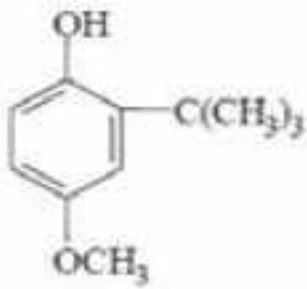
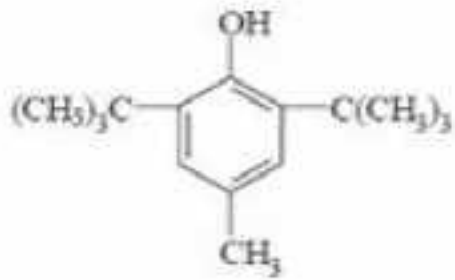


# Antioxidants

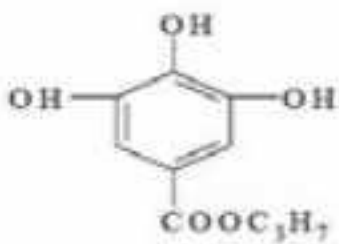
## Synthetic antioxidants



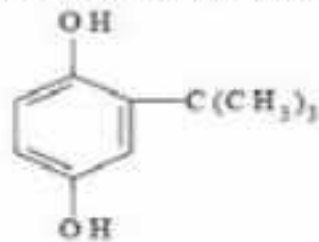
Butylatedhydroxyanisole (BHA)



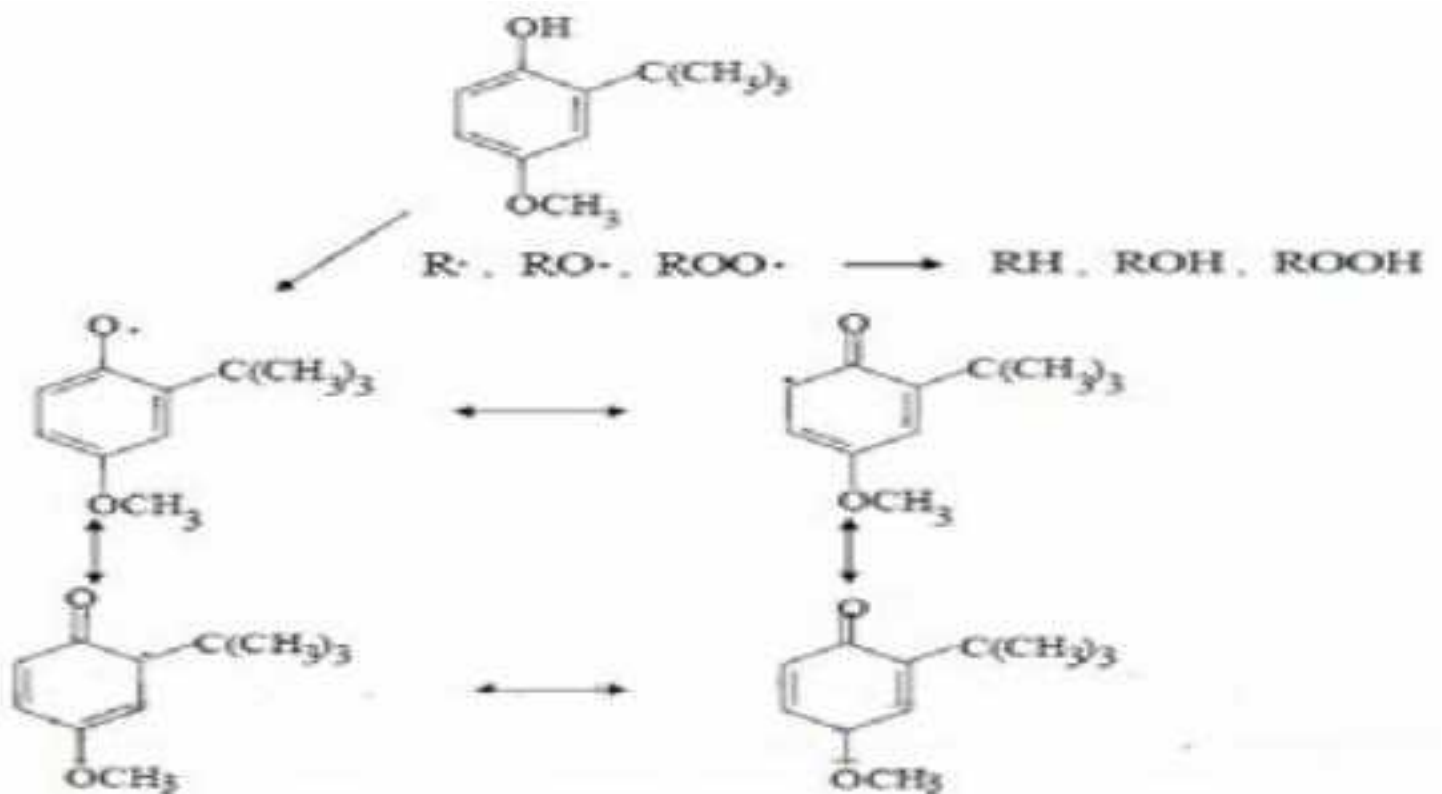
Butylatedhydroxytoluene (BHT)



Propyl gallate



Tertiary butylhydroquinone (TBHQ)

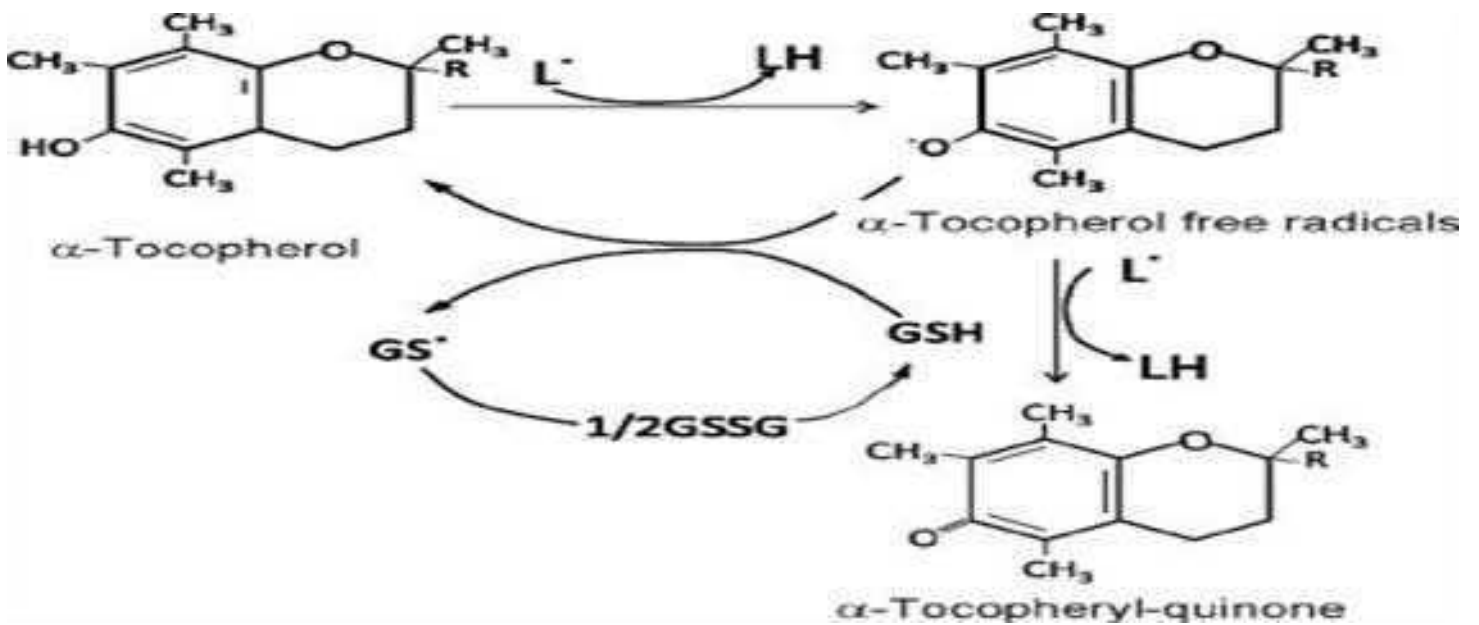
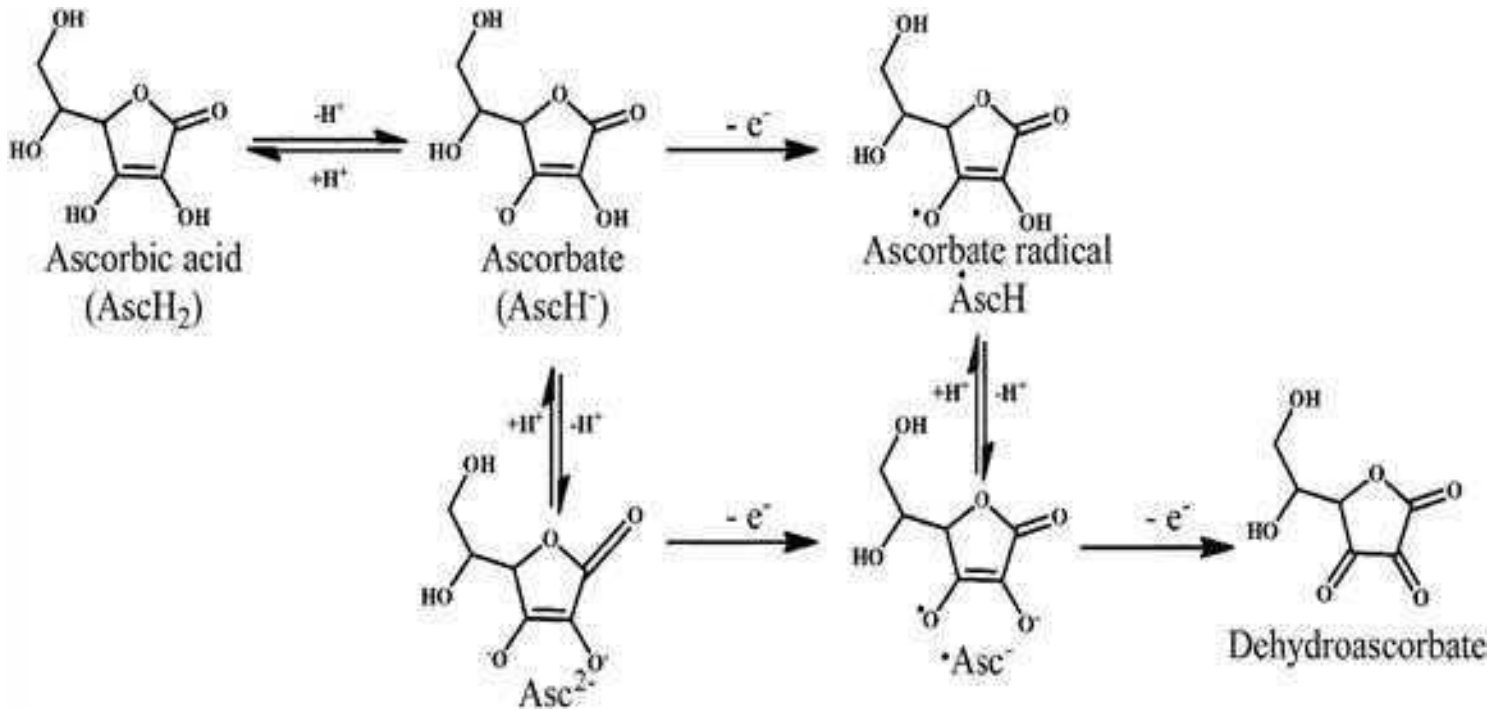


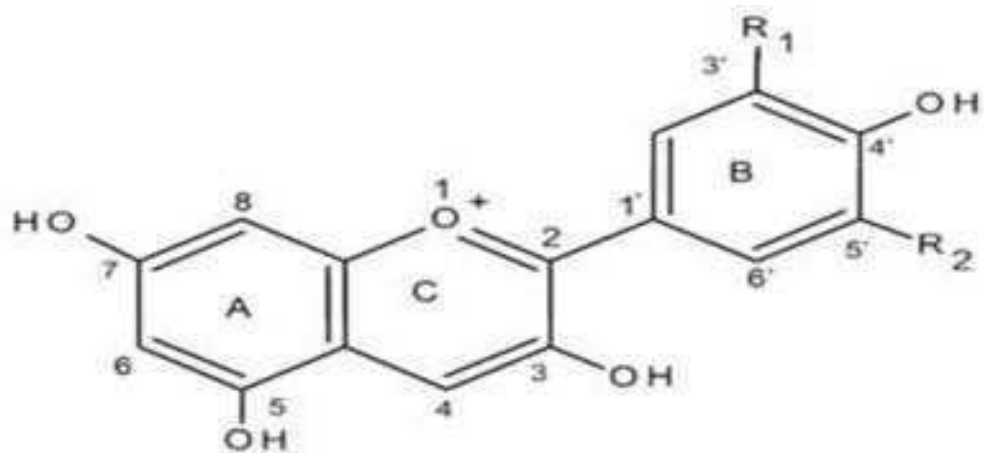
# Natural antioxidants

## Vitamin C

## Tocopherol

## Anthocyanin





Anthocyanidins	R1	R2
Pelargonidin (Pg)	H	H
Cyanidin (Cy)	OH	H
Delphinidin (De)	OH	OH
Peonidin (Pn)	OCH <sub>3</sub>	H
Petunidin (Pt)	OCH <sub>3</sub>	OH
Malvidin (Ma)	OCH <sub>3</sub>	OCH <sub>3</sub>

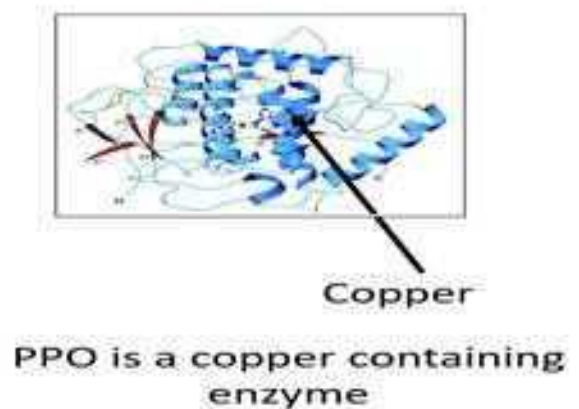
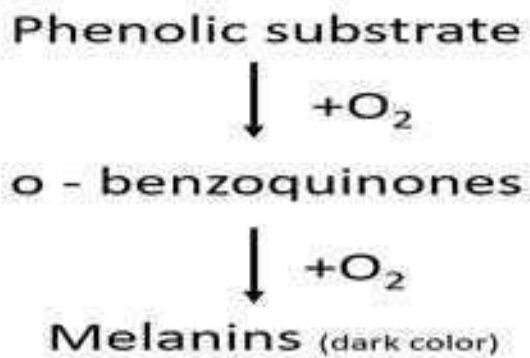
## Browning reactions

- Enzymic browning is one of the most important colour reactions that affects fruits, vegetables, and seafood.
- It is catalysed by the enzyme **polyphenol oxidase (PPO)**
- PPO is widely distributed in plants, fungi, arthropods and all mammals.
- Up to 50% of fruit and vegetable loses due to enzymic browning worldwide!



## General mechanism

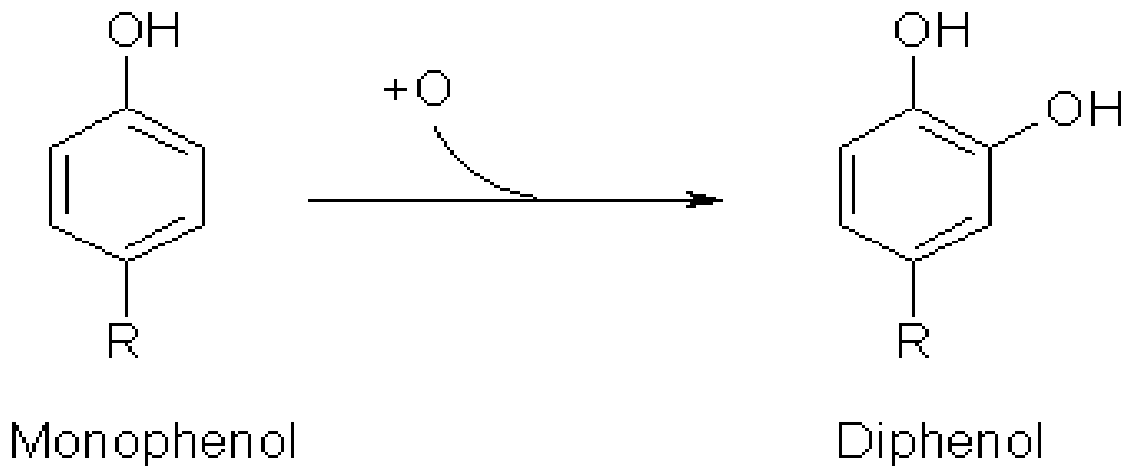
The reaction requires simultaneous presence of the enzyme, substrate and oxygen to occur. If one is absent the reaction does not proceed.



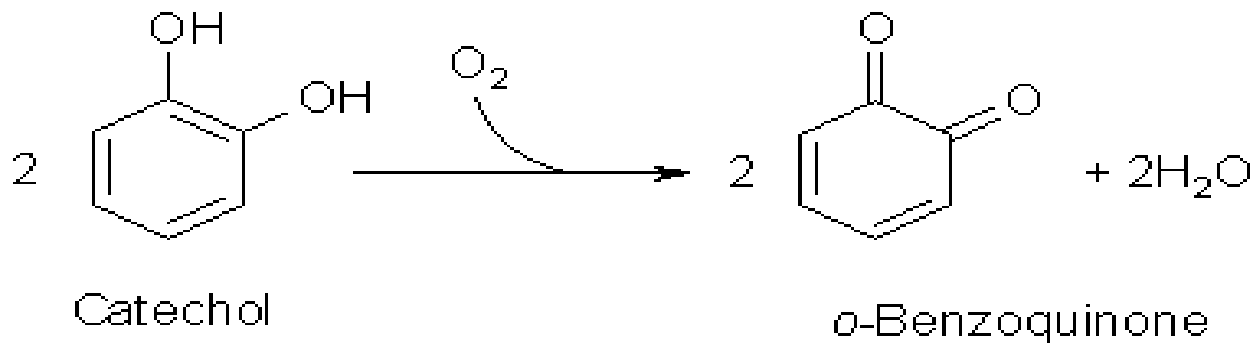
## Monophenol oxidase pathway

Hydroxylation to the o-position of the phenolic substrate (monophenol oxidase activity), and oxidation of di-phenol to o-benzoquinones (diphenol oxidase activity). Both reactions utilize oxygen as a co-substrate.

### Monophenol oxidase pathway



### Diphenol oxidase pathway



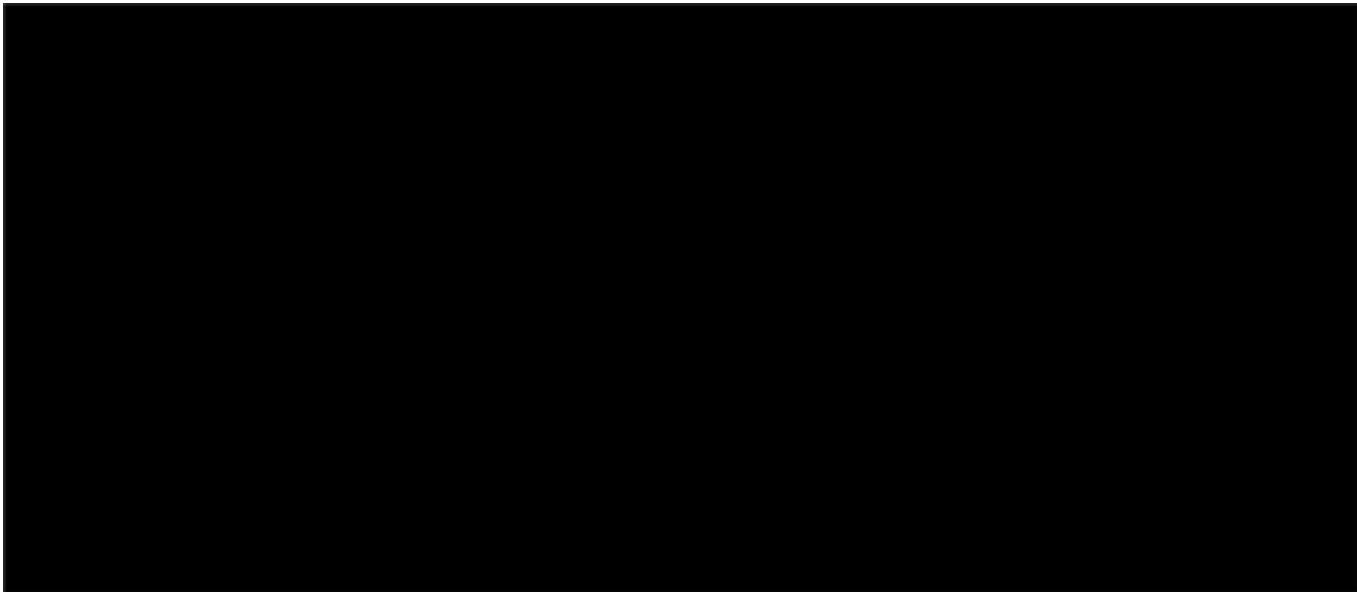
## **Formation of melanin from tyrosine in mushrooms**



The end product is melanin. Dark brown water insoluble compounds.

### **Laccases**

Laccase is a type of copper-containing polyphenol oxidase. It has the unique ability of oxidising p-diphenols, in contrast to PPO that oxidises o-diphenols. They have low specificity and oxidise a wide variety of substrates. Can be used to crosslink arabinoxylans and improve baking performance of dough.



## **Substrates**

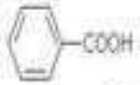
**Phenolic compounds** contain an aromatic ring bearing one or more hydroxyl groups. Enzymatic browning does not occur in intact plant cells since phenolic compounds are separated from PPO.

The polyphenolic composition of fruits varies in accordance with species, cultivar, degree of ripening and environmental conditions of growth and storage.

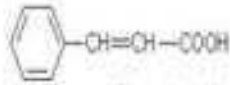
The substrate specificity of PPO varies in accordance with the source of the enzyme.

## Benzoic and Cinnamic acid derivatives

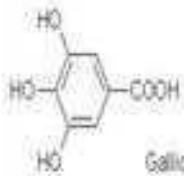
## Flavonoids Catechins



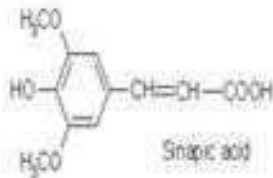
Benzoic acid



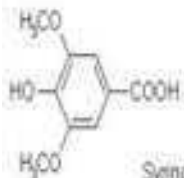
Cinnamic acid



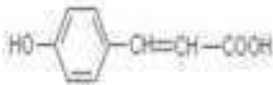
Gallic acid



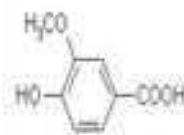
Sinapic acid



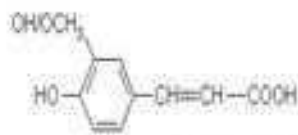
Syringic acid



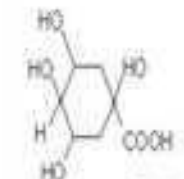
p-coumaric acid



Vanillic acid



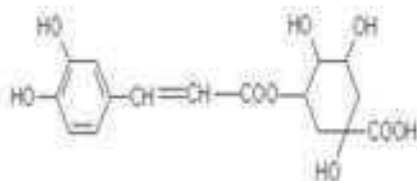
Caffeic acid/Feulic acid



Quinic acid



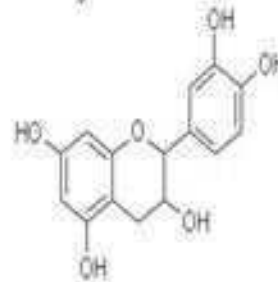
Coumarin



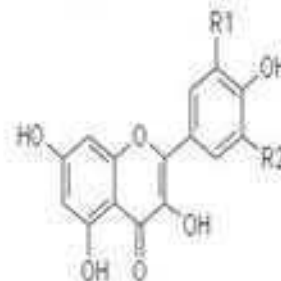
Chlorogenic acid



Flavonoid structure



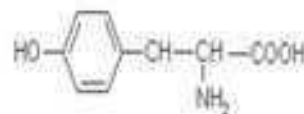
Catechins



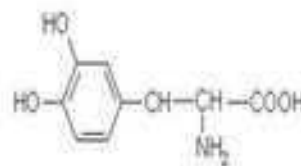
Quercetin (R1=OH, R2=H)

Myricetin (R1=R2=OH)

Kaempferol (R1=R2=H)



Tyrosine



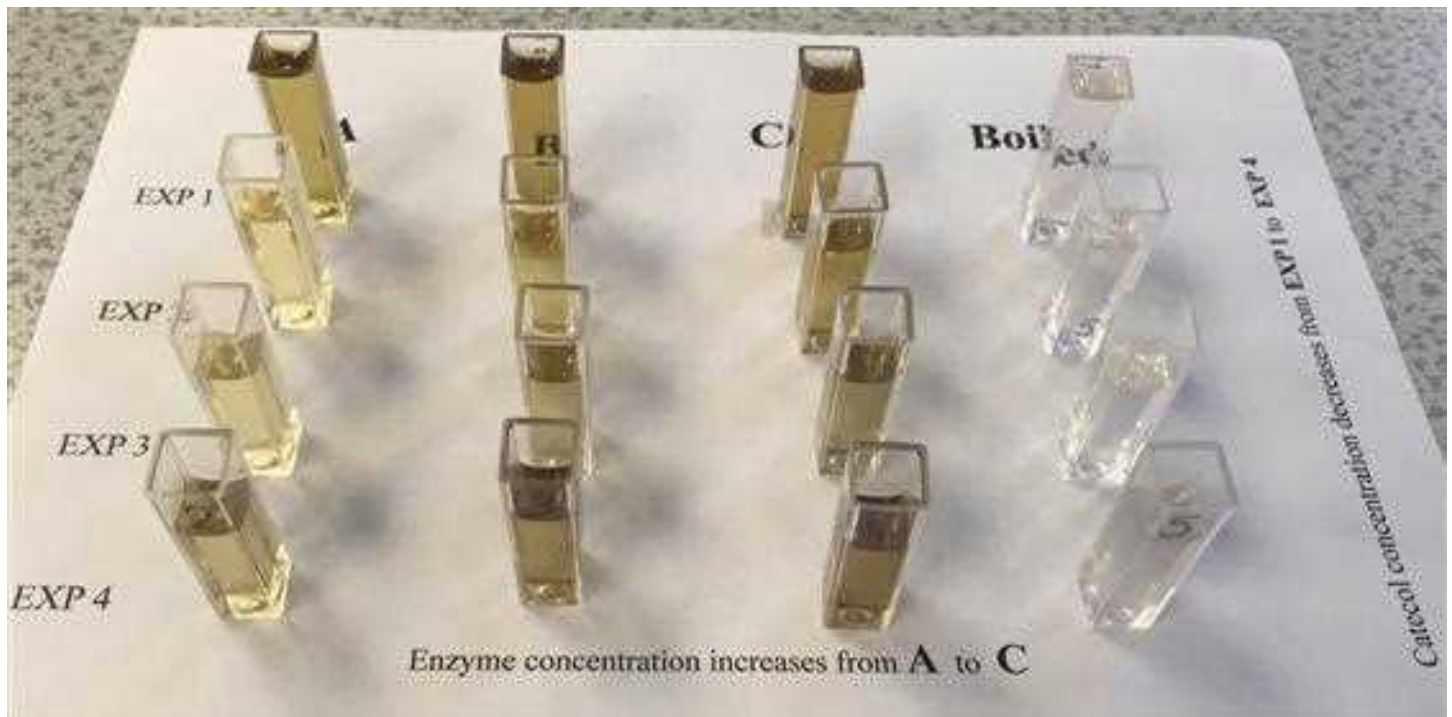
3,4-dihydroxyphenylalanine (DOPA)



Source	Phenolic compounds
Apple	chlorogenic acid (flesh), catechol, catechin (peel), caffeic acid, 3,4-dihydroxyphenylethylamine (DOPA), 3,4-dihydroxy benzoic acid, p-cresol, 4-methyl catechol, leucocyanidin, p-coumaric acid, flavanol glycosides
Avocado	4-methyl catechol, dopamine, pyrogallol, catechol, chlorogenic acid, caffeic acid, DOPA
Banana	3,4-dihydroxyphenylethylamine (Dopamine), leucodelphinidin, leucocyanidin
Eggplant	chlorogenic acid, caffeic acid, coumaric acid, cinnamic acid derivatives
Lettuces	tyrosine, caffeic acid, chlorogenic acid derivatives
Lobster	tyrosine
Mushroom	tyrosine, catechol, DOPA, dopamine, adrenaline, noradrenaline
Pear	chlorogenic acid, catechol, catechin, caffeic acid, DOPA, 3,4-dihydroxy benzoic acid, p-cresol
Potato	chlorogenic acid, caffeic acid, catechol, DOPA, p-cresol, p-hydroxyphenyl propionic acid, p-hydroxyphenyl pyruvic acid, m-cresol
Shrimp	tyrosine

## Influence of enzyme and substrate concentration on colour formation

Enzymatic browning does not occur in intact plant cells since phenolic compounds are separated from the polyphenol oxidase. Once tissue is damaged by processing formation of brown pigments begins. Both the organoleptic and biochemical characteristics of fruits and vegetables are altered by pigment formation. The rate of enzymatic browning in fruit and vegetables is controlled by PPO and substrate concentration, pH, temperature and oxygen availability within the tissue.



Colour variation with with increase in enzyme and substrate concentration. After a point colour formation levels off. The boiled enzyme has been denatured and browning does not take place.

## Strategies to control enzymatic browning

Eliminate one or more of the essential components (oxygen, enzyme, copper or substrate) from the reaction.

Low temperatures	<b>Refrigeration, freezing:</b> Reduce the rate of enzymic reactions but <b>DOES NOT</b> eliminate activity.
Water removal	<b>Dehydration:</b> Low $a_w$ the limiting factor for enzymatic activity.
Inhibition targeted toward the reaction products	Chemical compounds that react with the products of polyphenol oxidase activity and <b>inhibit the formation of coloured compounds</b> . Ascorbic acid, thiol compounds, and sulphites interfere with the reactions that lead to melanin formation
Inhibition targeted toward the substrate	<b>Elimination of oxygen</b> from the cut surface of fruits and vegetables. Vacuum packaging, immersion in water, syrup, or brine.
Action on the enzyme	<b>Chelating agents:</b> Copper removal from the active site (EDTA, polycarboxylic acids). Benzoic and cinnamic acid derivatives may act as <b>competitive inhibitors</b> of polyphenol oxidase, owing to their structural similarity with phenolic substrates. <b>Lowering the pH</b> with addition of polycarboxylic acids e.g., citric, malic, tartaric acids. Inactivation of the polyphenol oxidases by <b>heat treatments</b> or irradiation.

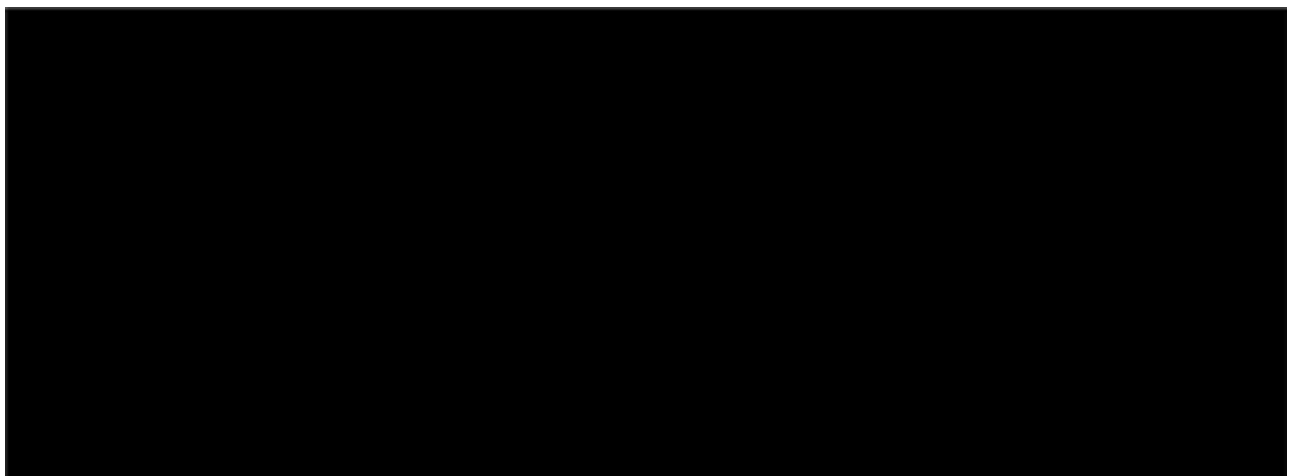
## Representative inhibitors of enzymatic browning

The use of browning inhibitors in food processing is controlled by considerations relevant to toxicity, freshness, and effect on taste, flavour, texture, and cost.

Browning inhibitors may be classified in accordance with their primary mode of action.

Reducing agents	Sulfur containing agents, ascorbic acid, cysteine, glutathione
Chelating agents	Phosphates, EDTA, organic acids
Acidulants	Citric acid, phosphoric acid
Enzyme inhibitors	Aromatic carboxylic acids
Enzyme treatments	Oxygenases, o-methyl transferase proteases
Complexing agents	Cyclodextrins

**Reducing agents** reduce pigment precursors (quinones) to colorless, less-reactive diphenols.



Mechanism of prevention of colour formation by ascorbic acid. Ascorbic acid also acts as an oxygen scavenger. Reduction of enzymatically formed o-quinones to diphenols.



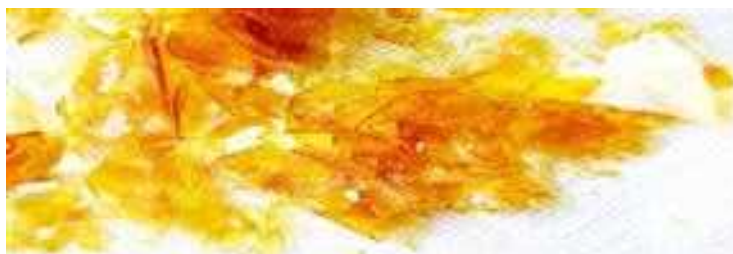
### **Non-Enzymic browning**

1. Caramelization
2. Oxidation of ascorbic acid
3. Maillard reaction

### **Caramelisation**

**Thermal degradation of sugars** (pyrolysis). Acid or base catalysed and generally requires temperatures  $\sim 150$  °C, low pH, low water content/high sugar.

Caramels are complex mixture of various high molecular weight components not well characterized



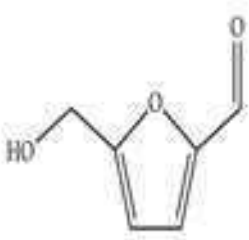
## Reactions during caramelization

- Anomeric equilibration (alpha, beta)
- Pyran, furan equilibration
- Sucrose inversion
- Hydrolysis
- Aldose, ketose interconversions
- Inter- and intramolecular condensation
- Dehydration
- Skeletal fragmentation

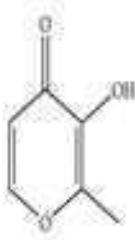
## Colour formation ranked from the highest to lowest rate of colour development

Fructose (~110 °C) < Glucose, Galactose, Sucrose (~160 °C) < Maltose (~180 °C) < Lactose (~203°C)

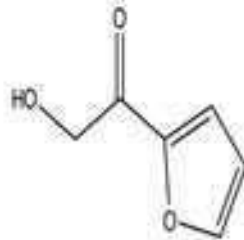
## Major products



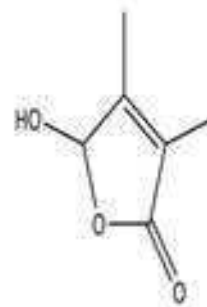
Hydroxymethyl  
furfural (HMF)



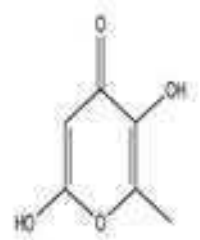
Maltol



Hydroxy acetyl  
furan (HAF)



Hydroxy dimethyl  
furanone (HDF)



Hydroxy maltol

**Diacetyl** is responsible for a buttery or butterscotch flavour.

**Esters** and **lactones** which have a sweet rum-like flavour.

**Furans** which have a nutty flavour.

**Maltol** has a toasty flavour.

If caramelisation continues too far the taste becomes bitter.

## Caramel colours

	No. 1	No. 2	No. 3	No. 4
Color	No. 1	No. 2	No. 3	No. 4
Taste	Slightly negative	Negative	Positive	Strongly negative
Flavor	-	+	-	-
Tannin	-	+	+	+
Acid	-	+	-	+
Typical applications	Desserts, cereals, baked products, pet food	Tea, liquor (negates mouth starchy)	Beer, juices, gravies	Soft drinks, carbonated beverages

**Class I** (Plain Caramel, Caustic Caramel): no ammonium or sulfite compounds are used.

**Class II** (Caustic Sulfite Caramel): sulfite compounds but no ammonium compounds.

**Class III** (Ammonia Caramel): ammonium compounds but no sulfite compounds.

**Class IV** (Sulfite Ammonia Caramel): both sulfite and ammonium compounds.

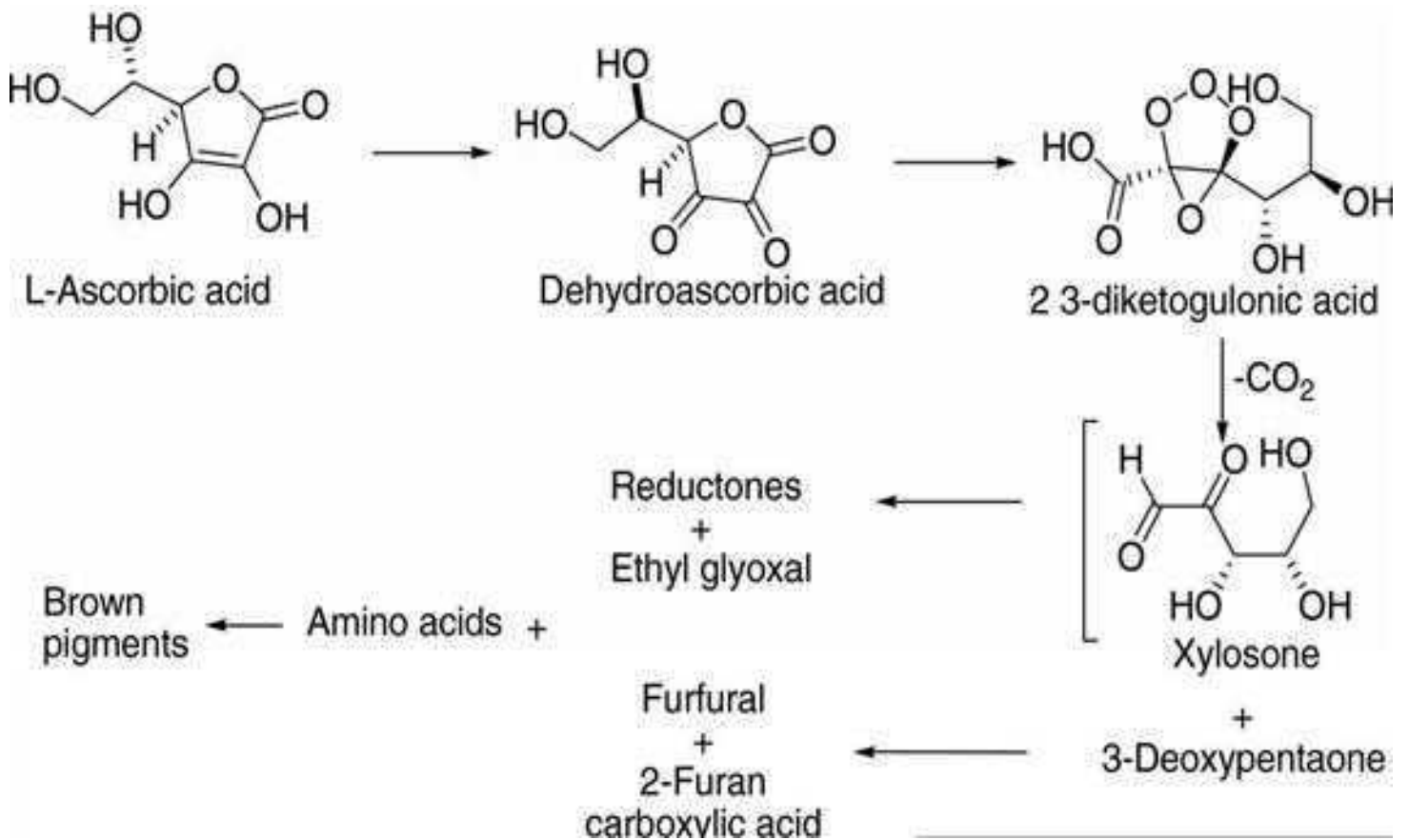
Caramels have positive or negative charge. Its application is related to the charge and the way it interacts with formulation ingredients.

Caramel colours are used in low concentrations and **do not give flavour** to the product. Their main purpose in food is as colouring agents.

### **Ascorbic acid browning**

Occurs in acidic foods such as fruit juices. Ascorbic acid is lost from foods lowering the nutritional value. Decomposition of vitamin C takes place when in contact with oxygen. The brown colour only starts appearing once all the vitamin C has been consumed in the reaction. May occur at relatively low temperatures and low pH. Presence of amino acids or proteins may facilitate colour production.





## Maillard Reaction

Is the reaction between a **reducing sugar** and an **amino acid** (free or in protein)

### Beneficial:

Bread, Soy sauce, Chocolate, Roasted, grilled meats, Coffee....

### Undesirable:

Dehydrated potatoes, Egg powder, Corn starch, Dried foods: milk, fruits

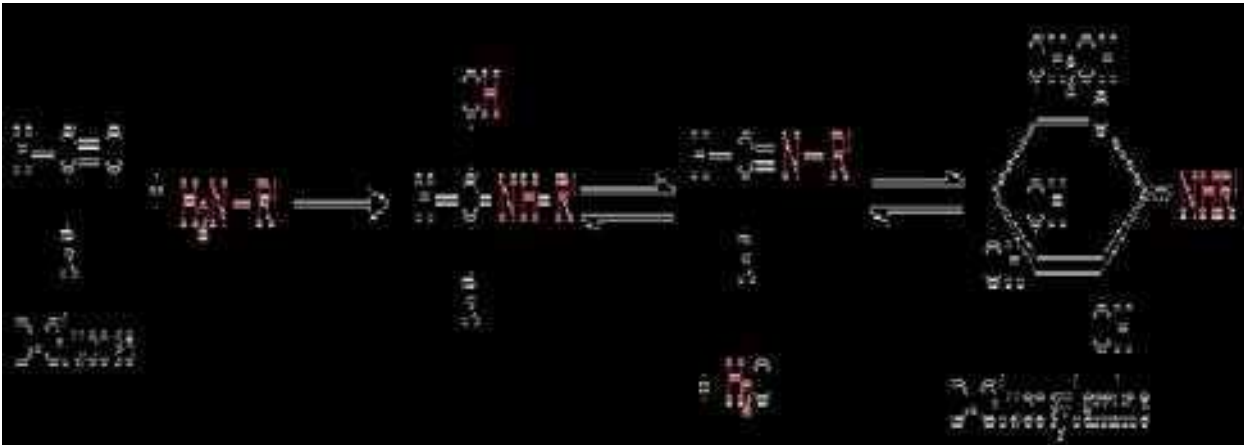
Maillard reaction **occurs every time a food is heated** and a reducing sugar and amino acid are present in the formulation. **This means nearly always.** Maillard reaction is the most important taste and flavour generating reaction that occurs in

food. **Loss of essential amino acids.** In products low in essential amino acids, this can become a significant problem.

1-Initial step: formation **N glycoside**

The **first reaction** is a condensation reaction i.e., water is produced .

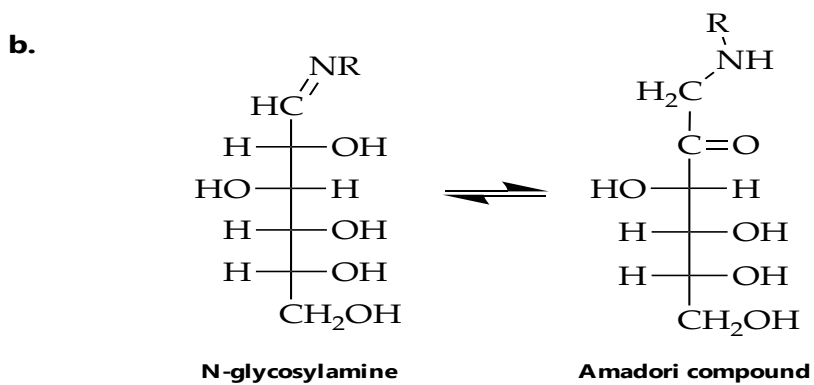
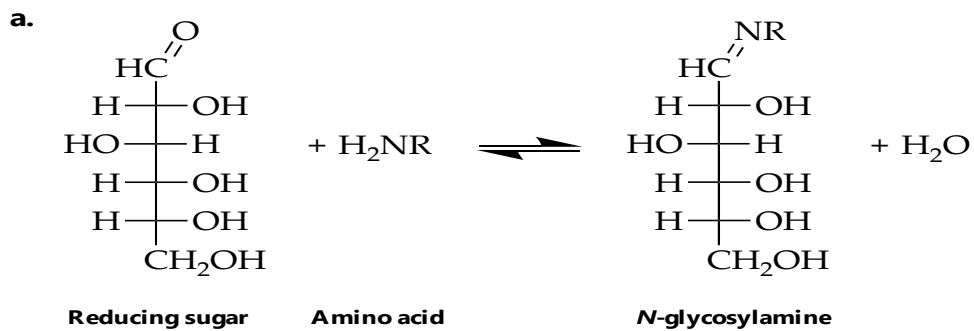
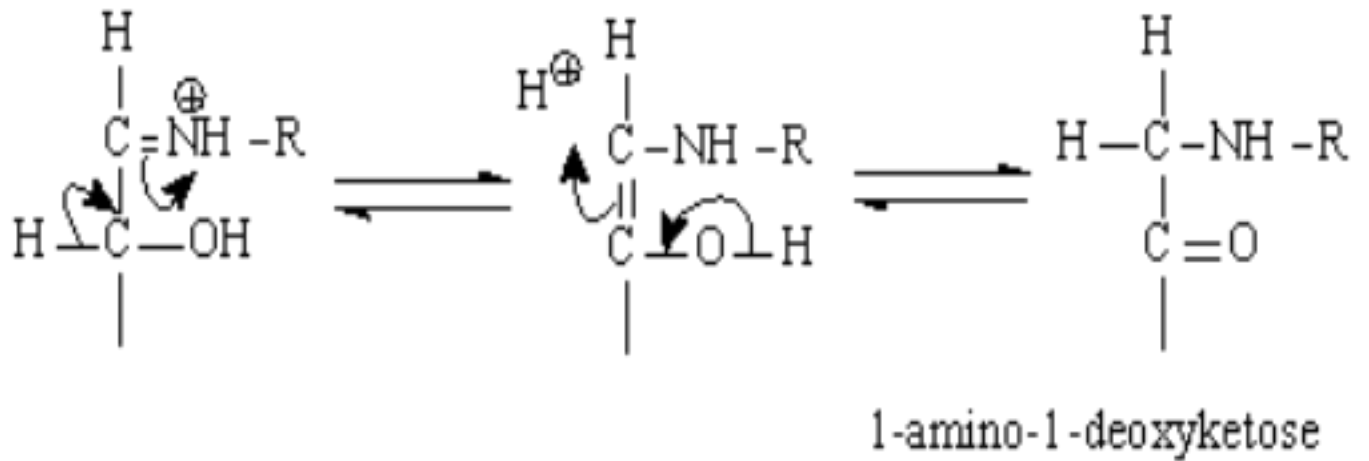
The first reaction in the sequence and is reversible.



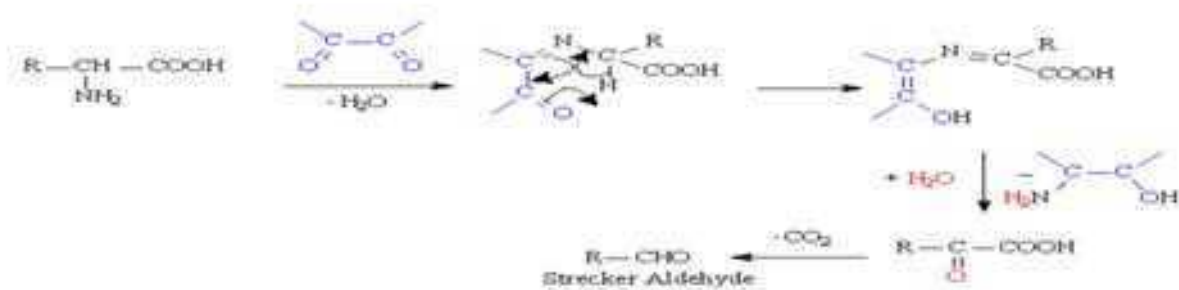
2-After formation of N glycoside the immonium ion is formed and then isomerize, this reaction is called **Amadori** rearrangement and forms a compound called **ketosamine**:

### **Rearrangements**

In the presence of a slightly acidic medium (**most foods**), the N-glycosylamine isomerises. The **Amadori rearrangement** describes the acid catalysed rearrangement reaction of the N-glycosylamine to the corresponding ketosamine.



3-The ketosamine products then either dehydrates into reductones and dehydro reductones, which are caramel, or products short chain hydrolytic fission products such as **diacetyl**, acetol or pyruvaldehyde which then undergo the **Strecker degradation**.



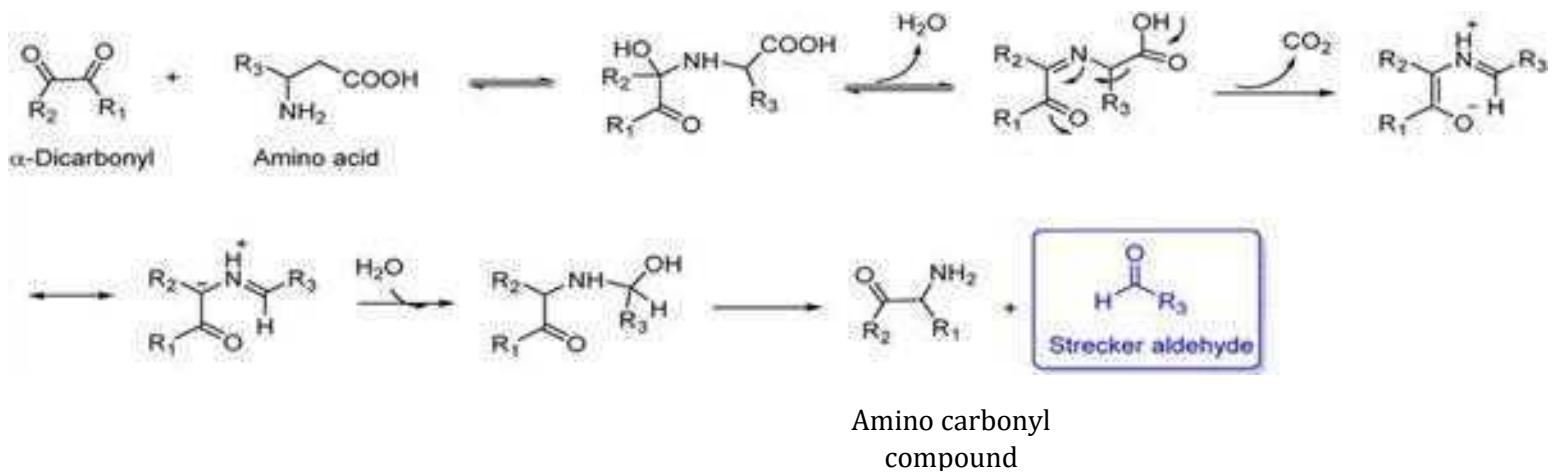
## Dehydration

The rearrangement products are eventually dehydrated in a series of reactions that are not reversible. Deamination (loss of amine) and further isomerisation leads to several **dicarbonyl compounds** that are very reactive. It is only at this point that colours start to develop, partly due to the appearance of hydroxymethyl furfural (HMF).

## 3-Strecker Degradation

**Reaction of  $\alpha$ -dicarbonyl with an amino acid to produce a “Strecker aldehyde”.**

Strecker aldehydes have very strong odour.



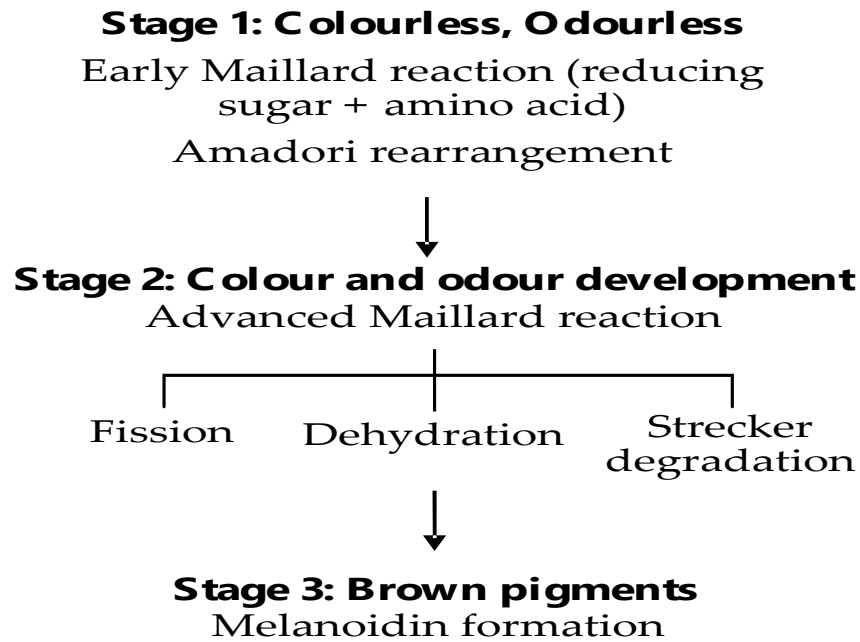
## 4-Polymerization

This occurs randomly between carbonyls and in many instances they become attached to partly degraded proteins in the mixture.

Although the polymerized pigments are initially water-soluble, as their size increase their solubility decreases.

Final reaction products are called **melanoidins**.

## Maillard reaction overview



## Factors affecting Maillard browning

### Effect of temperature:

As with all other chemical reactions, higher temperatures speed up Maillard browning.

### Effect of pH:

- The effect of pH is not as simple as that of temperature.
- Different reactions in the sequence are differently affected by changes in pH.
- A change in pH will also affect the mechanism of browning i.e. different end-products can be expected.

### Generalization:

Decrease in the rate of browning with a decrease in pH i.e. at acidic pH.

- Too much acid protonates amino acid and cannot react with the reducing sugar.

### Effect of moisture content:

- Browning occurs faster the lower the moisture content. This is normal for

most chemical reactions i.e. the higher the reactant concentrations, the faster the rate of reaction.

-As the moisture content decreases towards 30%, there is an increase in the rate of browning.

-Below 30% moisture, there is a decreased rate of browning since the solutes (reactants) become trapped and are not able to diffuse around freely. If the reactants are not able to meet each other, then no reaction will take place.

### **Effect of oxygen:**

-As oxygen is not involved in Maillard reaction it does not affect its rate.

### **accelerators and inhibitors**

-Phosphates, carboxylic acids and their salts can result in faster browning or more intense colour development.

-Many buffers are composed of these molecules and therefore any buffer added to a product may play a role in browning.

-Tin ions may, due to it causing strong reducing conditions, retard browning to a certain extent. Important of heating in tin cans.

### **Role of SO<sub>2</sub>**

-SO<sub>2</sub> is frequently used in a number of products, including different beverages, as a preservative.

-It can block carbonyls i.e. react with reducing sugars and in so doing prevent the condensation reaction of the Maillard reaction.

-In addition, it can also react with later products such as HMF and prevent further colour reactions from occurring.

### **Control of browning**

- Create unfavorable conditions for the reactions to take place
  - Water--low or high
  - pH--lower, decreases browning
  - Temperature-- the lower the temperature, the less the browning.
  - Treatment with SO<sub>2</sub>

-Removal of substrates:– Glucose oxidase--removes the open chain form by converting it to gluconic acid. This technology is used in preserving dried egg whites.

