

**FDE 303**  
**FOOD CHEMISTRY**  
**WEEK-7**

**Nonenzymatic Browning**

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# Nonenzymic Browning

## *Maillard reaction/ Maillard browning*

- Common browning of foods on heating or on storage
  - A chemical reaction between reducing sugars, mainly D-glucose, and a primary amino group (a free amino acid or amino group on a side chain of a protein molecule)
  - The overall process is sometimes designated Maillard browning
  - It is also called nonenzymic or nonenzymatic browning
- 
- ✓ When aldoses or ketoses are heated with amines, a variety of reactions ensue, producing numerous compounds
    - ✓ Flavors, aromas, and dark-colored polymeric materials
      - may be either desirable or undesirable
      - may be produced slowly during storage and much more rapidly at the high temperatures encountered during frying, roasting, or baking

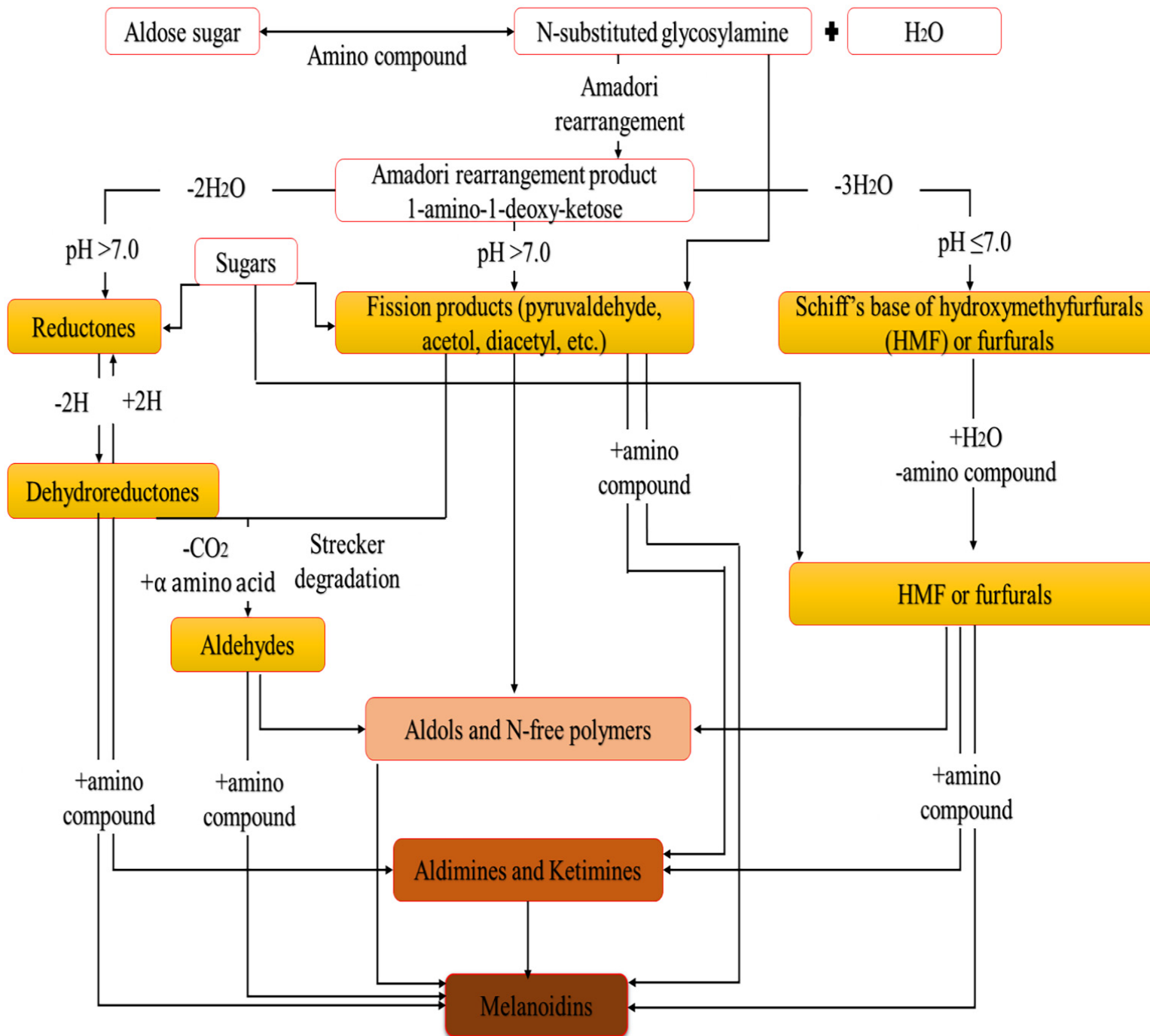
# Maillard browning reaction

- ✓ The Maillard reactions are sometimes called nonenzymic browning because the end products are brown colored melanoidins.
- ✓ Nonenzymic browning is also caused by
  - caramelization of sugars
  - ascorbic acid oxidation
  - interactions of quinones with amines and amino acids
  - interactions of oxidized lipids with protein
- ✓ The most important saccharides participating in the Maillard reactions;
  - glucose and fructose
  - sucrose is easily cleaved into glucose and fructose, especially on heating. Therefore, it can participate in nonenzymic browning quite easily.
  - in meat it may be ribose
  - lactose is an important browning precursor in dairy products
  - maltose in cereal products, such as malt

# Maillard browning reaction

- ✓ Sugars bound as glycosides, for example, in glycoproteins, glycolipids, and in heteroglycosides are less reactive
  - but the aglycones may be released during heating to yield free reducing saccharides.
- ✓ The other reaction partners are proteins, peptides, amino acids, and other amine compounds.
- ✓ The reactive group of proteins is mainly the  $\epsilon$ -amino group of the lysine residue.
- ✓ The end amino groups of peptidic chains may also participate in the reaction, but their concentration in proteins is at least several times lower than that of lysine.

- ✓ The guanidyl group of arginine or the thiol group of cysteine can also participate in Maillard browning.
- ✓ In some food products, especially in cheeses and fish, biogenic amines are also precursors of brown products.
- ✓ Ammonium hydroxide and ammonium salts are reactive as well as amines.
- ✓ Aldehydes and other carbonyls also participate in the browning reaction.
  - They may originate from sugar derivatives, such as ascorbic acid, but they may also be produced from other precursors, predominantly oxidized lipids.

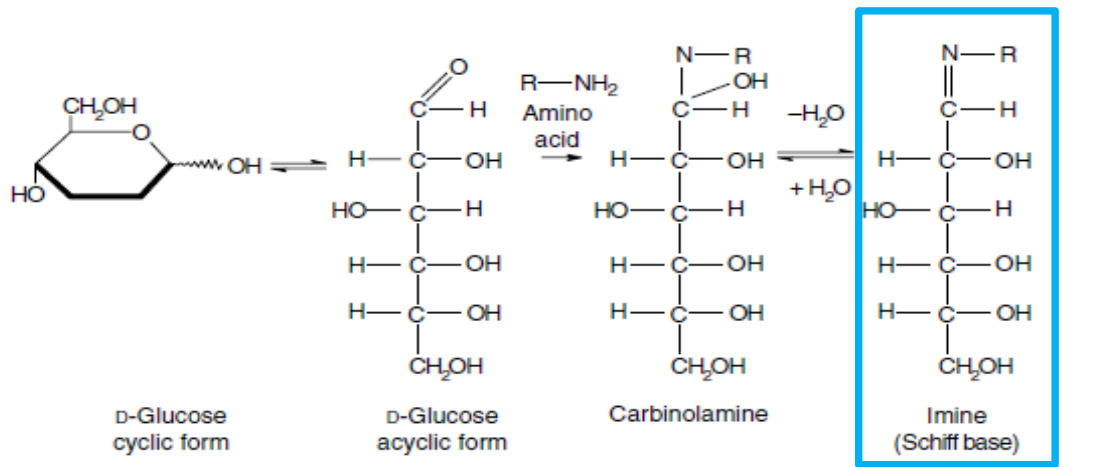


## Stages in the Maillard browning reaction

- ✓ The Maillard reaction comprises three stages including early, intermediate, and final stages

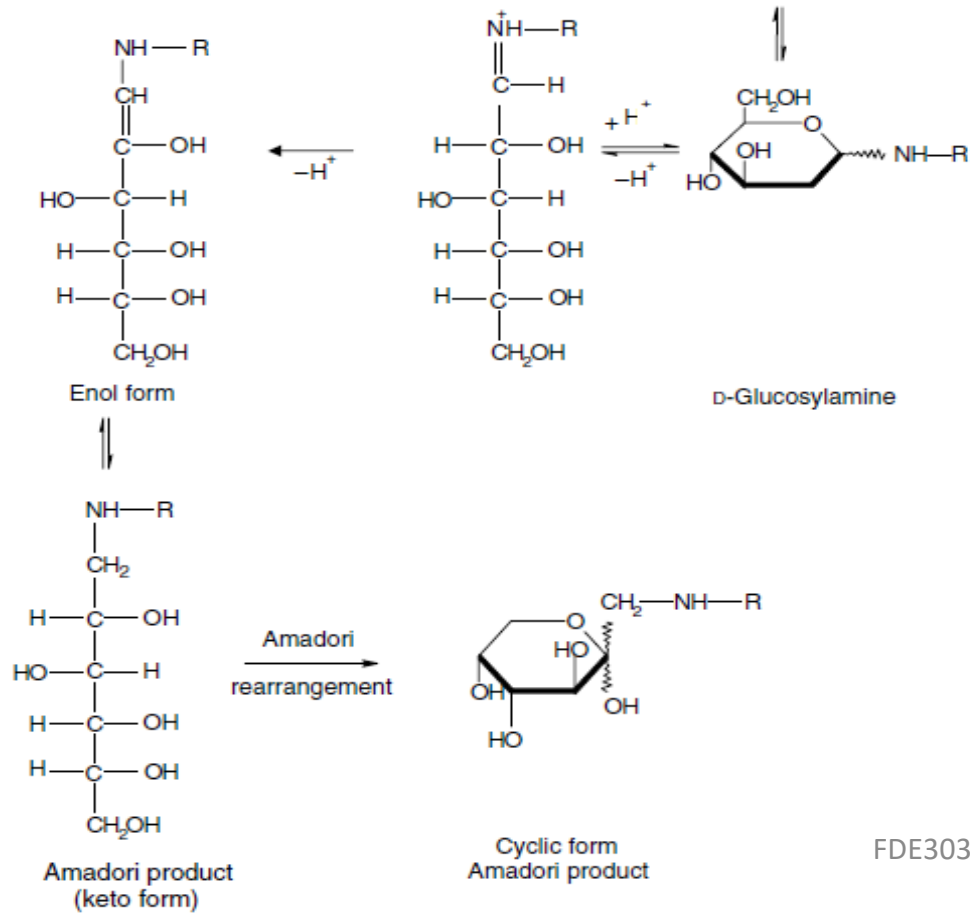
# Stages in the Maillard browning reaction

- ✓ Three stages in the Maillard browning reaction are;
- 1. STAGE 1:** Reaction of an amine with a reducing sugar with the formation of glycosyl amine, followed by the Amadori rearrangement
- 2. STAGE 2:** Dehydration of the intermediary products, fragmentation of the saccharidic moiety, and the Strecker degradation of the products
- 3. STAGE 3:** Reactions of intermediary products resulting in the formation of heterocyclic flavor compounds, that is, high molecular weight brown pigments, which are responsible for the typical flavor of brown products

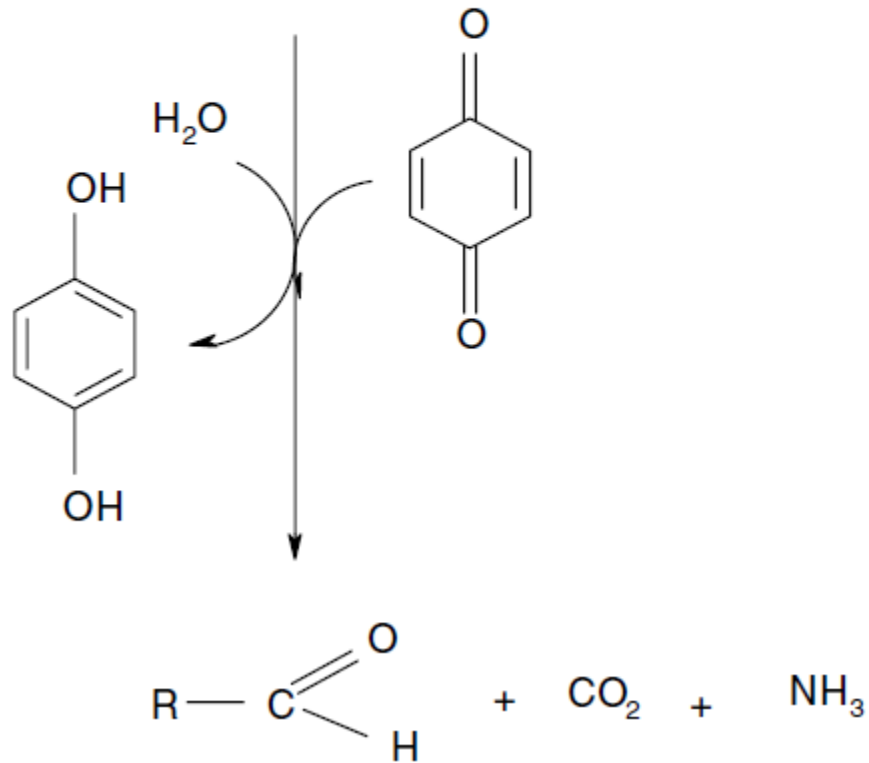
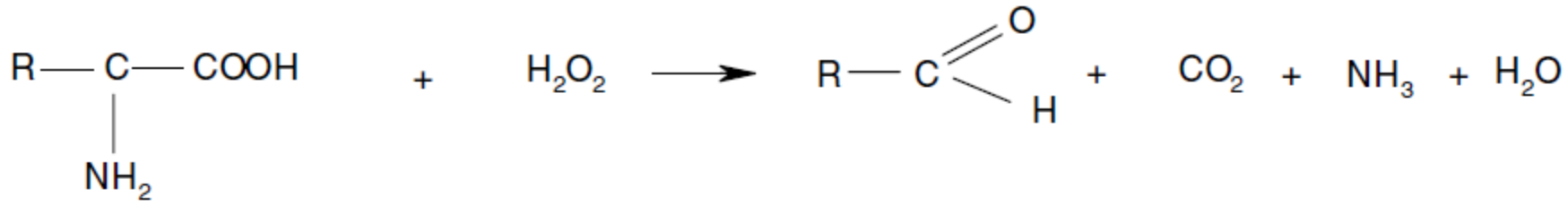


# The Maillard reaction- Stage 1

- ✓ The Maillard reaction starts with the addition of a nonprotonated amine group to the electrophilic carbonyl carbon of a reducing sugar.
- ✓ The addition product, a monotropic carbonylamine or similar compound, is dehydrated with the formation of an imine or a Schiff base (or an azomethine).
- ✓ The addition rate increases with increasing electronic density of the nucleophilic amine.
- ✓ The inductive effect of the carbonyl group substituents and steric factors are also important.



# The Maillard reaction- Stage 2

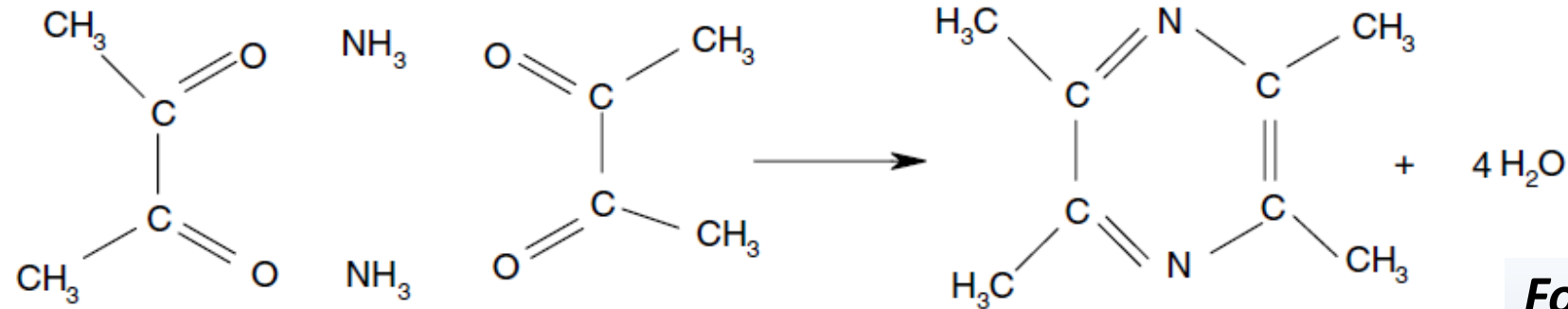


## Strecker degradation of amino acids with oxidizing agents

- ✓ Some of the carbonyl derivatives from the nonenzymatic browning sequence react readily with free amino acids.
- ✓ This results in degradation of the amino acids to aldehydes, ammonia, and carbon dioxide and the reaction is known as *Strecker degradation*.
- ✓ The aldehydes contribute to aroma development during the browning reaction.
- ✓ Strecker degradation of each amino acid produces a specific aldehyde with a distinctive aroma.

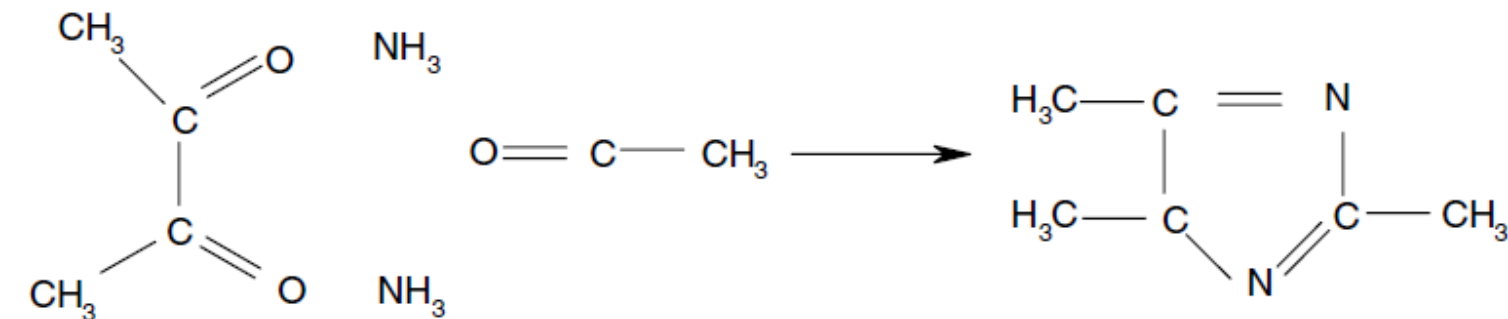


# The Maillard reaction- Stage 3



Biacetyl

Tetramethylpyrazine



Trimethylhydroxylamine

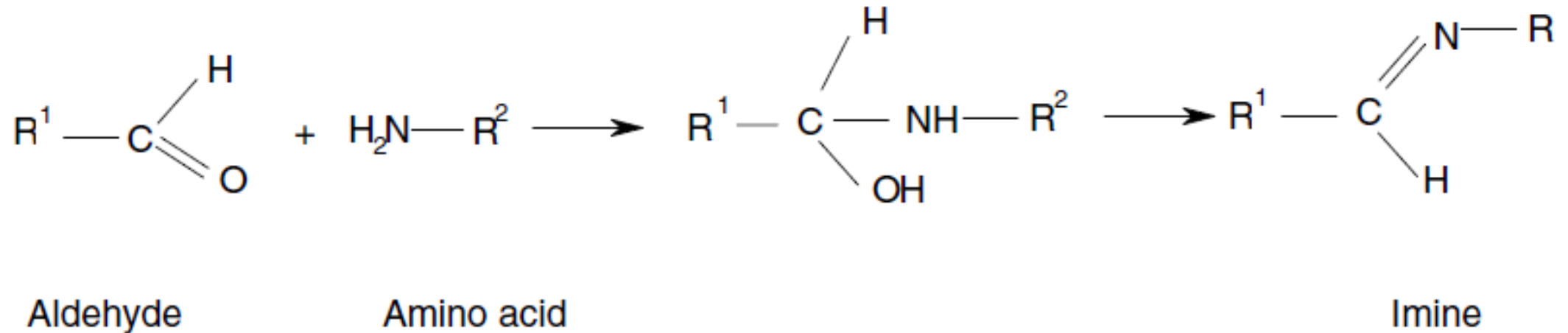
## ***Formation of heterocyclic flavor compounds***

- ✓ Several compounds, such as furans, pyrroles, pyridines, pyrazines, and carbonyl components, were detected in melanoidins isolated from model and some real food.
- ✓ Melanoidins are found in large amounts in cooked, fried, and roasted foods, which are consumed daily.

# The reactivity of carbonyl and amino groups

- ✓ The reactivity of a carbonyl group decreases in the following series:
  - ✓ Aldoses are more reactive with amino acids than ketoses.
  - ✓ Trioses react with amino acids more rapidly than tetroses, tetroses more rapidly than pentoses, which are more reactive than hexoses and disaccharides.
  - ✓  $\alpha$ -Dicarbonyl derivatives react with amino acids more easily than aldehydes, which are more reactive than ketones; reducing sugars are still less reactive, followed by oxo-acids.
  - ✓ Acyclic form of sugars reacts more easily with amino acids than cyclic form of sugars.
- ✓ The reactivity of amino compounds depends on their basicity: ammonium hydroxide and ammonium ions react with reducing sugars more easily than amines, whose reactivity decreases with their decreasing basicity.

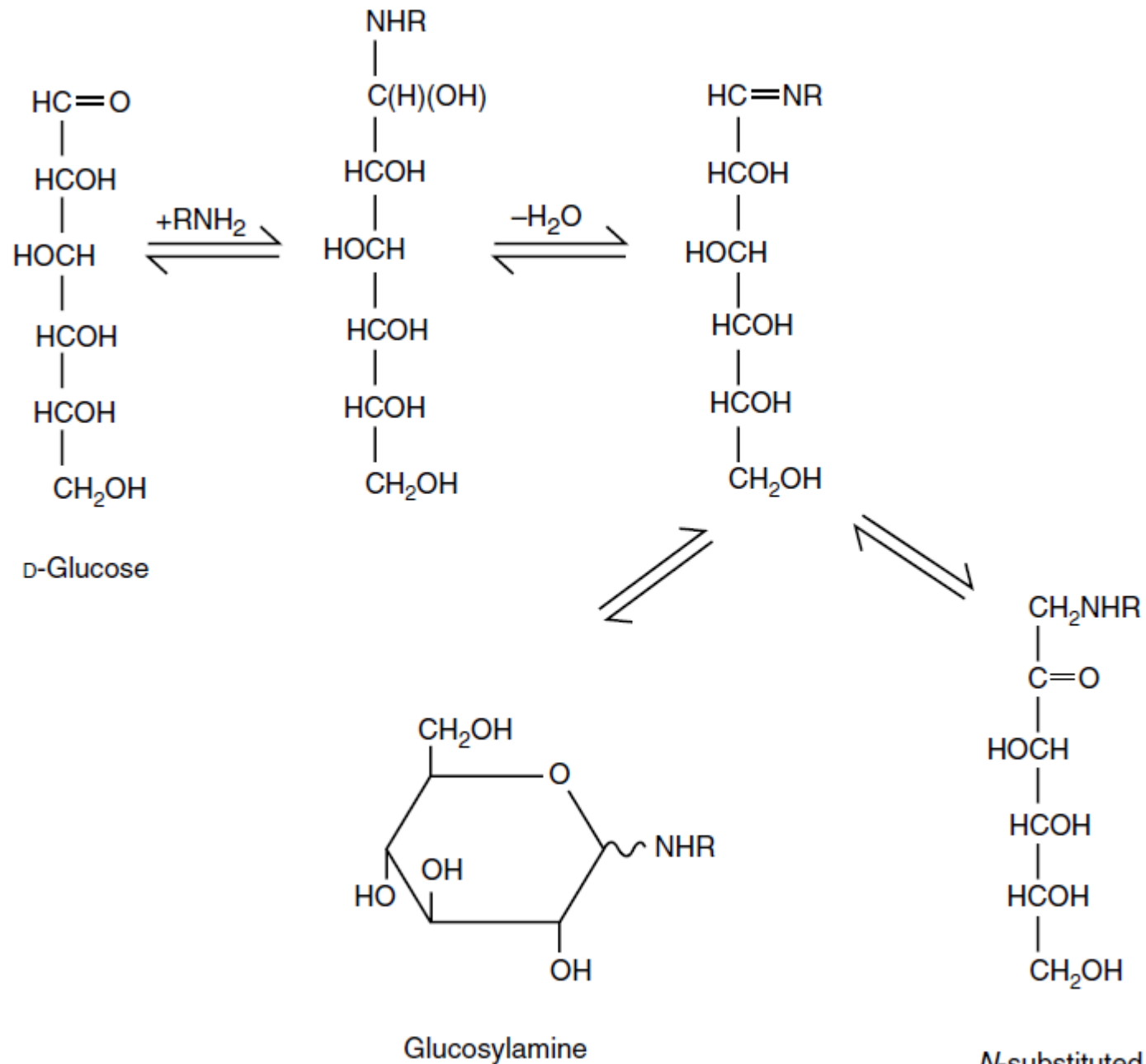
# Reaction of carbonylic compounds with amine groups



- ✓ With decreasing pH, the concentration of protonated carbonyl group increases, but the concentration of nonprotonated amine group decreases.
- ✓ As a result, the reaction rate reaches a maximum in a slightly acidic medium in case of reaction with amines, while this occurs in a slightly basic medium in the case of reaction with amino acids

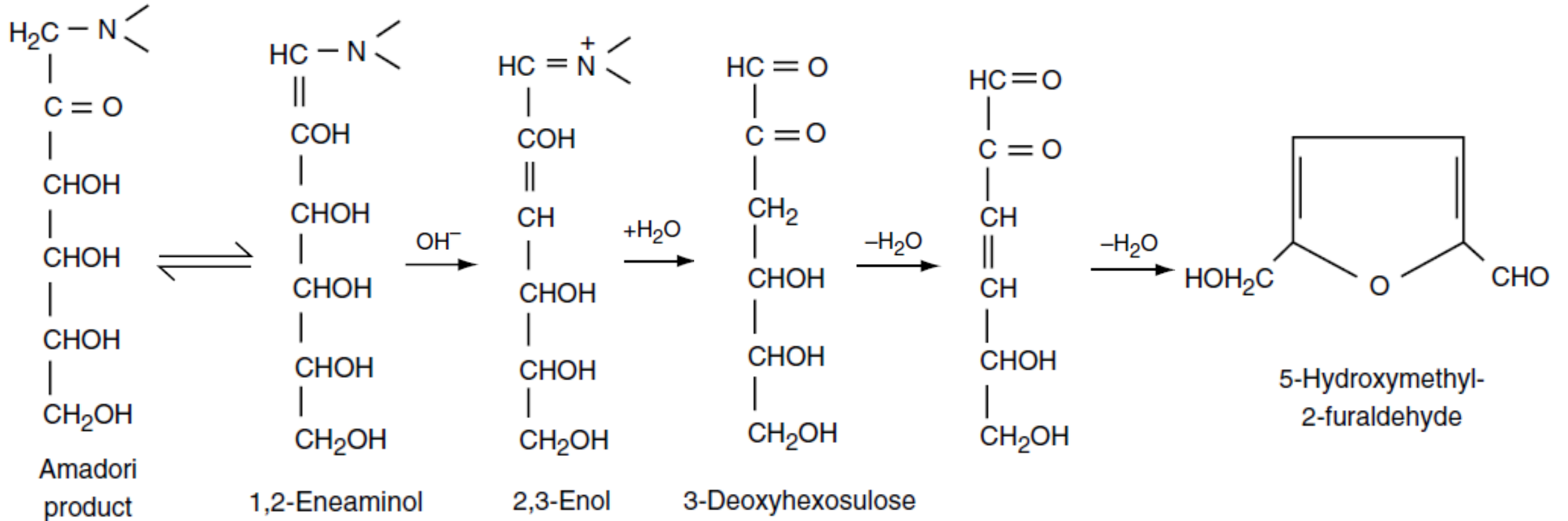
# The Maillard reaction

Products of reaction of D-glucose with a primary amine (RNH<sub>2</sub>)



N-substituted  
1-amino-1-deoxy-D-fructose

# The Maillard reaction



✓ Conversion of the Amadori product into HMF

# The Maillard reaction-The Effect of Reaction Conditions

- ✓ **Temperature and reaction time:** are the most important and interdependent factors that influence the glycation degree of the Maillard reaction in both dry and wet heating methods.
  - ✓ The rate of non-enzymatic reaction is slow at 35°C and increases at temperatures over 55°C.
  - ✓ The range of reaction time can vary from 30 min to 24 h and also up to several days.
- ✓ **The water content, water activity:** has great influence on the activation energy.
  - ✓ The browning rate increases with the increasing temperature reaching its maximum in the range of water activities of 0.3–0.7.
  - ✓ At low water content, the reaction is slow; it increases with increasing concentration of water, but at much higher water contents, the concentration of reactants is low so that the reaction rate starts to decrease again.
- ✓ **The pH:** the rate of the Maillard reaction increases with increasing pH value, and attains its maximum in slightly alkaline medium (pH > 7.0).
  - ✓ the solubility of proteins is higher at basic pH values and consequently more soluble protein is available to react with reducing sugars during the non-enzymatic glycation reaction.

# The Maillard reaction- The Effect of Reaction Conditions

- ✓ **Reactant type and concentration:** The glycation degree and subsequently the antioxidant capacity of Maillard-type conjugates can be influenced by the type of reactants (protein or carbohydrate) and their molar ratios.
- ✓ The reactivity of low molecular weight compounds is greater than high molecular weight ones.
  - ✓ aldopentoses possess more reactivity than aldohexoses and monosaccharides exhibit greater reactivity than di- or oligosaccharides.
  - ✓ aldose sugars have higher reactivity than ketose sugars because the carbonyl group of the latter is sterically more hindered than the former.
- ✓ Secondary amines give different reaction products than do primary amines.
- ✓ Browning is inhibited by sulfur dioxide and sulfites or sulfur-containing compounds, as sulfites react with aldehyde and keto group or sugars, decreasing their reactivity.

# The Maillard reaction- Nutritional Significance

- ✓ Reaction products from the Maillard reaction may alter nutritional value of foods by reducing the digestibility of proteins and generating some toxic and mutagenic compounds, but antioxidant products can also be formed.
- ✓ **Nutritional availability of essential amino acids:** The early products of the Maillard reaction involving the lysine residue can be nutritionally utilized in the human organism.
  - ✓ Further changes, however, gradually make the amino acid unavailable.
- ✓ **Lysine** is affected mainly in the outer parts of conventionally heated products, exposed to high temperature during baking, grilling, or frying, whereas in the center of the bread loaf or cooked meat, the yield of the reaction products is negligible.
  - ✓ However, in various liquid and powdered enteral proteinaceous food formulas produced commercially, the lysine availability may be decreased by up to 25%.
- ✓ **Acrylamide** may be formed in Maillard-type reactions between glucose and asparagine.



# The Maillard reaction-Summary

## *Desirable:*

- ✓ Maillard browning products, including soluble and insoluble polymers, are found where reducing sugars and amino acids, proteins, and/or other nitrogen-containing compounds are heated together, for example, in soy sauce and bread crusts.
- ✓ Browning is desired in baking--- in bread crusts and cookies, and roasting of meats.
  - ✓ The volatile compounds produced by nonenzymic browning (the Maillard reaction) during baking, frying, or roasting often provide desirable aromas.
- ✓ Maillard reaction products are also important contributors to the flavor of milk chocolate, caramels, toffees, and fudges, during which reducing sugars react with milk proteins.
- ✓ The Maillard reaction also produces flavors, especially bitter substances, which may be desired, for example, in coffee.

## *Undesirable:*

- ✓ Off-flavors and off-aromas are most likely to be produced during pasteurization, storage of dehydrated foods, and grilling of meat or fish.
- ✓ Application of heat to intermediate moisture foods is generally required for nonenzymic browning.

# Acrylamide Formation

- ✓ The Maillard reaction has been implicated in the formation of acrylamide in many foods that have been heated to high temperatures during processing or preparation.
- ✓ Acrylamide is *derived primarily from the second-order reaction between reducing sugars (carbonyl moiety) and the  $\alpha$ -amino group of free L-asparagine.*
- ✓ The reaction most likely occurs via a Schiff base intermediate, which then undergoes decarboxylation, followed by carbon–carbon bond cleavage to form acrylamide, whose atoms are known to be derived solely from L-asparagine.
- ✓ Acrylamide is a known neurotoxicant and probably a weak human carcinogen at exposure levels much higher than are obtained from food.
- ✓ Acrylamide is not detected in unheated or even boiled foodstuffs, such as boiled potatoes, because the temperature during boiling does not go above  $\sim 100^\circ\text{C}$ .

# Acrylamide Formation

- ✓ Levels of acrylamide (typically <1.5 ppm) have been reported in a wide range of food products that are made by frying, baking, puffing, roasting, or other elevated-temperature processing schemes during production or preparation
- ✓ Acrylamide is undetected or detected at only very low levels in canned or frozen fruits, vegetables, and vegetable protein products (vegetable burgers and related products) with the exception of pitted ripe olives, in which the measured levels ranged from 0 to 1925 ppb.
- ✓ Fried potato products, such as potato chips and French fries, are particularly susceptible to acrylamide formation because potatoes contain both free d-glucose and free l-asparagine.

## Ranges of Acrylamide Found in Common Food Products Containing High Levels

Food	ppb Acrylamide <sup>a</sup>
Almonds (roasted)	236–457
Bagels	0–343
Breads	0–364
Breakfast cereals (RTE)	34–1057
Cocoa	0–909
Coffee (unbrewed)	3–374
Coffee with chicory	380–609
Cookies	36–432
Crackers and related products	26–1540
French fries	20–1325
Potato chips	117–196 <sup>b</sup>
Pretzels	46–386
Tortillas	10–33
Tortilla chips	117–196

<sup>a</sup> Extreme values, especially extremely high values, are usually representative of only a small number of sampled products.

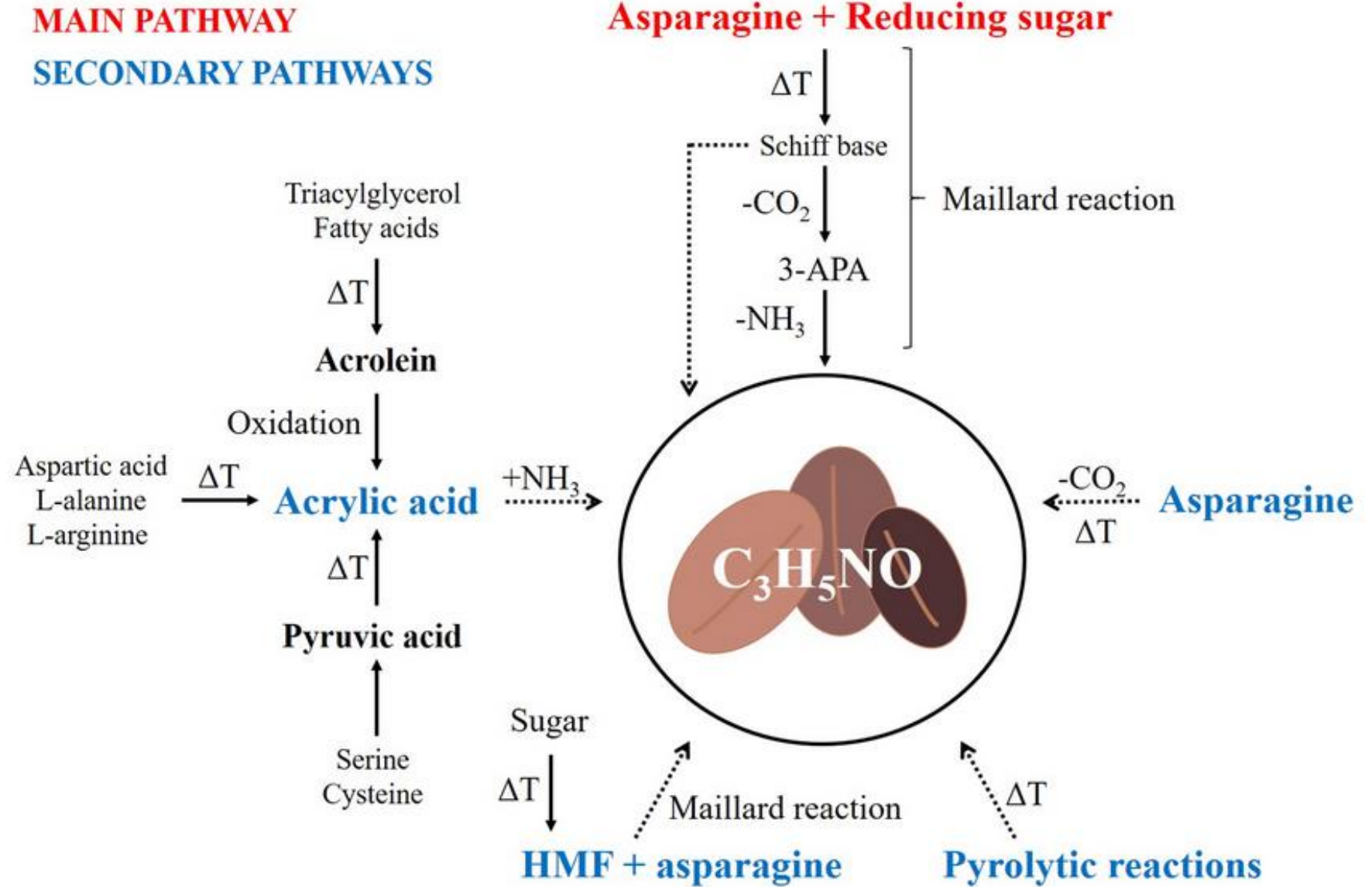
<sup>b</sup> A sample of sweet potato chips contained 4080 ppb acrylamide.

*Source:* Center for Food Safety and Applied Nutrition, USDA.

# Acrylamide Formation

- ✓ Acrylamide formation requires a minimum temperature of 120°C, which means that it cannot occur in high-moisture foods, and is kinetically favored with increasing temperatures approaching 200°C.
- ✓ With extended heating at temperatures above 200°C, acrylamide levels may actually decrease via thermal elimination/degradation reactions.
- ✓ Food levels of acrylamide are also impacted by pH. Acrylamide formation is favored as the pH is increased over the range of 4–8.
  - ✓ Reduced acrylamide formation as the pH decreases
- ✓ Products with high amounts of surface area, such as potato chips, are among those high-temperature processed foods that exhibit the highest acrylamide levels.

- ✓ Simplified scheme of possible routes of acrylamide ( $C_3H_5NO$ ) formation during roasting in coffee



Source : Schouten et al. (Critical Reviews in Food Science and Nutrition2020, 1-15.<https://doi.org/10.1080/10408398.2019.1708264>)

# Acrylamide Formation-Mitigation

## *Efforts to minimize formation of acrylamide in food:*

- ✓ Removal of either one or both of the substrates
- ✓ Alteration of processing conditions
- ✓ Acrylamide removal from food following its formation
  - ✓ Through blanching or soaking in water, it is possible to achieve up to a 60% reduction in acrylamide levels within processed potato products through removal of reaction substrates (reducing sugars and free asparagine).
- ✓ Reagent modification (e.g., protonation of asparagine by lowering the pH or conversion of asparagine to aspartic acid with asparaginase), addition of competing substrates that do not yield acrylamide (e.g., amino acids other than asparagine or protein)
- ✓ Incorporation of salts have been shown to mitigate acrylamide formation.

# Caramelization

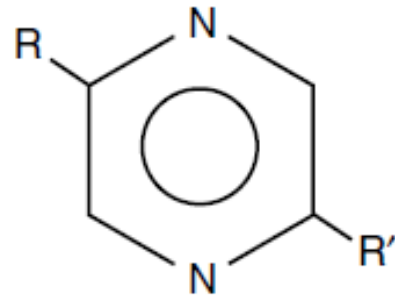
- ✓ Heating of carbohydrates, in particular sucrose and reducing sugars, without nitrogen containing compounds affects a complex group of reactions involved in caramelization.
- ✓ Reaction is facilitated by small amounts of acids and certain salts.
- ✓ Although it does not involve amino acids or proteins, caramelization is similar to nonenzymic browning.
- ✓ The final product, caramel (as in Maillard browning) contains a complex mixture of polymeric compounds, formed from unsaturated, cyclic (five- and six-membered ring) compounds.
- ✓ Formation of flavor and aroma compounds
  - ✓ heating causes dehydration of the sugar molecule with introduction of double bonds or formation of anhydro rings.
  - ✓ intermediates such as 3-deoxyosones and furans are formed.
- ✓ The unsaturated rings may condense to form useful, conjugated double-bond-containing, brown-colored polymers.

# Caramelization

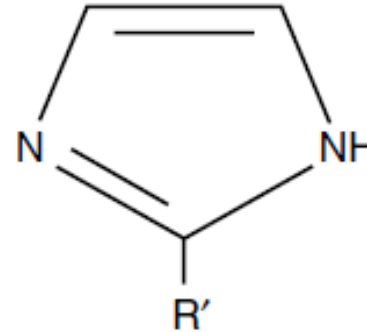
- ✓ **Caramel** is produced commercially both as a coloring material and as a flavoring material.
- ✓ To make caramel, a carbohydrate is heated alone or in the presence of an acid, a base, or a salt.
- ✓ The carbohydrate most often used is sucrose, but d-fructose, d-glucose (dextrose), invert sugar, glucose syrups, HFSs, malt syrups, and molasses may also be used.
- ✓ Acids that may be used are food-grade sulfuric, sulfurous, phosphoric, acetic, and citric acids.
- ✓ Bases that may be used are ammonium, sodium, potassium, and calcium hydroxides.
- ✓ Salts that may be used are ammonium, sodium, and potassium carbonates, bicarbonates, phosphates (both mono- and dibasic), sulfates, and bisulfites.
- ✓ Ammonia may react with intermediates, such as 3-deoxyosones, produced by thermolysis to produce pyrazine and imidazole derivatives.



# Caramelization



Left



Right

- ✓ Pyrazine (left) and imadazole (right) derivatives formed during carmelization in the presence of ammonia  $R = \text{--CH}_2\text{--(CHOH)}_2\text{--CH}_2\text{OH}$ ,  $R = \text{--(CHOH)}_3\text{--CH}_2\text{OH}$ .

# Nonenzymic Browning- *The role of lipids in browning*

- ✓ Reactions of oxidized lipids with proteins result in the formation of colored compounds similar to those produced in Maillard or enzymic browning reactions.
- ✓ Lipid peroxy radicals, lipid hydroperoxides, or their decomposition products interact with free amino acids forming yellow to brown products.
- ✓ The browning rate is particularly high in reactions with cysteine, methionine, and tryptophan.
- ✓ Similar reactions can also occur between oxidizing lipids and proteins.
- ✓ Since unsaturated lipids are more prone to oxidation than saturated ones, the browning reaction is more intense in tissues rich in fats containing polyenoic fatty acids, such as fish muscle.
- ✓ The browning discoloration is objectionable especially in white poultry or fish muscle, where brown spots are produced even under refrigerated or frozen storage.

# Nonenzymic browning and black spot formation

- ✓ The compounds formed in the early stages of Maillard reactions are essentially colorless or slightly yellowish.
  - ✓ They are called premelanoidins as they are precursors of brown-colored melanoidins.
- ✓ The colorless low-molecular weight products (<1 kDa) are produced mainly by reactions with free amino acids.
- ✓ Brown-colored macromolecular polymeric Maillard products—melanoidins— are generated predominantly by polymerization of intermediary products and/or by reactions with the reactive groups of amino acid residues in proteins.
- ✓ Reactions of oxidized lipids with proteins result in the formation of colored compounds similar to those produced in Maillard or enzymic browning reactions.

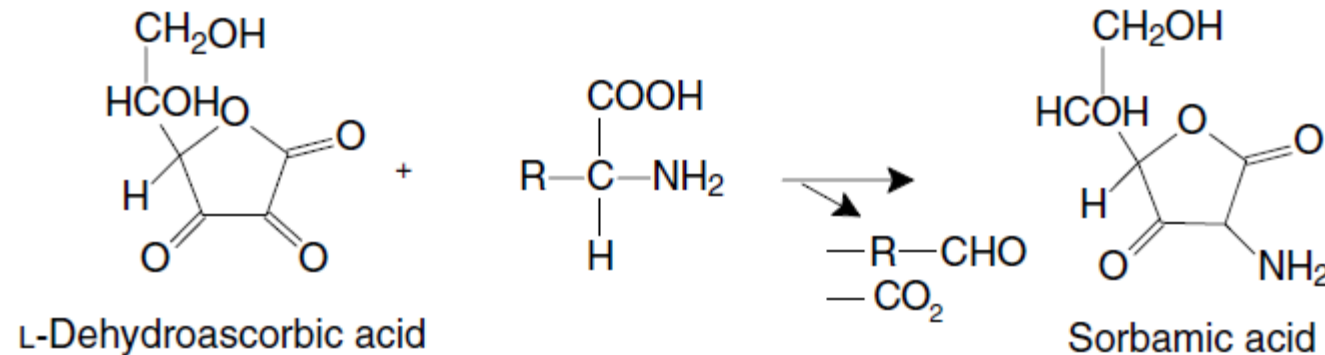
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# Nonenzymic Browning- *Oxidation of ascorbic acid*

- ✓ Ascorbic acid degradation is associated with discoloration reactions in both the presence and absence of amines.
- ✓ L-Dehydroascorbic acid, as well as the dicarbonyls formed during its degradation
  - ✓ These can participate in Strecker degradation with amino acids.
- ✓ Following Strecker degradation of dehydroascorbic acid with an amino acid, the sorbamic acid product can form dimers, trimers, and tetramers, several of which are reddish or yellowish in color.

## *Participation of dehydroascorbic acid in the Strecker degradation reaction*



# Nonenzymic Browning- *Oxidation of ascorbic acid*

- ✓ In addition, 3,4-dihydroxy-5-methyl-2(5H)-furanone, an intermediate product of dehydration following decarboxylation during anaerobic degradation of ascorbic acid, has a brownish color.
- ✓ Further polymerization of these or other unsaturated products forms either *melanoidins (nitrogenous polymers)* or *nonnitrogenous caramel-like pigments*.
- ✓ Nonenzymatic browning of citrus juices and related beverages is a complex process and the contribution of ascorbic acid to browning is clear.
- Juice manufacturers have developed strategies for mitigation, primarily utilizing additives or supplemental processes.

# Overview of mechanisms for the oxidative and anaerobic degradation of ascorbic acid

- AH<sub>2</sub>, fully protonated ascorbic acid;
- AH<sup>-</sup>, ascorbate monoanion;
- AH•, semidehydroascorbate radical;
- A, dehydroascorbic acid;
- FA, 2-furoic acid;
- F, 2-furaldehyde;
- DKG, diketogulonic acid;
- DP, 3-deoxypentosone;
- X, xylosone;
- Mn<sup>+</sup>, metal catalyst;
- HO•<sub>2</sub>, perhydroxyl radical.

