Class Changes for Online Delivery

- https://tamu.zoom.us/j/7545042696
  - We will meet online at least once per week
- DTE assignment now due Tuesday April 7th
- We will not have a Fact Check Quiz #2.
- Your final exam will be a take-home “exam” on a critical thinking subject. I will give you at least a week to complete this project and it will replace Exam #2. Due date is April 23rd at 5 pm.

Proteins

- Many important functions
  - Functional
  - Nutritional
  - Biological
- Enzymes
- Structurally complex and large compounds
- Major source of nitrogen in the diet
  - By weight, proteins are about 16% nitrogen

Protein Content of Foods

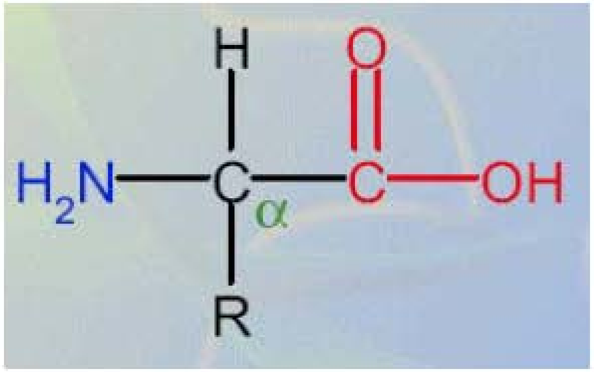
- Beef -- 16.5%
- Pork -- 10%
- Chicken -- 23.5%
- Milk -- 3.6%
- Eggs -- 13%
- Bread -- 8.5%
- Cooked beans -- 8%
- Potato -- 2%

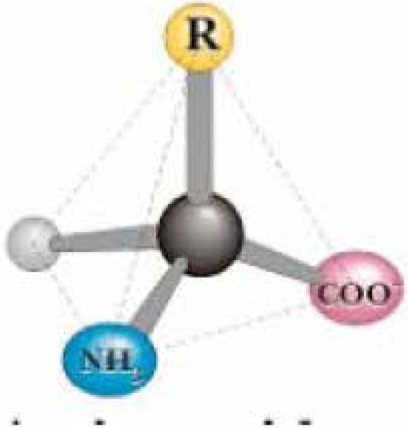
Proteins

- Proteins are polymers of amino acids joined together by peptide bonds
- Structure, arrangement, and functionality of a protein is based on amino acid composition
- All amino acids contain nitrogen, but also C, H, O, and S

Protein Structure

- Protein are polymers of α-amino acids
- The amino acids used to make proteins are 2-amino-carboxylic acids.

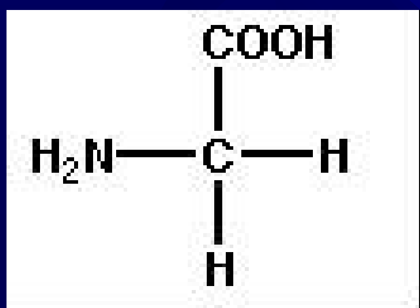


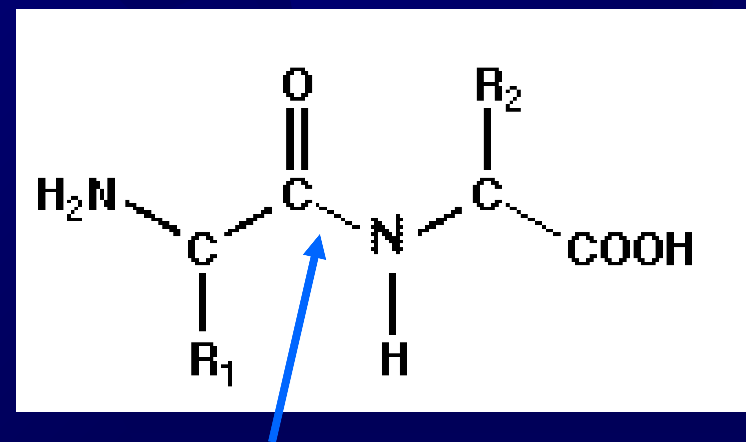


- The α (alpha) carbon is the carbon to which a functional group is attached.

Proteins

Proteins are composed of amino acids which are carboxylic acids also containing an amine functional group.





The amino acids are linked together by peptide bonds (amide bonds) forming long chains

Short chains of amino acids are commonly called polypeptides (eg. dipeptide, tripeptide, hexapeptide, etc)

Longer chains of amino acids normally called proteins.

Proteins

- <u>**Peptide bonds**</u> **are strong covalent bonds that connect 2 amino acids**

- <u>**Dipeptide-**</u> **2 amino acids joined together by a peptide bond**

- <u>**Polypeptide**</u>**- 3 or more amino acids joined together by peptide bonds in a specific sequence**

20 Amino Acids

- Alanine (Ala)
- Arginine (Arg)
- Asparagine (Asn)
- Aspartic acid (Asp)
- Cysteine (Cys)
- Glutamine (Gln)
- Glutamic acid (Glu)
- Glycine (Gly)
- Histidine (His)
- Isoleucie (Ile)
- Leucine (Leu)
- Lysine (Lys)
- Methinine (Met)
- Phenylalanine (Phe)
- Proline (Pro)
- Serine (Ser)
- Threonine (Thr)
- Tryptophan (Trp)
- Tyrosine (Try)
- Valine (Val)

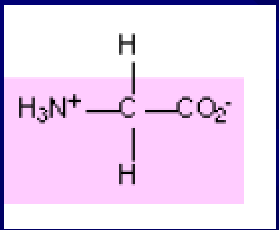
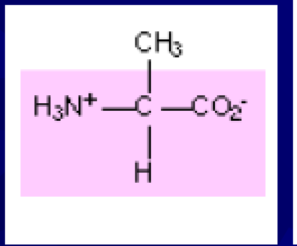
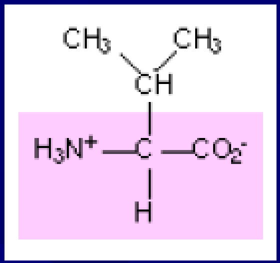
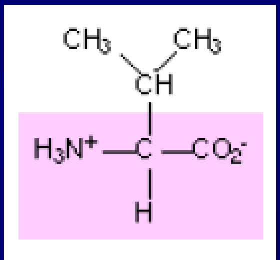
Proteins

- Composed of amino acids
- 20 common amino acids
- Polymerize via peptide bonds
- Essential vs. non-essential amino acids
- Essential must come from diet
- Essential amino acids:
  - "Pvt. T.M. Hill”
  - phenylalanine, valine, threonine, tryptophan, methionine, histidine, isoleucine, leucine, lysine

Properties of Amino Acids

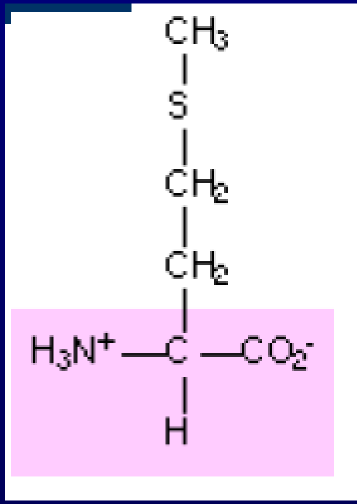
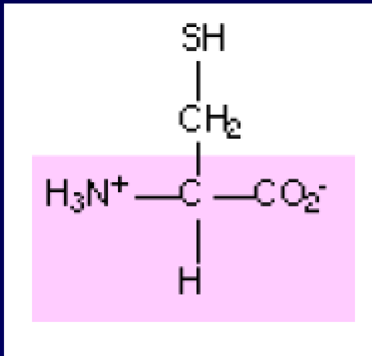
- Aliphatic chains: Gly, Ala, Val, Leucine, Ile
- Hydroxy or sulfur side chains: Ser, Thr, Cys, Met
- Aromatic: Phe, Trp, Try
- Basic: His, Lys, Arg
- Acidic and their amides: Asp, Asn, Glu, Gln

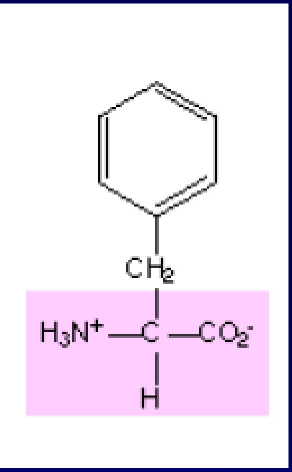
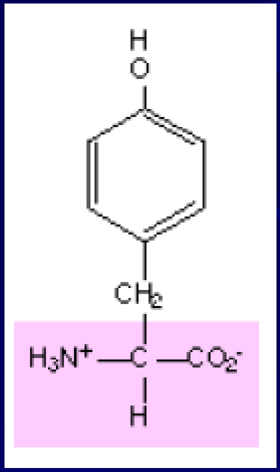
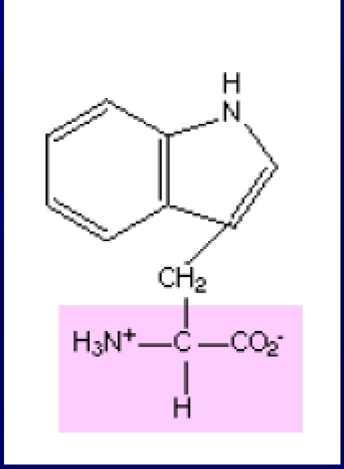
Properties of Amino Acids:

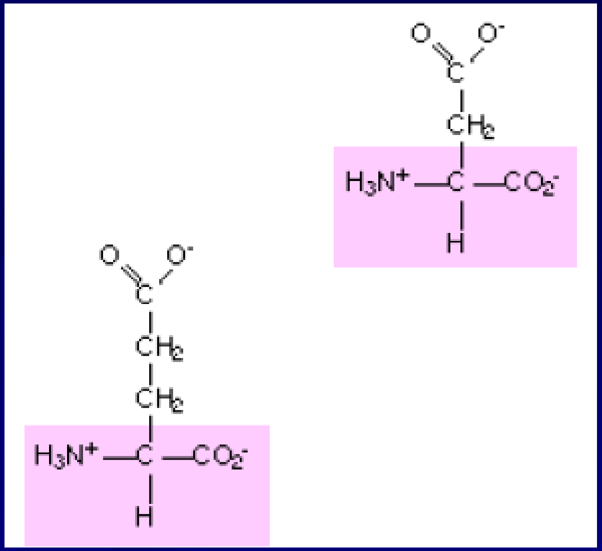
Aliphatic Side Chains

Sulfur Side Chains

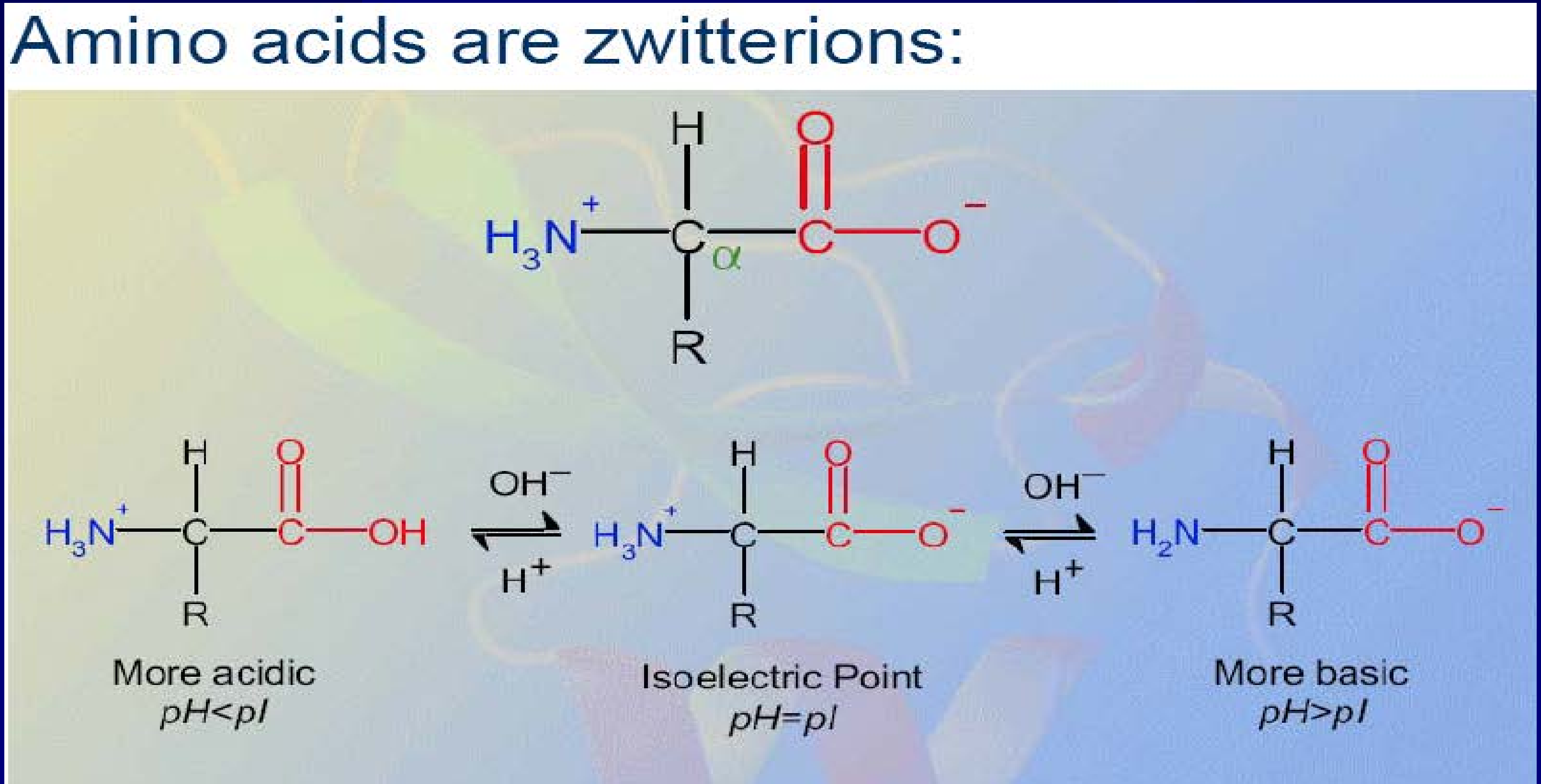
Aromatic Side Chains



Acidic Side Chains

Properties of Amino Acids:

- Zwitterions are electrically neutral, but carry a "formal" positive or negative charge.
- Give proteins their water solubility



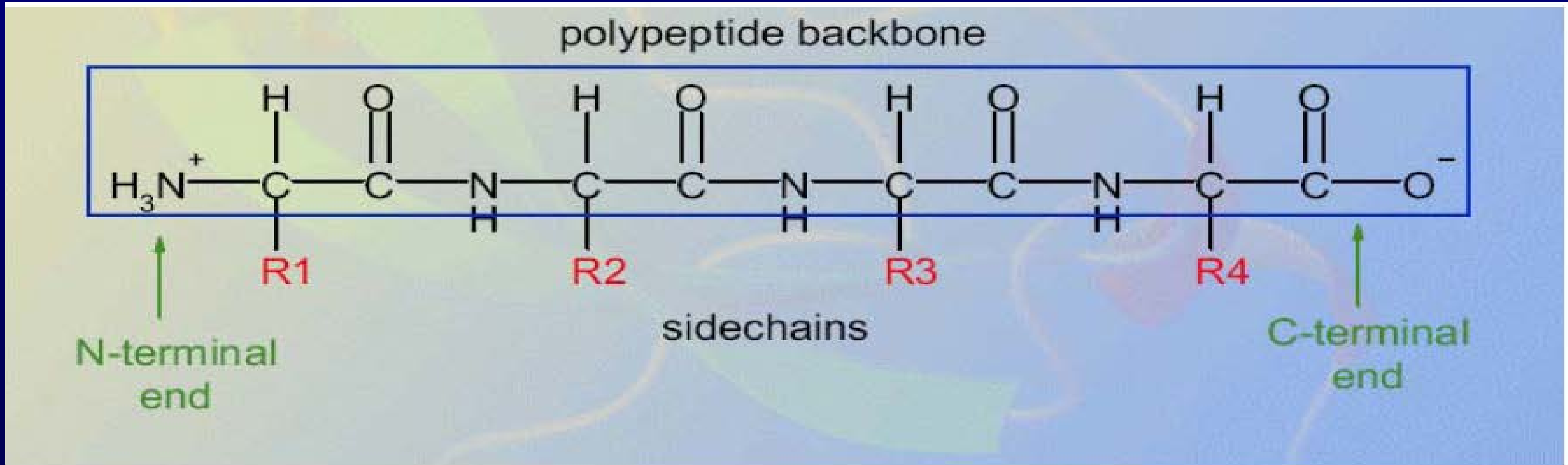
The Zwitterion Nature

- Zwitterions make amino acids good acid-base buffers.
- For proteins and amino acids, the pH at which they have no net charge in solution is called the Isoelectric Point of pI (i.e. IEP).
- The solubility of a protein depends on the pH of the solution.
- Similar to amino acids, proteins can be either positively or negatively charged due to the terminal amine -NH2 and carboxyl (-COOH) groups.
- Proteins are <u>positively</u> charged at low pH and <u>negatively</u> charged at high pH.
- When the net charge is zero, we are at the IEP.
- A charged protein helps interactions with water and increases its solubility.
- As a result, protein is the <u>least soluble</u> when the pH of the solution is at its isoelectric point.

Protein Structures

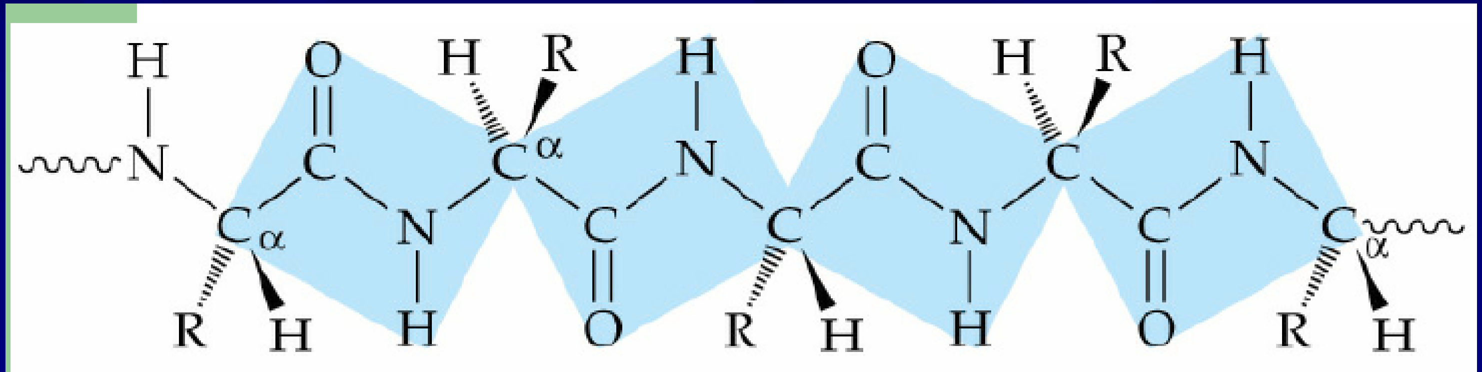
- Primary = sequence of amino acids
- Secondary = alpha helix, beta pleated sheets
- Tertiary = 3-D folding of chain
- Quaternary = "association" of subunits and other internal linkages

Primary Sequence



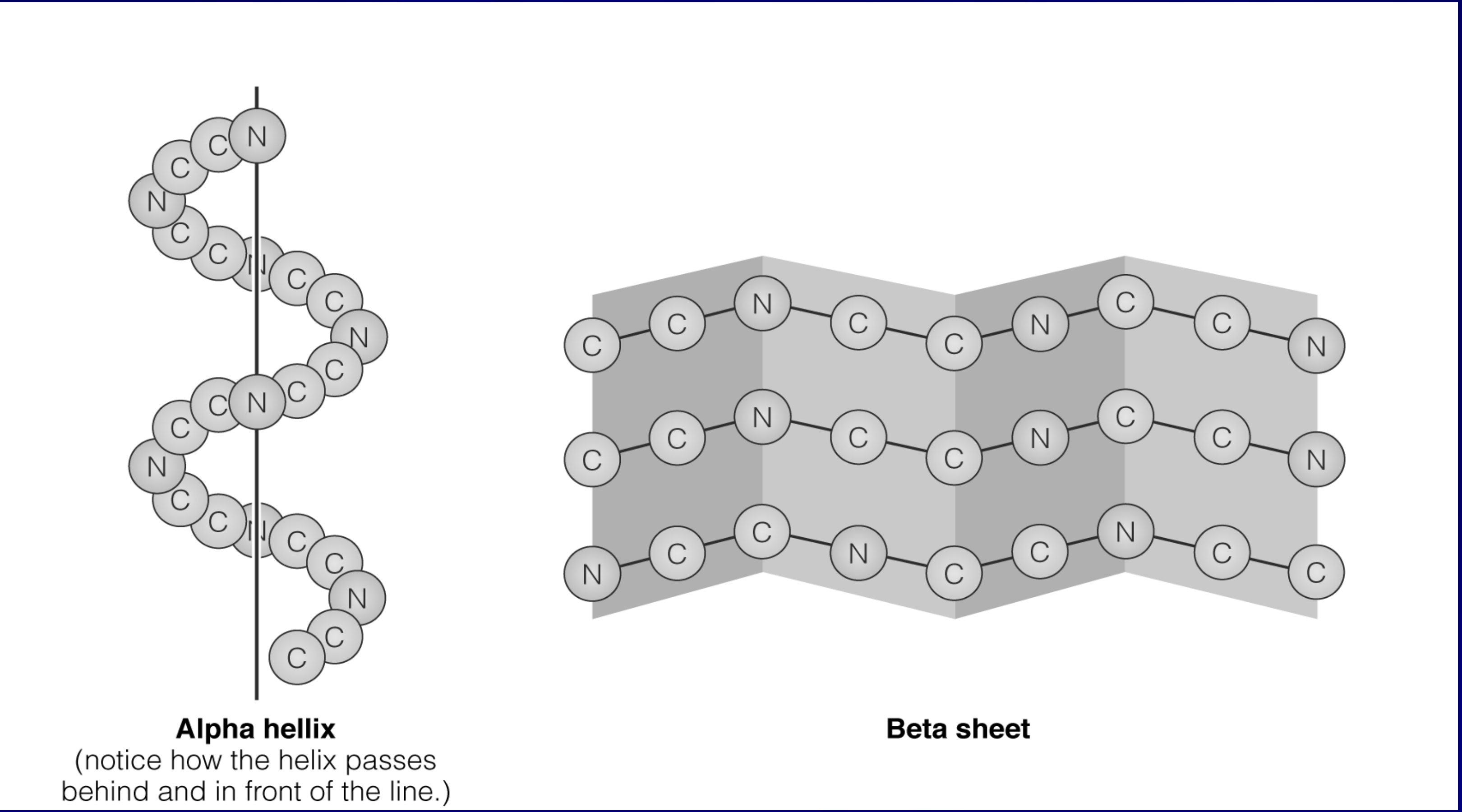
•The order of amino acids in a protein is genetically determined

•Contains all the information to assume its correct 3-D structure



Secondary protein structure

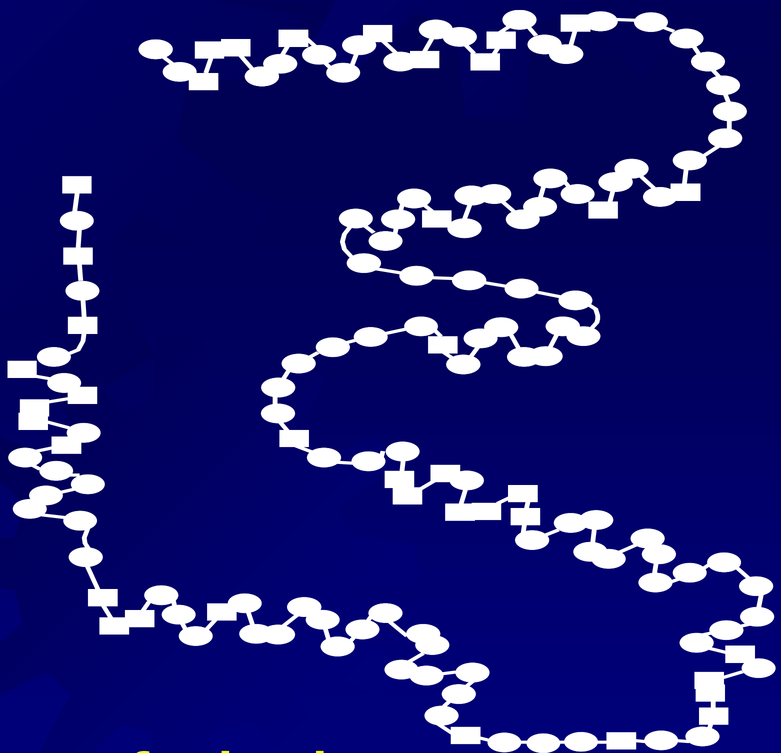
- The spatial structure the protein assumes along its axis (its “native conformation” or min. free energy)



This gives a protein functional properties such as flexibility and strength

Tertiary Structure of Proteins

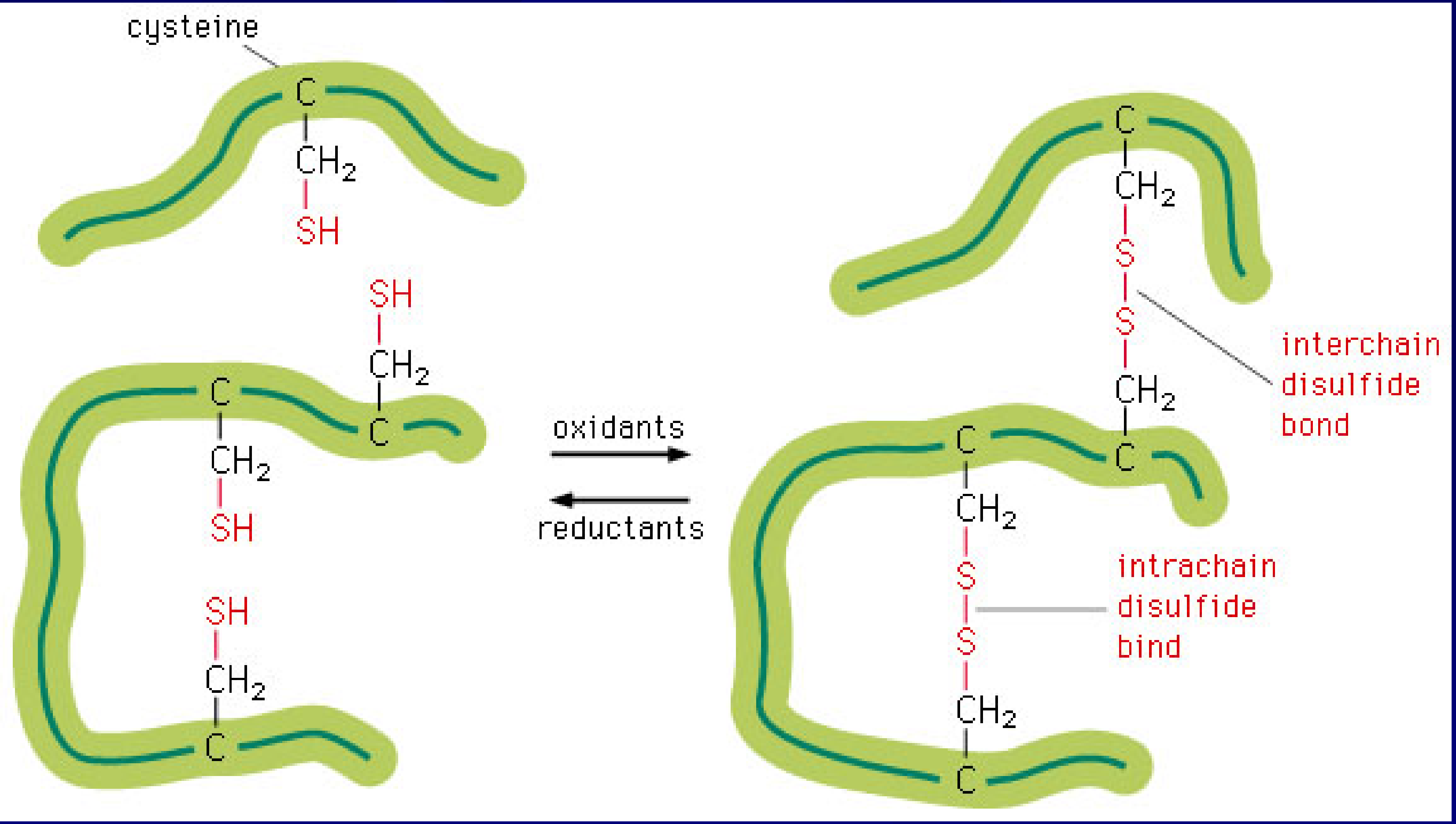
- 3-D organization of a polypeptide chain
- Compacts proteins
- Interior is mostly devoid of water or charge groups



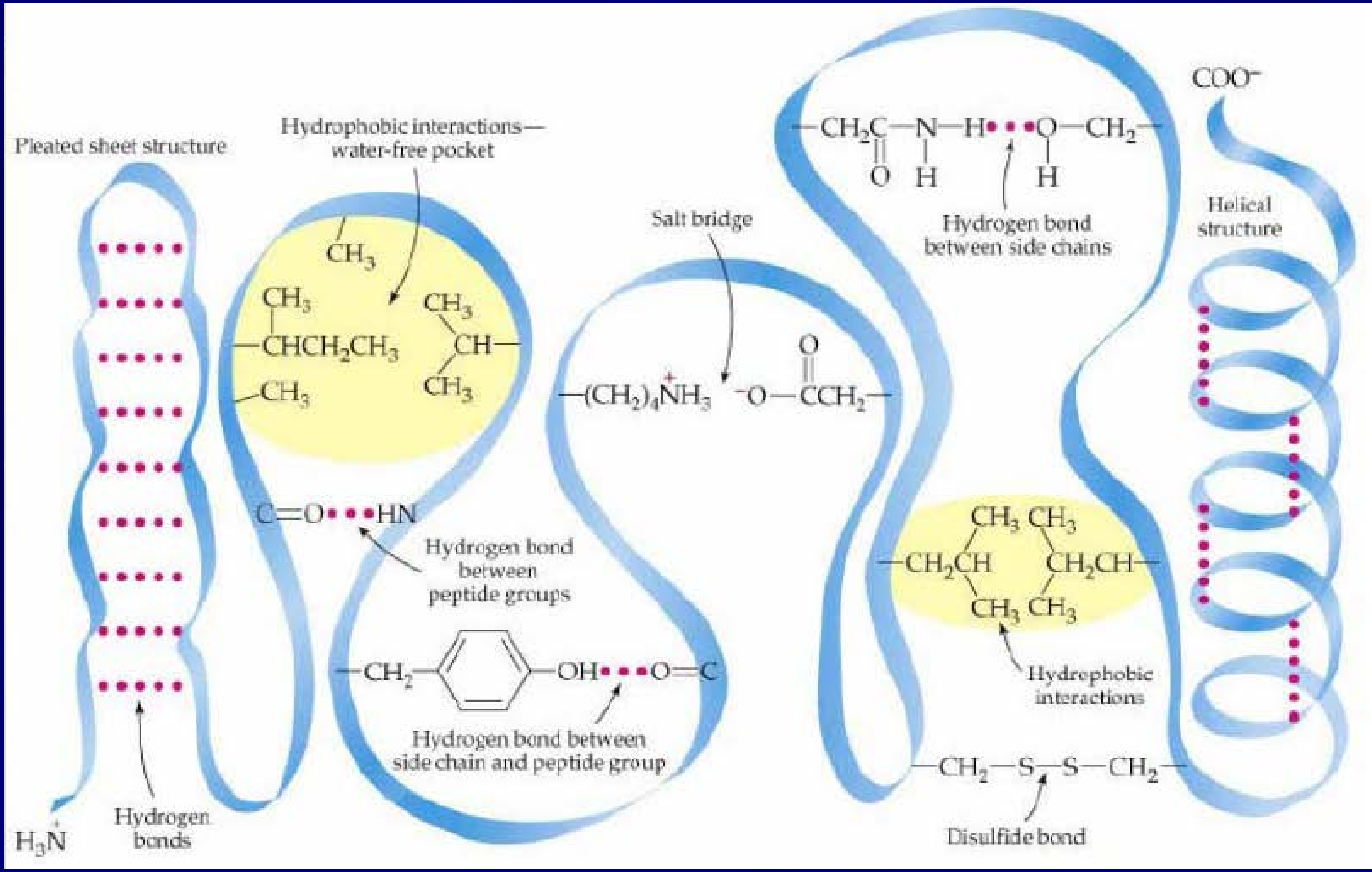
3-D folding of chain

Quaternary Structure of Proteins

- Non-covalent associations of protein units



Shape Interactions of Proteins



Protein Structure

- **<u>Globular</u> - polypeptide folded upon itself in a spherical structure**

- **<u>Fibrous</u> – polypeptide is arranged along a common straight axis**

Classification of simple proteins

- Composed of amino acids and based on solubility. Every food has a mixture of these protein types in different ratios.
- Albumins – soluble in pure water
- Globulins – Soluble in salt solutions at pH 7.0, but insoluble in pure water
- Glutelins – soluble in dilute acid or base, but insoluble in pure water
- Prolamins – soluble in 50-90% ethanol, but insoluble in pure water
- Scleroproteins – insoluble in neutral solvents and resistant to enzymatic hydrolysis
- Histones – soluble in pure water and precipitated by ammonia; typically basic proteins
- Protamines – extremely basic proteins of low molecular weight

Classification of complex proteins

A protein with a non-protein functional group attached

- Glycoproteins- carbohydrate attached to protein
- Lipoproteins – lipid material attached to proteins
- Phosphoproteins- phosphate groups attached
- Chromoprotein- prosthetic groups associated with colored compounds (i.e. hemoglobin)

Emulsoids and Suspensiods

- Proteins should be thought of as solids
  - Not in true solution, but bond to a lot of water
- Can be described in 2 ways:

- Emulsoids- have close to the same surface charge with many shells of bound water

- Suspensoids- colloidal particles that are suspended by charge alone

Functional Properties of Proteins

3 major categories

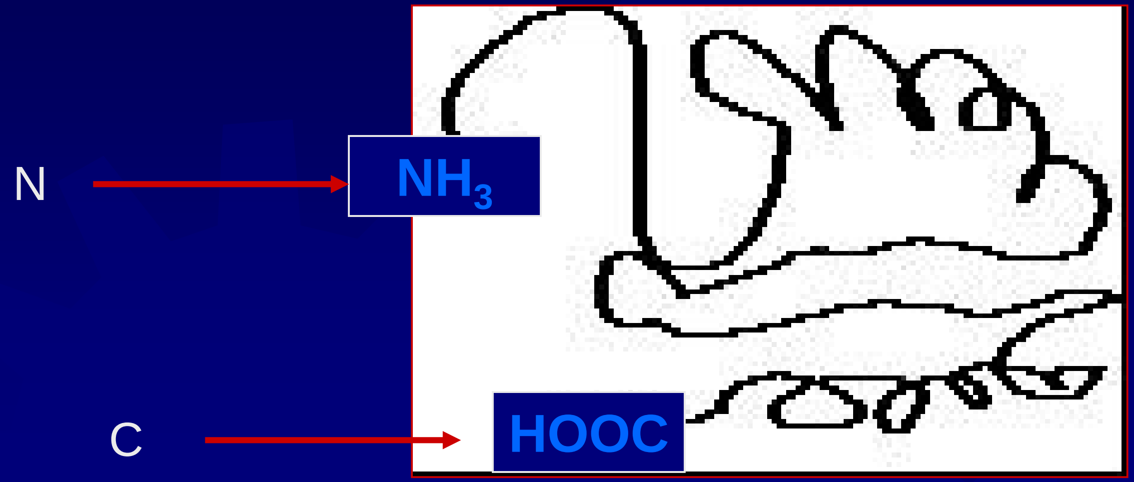
- **Hydration properties**
  - **Protein to water interactions**
  - **Dispersibility, solubility, adhesion,**
  - **Water holding capacity, viscosity**
- **Structure formation**
  - **Protein to protein interactions**
  - **Gel formation, precipitation,**
  - **Aggregation**
- **Surface properties**
  - **Protein to interface interactions**
  - **Foaming, emulsification**

Proteins and peptide chains are "directional".

That means the chain has a free alpha amino group and a free carboxyl group.

The **Amino Terminus** (N-Terminus) is the end of the chain containing the free alpha amino function.

The **Carboxy Terminus** (C-Terminus) is the end of the chain containing the free carboxyl group.



Proteins: more than just energy

"Functional" properties

- Emulsifier
- Foaming = egg whites
- Gel formation = jello
- Water binding or thickening
- Participation in browning reactions

Enzymes (more on this next week)

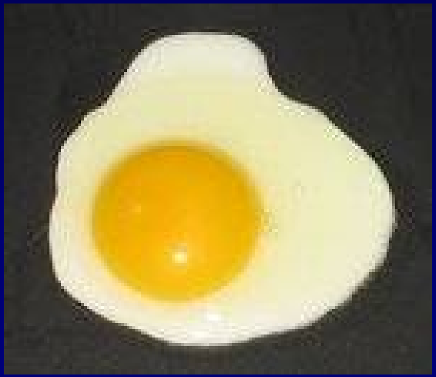
Enzymes

- Proteins that act as catalysts
  - Can be good or bad
- Ripening of fruits, vegetables
- Meat tenderization
- Destruction of color, flavor
- Heat preservation, inactivates
  - Blanching, cooking

Proteins

Changes in structure

- Denaturation
  - Breaking of any structure except primary
- Reversible or irreversible, depending on severity of the denaturation process
- Examples:
  - Heat - frying an egg
  - High salt content
  - High alcohol content
  - Low or High pH
  - Extreme physical agitation
  - Enzyme action (proteases)



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Quick Application: Food Protein Systems

- Milk- Emulsoid and suspensoid system
  - Classified as whey proteins and caseins
  - Casein - a phosphoprotein in a micelle structure
  - Suspensoid - coagulates at IEP (casein)
- Egg (Albumen) – Emulsoid
  - Surface denatures very easily
  - Heating drives off the structural water and creates a strong protein to protein interaction
  - Cannot make foam from severely denatured egg white, requires bound water and native conformation

Functional Properties of Proteins

3 major categories

- **Hydration properties**
  - **Protein to water interactions**
    - **Dispersion, solubility, adhesion, viscosity**
    - **Water holding capacity**
- **Structure formation**
  - **Protein to protein interactions**
  - **Gel formation, precipitation, aggregation**
- **Surface properties**
  - **Protein to interface interactions**
  - **Foaming and emulsification**

1. Hydration Properties (protein to water)

- Most foods are hydrated to some extent.
  - Behavior of proteins are influenced by the presence of water and water activity
  - Dry proteins must be hydrated (food process or human digestion)
- Solubility- as a rule of thumb, *denatured* proteins are less soluble than native proteins
- Many proteins (particularly suspensoids) aggregate or precipitate at their isoelectric point (IEP)
- Viscosity- viscosity is highly influenced by the size and shape of dispersed proteins
  - Influenced by pH
  - Swelling of proteins
  - Overall solubility of a protein

2. Structure Formation (protein to protein)

- Gels - formation of a protein 3-D network is from a balance between attractive and repulsive forces between adjacent polypeptides
- Gelation- denatured proteins aggregate and form an ordered protein matrix
  - Plays major role in foods and water control
  - Water absorption and thickening
  - Formation of solid, visco-elastic gels
- In most cases, a thermal treatment is required followed by cooling
  - Yet a protein does not have to be soluble to form a gel (emulsoid)

- Texturization – Proteins are responsible for the structure and texture of many foods
  - Meat, bread dough, gelatin
  - Proteins can be "texturized" or modified to change their functional properties (i.e. salts, acid/alkali, oxidants/reductants)
    - Can also be processed to mimic other proteins (i.e. surimi)

3. Surface Properties (protein to interface)

- **Emulsions-** Ability for a protein to unfold (tertiary denaturation) and expose hydrophobic sites that can interact with lipids.
  - Alters viscosity
  - Proteins must be “flexible”
  - Overall net charge and amino acid composition
- **Foams-** dispersion of gas bubbles in a liquid or highly viscous medium
  - Solubility of the protein is critical; concentration
  - Bubble size (smaller is stronger)
  - Duration and intensity of agitation
  - Mild heat improves foaming; excessive heat destroys
  - Salt and lipids reduce foam stability
  - Some metal ions and sugar increase foam stability

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Changes to Proteins

- Native State
  - The natural form of a protein from a food
  - The unique way the polypeptide chain is oriented
- There is only 1 native state; but many altered states
- The native state can be fragile to:
  - Acids
  - Alkali
  - Salts
  - Heat
  - Alcohol
  - Pressure
  - Mixing (shear)
  - Oxidants (form bonds) and antioxidants (break bonds)

Changes to Proteins

- Denaturation
  - Any modification to the structural state
  - The structure can be re-formed
  - If severe, the denatured state is permanent
- Denatured proteins are common in processed foods
  - Decreased water solubility (i.e. cheese, bread)
  - Increased viscosity (fermented dairy products)
  - Altered water-holding capacity
  - Loss of enzyme activity
  - Increased digestibility

Changes to Proteins

- Temperature is the most common way to denature a protein
  - Both hot and cold conditions affect proteins
    - Every tried to freeze milk? Eggs?
- Heating affects the tertiary structure
  - Mild heat can activate enzymes
- Hydrogen and ionic bonds dissociate
- Hydrophobic regions are exposed
- Hydration increases, or entraps water
- Viscosity increases accordingly

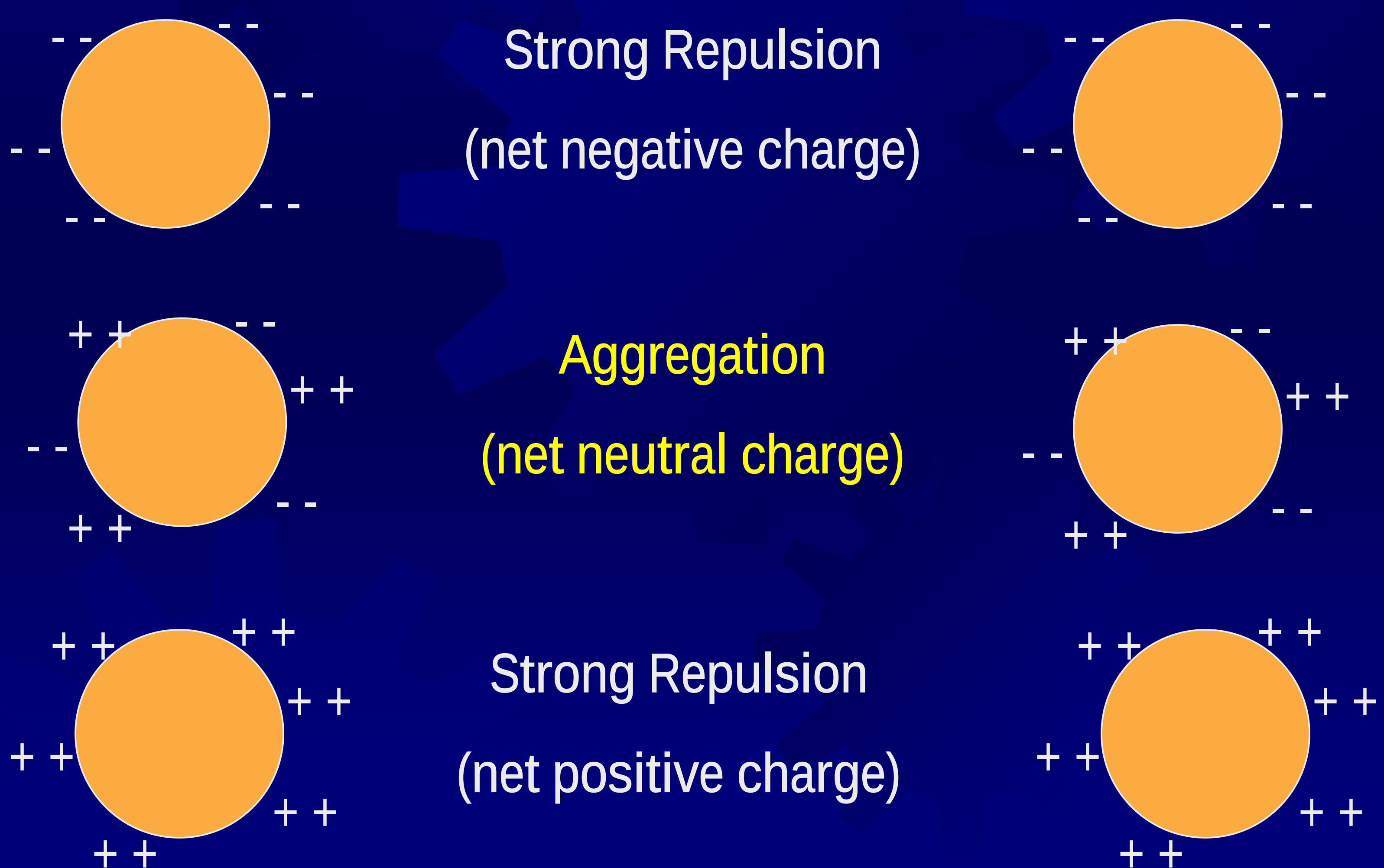
Changes to Proteins

- We discussed protein solubility characteristics
- Solubility depends on the nature of the solution

- Water-soluble proteins generally have more polar amino acids on their surface.
- Less soluble proteins have less polar amino acids and/or functional groups on their surface.

Isoelectric Precipitations

- Proteins have no net charge at their IEP



Isoelectric Precipitations

- Proteins can be “salted out”, adding charges

Measuring IEP Precipitations

- Empirical measurements for precipitation
- A protein is dispersed in a buffered solution
  - Add salt at various concentrations
  - Add alcohols (disrupt hydrophobic regions)
  - Change the pH
  - Add surfactant detergents (i.e. SDS)
- Centrifuge and measure quantitatively
  - The pellet will be insoluble protein
  - The supernatant will be soluble protein

Gel Formation

- Many foods owe their physical properties to a gel formation. Influences quality and perception.
  - Cheese, fermented dairy, hotdogs, custards, etc
- As little as 1% protein may be needed to form a rigid gel for a food.
- Most protein-based gels are thermally-induced
  - Cause water to be entrapped, and a gel-matrix formation
- Thermally irreversible gels are most common
  - Gel formed during heating, maintained after cooling
  - Will not reform when re-heated and cooled
- Thermally reversible gels
  - Gel formed after heating/cooling. Added heat will melt the gel.

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Processing and Storage

- Decreases spoilage of foods, increases shelf life
- Loss of nutritional value in some cases
  - Severity of processing
- Loss of functionality
  - Denatured proteins have far fewer functional aspects
- Both desirable and undesirable flavor changes

Processing and Storage

- Proteins are affected by
  - Heat
  - Extremes in pH (remember the freezing example?)
  - Oxidizing conditions
    - Oxidizing additives, lipid oxidation, pro-oxidants
  - Reactions with reducing sugars in browning rxns

Processing and Storage

- Mild heat treatments
  - May slightly reduce protein solubility
  - Cause some denaturation
  - Can inactive some enzyme
  - Improves digestibility of some proteins
- Severe heat treatments (for example: >100°C)
  - Some sulfur amino acids are damaged
    - Release of hydrogen sulfide, etc (stinky)
  - Deamination can occur
    - Release of ammonia (stinky)
- Very high temperatures (>180°C)
  - Some of the roasted smells that occur with peanuts or coffee

Enzyme Influencing Factors

- Enzymes are proteins that act as biological catalysts
- They are influenced in foods by:
  - Temperature
  - pH
  - Water activity
  - Ionic strength (ie. Salt concentrations)
  - Presence of other agents in solution
    - Metal chelators
    - Reducing agents
    - Other inhibitors

Also factors for Inhibition, including:

Oxygen exclusion
and
Sulfites

pH

- Like temp, enzymes have an optimal pH where they are maximally active
- Generally between 4 and 8
  - with many exceptions
- Most have a very narrow pH range where they show activity.
- This influences their selectivity and activity.

Water Activity

- Enzymes need free water to operate
- Low Aw foods have very slow enzyme reactions

Ionic Strength

- Some ions may be needed by active sites on the protein
  - Ions may be a link between the enzyme and substrate
  - Ions change the surface charge on the enzyme
  - Ions may block, inhibit, or remove an inhibitor
  - Others, enzyme-specific

Enzymes

Common Enzymes in Foods

- Polyphenol oxidase
- Plant cell wall degrading enzymes
- Proteases
- Lipases
- Peroxidase/Catalase
- Amylase
- Ascorbic acid oxidase
- Lipoxygenase

Enzyme Influencing Factors

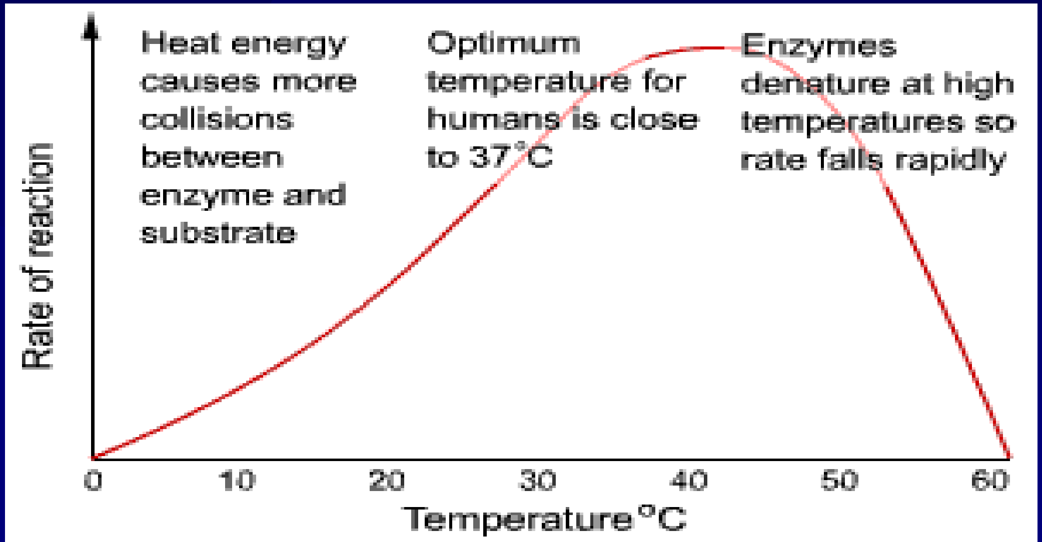
- Temperature-dependence of enzymes
- Every enzyme has an optimal temperature for maximal activity
- The effectiveness of an enzyme: Enzyme activity
- For most enzymes, it is 30-40°C
- Many enzymes denature >45°C
- Each enzyme is different, and vary by isozymes
- Often an enzyme is at is maximal activity just before it denatures at its maximum temperature

Enzymes

The effect of temperature is two-fold

- **From about 20, to 35-40**°C **(for enzymes)**
- **From about 5-35**°C for other reactions
  - **Q_{10}-Principal: For every 10**°C increase in temperature, the reaction rate will double
  - Not an absolute "law" in science, but a general "rule of thumb"

At higher temperatures, some enzymes are much more stable than other enzymes

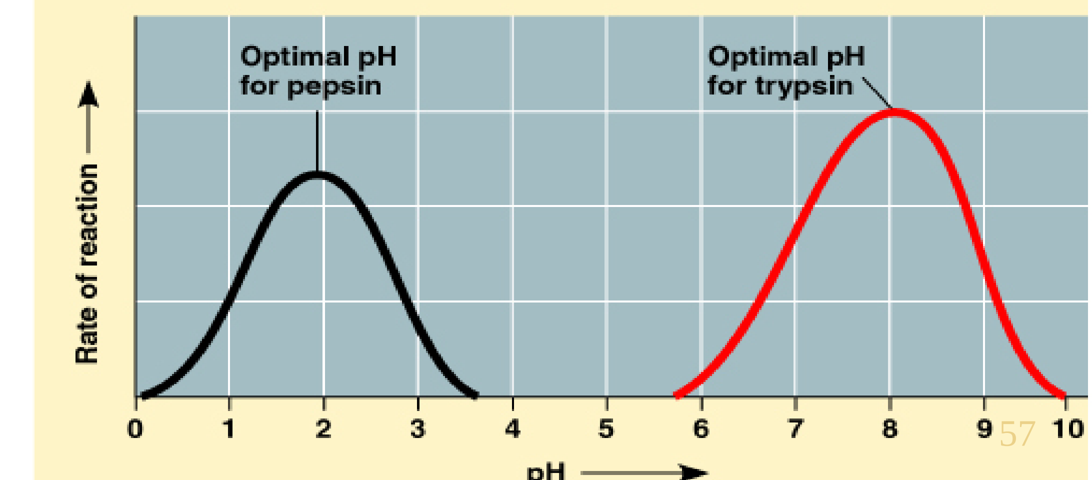


Enzymes

- **Enzymes are sensitive to pH – most enzymes active only within a pH range of 3-4 units (catalase has max. activity between pH 3 & 10!)**

- **The optimum pH depends on the nature of the enzyme and reflects the environmental conditions in which enzyme is normally active:**
  - Pepsin pH 2; Trypsin pH 8; Peroxidase pH 6

- **pH dependence is usually due to the presence of one or more charged AA at the active site.**



Nomenclature

Each enzyme can be described in 3 ways:

- Trivial name: α-amyl<u>ase</u>
- Systematic name: <u>α-1,4-glucan</u>-<u>glucono-hydrolase</u>

  substrate reaction

- **Number of the Enzyme Commission: E.C. 3.2.1.1**
  - **3- hydrolases (class)**
  - **2- glucosidase (sub-class)**
  - **1- hydrolyzing *O*-glycosidic bond (sub-sub-class)**
  - **1- specific enzyme**

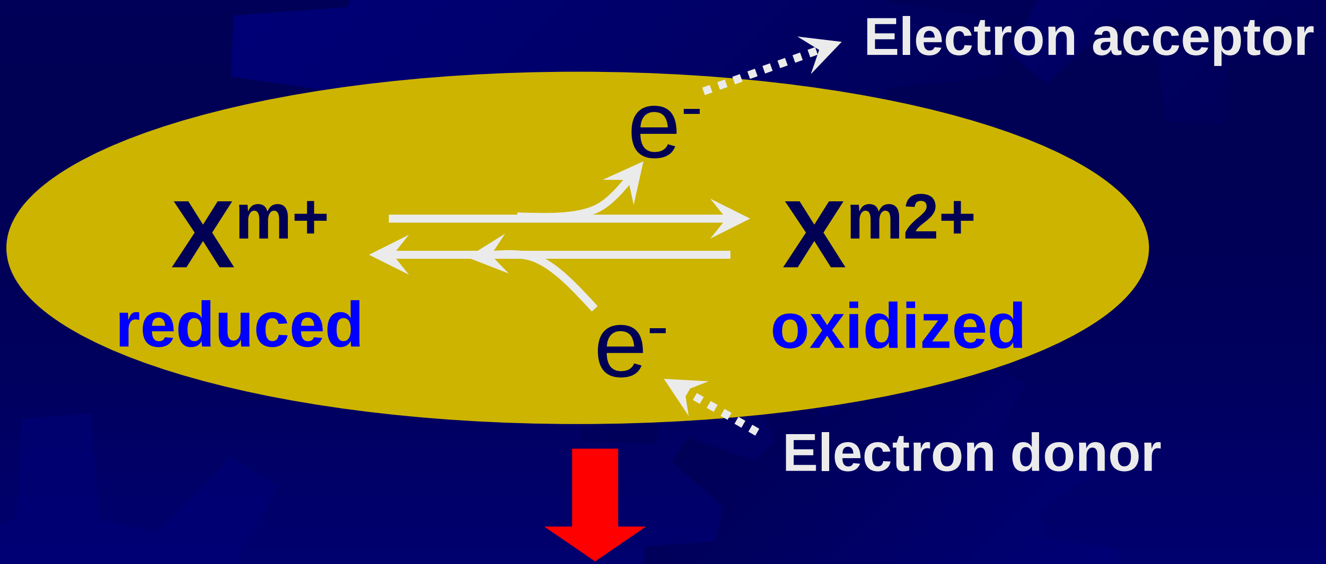
Enzyme Class Characterizations

1. Oxidoreductase
   Oxidation/reduction reactions
2. Transferase
   Transfer of one molecule to another (i.e. functional groups)
3. Hydrolase
   Catalyze bond breaking using water (ie. protease, lipase)
4. Lyase
   Catalyze the formation of double bonds, often in dehydration reations
5. Isomerase
   Catalyze intramolecular rearrangement of molecules
6. Ligase
   Catalyze covalent attachment of two substrate molecules

1. OXIDOREDUCTASES

Oxidation
Is
Losing electrons

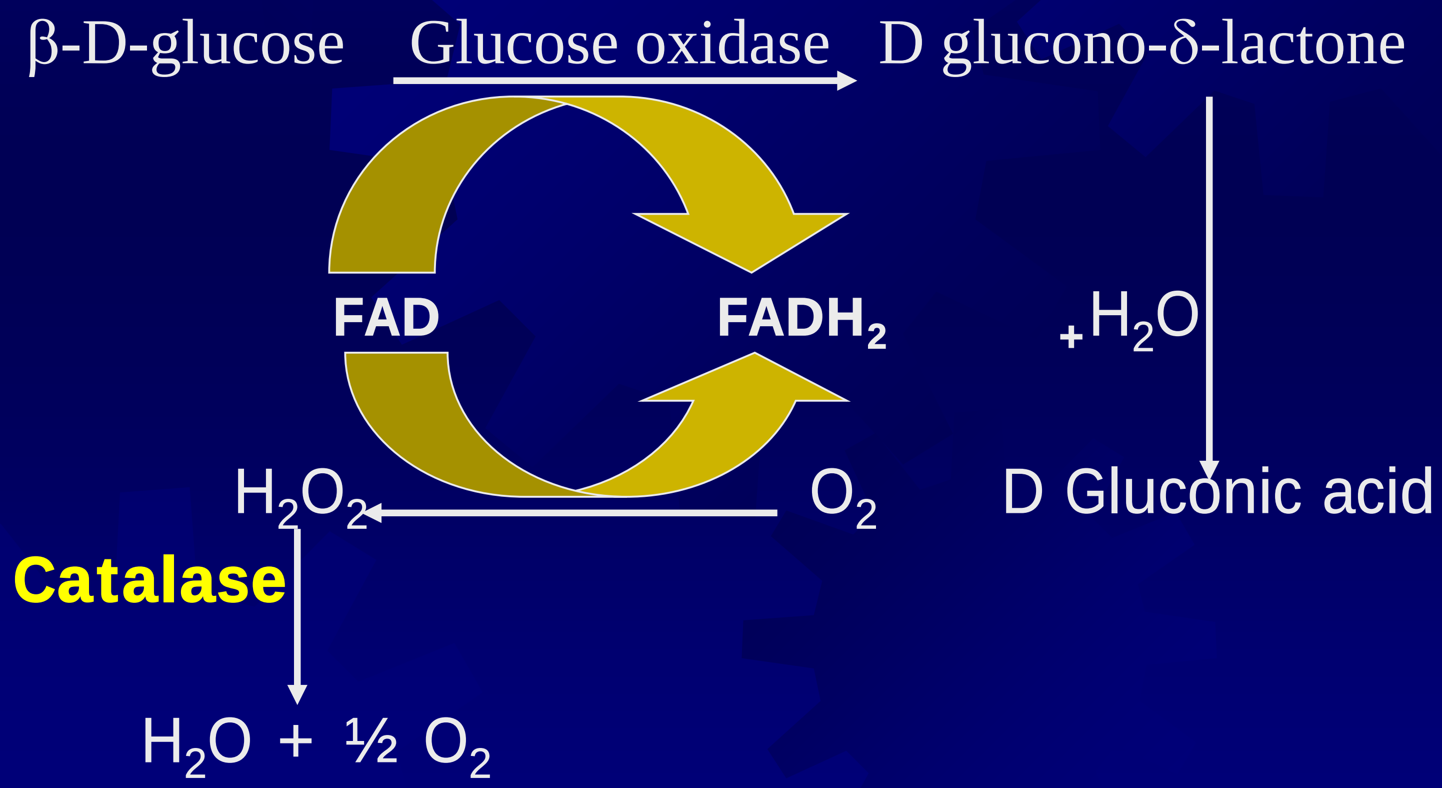
Reduction
Is
Gaining electrons



Redox active (Transition) metals
(copper/ iron containing proteins)

1. Oxidoreductases: GLUCOSE OXIDASE

- β-D-glucose: oxygen oxidoreductase
- **Catalyzes oxidation of glucose to glucono- δ -lactone**



Oxidation of glucose to gluconic acid

1. Oxidoreductases: Catalase

hydrogenperoxide: hydrogenperoxide oxidoreductase

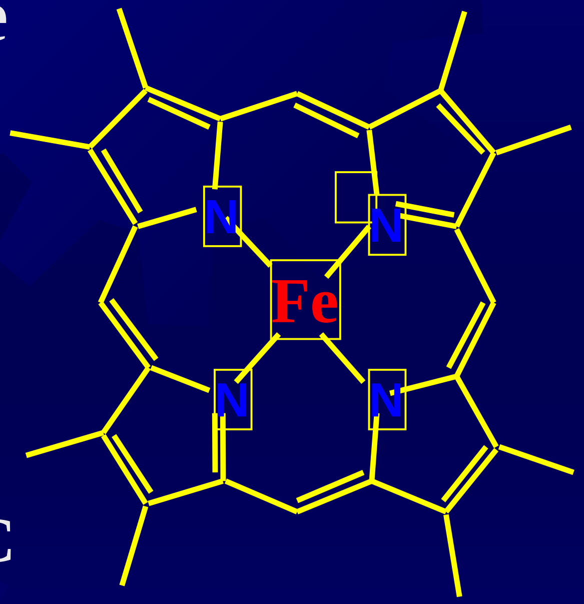
- **Catalyzes conversion of 2 molecules of H_2O_2 into water and O_2:**

$$H_2O_2 \text{ ------------------}\rightarrow H_2O + 1/2\ O_2$$

  - Uses H_2O_2
  - When coupled with glucose oxidase → the net result is uptake of ½ O_2 per molecule of glucose
  - Occurs in MO, plants, animals

1. Oxidoreductases: PEROXIDASE (POD)

donor: hydrogenperoxide oxidoreductase

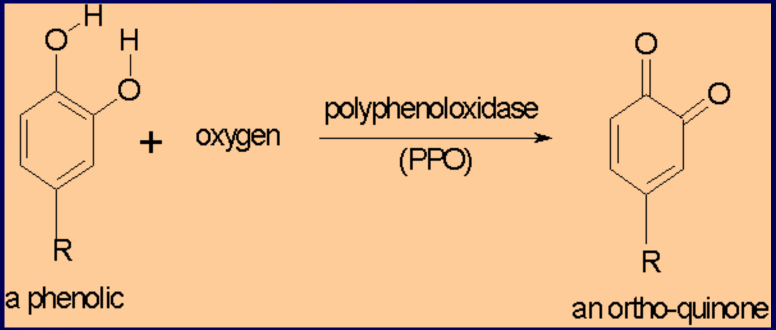


- **Iron-containing enzyme. Has a heme prosthetic group**
- **Thermo-resistant – denaturation at ~ 85ºC**
- **Since is thermoresistant - indicator of proper blanching (no POD activity in blanched vegetables)**

1. Oxidoreductases: POLYPHENOLOXIDASES (PPO)

Phenolases, PPO

- Copper-containing enzyme
- Oxidizes phenolic compounds to *o*-quinones:
- Catalyze conversion of mono-phenols to *o*-diphenols
- In all plants; high level in potato, mushrooms, apples, peaches, bananas, tea leaves, coffee beans



Tea leaf tannins

Catechins
Procyanidins
Gallocatechins
Catechin gallates

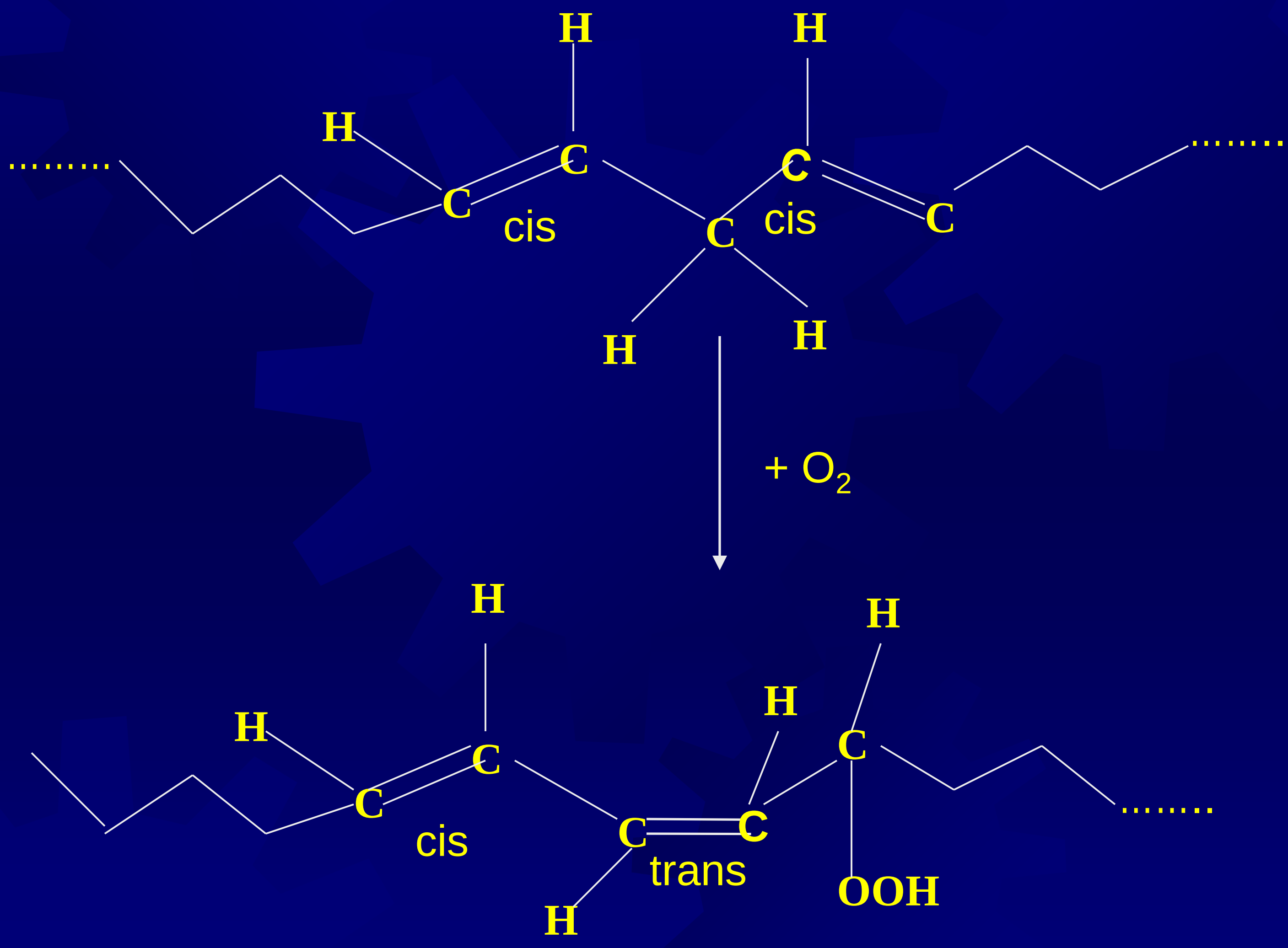
PPO, O_2 → *o*-Quinone + H_2O

↓

Colored products

Action of PPO during tea fermentation; apple/banana browning

1. Oxidoreductases: LIPOXYGENASE



Oxidation of lipids with cis, cis groups to **conjugated** cis, trans hydroperoxides.

Enzymes !!!

- We have observed carbohydrate hydrolysis
  - Sucrose into glu + fru
  - Starch into dextrins, maltose, and glucose
- We *will* observe lipid hydrolysis
  - Break-down of fats and oils
  - Enzyme-derived changes
- So….the enzyme discussion is not over yet.

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Worthington Enzyme Manual

http://www.worthington-biochem.com/index/manual.html

IUPAC-IUBMB-JCBN
http://www.chem.qmul.ac.uk/iubmb/enzyme

Browning reactions in foods

- Some foods are *naturally* brown
- Some foods are *expected* to be brown
- Some are expected not to be brown

Browning can be…

- Desirable:
  - Cooking meat, bread crust, coffee, chocolate
- Undesirable:
  - Fruits, vegetables, sauces
- Much of the undesirable browning occurs during cooking and subsequent storage
  - Affects consumer quality

Browning Reactions

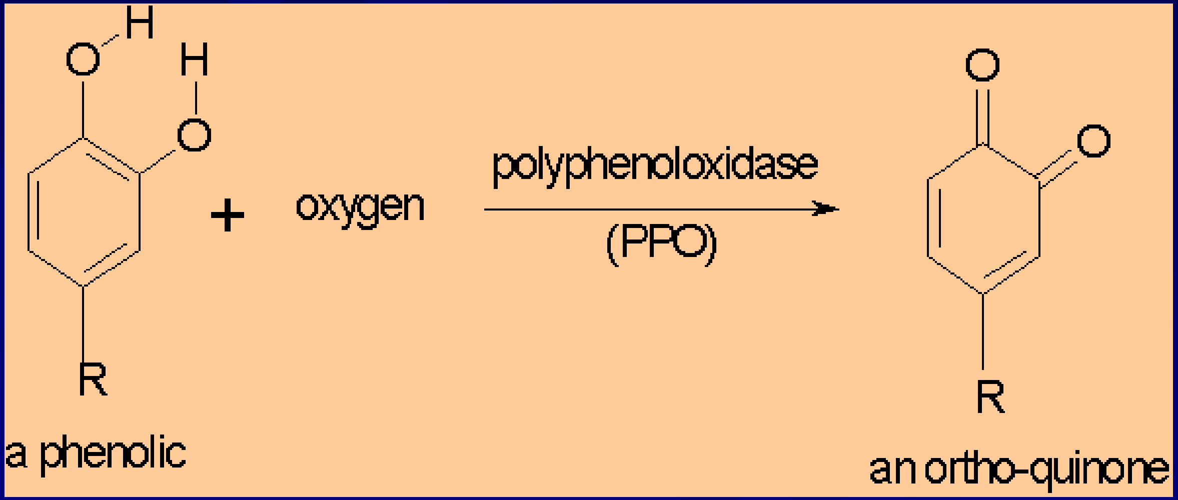
- Caramelization
  - Sugar at high temperatures → Brown pigments + flavors
- Enzymatic
  - Phenolics with PPO → Brown pigments + flavors
- Maillard
  - Reducing sugars + amine → Brown pigments + flavors
- Ascorbic acid oxidation → Brown pigments
- Frying oils → Brown pigments from polymers

BROWNING REACTIONS in CARBOHYDRATES

- CARAMELIZATION occurs when sucrose is heated >150-170°C (high heat!) via controlled thermal processing
  - Dehydration of the sugar leads to structural re-arrangements in the sugar, polymerization, and development of visible colors
- Complex mixture of polymers and fragments of sugar decomposition
  - Caramelans (24, 36, or 125 carbon lengths)
- (+) charged caramel in brewing and baking
- (-) charged caramel in beverage/ soft drinks

Enzymatic Browning

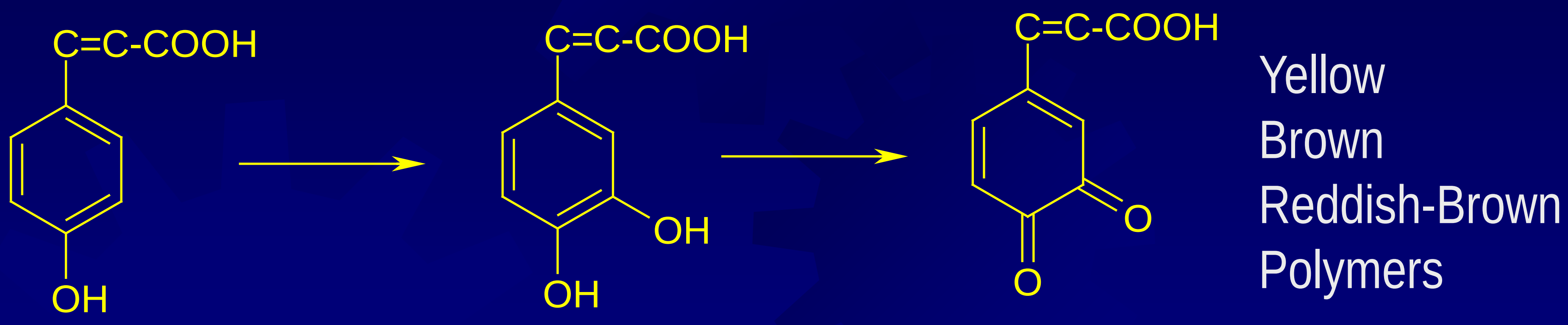
- Polyphenol oxidase is the most common enyzmes leading to browning in foods
- Polymerization of phenolic substrates by PPO enzymes
- "PPO" describes all enzymes with the capacity to oxidize phenolic compounds



OXIDOREDUCTASE

Phenolase, PPO

- Copper-containing enzyme
- Oxidizes phenolic compounds to *o*-quinones.
- Catalyzes the conversion of mono-phenols to *o*-diphenols
  - Hydroxylation reaction….followed by oxidation
- In all plants; high levels in potato, mushrooms, apples, peaches, bananas, tea leaves, coffee beans, shrimp



PPO

- **Important in plant tissues**
  - **Especially wounded tissues**
  - **Packaged foods**
  - **Bruised fruit**
- **Some bitterness can result from *severe* action**
- **Primary impact is loss of food quality**
  - **Potato, apple, banana, avocado**
- **Beneficial in coffee and tea production**

Slowing Down PPO

- **Minimize damage to tissues**
- **Exclude or remove molecular oxygen**
- **Acidification**
- **Water soluble antioxidants (ascorbic acid, cysteine, etc)**
- **Blanching- heat treatment to deactivate PPO**
- **Proteases**
- **Sugar or salt**
- **Vacuum packaging**
- **Metal complexing agents can block active site**
- **Sulfites can prevent reactions with enzymes**
- **Natural extracts**
  - **House flies**
  - **Cockroaches**

Browning Reactions

Non-Enzymatic Browning

- **The Maillard reaction is a classical browning reaction with special implications in the food industry**
- **Highly desirable in cooking and baking**
- **Highly undesirable in cooking and storage**
- **The reaction can not be stopped, but can only be limited or controlled.**
- **Cannot stop it, but can limit / control reaction rate**
- **Reactants are prevalent in foods, just need to get the conditions right**

Maillard Reaction

- The Maillard reaction is a NEB browning reaction
- Results from a condensation of an amino group and a reducing sugar.
- The system is catalyzed by heat, Aw, and pH.
- The result is a complex series of chemical changes to a food system.
- First described by Louis Maillard in 1912.
- The reaction occurs mostly during heating and cooking, but also during storage.
- Many of the reaction products are desirable, such as brown color, caramel aroma, and roasted aromas.
- But excessive browning, non-desired browning, and development of off-flavors can affect product quality.

Maillard Reaction Products

- Maillard aromas are extremely complex.
- From the primary reactants, hundreds of compounds can be formed.
- The formation of a specific, targeted flavor may require the simultaneous generation of hundreds of individual chemicals in the proper concentration and delicate balance.
- So it is a delicate balance during heating and/or storage that influences the reaction by-products.

- Color develop is also an important consequence of the reaction.
- Like caramel colors, Maillard-derived colors are poorly understood.
- Color development in seared meats and baked bread is desirable while browning of dry milk or dehydrated products is undesirable.

What Drives the Maillard Reaction

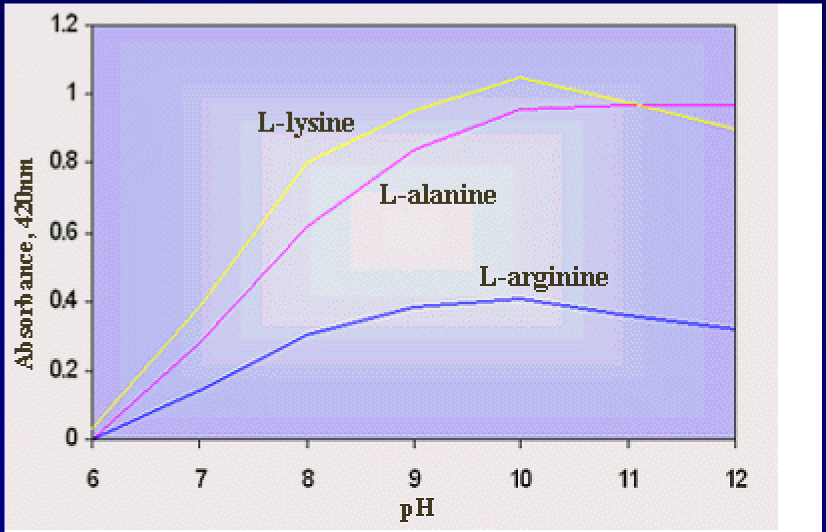
- Water activity
- Water is a by-product of the reaction and acts to slow down the overall reaction.
- Generally, the higher the Aw the slower the overall reaction.
- At lower Aw levels, the mobility of the reactants is reduced (proximity of the reactants) or their concentrations are increased as water is removed.
- Therefore, Maillard reactions commonly occur in dry or intermediate moisture foods (Aw 0.5 to 0.8) that experience a heat treatment.

What Drives the Maillard Reaction

- Acidity (pH)
- Many of the by-products of the reaction can alter the pH of the system (ie. buffering capacity).
- Therefore, evaluating pH on overall reactions is challenging and strong buffers are needed.
- Generally, the lower the pH the slower the reaction.
- However, acidifying food systems will not completely stop the reaction and characteristic colors and aromas may be preferably formed under slightly acidic conditions.
- I.e Lemon juice can brown during storage by the Maillard reaction

What Drives the Maillard Reaction

- The effect of pH on the reaction.
- L-lysine, L-alanine, and L-arginine
- Heated with D-glucose
- 121C for 10 min.
- Increasing the pH with a basic amino acid (Lys) will drive the reaction and increase browning (Abs @ 420 nm).



What Drives the Maillard Reaction

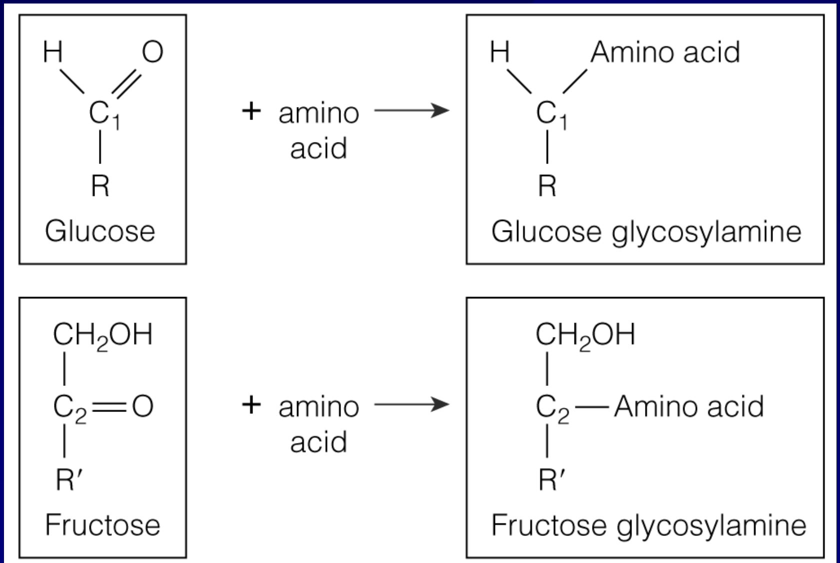
- Temperature
- The activation energies of most chemical reactions are over-come under most food processing conditions.
- Temperature is a major driving factor for the reaction, but the reaction required other contributors
- Heat, in combination with high pH and low Aw, are the perfect criteria for the reaction.

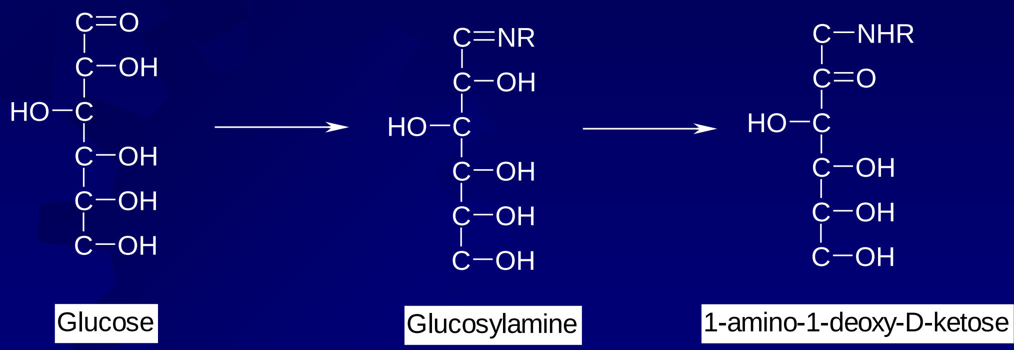
Anti-Nutritional Effects

- There is a trade-off for many chemical changes that occur in foods.
- Since reducing sugars and amino acids participate in the reaction, there will be a loss of these substrates from a food system.
- The reaction may impact the bioavailability of some proteins and can destroy amino acids such as Lys, Arg, and His.
- Reaction products may also bind to micronutrients and contribute to vitamin destruction or inhibit digestive enzymes.
- Some reaction products may be toxic or mutagenic (ie. pyrazines or heterocyclic amines).
- The melanoidin pigments have been shown to inhibit sucrose uptake in the intestine.
- However, some products were shown to be antioxidants

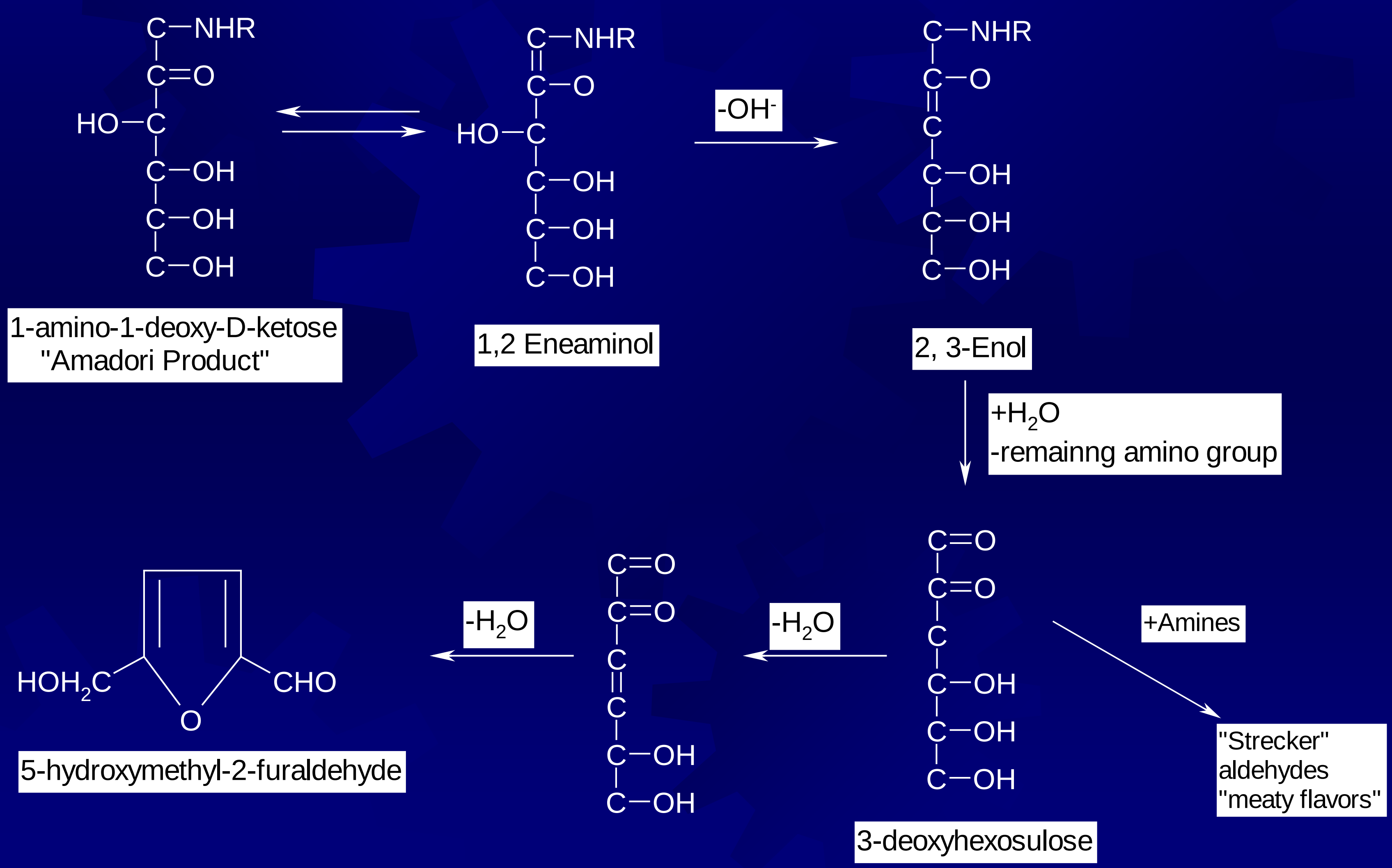
Progression of the Maillard Reaction

- An amino group of an amino acid (-NH2) reacts with,
- An aldose or ketose sugar to form,
- An N-substituted glycosylamine (colorless) plus water.
- This is altered in what is known as an Amadori rearrangement to form an N-substituted-1-amino-1-deoxy-2 ketose compound.
- This is an isomerization reaction and is *essential* for browning





Creation of Just ONE by-product



Compounds Formed by Maillard Reaction

Reducing Sugars and α-amino acids

↓

N-glycosylamine or N-fructosylamine

↓

1-Amino-1-deoxy-2-ketose (Amadori intermediate) or
2-Amino-2-deoxy-1-aldose (Heynes intermediate)

↓

Reductones and Dehydroreductones

+ H_2S, NH_3 → Furans, Thiophenes, Pyrroles

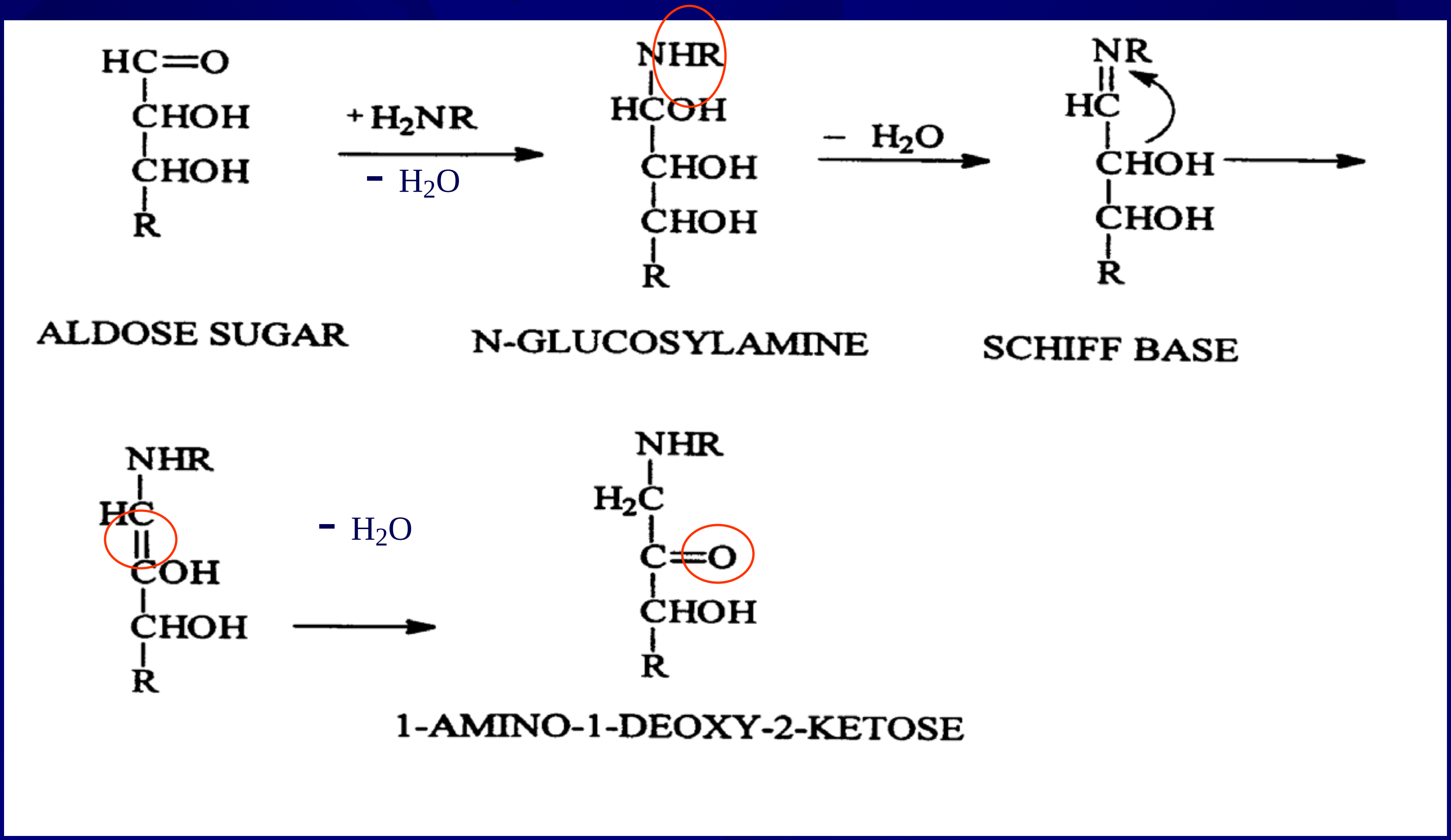
↓ Retroaldol Reaction → Hydroxyacetone, Hydroxyacetylaldehyde, Acetoin, Acetylaldehyde; Glyoxal, Pyruvaldehyde, Glycerolaldehyde

+ Amino Acids → Strecker degradation → Strecker Aldehydes + CO_2 + α-aminoketone (Methional, NH_3, H_2S)

↓ Heterocyclizaion

Pyrazines
Pyridines
Oxazoles
Thiazoles
Pyrroles

A Closer Look: Amadori Rearrangement



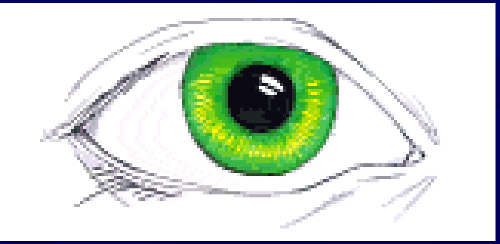
Limiting Maillard Reaction in Foods

- Keep product cool
- Know the limited substrate in a given food
- Optimize pH and moisture during processing
- Add inhibitors
  - Some antioxidants (ie. sulfur dioxide) reacts with intermediate products to prevent polymerization

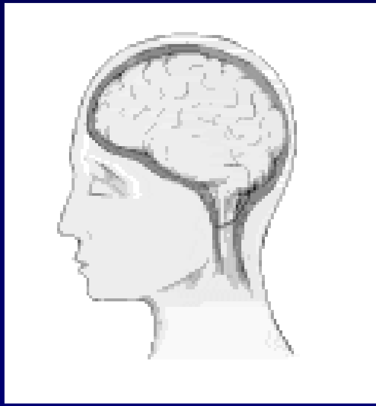
Color

What is color?

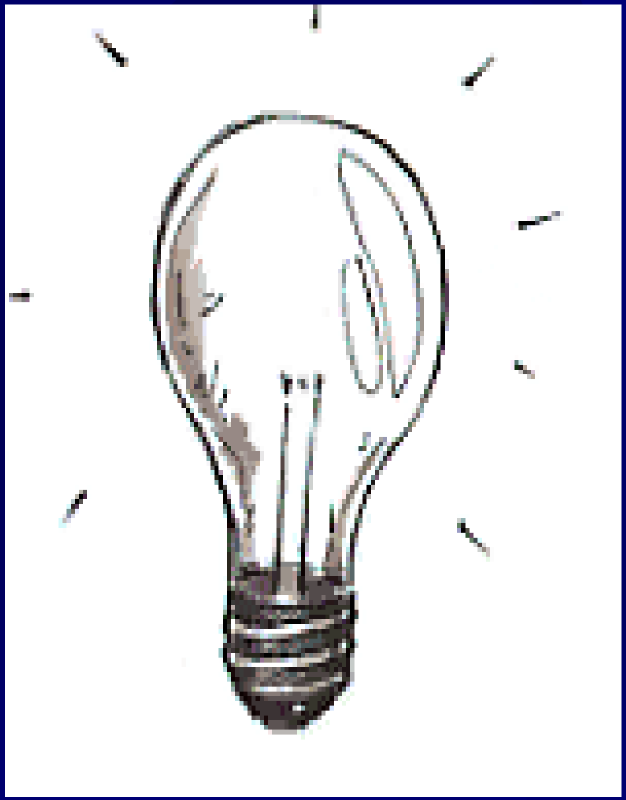
- Color is the human response to light



- Color is the human judgment of the color response.

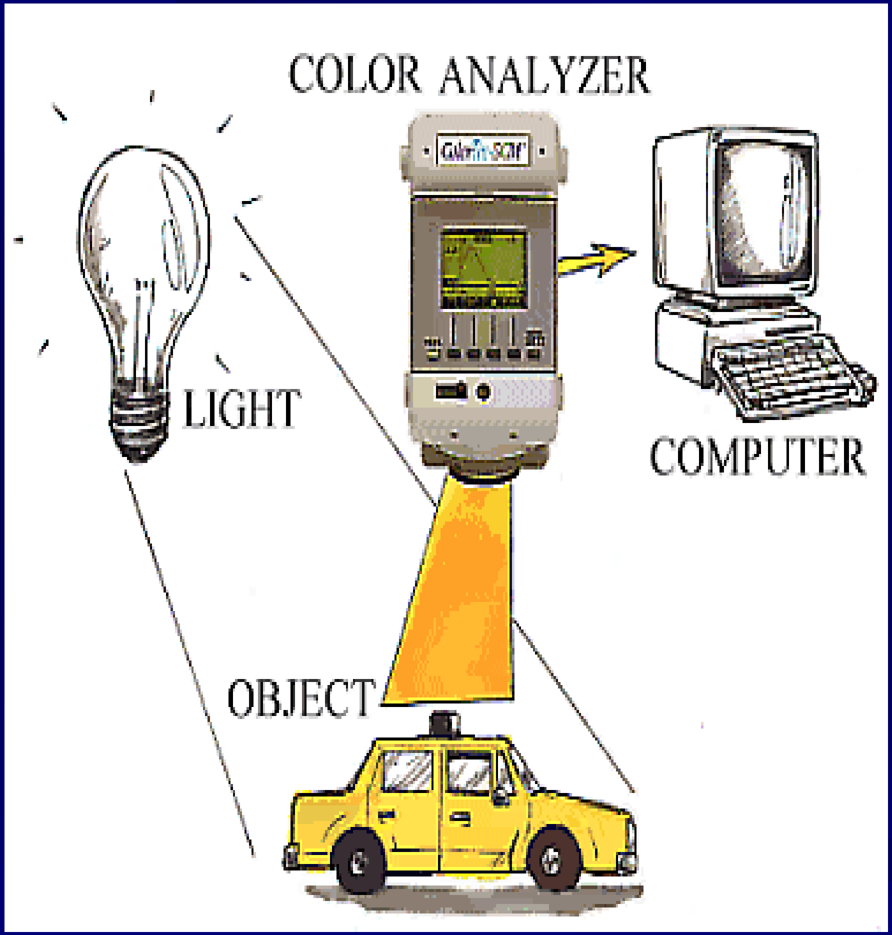


Instrumental Measurement of Color



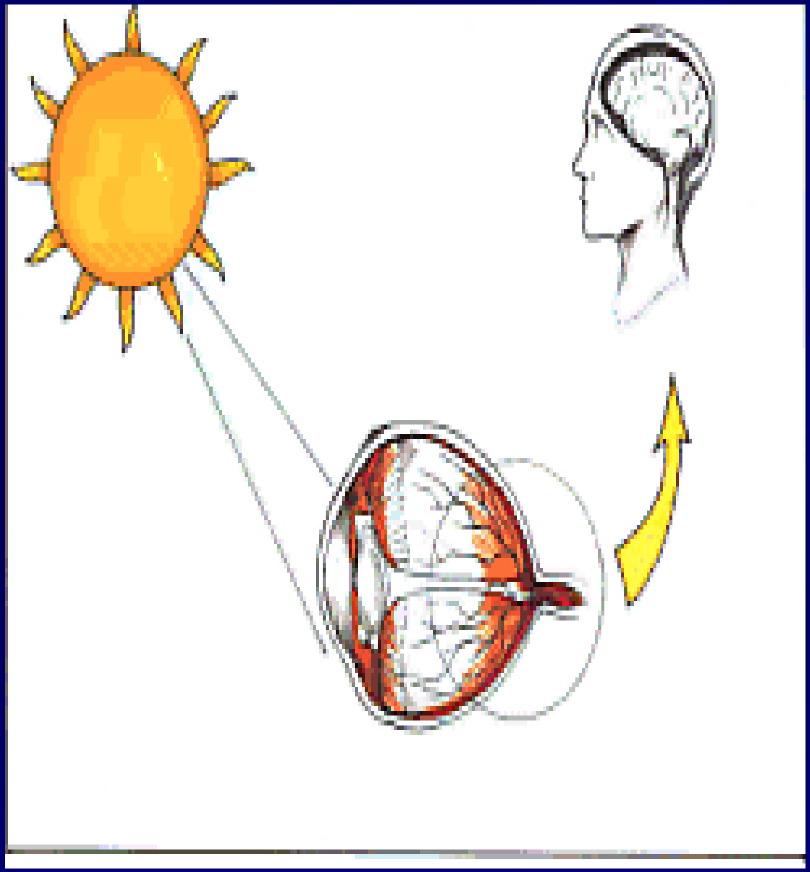
- Goal was to create a computerized device that would measure and describe color like humans see and judge color
- To simulate a system that creates the human color response:
- Color instruments need a controlled light source that we can define mathematically

What is a Color Computer



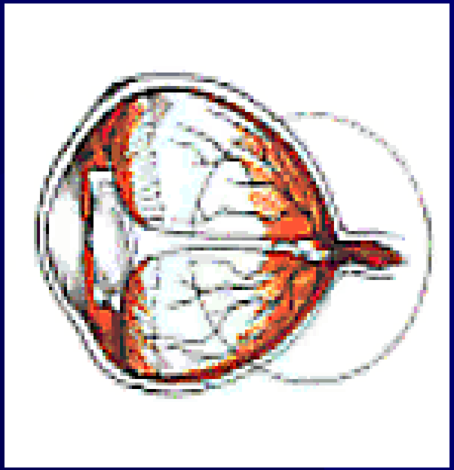
- Computer programs relate the data from the color instrument to the human response to color using mathematical simulations of light, human vision, and judgment.

HUMAN VISION

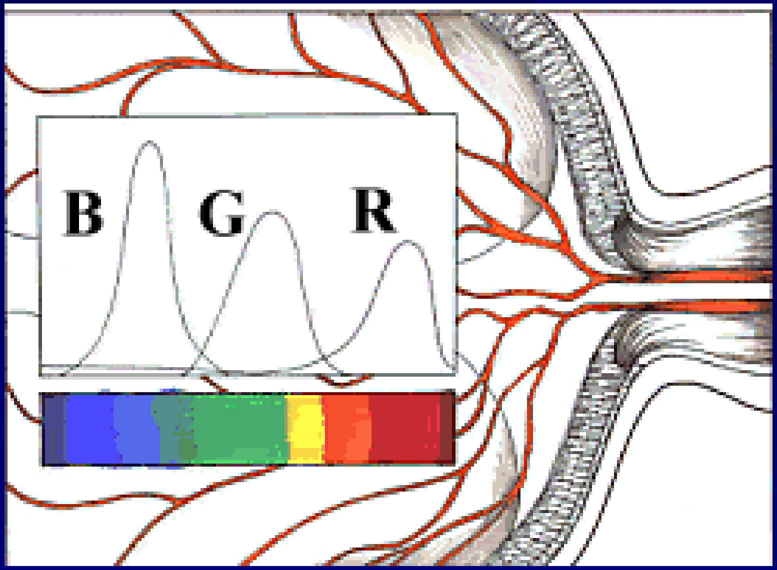


- The eye is the window to the color experience.
- Light from a source can be
  - A direct source
  - Additive light mixing
  - Reflected
  - Transmitted
- The brightness and balance of the light energy creates the color stimulus

HOW THE EYE SENSES COLOR

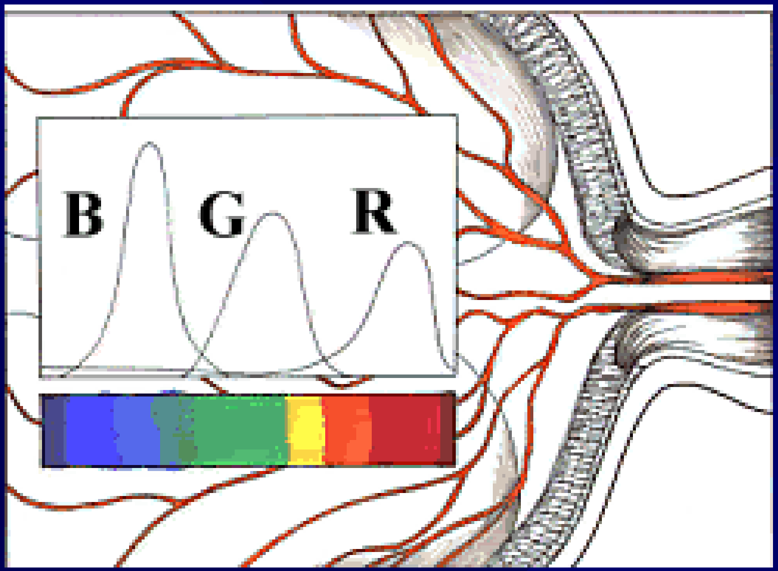


- Light enters the eye, passing through the cornea, aqueous humor, the lens, through the vitreous humor, and falls on the light-sensitive retina.



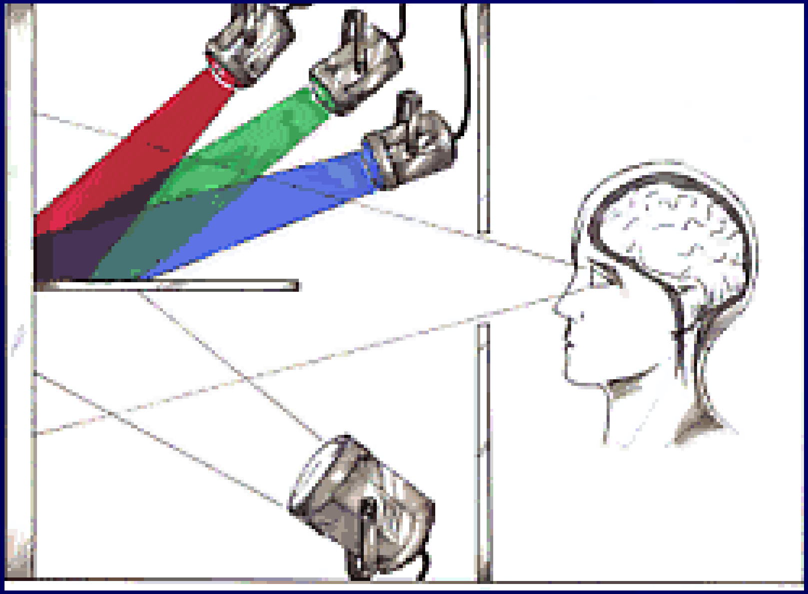
- Three types of cone cells in the retina respond to the color balance of the light stimulus. There are red cone cell responders, green cone cell responders and blue cone cell responders.

HOW THE EYE SENSES COLOR

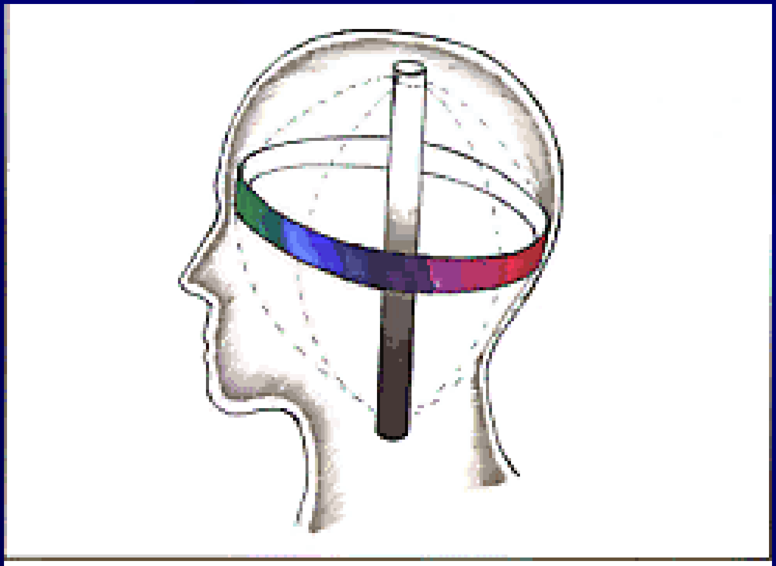


- Since there are more than 7 million cone cells in the retina, we can see many different colors in one scene at the same time. Rod cells relate to the brightness of light (white to black). There are over 17 million rod cells
- Note that each of the cone cell responses which are diagrammed as the R, G, B curves covers a broad band of color space.
- The responses overlap and have areas where they are more sensitive than other areas.

How can the human response be programmed in a computer?



- Experiments were done in the 1920's using observers working with devices that allowed the observer to mix red, green, and blue filtered light to match target colors created by another filtered light.
- The data was gathered after thousands of tests, and the results were calculated to define the human red, green, and blue response



- The vision sensations that are sent to the brain create the three dimensions (tristimulus values) of color judgment response
- Often referred to as three-dimensional color space.

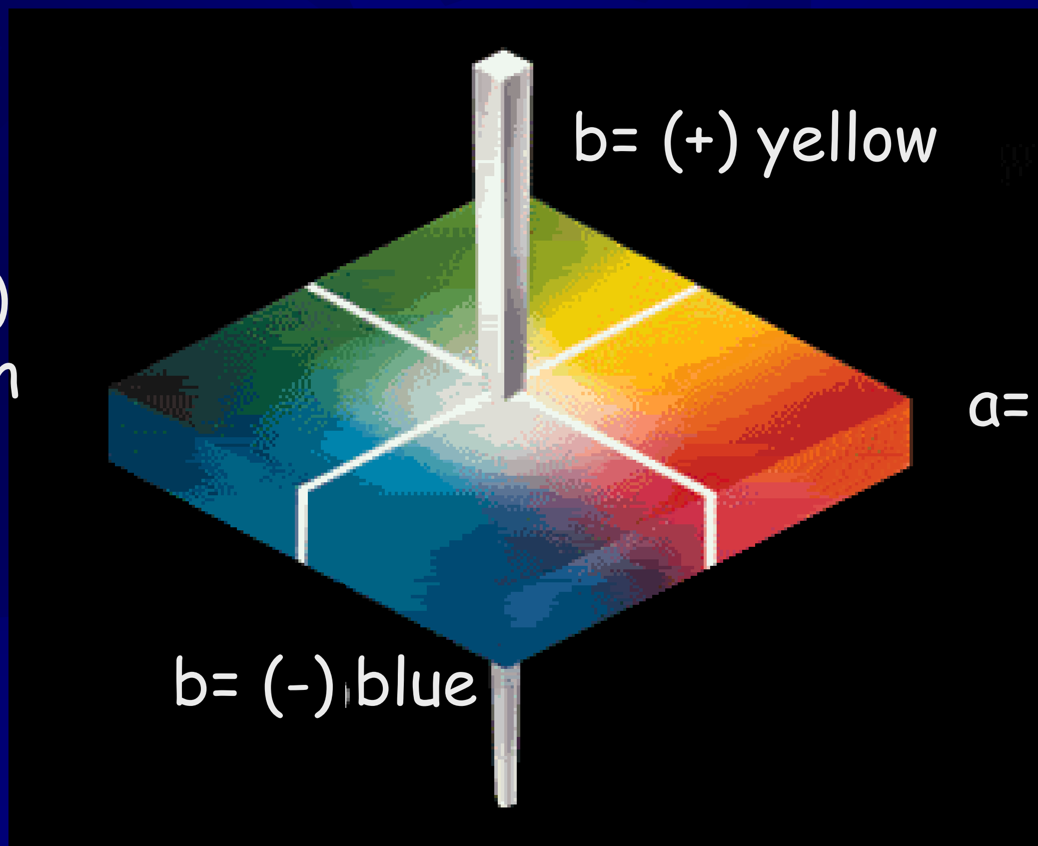
- The dimensions are:
- Light to dark (L)
- Reddish to greenish (a)
- Yellowish to bluish (b)

Color Space

HunterLab
The Color Management Company®

L= lightness (white= 100)

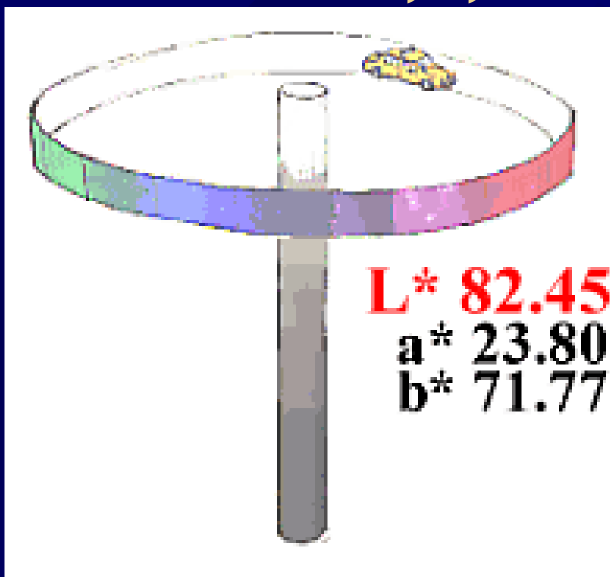
a= (-) green

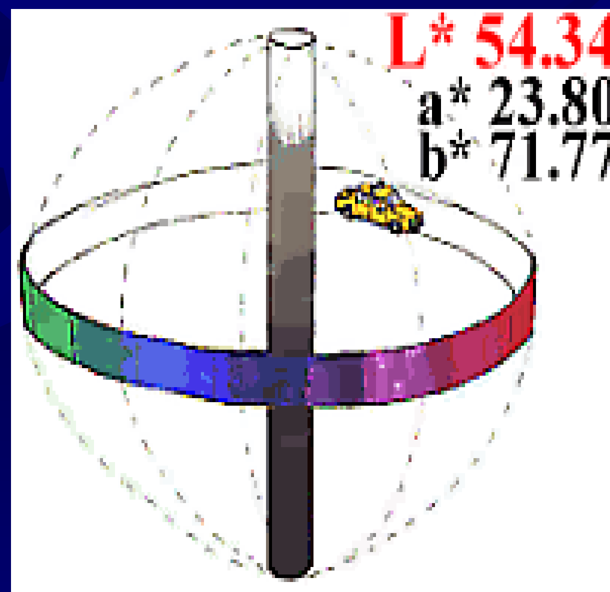


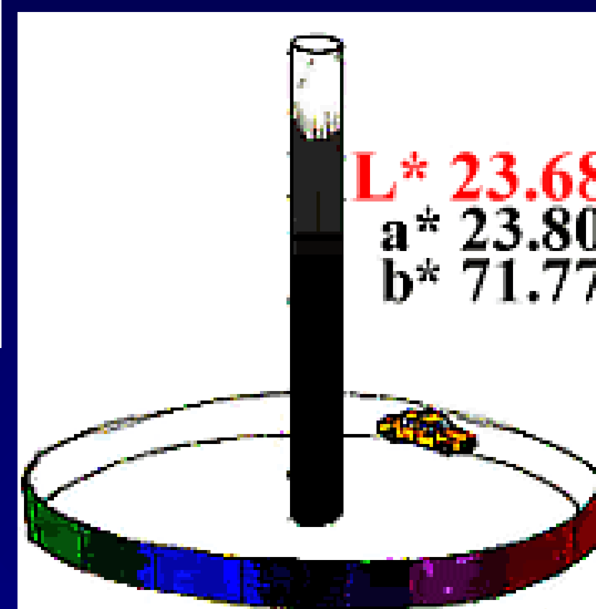
a= (+) red

L= lightness (black=0)

We express this data in the three dimensions of human color response. The mathematics is expressed as L, a, b factors defined as either Hunter L,a,b or CIE L,a,b:







L = Lightness
(black= 0 and white = 100)

COLOR WHEEL

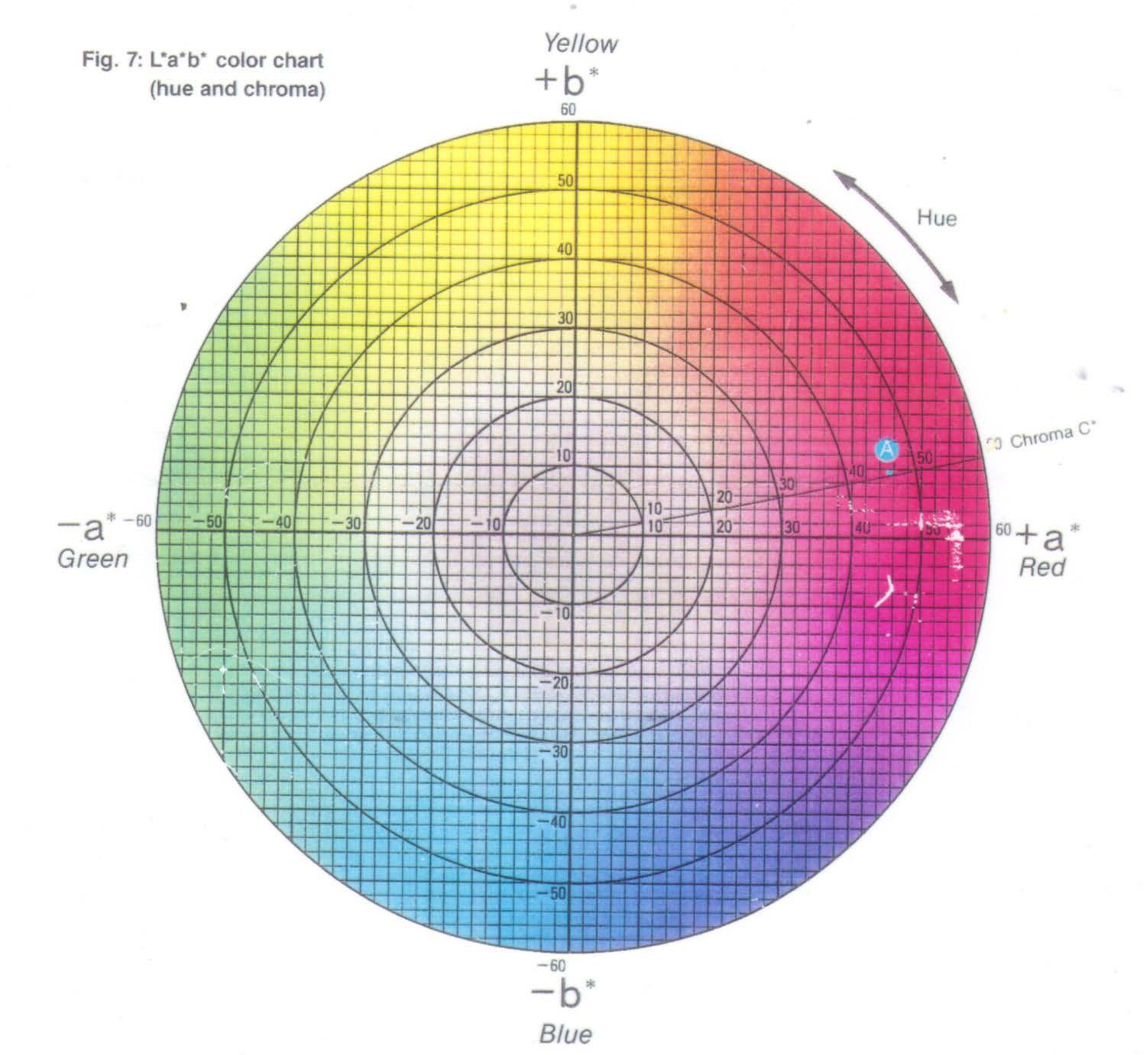
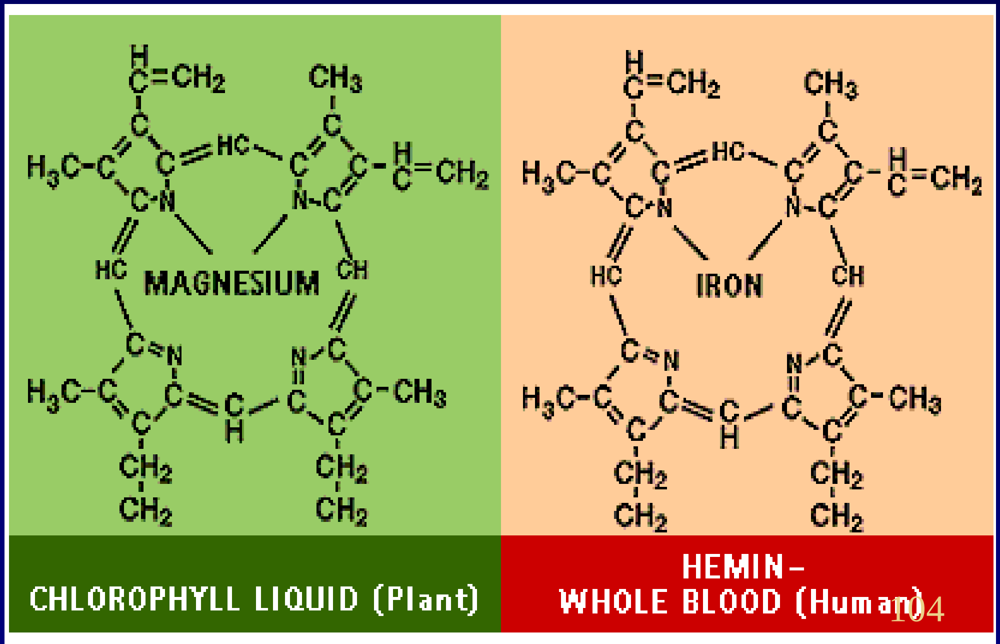


Fig. 7: L*a*b* color chart
(hue and chroma)
Yellow
+b*
Hue
Chroma C*
−a*
Green
+a*
Red
−b*
Blue

CHLOROPHYLLS

Chlorophyll

- Major light harvesting pigments in green plants, algae and photosynthetic bacteria
- Located in the lamellae of intercellular organelles of green plants known as chloroplast
- Associated with carotenoids, lipids and lipoprotiens



Chlorophyll Biosynthesis

Tertrapyrrole pigments – 4 pyrrole units joined in porphyrin ring

Mg

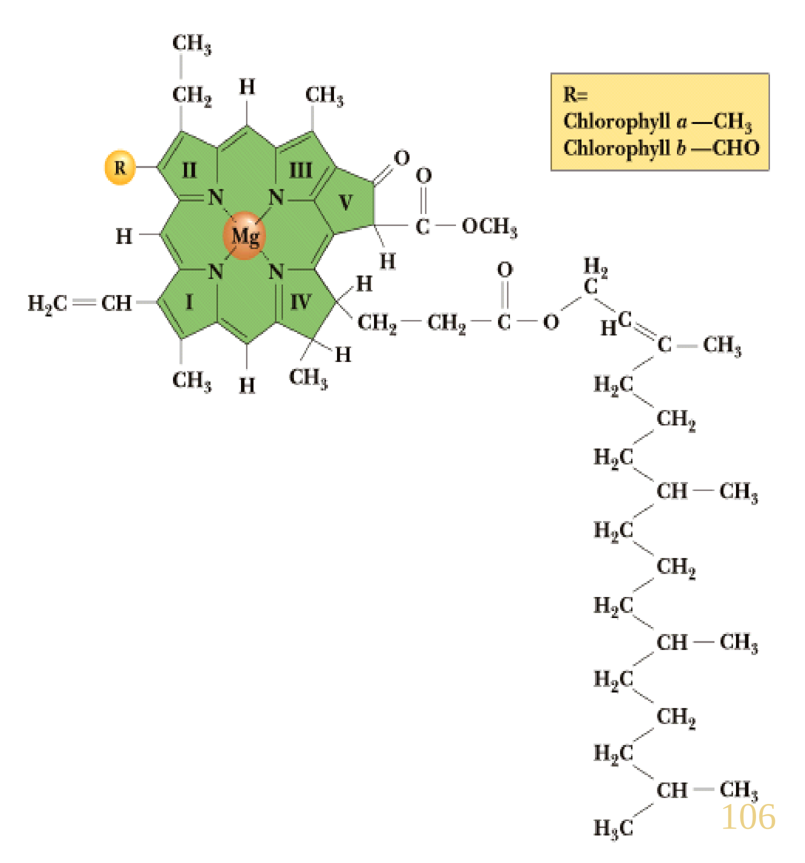
Protoporphyrin → **Chlorophyll**

Porphyrin ring

Phytol chain

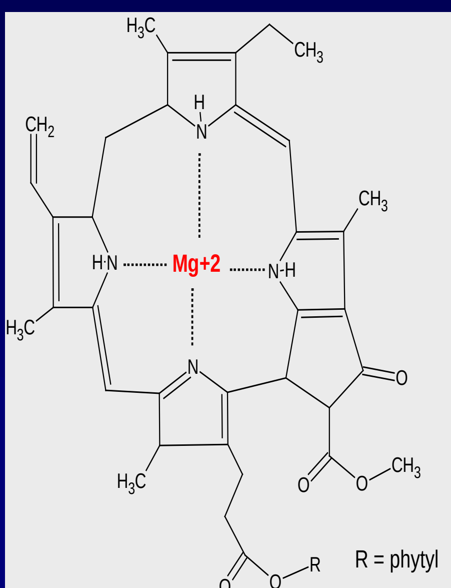
Degradation of Chlorophyll

- Enzymatic – chlorophyllase
- Heat and acidity – hydrolyze compound reducing color
- pH – alkaline stable
- Cleavage of phytol chain

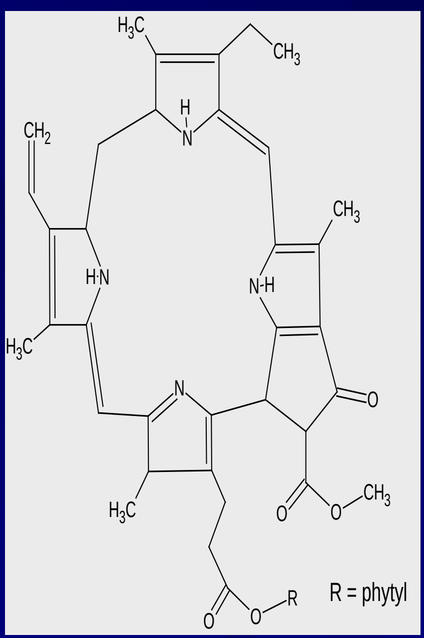


CHLOROPHYLL – effects of pH

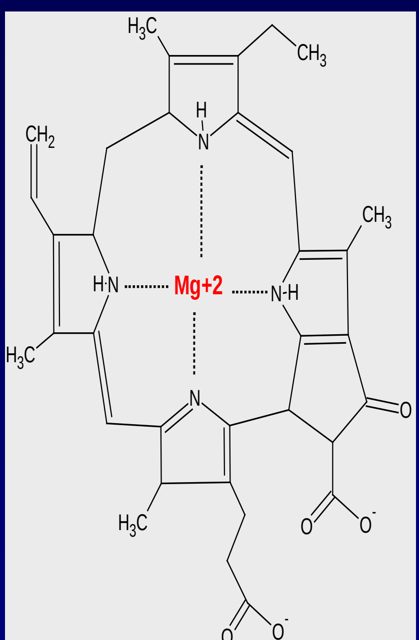
- pH 5: **chlorophyll** has its normal vegetable green color
- pH < 5: Mg^{+2} is lost and the color changes to the characteristic **pheophytin** olive green color
- pH >7: the methyl and phytyl esters are removed, producing **chlorophyllin** which is a bright green color.



Chlorophyll



Pheophytin



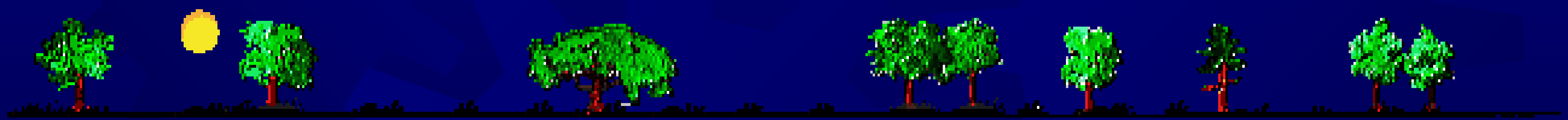
Chlorophyllin

- **CHLOROPHYLL** – effects of heating
  - heating → loss of Mg^{++} → pheophytin

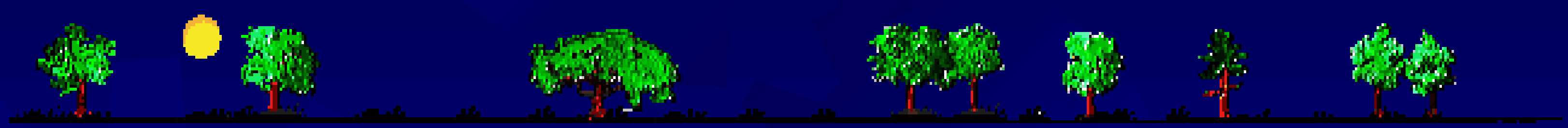
- **CHLOROPHYLL** – effects of enzymes
  - chlorophyllase – removes the phytol group (even under conditions of frozen storage)

- CHLOROPHYLL – **effects of light and oxygen**
  - photodegradation → irreversible bleaching

- If Mg++ ion is replaced with either zinc or copper → stable **green** complex at low pH



Chlorophyll → Carotenoids



Carotenoids

- Function as secondary pigments to harvest light energy
- Photoprotection role
- Precursors of Vitamin A
- Prevention of chronic diseases
- ß-carotene
  - converted to Vit.A by the body
  - reduce risk of lung and stomach cancers
- Protecting LDL oxidation

Carotenoid Biosynthesis

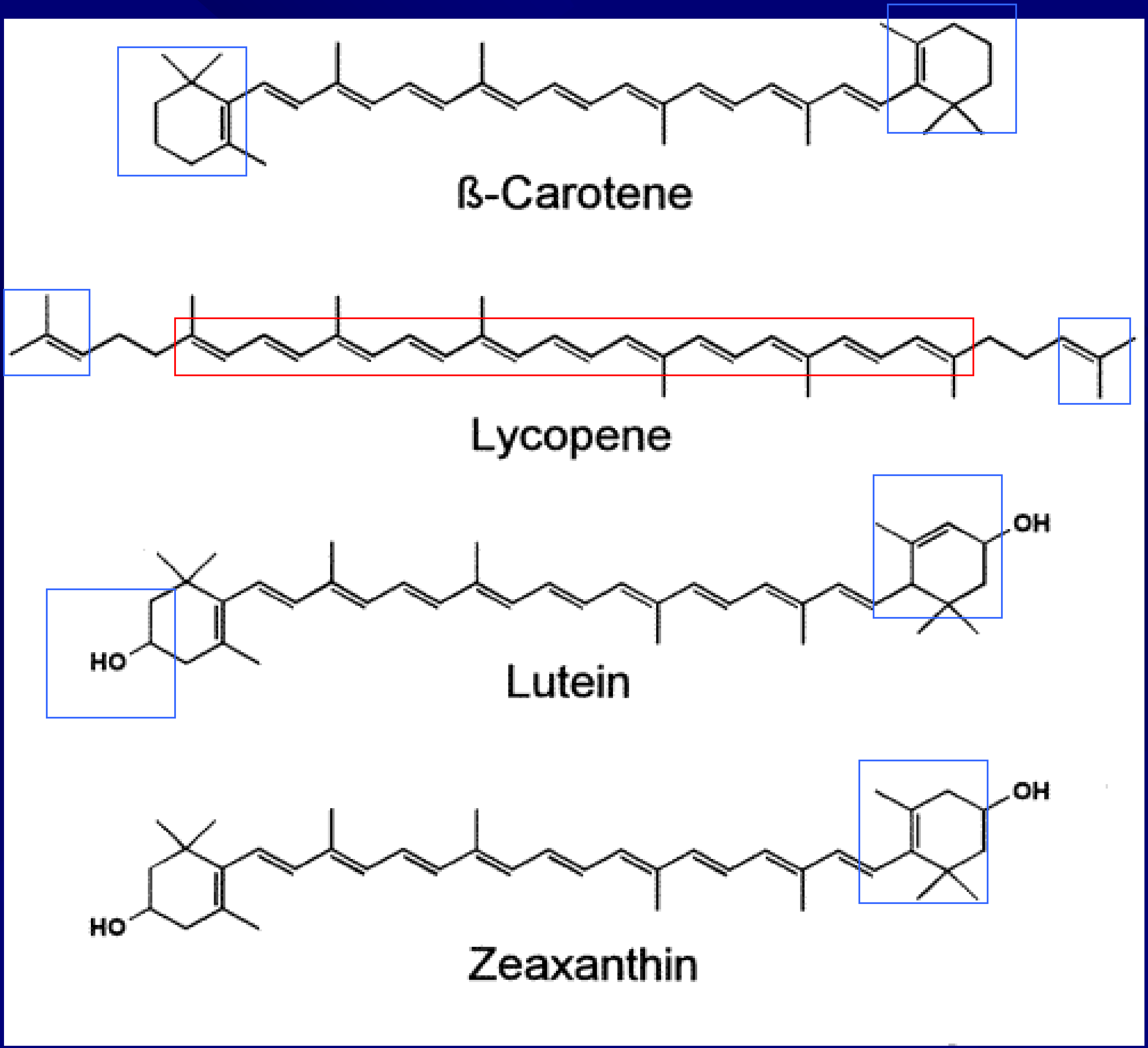
Mevalonic acid pathway

↓

Carotenoids C_{40}

↓

Made of polymers of isoprene

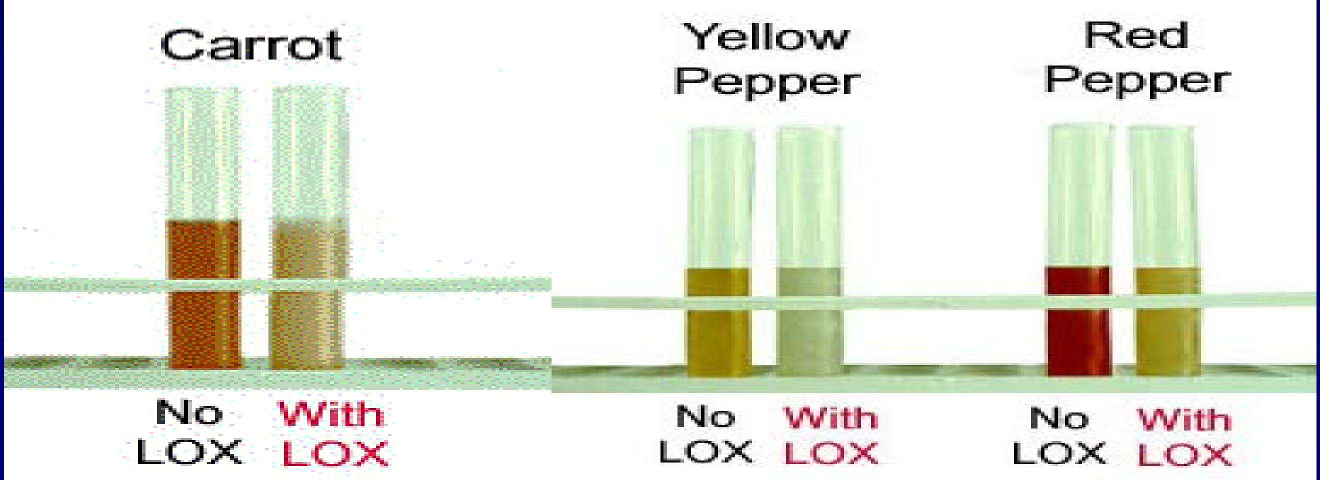


Carotenes

Hydroxy-Carotenoids
or
Xanthophylls

CAROTENOIDS: Effects of processing

- Canning → ~10% loss of provitamin A activity because of isomerization of *trans* configuration to the *cis* conf.
- Storage of dried carrots → off flavor due to carotene oxidation
- O_2 and light major factors in carotenoids breakdown (good stability to thermal treatments if O_2 and light are not present)
- Blanching prevents enzymatic oxidation reactions



Carrot Breeding

White	Orange	Purple-Orange	Red	Yellow
None	Carotene	Carotene Anthocyanin	Lycopene	Xanthophyll

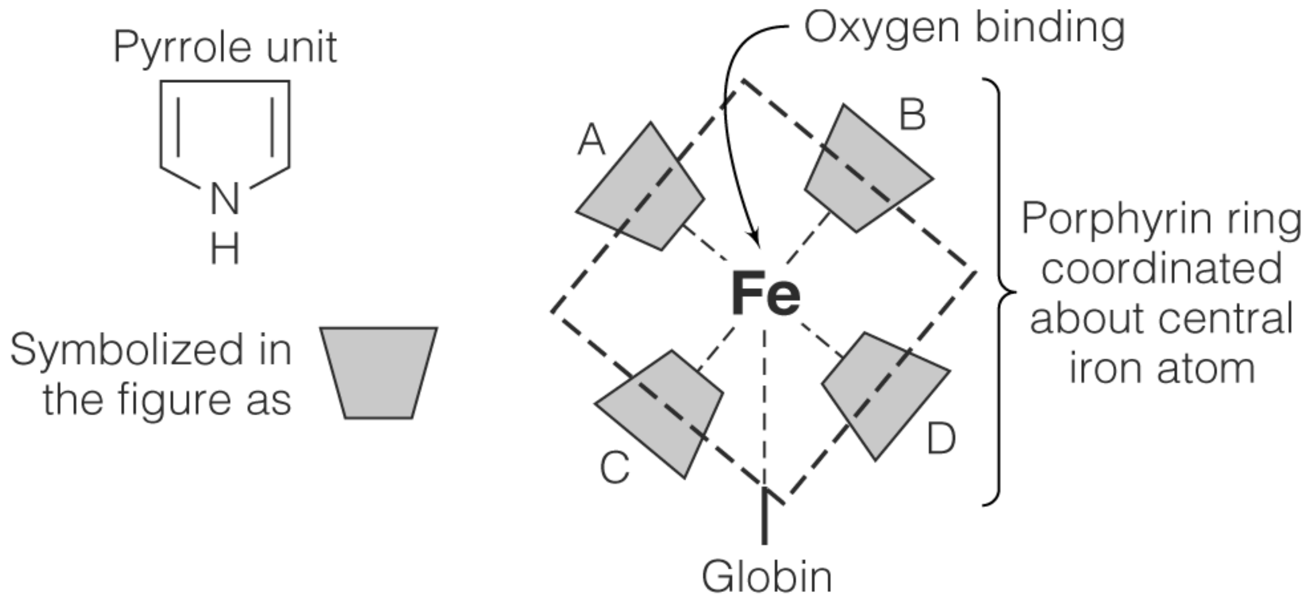
Source: USDA-ARS

Beef

What's for dinner tonight?

- Meat contains both hemoglobin and myoglobin that bind oxygen
- The bright red color of fresh cut meat is due to oxymyoglobin (*oxygenation*)
- The red color fades as *oxidation* occurs, converting Fe^{+2} to ferric (Fe^{+3}) state

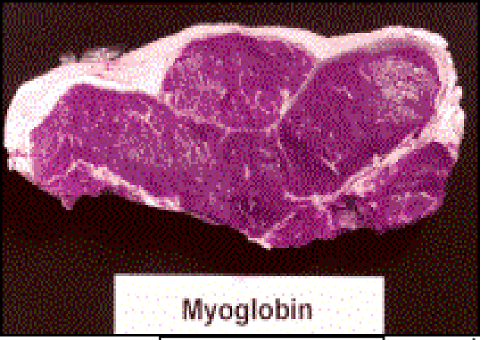
The Structure of Myoglobin

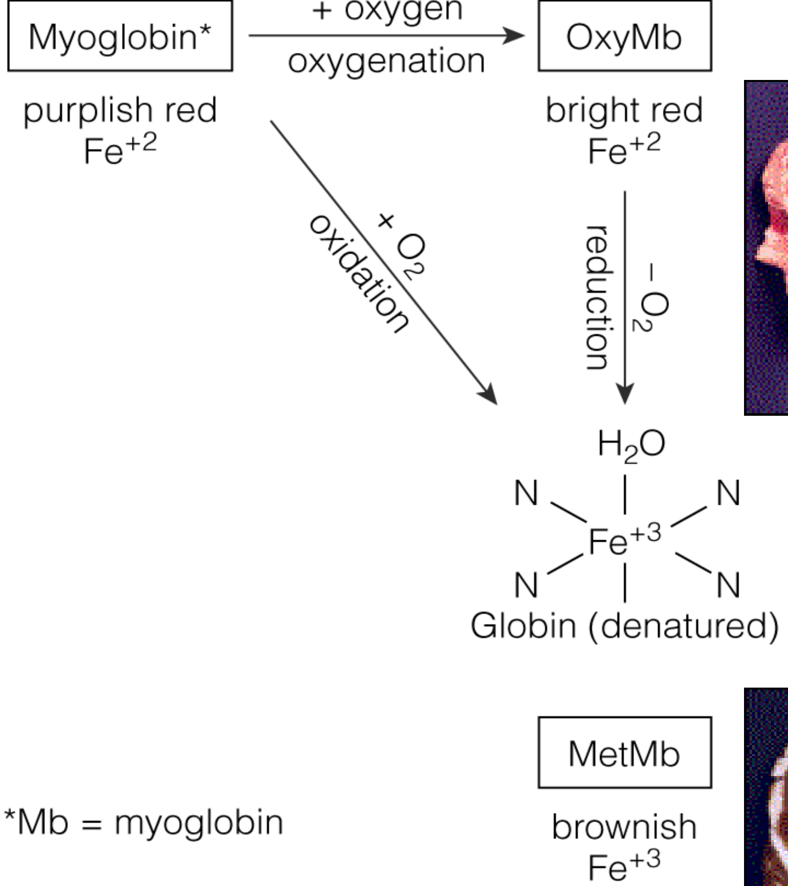


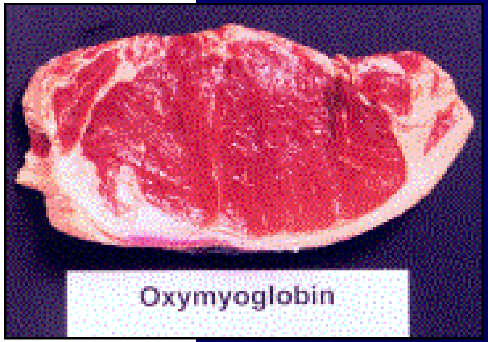
Myoglobin
contains 4 pyrrole units (A, B, C, D) that form a porphyrin ring, attached to the globin polypeptide

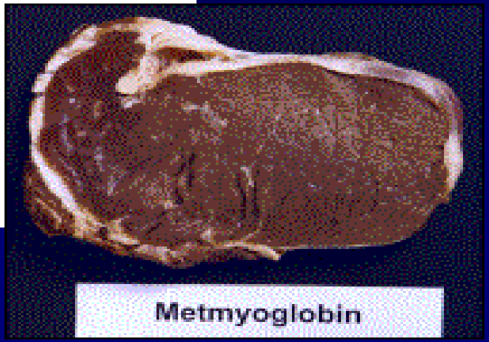
Myoglobin (MW= 17,000) is the pigment in muscle tissue, whereas hemoglobin (MW= 68,000) is the heme pigment in blood

Forms of Myoglobin in Meat









Colors in Fruits and Vegetables

Natural Colors

- Anthocyanins (grapes, blueberries, etc)
- Betalains (beets)
- Carotenoids (carrots, peach, tomato)
- Chlorophyll (broccoli, spinach)

Other Colors

- FD&C
- Exempt

Pigments and Colors

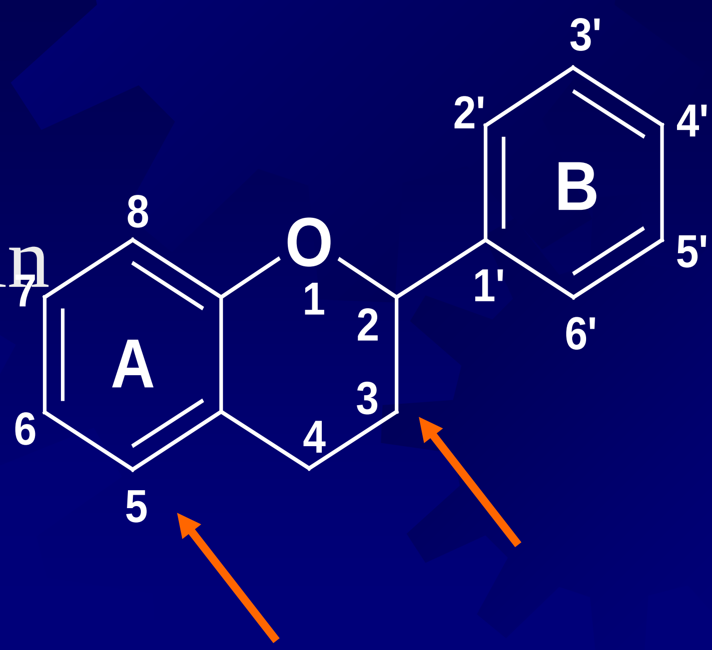
- Pigments can be degraded
- Heat, air, enzymes, etc.
- Brown pigment formation
  - Carmelization of sugars
  - Maillard reaction: reducing sugars and amino acids
  - Enzymes and oxidation

Anthocyanins

- Natural, water-soluble plant pigments
- Display a variety of pH dependent colors
- Polyphenolic compounds (flavonoid)
- Used as food colorants
- Numerous "functional" components

Anthocyanins in the Foods We Eat

- Common anthocyanin aglycones:
  - Delphinidin
  - Cyanidin
  - Petunidin
  - Pelargonidin
  - Peonidin
  - Malvidin

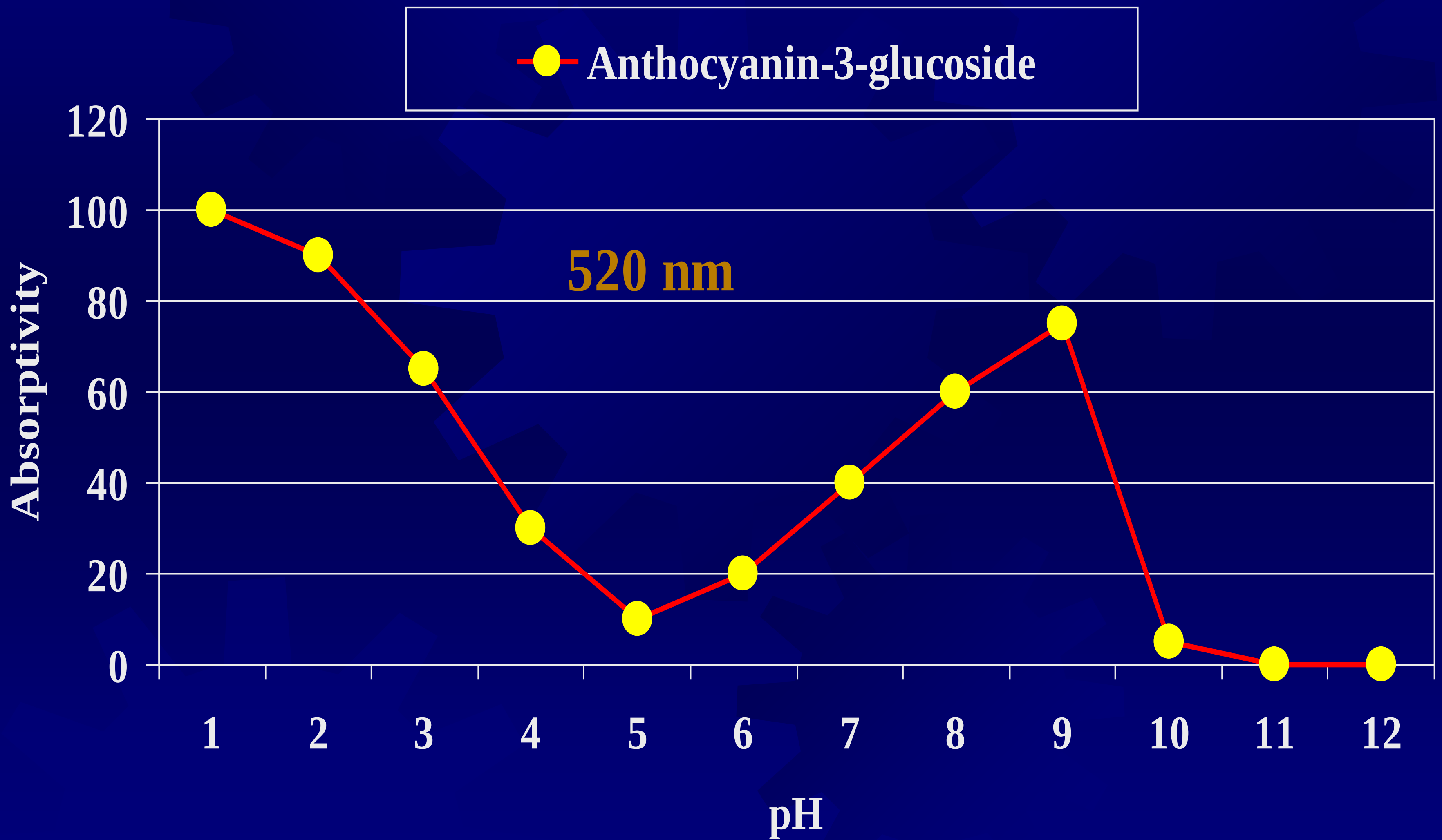


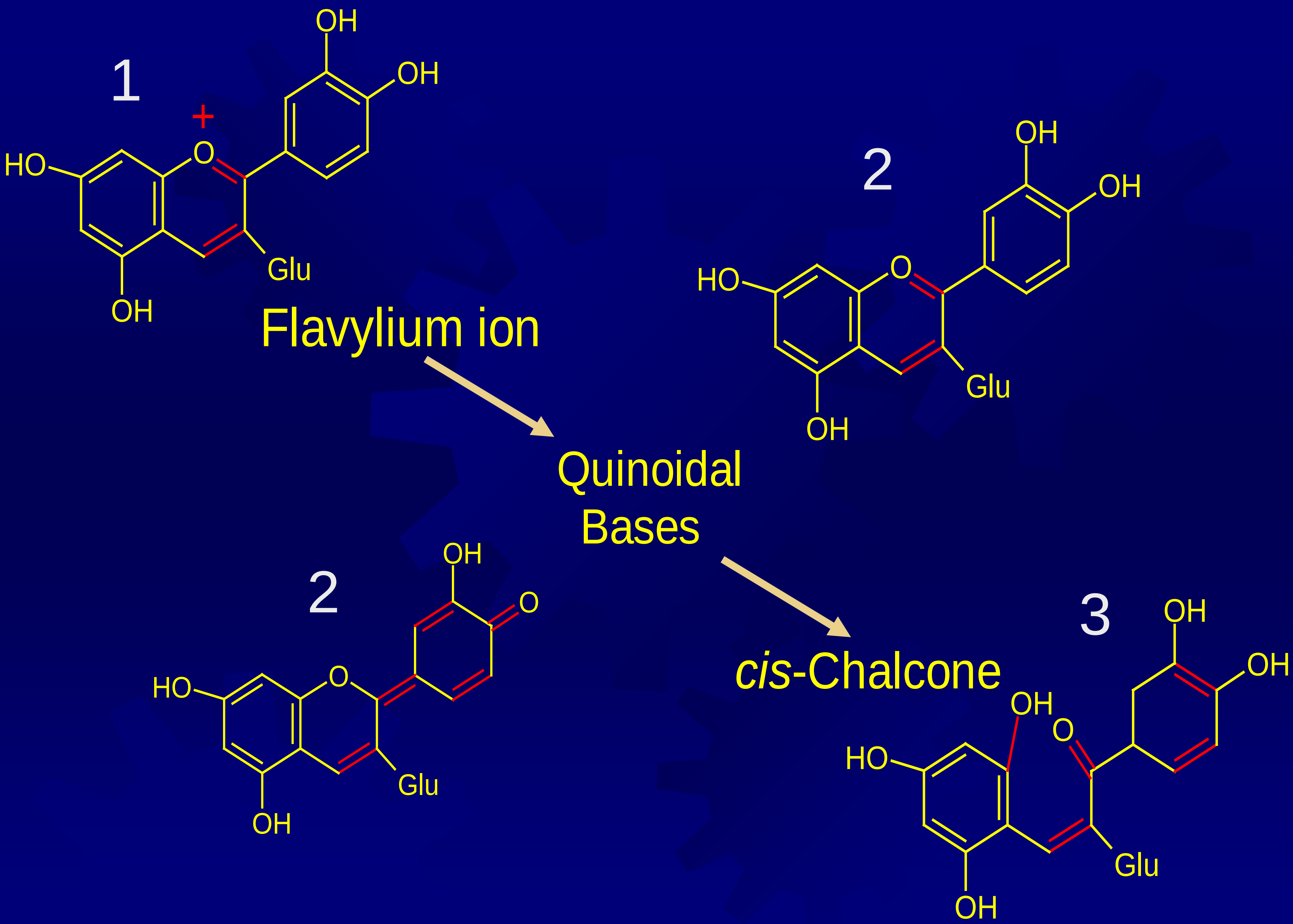
- Common sugar substitutions:
  - Glucose
  - Rhamnose
  - Galactose
  - Xylose
  - Arabinose

Altering Functional Properties

- Natural pigments have low stability compared to synthetic colorants (Red 40).
- Application range in food is limited due by pH, temperature, and complexing factors.
- High raw product costs

Anthocyanin Color at Varying pH




1
Flavylium ion
2
Quinoidal
Bases
2
cis-Chalcone
3
OH
HO
O
Glu

Traditional Anthocyanins

Sources and Applications

Sources

- Grape skin
- Red cabbage
- Elderberry
- Purple carrots
- Purple potatoes
- Red radish
- Strawberry, blueberry, blackberry, bilberry, chokeberry, cranberries, black current, hibiscus, roselle

Applications

- Soft drinks
- Instant drinks
- Fruit drinks
- Liquors
- Confectionery
- Fruit jellies
- Jams

Natural, Non-Certified or Exempt Colors

- Consist of ~26 colorants made up of dyes, pigments or other substances capable of coloring a food that are obtained from various plant, animal or mineral sources

Must be proven safe and meet FDA approval

- Caramel (brown)
- Annatto extract (red/orange/yellow; achiote)
- ß-carotene (yellow/orange; paprika)
- Beet powder (red)
- Cochineal extract (red; carmine)
- Grape skins (red/purple)
- Ferrous gluconate (black)

Synthetic or Certified

Widely used, some controversy with consumers

- *Each batch* certified by FDA
- Less than 10 synthetic colors are actually certified
- The FDA has approved certain dyes for use in foods: FD&C Colorants
  - Blue #1 (Brilliant blue)
  - Blue #2 (Indigotine)
  - Green #3 (Fast green)
  - Yellow #5 (Tartrazine)
  - Yellow #6 (Sunset yellow)
  - Red #3 (Erythrosine)
  - Red #40 (Allura red)
  - Orange B
  - Citrus Red #2
- Another class of certified colors: FD&C lakes.
  - Lakes are aluminum or calcium salts of each certified color
  - Lakes of all of the FD&C dyes *except* Red #3 are legal

21 CFR

- Search Term: “Color” or “Color Additives”
  - 70: Color additives
  - 71: Color petitions
  - 73: Exempt colors
  - 74: Certified colors

Flavor

Flavor Chemistry

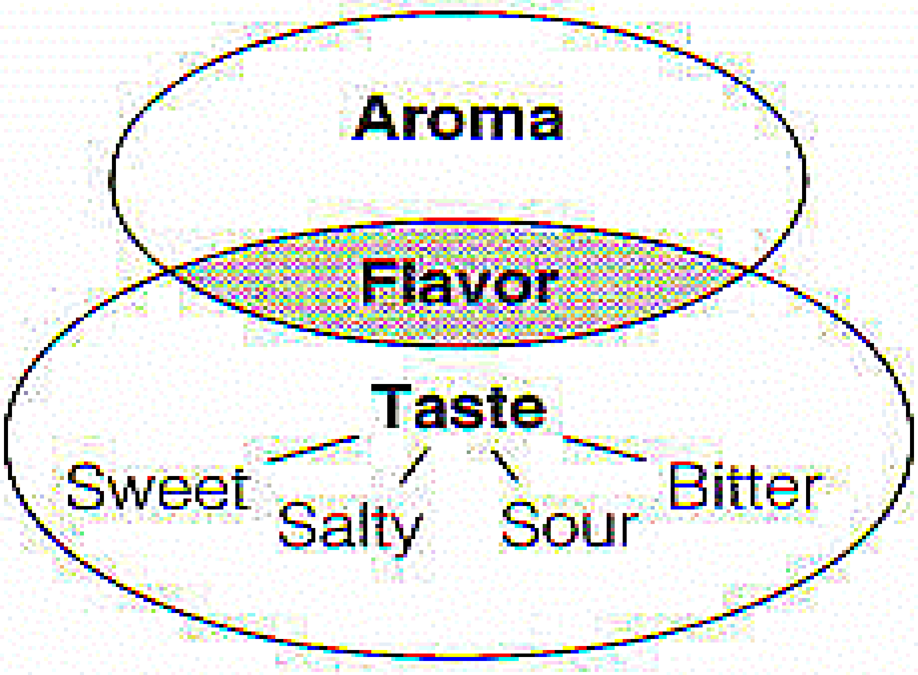
Flavor is a combination of taste and aroma

Taste

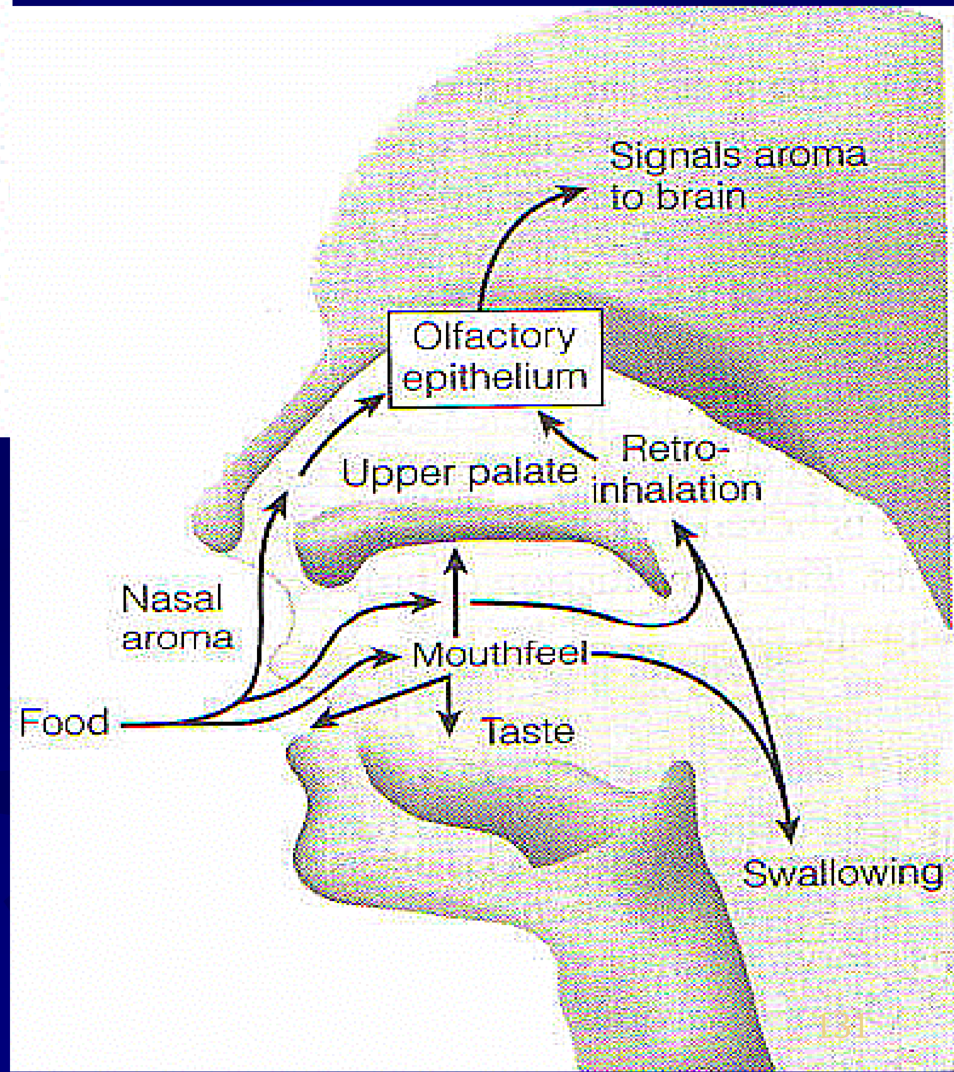
- sweet, sour, bitter, salty
- only what can be sensed on the tongue
- nerve sensations for metallic and astringent

Aroma

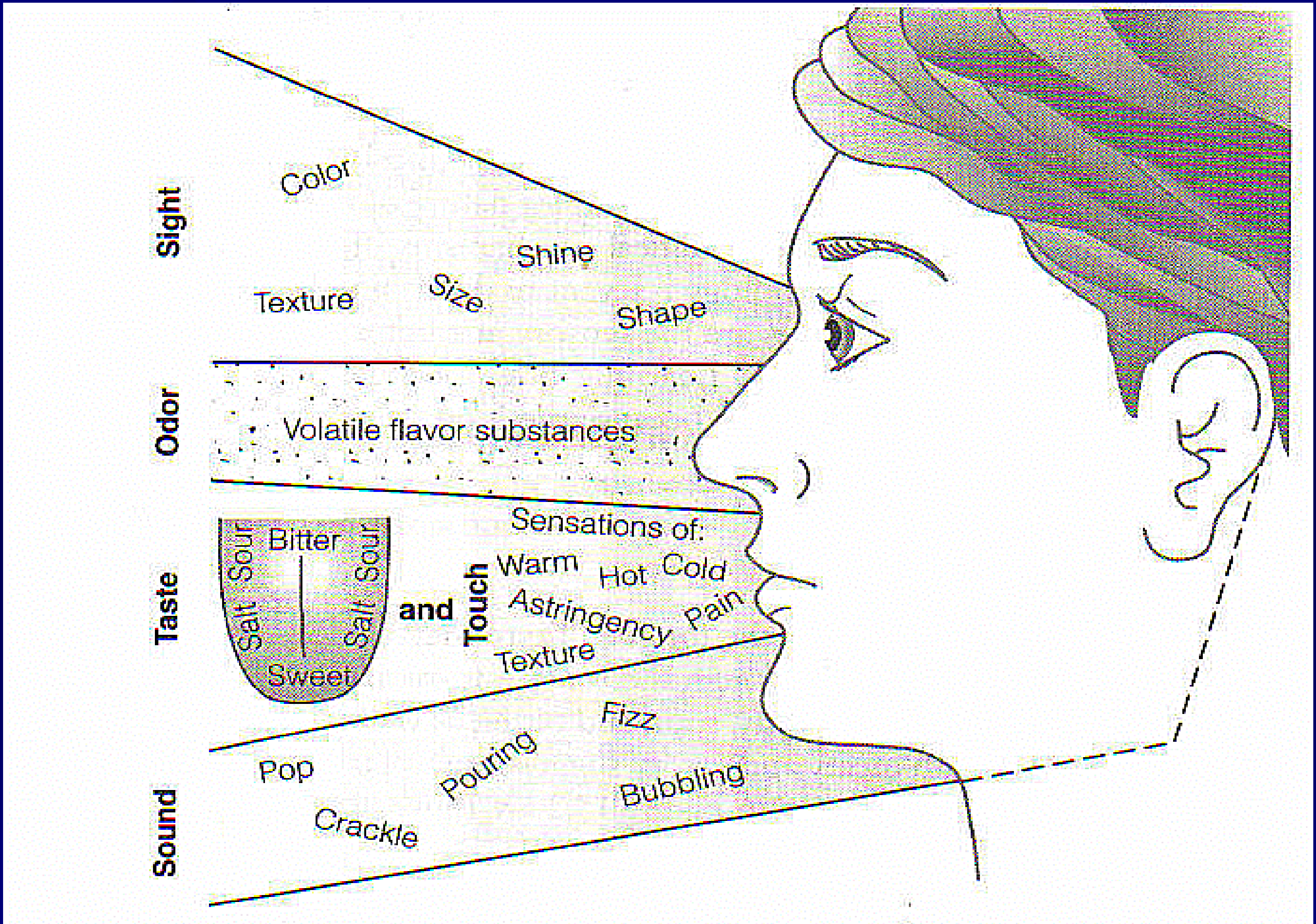
- volatiles are released in mouth and then sensed in the nasal cavity



Aroma
Flavor
Taste
Sweet
Salty
Sour
Bitter



Signals aroma to brain
Olfactory epithelium
Upper palate
Retro-inhalation
Nasal aroma
Mouthfeel
Food
Taste
Swallowing



Sight
Color
Shine
Texture
Size
Shape
Odor
Volatile flavor substances
Taste
Bitter
Salt Sour
Salt Sour
Sweet
and
Touch
Sensations of:
Warm
Hot
Cold
Astringency
Pain
Texture
Sound
Fizz
Pop
Pouring
Bubbling
Crackle

Food flavors

Mixtures of natural and/or artificial aromatic compounds designed to impart a flavor, modify a flavor, or mask an undesirable flavor

Natural versus Artificial

Natural - concentrated flavoring constituents derived from plant or animal sources

Artificial - substances used to impart flavor that are not derived from plant or animal sources

Most **natural flavors** are concentrated from botanicals -plants, trees, fruits, and vegetables

Most **artificial flavors** are synthesized with high purity - pharmaceutical flavors

Isolation techniques

- Steam distillation - mint and herbal oils
- Solvent extraction - vanilla & oleoresins
- Expression - citrus oils
- Supercritical fluid extraction – targeted extractions

Natural flavors can also be **enzymatically** or **chemically** produced

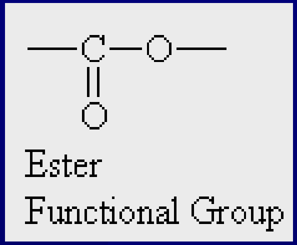
- **Fermentation** reactions
- **Microbial** enzymes
  - *Saccharomyces* Sp.
  - *Lactobacillus* Sp.
  - *Bacillus* Sp.
  - Molds

Maillard flavor compounds

Glucose	+	Glutamic acid	=	chicken
Glucose	+	Lysine	=	burnt or fried potato
Glucose	+	Methionine	=	cabbage
Glucose	+	Phenylalanine	=	caramel

Fructose	+	Glutamic acid	=	chicken
Fructose	+	Lysine	=	fried potato
Fructose	+	Methionine	=	bean soup
Fructose	+	Phenylalanine	=	wet dog

Artificial Flavors



Typically are **esters**

Esters have pleasant fruity aromas, derived from acids

a **condensation** reaction

ACID + ALCOHOL --> ESTER + WATER

Most artificial flavors are simple mixtures of esters

i.e.
Isobutyl formate + isobutyl acetate = raspberry

FERMENTATION and FLAVOR

$$CH_3-\overset{\displaystyle O}{\overset{\|}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}-CH_3$$

Diacetyl ($CH_3 – C - C – CH_3$) is a compound produced by Yeasts via fermentation of carbohydrates

Major compound in the flavor of cultured dairy products
Butter and butter-like flavor

Compounds potentially used for diacetyl formation

Lactic acid	Oxalacetic acid
Pyruvic acid	acetyl lactic acid
Acetaldehyde	Citric acid

Flavor stabalization

- Need to protect from light, heat, oxygen, water

- **Liquid flavors** are typically dissolved in solvents

Partially hydrogenated oil or brominated vegetable oil

Ethanol, propylene glycol, glycerin

Dry flavors are typically encapsulated

- **Spray drying**
- Use of excipients

Plating - coat flavor onto sugar or salt

Extrusion - glassy sugar film

Inclusion complex - beta cyclodextrins

Secondary coatings - high melting temperature fat

Flavor interactions

pH, tartness of acids dependent on acid

Acidulant, the type of acid used influences intensity of other flavors

Carbohydrates, can bind flavor compounds, so less flavor may be needed at low sugar levels

Sweeteners, sweetness can impact flavor intensity

Lipids, flavors partition, fat helps flavor impact

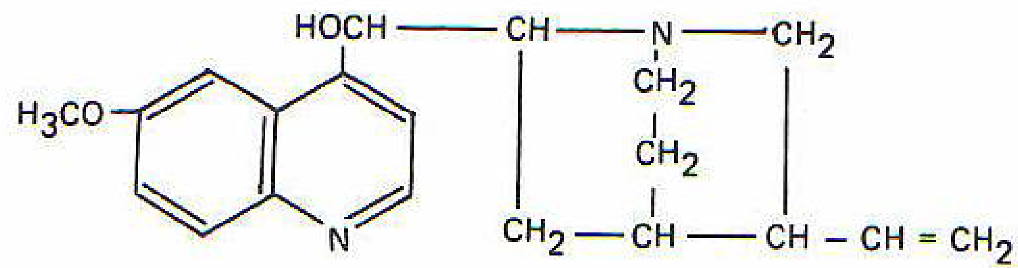
Protein, selective binding of flavor compounds

BITTERNESS cqn be attributed to several inorganics and organics

$KI \quad CsCl \quad MgSO_4$

Certain amino acids and **peptides** (dipeptide leucine-leucine)

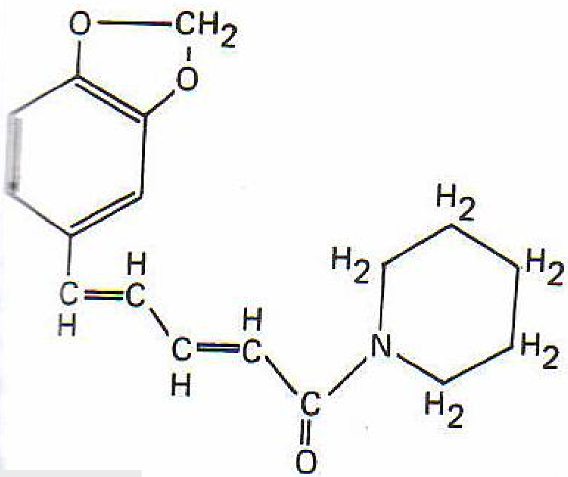
Alkaloids derived from pyridine (N-containing 6-membered ring) and purines



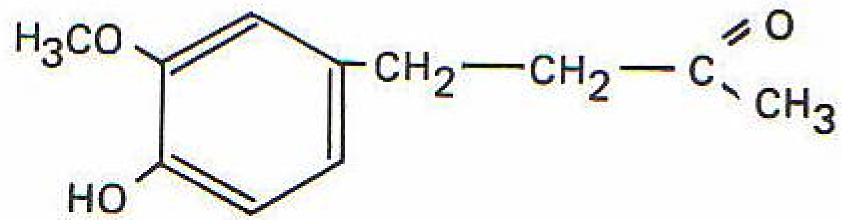
Structure of Quinine. This has an intensely bitter taste.

A = caffeine (1, 3, 7 trimethylxanthine)　　B = theobromine (from cacao)

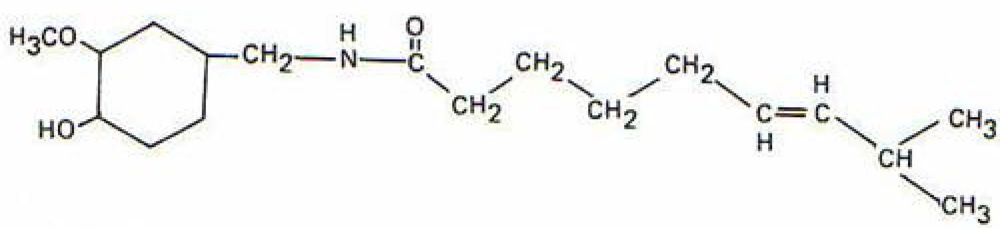
“HOTNESS” (pungency) is characteristic of piperine in black pepper and capsicum in red pepper and gingerols in ginger



Piperine, Responsible for the Hot-
of Pepper



Zingerone, the Pungent Principle of
Ginger



Capsaicin, the Pungent Principle of Red Pepper

Sunlight Flavor

Sunlight will induce oxidized flavor and sunlight flavor and hay-like flavor.

Oxidized flavor
Sunlight flavor: burnt and / or cabbage

Riboflavin Effect on Sunlight Flavor

Riboflavin is a catalyst for production of the sunlight flavor.

1) Milk protein and riboflavin $\xrightarrow{\text{sunlight}}$ sunlight flavor

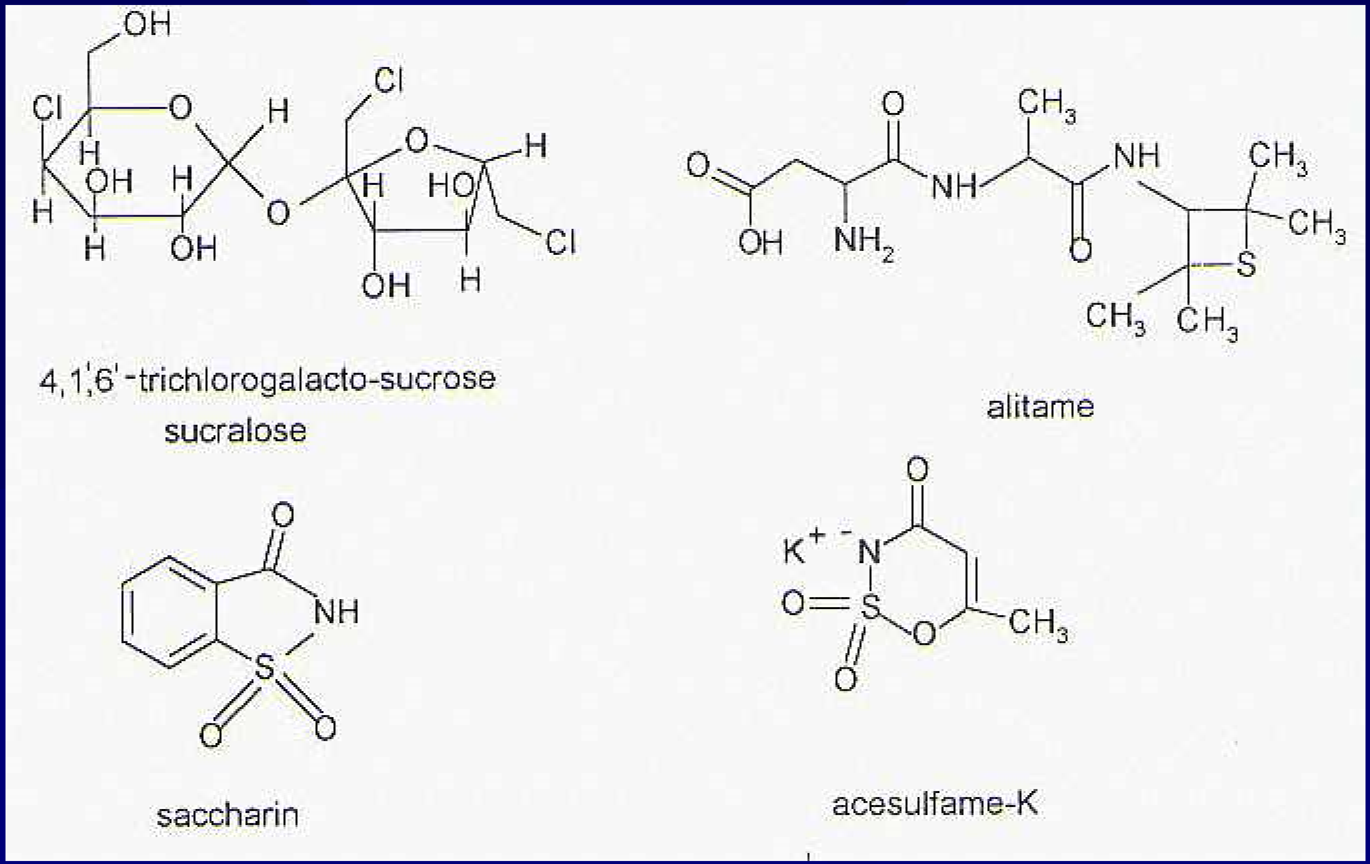
2) Riboflavin increase in milk will increase the sunlight flavor

3) Riboflavin removal prevent the sunlight flavor

According to the TG Lee Website:

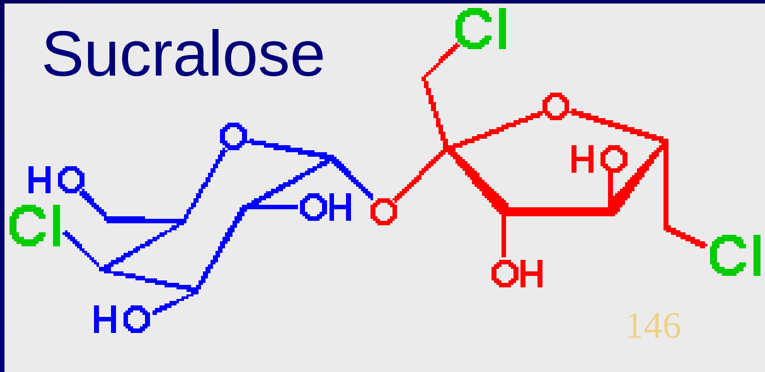
- Studies at the Silliker Laboratories in Illinois, the University of Michigan and other leading labs and universities concluded that both sunlight and the fluorescent lighting in stores could decrease the freshness and flavor of milk and the potency of vital vitamins in it. But this research also showed that the majority of natural and artificial light could be blocked by containers that were yellow instead of white.

Artificial and Alternative Sweeteners



Sweeteners

- Non-nutritive (no calories)
- Cyclamate (banned in 1969)
- Saccharin (Sweet ‘N Low, 300-fold)
- Aspartame (warning label) = aspartic acid and phenylalanine (180-fold)
- Acesulfame-K (Sunette, 200-fold)
  - Alitame (Aclame, 2,000-fold)
- Sucralose (Splenda, 600-fold)



The perception of sweetness is proposed to be due to a chemical interaction that takes place on the tongue Between a tastant molecule and tongue receptor protein

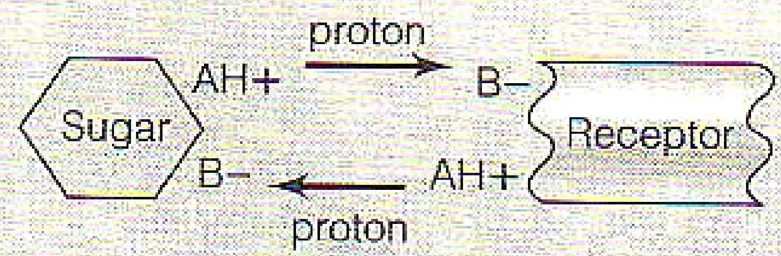
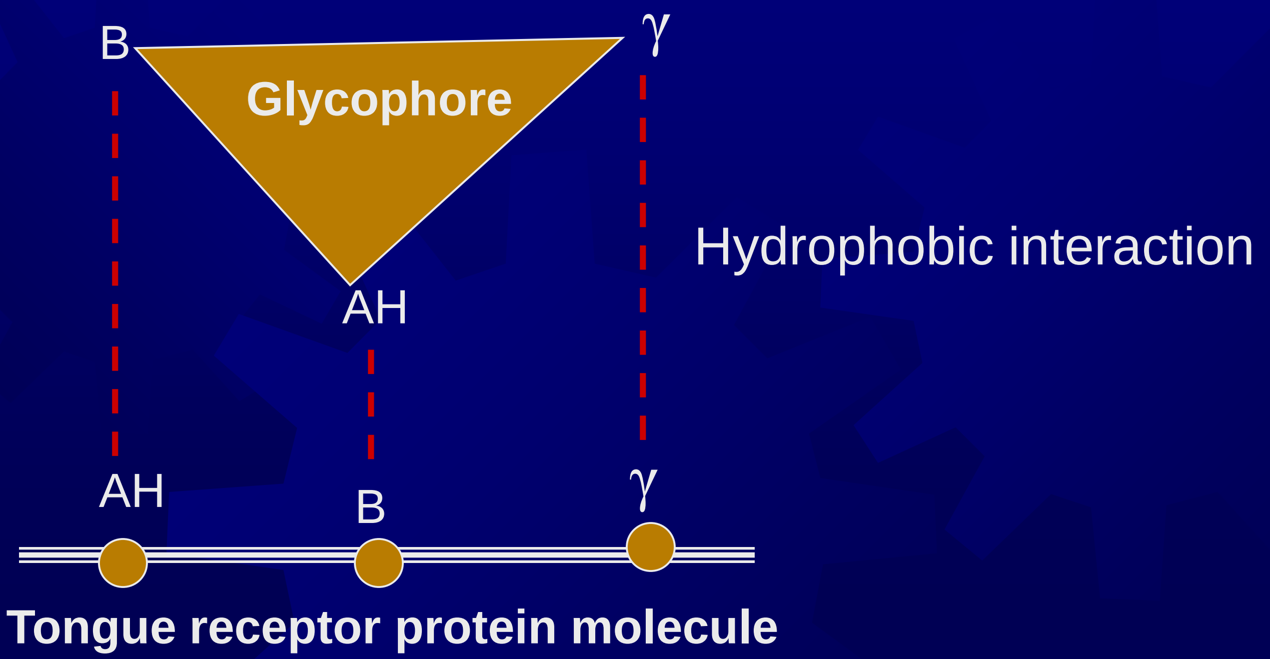


FIGURE 6.14 Sweetness theory. *Tastant sugar molecule having AH+ and B– sites is attracted to the complementary regions of the tongue protein receptor molecules through donation of a proton.*

THE AH/B THEORY OF SWEETNESS

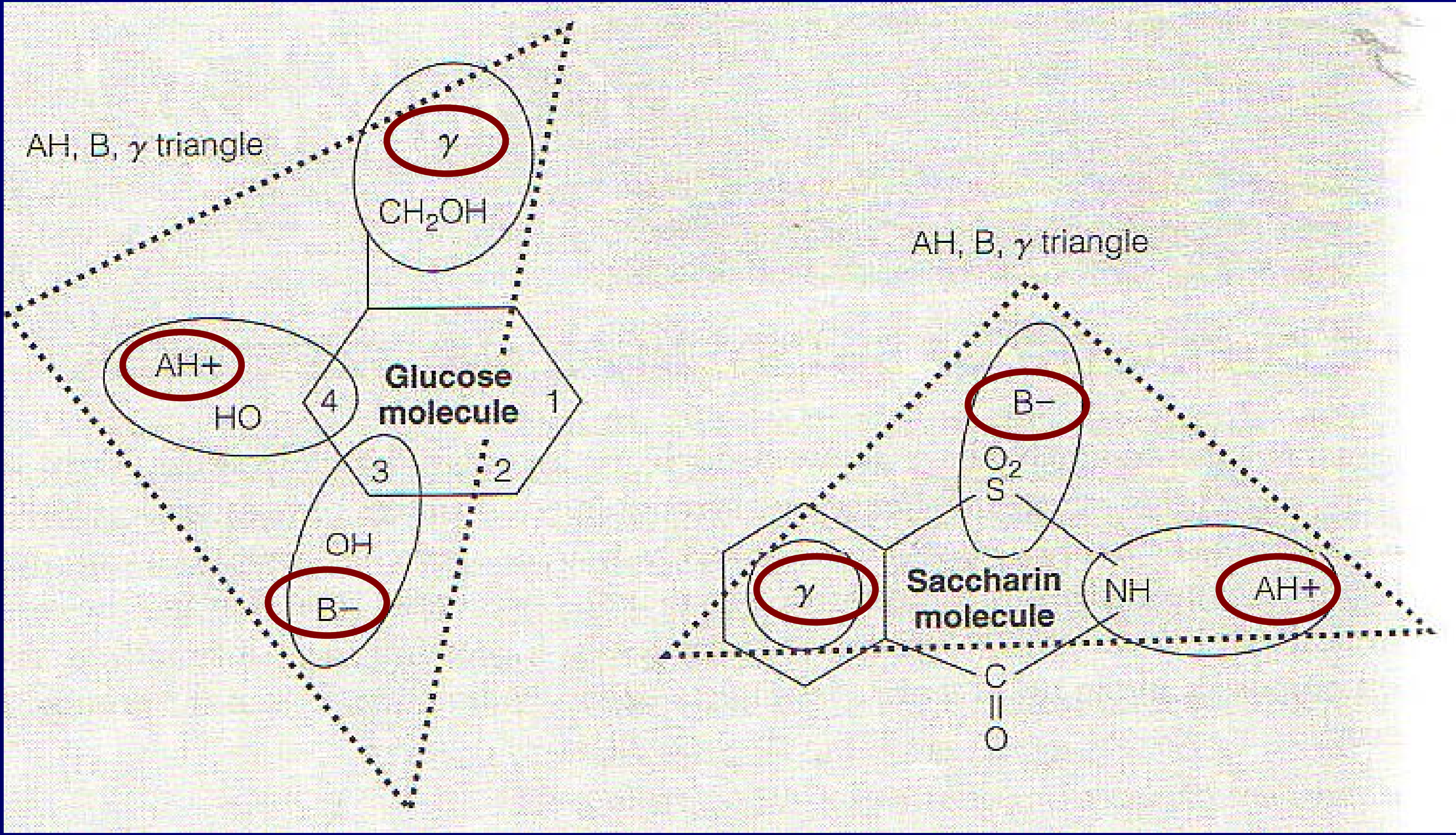
A sweet tastant molecule (i.e. glucose) is called the AH+/B- "glycophore".
It binds to the receptor B-/AH+ site through mechanisms that include H-bonding.

AH+ / B-

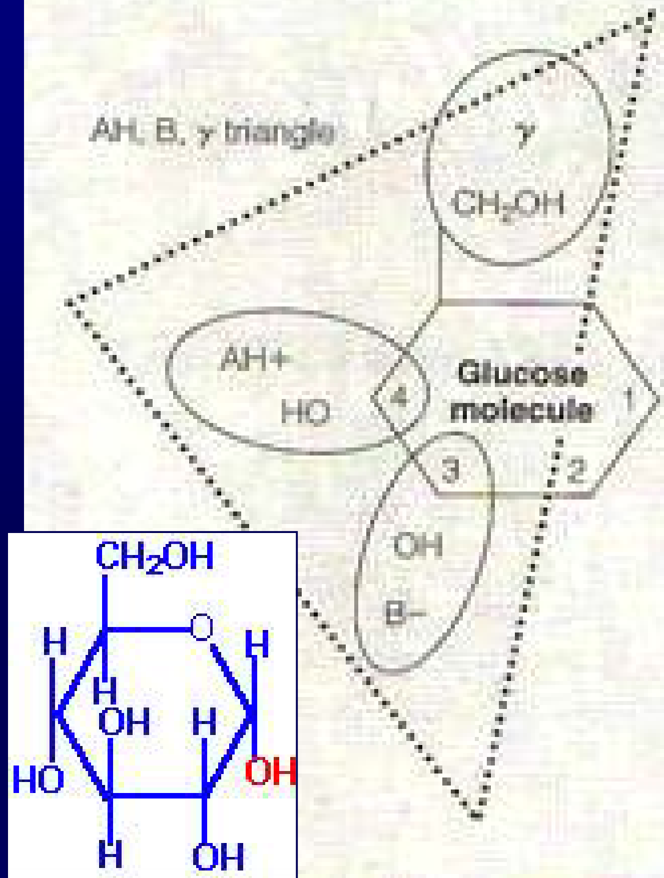


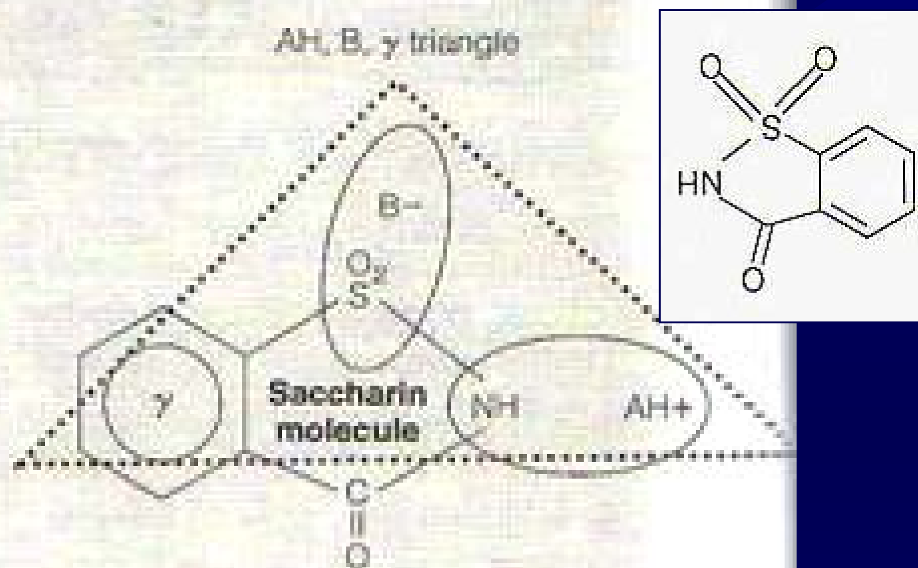
For sweetness to be perceived, a molecule needs to have certain requirements. It must be soluble in the chemical environment of the receptor site on the tongue. It must also have a certain molecular shape that will allow it to bond to the receptor protein.

Lastly, the sugar must have the proper electronic distribution. This electronic distribution is often referred to as the AH, B system. The present theory of sweetness is AH-B-X (or gamma). There are three basic components to a sweetener, and the three sites are often represented as a triangle.


AH, B, γ triangle
γ
CH2OH
AH+
HO
4
Glucose
molecule
1
3
2
OH
B−
AH, B, γ triangle
B−
O2
S
γ
Saccharin
molecule
NH
AH+
C
O

Identifying the AH+ and B- regions of two sweet tastant molecules: glucose and saccharin.





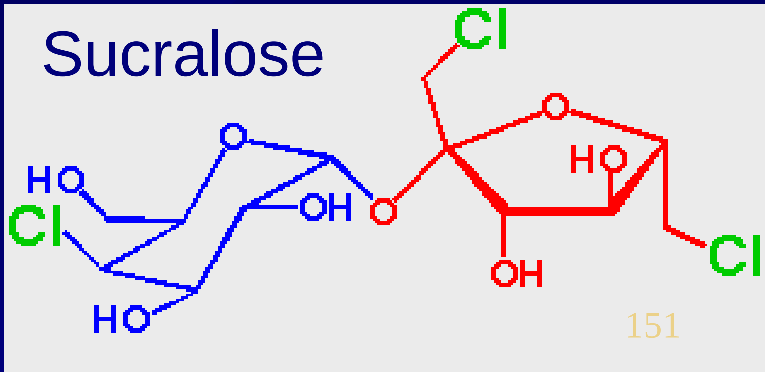
Gamma (γ) sites are relatively **hydrophobic** functional groups such as benzene rings, multiple CH_2 groups, and CH_3

WHAT IS SUCRALOSE AND HOW IS IT MADE?

Sucralose, an intense sweetener, approximately 600 times sweeter than sugar.

In a patented, multi stage process three of the **hydroxyl groups** in the sucrose molecule are selectively substituted with 3 atoms of chlorine.

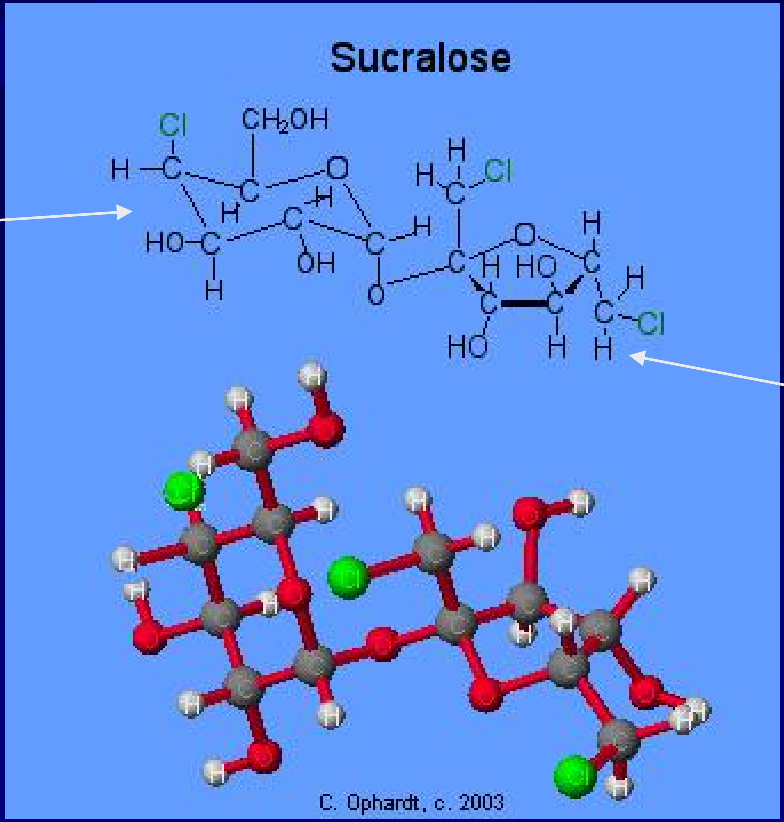
This intensifies the sugar like taste while creating a safe, stable sweetener with zero calories.



Developers found that selective **halogenations** changed the perceived sweetness of a sucrose molecule, with chlorine and bromine being the most effective.

Chlorine tends to have a higher water solubility, so chlorine was picked as the ideal halogen for substitution.

Sucrose portion



Fructose portion

Sucralose

- Splenda
- 1998, approved for table-top sweetener and use in various foods
- Approved already in UK, Canada before US
- Only one “made from sugar”
  - There was a law suit last year of this claim
  - Splenda lost….not a natural compound and not really made from sugar….a bit of a deceptive marketing.
- Clean, sweet taste and no undesirable off-flavor

Saccharin

- Sweet'n Low, The 1st artificial sweetener
- Accidentally found in 1879 by Remsen and Fahlberg
- Saccharin use increased during wars due to sugar rationing
- By 1917, common table-top sweetener in America
- Banned in 1977 due to safety issue
- 1991, withdrawal banning, but remained warning label
- 2000, removed warning label
- Intensely sweet, but bitter aftertaste

Aspartame

- Nutrasweet, Equal
- Discovered in 1965 by J. Schlatter
- Composed of aspartic acid and phenylalanine
- 4 kcal/g, but 200 times sweeter
- Approved in 1981 for table-top sweetener and powdered mixes
- Safety debating
- 1996, approved for use in all foods and beverage
- Short shelf life, not stable at high temperature

Acesulfame K

- Sunette, Sweet One
- Discovered in 1967 by Hoechst
- 1992, approved for gum and dry foods
- 1998, approved for liquid use
- Blending with Aspartame due to synergistic effect
- Stable at high temperature and long shelf life (3-4 years)
- Bitter aftertaste

Neotame

- Brand new approved sweetener (Jan. 2000)
- 7,000 ~ 13,000 times sweeter than sugar
- Dipeptide methyl ester derivative structurally similar to Aspartame
- Enhance sweetness and flavor
- Baked goods, non-alcoholic beverages (including soft drinks), chewing gum, confections and frostings, frozen desserts, processed fruits and fruit juices, toppings and syrups.
- Safe for human consumption

Food Toxicology 101

Food Toxicology

- The study of the nature, properties, effects, and detection of toxic substances in food, and their impact on humans.
- Early on, people were aware that some plants are poisonous and should be avoided as food.
- Other plants were found to contain chemicals that have medicinal, stimulatory, hallucinatory, or narcotic effects.

The Dose Makes the Poison

Attributed to Pericles

-a Greek statesman.

“Toxic” to One….not to Another

- Food Allergens
  - Cows milk
  - Crustacea
  - Eggs
  - Fish
  - Peanuts
  - Soybean
  - Tree nut
  - Wheat

Celiac Disease

- Ingestion of wheat, barley, rye
- Proline-rich protein - gliadins
- Triggers immune damage to small intestine
- Impairs absorption of nutrients
- Diarrhea, bloating, weight loss, bone pain, anemia, chronic fatigue, weakness, muscle cramps

Scombroid Poisoning

- Anaphylactic shock
- Eating fish with high histamine levels
  - Tuna, mackerel, other pellagic fish species
- Histamine from spoilage bacteria in fish
  - Also from putrecine and cadaverine
- Everyone is susceptible
- Some more sensitive (allergy?) than others.

Sensitivity or Allergy to:

- Lactose
- Sulfites
- Strawberries (internal histamine release)
- Fava beans (enzyme deficiency)
- Asparagus (sulfur compounds)
- Red wine (low levels of histamine)
- Fructose intolerance
- Aspartame
- Tartarazine (FD&C Yellow #5)

Mycotoxins

- Substances produced by fungi that are harmful to animals and humans
- > 100,000 species of fungi
- > 300 mycotoxins isolated; 30 with food issues
- Plant specific
  - Environmental
  - Temperature
  - Humidity
  - Moisture
  - Oxygen

Claviceps purpurea

- Grows in wet and over-wintered grains:
  - rye, barley, wheat
- Sclerotia or "ergots" (Hard-packed mycelium)
- "Ergotism"
  - Convulsions and gastrointestinal symptoms
- Ergotamine is an analogue of lysergic acid (LSD)
- Vasoconstrictor that may cause hallucinations
- *St. Anthony's fire*
- Potential cause of Salem, MA witch trials, 1692.

Aspergillus flavus and A. *paraciticus*

- Universal food contaminant
  - Corn, peanuts, wheat, rice, pecans, walnuts, etc
- Animal carcinogen at 5 ppb
- Human liver carcinogen
- Problem in food industry and grain handling
  - Harvesting, transport, storage
- 4 main aflatoxins: B1, B2, G1, G2
- In animal feed, B1 and B2 can be converted to M1 and M2 and secreted in milk (ie. cow or human)

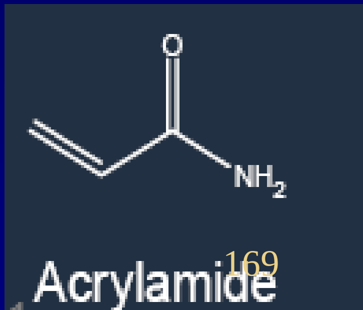
Toxins formed during Food Processing

- We previously covered the Maillard reaction
  - Polycyclic aromatic hydrocarbons (benzo(a)pyrine)
  - Carcinogenic agents in almost every model tested

- Nitrite used in curing meat and fish
  - Antimicrobial agent
  - Reacts with myoglobin and hemoglobin to form red nitrosyl compounds
  - Nitrite may also react with amines to form nitrosoamines.
  - Carcinogenic, mutagenic…..but really harmful??

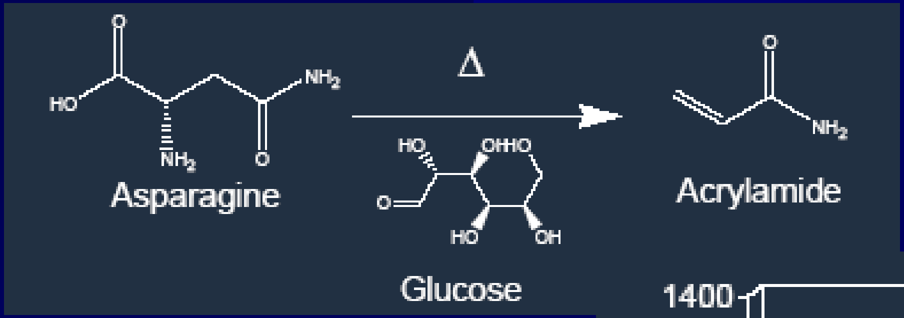
Toxins formed during Food Processing

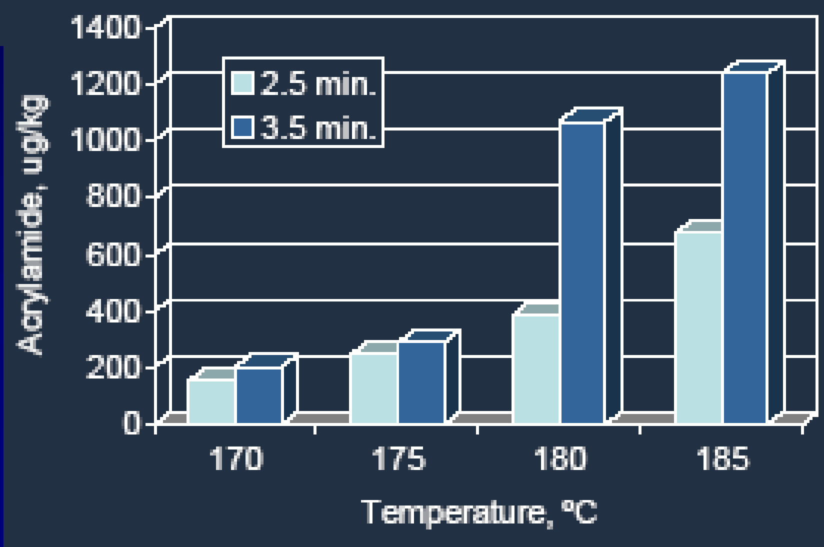
- Acrylamide in foods
- In 2000-2002 Swedish researchers identified acrylamide in foods and residues from human samples.
- Acrylamide is a neurotoxin and carcinogen.
- Used as:
  - Cement binder, plastic manufacture, waste water treatment agent, soil conditioner, thickening agent for pesticides, cosmetics, laboratory gels, etc)
- Broad range of foods with significant levels of acrylamide.
  - Foods prepared at high temperatures.
  - Fried and baked, but not boiled.
  - Higher in high carbohydrate foods.



Acrylamide in Potatoes

- Acrylamide derived from asparagine in the presence of sugar.
- Carbonyl carbon in glucose facilitates the reaction
- Asparagine + Sugar + Heat = Acrylamide





Changes in Acrylamide levels in French-fries with increasing temperature and frying time

Phytoalexic Toxins In Our Food

Potato

Stress Metabolites

- Cinnamates, scopolin, quinic acid, sesquiterpenoids, solanine, chaconine

Solanine (Glycoalkaloid)

- Sunburned spuds or growth shoots (periderm)
- 10-50 ppm is normal, increases 7-fold during stress
- Natural pesticide (cholinesterase inhibitor)
  - Acetylcholine is a neurotransmitter
- Extremely bitter, not soluble in water
- Heat stable

Tomato

Stress Metabolites

- Cinnamates, rishitin, falcarindiol, tomatine

Tomatine (Alkaloid)

- High in immature fruit
- Ripe tomato contains ~30-40 ppm
- Natural pesticide
- Heat labile

Carrot

Stress Metabolites

- Cinnamates, falcarinol, falcarindiol, isocoumarin

Isocoumarin (neutral phenolic)

- Anti-microbial (~10 ppm)
- Extremely bitter, not soluble in water
- Heat stable
- Ethylene sensitive synthesis

“Natural” Carcinogens in Coffee

- Acetaldehyde
- Benzaldehyde
- Benzene
- Benzofuran
- Benzo[a]pyrene
- Caffeic acid
- Catechol
- 1,2,5,6 Dibenzanthracene
- Ethanol
- Ethylbenzene
- Formaldehyde
- Furan
- Furfural
- Hydrogen peroxide
- Hydroquinone
- Limonine
- Styrene
- Toluene
- Xylene

Other Commodities

Peppers- Capsidiol

Sweet potato- Ipomeamarone

Celery, parsnips, parsley- Psoralens (furanocoumarins)

Grapes- Resveratrol, stilbene

Alfalfa- Medicarpin (Isoflavonoid)

Soybean roots- Glyceollin

Peas- Pisatin

Bean pods- Phaseollin

Phyto= plant…chemicals

- Organosulfides
- Isothiocynates
- Indoles
- Carotenoids
- Saponins
- Tocopherols
- Amino acids/Proteins
- Lipids
- Carbohydrates
- Polyphenols
  - Flavonoids
  - Tannins
  - Isoflavones
- Vitamins/Minerals
- Coumarins
- Dietary Fiber
- Enzymes

How Did They Get There ??

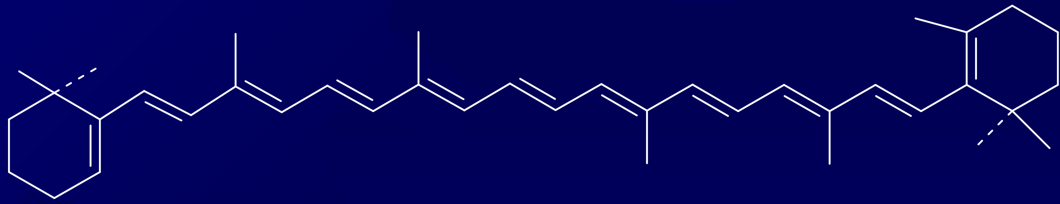
Selective Biosynthesis

How are Phytoalexins Formed?

The "-noids"

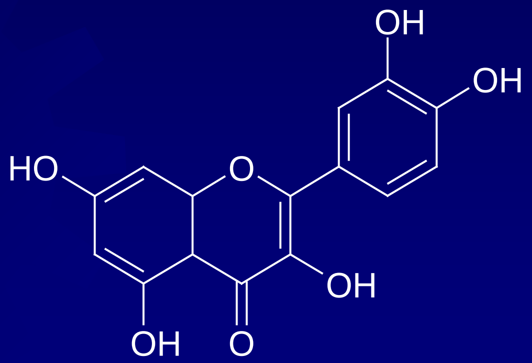
- Shikimic acid pathway (phenylpropanoids)
  - Hydroxycinnamic acids
  - Coumarins
  - Hydroxybenzoic acids

- Mevalonic acid pathway (Isoprenoids)
  - Carotenoids
  - Terpenoids



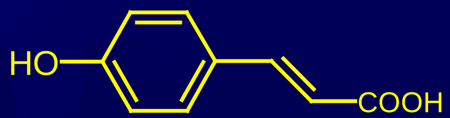
- Combination of Pathways

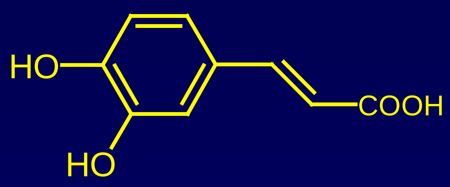
  (Shikimic-Polymalonic)
  - Flavonoids and anthocyanins

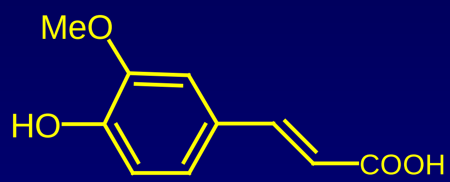


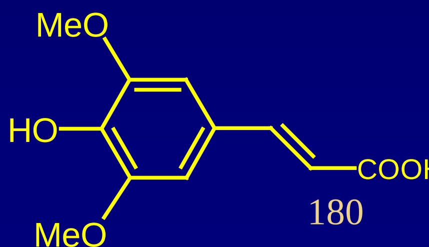
Shikimic Acid Pathway- Phenolics

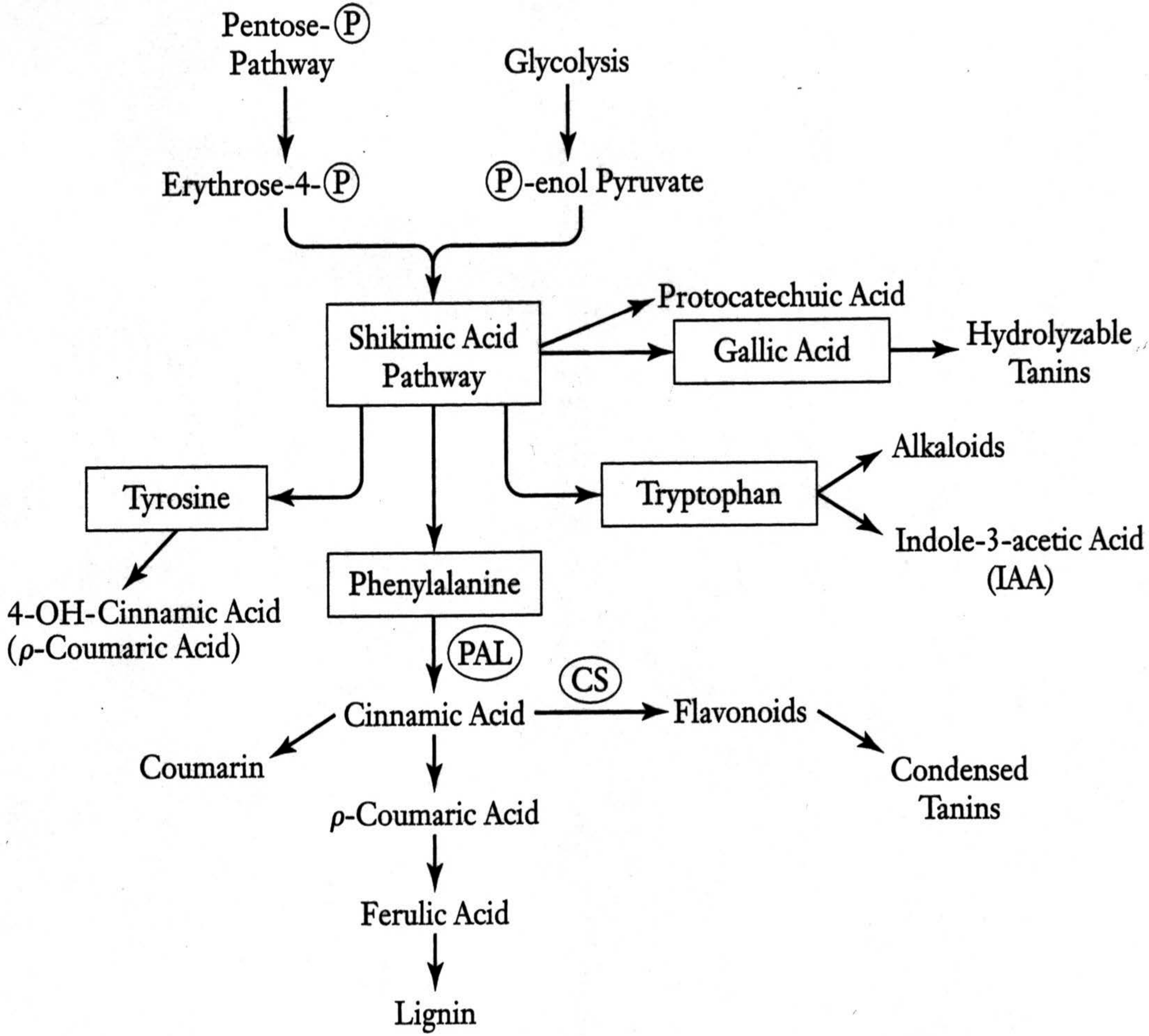
- PEP from glycolysis + erythrose 4-P from PP-pathway forms skikimic acid which are the precursor to phenylalanine and tyrosine.
- PAL and TAL (ammonia lyases)
  - Cinnamic acid
  - Coumarin
  - *p*-Coumaric acid
  - Caffeic acid
  - Ferulic acid
  - Sinapic acid





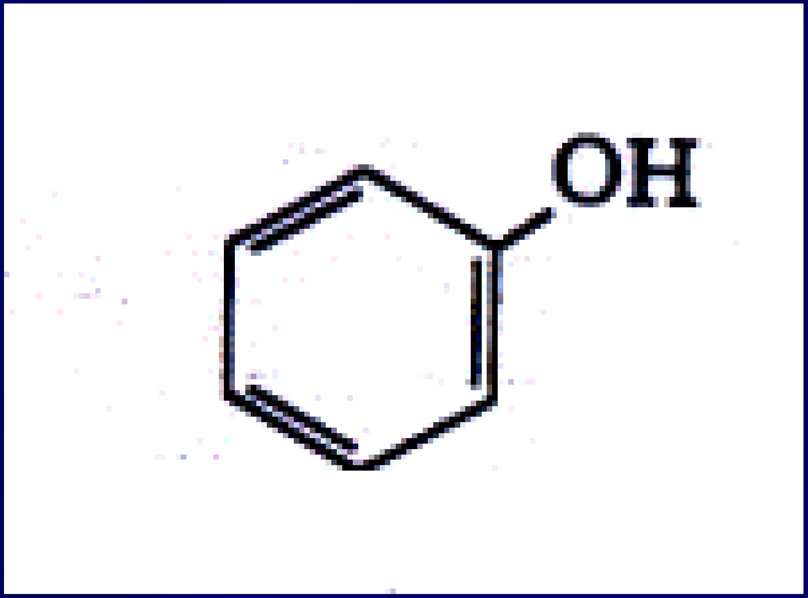





Pentose-Ⓟ Pathway
Glycolysis
Erythrose-4-Ⓟ
Ⓟ-enol Pyruvate
Shikimic Acid Pathway
Protocatechuic Acid
Gallic Acid
Hydrolyzable Tanins
Tyrosine
Tryptophan
Alkaloids
Indole-3-acetic Acid (IAA)
4-OH-Cinnamic Acid (ρ-Coumaric Acid)
Phenylalanine
PAL
CS
Cinnamic Acid
Flavonoids
Coumarin
Condensed Tanins
ρ-Coumaric Acid
Ferulic Acid
Lignin

Phenolics

- Plants produce a variety of compounds that contain one or more phenol groups - called phenolics
- Thousands of phenolics occur in plants



Phenolics

- Large group of diverse compounds
- Many serve as defense compounds against herbivores and pathogens
- Some attract pollinators
- Some absorb UV light
- Some reduce growth of competitors

Background

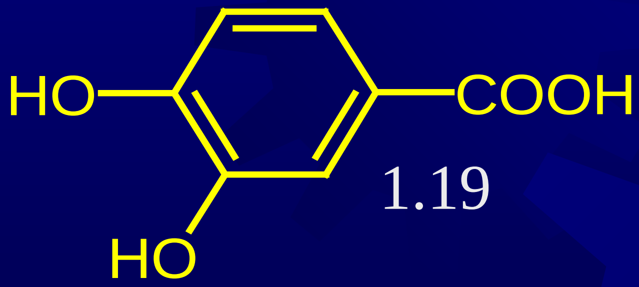
- Flavonoids are non-nutrients
- 1936-Szent-Gyorgyi, called flavonoids Vitamin P.
- 1950's disproved the theory
- Late seventies-mutagenecity of quercetin
- Recent research-anticarcinogenic
- Current research-metabolism by gut bacteria

Structure-Based Antioxidant Activity of Phytochemicals

0.08

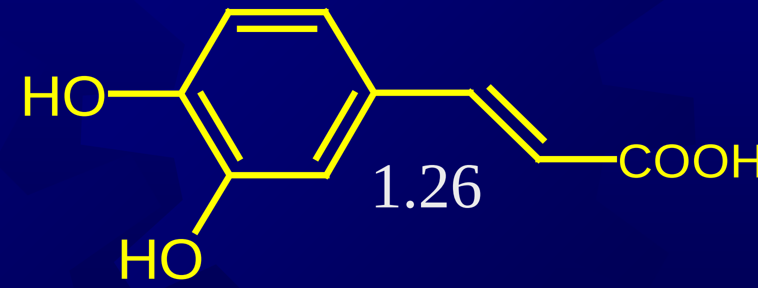
p-OH-benzoic
p-coumaric

2.22

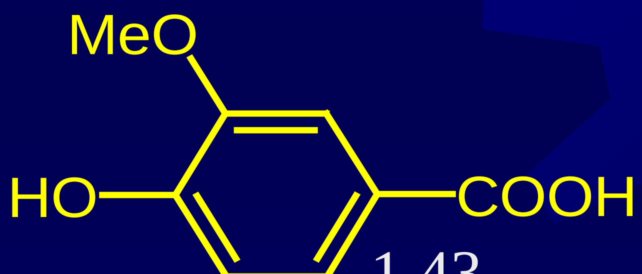


1.19

Protocatechuic
Caffeic

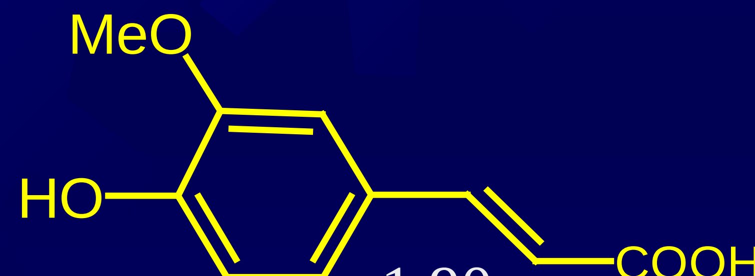


1.26



1.43

Vanillic
Ferulic

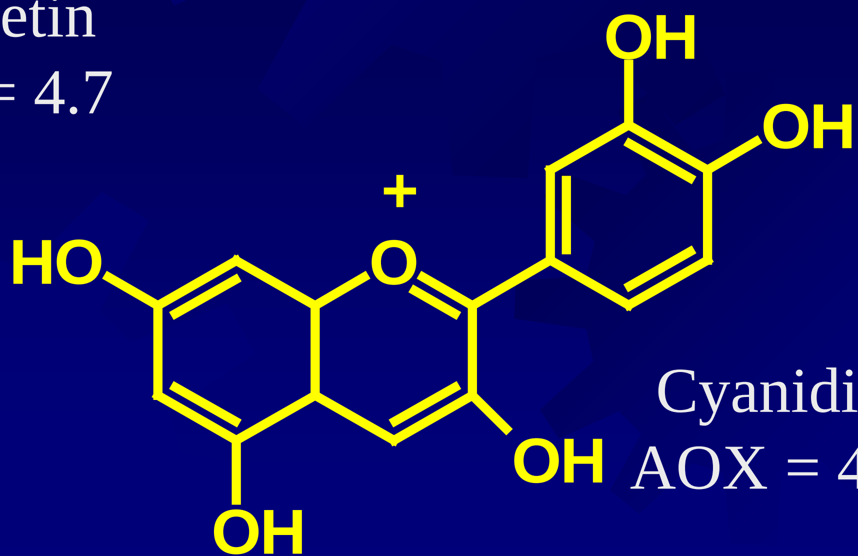


1.90

Structurally Similar Compounds

Quercetin
AOX = 4.7

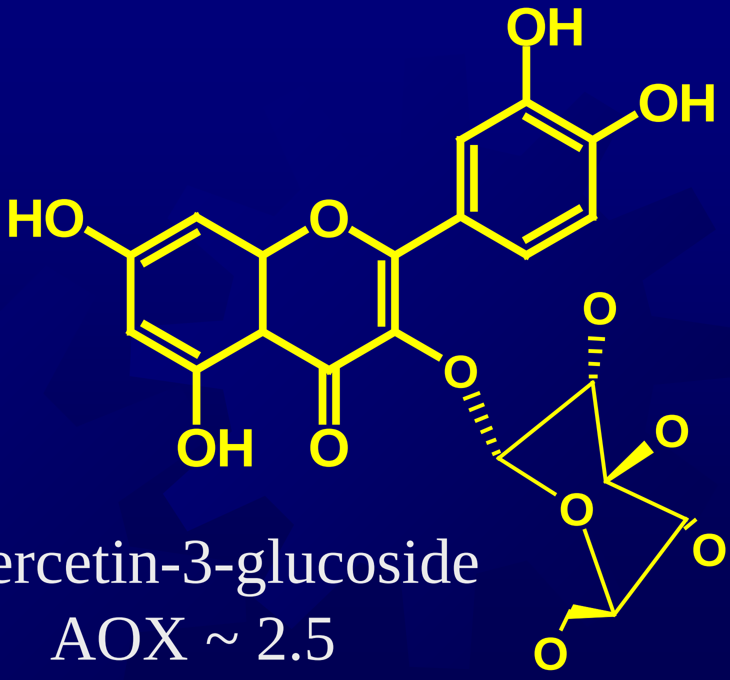
Catechin
AOX = 2.4



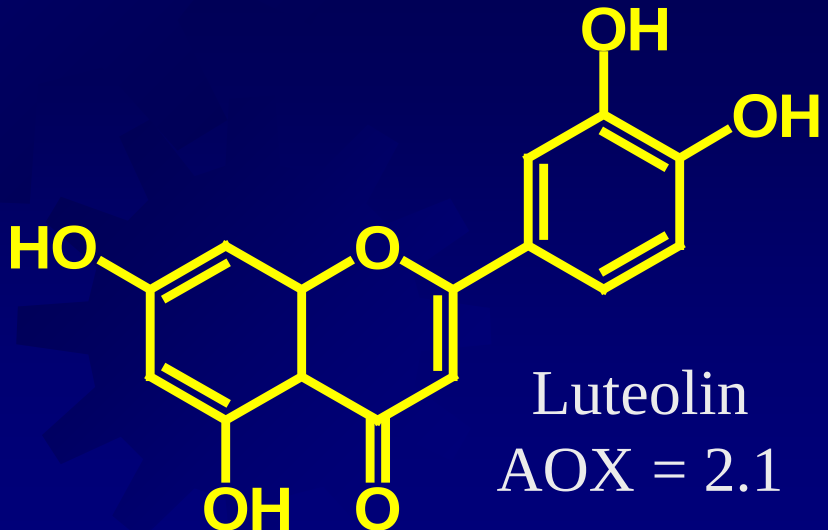
Cyanidin
AOX = 4.4

Importance of the 3-OH group

Quercetin
AOX = 4.7



Quercetin-3-glucoside
AOX ~ 2.5



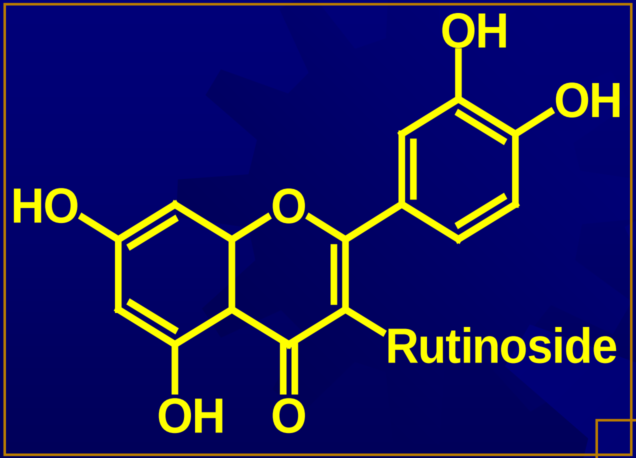
Luteolin
AOX = 2.1

Importance of the 2-3 db

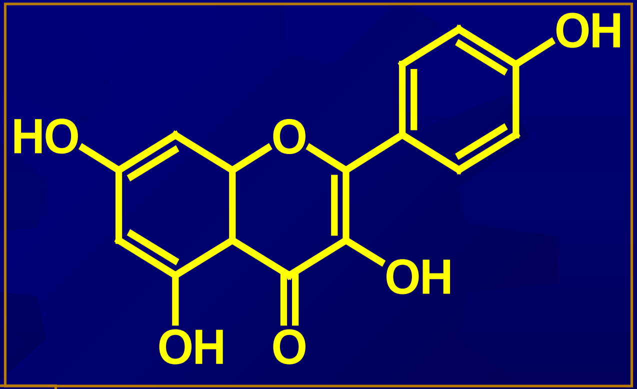
Quercetin
AOX = 4.7

Taxifolin
AOX = 1.9

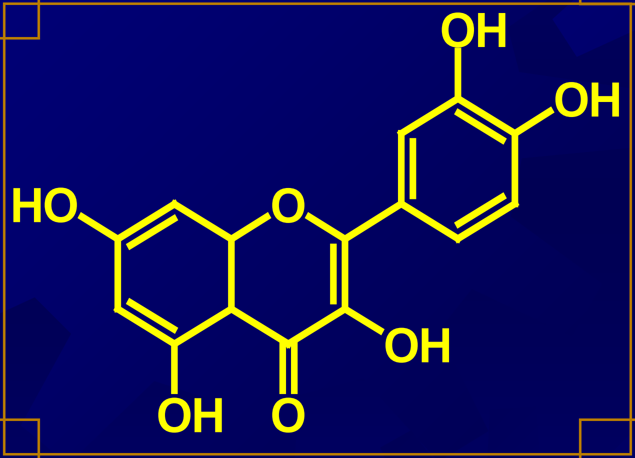
Summation



Rutin
AOX = 2.4

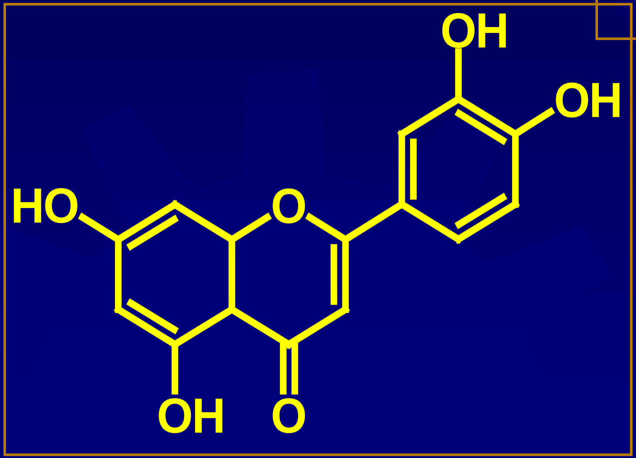


Kaempferol
AOX = 1.3

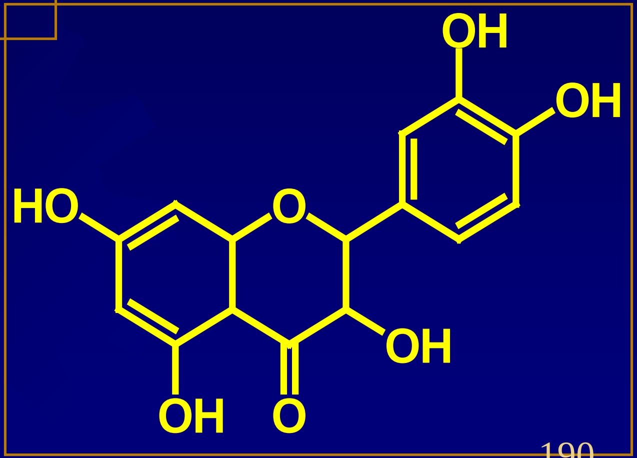


Quercetin
AOX = 4.7

Luteolin
AOX = 2.1



Taxifolin
AOX = 1.9



Food Additives

Primary Laws and Regulations

Federal Food, Drug and Cosmetic Act of 1938

- Gives FDA authority to:
  - Regulate food purity, safety, and wholesomeness
  - Regulate labeling and packaging
  - Inspect processing facilities
  - Examine and seize foods in interstate commerce

Laws and Regulations

FFDCA of 1938

- Gave FDA authority to:
  - Supervise product recalls
  - Regulate food additives
  - Regulate food colors
  - Inspect imports and exports
  - Regulate standards of identity
  - Work with state and local agencies

Laws and Regulations

Highlights of FFDCA of 1938

Adulteration

- Poisons or harmful substances
- Filth or decomposed food
- Unsanitary conditions
- Food from diseased animals
- Substituting non-specified ingredients
- Not meeting standards of identity
- Inferiority concealed
- Non-approved food additive or color
- Non-approved or high pesticide residues

Laws and Regulations

Highlights of FFDCA of 1938

Misbranding

- Labeling false or misleading
- Sold under name of another food
- Container made or filled in misleading manner
- Doesn't contain address, net contents, name, ingredients, nutrition label
- Information not legible
- Doesn't conform to standards of identity
- Makes non-approved health claim
- Improper nutritional descriptor

Laws and Regulations

Amendments to FFDCA of 1938

Color Additive Amendment, 1960

- Safety
- Approval & *certification* process

Laws and Regulations

Amendments to FFDCA of 1938

Food Additive Amendment, 1958

- For intentional & non-intentional additives
- Safety requirements

GRAS vs non-GRAS

A food additive is deemed:

"Generally Recognized As Safe"

Delaney Clause – Can't cause cancer at any concentration

Established a "zero-cancer-risk"

An extremely difficult issue facing new food additives

Negligible Risk

- “Margin of Safety”
- Better science and analytical detection
- “Safe” = a reasonable certainty of no harm
- 1996…Delaney Clause….ousted
- “Negligible Risk” or *de minimus*
- No more “zero tolerance”
- 70-year risk is 1 : 1,000,000 risk of cancer

Laws and Regulations

Additional requirements of additives

- Useful function
- Cannot cover up
- Cannot reduce nutritional value
- Cannot replace good manufacturing practices
- Compound must be able to be analyzed for

Food Additives

- Generate lot of debate
- Our food supply would change drastically without them
- Regulated by FDA (Food and Drug Administration)

Quick examples:

- Vitamin A and D
- Nitrates for cured meats
- BHT for fats and oils
- Citric acid
- Salt/Pepper

Food Additive: Any substance added to foods

Food Additives

<u>Categories of Food Substances</u>

- GRAS substances (long history)
- Food Additive Status List (from 21 CFR)
  - Center for Food Safety and Applied Nutrition
- Food additives
  - Intentional <u>and</u> unintentional
  - Thousands of compounds, and growing
  - New ones have to be proven safe
- Color additives (special cases)

- Acacia
- Acetic acid
- Aconitic acid
- Adipic acid
- Agar-agar
- Alginic acid
- Ammonium alginate
- Ammonium bicarbonate
- Ammonium carbonate
- Ammonium chloride
- Ammonium citrate, dibasic
- Ammonium hydroxide
- Ammonium phosphate, dibasic
- Ammonium sulfate
- Animal lipase
- Bakers yeast extract
- Beeswax
- Bentonite
- Benzoic acid
- Benzoyl peroxide
- Beta-carotene
- Bromelain
- Brown algae
- n-Butane and iso-butane
- Calcium acetate
- Calcium alginate
- Calcium carbonate
- Calcium chloride
- Calcium citrate
- Calcium gluconate
- Calcium glycerophosphate
- Calcium hydroxide
- Calcium iodate
- Calcium lactate
- Calcium oxide
- Calcium pantothenate
- Calcium propionate
- Calcium stearate
- Calcium sulfate
- Candelilla wax
- Caprylic acid
- Carbon dioxide
- Carnauba wax
- Catalase (bovine liver)
- Citric acid
- Clove and its derivatives
- Cocoa butter substitute
- Copper gluconate
- Copper sulfate
- Corn gluten
- Corn silk and corn silk extract
- Corn sugar
- Corn syrup
- Cuprous iodide
- L-Cysteine
- L-Cysteine monohydrochloride
- Dextrin
- Diacetyl
- Dill and its derivatives
- Enzyme-modified fats
- Enzyme-modified lecithin
- Ethyl alcohol
- Ethyl formate
- Ferric ammonium citrate
- Ferric chloride
- Ferric citrate
- Ferric phosphate
- Ferric pyrophosphate
- Ferric sulfate
- Ferrous ascorbate
- Ferrous carbonate
- Ferrous citrate
- Ferrous fumarate

- Ferrous gluconate
- Ferrous lactate
- Ferrous sulfate
- Ficin
- Garlic and its derivatives
- Glucono delta-lactone
- Glyceryl behenate
- Glyceryl monooleate
- Glyceryl monostearate
- Glyceryl palmitostearate
- Ground limestone
- Guar gum
- Gum ghatti
- Gum tragacanth
- Helium
- High fructose corn syrup
- Hydrogen peroxide
- Inositol
- Insoluble glucose isomerase enzyme preparations
- Invert sugar
- Iron, elemental
- Isopropyl citrate

- Karaya gum (sterculia gum)
- Lactic acid
- Lecithin
- Licorice and licorice derivatives
- Linoleic acid
- Locust (carob) bean gum
- Magnesium carbonate
- Magnesium chloride
- Magnesium hydroxide
- Magnesium oxide
- Magnesium phosphate
- Magnesium stearate
- Magnesium sulfate
- Malic acid
- Malt
- Malt syrup (malt extract)
- Maltodextrin
- Manganese chloride
- Manganese citrate
- Manganese gluconate
- Manganese sulfate
- Menhaden oil
- Methylparaben
- Microparticulated protein product

- Mono- and diglycerides
- Monosodium phosphate
- Niacin
- Niacinamide
- Nickel
- Nisin preparation
- Nitrogen
- Nitrous oxide
- Oil of rue
- Ox bile extract
- Ozone
- Pancreatin
- Papain
- Pectins
- Pepsin
- Peptones
- Potassium acid tartrate
- Potassium alginate
- Potassium bicarbonate
- Potassium carbonate
- Potassium chloride
- Potassium citrate
- Potassium hydroxide

- Potassium iodate
- Potassium iodide
- Potassium lactate
- Potassium sulfate
- Propane
- Propionic acid
- Propyl gallate
- Propylene glycol
- Propylparaben
- Pyridoxine hydrochloride
- Rapeseed oil
- Red algae
- Reduced lactose whey
- Reduced minerals whey
- Rennet
- Riboflavin
- Riboflavin-5-phosphate (sodium)
- Rue
- Sheanut oil
- Sodium acetate
- Sodium alginate
- Sodium benzoate
- Sodium bicarbonate
- Sodium carbonate
- Sodium citrate
- Sodium diacetate
- Sodium hydroxide
- Sodium hypophosphite
- Sodium lactate
- Sodium metasilicate
- Sodium potassium tartrate
- Sodium propionate
- Sodium sesquicarbonate
- Sodium tartrate
- Sodium thiosulfate
- Sorbitol
- Stannous chloride (anhydrous and dihydrated)
- Starter distillate
- Stearic acid
- Stearyl citrate
- Succinic acid
- Sucrose
- Sulfuric acid
- Tannic acid
- Tartaric acid
- Thiamine hydrochloride
- Thiamine mononitrate
- α-Tocopherols
- Triacetin
- Tributyrin
- Triethyl citrate
- Trypsin
- Urea
- Urease enzyme preparation from Lactobacillus fermentum
- Vitamin A
- Vitamin B_{12}
- Vitamin D
- Wheat gluten
- Whey
- Whey protein concentrate
- Zein

Types of Food Additives

- Microbial inhibitors
- Antioxidants
- Sequestrants and chelating agents
- Emulsifiers
- Stabilizers and thickeners
- Bleaching and maturing agents
- pH control agents
- Food colors
- Sweeteners
- Flavoring agents
- Nitrites

Food Additives Serve To:

- Maintain product consistency
- Improve or maintain nutritional value
- Improve palatability or wholesomeness
- Control of acidity
- Enhance flavor
- Impart color
- Inhibit micro-organisms

Microbial inhibitors

- Slows or inhibits microbial spoilage
- Sodium benzoate - soft drinks
- Sodium or calcium propionates - breads
- Sorbates - cheese, moist dog food, juices
- Sulfur dioxide - wines

Antioxidants

- Prevent color, flavor, other changes
- BHA
- BHT
- TBHQ
- Propyl gallate
- Ascorbic acid
- Tocopherols
- Sulfur dioxide (dual function)

Sequestrants and chelating agents

- Bind ions (mostly metal ions: Cu or Fe)
- Help limit oxidation
- EDTA
- Polyphosphates
- Citric acid

Emulsifiers

- Stabilize emulsions (O/W or W/O)
- Egg yolks (contains lecithin and protein) - mayonnaise, ice cream
- Lecithin (a phospholipid)
- Mono- and diglycerides - margarine, peanut butter

Stabilizers and Thickeners

- Improve consistency or texture
- Gelatin (Jell-O)
- Gums (Guar, locust bean, arabic, tragacanth)
- Pectin (jam and jelly)
- Starch
- Vegetable proteins
- Carboxymethyl cellulose (CMC)

Bleaching and Maturing Agents

- Used a lot to improve baked goods as "dough conditioning agents"
- Benzoyl peroxide - bleaches flour and alters starch and protein
- Hydrogen peroxide - same

pH Control Agents

- Lower, raise, or maintain pH
- Organic acids: citric, phosphoric, malic, etc.
- Alkali: sodium bicarbonate

Flavoring Agents

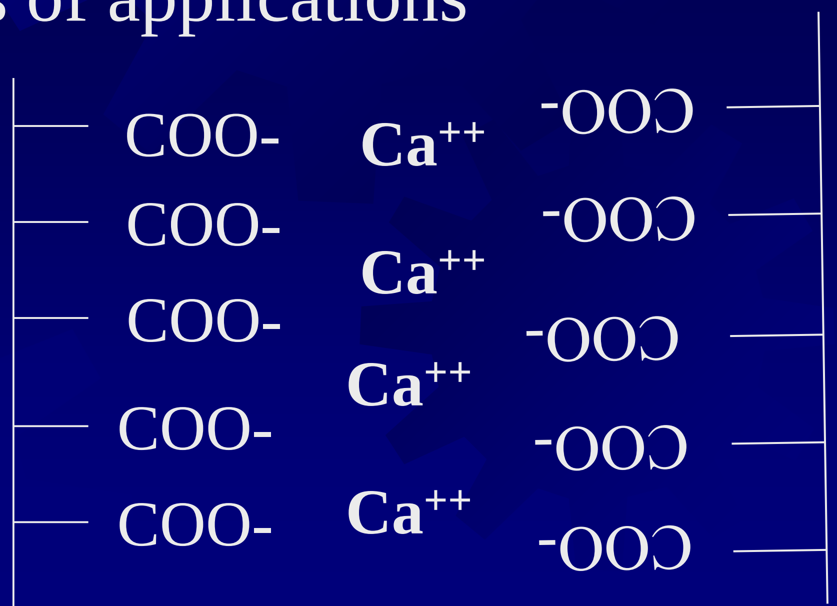
- Largest category, by far
- Artificial and natural
- Do not have to put full name on label
- Flavor enhancers
- MSG (monosodium glutamate), others

Nitrites

- Added to cured meats (hot dogs, lunch meats, bacon, etc.)
- Nitrates and nitrites
- Protects color and gives color (pink)
- Antimicrobial

Other Additives

- Firming agents (calcium chloride) in fruits and vegetables
- Anticaking agents (calcium silicate) in powders
- Humectants - retain moisture
- Clarifying agents - bentonite in wines
- Enzymes - lots of applications
- Many others



Calcium as a Firming Agent