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Module on

Pigments and their significance

By

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INTRODUCTION

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Pigments can be classified by their origin as natural, synthetic, or inorganic. Natural pigments are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic pigments are obtained from laboratories. Natural and synthetic pigments are organic compounds. Inorganic pigments can be found in nature or reproduced by synthesis. The energy range to which the eye is sensitive is referred to as visible light. Visible light, depending on an individual's sensitivity, encompasses wavelengths of approximately 380–770 nm. This range makes up a very small portion of the electromagnetic spectrum (Fig. 1). Pigments are natural substances in cells and tissues of plants and animals that impart color. Dyes are any substances that lend color to materials. The term dye is commonly used in the textile industry. In the U.S. food industry, a dye is a food-grade water soluble colorant certified by the U.S. Food and Drug Administration (FDA). Color and appearance are major, if not the most important, quality attributes of foods. It is because of our ability to easily perceive these factors that they are the first to be evaluated by the consumer when purchasing foods. Many food pigments are, unfortunately, unstable during processing and storage. Prevention of undesirable changes is usually difficult or impossible. Depending on the pigment, stability is influenced by factors such as the presence or absence of light, oxygen, heavy metals, and oxidizing or reducing agents; temperature and water activity; and pH. Because of the instability of pigments, colorants are sometimes added to foods.

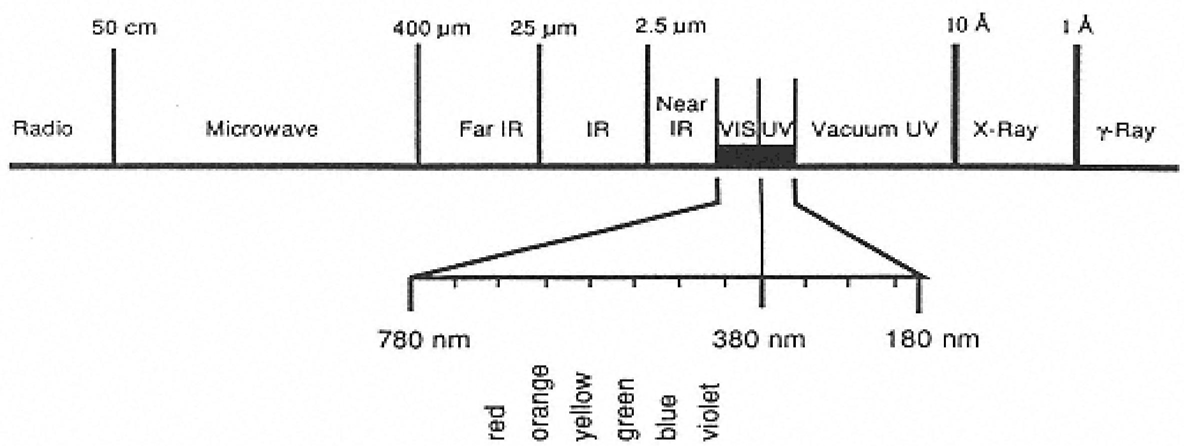


Fig.1. Electromagnetic spectrum

Pigments in Animal and Plant Tissue:

1. HEME COMPOUNDS

Heme pigments are responsible for the color of meat. Myoglobin is the primary pigment and hemoglobin, the pigment of blood, is of secondary importance. Most of the hemoglobin is removed when animals are slaughtered and bled. Thus, in properly bled muscle tissue myoglobin is responsible for 90% or more of the pigmentation. The myoglobin quantity varies considerably among muscle tissues and is influenced by species, age, sex, and physical activity. For example, pale-colored veal has lower myoglobin content than red-colored beef. Other minor pigments present in muscle tissue include the cytochrome enzymes, flavins, and vitamin B12.

Myoglobin/hemoglobin

Myoglobin is a globular protein consisting of a single polypeptide chain. Its molecular mass is 16.8 kD and it is comprised of 153 amino acids. This protein portion of the molecule is known as globin. The chromophore component responsible for light absorption and color is a porphyrin known as heme. Within the porphyrin ring, a centrally located iron atom is complexed with four tetrapyrrole nitrogen atoms. Thus, myoglobin is a complex of globin and heme. The heme porphyrin is present within a hydrophobic pocket of the globin protein and bound to a histidine residue (Fig. 2). The centrally located iron atom shown possesses six coordination sites, four of which are occupied by the nitrogen atoms within the tetrapyrrole ring. The fifth coordination site is bound by the histidine residue of globin, leaving the sixth site available to complex with electronegative atoms donated by various ligands.

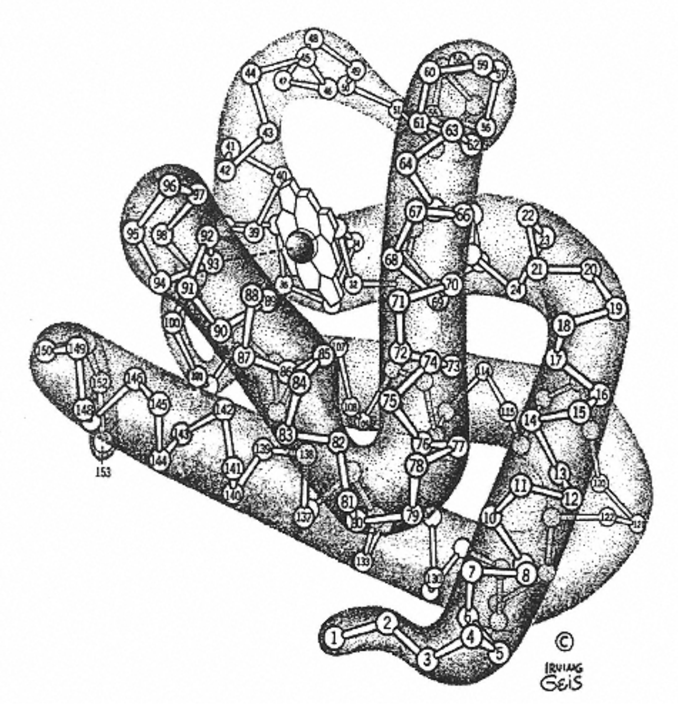


Fig.2. Tertiary structure of myoglobin

Hemoglobin consists of four myoglobins linked together as a tetramer. Hemoglobin, a component of red blood cells, forms reversible complexes with oxygen in the lung. This complex is distributed via the blood to various tissues throughout the animal where oxygen is absorbed. It is the heme group that binds molecular oxygen. Myoglobin within the cellular tissue acts in a similar fashion, accepting the oxygen carried by hemoglobin. Myoglobin thus stores oxygen within the tissues, making it available for metabolism. Meat color is determined by the chemistry of myoglobin, its state of oxidation, type of ligands bound to heme, and state of the globin protein. The heme iron within the porphyrin ring may exist in two forms: either reduced ferrous (+2) or oxidized ferric (+3) form. This state of oxidation for the iron atom within heme should be distinguished from oxygenation of myoglobin. When molecular oxygen binds to myoglobin, oxymyoglobin (MbO_2) is formed and this is referred to as oxygenation. When oxidation of myoglobin occurs, the iron atom is converted to the ferric (+3) state, forming metmyoglobin (MMb). Heme iron in the +2 (ferrous) state, which lacks a bound ligand in the sixth position, is called myoglobin. The fig.3.shows various reactions occurring in myoglobin in fresh and cured meat.

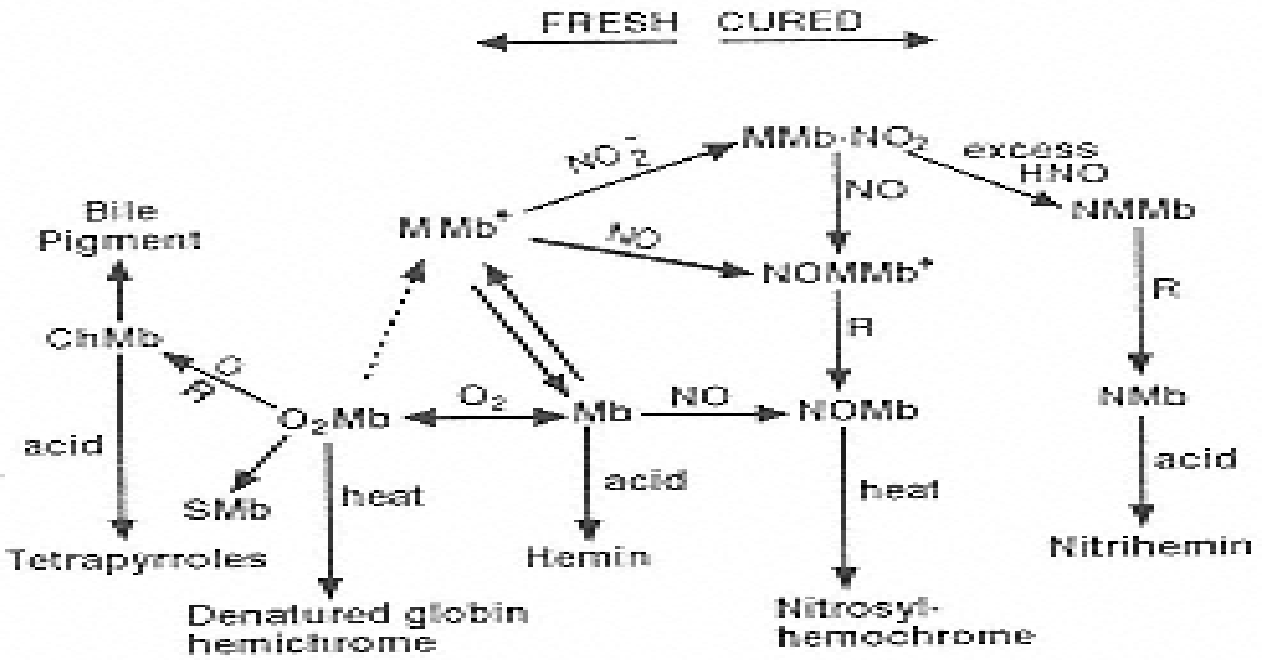
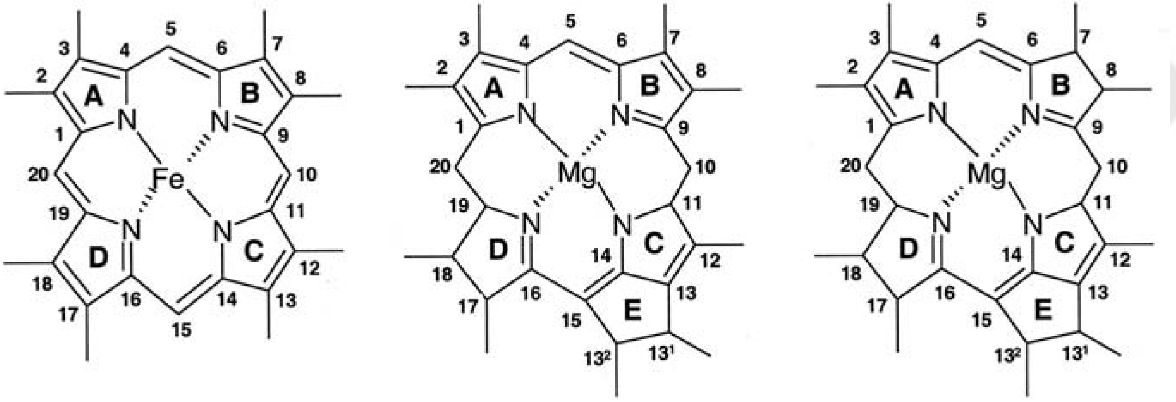


Fig.3. Myoglobin reactions in fresh and cured meats. ChMb = cholemyglobin (oxidized porphyrin ring); O_2Mb = oxymyog lobin (Fe2+); MMb = metmyog lobin (Fe 3+); Mb =myoglobin (Fe 2+); MMb-NO_2 = metmyog lobin nitrite; NOMMb = nitrosylmetmyoglobin; NOMb = nitrosylmyog lobin; NMMb = nitrometmyoglobin; NMb = nitromyog lobin, the latter two being reaction products of nitrous acid and the heme portion of the molecule; R = reductant; O = strong oxidizing conditions.

An important means of stabilizing meat color is to store it under appropriate atmospheric conditions. The problem of discoloration caused by heme oxidation (Fe^{2+} Fe^{3+}) can be resolved by using modified atmosphere (MA) packaging. This technique requires the use of packaging films with low gas permeabilities. After packaging, air is evacuated from the package and the storage gas is injected. By employing oxygen-enriched or -devoid atmospheres, color stability can be enhanced.

2. CHLOROPHYLL

Chlorophylls are the major light-harvesting pigments in green plants, algae, and photosynthetic bacteria. They are magnesium complexes derived from porphin. Porphin is a fully unsaturated macrocyclic structure that contains four pyrrole rings linked by single bridging carbons. Pyrrole carbon atoms on the periphery of the porphin structure are numbered 1 through 8. Carbon atoms of the bridging carbons are designated alpha, beta, gamma, and delta. The IUPAC numbering system for porphin is shown in Fig. 4. Porphyrinogens are cyclic tetrapyrrole precursors which give rise to three basic groups of molecules, discriminated by their state of oxidation: porphyrins, dihydroporphyrins and tetrahydroporphryins; the basic structures are depicted in the below shown figure. Porphyrins have the highest degree of unsaturation among the tetrapyrrole macrocycles.



haem
porphyrins

chlorophyll a
dihdroporphyrins
or chlorins

bacteriochlorophylls
tetrahydroporphyrins

Fig. 4. Basic structures of porphyrins, dihydroporphyrins, and tetrahydroporphyrins.

Chlorophylls are located in the lamellae of intercellular organelles of green plants known as chloroplasts. They are associated with carotenoids, lipids, and lipoproteins. Weak linkages (non-covalent bonds) exist between these molecules. The bonds are easily broken; hence chlorophylls can be extracted by macerating plant tissue in organic solvents. It is well known that chlorophyll contents in plants vary greatly, depending upon metabolic processes that are reliant primarily on physiological, genetic, and biochemical factors but also on external influences: climatic factors, agricultural practices, season, geographic growing region, post-harvest handling, storage, and plant part considered. There are pronounced differences in chlorophyll contents among stems, leaves, peels, and pulps of vegetables and fruits. In general, it can be stated that higher chlorophyll contents are mainly found in leaves and, the more colored they are, the higher amounts of chlorophyll they have. Therefore, the highest amounts (as much as 1.5 to 2.0% fresh weight) can be found in fully developed leaves of spinach, parsley, kale, and green cabbage, for example. Senescence of plants and ripening of fruits causes a sharp decrease in chlorophyll content due to the programmed natural biochemical process of chlorophyll breakdown, which ensures their complete transformation into colorless catabolites. High-performance liquid chromatography (HPLC) today is the method of choice for separating individual chlorophylls and their derivatives. In the case of chlorophyll *a* and *b,* for example, the increase in polarity contributed by the C-3 formyl substituent of chlorophyll *b* causes it to be more strongly adsorbed on a normal-phase column and more weakly absorbed on a reverse-phase column than chlorophyll *a.* The Table 1 list various below gives the nomenclature of various chlorophyll derivatives

Table.1. Nomenclature of Chlorophyll Derivatives

Phyllins:	Chlorophyll derivatives containing magnesium
Pheophytins:	The magnesium free derivatives of the chlorophylls
Chlorophyllides:	The products containing a C-7 propionic acid resulting from enzymic or chemical hydrolysis of the phytyl ester
Pheophorbides:	The products containing a C-7 propionic acid resulting from removal of magnesium and hydrolysis of the phytyl ester Methyl or ethyl
Pheophorbides:	The corresponding 7-propionate methyl of ethyl ester
Pyrocompounds:	Derivatives in which the C-10 carbomethoxy group has been replaced by hydrogen
Meso compounds:	Derivatives in which the C-2 vinyl group has been reduced to ethyl
Chlorins e:	Derivatives of pheophorbide resulting from cleavage of the isocyclic ring
Rhodins g :	The corresponding derivatives from pheophorbide

The chlorophylls can be altered by various methods like enzymatic, heat and acid treatment, metallo complex formation and allomerization and the various derivatives of which are shown in fig.5.

1. ***Enzymatic***

Chlorophyllase is the only enzyme known to catalyze the degradation of chlorophyll. Chlorophyllase is an esterase, and in vitro, it catalyzes cleavage of phytol from chlorophylls and its Mg-free derivatives (pheophytins), forming chlorophyllides and pheophorbides, respectively

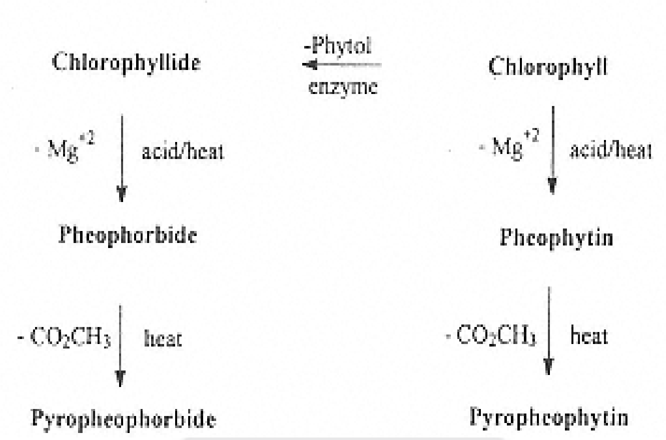


Fig.5. Chlorophyll and its derivatives.

2. *Heat and Acid*

Chlorophyll derivatives formed during heating or thermal processing can be classified into two groups based on the presence or absence of the magnesium atom in the tetrapyrrole center. Mg-containing derivatives are green in color, while Mg-free derivatives are olive-brown in color. The latter are chelators and when, for example, sufficient zinc or copper ions are available they will form green zinc or copper complexes. Chlorophyll alteration during heating is sequential, and proceeds according to the following kinetic sequence:

Chlorophyll → pheophytin → pyropheophytin

3. *Metallo Complex Formation*

The two hydrogen atoms within the tetrapyrrole nucleus of the magnesium-free chlorophyll derivative are easily displaced by zinc or copper ions to form green metallo complexes. Formation of metallo complexes from pheophytins *a* and *b* causes the red maximum to shift to a shorter wavelength and the blue maximum to a longer wavelength.

4. ***Allomerization***

Chlorophylls oxidize when dissolved in alcohol or other solvents and exposed to air. This process is referred to as allomerization. It is associated with uptake of oxygen equimolar to the chlorophylls present. The products are blue-green in color.

5. ***Photodegradation***

Chlorophyll is protected from destruction by light during photosynthesis in healthy plant cells by surrounding carotenoids and other lipids. Once this protection is lost by plant senescence, by pigment extraction from the tissue, or by cell damage caused during processing, chlorophylls are susceptible to photodegradation. When these conditions prevail and light and oxygen are present, chlorophylls are irreversibly bleached. It is believed that photodegradation of chlorophylls results in opening of the tetrapyrrole ring and fragmentation into lower molecular weight compounds.

3. ***CAROTENOIDS***

Carotenoids are nature's most widespread pigments, with the earth's annual biomass production estimated at 100 million tons. A large majority of these pigments are biosynthesized by the ocean algae population. In higher plants, carotenoids in chloroplasts are often masked by the more dominant chlorophyll pigments. In the autumn season when chloroplasts decompose during plant senescene, the yellow-orange color of carotenoids becomes evident. In all chlorophyll-containing tissues, carotenoids function as secondary pigments in harvesting light energy. The photoprotection role of carotenoids stems from their ability to quench and inactivate reactive oxygen species formed by exposure to light and air. In addition, specific carotenoids present in roots and leaves serve as precursors to abscisic acid, a compound that functions as a chemical messenger and growth regulator. The most prominent role of carotenoid pigments in the diet of humans and other animals is their ability to serve as precursors of vitamin A.

Carotenoids are comprised of two structural groups: the hydrocarbon carotenes and the oxygenated xanthophylls. Oxygenated carotenoids (xanthophylls) consist of a variety of derivatives frequently containing hydroxyl, epoxy, aldehyde, and keto groups. In addition, fatty acid esters of hydroxylated carotenoids are also widely found in nature. Thus, over 560 carotenoid structures have been identified and compiled. The basic carotenoid structural backbone consists of isoprene units linked covalently in either a head-to-tail or a tail-to-tail fashion to create a symmetrical molecule. Other carotenoids are derived from this primary structure of forty carbons. The most common carotenoid found in plant tissues is β-carotene. This carotenoid is also used as a colorant in foods. Both the naturally derived and synthetic forms can be added to food products. Some carotenoids found in plants are shown in Fig. 6. which include a-carotene (carrots), capsanthin (red peppers, paprika), lutein, a diol of α-carotene, its esters (marigold petals), and bixin (annatto seed). Other common carotenoids found in foods include zeaxanthin (a diol of β-carotene), violaxanthin (an epoxide carotenoid), neoxanthin (an allenic triol), and β-cryptoxanthin (a hydroxylated derivative of β-carotene). All classes of carotenoids (hydrocarbons: carotenes and lycopene, and oxygenated xanthophylls) are lipophilic compounds and thus are soluble in oils and organic solvents. They are moderately heat stable and are subject to loss of color by oxidation. Carotenoids can be easily isomerized by heat, acid, or light. Since they range in color from yellow to red, detection wavelengths for monitoring carotenoids typically range from approximately 430 to 480 nm.

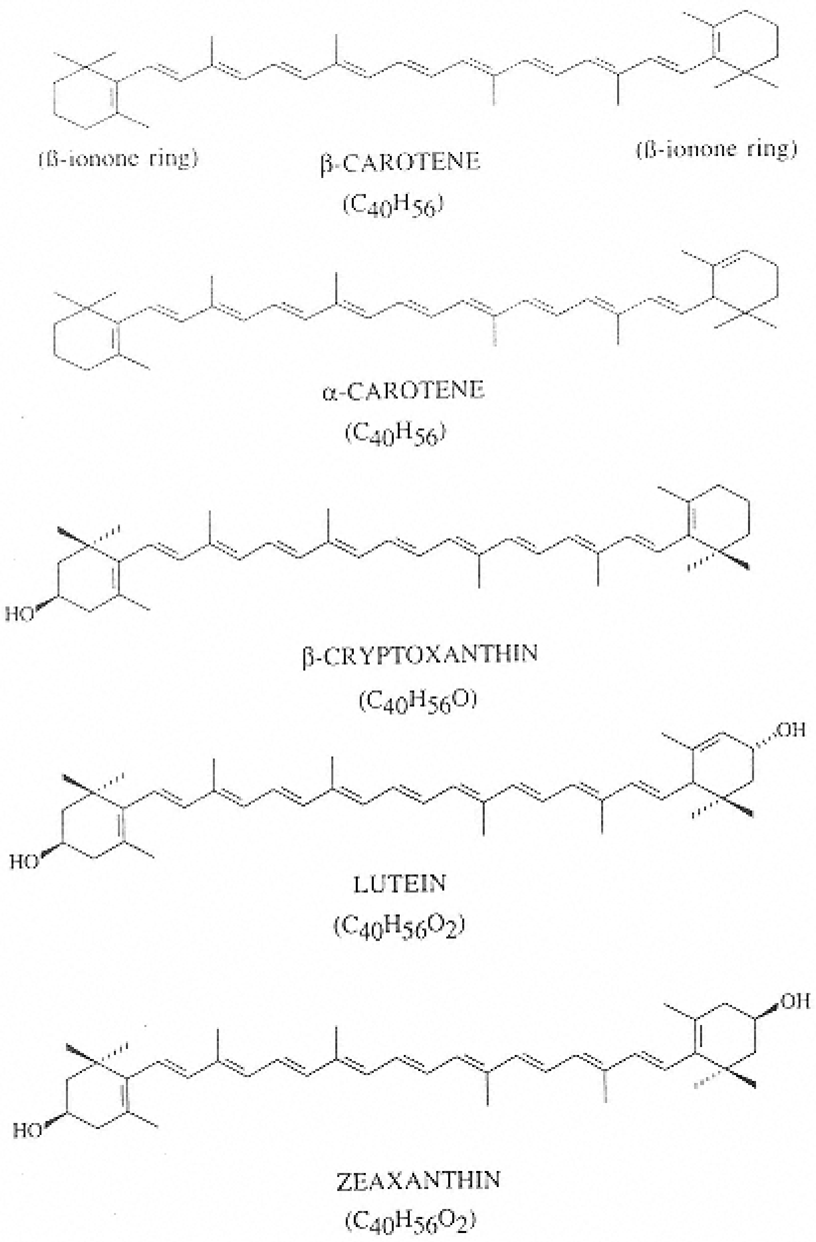
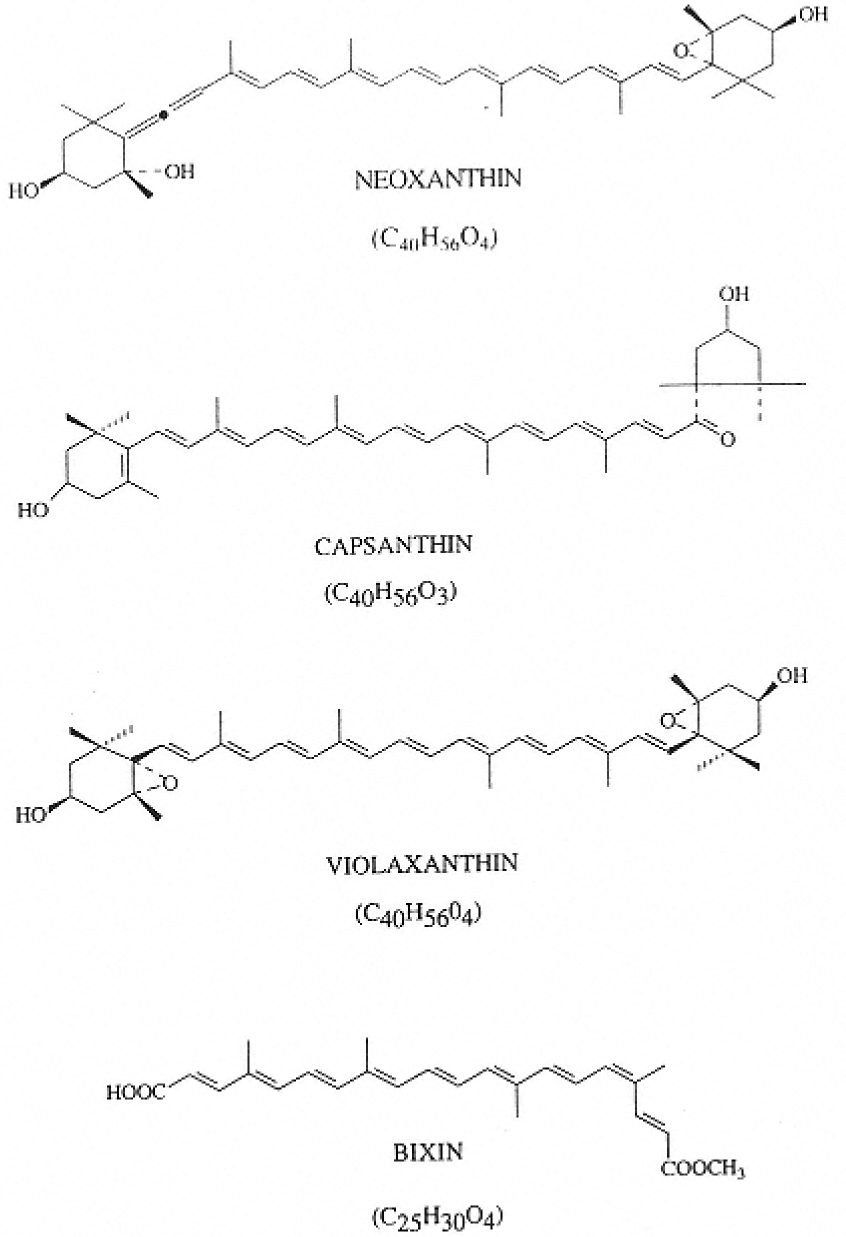


Fig.6. Structures of commonly occurring carotenoids.



NEOXANTHIN

($C_{40}H_{56}O_4$)

CAPSANTHIN

($C_{40}H_{56}O_3$)

VIOLAXANTHIN

($C_{40}H_{56}O_4$)

BIXIN

($C_{25}H_{30}O_4$)

Fig.6. continued…..

4. ***Flavonoids and Other Phenols***

4.1Anthocyanins

Anthocyanins are responsible for a wide range of colors in plants, including blue, purple, violet, magenta, red, and orange. The word anthocyanin is derived from two Greek words: *anthos,* flower, and *kyanos,* blue. These compounds have attracted the attention of chemists for years, two of the most notable investigators being Sir Robert Robinson (1886–1975) and Professor Richard Willstätter (1872–1942). Both were awarded Nobel prizes in chemistry, in part for their work with plant pigments. Anthocyanins are considered flavonoids because of the characteristic C6C3C6 carbon skeleton. The basic chemical structure of the flavonoid group and the relationship to anthocyanin are shown in Fig. 7.

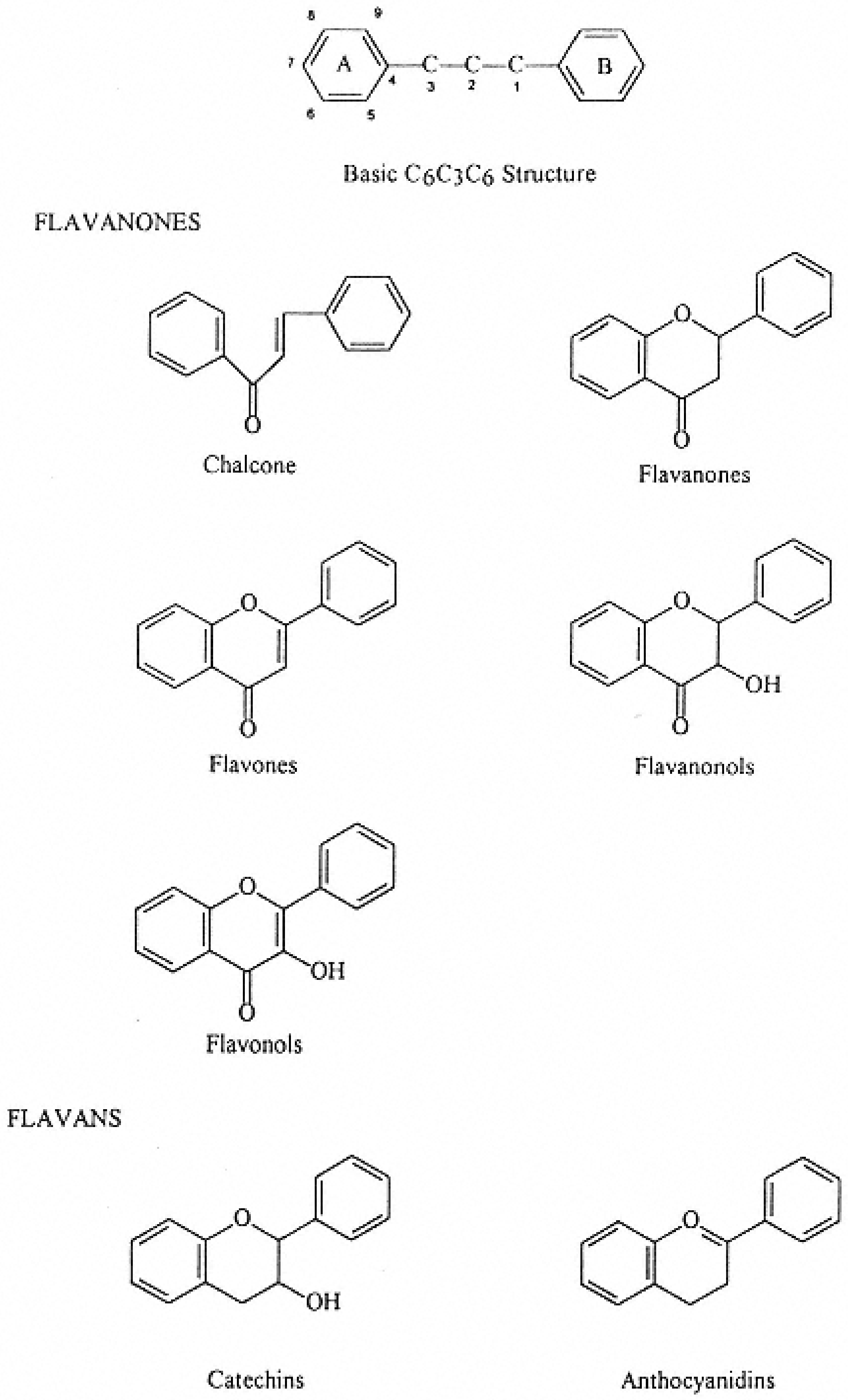


Fig. 7. Carbon skeleton of the flavonoid group.

Anthocyanin pigments are relatively unstable, with greatest stability occurring under acidic conditions. Both the hue of the pigment and its stability are greatly impacted by substituents on the aglycone. Degradation of anthocyanins occurs not only during extraction from plant tissue but also during processing and storage of foods tissues. Anthocyanins, as previously mentioned, are the most prevalent flavonoids. Although most yellow colors in food are attributable to the presence of carotenoids, some are attributable to the presence of nonanthocyanin-type flavonoids. In addition, flavonoids also account for some of the whiteness of plant materials, and the oxidation products of those containing phenolic groups contribute to the browns and blacks found in nature. The term anthoxanthin (Gr. *anthos,* flower; *xanthos,* yellow) is also sometimes used to designate certain groups of yellow flavonoids. Differences among classes of flavonoids relate to the state of oxidation of the 3-carbon link. Structures commonly found in nature vary from flavan-3-ols (catechin) to flavonols (3- hydroxyflavones) and anthocyanins. The flavonoids also include flavanone, flavononols or dihydroflavonol, and flavan-3, 4-diols (proanthocyanidin). In addition, there are five classes of compounds that do not possess the basic flavonoid skeleton, but are chemically related, and therefore are generally included in the flavonoid group.

4.2. Other Flavonoids

Anthocyanins are the most prevalent flavonoids, and most yellow colors in food are attributable

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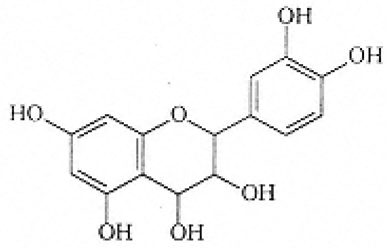
Non-anthocyanin (NA) flavonoids make some contribution to color in foods; however, the paleness of most NA-flavonoids generally restricts their overall contribution. The whiteness of vegetables such as cauliflower, onion, and potato is attributable largely to NA- flavonoids, but their contribution to color through copigmentation is more important. The chelation characteris can be converted to colored products during food processing. Proanthocyanidins are also referred to as leucoanthocyanidins or leucoanthocyanins. Other terms used to describe these colorless compounds are anthoxanthin, anthocyanogens, flavolans, flavylans, and flaylogens. The term leucoanthocyanidin is appropriate if it is used to designate the monomeric flaven-3, 4-diol (Fig. 7 a), which is the basic building block proanthocyanidins.



flavan-3,4-diol (leucoanthocyanidin)

Fig.7 a. Basic structure of proanthocyanidin.

5. BETALAINES

Plants containing betalaines have colors similar to plants containing anthocyanins. Betalaines are a group of pigments containing betacyanins (red) and betaxanthins (yellow) and their color is not affected by pH, contrary to the behavior of anthocyanins. They are water soluble and exist as internal salts (zwitter ions) in the vacuoles of plant cells. Plants containing these pigments are restricted to 10 families of the order *Centrospermae.* The presence of betalaines in plants is mutually exclusive of the occurrence of anthocyanins. The general formula for betalaines (Fig. 8.) represents condensation of a primary or secondary amine with betalamic acid. Betalains are vacuolar plant pigments. Hence their hydrophilic nature is comprehensible. Although they are slightly soluble in ethanol and methanol, water is the best suited solvent both for stability and solubility reasons. In contrast to the anthocyanins, the betalains are even more polar as can be demonstrated by shorter retention times in RP-HPLC and lower solubilities in alcoholic solutions. Betalains are considered to be most stable at near neutral conditions in foods that are devoid of sulfites, protected from oxygen and light, and stored for short times at cooling temperatures. Hence, typical food commodities colored with betalains include dairy products, fruit fillings for bakery products, relishes, various instant products, confectionary, meat substitutes, and sausages.

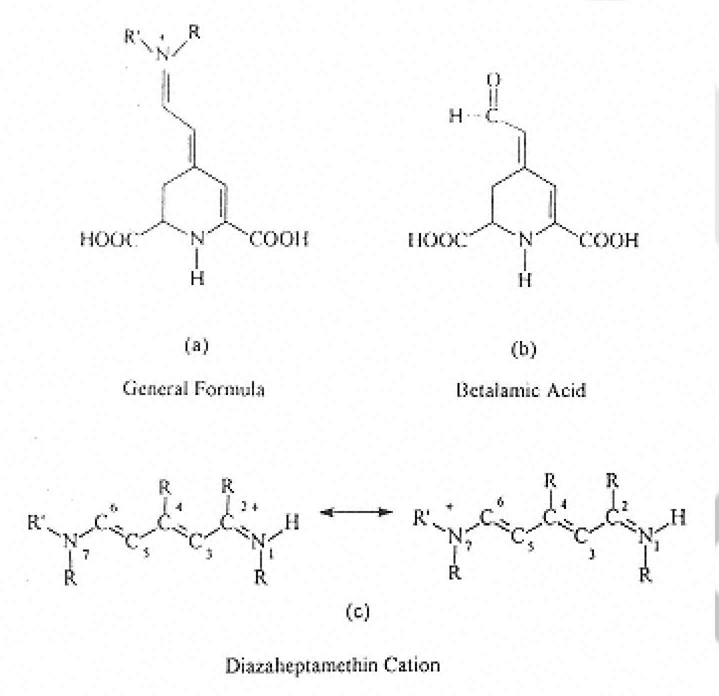
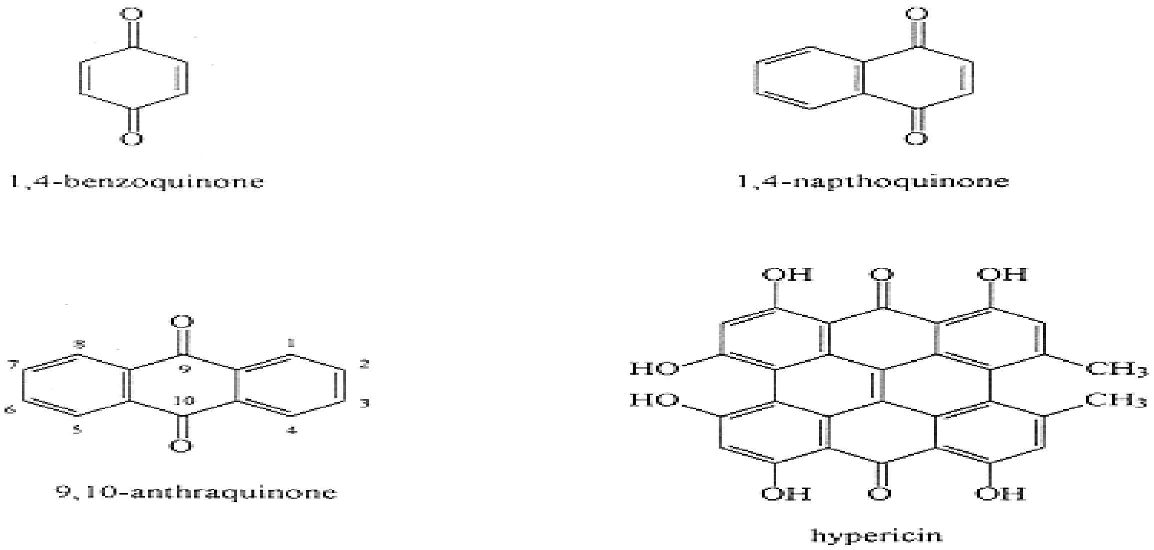


Fig. 8. General formula of betalaines.

6. QUINONES

Quinones constitute a large group of natural pigments, widely distributed in higher plants but also present in fungi, lichens, and some invertebrate animals. The common structural feature of quinones is the presence of two ketone groups on a monocyclic or polycyclic aromatic skeleton. Quinones cover a wide range of colors, from pale yellow, to orange, red, purple, and brown. Quinones are among the most widespread pigments in nature, characterized by great structural diversity. Prenylated quinones such as ubiquinones, menaquinone, and plastoquinone serve important biological functions in animal and plant tissues. Ubiquinones are ubiquitous molecules. Plastoquinone is located in chloroplasts; menaquinone (vitamin K2) is produced by microorganisms, and phylloquinone (vitamin K1) by plants. In higher plants, quinones can be found in leaves, flowers, fruits, roots, bark and heartwood. There is a long history of utilization of quinones in dyes. The best-known example is the lawsone napththoquinone present in henna, a natural dye obtained by crushing leaves of *Lawsonia alba.* Another well known naphthoquinone is juglone, isolated from buds, nut hulls, and roots of the walnut tree (*Juglans regia*). Juglone can also occur in glycosylated form, hydrojuglone β-D-glucopyranoside, representing 6 to 8% dry weight in young organs. The figure 9. shows the structure of various quinones.



1,4-benzoquinone

1,4-napthoquinone

9,10-anthraquinone

hypericin

Fig.9. Structure of quinones

Functions of quinines: Quinones play an important role in the coloration of some fungi, lichens, insects (Coccidae), and echinoderms, but they rarely contribute to the external colors of higher plants. Ubiquinone, known as coenzyme Q, plays a crucial role as a respiratory chain electron carrier transport in inner mitochondrial membranes. It exerts this function through its reversible reduction to semiquinone or to fully hydrogenated ubiquinol, accepting two protons and two electrons. Because it is a small lipophilic molecule, it is freely diffusable within the inner mitochondrial membrane. Ubiquinones also act as important lipophilic endogenous antioxidants and have other functions of great importance for cellular metabolism. Plastoquinone in plant chloroplasts and menaquinone in bacteria play similar roles in electron transport. Phylloquinone (vitamin K1) and menaquinone (vitamin K2) are essential for humans. They function mainly as lipid co-factors in blood clotting.

7. MELANINS

Melanins are complex polymeric structures, which are usually mixtures of macromolecules. Melanins are produced in mammals in two types of cells of different developmental origin: (1) the melanocytes of the skin, hair, choroids and iris and (2) the retinal pigment epithelium (RPE). Specialized organelles of the melanocytes, the melanosomes, synthesize and store eumelanins and phaeomelanins. Melanin biosynthesis in animals is a complex process starting with the L-tyrosine amino acid. In the first step, L-tyrosine is converted first into DOPA and then into dopaquinone, a process catalyzed by tyrosinase. In the biosynthesis of eumelanins, dopaquinone undergoes a cyclization to form dopachrome and subsequently a tautomerization into 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Melanins are produced in mammals in two types of cells of different developmental origin: (1)

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Melanins are classified as eumelanins, phaeomelanins and allomelanins

Eumelanins — These melanins are considered polymers derived from tyrosine derivatives, mainly 5,6-dihydroxyindole-2-carboxylic acid (DHCIA) and dihidroxyindole (DHI), with high degrees of cross-linking. *In vivo* eumelanins are associated with proteins and with metals, most frequently copper, zinc, or iron.

Phaeomelanins—This group consists of sulfur-containing polymers composed of benzothiazines, derived from cysteinyl-DOPA.

Allomelanins — These are structurally different compounds containing little or no nitrogen. They are considered polymers of phenolic compounds like catechol. Fungi produce melanin pigments, predominantly dihydroxyphenylalanine (DOPA) - melanin and dihydroxynaphthalene (DHN)-melanin.

Eumelanins are produced mainly by animals, but sometimes they also occur in plants. Eumelanins provide black and brown colors of animals and can be found as granules in hair, feather, fur, skin, scales, and integuments. They are also produced in inner tissues such as choroid, iris and inner ear.

Functions of melanin: The most evident function of melanins is providing pigmentation to mammals, invertebrates, and plants as well. In animals, melanin pigmentation is important for camouflage and display and even in defense (e.g., cuttlefish ink). In invertebrates, melanin is involved in immunity, wound healing, and sclerotization (cuticular hardening). Accumulation of melanin in the skin was considered a protective response to the damages induced by UV radiation. Several studies covered the photoprotective role of melanins against UV light. Their photoprotective effect was proven also in RPE cells by an EPR (electron paramagnetic resonance) study. It seems that melanin protects cells by absorbing the photodamaging blue light and scavenging the reactive oxygen species. Melanins can bind toxic metals, and this property may have a role in antioxidant defense and also in transcutaneous metal excretion. The role of allomelanins is not completely understood but it seems that their accumulation is related to the maturation of truffles. Plant melanins show antioxidant, liver protecting, and antitumor properties when tested in various experimental models.

Recently it was shown that fungal melanins are linked to virulence in some human pathogenic and phytopathogenic fungi and they protect fungal cells.