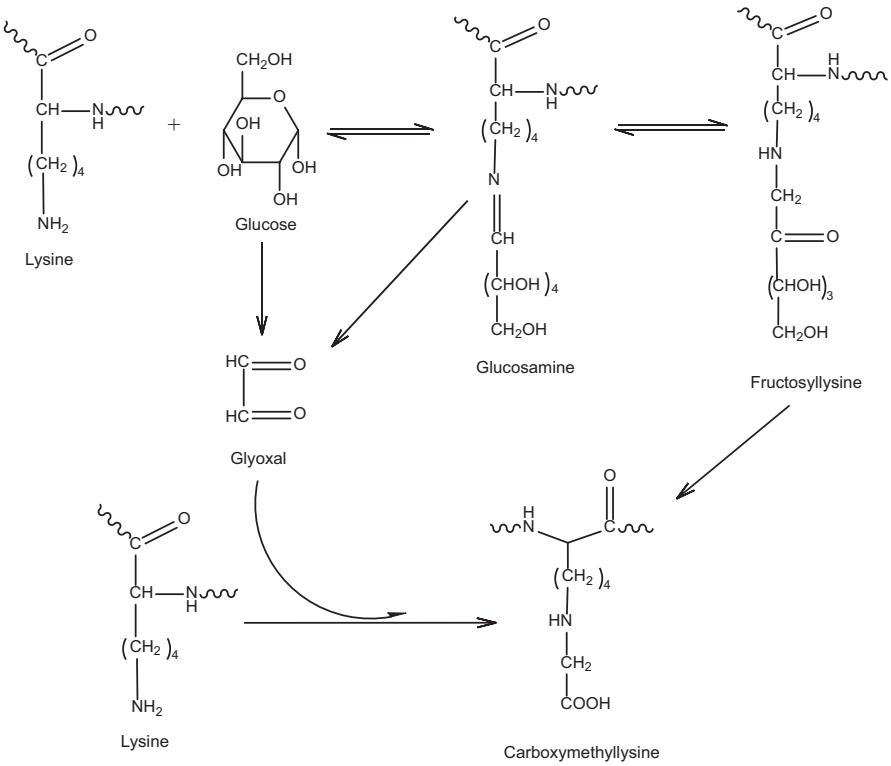

Fig. 6.5 Pathways of CML formation. (Adapted from Han et al. [69])

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].

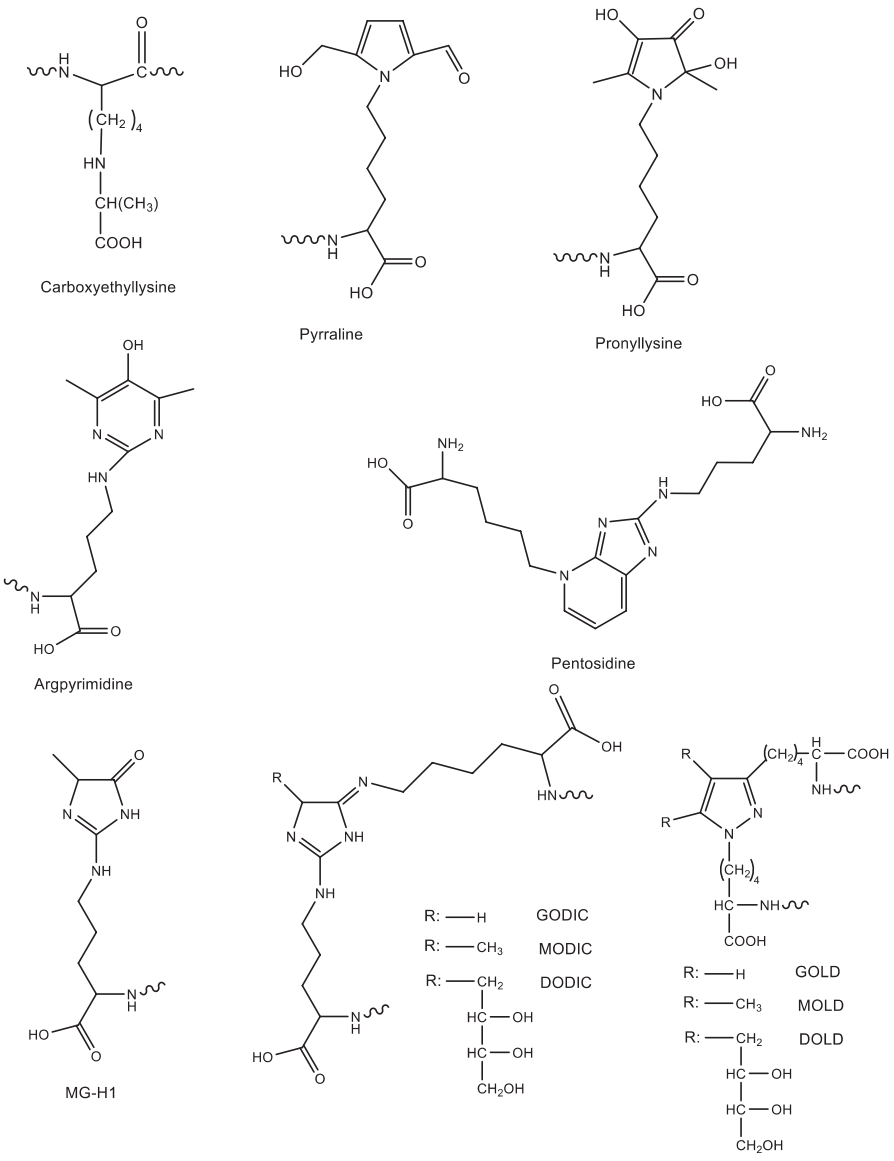

Fig. 6.6 Examples of AGEs found in foods

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-

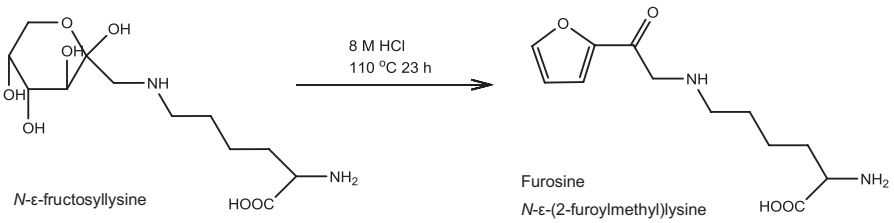

Fig. 6.7 Formation of furosine during acid hydrolysis of Amadori compounds

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.

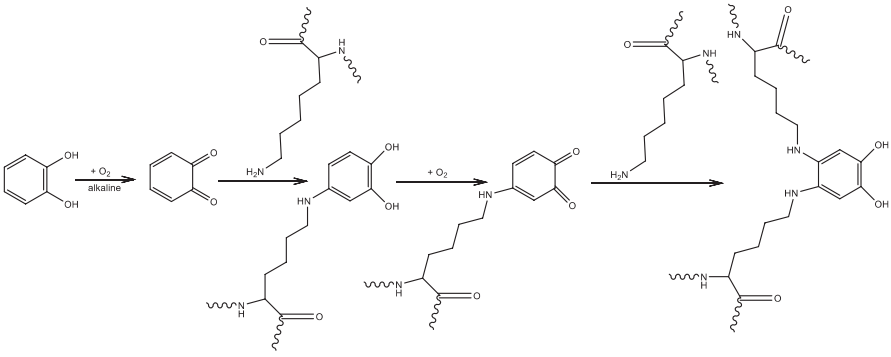

Fig. 6.8 Reaction between green tea polyphenols and proteins at alkaline condition

[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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Chapter 7
Other Chemical Hazards

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This chapter reviews five groups of chemical hazards, including 5-HMF, trans-fatty acids, MCPDs and their esters, glycidol and its esters, and acrolein and other alkenals. Their analytical methods, formation mechanisms, and mitigation strategies are discussed. Understanding these chemical hazards may improve our knowledge about the whole thermal-processing-induced hazards, then improving food safety and quality in food industry.

7.1 5-HMF

5-HMF (IUPAC name, 5-(hydroxymethyl)furan-2-carbaldehyde; Fig. 7.1) was a ubiquitous food contaminant formed during processing of sugary foods. 5-HMF is mainly generated from hexose under high temperature or during long-term storage at room temperature, regardless of the presence of oxygen or UV light (Maillard reaction). Aberrant crypt foci were induced in a dose-dependent matter in F344 rats by 5-HMF [1] and skin tumor was reported in mice [2]. However, no genotoxicity could be detected from 5-HMF in standard in vitro assays, such as gene mutation assay [3], the rec assay [4], or comet assay [5]. To conform the acute, subacute, and

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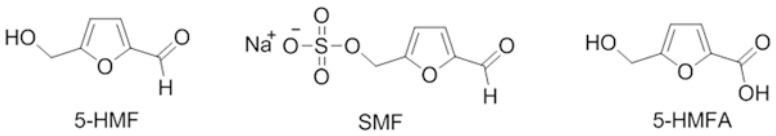


Fig. 7.1 The structure of 5-HMF, SMF, and 5-HMFA

chronic toxicity of 5-HMF, the National Toxicology Program conducted a toxicological evaluation on 5-HMF using F344/N rates and B6C3F1 mice for 3 weeks, 3 months, and 2 years. They concluded that 5-HMF caused liver cancer in female mice and was related with gradually enhanced injury of the respiratory epithelium and olfactory regardless of the gender and rats or mice [6].

In recent years, 5-sulphooxymethylfurfural (SMF, Fig. 7.1), the metabolite of 5-HMF, was confirmed with mutagenicity and genotoxicity by in vitro and in vivo studies [7, 8]. At the same time, 5-HMF was linked with the formation of 5-hydroxymethyl-2-furoic acid (5-HMFA) (Fig. 7.1) [9], acrylamide [10], dicarbonyl compounds [11], and melanoidins [12]. So, it is necessary to detect the amount, illuminate the formation mechanism, and discover the mitigation strategies of 5-HMF in food processing.

7.1.1 Analytical Methods

5-HMF is a needle-like crystalline solid with a slight odor of chamomile flowers. It tastes buttery, caramellic, and musty (data from HSDB) and is easily dissolved in water and methanol. 5-HMF has a maximum UV absorption wavelength at 283 nm. To remove the interference of protein which has strong absorption wavelength at 280 nm, Carrez clarification reagents were used [13]. The analytical methods for 5-HMF in foods will be discussed as two major approaches, the direct approaches and the indirect approaches [14–16].

7.1.1.1 Direct Approaches in Detecting 5-HMF

Direct approaches aim at analyzing 5-HMF directly without breaking the structure of 5-HMF. In 2009, melamine and 5-HMF were firstly detected by CE-DAD (capillary electrophoresis-diode array detection) in milk samples; the linearity range of 5-HMF was 0.1–100 μg/mL. The limit of detection (LOD) was 0.067 μg/mL for 5-HMF [17]; in 2010, Gloria del Campo et al. used ^{1}H NMR spectrometry to detect the 5-HMF and caffeine, formic acid, and trigonelline in soluble coffees; the LOD of 5-HMF was 0.30 mg/g; this method was no need for derivative procedure [18]; in 2013, Ales Rajchl used a novel technique series connection of direct analysis in real time (DART) ion source and time-of-flight mass spectrometry (TOFMS) to develop a rapid determination of 5-HMF. Linearity was measured in the range 1–1000 mg/L;

the calibration plot was linear only within the range 1–20 mg/L (R^2 = 0.9985). Repeatability of the measurement was 66% and 11% (RSD) without and with the internal standard (isotope-labeled 5-HMF), respectively. For the honey and caramel samples, the LOD was 2 and 3 mg/kg, respectively; the LOQ was 3 and 4 mg/kg, respectively. Recovery was 98% (concentration 10 mg/kg) and 101% (concentration 10 mg/kg) for honey and caramel samples, respectively [19]. This method requires isotope-labeled 5-HMF as internal standard and needs expensive TOF mass spectrometry. So, this method is difficult to have a wide application.

In 2016, Jucimara developed an HPLC-DAD method for quantitative analysis of HMF in cane syrups and corn. In this method, the column was C18 at 30 °C and DAD was set at 285 nm, and the mobile phase was acetonitrile/water (1/9, v/v, added with 0.5% formic acid) isocratic elution, and flow rate was 0.8 mL/min. The LOD was 0.09 mg/L and LOQ was 0.26 mg/L; the recovery rates were between 100% and 104%; RSD was 0.57–6.43%. The contents of HMF were 109.2–893.1 mg/kg and 406.6–2121.3 mg/kg in cane syrup and corn syrup, respectively [20]; in 2017, Terra and colleagues developed UV-MCR-ALS methods for quantification of 5-HMF with the use of ultraviolet (UV) spectroscopy technique in a combination with data analysis technology of multivariate curve resolution (MCR) coupled with alternating least squares (ALS). This model was evaluated by analyzing statistical parameters of quality such as root mean square error (0.68 mg/L) and correlation coefficient (R = 0.988). This method was easy and quick, and no pretreatment and chromatographic separations were required. In addition, it would achieve the accurate determination of 5-HMF content [21]. However, solid and fluid cannot be directly measured using this method. The food matrix is complex and different, so each kind of sample needs a separate modeling analysis. This method shows great potential in automation applications but still requires deepgoing research.

7.1.1.2 Indirect Approaches in Detecting 5-HMF

5-HMF has both hydroxyl and aldehyde groups, which could easily react with derivatization reagents to get specific target products. Colorimetric method was used to detect 5-HMF in tomato with the use of toxic derivatization reagents barbituric acid and *p*-toluidine. Now this method is gradually abandoned, because of the poor repeatability and anti-interference. In 2009, Bernhard et al. used UPLC-MS/MS to detect 5-HMF in plasma samples. Derivative reagent was 2,4-dinitrophenylhydrazine (DNPH) dissolved in acetonitrile/HCl (1 M, 3/1, v/v), equivalent volume mixed with samples and incubated for 1 h at 37 °C; used potassium hydroxide which dissolved in equivalent volume of water and ethanol to neutralize the redundant acid; finally diluted by water. After centrifugation, the upper layer was used for analysis. The derivative compounds of DNPH-HMF were analyzed by water MS in the positive ion mode with ESI [22]. The derivative process was complex and time-consuming and derivatization reagent is toxic for the analyst. So now the application of this method is relatively narrow.

7.1.2 Formation Mechanisms

5-HMF was found at high levels in many foods, especially from sugar-containing products processed under high temperature. HMF in foodstuff was mainly produced by two types of chemical reactions. The first reaction includes caramel products, instant coffee, biscuit, and other roasting foods. This group was considered as major sources to form 5-HMF mainly through caramelization reaction, whereas the second one includes dried fruits, fruit juices, and paste, which can form 5-HMF by Maillard reaction.

Antal and colleagues demonstrated a formation mechanism of 5-HMF from fructose using isotope labeling [24]. Fructose (A1) or sucrose was dehydrated at C5 position to form the fructofuranosyl oxocation, and then a derivative like enol of 2,5-anhydro-D-mannose was formed by losing a proton through enolization. In the next step, the structure was isomerized to aldehyde group and a second water molecule was released at C4 to form the double bond in the furan ring. When the third water molecule was eliminated at C3 position, 5-HMF was formed [25]. This pathway was shown in Fig. 7.2.

When the hexose was glucose, the mechanism of 5-HMF formation was more complex. Glucose may undergo complex transformation in which the intermediate was tautomeric enediol and isomerized into fructose through a tautomeric 1,2-enediol (A2), which was subjected to a dehydration step at C3 to form unsaturated hydroxyaldehyde (structure 6). 3-Deoxy-2,3-diulose (structure 7) might be automatically converted to unsaturated hydroxyaldehyde (structure 6), which was associated with numerous side reactions. Structure 7 released the second water molecule at C4 forming dicarbonyl compound (structure 8). Then, cyclization and dehydration resulted in 5-HMF formation [24]. This pathway was shown in Fig. 7.2.

The proteins and amino acids can take part in the formation of 5-HMF in food processing [26]. It is not clear whether fructose reacts with amino acid to form 5-HMF through a prolix pathway [27] or through isomerization into glucose. The formation mechanism of 5-HMF from glucose and amino acid was widely accepted (Fig. 7.3). This was similar to the beginning and middle stages of the Maillard reaction [23, 28].

7.1.3 Mitigation Strategies

For now, only honey and dairy products limited the amount of 5-HMF by legislation. For the foods such as coffee, biscuit, dried fruit, etc., there is no limit to the content of 5-HMF. At the same time, the toxicity mechanism of 5-HMF has not yet been elucidated. Although 5-HMF is common and abundant in the foods, it does not attract enough attention. So, the literature associated with mitigation strategies of 5-HMF is limited. The formation of 5-HMF was linked with Maillard reaction directly; as such the researchers pay more attention to mitigation strategies for reducing Maillard reaction and its toxicity derivate such as anti-Maillard agents [29]

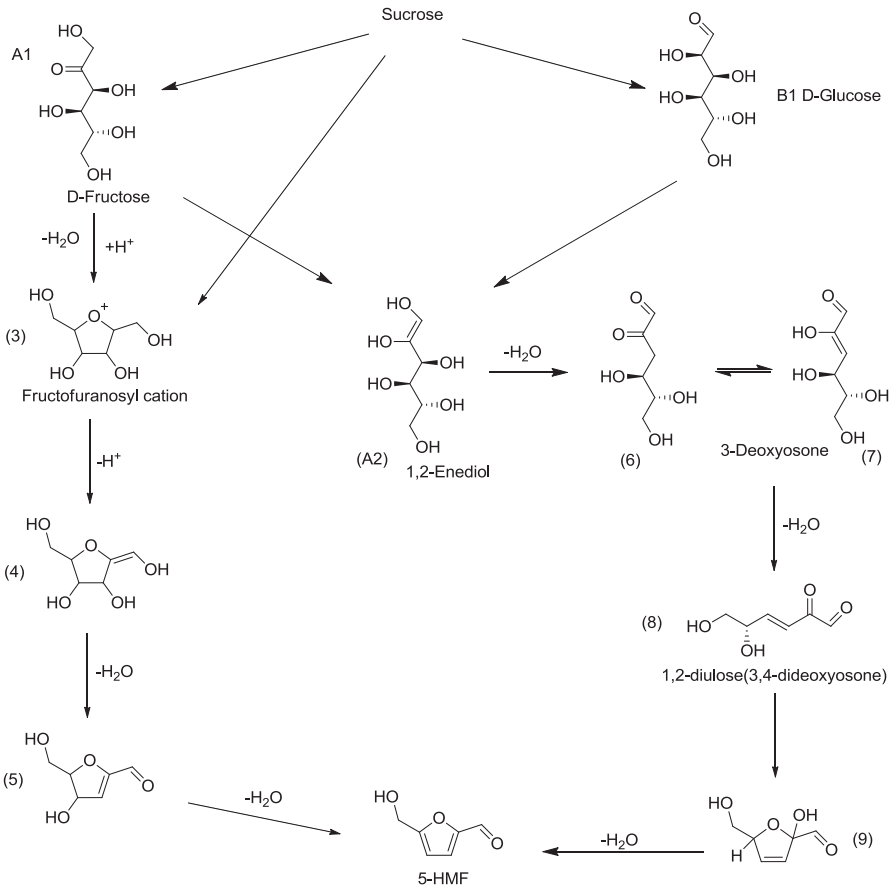


Fig. 7.2 Formation mechanism of 5-HMF from sucrose. (Adapted from Perez Locas (2008) and Stanistaw Kowalski (2013) [23])

or advanced glycation end product (AGE) inhibitor in recent years [30, 31]. These methods and strategies are worth being used for reference. Next, we will summarize the mitigation strategies to reduce 5-HMF in food matrix.

7.1.3.1 Optimization of Food Processing Conditions

The content of 5-HMF in food matrix was determined by temperature, time, pH, and activity of the water. During baking, when the temperature at the surface significantly increases, the 5-HMF could be rapidly accumulated [32]. The heating temperature and time are the decisive factors. When the pH value rises from 3.28 to 4.37 and then to 7.40, the level of formation of 5-HMF showed a significant decrease during baking at more than 200 °C [33]. Typically, relatively lower pH value will increase the formation of HMF in the biscuit during baking. In 2016, cocoa bean was immersed in 7.5% Na_2CO_3 containing the alkaline solution for 30 min, while

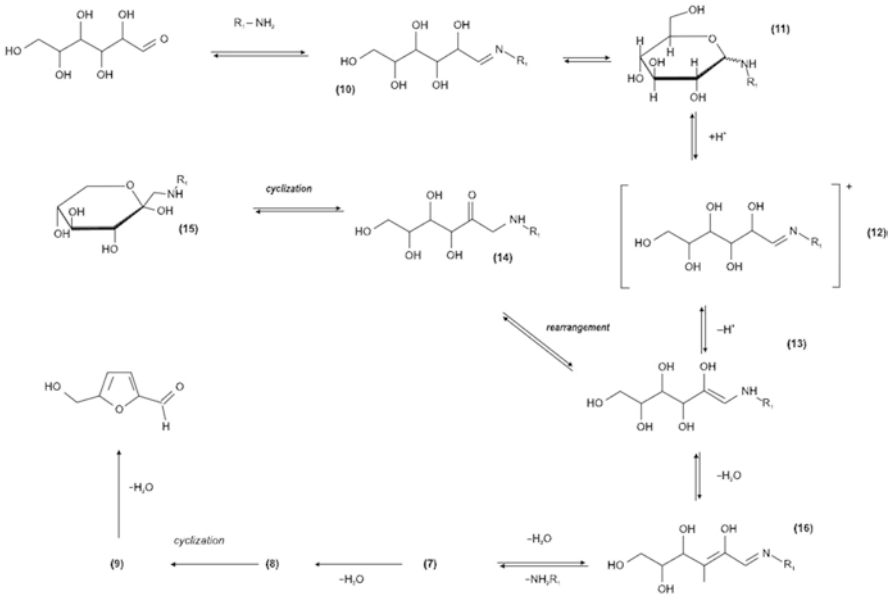


Fig. 7.3 The formation mechanism of 5-HMF from glucose and amino acid or protein. (Adapted from Kowalski (2013) [23])

water treatment and no treatment were used as a comparison. After being roasted for 1 h, 5-HMF concentrations were 0.34 mg/kg, 0.41 mg/kg, and 0.73 mg/kg in alkaline-treated samples, water immersed, and non-treated, respectively. The concentration of α-dicarbonyl compounds and N-carboxymethyl-L-lysine was higher for alkaline-immersed samples [34]. Both of them were more toxic than 5-HMF, so it needs comprehensive consideration when food processing conditions need optimization.

7.1.3.2 To Change Reactants from the Raw Materials

The sugar was also a key factor. Sucrose, glucose, fructose, and starch were common sugars used in food processing. At the baking temperature from 160 to 230 °C, the dough containing glucose produced more 5-HMF compared to sucrose [33]. When the temperature of baking biscuits exceeds 250 °C, using sucrose instead of glucose or fructose would result in more 5-HMF production [35]. In 1996, J. O'Brien compared the stability of trehalose, sucrose, and glucose in freeze-dried systems, with the presence of lysine at pH 2.5 and water activity of 0.33. After being heated at 90 °C for 194 h, trehalose system showed the lowest absorption at 420 nm and

280 nm [36], which means the trehalose was more stable than sucrose and glucose and less stable than furans (e.g., 5-HMF) generated. It is worth considering replacement of sucrose and glucose with trehalose in the processing of coffee, roasting foods, and dried fruit.

7.1.3.3 To Remove 5-HMF from Foods

Once 5-HMF is generated in the process of food processing, it is difficult to be removed. In 2017, T. Silva-Fernandes used two kinds of biopolymer, in which commercial names were Aquapol (tannin 18%, biopolymer A) and Bioclin (tannin 6.5–7.3%, biopolymer B) to remove 5-HMF from sugarcane bagasse hemicellulosic hydrolysates. The highest relative content removed by A and B biopolymer was 57% and 40%, respectively, after optimizing the operation parameters. From the above results, it can be seen that tannin-based biopolymers (TBBs) were efficiently and selectively adsorbed for removing 5-HMF. Because of the wide range of sources of tannins, TBBs were simple to prepare, low in cost, and environmentally friendly [37]. Therefore, other polymeric materials can be developed in the future to specifically adsorb 5-HMF.

7.2 Trans-Fatty Acid

Trans-fatty acids, as the name implies, have one or more double bonds in which the adjacent hydrogen is substituted by the opposite sides of the hydrocarbon chain [38]. Mono- and polyunsaturated fatty acid and conjugated linoleic acids are the important sources of dietary trans fats [39]. Fatty acid components seem to be a perennial concern of nutritionists and persons concerned with healthful diets. Previous researches have suggested a possible association of the intake of trans-fatty acids with the risk of coronary heart diseases. The concentrations of LDL cholesterol are increased and the contents of HDL cholesterol are reduced by the intake of trans-fatty acids. This has a negative impact on blood lipids [40].

Due to trans-fatty acid's higher melting point, the fluidity and permeability of cell membranes were altered and affected when trans fat incorporated into cell membranes [38]. The main target of this section is to provide a clear description of the relevant studies in trans-fatty acid, including their analytical detection method, formation mechanisms, and mitigation strategies.

7.2.1 The Analytical Detection Method of Trans-Fatty Acid

The negative impact on trans isomers of unsaturated fatty acids raised lots of concerns since the research consequence of controlled dietary intervention performed in 1990 [41–44]. These results indicated that *trans*-fatty acid (TFA) had a detrimental effect on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. Based on a population-based studies in the USA, the consumption of trans isomers might affect the rate of coronary heart disease [45, 46]. Therefore, in order to understand the presence and content of TFA, it is particularly vital to develop different monitoring technologies. Owing to the complexity of the possible fat mixtures, TFA analyses were still challenges. Researchers used several techniques including gas chromatography (GC) [47], infrared spectroscopic (IR) [48], capillary zone electrophoresis (CZE) [49], and silver ion chromatography to determine TAF [50]. Among these detection technologies, GC is the most popular and common technique.

7.2.1.1 Gas Chromatography (GC)

Gas chromatography (GC) has been widely used in the fatty acid measurement of oilseed plant, human metabolism, and so on [47]. GC methods equipped with high-quality capillary columns for fatty acid analyses are allowed to get sensitive and reproducible results [47]. The fatty acid is transformed into suitable derivatives such as methyl esters, as required by analysis GC [51]. In principle, gas chromatography equipped with flame ionization detection (GC-FID) as the official method is applied to detect fatty acid because of its high resolution and sensitivity [47]. The authors proposed a quantitation method of TFA in vegetable and nonruminant fats and oils; the separation of *cis/trans*-octadecenoic (18:1) in hydrogenated vegetables was performed by the optimization parameters using the CP-Sil 88 and SP 2560 capillary gas chromatographic columns [52]. Because frying process is considered to be a source of TFA, Romero et al. compared TFA profile of potatoes fried in the extra virgin olive oil, high oleic sunflower oil, and sunflower oil from the frying 8 and 20 with frequent replenishment or without replenishment of used oil with fresh oil during the frying by GC [53]. Results represented that elaidic acid showed the greatest amount in fried potatoes. Huang et al. also measured the TFA content both in fried potatoes and in frying oils by GC [54]. The gas chromatography-mass spectrometer using Alltech AT™-Silar-90 capillary column could completely separate *trans*-fatty acid from *cis* standard. Moreover, under the optimized condition, shortening samples were analyzed to check the feasibility of this method; the predominant fatty acids detected in the sample were trans-18:1, cis-18:1, cis-18:2, and cis-18:3. In general, prior to GC analysis, the fractionation of fatty acids by liquid chromatography is applied to eliminate co-elution problems.

7.2.1.2 Capillary Zone Electrophoresis with UV Detection (CZE-UV)

Capillary zone electrophoresis (CE) is an identification and separation technique on the basis of solvated ions, neutral compounds, or ionizable species [55]. The technique as an attractive analysis method for fatty acid has been sought by the hundreds of other scientists. Compared with GC, CE is a throughput analytical method that employs the short analytical time and separated the analyses at lower temperatures without derivatization step [56]. According to the previous study, Oliveira and colleagues first reported a novel capillary electrophoresis with UV indirect detection for the TFA in hydrogenated oils [49]. It is suggested that the formation of TFA could be monitored by the optimized method. Later, CZE was applied for the different FA in different samples by the 2^3 central composite design optimization, owing to factors Brij 35, acetonitrile, and 1-octanol [57]. Moreover, the results were comparable with AOCS GC official method by t-test. However, these methods had the problems about partial dissolving of the capillary coating polymer in the background electrolyte (BGE), which could cause a significant interference in the separation of TFA. In this case, Porto and colleagues proposed a CZE methodology coupled with contactless conductivity detection using two different cyclodextrins in the BGE; it was used for the determination and quantification of TFA of processed food including in 13 min [58]. Furthermore, there were no marked differences of statistical results between CZE-UV and the classical GC method within the 95% confidence interval.

7.2.1.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FT-IR), as a simplest analytical approach, is commonly applied for the measurement of TFA in the edible oils and fats. In general, the isolated TFA were quantified by FT-IR spectroscopy on the basis of the determination of *trans* peak in the area from 991 to 945 cm^{-1}, indicating CH out of deformation absorption [56]. Sherazi and colleagues used FT-IR techniques for the detection of TFA in cooking oil and hydrogenated oil samples [48]. The results obtained by the transmission FT-IR were comparable to GC-FID technique results and have indicated slightly better sensitivity and higher accuracy for low TFA values in the tested edible oil samples. Moreover, FT-IR with an attenuated total reflectance combined with partial least squares models was applied to analysis of TFA concentration of cereal products without oil extraction [59]. The predicted model of TFA has a higher coefficient of determination and lower standard error of performance, suggesting the superiority and robustness of predicting models for screening. The model-based FT-IR was closely related to the fingerprint region, which showed that the unique characteristic of TFA configuration at 966 cm^{-1} has the main contribution to the development of the PLS model [59]. The FT-IR of fats extracted from lipid samples extracted from cereal-based foods, analyzed with a

single-bounce horizontal attenuated total reflectance (SB-HATR), was developed to determine TFA content [60]. It can be verified that FT-IR combined with PLS model might be an effective method to predict TFA content in food.

7.2.1.4 Silver Ion Chromatography

Silver ion chromatography is recognized as one of the attractive techniques for *trans* monoenes profiling giving a good separation and reproducibility of TFA in dairy samples [61], which is most commonly used in the separation of saturated, *trans* monounsaturated, and *cis*-monounsaturated fatty acids along with *cis/trans* conjugated linoleic acid isomers [51]. Indeed, scholars generally believe that silver ion chromatography has no rival for discrimination of liquid species differing in the number and especially liquid with the configuration of double bonds [62]. Thin-layer chromatography, high-performance liquid chromatography, and supercritical fluid chromatography in the silver ion mode are rather powerful tools for the separation and determination of geometrical fatty acid composition [50]. Ag-TLC was performed to pre-fractionate *cis* and *trans*-fatty acids prior to accurately quantifying TFA by infrared spectroscopy and gas-liquid chromatography (GLC) [63]. The *cis* and *trans* geometric and positional fatty acid methyl ester (FAME) and triacylglycerol (TAG) isomer are separated and isolated by Ag-HPLC as alternative techniques [50]. Adlof investigated the profile of the *cis* and *trans* unsaturated FAME by Ag^+ high-performance liquid chromatography; the isocratic elution solvent was acetonitrile in hexane [64]. The other researchers also use Ag-HPLC with two commercially available columns connected in series as a separation method to analyze a mixture of conjugated 18:2 isomers [65]. The method provided an improved resolution of the *cis* and *trans* 18:2 isomer pair, but it could not resolve *trans* form of linoleic acid and linolenic acid.

7.2.2 The Formation Mechanism of Trans-Fatty Acid

The formation mechanisms of TFA are derived from biological hydrogenation in the stomach of ruminants and the industrial process of catalytic hydrogenation of fats. Of the dietary TFA, 80–90% are originated from the latter source, while 2–8% are offered by dairy products [66].

7.2.2.1 The Natural Sources from Ruminant Animals

It is well known that the digestion and absorption of dietary lipid for ruminant animals occurs in the reticulo-rumen [67]. Its metabolism process was comprised of hydrolysis of lipids, biohydrogenation of unsaturated fatty acids by rumen bacteria, and synthesis de novo of microbial lipids [67]. According to the previous studies

[68], the free fatty acids were liberated from hydrolysis of the dietary acryl lipids by microbial lipases in the rumen. Then unsaturated fatty acids suffered from biohydrogenation by rumen bacteria, and the end product of the hydrogenation becomes stearic acid (18:0), saturated fatty acids, *trans*-fatty acids, conjugated fatty acids, and its isomer [68]. The several studies basically confirmed that in hydrogenation pathways of rumen bacteria, the most common is α-linolenic and linoleic acid hydrogenation [67], as shown in Figs. 7.4 and 7.5. At first, both of them are formed to a conjugated *cis*-9, *trans*-11 acid by an initial isomerization step. Then, *cis*-9, *trans*-11 acid as intermediate product undergoes hydrogenation of its *cis* double bonds, while *trans*-11-octadecenoic acid is liberated as penultimate. In the end, they are hydrogenated to stearic acid. It is found that both α-linolenic and linoleic acid could be hydrogenated to *trans*-octadec-15-enoic acid by a rumen bacterium called *Butyrivibrio fibrisolvens*, which was directly involved in the metabolism of fatty acid [69]. In addition, the *Ruminococcus albus* could convert linoleic and linolenic acids to a mixture of octadecenoic acids and *trans*-octadec-11-enoic acids [68].

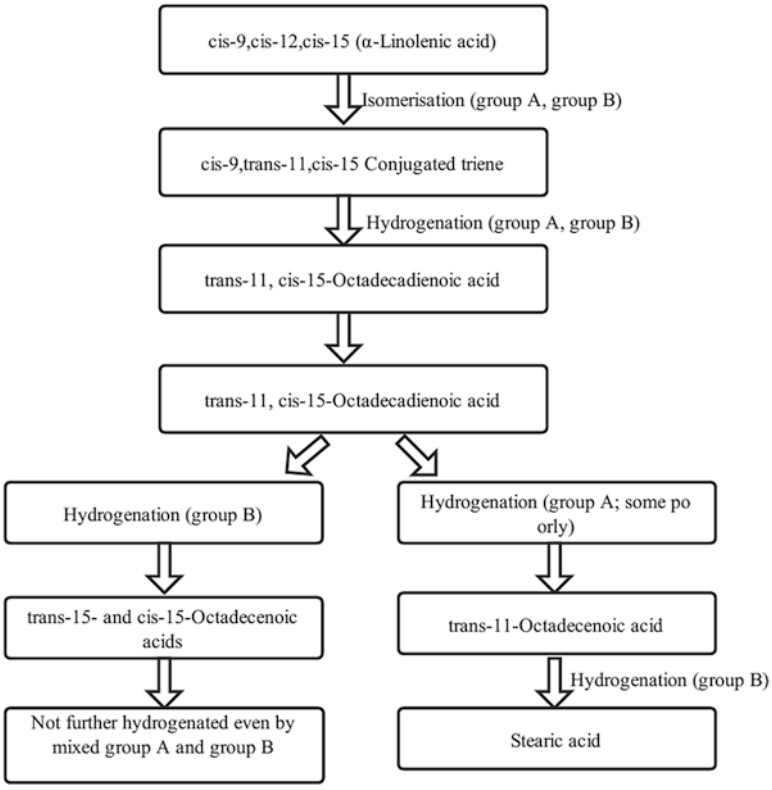


Fig. 7.4 Scheme for the biohydrogenation of linoleic acid; group A and B present the two types of biohydrogenating bacteria. (Adapted from Harfoot and Hazlewood [67])

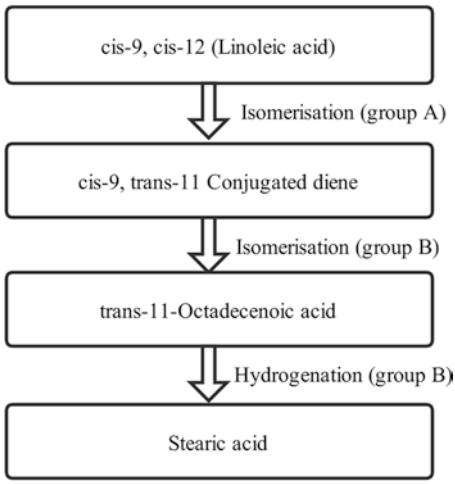


Fig. 7.5 Scheme for the biohydrogenation of linoleic acid; group A and B present the two types of biohydrogenating bacteria. (Adapted from Harfoot and Hazlewood [67])

7.2.2.2 The Processing of Hydrogenated Oil

Some fats and oils are susceptible to autoxidation or thermal degradation and other reactions during thermal food processing on the account of the presence of two isolated double bonds, in order to improve the stability and utility of fat or oils, which are often subjected to the process of hydrogenation, forming shortenings or margarine [70]. Therefore, the fat and oil hydrogenation is an indispensable operation in food and chemical industry. The process can convert a liquid oil to a solid or semisolid product by means of a multiphase catalytic reaction with hydrogen [71]. However, the occurrence of *trans*-fatty acid is a severe challenge in the process of oil hydrogenation.

According to the previous studies [72], the mechanism of geometric and positional isomers as described by Min, a free radical site, is formed when a hydrogen first entered either side of the double bond of unsaturated fatty acid, especially bound to the catalyst, while the free radical site is relatively unstable. A hydrogen atom neighboring carbon could be eliminated with the catalyst partially covered by hydrogen, thus regenerating the double bond or resulting from the formation of a positional isomer. The formed double bond may present as either *cis* or *trans* configuration because of free rotation properties of a free radical site (Fig. 7.6).

7.2.3 The Mitigation Strategies of Trans-Fatty Acid

The contents of TFA in some animal products occurred naturally in the fermentation step, and the amounts were relatively low. Thus, diet content of a large proportion of TFA is generally derived from partial hydrogenation of fats. In addition, the main dietary sources of TFA are fried and baked foods, traditional vegetable shortenings, and solid margarine. Excessive consumption of TFA can increase risk of heart

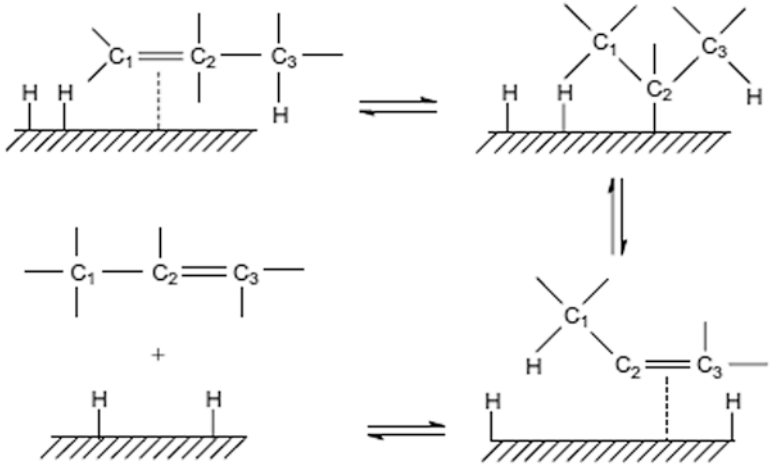


Fig. 7.6 Formation mechanism of geometric and positional isomers in the hydrogenation process

disease [73]. Therefore, food producers try their best to decrease the level of TFA in related products. Meanwhile, the authorities have made law and take some measure to prevent or decrease the damage of TFA.

7.2.3.1 The Method of Reduced TFA

Recently, several food manufacturers voluntarily removed TFA from their products. For example, in 1994, Unilever set about removing TFA from all retail margarine by introducing a worldwide policy [74], triggering by media coverage that TFA was more unhealthy than saturated fats [45]. In addition, many manufacturers have sought to TFA substitutes. In order to minimize the TFA content of the products, numerous technologies have been performed by the food and edible oil industries. Firstly, the partial hydrogenations were replaced by full hydrogenation to obtain fats with low TFA levels. Secondly, the oil seeds with modified fatty acid components are produced by traditional plant breeding and modern genetic engineering techniques for the sake of altering the oil properties [75]. Thirdly, the fractionated tropical oils are used in the manufacturing process [75]. Finally, the chemical and enzymatic interesterification of hydrogenated fats with liquid oils is obtained to a range of melting point customized fats to fit different food industry applications [75].

7.2.3.2 Regulating the Concentrations of TFA in Foods

Regarding the need for public health action of international policy consensus, TFA intake should be no more than 1% of total energy intake according to Physical Activity and Health, 2004 WHO Global Strategy on Diet [76]. Moreover, to reduce

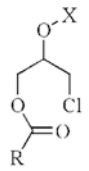
the health risk associated to TFA, no more than 2 g/100 g fat on industrially produced TFA is imposed in Denmark [77], as labeling was inadequate to protect the consumers, particularly for children or people with high intake of fast foods. The Danish Nutrition Council requests a reduction of TFA in the cooking oil for major fast food. Based on Denmark's legislation introduced and enforced on June 1, 2003, the industrially produced TFA content of all food products and ready meals is restricted to a maximum of 2% [78]. Afterward, hydrogenated oils or margarine containing trans fats are illegally used to prepare food, except for foods that contain no more than 0.5 grams of artificial *trans* fat per serving of food [79]. In 2008, Switzerland begins to regulate TFA content. A survey determined by the Federal Institute of Technology has found that almost one-third of 120 food items contained excessive amounts of TFA. This finding was a key factor in attracting attention of the authorities and food industry to the problem [80].

7.3 MCPDs and Their Fatty Esters

Monochloropropane-1,2-diols (MCPDs), including 3-monochloropropane and 2-monochloropropane, are one type of chemical compounds mainly produced during thermal processing of food. MCPDs were recognized as potential food source toxicants in the past decades. Monochloropropane-1,2-diol fatty acid esters (MCPD esters) are a group of toxicants formed during food thermal processing. These compounds have been detected in many food categories, including refined edible oil, fried food, infant foods, and even in human breast milk. In 2004, 3-MCPD esters, as well as the free 3-MCPD, were firstly reported in processed foods. The ester form of 3-MCPD occurs at a much higher concentration in foods, especially in some high-lipid content foods after high-temperature processing. Therefore, a tolerable daily value calculated as the amount of free 3-MCPD at 2 μg/kg body weight was estimated by the European Food Safety Authority in 2013. These facts suggested that MCPDs together with their fatty acid esters are an important food safety concern and warrant research of their analytical methods, formation mechanisms, and the approaches for mitigation (Fig. 7.7).

7.3.1 Analytical Methods

Since MCPDs and their fatty acid esters were reported and considered as potential food source toxins in 2006 [81], it is important to develop accurate, fast, and high-sensitivity chemical analytical methods to detect this group of chemical components in different types of food samples. All of the reported analytical methods can be separated into two major types. The first method is to hydrolyze all the fatty ester forms of MCPDs to free MCPD first, quantify the amount of free MCPD, and then use the concentration of free MCPD to represent the total amount of all the MCPD



3-MCPD ester X = H, OR

Fig. 7.7 Chemical structure of 3-MCPD and 3-MCPD esters

esters indirectly. This approach is much convenient to process. Another possible method is to characterize and quantify every MCPD ester in food samples directly. This method is straightforward and easy to understand, but is also much difficult for sample purification and method development. In the following paragraph, a systematic review of these two methods will be given to improve the understanding of the chemical analytical methods about MCPD esters.

7.3.1.1 Indirect Approaches in Detecting MCPD Esters

The basic working mechanism of the indirect analytical method starts with the transesterification of all of the MCPD esters into free MCPD under acidic or alkaline conditions, then free MCPD is transformed into a stable voltaic derivate, and, finally, the amount of free MCPDs is detected using GC or GC-MS [82]. This approach could also be used to analyze the free MCPDs. The major differences between these methods are the choices of different transesterification conditions and derivatization agents. In 2006, Zelinková and colleagues reported their study results about the quantification of 3-MCPD esters and free 3-MCPD in 25 vegetable oil samples using the indirect analytical approach [81]. In this study, 3-MCPD ester extracts were hydrolyzed using sulfuric acid, neutralized with a saturated $NaHCO_3$ solution, derivatized by using the phenylboronic acid, and then analyzed with GC-MS. The results indicated that most of 3-MCPD that existed in oil samples is bounded 3-MCPD (3-MCPD esters) rather than free 3-MCPD and 3-MCPD diesters are the major form of 3-MCPD esters. The results in this study also made the research about ester form of 3-MCPD become more and more important, since they are the major existence form of 3-MCPD in almost all types of foods. In 2008, Zelinková reported the occurrence of 3-MCPD esters, firstly, in human breast milk using a similar analytical method, which confirmed the stability of this method [83]. In the same year, Seefelder designed and processed a novel enzymatic hydrolysis method to hydrolyze the 3-MCPD esters to the dissociated 3-MCPD by using the intestinal lipase, followed by the GC-MS analysis [84]. The results represented the fact that only equal or less than 15% of 3-MCPD bounded in esters are monoesters and the rest of the parts are diesters. In 2008, Weißhaar reported a method to quantify 3-MCPD esters in edible fat and oil samples by transesterifying 3-MCPD esters with NaOCH3/methanol and derivatizing with phenylboronic acid, and finally, 3-MCPD was determined by GC-MS [85]. In 2010, Baer and colleagues published

a review article about 3-MCPD in food [82]. In this review, different derivatives including heptafluorobutyryl imidazole (HFBI) [86], phenylboronic acids (PBA) [87–89], and dioxolane were utilized to increase the volatility of hydrolyzed 3-MCPD. In 2016, Samaras and colleagues reported an indirect analytical method for the quantification of 3-MCPD esters, 2-MCPD esters, and glycidol esters in different types of food samples which were purified with pressurized liquid extraction (PLE) and determined using GC-MS. In order to differentiate glycidol esters from MCPD esters, all the glycidol esters were converted to monobromopropanediol esters (MBPD esters) in acidic solution first. Then MCPD esters and MBPD esters were hydrolyzed to release their free forms in ethyl acetate in the environment of phenylboronic acid. And the concentrations were finally quantified using isotopic labeled MCPD esters by GC-MS [90]. Also in 2016, an indirect analytical method using the enzymatic mechanism was applied to analyze 3-MCPD esters, 2-MCPD esters, and glycidol esters in vegetable oils and fats. This enzymatic method utilized *Candida rugosa* lipase to hydrolyze the ester form of MCPDs and glycidols to their free form at an ambient temperature for 30 min. Then the free form of MCPDs and glycidols was analyzed using GC-MS [91]. All these results above indicated that the indirect approaches required less analytical standards, thus recommended to be used to describe the total amount of 3-MCPD-related components in food matrix. But, on the other hand, the indirect methods need more sample preparation approaches with the use of some toxic chemical reagents. Also, the indirect method could only be used to quantify the total amount of 3-MCPD esters instead of every specific 3-MCPD ester. These defects made the indirect analytical methods more and more uncommon after 2010, and increasing researches turned to develop direct analytical methods to determine the concentrations of each 3-MCPD ester individually.

7.3.1.2 Direct Approaches in Detecting 3-MCPD Esters

Direct approaches aimed to develop chemical analytical methods to analyze 3-MCPD esters directly without hydrolyzing them to free 3-MCPD. This method was not widely applied before 2010. It might be majorly due to the nonpolar and relatively low volatile properties of 3-MCPD esters. As such they are not suitable for either LC or GC analysis. However, with the development of analytical techniques and new types of LC columns, there is increasing research about the direct approaches of analyzing 3-MCPD esters directly in different food matrixes. In 2012, Dubois and colleagues made a comparison of previous indirect methods with their novel developed a direct method to analyze 3-MCPD esters in edible vegetable oils [92]. In this study, double solid-phase extraction (SPE) and silicone gel column were utilized to separate and concentrate 3-MCPD esters; an HSS T3 normal-phase LC column was utilized with the combination of ESI time-of-flight (TOF) mass spectrometry. The comparison between indirect and direct methods represented that these two types of analytical methods showed similar results when analyzing 29 oil samples. The indirect approach needs fewer chemical standards and is relatively

easier for sample preparation and is applicable to all types of commodities compared to the direct approach which determines the total 3-MCPD ester content. On the other hand, direct method can provide detailed information about the identification and concentration of every 3-MCPD ester, though with a longer sample pretreatment time. After 2012, increasing research articles reported modified direct method of analyzing the types and concentrations of 3-MCPD esters in food samples or organ/tissue samples in in vivo studies [93–96]. All these studies selected high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) combined with high-resolution mass spectrometry to detect 3-MCPD esters directly; these methods represented the development of 3-MCPD ester analysis research and played an important role in the analysis of MCPD and its esters.

7.3.2 Formation Mechanisms

The formation mechanisms about MCPDs were first described as protein hydrolysates in 1978 [97]. In these years, increasing studies indicated that thermal processing might be closely related to the amount of MCPD esters in food products. And the ester form of MCPDs can be degraded to the free form of MCPDs. During this progress, the temperature was the most important factor that controls the formation of MCPDs. Take the refined edible oil as an example; deodorization was recognized as the key step in forming MCPD esters during processing, with the working temperature at around 260 °C. Reducing the temperature significantly decreased the concentrations of MCPDs. In order to clarify the formation mechanisms of MCPDs during food processing, different formation pathways have been derivated to explain the possible ways of ester form of MCPDs formed during thermal processing.

The primary aim about the studies of 3-MCPD esters is to investigate the chemical and biochemical formation mechanisms during food processing, since it is the fundamental of further research of 3-MCPD esters. Several previous researches investigating the possible formation mechanisms of 3-MCPD mono- and di-fatty acid esters were reviewed and summarized in four major possible types [98], including the direct nucleophilic substitution of chlorine anion of a hydroxyl (pathway 1) or a fatty acid ester group at sn-3 carbon atom in the glycerol (pathway 2) and through the formation of an acyloxonium cation (pathway 3) or through an epoxide cation intermediate (pathway 4) and then the intermediate cation attacked by chlorine anion to open the three- and five-membered rings to form MCPD esters (Fig. 7.8).

In 2013, a novel research approach about 3-MCPD ester formation from diacylglycerol was reported [99]. For the first time, this study reported a free radical intermediate reaction mechanism of 3-MCPD diester. In 2015, possible formation mechanisms of 3-MCPD monoesters and diesters from triglycerides were derivated and involve either a glycidol ester radical or a cyclic acyloxonium radical intermediate in a high-temperature and low-moisture condition [100]. In 2016, a further study

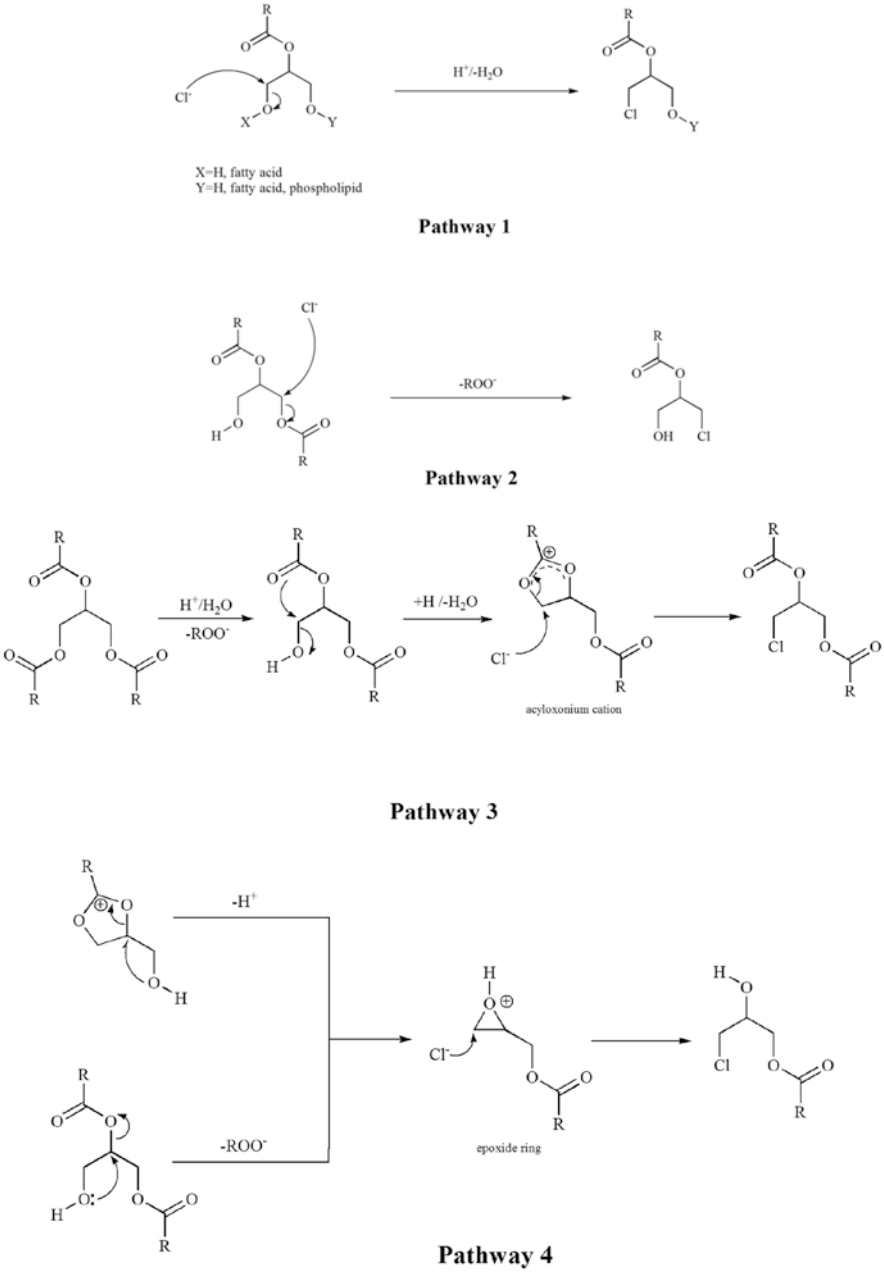


Fig. 7.8 Hypothesis of formation mechanisms of 3-MCPD esters

about the formation of 3-MCPD esters from monoglycerides was reported [101]. The results of this study confirmed the free radical intermediate reaction as one of the mechanisms for 3-MCPD ester formation. This study demonstrates for the first time that five- or six-membered cyclic acyloxonium structures might work as an intermediate in this free radical reaction, which could be used to explain the high-temperature and low-moisture formation environment of 3-MCPD and 2-MCPD esters during thermal processing. Besides, results also represented that the monoester of 3-MCPD could degrade to form monoacylglycerol or free 3-MCPD and the ferric ion might play an important role in the thermal degradation of 3-MCPD monoesters.

7.3.3 Mitigation Strategies

After clarifying the formation mechanisms of MCPD and 3-MCPD esters during thermal processing, the strategies for reducing the content of these compounds in foods become necessary and important. To our best knowledge, eliminating chlorine-related compounds in the deodorization is one of the most important steps in developing a mitigation strategy to reduce the existence of 3-MCPD ester during food processing. All these strategies could be summarized into two types: to remove the reactants (either the glyceride or the chlorine substances) before the formation of MCPD/MCPD esters or remove the MCPD/MCPD esters after their formation.

Another possible approach to mitigate the formation of MCPD esters is to remove or reduce major reactants from the raw food materials before thermal processing. For example, monitoring and controlling the existence of chlorine-related components in food materials could effectively mitigate the content of the MCPD esters.

Chelating agents are another group of compounds that can theoretically mitigate MCPD esters during thermal processing. Based on the free radical formation mechanism of MCPD esters, the existence of free radical intermediate is the key step for the formation of MCPD esters. Chelating agents could competitively react with the free radical intermediate products and reduce the amount of final products. Zhang and colleagues reported the results about using EDTA-2Na to competitively inhibit the generation of 3-MCPD esters during thermal processing [100]. These results represented a fact that chelating agents might be used in selected processing steps to effectively reduce the content of 3-MCPD esters in the thermal processed food.

Although MCPD esters were formed during thermal processing, latest studies reported a potential catalytic role of ferric ion in degradation of 3-MCPD monoesters during thermal treatment [101]. In this study, 3-MCPD monoesters could be degraded at a greater ratio with the existence of ferric ion; meanwhile glycidyl esters and monoglycerides were formed significantly.

Besides, there are some other strategies that might be used to reduce the concentrations of MCPD/MCPD esters in food during thermal processing, such as manipulating the deodorization conditions or using lower chlorine-contaminated fats instead of higher chlorine-contaminated fats [102]. All these approaches aim to mitigate the existences of MCPD/MCPD esters in thermal processed foods.

7.4 Glycidol and Its Fatty Acid Esters

Glycidol has been classed as a probable carcinogen in humans (2A) (IARC, 2000), due to the alkylation properties of the epoxide prone to react with cellular nucleophiles which induced the mutagenic and carcinogenic effects in rodents. Glycidol fatty acid esters (GEs) (Fig. 7.9) have been detected in a variety of thermal processing food, especially in refined oil [103–105]. In the gastrointestinal tract, GEs can hydrolyze to form the free glycidol [106, 107]. Therefore, GEs are also probable carcinogen in humans, which need significant effort to inhibit and eliminate. The section below aims at the following topics: (i) analytical method for glycidyl and its ester detection, (ii) chemical mechanism for the formation of glycidyl and its esters, and (iii) possible approaches to reduce glycidyl and its ester levels in foods.

7.4.1 Analytical Method for Glycidyl and Its Ester Detection

Glycidol can be detected in gas chromatography combined with flame ionization detection [108]. And the analytical method for GE detection could be summarized into two major approaches, indirect methods and direct methods.

7.4.1.1 Indirect Analysis Methods

In the indirect analysis methods of GEs, the GEs are hydrolyzed into glycidol and then glycidol derivatized with derivatizing agents such as phenylboronic acid (PBA), heptafluorobutyryl imidazole (HFBI), bis(trimethylsilyl)trifluoroacetamide (BSTFA), or heptafluorobutyric anhydride (HFBA).

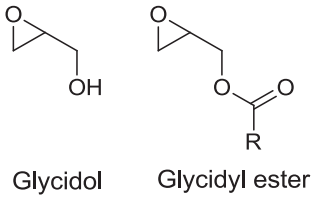


Fig. 7.9 Structure of glycidyl and glycidyl esters

The German Society for Fat Science (DGF) official method (DGF Standard Methods C III 18 (09) 2009) is an indirect analytical method. This indirect analysis method consists of two pretreat options (options A and B) in different analytical mechanisms. In brief, GE samples are hydrolyzed in either acidic or alkaline conditions to free the glycidol and purified by a liquid-liquid extraction, derivatization with derivative reagents, and followed by the GC-MS quantification. Finally, the total GE amounts are calculated as the measured amounts multiplied by 0.67 (stoichiometric factor). Based on the DGF official method, a lipase from *Candida rugosa*-catalyzed hydrolysis of GEs for 30 min at room temperature shortens detection time [91, 109].

Three indirect analysis methods of GEs in fats and oils have been published by American Oil Chemists' Society (AOCS), namely, AOCS Cd29a-13, Cd29b-13, and Cd29c-13 [110–112]. In mildly alkaline or lipase-catalyzed conditions, GE samples transesterification, then transformation into monobromopropanediol (MBPD), derivatization of MBPD with derivative reagents, and followed GC-MS analysis.

Kuhlmann reported a new type of indirect analysis methods of GEs and 3-MCPD esters, which could analyze the glycidol derivatives and 3-MCPD derivatives independently [113].

Although the lipase-catalyzed conditions in indirect determine methods enables GEs, 3- and 2-MCPD esters to be analysis simultaneously with and the lipase use for the hydrolysis of esters, averting the transformation of MCPD esters or partial acylglycerols into GEs [109, 114], but in the bromination process, an incomplete bromination condition may still produce an underestimation amount of GEs in the tested oils samples. All these reasons above made the direct determination method without transesterification or derivatization necessary.

7.4.1.2 Direct Analysis

The direct methods include liquid chromatography-mass spectrometry (LC-MS), GC-MS, and nuclear magnetic resonance (NMR). Haines and his colleagues described the determination of seven kinds of GEs in edible oils by using LC-TOFMS in the positive ion mode [106]. And the sample preparation was the simplest to implement: the edible oil samples were diluted in a mixed solvent (methanol-sodium acetate solution (0.26 mM)/methylene chloride/acetonitrile = 1/8/1) prior to analysis. The LOD of glycidyl myristate in refined edible oil was estimated to be 0.29 mg/kg, and the LODs of other six GEs were 0.1 mg/kg. Based on the LC-MS method, Shiro and his colleagues reported a more sensitive LOD, which is only 1/3 compared to that reported by Haines and his colleagues [115]. Leigh and his colleagues developed an LC-MS/MS method to detect seven kinds of GEs [116] by liquid-liquid extraction. In increasing the formation of sodiated adducts, Hori and his colleagues added the sodium salts in the mobile phase, which might induce a significant negative impact on MS instruments [117]. Prior to LC-MS analysis, several purification techniques are used to remove the large amount of tri- and partial acyl

glycerides, including gel permeation chromatography (GPC) [118] and two-step SPE [119]. Steenbergen and his colleagues have reported a novel direct analysis approach for intact GEs in edible fats and oils: the sample was extracted by acetonitrile and heptane and then purification by normal-phase liquid chromatography, followed by GC-MS analysis [120]. The direct analysis method for GEs based on ^{1}H NMR spectroscopy was reported by Song and his colleagues [121]. In this study, the quantification formula of GEs was deduced from the characteristic diagnostic signals of two epoxy methylene (CH_2) protons, in which chemical shifts were 2.56 and 2.76 ppm, respectively.

To sum up, the advantage of the indirect analysis method of GEs is the simple and unique standard. On the other hand, the direct methods could supply more detail information about the chemical profiles of GEs in samples directly. Therefore, the direct analysis method could be an alternative method for the routine analysis of GEs.

7.4.2 Chemical Mechanism for the Formation of GEs

Till now, no systemic study was reported about the formation mechanism of glycidol in the thermal processing of food. But there are a few studies which reported the theoretical approaches about the chemical mechanisms for the formation of GEs using standard chemical compounds as a model.

DAGs and MAGs are common substrates for the formation of GEs; TAGs and MCPD esters also transferred to GEs under some condition. This chapter will review the formation mechanisms of GEs from MAGs, DAGs, TAGs, and MCPD esters.

There are three proposed chemical mechanisms of the formation of GE from MAGs (Fig. 7.10). One of them considers a direct elimination of water for 1-MAGs [101]. Another one of them considers an elimination of water after a direct intramolecular rearrangement for 1-MAGs [122]. The last mechanism involves the formation of the cyclic acyloxonium ion (CAI) by dehydration of either 1-MAGs or 2-MAGs (MAGs) and then elimination of water for CAI after the intramolecular rearrangement [98, 123, 124].

There are also three proposed chemical mechanisms for the formation of GE from DAGs (Fig. 7.11). All three proposed mechanisms involve an intramolecular rearrangement via charge migration, and differences among the three mechanisms are the nature of the intermediate and the leaving group. One of them considers an elimination of fatty acid for either 1,2-DAGs or 1,3-DAGs (DAGs) after a direct intramolecular rearrangement (Fig. 7.11, pathways a and a′) [125]. The other mechanism involves the formation of the CAI by deacidification of 1,2-DAGs (Fig. 7.11, pathway b) [98, 123, 124]. The last proposed mechanism involves the formation of

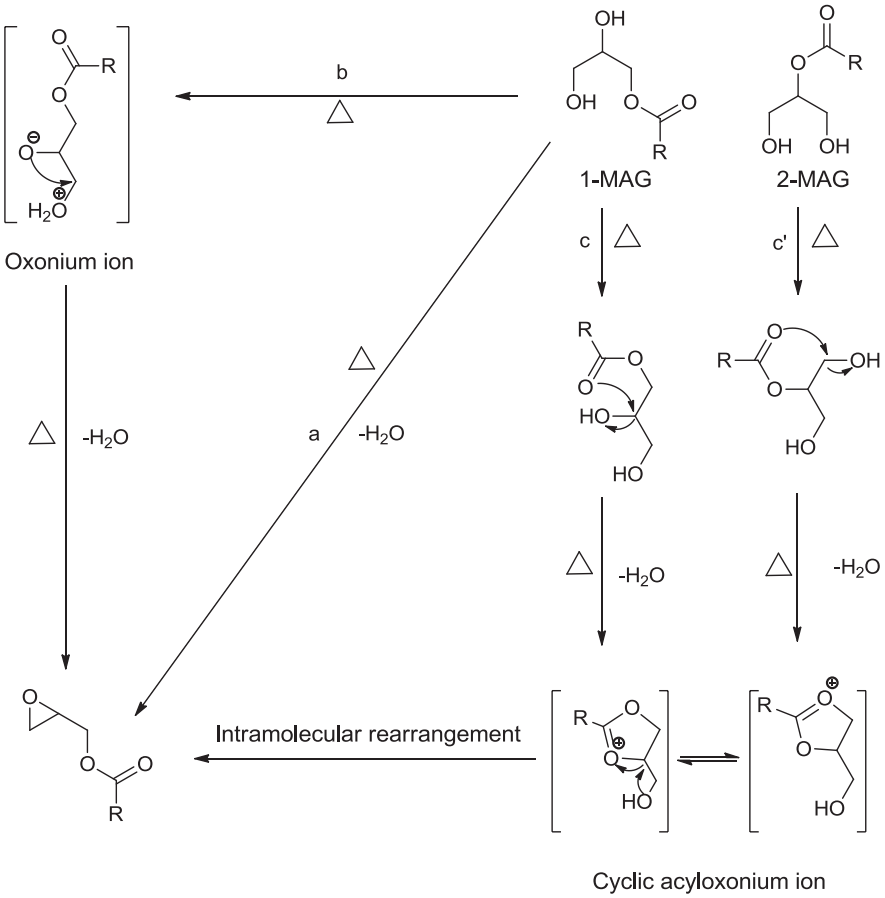


Fig. 7.10 Summary of proposed chemical mechanisms of GE formation from MAGs. Pathway (a), direct elimination of water for 1-MAGs [101]. Pathway (b), direct intramolecular rearrangement form of oxonium ion followed by elimination of water from 1-MAGs. Pathways (c) and (c′) involve the formation of the cyclic acyloxonium ion by dehydration of either 1-MAGs or 2-MAGs (MAGs) and then elimination of water for CAI after the intramolecular rearrangement [98, 123, 124]

an intermediate which is known as cyclic acyloxonium free radical (CAFR) by either deacidification (Fig. 7.11, pathway c) or dehydration (Fig. 7.11, pathway c′) of 1,2-DAGs [124].

There are two proposed chemical mechanisms for the formation of GE from TAGs (Fig. 7.12). One of the mechanisms involves the formation of either DAGs or MAGs derived from TAGs and then transforms GE by known pathway of DAGs or MAGs (Fig. 7.12, pathways a and a') [98, 124]. The other proposed mechanism involves the formation of the CAFR intermediate by deacidification of sn-2 and sn-3 fatty acid (Fig. 7.12, pathway b) [100].

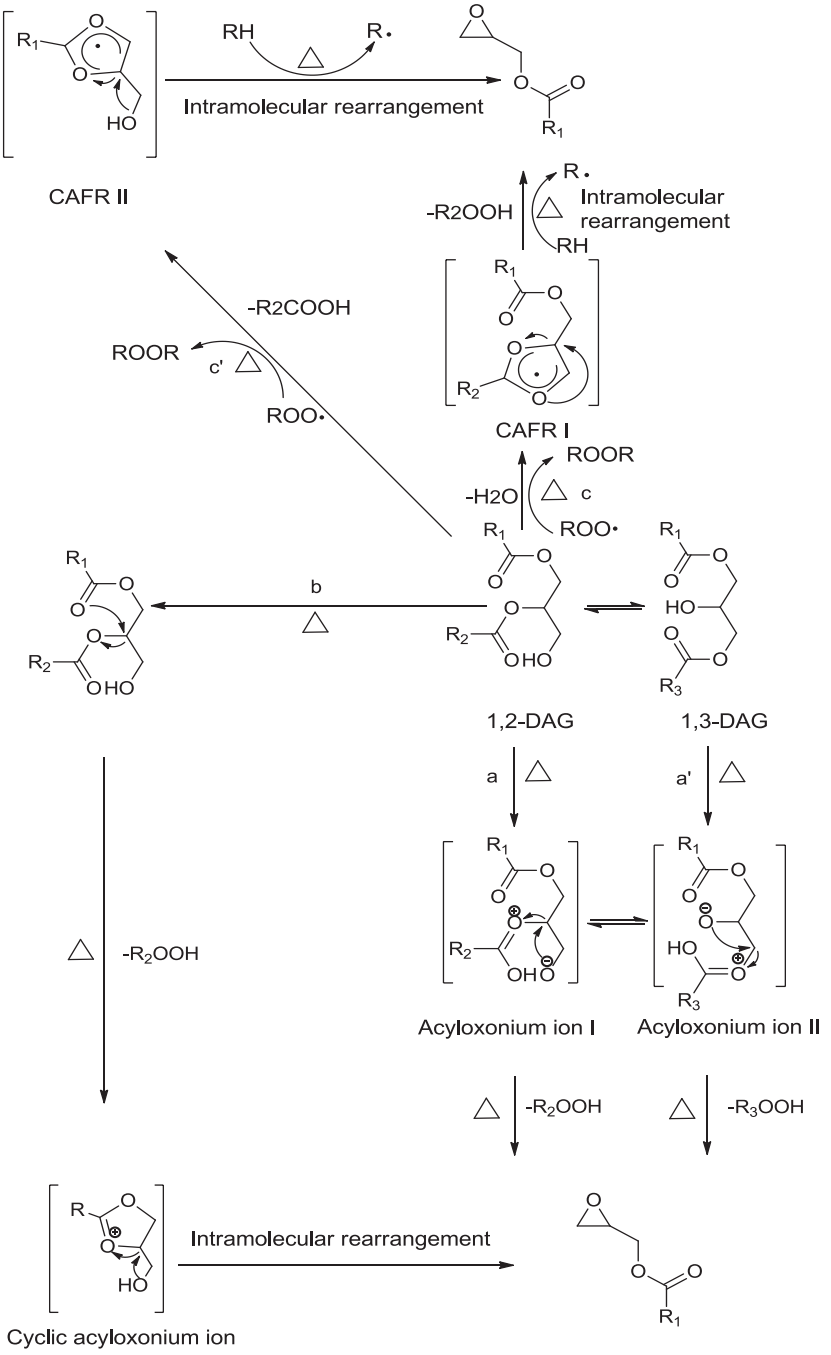


Fig. 7.11 Summary of three proposed mechanisms of GE formation from DAGs. Pathways (a) and (a′), an elimination of fatty acid for either 1,2-DAGs or 1,3-DAGs after a direct intramolecular rearrangement [125]. Pathway (b) involves the formation of the intermediate which is known as cyclic acyloxonium ion (CAI) by deacidification of 1,2-DAGs [98, 123, 124]. Pathways (c) and (c′) involve the formation of an intermediate which is known as cyclic acyloxonium free radical (CAFR) by either deacidification (pathway c) or dehydration (pathway c′) of 1,2-DAGs [124]

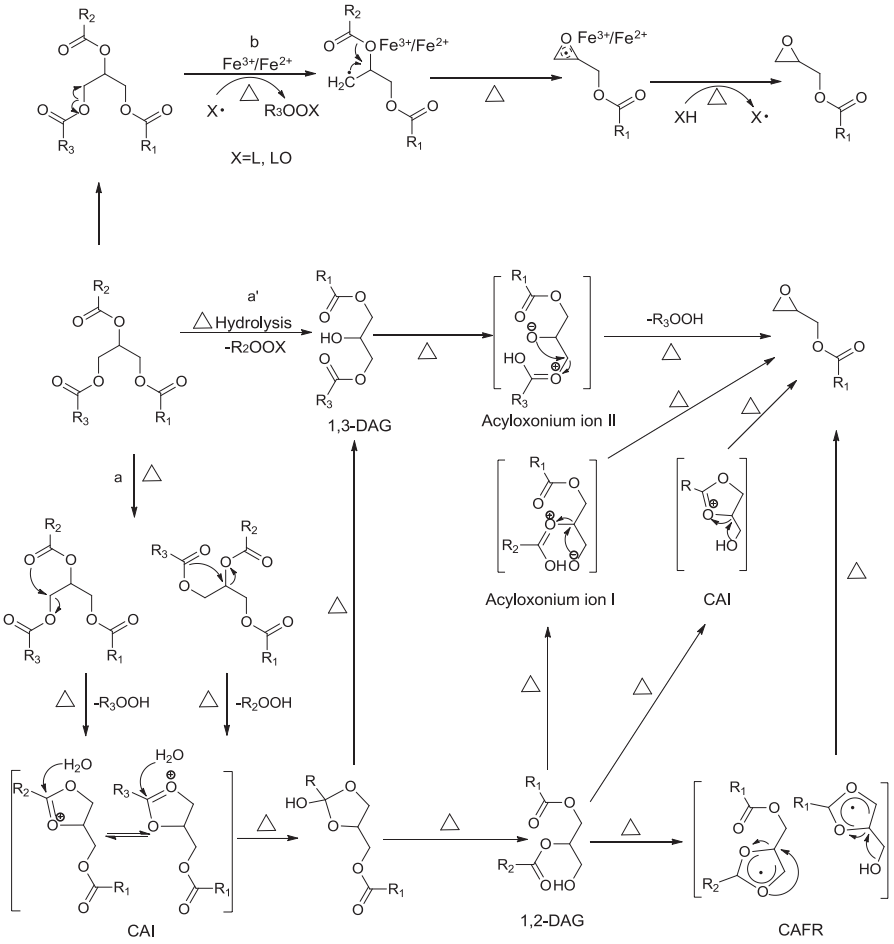


Fig. 7.12 Summary of proposed mechanisms of GE formation from TAGs. Pathways (a) and (a′) involve the formation of either DAGs or MAGs derived from TAGs and then transforms GE by known pathway of DAGs or MAGs [98, 124]. Pathway (b) involves the formation of the CAFR intermediate by deacidification of sn2 and sn-3 fatty acid [100]

There are two proposed chemical mechanisms for the formation of GE from 3-MCPD esters (Fig. 7.13). One of the mechanisms involves the formation of the alcoholate intermediate derived from either monoesters of 3-MCPD (a) or monoesters of 2-MCPD (a′) in alkaline media (Fig. 7.13, pathways a and a′) [106, 124]. The other proposed mechanism involves the formation of the carbocation intermediate derived from 3-MCPD monoesters in neutral and acidic media (b) or 2-MCPD monoesters (b′) (Fig. 7.13, pathways b and b′) [106, 124].

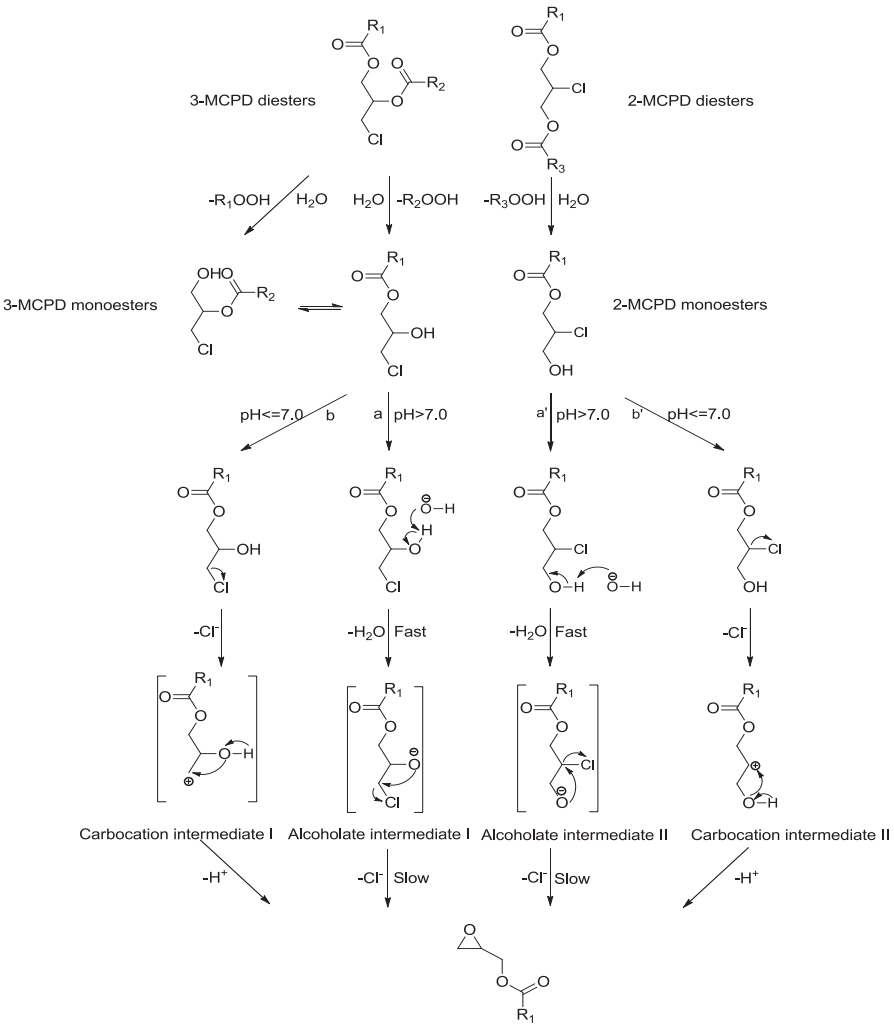


Fig. 7.13 Summary of proposed mechanisms of GE formation from MCPDs. Pathways (a) and (a′) involve the formation of the alcoholate intermediate derived from either monoesters of 3-MCPD (a) or monoesters of 2-MCPD (a′) in alkaline media [106, 124]. Pathways (b) and (b′) involve the formation of the carbocation intermediate derived from 3-MCPD monoesters in neutral and acidic media (b) or 2-MCPD monoesters (b′) [106, 124]

7.4.3 Possible Approaches to Reduce Glycidyl Ester Levels in Foods

7.4.3.1 Inhibition and Removal of Reagents

As shown in formation mechanism parts, the reagents of GEs are mainly DAGs and MAGs, and the TAG and MCPD esters should not be ignored. There are only a few approaches about reducing DAGs and MAGs to inhibit GE formation.

The removal of the main reagents (DAGs and MAGs) is the most efficient method to control the formation of GEs. For edible vegetable oil producers, the climatological locations, soil fertility and planting conditions, the harvest and processing practices of oil fruits/seeds, damaged oil seeds/fruits, and postmature fruits are essential to activate lipase and then change the content of DAGs and MAGs in oil seeds/fruits [104, 126–128]. All these factors could reduce the DAG and MAG content in oil. For the reason of inhibiting the lipase activity, the sterilization temperature during the milling of oil fruits/seeds should be kept at or below 120 °C [129, 130]. Aniołowska and Kita also found that the composition of oil fruits/seeds had a greater influence on polymerization transformation and GE formation than on hydrolytic and oxidative reaction in the frying oil [131]. Another strategy is removing some of the DAGs and MAGs in the refining steps (degumming, neutralization, bleaching, and deodorization) [105]. Some adsorption materials were used for the removal of DAGs, MAGs, and the other polar components from frying oil, suggesting that the same process could be established for the removal of DAGs and MAGs from raw materials [132, 133]. Strijowski and his colleagues reported that approximately 25% of DAGs, MAGs, and the other polar components could be removed by amorphous magnesium silicate and calcined zeolite [134]. Craft and his colleagues reported that refined, bleached palm oil by either ethanol or glycerol during oil deodorization could remove the DAGs and FFAs, which resulted in a significant reduction of GEs and 3-MCPD esters [129].

7.4.3.2 Modification of Reaction Conditions

Temperature and reaction time represent the key factor of the reaction condition for the formation of GEs [123–125, 130].

Properly adjusting the temperature and reaction time of deodorization is critical in reducing the formation of GEs. Pudel and his colleagues reported that the GE content reduced to less than 5 ppm at a deodorization temperature lower than 240 °C [105]. However, when deodorization temperature increases to over 250 °C, the concentrations of GEs significantly increased in a time-dependent manner. Craft and

his colleagues confirmed that the formation of GEs significantly increased at a deodorization temperatures above 230 to 240 °C [135]. So, one possible method to inhibit the formation of GEs is to keep the deodorization temperature lower than 240 °C. Pudel and his colleagues carried out an experiment with a short-path distillation with a 60 °C condenser temperature, a 170 °C evaporator temperature, a 100 rpm stirrer speed, and a 20 Hz pump frequency [136]. The results of this study represented that with the mild deodorization condition, the content of GEs in refined edible oil decreased and the sensory quality (taste and odor) improved. Besides the temperature, reducing the reaction time is another important strategy. Pudel and his colleagues carried out an experiment with a two-stage deodorization; that is, the first step at a temperature of 250–270 °C in a short time and the second step at a temperature of 200 °C in a longer time [136]. Results represented that GE concentrations significantly reduced in this condition.

In addition, the oil fruit/seed pressing process also involves a roasting at a temperature of over 200 °C, which might induce GE formation and increase DAG and MAG contents [124]. Wong and his colleagues found that low levels of DAGs and MAGs and high temperature during the deep-frying reduced the formation of GEs in frying chicken breast meat [137]. Šmidrkal and his colleagues reported that the level of GEs formed is dependent on the pH value in oils [138]. An increase in pH value by addition of Na_2CO_3, $NaHCO_3$, or other alkaline substances could reduce the level of GEs formed in refined oils [107, 138–141]. However, the chemical mechanisms of pH in the reduction of GEs formed are still unclear and further experimental data are required.

Cheng and his colleagues found that the free radicals produced in the process of oil oxidation increase the GE formation [124]. And the 3-MCPD esters formed were reduced by using antioxidants in scavenging the free radicals [94, 142], based on the free radical formation mechanisms [99, 100]. Further studies are necessary to provide more evidence to support this hypothesis.

7.4.3.3 Elimination of Formed GEs

The elimination of formed GE methods includes two methods: physical adsorption and chemical degradation. The physical elimination methods are scavenging of the formed GEs by using activated carbon, magnesium silicate, zeolite, activated bleaching earth, or other adsorption materials [124]. Strijowski and his colleagues have reported the possibilities of a removal of GEs from palm oil by using different adsorption materials [134]. The results indicated that two of the adsorption materials (calcined zeolite and synthetic magnesium silicate) could reduce the formation of 3-MCPD esters and GEs up to 40%, and this physical elimination method did not show the adverse effect in palm oil, including oxidative stability and sensory properties.

Chemical degradation methods are adopted to transform GEs into other compounds, such as changing distillation process parameters or adding nontoxic reagents to refined oils to degrade GEs transforming GEs to glycerol, DAGs, MAGs,

or other nontoxic intermediates. Craft and his colleagues reported that adjusting the parameters of steam distillation steps could remove the high amounts of GEs (>100 mg/kg) in refined oils [143]. Özdikicierler and his colleagues investigated the effects of stripping steam rate, temperature, and pressure in the removal of the formation of GE in edible oil during the steam distillation process [144] and found that the level of GEs in olive oil and olive pomace oil decreases in the steam distillation temperature of 230 and 230 °C, the water flow rate of 1.2 mL/min and 1.0 mL/min, and the pressure of 4 mbar and 2 mbar, respectively. Besides, the new generated GEs are instable in acidic environment. Matthäus and his colleagues investigated the effects of acidic strip steam on GE formation by implementing formic acid in the oil deodorization step [104]. The results indicated that the content of GEs in refined edible oils decreases about 35% by the addition of higher concentration of formic acid in strip steam. Shimizu and his colleagues carried out an experiment to eliminate GEs by the addition of activated bleaching earth in DAG-rich oils in a model system [145]. And the results represented that the GE elimination effect of activated bleaching earth was not because of its physical adsorption effect but through the chemical conversion of GEs involving a ring-opening reaction.

In the past decades, a great deal of research had focused on the contamination of glycidol and GEs in food and food ingredients. The chemical mechanism research had shown the formation of GEs from various reagents during the food process. The analytical technique studies developed the effective and accurate quantification method for glycidol and GEs in food and food ingredients, allowing reliable assessments on glycidol and GE occurrence in the food processing.

7.5 Acrolein and Other Alkenals

Acrolein and other alkenals are a kind of electrophilic α,β-unsaturated aldehydes. The basic chemical structure of these compounds is an acrolein CH_2 = CHCHO (Fig. 7.14). Acrolein is water soluble and could also be dissolved well in ethanol, acetone, and other organic solvents. Acrolein is colorless or pale yellow liquid and has a pungent odor like oil burning [146]. Acrolein and other alkenals are the important industrial raw materials, the major components of tobacco smoke, and could be released from the burned manufactured goods from the petroleum industry. Also, these compounds could be formed from overheated food lipids. Overcooked, fried, or charred foods are the major sources of acrolein and other alkenals. The TDI value (tolerable daily intake value) for acrolein is 7.5 μg/kg body weights [147].

Acrolein

Fig. 7.14 Chemical structure of acrolein and its fatty acid esters

Inhalation of acrolein can cause watery eyes, sore eyes, headaches, dizziness, coughing, and breathing problems. Moreover, acrolein could induce several diseases, including multiple sclerosis, spinal cord injury, Alzheimer's disease, diabetes mellitus, cardiovascular disease, and hepato-, nephro-, and neurotoxicity. On the cellular level, acrolein exposure could lead to DNA and protein adduction, oxidative and endoplasmic reticulum stress, mitochondria and membrane damage, and immune dysfunction [148]. Due to their wide existence of potential toxic effects, a systemic review about the analytical methods, formation mechanisms, and mitigation strategies of acrolein and other alkenals will be discussed in this section.

7.5.1 Analytical Methods

The analysis of acrolein in foods is extremely complex, so it requires great efforts. Early methods were based on thin and paper layer chromatography back to the 1960s or using spectroscopic techniques, such as UV-Vis and fluorescent spectroscopy, to detect the derivations of acrolein with hydroxylamine, N-methylhydrazine, morpholine, or sodium bisulfate. In the 1980s, HPLC-UV, GC-MS, and LC-MS have also been used for the determination of acrolein formed during heat processing of foods [147].

7.5.1.1 Chromatographic Methods in Detecting Acrolein

In 1987, acrolein from heated beef fat and cooking oils was analyzed by a gas chromatograph with a fused silica capillary column and a thermionic detector [149]. In 2009, Seaman and colleagues establish a method, which used gas chromatography-negative chemical ionization mass spectrometry and a labeled standard acrolein-d4 to quantify acrolein [150]. In 2011, two stable isotope dilution assays were developed for quantifying acrolein in fats and oils, which were a direct GC-MS headspace method and an indirect GC-MS method using derivation with pentafluorophenylhydrazine [151]. Also, in 2011, Osório and colleagues developed a GC-MS method to determine acrolein in French fries using SPME as the sampling technique after derivatization with 2,4-dinitrophenylhydrazine [152]. In 2012, an automatic method based on derivatization with 2,2,2-trifluoroethylhydrazine (2,2,2-TFEH) and consecutive headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS) to detect acrolein in surface and drinking water are described [153]. In 2012, a fast qualitative and quantitative determination of acrolein in water was established by the portable headspace GC-MS analysis [154]. In 2013, Osório and colleagues used a method of solid-phase microextraction (SPME) coupled with gas chromatography and mass spectrometry to evaluate acrolein productions during frying the cassava and pork sausage in different vegetable oils [155]. In 2017, since using one-dimensional GC to analyze acrolein was difficult, a validated method using headspace solid-phase

microextraction (HS-SPME) combined with the comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC × GC/TOFMS) showed appropriate separation and identification abilities of the acrolein in wines [156]. In 2004, the liquid chromatographic separation equipped with pulsed electrochemical detection is described as a selective and sensitive analytical method for the determination of acrolein in heated vegetable oils [157]. In 2008, Uebori and colleagues determined acrolein in ambient air as its CNET derivative by LC/MS/MS [158].

7.5.1.2 Other Methods in Detecting Acrolein

In 1992, a membrane introduction mass spectrometry was used to directly quantify the acrolein in aqueous solution at low levels [159]. In 2016, Zheng and colleagues used nuclear magnetic resonance (NMR) spectroscopy to analyze acrolein degradation and established a quantitative headspace solid-phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS) determination of acrolein in alcoholic beverages [160]. In 2017, a needle trap device (NTD) equipped with nanoporous silica aerogel (NPSA) as a sorbent was used as a new technique for analysis of acrolein. A 21-G needle was applied for extraction of gas in the sample headspace [161]. Also, in 2017, another NTD equipped with NPSA was developed as a technique for the rapid determination of acrolein and formaldehyde in air. Thus, this technique proved to be a reliable and effective method [162].

7.5.2 Formation Mechanisms

In 1916, the formation of acrolein was related with the oxidation of unsaturated fatty acids, particularly the linseed oil and linolenic acid [163]. In 1976, methional, the three-substituted propanal derived from methionine, was confirmed to easily decompose with the formation of acrolein with/without oxygen. Homoserine, homocysteine, and cystathionine could generate significant amounts of acrolein in aerobic interactions at neutral pH and 100 °C [164].

In 1992, acrolein was detected from cod liver oil upon ultraviolet irradiation (λ_{max} = 300 nm). It was derivative into nitrogen or sulfur-containing compounds and then analyzed by capillary gas chromatography equipped with a nitrogen-phosphorus detector or a flame photometric detector [165]. In 2010, a study reported that glycerol could be dehydrated to form acrolein, which has been mentioned in Alhanash's research [166] (Figs. 7.15, 7.16, 7.17 and 7.18).

In 2013, several vegetable oils, including perilla, high-oleic sunflower, rice bran, soybean, and rapeseed oils, were heated at 180 °C for 480 min and then the concentration of acrolein was determined by gas-liquid chromatography.

In 2014, enzymatically synthesized linolenic and linoleic acid hydroperoxides were the key intermediates in acrolein formation.

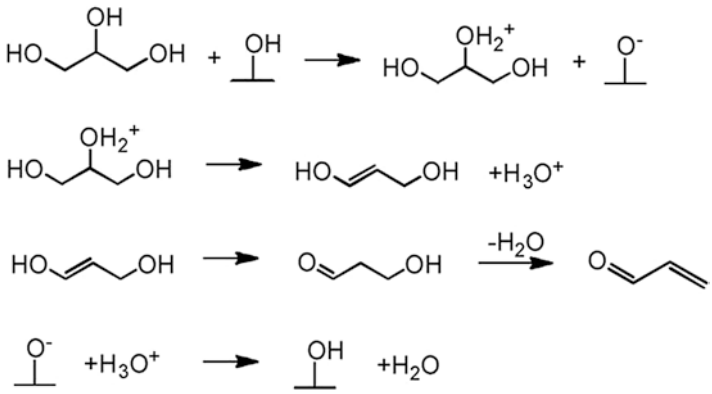


Fig. 7.15 Formation mechanism of acrolein esters from glycerol [166]

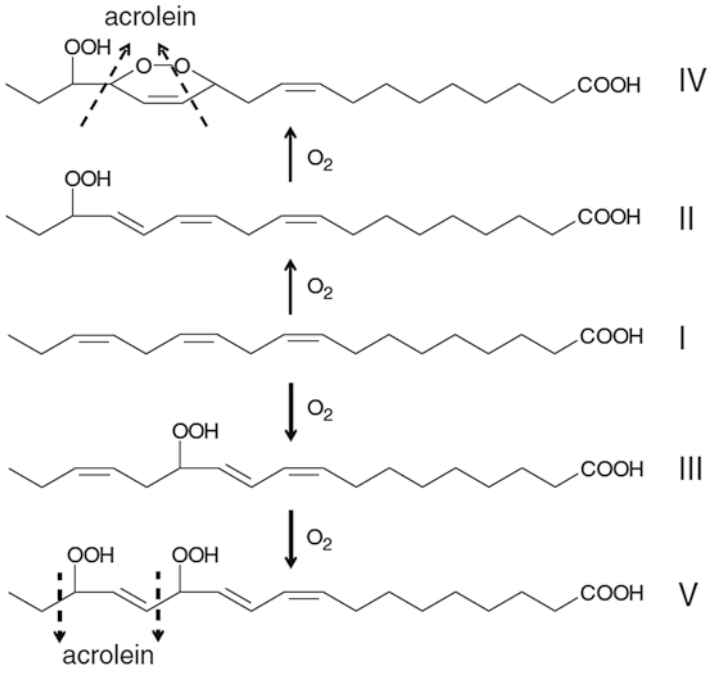


Fig. 7.16 Formation mechanism of acrolein esters from oil [167]

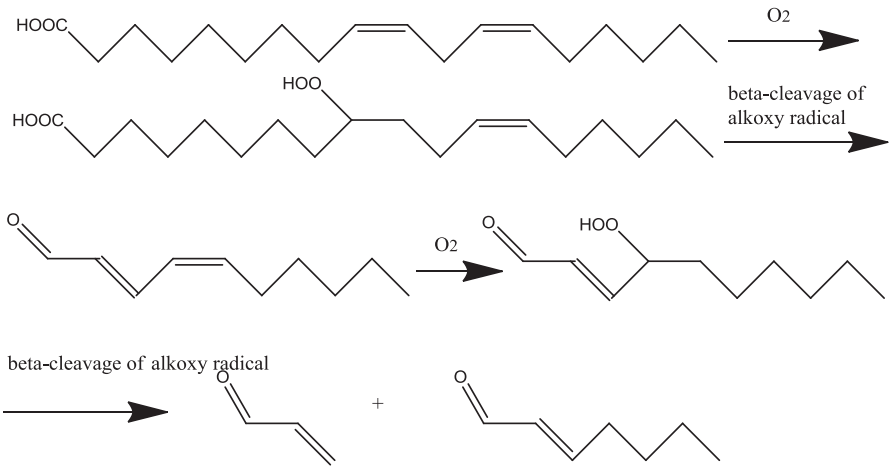


Fig. 7.17 Formation mechanism of acrolein esters from fatty acid [168]

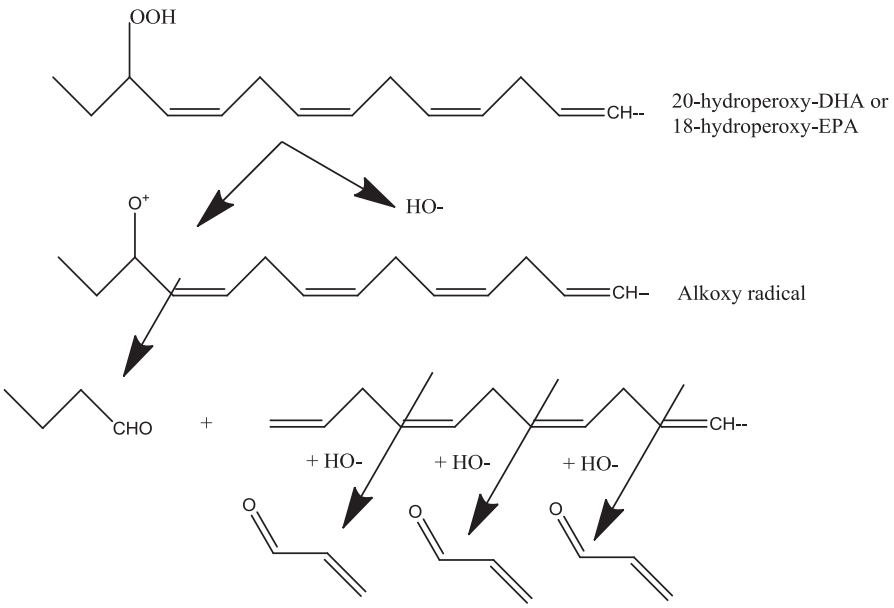


Fig. 7.18 Formation mechanism of acrolein esters from triacylglycerols [169]

In 2015, the high quantities of acrolein, in linseed, echium, fish, and soybean oil triacylglycerols oxidized at 50 or 60 °C, were detected by the static headspace gas chromatography method.

7.5.3 Mitigation Strategies

Acrolein, as a harmful and toxic substance in food, is a potential threat to human health. So, the removal of acrolein is extremely important. The mitigation strategies of acrolein could be separated into two major approaches, the direct removal and indirect removal.

7.5.3.1 Direct Removal Approaches

Direct removal approaches are the methods that reduce the acrolein with the acrolein scavenger directly. In 2004, Kaminskas and colleagues found the chemical mechanisms of acrolein trapping by hydralazine, and the study represented that together with its structural analogue dihydralazine, it also readily traps crotonaldehyde [170]. In 2006, Li and colleagues found that the nanoparticle dye is effective as an oxidant for the conversion of carbon monoxide to carbon dioxide and/or as a catalyst for the conversion of carbon monoxide to carbon dioxide and/or catalyst for conversion of aldehydes such as acetaldehyde and acrolein [171]. In 2011, Shi and colleagues also found that hydralazine serves as an excellent acrolein scavenger, since hydralazine can reduce acrolein concentrations and inhibit acrolein pathologies in vivo [115]. In 2012, PS-NH_2 was used to make active pharmaceutical ingredients (API) in the acrolein to get the most complete clearance. PS-NH_2 can remove 97.8% of acrolein without any substantial removal of the API during 20 min of reaction time. And in their API process solution system, scavenging of acrolein was seen to be quite fast and effective using both polymer- and silica-based scavengers. In addition, there are some nitrogen compounds that are capable of binding and inactivating acrolein [172]. In 2017, dimercaprol, which possesses thiol functional groups, was found to bind and trap acrolein and dimercaprol is not known to elicit a reduction in blood pressure. Therefore, in the event that hydralazine is not applicable in a subset of patients as a result of the risk of hypotension, dimercaprol could be a viable alternative treatment [173].

7.5.3.2 Indirect Removal Approaches

Indirect removal approaches refer to the inhibition of acrolein by not directly acting with acrolein, but by affecting its production or inhibiting the harmful physiological changes it produces. In 2011, Abraham and colleagues emphasized the strict control of the content of acrolein in food. For the exposure data, some improvements would be

necessary: development of valid analytical methods for the concentration of acrolein in foods, as well as the examination of foods in ready-to-eat form with high acrolein contents. The effective detection of acrolein in food could improve the control of acrolein by adjusting the processing materials [147]. In 2017, Gu and colleagues found that a squid solution polysaccharide (SIP) can effectively inhibit the Leydig cells in acrolein-mediated autophagy and apoptosis and thus play a role in scavenging [174].

This chapter discussed the analytical methods, formation mechanisms, and mitigation strategies of five groups of chemical hazards, including 5-HMF, trans-fatty acids, MCPDs and their esters, glycidol and its esters, acrolein, and other alkenals. The knowledge about these chemical hazards reported in food science in the past decades may improve our knowledge about these thermal processing-induced hazards and finally improve the food safety and quality in the thermal processing of foods.

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Chapter 8
Conclusions and Future Directions

Shujun Wang and Yuan Zhuang

8.1 Conclusions

Thermal processing is an important operation in food industry to ensure microbiological safety, eliminate anti-nutritional factors, and enable the development of desirable profiles of color and flavor attributes of food. However, thermal processing also gives rise to potentially hazardous by-products which may be a significant health risk for humans in the long run. With the development of analytical technology, significant improvements have been achieved in the area of hazardous substances produced during thermal processing of foods, including the detection methods, the formation mechanisms, and the mitigation strategies. More and more rapid and sensitive analysis methods have been developed for the detection of hazardous chemicals produced during thermal processing. Meanwhile, the developments of pretreatment technologies give us more choices in detection of hazardous substances for different food matrices. Hitherto, LC and GC coupled to MS appear to be acknowledged as the most extensively published and authoritative methods for the determination of trace α-dicarbonyl compounds, acrylamide, AGEs, and other hazardous substances. Many research efforts have been devoted to the establishment of strategies which could significantly reduce the content of chemical hazards without undesirable impact on the quality of final food products. Because of the obvious matrix affects in the food system and various formation mechanisms, several factors are needed to consider for reducing the content of chemical hazards in foods. The widely used methods of inhibiting hazardous compounds are mainly in the following three ways. The first one is to reduce or eliminate the formation precursors to decrease the content of chemical hazards formed in the end products. The second is to optimize the processing technology, such as adjusting the processing

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mode, processing temperature, processing time, pH values, moisture content, and other factors. The third is to add exogenous compounds to inhibit the formation of chemical hazards. The knowledge about these chemical hazards reported in food science in the past decades may increase our knowledge about these thermal processing-induced hazards and finally improve safety and quality of thermally-processed foods.

8.2 Future Directions

While the great advancement is achieved in this area, there are still many concerns to be addressed in the future. The development of rapid detection methods has satisfied the need of food industries, regulatory agents, and customers. However, they still need further improvements to make themselves more accurate, sensitive, repeatable, reproducible, and portable to achieve online and real-time detection of trace chemical hazards. Besides, simplified pretreatment is essential in the detection of hazardous compounds using a rapid method. Further researches in this area should focus on the following aspects: development of standardized quantitative methods to facilitate the proper and accurate evaluation of levels in foods, building up a corresponding database worldwide, and the development of new technologies to successfully reduce chemical hazards in food at a commercial scale.

Different food matrices imply different reaction mediums with numerous variables, such as composition (presence of precursors, i.e., sugars, amino acids, ascorbic acid, PUFA), pH, oxygen concentration, and water content, and might need specific approach. On the other hand, different food products require and undergo relevant thermal process, which ultimately determines the final hazardous compound content of food. Despite the complexity of reaction conditions, the mechanisms underlying these reactions are still far from being revealed. Some substances have not yet been discovered during the Maillard reaction, and new products and intermediates are constantly being reported. Future studies on the discovery of new chemical hazards and development of the corresponding detection methods are therefore needed.

Meanwhile, the development of mitigation strategies for the chemical hazards is also much required. Eliminating precursors of hazardous compounds or modifying formulation and thermal processes may not always be easy approaches since desired sensory property and microbiological safety of the food should be attained at the same time. For example, decreasing thermal process temperature of canned foods, particularly baby foods, would not ensure the inactivation of pathogens. As complex systems, foods may have more than one potential precursor leading to formation through different mechanisms during processing. Generally, addition of a functional food ingredient is preferred rather than changing the process conditions, in order to retain 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 the food and the kinetics of the reactions taking place in the presence of the inhibitor agent. Unfortunately, most of the studies undertaken up to now do not give concentration-dependent inhibition information. Further studies are needed in this regard.