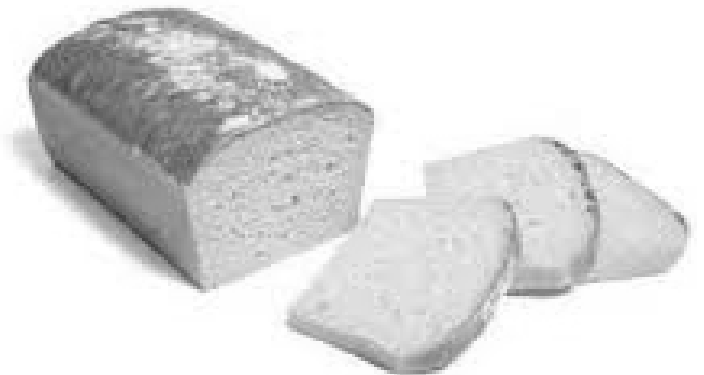




# ENZYMES



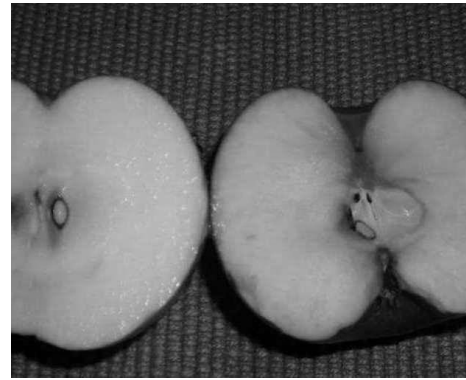
# Introduction

- Minor constituents of many foods.
- They play a major and manifold role in foods.
- They are naturally present in foods and may change the composition of those foods.
- Some changes are desirable, but in most instances are undesirable, so the enzymes must be deactivated.
- Enzymes → food science deals with decompositions, hydrolysis, and oxidation.

# Introduction

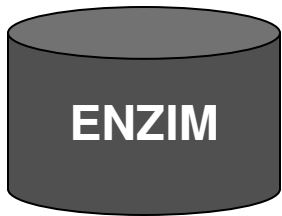
## UNDESIRABLE

- In vegetables/ fruits – should be blanching



## DESIRABLE

- Indicator for analysis methods – Phosphatase (for milk pasteurization)
- Coagulant for milk in cheese production - Rennin



# Nature and Function

## Enzymes:

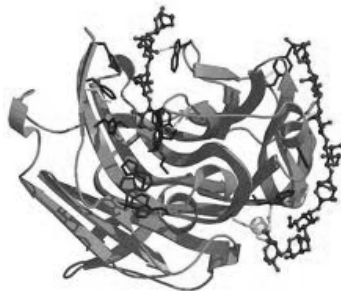
Proteins with catalytic activity due to their power of specific activation and conversion of substrates to products



Some enzymes consist only of protein, but most enzymes contain additional non-protein components such as carbohydrates, lipids, metals, phosphates, or other organic moiety.

# Nature and Function

Size: 2,000 MW  
up to 1,000,000 MW



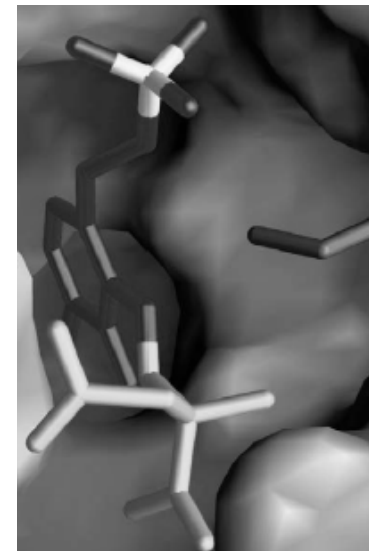
**ENZIM**

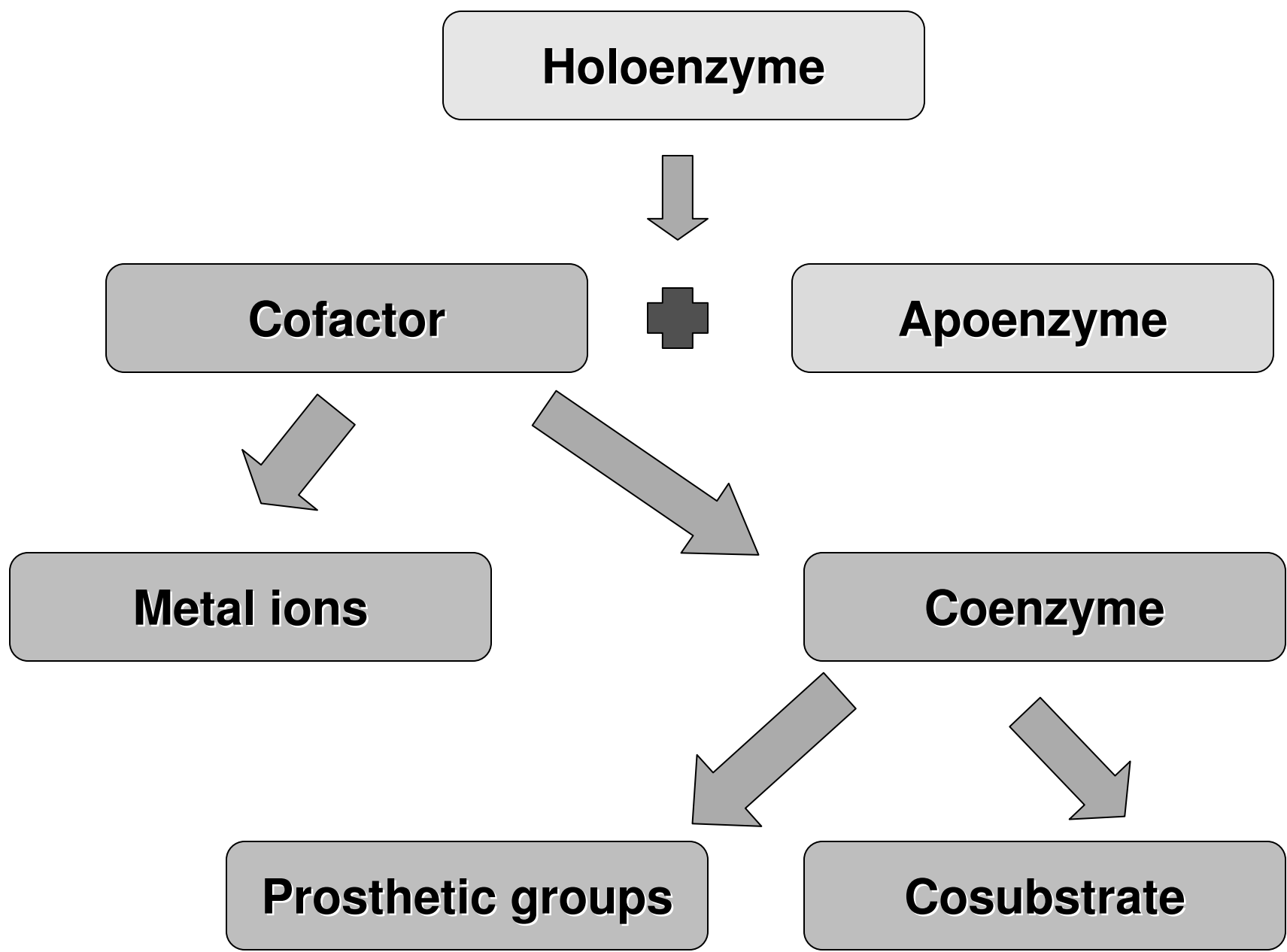
**Holoenzyme: the complete enzyme**

**Apoenzyme: the protein part**

**Cofactor: the non-protein part**

**Coenzyme**





# Common Coenzymes

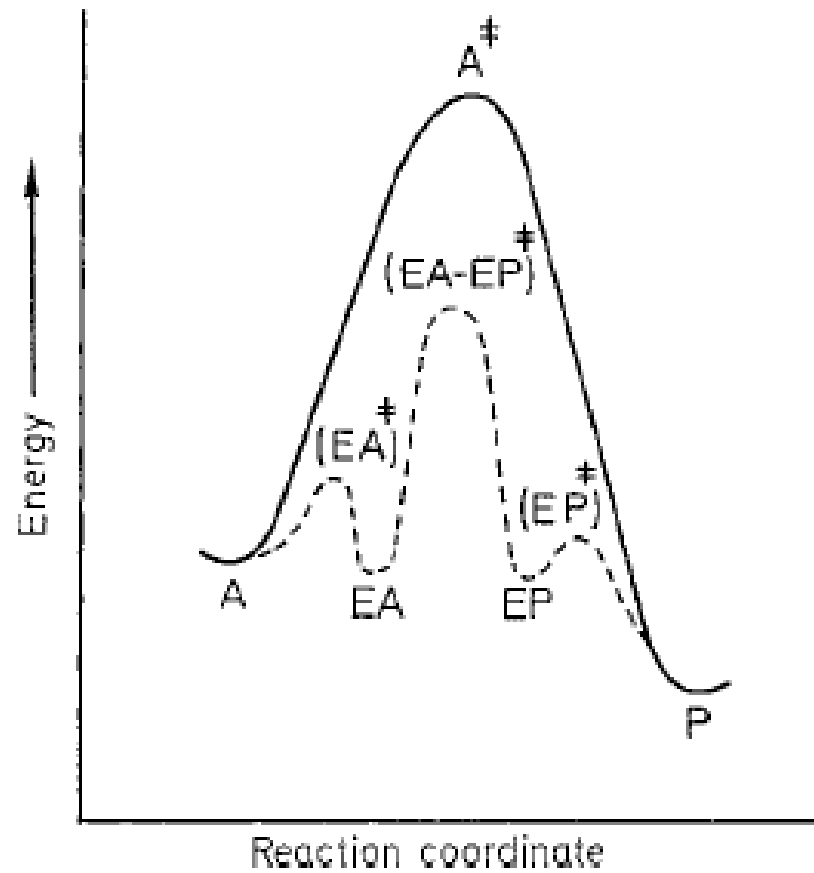
| Coenzyme               | Reaction Catalyzed         |
|------------------------|----------------------------|
| Biotin                 | Carboxylation              |
| Cobalamin (B12)        | Alkylation transfer        |
| Coenzyme A             | Acyl transfers             |
| Flavin                 | Oxido-Reduction            |
| Lipoic acid            | Acyl transfers             |
| Nicotinamide           | Oxido-Reduction            |
| Pyridoxal phosphate    | Amino group transfers      |
| Tetrahydrofolate       | One carbon group transfers |
| Thiamine pyrophosphate | Aldehyde transfer          |

# Nature and Function

- Enzymes are not different from all other proteins found in nature and they comprise a small part of our daily protein intake of our food.
- However, they are highly specific catalyst for the thousand of chemical reactions required by living organisms.
- Enzymes accelerate reactions by factors of  $10^3$  to  $10^{11}$  times that of non-enzyme catalyzed reactions.



- Catalytic activity?



Energy profile of an exergonic reaction

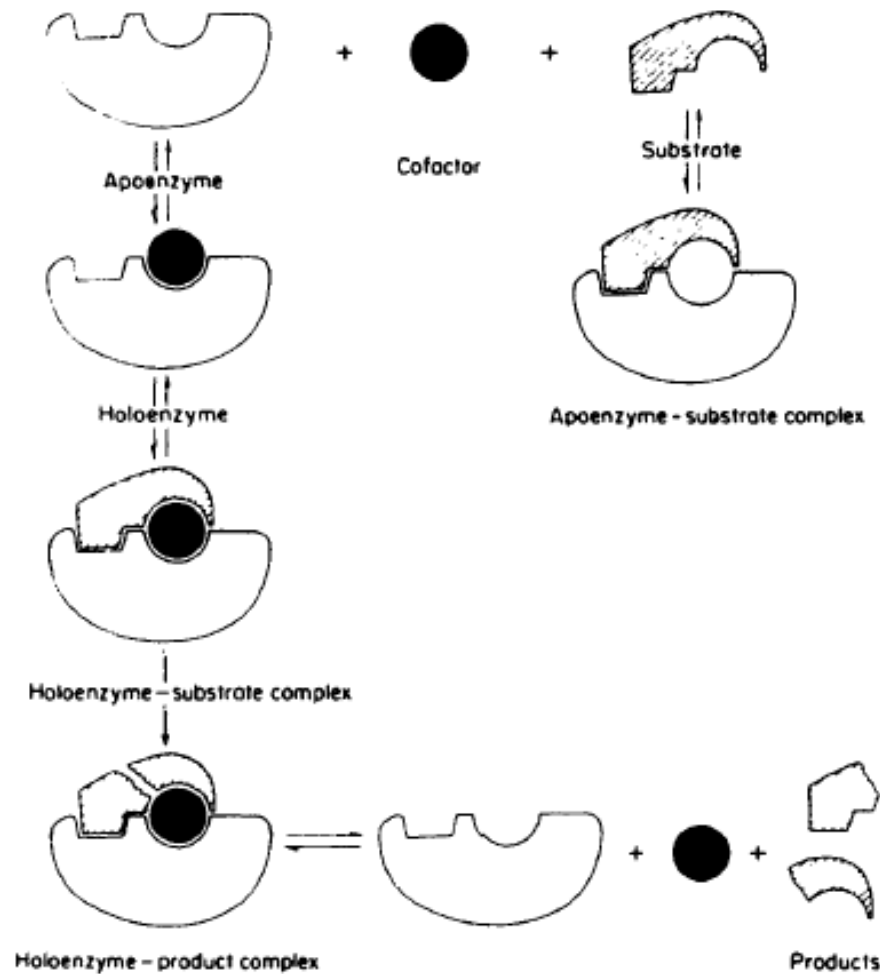
$A \rightarrow P$ ; — without and - - - with catalyst  $E$

Belitz et al., 2009

## Examples of catalyst activity

| Reaction   | Catalyst         | Activation energy (kJ · mol <sup>-1</sup> ) | k <sub>rel</sub> (25 °C) |
|--|------------------|---|--------------------------|
| 1. H <sub>2</sub> O <sub>2</sub> → H <sub>2</sub> O + 1/2 O <sub>2</sub> | Absent           | 75  | 1.0                      |
|  | I <sup>⊕</sup>   | 56.5  | ~ 2.1 · 10 <sup>3</sup>  |
|  | Catalase         | 26.8  | ~ 3.5 · 10 <sup>8</sup>  |
| 2. Casein + n H <sub>2</sub> O →<br>(n+1) Peptides                       | H <sup>⊖</sup>   | 86  | 1.0                      |
|  | Trypsin          | 50  | ~ 2.1 · 10 <sup>6</sup>  |
| 3. Ethylbutyrate<br>+ H <sub>2</sub> O → butyric acid + ethanol          | H <sup>⊖</sup>   | 55  | 1.0                      |
|  | Lipase           | 17.6  | ~ 4.2 · 10 <sup>6</sup>  |
| 4. Saccharose + H <sub>2</sub> O →<br>Glucose + Fructose                 | H <sup>⊖</sup>   | 107   | 1.0                      |
|  | Invertase        | 46  | ~ 5.6 · 10 <sup>10</sup> |
| 5. Linoleic acid<br>+ O <sub>2</sub> → Linoleic acid<br>hydroperoxide    | Absent           | 150–270                                     | 1.0                      |
|  | Cu <sup>2+</sup> | 30–50                                       | ~ 10 <sup>2</sup>        |
|  | Lipoxygenase     | 16.7  | ~ 10 <sup>7</sup>        |

- The compound that is being converted in an enzymic reaction is called *substrate*.
- The substrate combines with the holoenzyme and is released in a modified form.

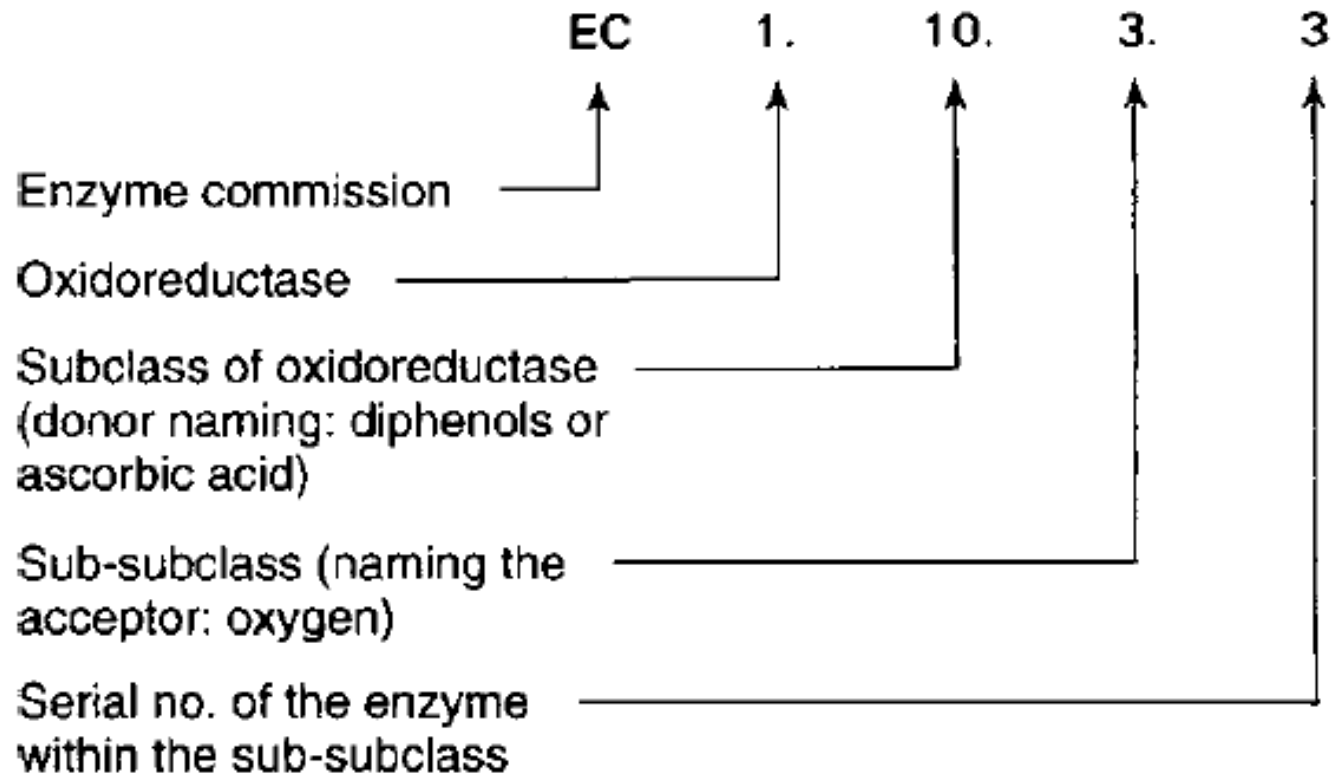


## Substrate Reactions (deMan)

# Enzyme Nomenclature

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

L-ascorbate: oxygen oxidoreductase,  
and its systematic number is E.C. 1.1.10.3.3



# Specificity of Enzymes

- The nature of the enzyme-substrate reaction requires that each enzyme reaction is highly specific.
- There is a unique enzyme property related to its high specificity for both the compound to be converted (substrate specificity) and for the type of reaction to be catalysed (reaction specificity).
- The shape and size of the active site of the enzyme, as well as the substrate, are important.

# Specificity of Enzymes

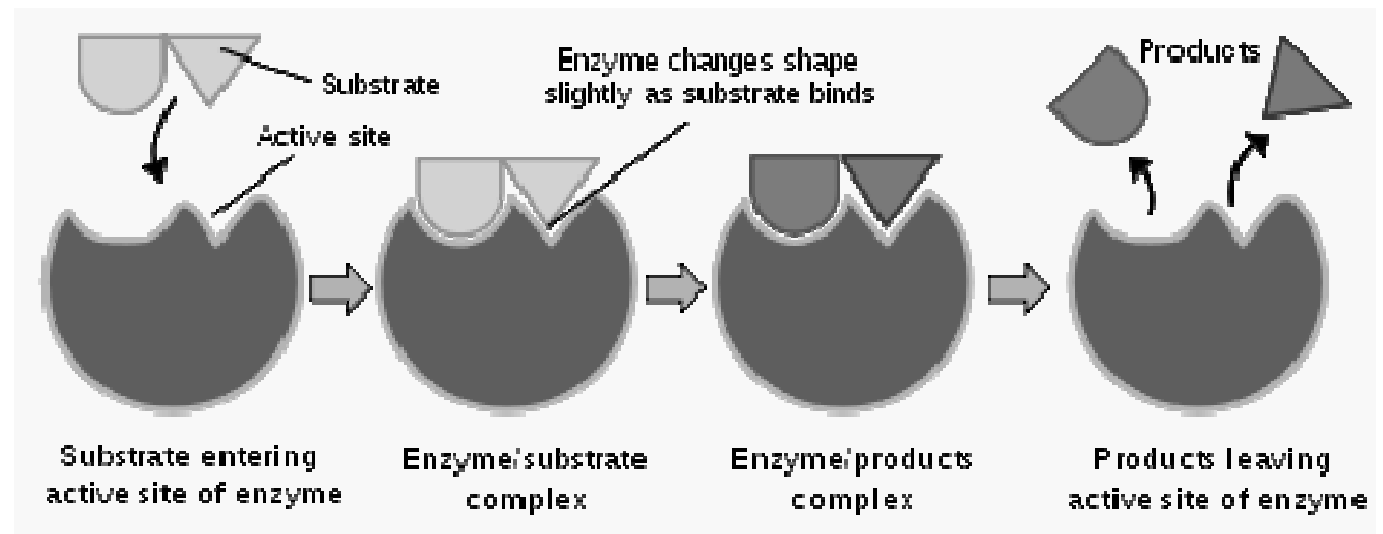
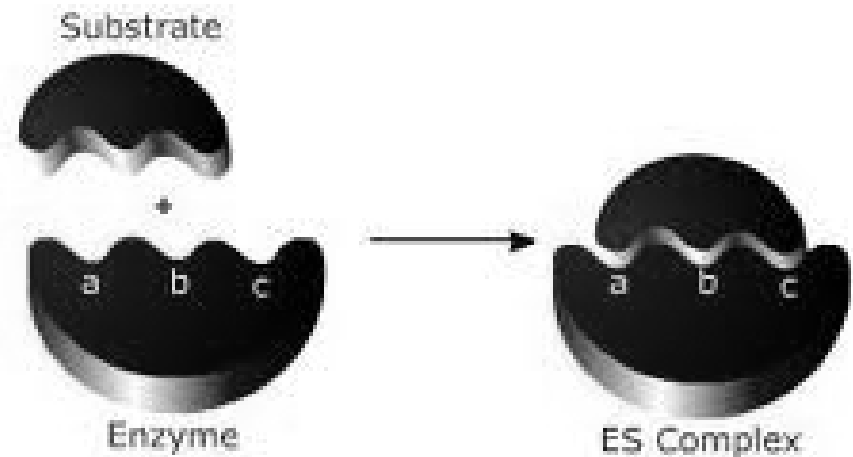
- Substrate specificity
  - The occurrence of a distinct functional group in the substrate is the only prerequisite for a few enzymes. For example: nonspecific lipases or peptidases which generally act on an ester or peptide covalent bond.
  - More restricted specificity is found in proteinases trypsin and chymotrypsin which cleave only ester or peptide bonds with the carbonyl group derived from lysyl or arginyl (trypsin) or tyrosyl, phenylalanyl or tryptophanyl residues (chymotrypsin).

# Specificity of Enzymes

- Many enzymes activate only one single substrate or preferentially catalyze the conversion of one substrate while other substrates are converted into products with a lower reaction rate.
- Special interest for enzymatic food analysis. They can be used for the selective analysis of individual food constituents.

# Substrate Binding

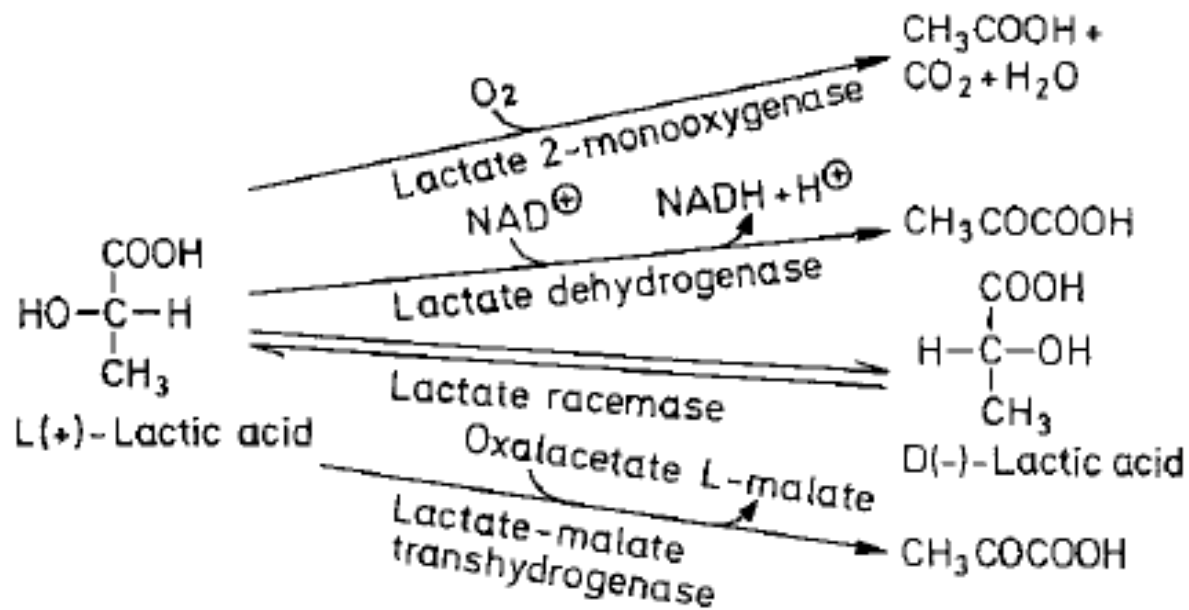
- Lock and Key Model (Emil Fischer, 1894)
- Induced Fit Model (Daniel Koshland, 1958)





# Specificity of Enzymes

- Reaction specificity
  - The substrate is specifically activated by the enzyme so that, among the several thermodynamically permissible reactions, only one occurs.



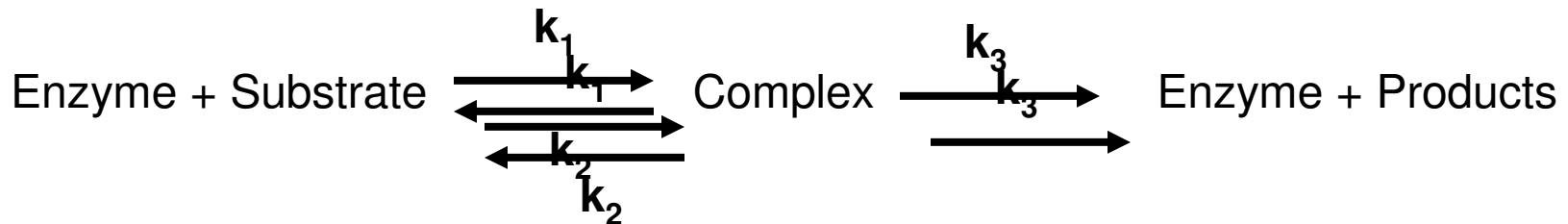
Examples of reaction specificity of some enzymes

# Specificity of Enzymes

- The enzyme's reaction specificity as well as the substrate specificity are predetermined by the structure and chemical properties of the protein moiety of the enzyme.

# Nature and Function

- Enzyme reaction:



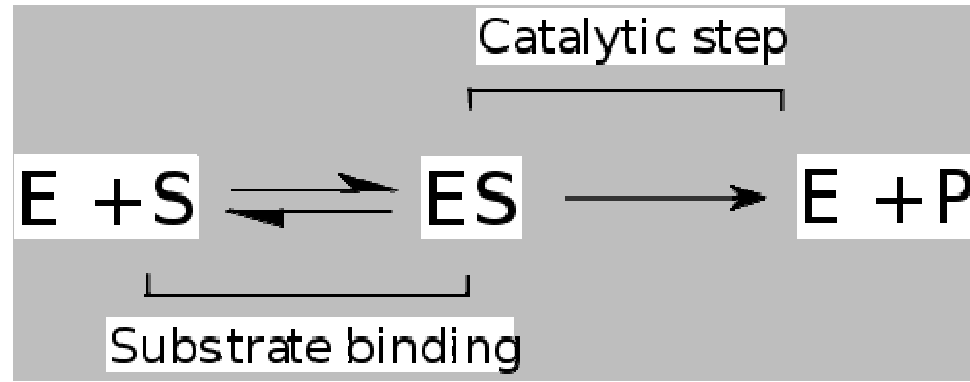
- The equilibrium for the formation of complex:

$$K_m = \frac{[E][S]}{[ES]}$$

$K_m$  = equilibrium constant, E = enzyme, S = substrate, ES = complex

# Kinetics of Enzyme-Catalyzed Reactions

- Single substrate



- Enzyme reactions follow either zero-order or first-order kinetics.
- When the substrate concentration is relatively high, the concentration of the enzyme-substrate complex will be maintained at a constant level and amount of product formed is a linear function of the time interval.

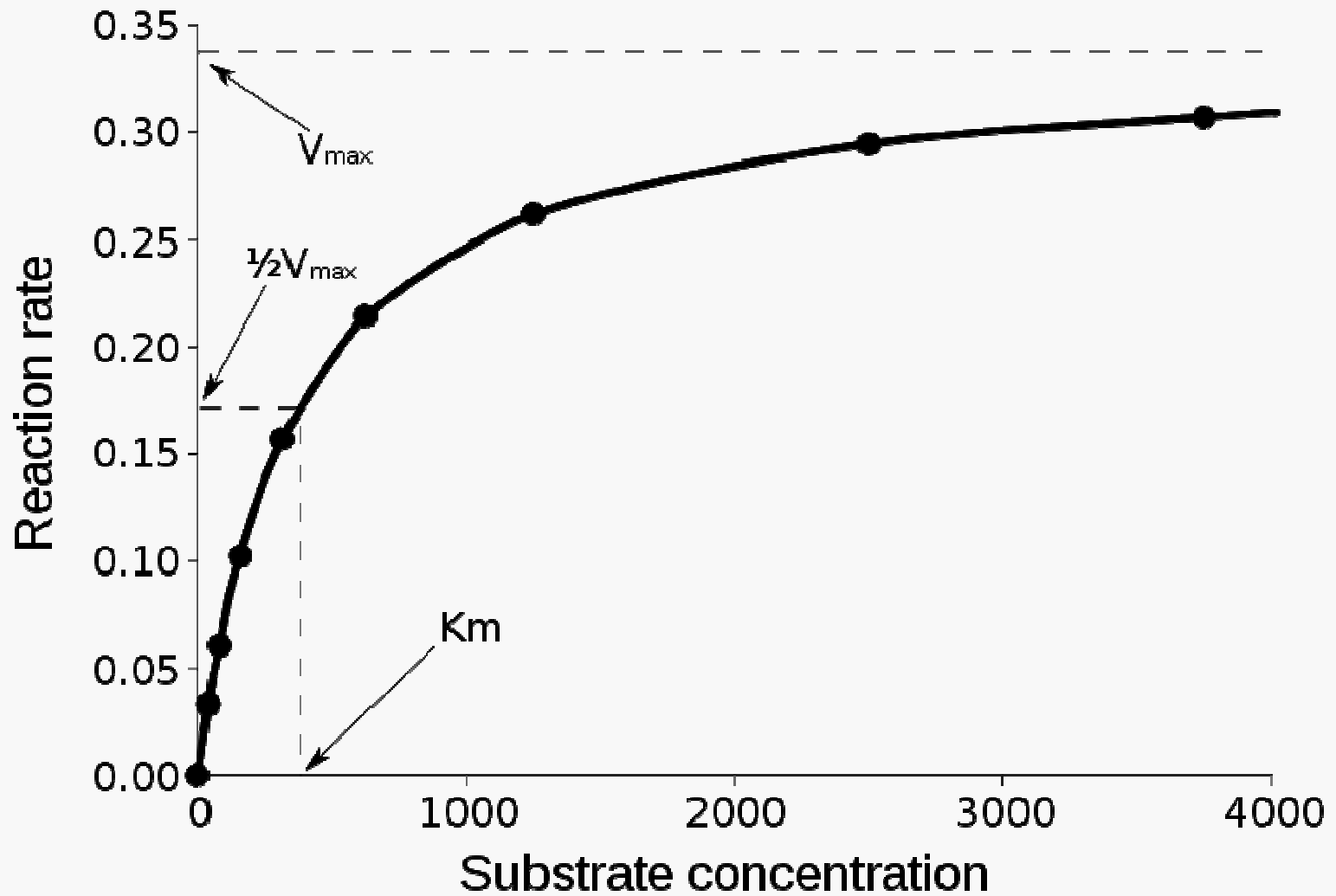
# Kinetics of Enzyme-Catalyzed Reactions

- This can be expressed in the form of the **Michaelis-Menten equation**, as follows:

$$v = V \frac{[S]}{[S] + K_m}$$

- $v$  is the initial short-time velocity of the reaction at substrate concentration  $[S]$
- $V$  is the maximum velocity that can be attained at a high concentration of the substrate where all of the enzyme is in the form of the complex

# Kinetics of Enzyme-Catalyzed Reactions



# Kinetics of Enzyme-Catalyzed Reactions

- Zero-order reactions can be described as follows:

$$k^0 = \frac{d[S]}{dt} \quad S = \text{substrate}$$

- First-order reaction kinetics are characterized by a graduated slowdown of the formation of product.
- This is because the rate of its formation is a function of the concentration of unreacted substrate, which decreases as the concentration of product increases.

# Kinetics of Enzyme-Catalyzed Reactions

- First-order reaction kinetics follow the equation:

$$\frac{d[S]}{dt} = k^1 ([S] - [P])$$

P is product and  $k^1$  is the first-order reaction constant

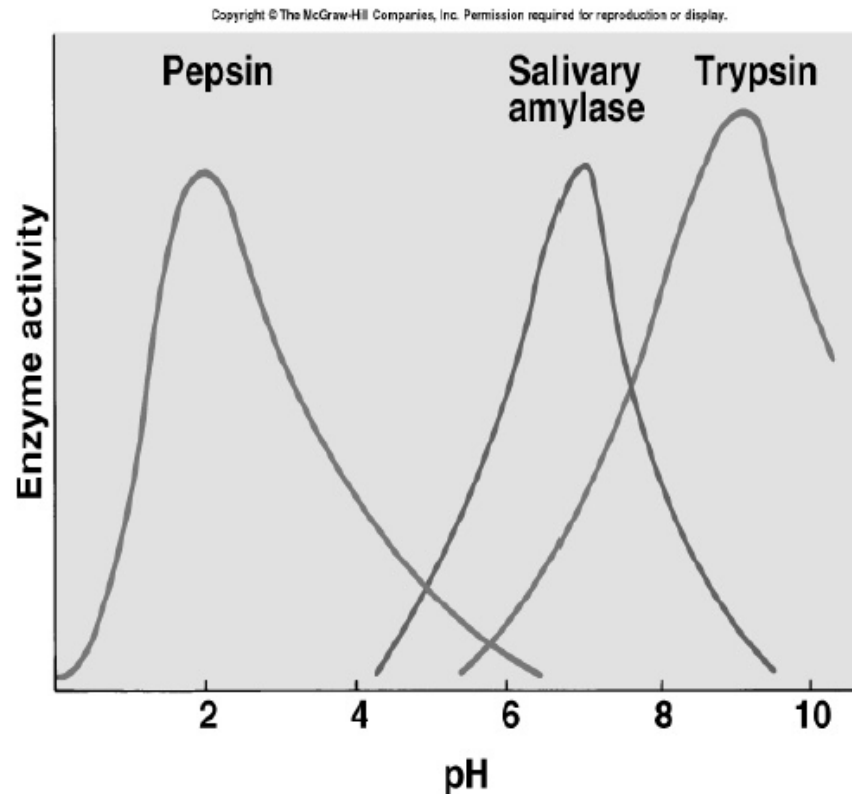


# Factors affecting the effectiveness of enzymes in food processing

- **pH**

- Each enzyme is catalytically active only in a narrow pH range
- Each enzyme has one – and some enzymes have more – optimum pH values.
- For most enzymes this is in the range of 4.5 – 8.0 (max activities). Example:
  - Amylase – 4.8
  - Invertase – 5.0
  - pancreatic  $\alpha$ -amylase – 6.9

- Extremes pH generally inactivate enzymes
- The pH optimum is usually quite narrow, although some enzymes have a broader optimum range.
- Exceptions
  - Pepsin: optimum pH is 1.8
  - Trypsin: optimum pH is 9.8

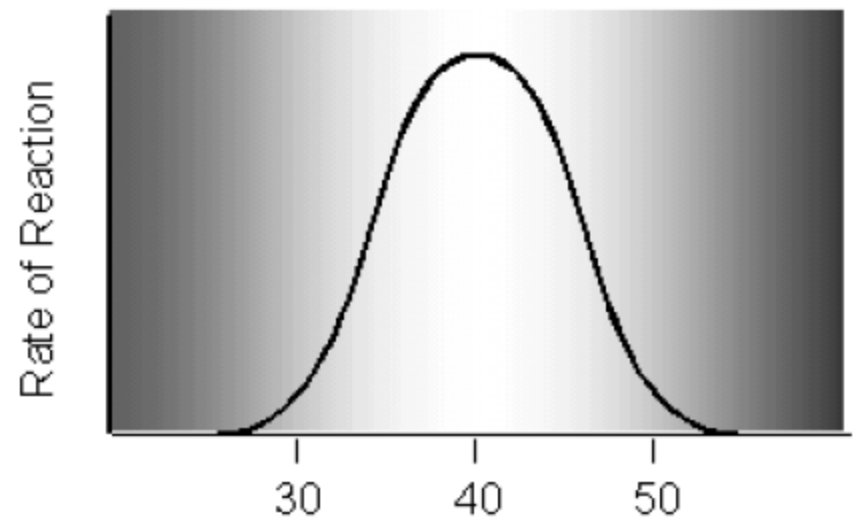


- The optimum pH is affected by the type and ionic strength of the buffer used in the assay.
- The reasons for the sensitivity of the enzyme to changes in pH are two-fold:
  - a. sensitivity is associated with a change in protein structure leading to irreversible denaturation,
  - b. the catalytic activity depends on the quantity of electrostatic charges on the enzyme's active site

# Factors affecting the effectiveness of enzymes in food processing

- **Temperature**

- Enzymes function very slowly at sub-freezing temperatures.
- Optimal activity in the 30-40°C range.
- Denature above 45°C.  
→ the activity quickly decreases



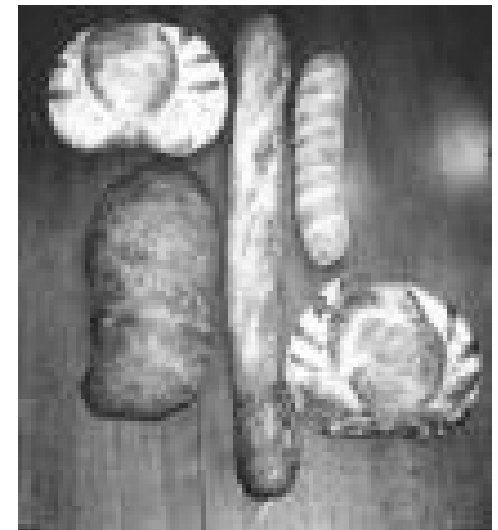
- Freezing
  - Activity depends on the enzyme (0 –10°C)
  - Below –10°C almost always decrease activity
- Heat treatment may either accelerate desirable chemical or enzymatic reactions or inhibit undesirable changes by inactivation of enzymes or microorganisms.

## Thermal inactivation of enzymes to prevent deterioration of food quality

| Food product                       | Enzyme                            | Quality loss  |
|------------------------------------|-----------------------------------|---|
| Potato products,<br>apple products | Monophenol<br>oxidase             | Enzymatic browning  |
| Semi-ripe peas                     | Lipoxygenase,<br>peroxidase       | Flavor defects;<br>bleaching                              |
| Fish products                      | Proteinase,<br>thiaminase         | Texture (liquefaction),<br>loss of vitamin B <sub>1</sub> |
| Tomato purée                       | Polygalacturonase                 | Texture (liquefaction)                                    |
| Apricot products                   | $\beta$ -Glucosidase              | Color defects   |
| Oat flakes                         | Lipase,<br>lipoxygenase           | Flavor defects<br>(bitter taste)                          |
| Broccoli                           | Cystathionine                     | Off-flavor  |
| Cauliflower                        | $\beta$ -Lyase<br>(cystine-lyase) |   |

# Water Activity & Enzymatic Activity

- Enzymatic reaction requires water  $\rightarrow a_w \downarrow$   
 $\rightarrow$  reaction  $\downarrow \downarrow$   
 $a_w \downarrow$  activity is still present  $\rightarrow$  blanching prior to drying of vegetables & fruits is necessary
- The activity of nonhydrolytic enzyme are also influenced by  $a_w$ . E.g. In whey protein concentrate containing lipoxigenase. Water serves as a medium for the reaction & a vehicle for the substrate  $\rightarrow$  when water is tightly bound, enzymatic oxidation is slow or impossible
- Enzymes are more stable in dry state. The higher the moisture content  $\rightarrow$  The higher tendency of inactivation by heat  
E.g. Not all enzymes are inactivated in baking of some baked goods



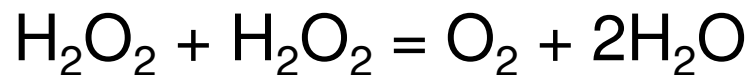
- **Electrolytes and ionic strength**
  - Ions may be required components in the active site
  - Cation requirements of enzymes is sometimes specific
- **Chemicals**
  - Chelating agents
  - Reducing agents
  - Alterations of substrates



# Six Main Types of Enzymes (1)

- **Oxidoreductases**

- Enzymes that oxidize or reduce substrates by transfer of hydrogens or electrons or by use of oxygen.
- A number of oxidoreductases have been suggested particularly for aroma improvement
- The systematic name is formed as “ donor:acceptor oxidoreductase”.
- Example: hydrogen peroxide: hydrogen peroxide oxidoreductase (catalase, EC 1.11.1.6)



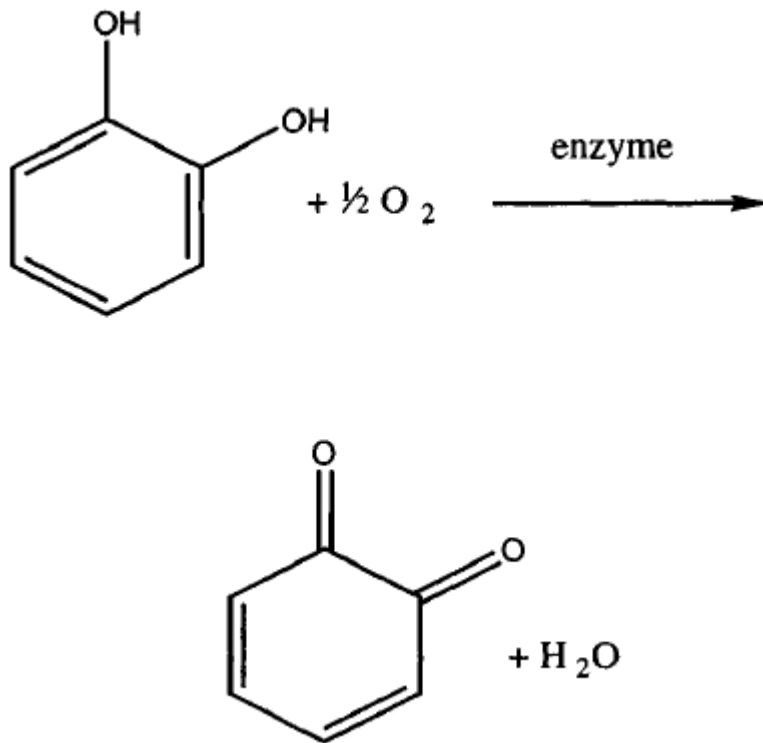
# The examples of oxidoreductase

## 1. Phenolases

- The enzymes involved in enzymic browning are known by the name polyphenoloxidase / polyphenolase/ phenolase.
- These terms include all enzymes that have the capacity to oxidize phenolic compounds to *o*-quinones.
- This can be represented by the conversion of *o*-dihydroxyphenol to *o*-quinone.



## Phenolase



- The action of polyphenolases is detrimental when it leads to browning in bruised and broken plant tissue but is beneficial in the processing of tea and coffee.

The enzyme occurs in almost all plants, but relatively high levels are found in potatoes, mushrooms, apples, peaches, bananas, avocados, tea leaves, and coffee beans.

## Phenolase

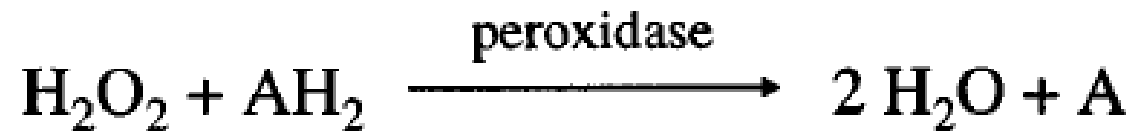
- The substrates of the polyphenol oxidase enzymes are phenolic compounds present in plant tissues, mainly flavonoids.
- These include catechins, anthocyanidins, leucoanthocyanidins, flavonols, and cinnamic acid derivatives.
- Some specific examples of polyphenolase substrates are chlorogenic acid, caffeic acid, dicatechol, protocatechuic acid, tyrosine, catechol, dihydroxyphenylalanine, pyrogallol, and catechins.

## Phenolase

- To prevent or minimize enzymic browning of damaged plant tissue involves:
  - the exclusion of molecular oxygen
  - the addition of reducing agents that can prevent the accumulation of *o*-quinones, ex: the use of L-ascorbic acid as a reducing agent. The ascorbic acid reacts with the *o*-quinones and changes them back into *o*-diphenols.
  - heat treatment is effective in deactivating the enzymes.
  - metal complexing agents may deactivate the enzyme by making the copper unavailable.

## 2. Peroxidase

- The reaction type catalyzed by peroxidase involves hydrogen peroxide as an acceptor, and a compound AH<sub>2</sub> as a donor of hydrogen atoms.



- The peroxidases can be classified into the two groups, iron-containing peroxidases (peroxidases from plants such as horseradish, fig, turnip) and flavoprotein peroxidases (peroxidases from animal tissues and milk – lactoperoxidase).

## Peroxidase

- Because of the widespread occurrence of peroxidase in plant tissues, it has been suggested that peroxidase plays an important role in the development and senescence of plant tissues.
- It plays a role in biogenesis of ethylene; in regulating ripening and senescence; and in the degradation of chlorophyll.
- The peroxidase test is used as an indicator of satisfactory blanching of fruits and vegetables.
- However, the enzymes causing off-flavors during frozen storage can, under some conditions, be regenerated.

## Peroxidase

- The deactivation of peroxidase is a function of heating time and temperature.
- Lactoperoxidase is completely deactivated by heating at 85°C for 13 seconds.
- Lactoperoxidase can be regenerated under conditions of high temperature short time (HTST) pasteurization.
- The regeneration effect depends greatly on storage temperature; the lower the storage temperature, the smaller the regeneration effect.



### 3. Glucose oxidase

- The enzyme produced by fungi such as *Aspergillus niger* and *Penicillium notatum*.
- It catalyzes glucose oxidation by consuming oxygen from the air. Hence, it is used for the removal of either glucose or oxygen.
- The  $H_2O_2$  formed in the reaction is occasionally used as an oxidizing agent, but it is usually degraded by catalase.

## Glucose oxidase

- Application:
  - Removal of glucose during the production of egg powder using glucose oxidase prevents the *Maillard* reaction responsible for discoloration of the product and deterioration of its whippability.
  - It would enhance the golden-yellow color rather than the brown color of potato chips or French fries which is obtained in the presence of excess glucose.

## Glucose oxidase

- Removal of  $O_2$  from a sealed package system results in suppression of fat oxidation and oxidative degradation of natural pigments. For example, the color change of crabs and shrimp from pink to yellow is hindered by dipping them into a glucose oxidase solution.
- The combination of glucose oxidase and catalase will prolong the shelf life of citrus fruit juices, beer and wine since the oxidative reactions which lead to aroma deterioration are retarded.

## **Other examples of oxidoreductase**

- Catalase
- Lipoxygenase
- Aldehyde Dehydrogenase
- Butanediol Dehydrogenase

# Six Main Types of Enzymes (2)

- **Transferase**

- Enzymes that remove groups (not including H) from substrates and transfer them to acceptor molecule (not including water).
- The systematic name is formed as “donor:acceptor group – transferred-transferase”.
- Example: ATP:d-glucose 6-phosphotransferase (glucokinase, EC 2.7.1.2)



## Transferase

- Protein glutamine- $\gamma$ -glutamyl transferase (transglutaminase, TGase) catalyzes the acyl transfer between the  $\gamma$ -carboxyamide group of peptide-bound glutamine (acyl donor) and primary amines (acyl acceptor).
- TGases play an important role in the metabolism of animals and plants.
- The TGase from the actinomycete *Streptoverticillum mobaraense* is used for the production of protein gels.

## Transferase

- The pH optimum of TGase activity is 5 – 8.
- This enzyme can also be used at low temperatures and is rapidly denatured at 70 °C.
- The viscoelastic properties of the resulting protein gels depend not only on the type of proteins and the catalytic conditions (TGase concentration, pH, temperature, time), but also on the pretreatment of the protein, e. g., heat denaturation.

# Transferase

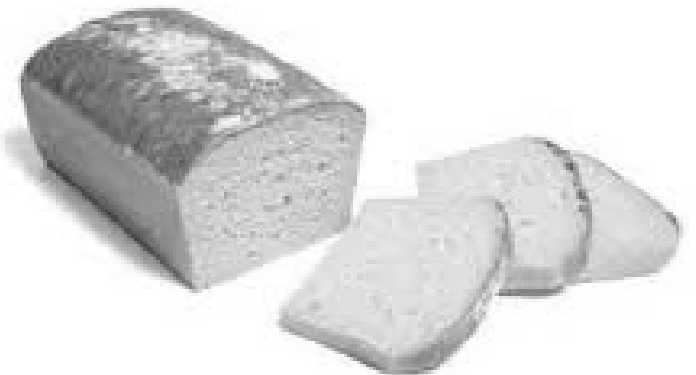
## Possible applications of transglutaminase

| Raw material | Application  |
|--------------|--|
| Meat         | Restructured meat from small pieces<br>Partial replacement of cutter aids in the production of boiling sausage (“Brühwurst”).  |
| Fish         | Production of fish gel (surimi, cf. 13.1.6.11)<br>Reducing water loss in the thawing of frozen fish.   |
| Milk         | Texture control of low-fat yoghurt to produce the palate feeling of a whole-fat product<br>Increasing the solubility of casein in the presence of $\text{Ca}^{2\oplus}$ ions or at a lower pH, e. g., for beverages.<br>Cross linking of casein with whey proteins to increase the protein yield in cheese making. |
| Wheat        | “Hardening” of soft wheat flour for the production of pasta.   |



## Transferase

- The application of TGase also includes in bakery products, especially in gluten free-bread formulation.
- In the formulation brown rice flour/ corn flour is used to replace wheat flour and there is an addition of transglutaminase (TGase).
- The role of TGase is to form cross link between protein from gluten free-cereals.



# Six Main Types of Enzymes (3)

- **Hydrolases**

- Most of the enzymes used in the food industry belong to the class of hydrolase enzymes.
- Enzymes in which water participates in the breakage of covalent bonds of the substrate.
- The systematic name is formed as ‘substrate hydrolase’.
- Water is not listed as a substrate, even though it is, since the concentration doesn’t change significantly during reaction.

– Example:

Triacylglycerol + H<sub>2</sub>O = diacylglycerol + a fatty  
acid anion

catalyzed by triacylglycerol acylhydrolase  
(triacylglycerol lipase, EC 3.1.1.3).

# The examples of hydrolase

## 1. Lipase

- Lipases are produced by microorganisms (bacteria and molds); by plants; present in animals (especially in the pancreas; and are present in milk.
- Undesirable effect:
  - Lipases may cause spoilage of food because the free fatty acids formed cause rancidity.
  - For instance, hydrolysis of milk fat in milk leads to very unpleasant off flavors at very low free fatty acid concentration.

– Desirable effect:

- The hydrolysis of triglycerides in cheese is an example of a desirable flavor-producing process.
- The extent of free fatty acid formation is much higher in blue cheese.
- This is most likely the result of lipases elaborated by organisms growing in the blue cheese, such as *P. roqueforti*, *P. camemberti*, and others.
- The extent of lipolysis increases with age, as is demonstrated by the increasing content of partial glycerides during the aging of cheese.

## 2. Amylases

- The amylases are the most important enzymes of the group of glycoside hydrolases.
- Amylases are either produced by bacteria or yeasts or they belong to the components of malt preparations.
- These enzymes can be divided into two groups, (1) that specifically hydrolyze the 1,6-linkages between chains, and (2) the enzymes that split the 1,4-linkages between glucose units of the straight chains.

# Amylases

- **Example *Alpha-amylase***

- Alpha-amylase ( $\alpha$ -1,4-glucan-4-glucanohydrolase) is an endoenzyme that hydrolyzes the  $\alpha$ -1,4-glucosidic bonds in a random fashion along the chain.
- It hydrolyzes amylopectin to oligosaccharides that contain two to six glucose units → leads to a rapid decrease in viscosity, but little monosaccharide formation.
- A mixture of amylose and amylopectin will be hydrolyzed into a mixture of dextrans, maltose, glucose, and oligosaccharides. Amylose is completely hydrolyzed to maltose.

## Amylases

- $\alpha$ -Amylases added to the wort in the beer production process accelerate starch degradation.
- These enzymes are also used in the baking industry, especially for binding water and enhancing the desirable browning.



### **3. Peptidases**

- These proteolytic enzymes are isolated from animal organs, higher plants or microorganisms.
- The application in bakery products:
  - Proteinases are added to wheat flour in the production of some bakery products to modify rheological properties of dough and, thus, the firmness of the end product.
  - During such dough treatment, the firm or hard wheat gluten is partially hydrolyzed to a soft-type gluten.

## Peptidases

- The application in dairy industry:
  - In cheese making, the formation of casein curd is achieved with chymosin or rennin or other proteinases.
  - Rennin is essentially free of other undesirable proteinases and is, therefore, especially suitable for cheese making.
  - Proteinases from *Mucor miehei*, *M. pusillus* and *Endothia parasitica* are a suitable replacement for rennin.

# Peptidases

- The application in meat industry:
  - Plant proteinases and also those of microorganisms are utilized for ripening and tenderizing meat.
  - The practical problem to be solved is how to achieve uniform distribution of the enzymes in muscle tissue.
  - An optional method appears to be injection of the proteinase into the blood stream immediately before slaughter, or rehydration of the freeze-dried meat in enzyme solutions.

## Other examples of hydrolases

- Glucan-1,4- $\alpha$ -D-Glucosidase (Glucoamylase)
- Pullulanase (Isoamylase)
- Endo-1,3(4)- $\beta$ -D-Glucanase
- $\alpha$ -D-Galactosidase
- $\beta$ -D-Galactosidase (Lactase)
- $\beta$ -D-Fructofuranosidase (Invertase)
- $\alpha$ -L-Rhamnosidase
- Cellulases & Hemicellulases
- Lysozyme
- Thioglucosidase
- Pectolytic Enzymes
- Tannases
- Glutaminase

# Six Main Types of Enzymes (4)

- **Lyases**

- Enzymes that remove groups from their substrate (not by hydrolysis) to leave a double bond, or which conversely add groups to double bonds.
- The systematic name is form as “substrate prefix-lyase”.
- Prefixes such as “hydro-” and “ammonia-” are used to indicate the type of reaction.
- Example: L-malate hydro-lyase (EC 4.2.1.2)  
Decarboxylases are named as carboxy-lyases.

- (S)-Malate = fumarate + H<sub>2</sub>O  
using the enzyme (S)-malate hydro-lyase (fumarate hydratase, EC 4.2.1.2, formerly known as fumarase)

# Six Main Types of Enzymes (5)

- **Isomerases**

- Enzymes that bring about isomerization of substrate.
- The systematic name is formed as “substrate prefix – isomerase”.
- The prefix indicates the type of isomerization involved, example: ‘ maleate *cis-trans*-isomerase’.
- Enzymes that catalyze an aldose-ketose are known as “ketol-isomerases”.
- Isomerases that catalyze inversions of assymmetric groups are termed “racemases” or “epimerases”.  
Ex: L-Alanine = D-alanine, with alanine racemase.

# Six Main Types of Enzymes (6)

- **Ligases**

- Enzymes that catalyze the covalent linking together of 2 molecules, coupled with the breaking of a pyrophosphate bond as in ATP.

- This group has previously been referred to as the “synthetases”.

- The systematic name is formed as “X:Y ligase (Z),” where X and Y are the 2 molecules to be joined together.

- Example:



pyrophosphate + L-asparagine

with L-aspartate:ammonia ligase (aspartate-ammonia ligase)



# Typical concentrations of enzymes in some foods

- Lipoxygenase
  - It is very high in concentration in soybeans.
- Polygalacturonase
  - Responsible for softening
  - Varies widely in plant sources like tomato, avocado, pear, and pineapple.

# Typical concentrations of enzymes in some foods

- Peroxidase
  - It is found in all fruits, but varies some sevenfold from English green peas to lima beans.
- Polyphenol oxidase
  - The most noticeable enzymes in plants.
  - It is present at high concentrations in some grapes, prune plums, figs, dates, tea leaves, and coffee beans.
  - It is present at moderate concentrations in peaches, apples, bananas, potatoes, and lettuce.

# The role of endogenous enzymes in food processing

- **COLOR**

- Oxidation of the Fe (II) present in oxymyoglobin and deoxymyoglobin, to Fe (III) producing metmyoglobin.
- Enzyme-catalyzed reactions in meat can compete for oxygen, can produce compounds that alter the oxidation-reduction state and water content → induce the color of meat

- The quality of fresh vegs and fruits → on ripening, the green color of many fruits decreases and is replaced with other colors.
- The maturity leads to a decrease in chlorophyll level.
- The all changes are a result of enzyme reaction.
- 3 key enzymes responsible for chemical alterations of pigments in fruits and vegs are lipoxygenase, chlorophyllase and polyphenol oxidase.

- **TEXTURE**

- In fruits and vegs, texture is due primarily to the complex carbohydrates.
- There are enzyme(s) that act on each of the complex carbohydrates that are important in food texture, such as pectic enzymes, cellulases, amylases, pentosanases, etc.
- Proteases are important in the softening of animal tissues and high-protein plant foods.

- **FLAVOR & AROMA**

- It is difficult to identify the enzymes instrumental in the biosynthesis of flavors typical of food flavors and in the development of undesirable flavors.
- Enzymes cause flavors and off aromas in foods, particularly during storage.
- Improperly blanched foods, develop very noticeable off flavors and off aromas during frozen storage.

- Peroxidase (heat resistant enzyme) is generally used as the indicator for adequate heat treatment of blanched foods.
- Lipoxygenase is responsible for off flavor and off aroma development in peas and corn.
- Cystine lyase is responsible for off flavor and off aroma development in broccoli and cauliflower.
- Naringin is responsible for the bitter taste of grapefruit and grapefruit juice → destroyed by naraginase.

- **NUTRITIONAL QUALITY**

- Lipoxygenase, oxidize linoleic, linolenic, and arachidonic acids → certainly decrease amount of these essential fatty acids in foods.
- The free radicals produced by lipoxygenase-catalyzed oxidation of PUFA decrease the carotenoids, tocopherols, vitamin C, and folate content of foods.
- The free radicals also are damaging to cysteine, tyrosine, tryptophan and histidine residues of proteins.



- Ascorbic acid is destroyed by ascorbic acid oxidase in some vegetables.
- Thiaminase destroys thiamine.
- Riboflavin hydrolase (found in some microorganisms) can degrade riboflavin.
- Polyphenol oxidase-caused browning decreases the available lysine content of proteins.

# Enzymes used as processing aids and ingredients

- Enzymes are ideal for:
  - producing key changes in the functional properties of food
  - removal of toxic constituents
  - producing new ingredients
  
- Because they are highly specific, act at low temperature (25-45°C) and do not produce side reactions.

- There are some major successes in the use of food-related enzymes. Ex: the production of high-fructose corn syrup and sweeteners.
- This involves a relatively heat stable  $\alpha$ -amylase, glucoamylase and glucose isomerase.



- Enzymatic production of valuable compounds
  - Proteases → surfactants; decrease ripening time of cheeses; tenderization of meat; meaty flavors
  - Lipases → flavor esters
  - Aspartase → aspartate
  - Tannase → antioxidants such as propylgallate
- Enzymatic removal of undesirable compounds
  - Raw food materials often contain toxic or anti-nutrient compounds that are sometimes removed by proper heat treatment, extraction or by enzymatic reactions.
  - Phytase → phytic acid
  - Catalase → hydrogen peroxide
  - Cyanidase → cyanide
  - Protease → phenylalanine

- Enzymes in baking
  - Amylase → to maximize fermentation process; prevent staling
  - Proteases → improve handling and rheological properties
  - Glutamyl transferase → improve dough elasticity, loaf volume
- Enzymes in milk and dairy products
  - Chymosin → milk coagulation
  - Proteases → flavor improvement
  - B-galactosidase → lactose removal
  - Sulfhydryl oxidase → removed cooked flavor