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. Examples?
- Enzymes → food science deals with decompositions, hydrolysis, and oxidation.

Introduction 2

- Undesirable
  - In vegetables/ fruits --- should be blanched
- Desirable
  - Indicator for analytical methods, such as phosphatase (for milk pasteurisation)
  - Rennin, as a coagulant for milk in cheese production

Nature and Function

- Enzymes are proteins with catalytic activity due to their power of specific activation and conversion of substrates to products:

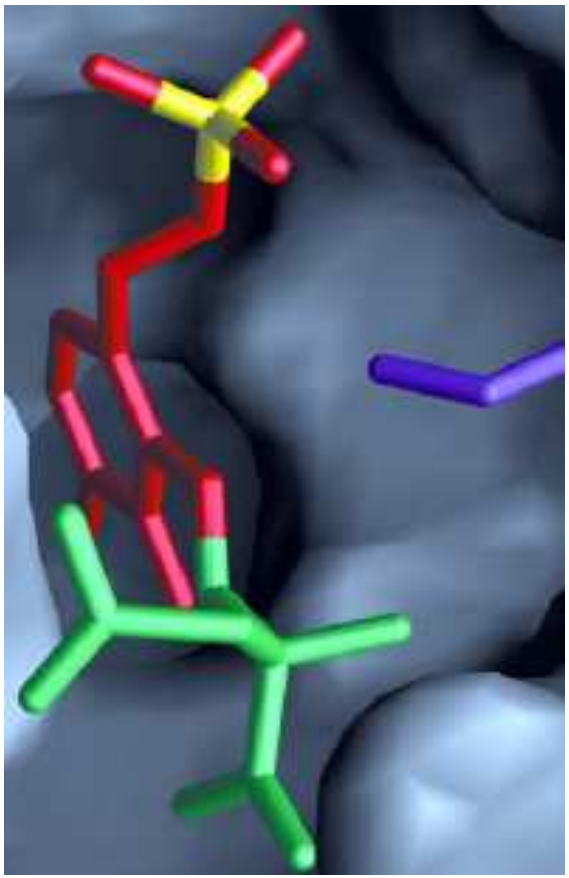
- Some enzymes consist only of protein, but most enzymes contain additional non-protein components such as carbohydrates, lipids, metals, phosphates, or other organic moiety.

Nature and Function

- The range of enzyme size starts from 12,000 MW up to 1,000,000 MW.
- Enzymes are proteins that are synthesized in the cells of plants, animals, or microorganisms.
- The complete enzyme is called *holoenzyme*; the protein part, *apoenzyme*; and the nonprotein part, *cofactor*.

Enzymes

- Cofactors
- Coenzymes
- Holoenzyme
- Apoenzyme



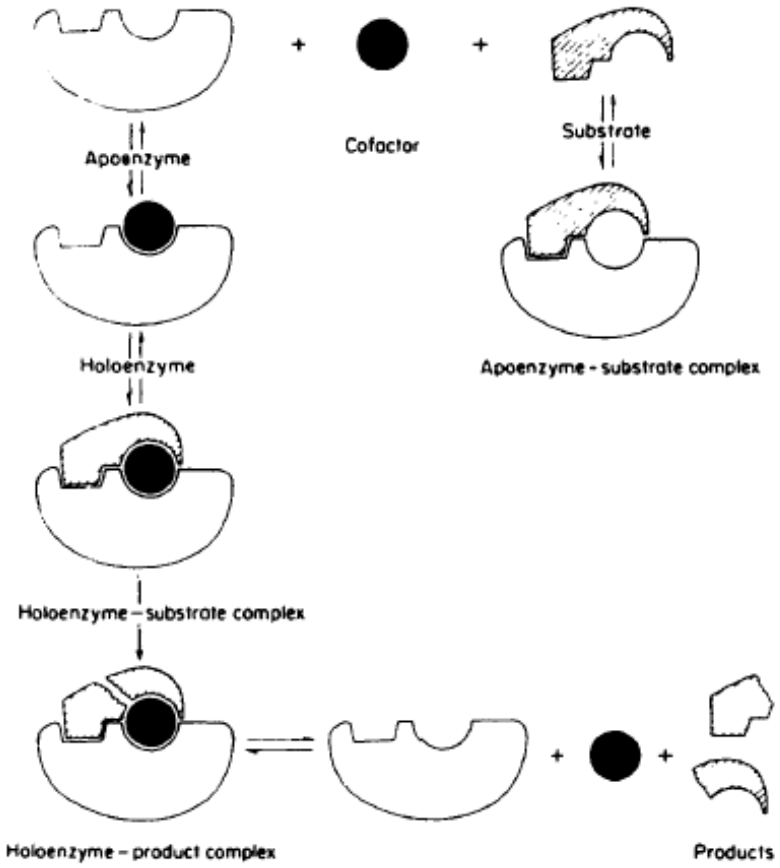
Common Coenzymes

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, unlike other groups of proteins, 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.

- 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)

Nature and Function 3

- Enzyme reaction:

- The equilibrium for the formation of complex:

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

Km = equilibrium constant, E = enzyme, S = substrate

ES = complex

- 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

- 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.
- Zero-order reactions can be described as follows:

$$k^o = \frac{d[S]}{dt}$$

S= 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.
- 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

Specificity of Enzymes

- The nature of the enzyme-substrate reaction requires that each enzyme reaction is highly specific.
- The shape and size of the active site of the enzyme, as well as the substrate, are important.
- Types of specificity may include group, bond, stereo, and absolute specificity, or some combination of these.

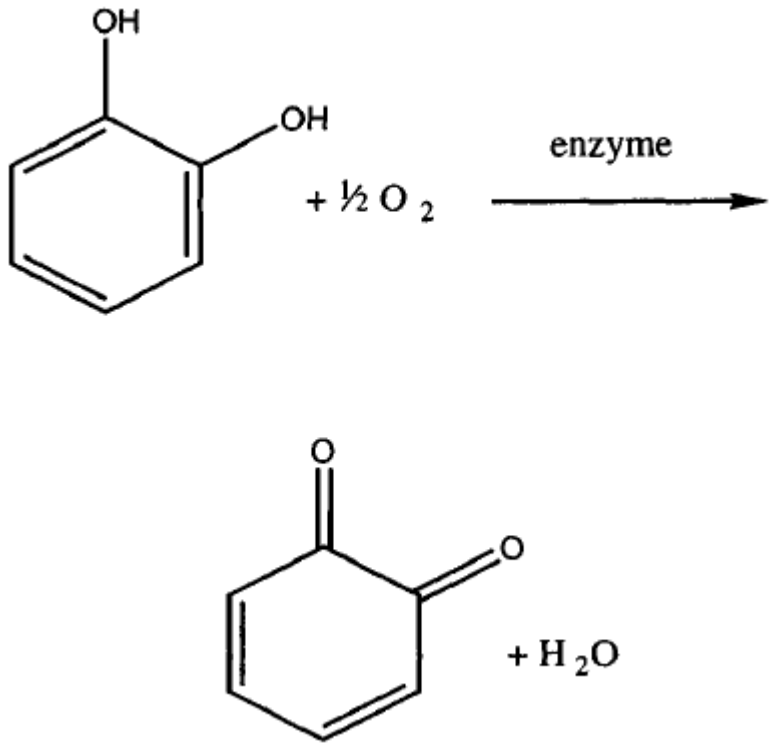
Six Main Types of Enzymes (1)

- Oxidoreductases
  - Enzymes that oxidize or reduce substrates by transfer of hydrogens or electrons or by use of oxygen.
  - The systematic name is formed as “ donor:acceptor oxidoreductase”.
  - Example: hydrogen peroxide: hydrogen peroxide oxidoreductase (catalase, EC 1.11.1.6)

$$H_2O_2 + H_2O_2 = O_2 + 2H_2O$$

The examples of oxidoreductase

- 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.



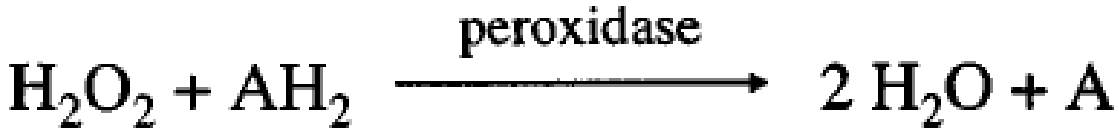
- The action of polyphenolases is detrimental when it leads to browning in bruised and broken plant tissue but is beneficial in the processing of tea and coffee.

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.

- 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.

- 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.

- Peroxidase
  - The reaction type catalyzed by peroxidase involves hydrogen peroxide as an acceptor, and a compound AH2 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).

- 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.

- The deactivation of peroxidase is a function of heating time and temperature.
- Lactoperoxidase is completely deactivated by heating at 85^0C 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.

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)

    ATP + D-glucose = ADP + D-glucose 6-phosphate

Six Main Types of Enzymes (3)

- **Hydrolases**
  - Enzymes in which water participates in the breakage of covalent bonds of the substrate, with concurrent addition of the elements of water to the principles of those bonds.
  - 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_2O = diacylglycerol + a fatty acid anion

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

The examples of hydrolase

- **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.

- **Amylases**
  - The amylases are the most important enzymes of the group of glycoside hydrolases.
  - These starch-degrading 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.

- **Example *Alpha-amylase***
  - Alpha-amylase (a-1,4-glucan-4-glucanohydrolase) is an endoenzyme that hydrolyzes the a-l,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 dextrins, maltose, glucose, and oligosaccharides. Amylose is completely hydrolyzed to maltose

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_2O

  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 assymetric 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:

    ATP + L-aspartate + NH3 = AMP + pyrophosphate + L-asparagine

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

Typical concentrations of enzymes in some foods

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

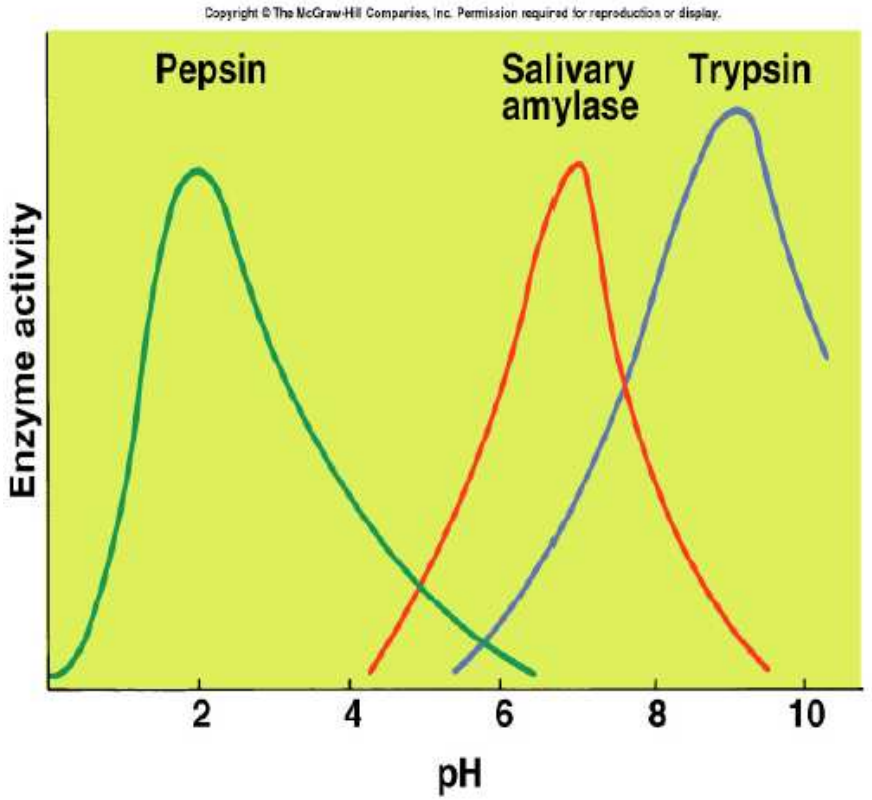
Typical concentrations of enzymes in some foods

- 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.

Factors affecting the effectiveness of enzymes in food processing

- **pH**
  - 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 α-amylase – 6.9

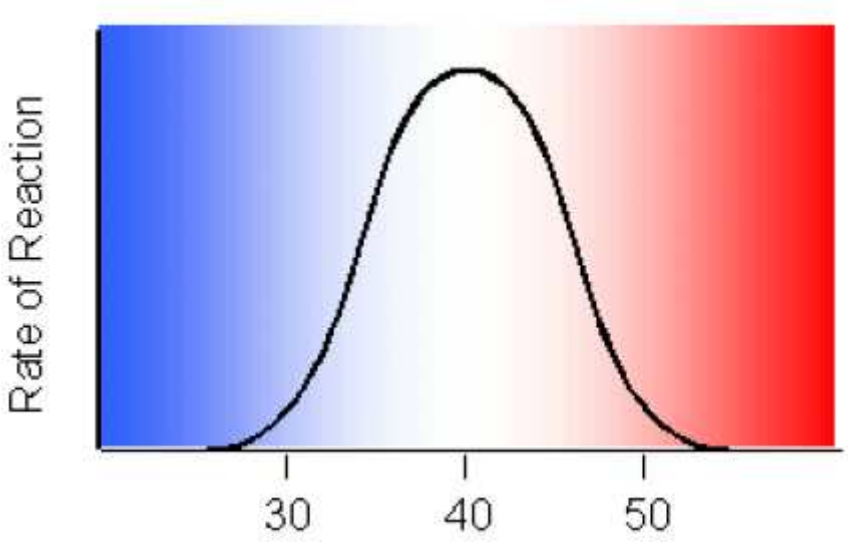
- Extremes generally inactivate enzymes
- The pH optimum is usually quite narrow, although some enzymes have a broader optimum range.
- Exceptions
  - Pepsin: optimum pH is 1.8
  - Trypsin: optimum pH is 9.8



Factors affecting the effectiveness of enzymes in food processing

- **Temperature**
  - Enzymes function very slowly at sub-freezing temperatures.
  - Optimal activity in the 30-40^{o}C range.
  - Denature above 45^{o}C.

    → the activity quickly decreases



- Freezing
  - Activity depends on the enzyme (0 –10^{o}C)
  - Below –10^{o}C almost always decrease activity
- Factors involved in inconsistent behavior:
  - Composition of medium
  - Rate and extent of freezing
  - Concentration effects
  - Viscosity
  - Changes in phase (crystallization of water, solidification of triacylglycerides)

- **Water activity**
  - Dried foods
    - Restricted water activity
    - Susceptible to enzymatic spoilage
  - The rate of enzymatic reactions in dried products is limited by the rate at which the substrate diffuses to the enzyme
  - Heat stability

- **Electrolytes and ionic strength**
  - Ions may be required components in the active site
  - Cation requirements of enzymes is sometimes specific
  - Salting in
  - Salting out

- **Chemicals**
  - Chelating agents
  - Reducing agents
  - Alterations of substrates

The role of endogenous enzymes in food processing

- COLOR, ex:
  - 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, ex:
  - 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, ex:
  - 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, ex:
  - 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, trypthophan 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^{o}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 a-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 prophylgallate
- 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

- Enzymes in brewing
  - Amylases → convert nonmalt starch to maltose and detrine; yeast fermentation
  - B-glucanase → hydrolyze glucans to reduce viscosity and aid filtration
  - Papain → chillproofing beer
- Enzymes for control of microorganisms
  - Enzymes have potential for destroying microbes.
  - Lipases → liberation of FFA, which toxic to protozoa *Giardia lamblia*
  - Chitinase → effective against chitin in cell wall of several fungi.